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Original Research Article

Prevalence and antibiogram of *Burkholderia* species isolated from a tertiary care hospital

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ABSTRACT

Background: The genus *Burkholderia* which was earlier considered as a soil saprophyte, is now gaining importance as a human pathogen. The pathogenic species include *B. pseudomallei*, *B.cepacia*, *B.mallei*, *B.gladioli* and *B.thailandensis*. Ongoing studies on *Bukholderia* spp. have resulted in the isolation of newer subspecies from human samples.
Aim: The study was undertaken to know the prevalence of *Burkholderia* spp among the clinical isolates in our hospital setup, to identify and also to evaluate their antibiogram.
Materials and Methods: Various clinical samples from patients were analysed along with their demographic data. All the specimens were processed according to the standard microbiology procedures.
Results: In a total of 4115 culture positive samples, 951 (23.11%) were identified as Non Fermenting Gram Negative Bacilli (NFGNB). 30 (3.2%) of these NFGNB were identified as *Burkholderia* spp. with an overall prevalence rate of 0.72%. 12 (40%) were further identified as *B.pseudomallei* and 18 (60%) as *B.cepacia* complex. 76.7% of the patients were above 40 years and a male preponderance was also observed (80%). Diabetes mellitus was found to be the major risk factor (60%) and fever was the commonest presentation (53.3%). Antibiotic sensitivity testing showed highest sensitivity to minocycline and cotrimoxazole and the least to imipenam.
Conclusion: This study provides a baseline data of the present scenario of *Burkholderia* infections in our hospital. A continuing study will be beneficial in identifying a number of cases as this is a grossly under-reported organism.

1. Introduction

Non-fermenting gram negative bacilli (NFGNB) are a diverse group of opportunistic pathogen that neither utilize carbohydrates as their source of energy nor utilize it oxidatively.[1] They have emerged as opportunistic pathogens causing health care associated infections (HAI) such as septicemia, meningitis, pneumonia, urinary tract infection (UTI) and surgical site infections (SSI) in the hospital settings.[2] Predominant among the NFGNB's are *Pseudomonas aeruginosa* followed by *Acinetobacter calcoaceticus baumanni complex, Pseudomonas fluorescence, Pseudomonas stutzeri, Stenotrophomonas maltophilia, Pseudomonas putida and Burkholderia cepacia complex*.[3] *Burkholderia* species belongs to the β proteobacteria and was first described in 1942 by Walter Burkholder as a phytopathogenic organism affecting carnation and onions. It belongs to the *Burkholderiaceae* family and consists of diverse species which include both "friends and foes".[4,5] Some of the friendly *Burkholderia* species are widely used in the biotechnological and agricultural industries for bioremediation and biocontrol. They are gram negative saprophytes living in the soil, water reservoirs and in the

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rhizosphere in the endemic regions such as Southeast Asia and the Northern Australia.[6] The genus is currently composed of more than 99 species but the 3 most notable pathogens to humans and animals are *Burkholderia cepacia complex* (*BCC*), *B. psuedomallei* and *B. mallei. BCC* is both a nosocomial pathogen and a cause of infection in cystic fibrosis patients, B.pseudomallei causes melioidosis and Burkholderia mallei cause glanders disease.[7] Apart from the above 3 species of *Burkholderia*, other species isolated from clinical samples include B*urkholderia thailandensis*, *B. fungorum* and *B. gladioli.*[8,9] Since this group of organism as causative agents are grossly under reported or wrongly reported as *Pseudomonas* species, we decided to study the prevalence of this organism in our hospital.

2. Materials and Methods

This is a prospective study conducted in the Department of Microbiology in a tertiary hospital from December 2014 to July 2016. Various clinical samples from patients were analyzed. All the specimen on receiving were microscopically examined by Gram staining and were inoculated onto Sheep Blood agar (5%) and Mac Conkey agar. The plates were incubated aerobically at 37^0C for a minimum of 5 days. The organisms were identified based on their colony morphology, growth and biochemical reactions. The isolates of NFGNBs were further identified as *Burkholderia* species based on oxidase test, resistance to Polymyxin B, Colistin and Gentamicin and other relevant conventional biochemical reactions like, oxidative utilization of glucose, lactose, mannitol and maltose, liquefaction of gelatin, reduction of nitrate and hydrolysis of arginine. Case records of patients having infection with *Burkholderia spp.* were further analysed for their clinical presentations, underlying risk factors and other demographic details.

2.1. Antibiotic sensitivity testing

The clinical isolates of *Burkholderia* spp. were subjected to antibiotic sensitivity testing according to the CLSI guidelines 2016.[10] The isolates were inoculated onto Mueller Hinton's agar and sensitivity testing was done by using Kirby Bauer's disk diffusion method with the relevant antibiotics.

3. Result

In our study, a total of 4115 culture positive samples were analysed, among which 951 were identified as Non Fermenting Gram Negative Bacilli. Out of these NFGNBs, 30 isolates were identified biochemically to belong to *Burkholderia* spp. The prevalence rate of *Burkholderia* spp. was 0.72%. Of the 30 *Burkholderia* isolates, 12 (40%) were further identified as *B.pseudomallei* and the remaining 18 (60%) were of *B. cepacia*.

The distribution of *B.pseudomallei* and *cepacia* isolates in the various clinical samples other than urine is as shown in the Table below. The urine samples analyzed over this period did not have any *Burkholderia* isolates, so that they were not included in the results. The patients from whom *Burkholderia* spp. was isolated, were further categorized as bacteremic (those which are blood culture positive with a single or no identifiable focus of infection) and nonbacteremic (localized) cases. Of the 12 isolates of *B.psuedomallei*, 4 (57.1%) were isolated from blood alone, 2 (28.6%) from blood and pus sample and 1 isolate (14.3%) from blood and knee aspirate. Amongst the *B.cepacia* 38.9% (7 cases) had bacteremia of which 6 (85.7%) were isolated from blood alone and 1 (14.3%) from blood and bronchoscopic washing.

Table 1: Distribution of *Burkholderia* spp. among various samples

Samples	*B. pseudomallei*	*B. cepacia*
Blood	4	6
Pus	3	5
Pus + Blood	2	-
Blood + Body fluids	1	1
Body fluids (stomach aspirate)	1	-
Sputum	1	3
Bronchoscopic washings and ET secretions	-	3

Male predominance was seen in this study as 24 (80%) of the patients were males. The age wise distribution studied also showed that 23 (76.7%) patients with Burkholderia infection were above 40.

Table 2: Gender distribution of *Burkholderia* spp. Infection

Gender	*B.pseudomallei*	*B.cepacia*
Male	11 (92%)	13 (72%)
Female	1 (8%)	5 (28%)

Table 3: Age wise distribution of *Burkholderia* infected patients

Age	*B. pseudomallei*	*B. cepacia*
0 - 20yr	1	2
21 - 40yr	2	2
>40yr	9	14

The factors that predispose to *Burkholderia* infection were studied and it was found that Diabetes Mellitus Type II (DM) was the major predisposing factor in 18 patients (60%) and 6 (23.3%) of the cases were smokers and alcoholics. No underlying risk factors were found in 4 (13.3%) patients. In most of the patients with infection, there was overlapping of risk factors.

Patients with *Burkholderia spp.* infection had varied clinical presentations of which fever (16 cases- 53.3%)

Table 4: Predisposing factors in the *Burkholderia* cases

Risk Factors	*B. pseudomallei*	*B. cepacia*
Diabetes Mellitus	8 (66.7%)	10 (55.6%)
Alcoholic/smoking	3 (25%)	3 (16.7%)
COPD/TB	1 (8.3%)	5 (27.8%)
CKD	1 (8.3%)	2 (11.1%)
Trauma	_	1 (5.6%)
Preterm	1 (8.3%)	_
Others	_	2 (13%)
No risk factors	1	3

was the major presentation. Respiratory tract infections were seen in 13 (43.3%) cases and their presenting symptoms included cough, breathlessness, respiratory distress, pneumonia and 1 case of hydropneumothorax. Abscess and soft tissue infections was seen in 8 (27.6%) of the patients. There was overlapping of presentations in many of the cases.

Table 5: Clinical presentation of patients with *Burkholderia* isolates

Presentations	*B.pseudomallei*	*B.cepacia*
Fever	6 (50%)	10 (55.6%)
Respiratory Symptoms	4 (33.3%)	9 (50%)
Abscess & soft tissue infections	5 (41.6%)	3 (16.7%)

Of the 30 isolates of *Burkholderia spp*, 23 (76.7%) were from patients who had presented during the heavy monsoon months from June to September (southwest monsoon). Among the infected patients, 17 (56.7%) of them were involved in various occupational activities like farming, mining etc. Of these 17, 6 patients presented with melioidosis (50%) and 11 had infection with *B.cepacia* (61.1%).

Antibiotic sensitivity of *B.pseudomallei* was done by Kirby Baeur's disc diffusion method using as per the guidelines of CLSI 2016. The antibiotic discs used for testing included Ceftazidime (CAZ), Imipenem (IPM), Meropenem (MRP), Minocycline (MI), Levofloxacin (LE), Amoxiclav (AMC) and Cotrimoxazole (COT). According to CLSI 2016 guidelines doxycycline should be used for sensitivity testing of *B.pseudomallei*. Since it was not there in the panel of antibiotics, the sensitivity of doxycycline could not be assessed. All the *B. pseudomallei* strains showed 100%

sensitivity to Minocycline and Cotrimoxazole, 91.6% sensitive to Levofloxacin, 83.3% sensitive to Amoxiclav and Ceftazidime, 75% sensitive to Meropenem and 58.3% sensitive to Imipenem.

For *B.cepacia*, antibiogram was done in Vitek II compact (bioMerieux) as per the guidelines of CLSI 2016. Antibiotic discs were chosen included Ceftazidime (CAZ), Imipenem (IPM), Meropenem (MRP), Minocycline (MI), Levofloxacin (LE), ticarcillin-clavulanate and Cotrimoxazole (COT). All the 18 strains showed 100% sensitivity to minocycline and Cotrimoxazole, 94.4% sensitivity to Levofloxacin, 77.8% sensitivity to Meropenam and Ceftazidime and 38.8% sensitivity to Imipenam. 3 strains (16.7%) showed resistance to Ceftazidime and 1 strain showed intermediate sensitivity.

Table 6: Antibiogram of *Burkholderia pseudomllei* isolates

Discs	Sensitive	Intermediate	Resistant
Ceftazidime (Caz)	10	2	_
Imipenem (Ipm)	7	1	4
Meropenem (Mrp)	9	_	3
Cotrimoxazole (Cot)	12	_	-
Amoxiclav (Amc)	10	_	2
Levofloxacin (Le)	11	1	_
Minocycline (Mi)	12	_	_

Table 7: Antibiogram of *Burkholderia* cepacia isolates

Discs	Sensitive	Intermediate	Resistant
Ceftazidime (Caz)	14	1	3
Imipenem (Ipm)	7	1	11
Meropenem (Mrp)	14	1	3
Cotrimoxazole (Cot)	18	-	-
Ticarcillin+clavulanate (Ti)	14	_	4
Levofloxacin (Le)	17	1	_
Minocycline (Mi)	18	_	_

4. Discussion

Burkholderia is a Non-fermenting gram negative bacilli (NFGNB) which belongs to rRNA group II. It can be differentiated from *Pseudomonads* by its property of showing resistance to the polymyxin group of antibiotics (polymyxin B 300μg and colistin 10μg). During our study, we had 30 isolates of (3.1%) *Burkholderia* spp. which included *B. pseudomallei* and *B. cepacia*. This was similar to a study done by Hu Yan Jian who had an isolation rate of 2.9% *Burkholderia* species.[11]

Sample wise prevalence of *B.pseudomallei* and *B.cepacia* was studied. Of the 12 isolates of *B. pseudomallei*, 7 patients i.e. 58.3% had bacteremia and the remaining 5 patients (41.7%) presented with features of localized melioidosis. This was similar to studies by Suputtamongkol[12] where the incidence of bacteremic melioidosis was 58% and according to the study by Cheng

and Currie the incidence of localised melioidosis was 44.8%.[13] Of the 18 isolates of *B. cepacia*, 7 (38.9%) patients had bacteremia. In a study by Reik and LiPuma, the isolation of *B.cepacia* presenting as bacteremia was 15.5%.[14] In our study *B.cepacia* isolates also showed localized infection and it was 61.1%.

Majority of our *Burkholderia* spp. were isolated during the monsoon season (June September) i.e. 23 cases (79.3%). This finding was consistent with the study by Vidyalakshmi et al., in the coastal regions of Kerala and Karnataka, where 80% melioidosis patients presented during monsoon[15] thus proving its seasonal distribution. The saprophytic nature of *B.pseudomallei* was 1st recognized in 1955 by French Indo China. Also sampling studies in Australia have suggested that bacterial counts are increased at a depth of 60-90 cms. The association of increased isolation of *Burkholeria* spp. during monsoon was explained by Currie and Jacups in their study, to the movement of the bacteria from the deeper soil layers to the surface with the rising water table.[16] Similarly Ramsay et al. in his study found the increased incidence of *B. cepacia* cases during the heavy rainfall.[17]

Studies by Cheng and Currie on the relationship between occupation and melioidosis showed that there was an increase in the incidence of melioidosis among the people with occupational and recreational exposure to surface water and soil particularly with flooding of rice fields and farming.[13] In our study on patients with melioidosis, occupational association was seen in 50% with the majority of them being agricultural workers, fishermen, construction workers etc. Vidyalaksmi et al.,[15] Beena et al[18] and Currie et al[13] in their studies also showed a higher incidence of exposure among agricultural workers and those involved in outdoor maintenance. There are no proven studies showing the association of occupation with the acquisition of *B. cepacia* infection. But 61.1% of our patients were involved in agricultural and construction activities. So this may be explained by Ludovic et al[19] as the fact that, *B.cepacia* being a soil saprophyte, the clinical strains of *cepacia* would have been acquired from natural environment. A higher proportion of *Burkholderia spp*. infection was seen among males (80%) in our study of which 45.8% males had *B.pseudomallei* infection and 54.2% had cepacia infection. This was explained by Vidyalakshmi et al[15] in her article, as the higher exposure of men to outdoor activities. In a study by Rahbar et al[20] on *B.cepacia*, female patients were more (58.3%) compared to males (41.7%) which is in variance with our findings where we had only 28% females with *B.cepacia* infection.

Age wise statistics in our study showed a median age of 52.2 with the youngest age presented being a 3-day-old child to the oldest being 79 years. The commonest age group presented was above 40 years (76.7%) which is consistent with other studies. 75% of melioidosis cases were above 40 years which is similar with the findings by Vidyalaksmi et al. (75.8%).[15] Among *B.cepacia* infected patients, 77.8% of cases were above 40 years.

Underlying risk factors associated with infection by *Burkholderia spp*. has been studied, as this organism is known to cause disease in the immunocompromised as well as the healthy.[21] In studies worldwide and in our study, it has been seen that Diabetes mellitus is a major underlying risk factor among patients infected with this organism. Increasing prevalence of this lifestyle disease, could be a contributing factor to the increase in infections by this bacteria. In our study it was seen that 80% of the patients infected with *Burkholderia spp*, had one or more of the risk factors with the major risk factor being diabetes mellitus (DM- 60%). Further the risk factors among melioidosis patients were studied where DM was found to be the leading risk factor. Studies from regions endemic for melioidosis also showed Diabetes mellitus as the major risk factor with rates varying in different regions. Vidyalakshmi et al.[15] in her study reported that 75.8% of her study subjects had diabetes which appears to be the highest reported so far. A study from Thailand associated this increased incidence of melioidosis among diabetic patients to defective polymorphonuclear leukocytes (PMNLS). This results in impaired phagocytosis of the organism, reduced migration in response to interleukin-8 and inability to delay apoptosis when compared to PMNLs in nondiabetic patients.[22]

Chronic obstructive pulmonary disease and tuberculosis was seen as a next important risk factor. In our study, 8.3% melioidosis patients had this risk factor whereas Saravu et al. in her study had 4% cases.[23] Chronic kidney disease (CKD) was also seen as a risk factor among our patients with Burkholderia infection. Mukhopadhyay et al. in his study had 8% cases with renal disease which was consistent with our finding of 8.3% of our melioidosis patient with the same.

Unlike studies where cystic fibrosis was found to be the major risk factor in *B.cepacia* infection, our study did not have any reported case of the same. In our study DM was found to be the major risk factor in patients infected with *B. cepacia* followed by COPD and TB to be the next in row (27.8%). In a study by Matthaiou et al.,[24] he explains that patients with COPD have pulmonary lesions which may be niches for chronic colonizers thus attributing *B.cepacia* to be a chronic colonizer, which may cause lung disease in patients other than with cystic fibrosis. CKD was seen among 11.1% of *B.cepacia* patients in our study while Bressler[25] had 20% *B.cepacia* patients with CKD as the risk factor.

The varying clinical presentation of infection with *Burkholderia spp*. was studied and fever was found to be the major presenting symptom (53.3%). Other presentations were respiratory symptoms (43.3%) and abscesses and soft tissue infection (27.8%). 50% of the melioidosis patients

presented with fever which was the similar finding in studies by Gopalakrishnan et al.[26] (65%) and Saravu (80%).[23] The next common presentation among melioidosis patients was abscesses and soft tissue infections- (41.6%) which includes one case of epidural abscess and 1 case of perigastric abscess. In a study by Mukhopadhyay et al.,[23] 32% of the melioidosis patient had skin and soft tissue involvement. Cheng has described in his article of melioidosis that abscesses may act as a source of systemic infection by hematogenous spread. Respiratory infection was the next prominent symptom in 33.3% (4 cases) patients with melioidosis. Out of the 4 with respiratory infections, only 2 had presented with radiographic evidence and the rest where sputum or blood culture positive. Positive sputum culture among melioidosis patients without radiologic changes has been previously reported in a Thai series in which 40% of melioidosis patients with normal chest radiograph had *B. pseudomallei* isolated from their sputum. These patients were not severely unwell. It is likely that this represents respiratory tract melioidosis at the milder end of the spectrum rather than incidental detection of colonizing B. pseudomallei.[27]

B. cepacia is associated with a wide variety of infections including pneumonia, bacteremia, skin and soft tissue infections. In our study, among the *B.cepacia* infected patients, 55.6% presented with fever as the major manifestation followed by respiratory symptoms in 50% and soft tissue infections in 16.7%. Our study also showed that, patients with *B.cepacia* infection had more respiratory symptoms when compared to patients with B. pseudomallei. The higher degree of colonization by *B. cepacia* in the upper respiratory tract accounts for the increased respiratory symptom which could be the reason for this finding.

The antibiogram of *Burkholderia* species was studied. It was found that the organism showed maximum sensitivity to Minocycline and Cotrimoxazole (100%) and the least to Imipenem (46.7%). Levofloxacin showed a sensitivity of 93.3%, ceftazidime and ticarcillin clavulanate (80%) and meropenem (76.7%). Most of the studies has mentioned ceftazidime as the drug of choice for infection with *Burkholderia*. Our study had 3 isolates (10%) that showed resistance to Ceftazidime. Because of the non-availability of doxycycline in the panel of antibiotics, its sensitivity pattern against *B. pseudomallei* could not be assessed. In our study, there were 5 deaths (16.7%) reported and all were *B. cepacia* infected patients. There were no deaths or relapse reported in melioidosis patients. Out of the 5 patients who expired, 4 patients (17.4%) were in the age group above 40 years and had risk factors like CKD, Malignancy, neurologic diseases and COPD. There was no enough clinical evidence to suggest that the deaths were due to infections by *B.cepacia* as all these patients had underlying risk factors which may also have contributed to the death. More studies on a larger sample size over a longer period of time will be required to pin point *Burkholderia* infection as the primary cause of death among those infected with this organism.

5. Conclusion

Despite advances made in understanding the basic biology and pathogenesis of this organism, our understanding of the epidemiology and ecology of the organism remains poor. *Burkholderia spp.* causes infection of the blood, soft tissue and respiratory tract. Since clinical diagnosis of infection caused by this bacteria is not possible, most cases worldwide probably go unrecognised because they occur in people who have limited access to diagnostic facilities.

Laboratory diagnosis with identification of these bacteria becomes mandatory. Many a times, there are no advanced diagnostic facilities to confirm the aetiological agent and are reported as NFGNB or simply as *Pseudomonas* species. To facilitate diagnosis, all NFGNB grown in clinical specimens should be speciated.

Several studies, including ours, showed that these organisms are still sensitive to many of the recommended antibiotics. The infections caused by them respond well to antibiotics with melioidosis requiring a prolonged therapy for upto 12 weeks or longer. A combination of a high index of suspicion, culture confirmation, and prompt and appropriate therapy with recommended drugs will result in an excellent outcome in majority of the patients.

6. Source of Funding

None.

7. Conflict of Interest

None.

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