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Original Article

Trends in laboratory detection of *Burkholderia pseudomallei* and their antibiotic susceptibility pattern from clinical samples over twenty one years from 2001 to 2021 in a tertiary care hospital of Bangladesh

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Abstract
Melioidosis, a highly fatal infectious disease caused by *Burkholderia pseudomallei*, is largely under-recognized and underreported due to lack of alertness of health care professionals. The real burden of melioidosis in our country is poorly understood. Here, we have documented laboratory isolation trends of *B. pseudomallei* from different clinical samples sent to our Microbiology department from 2001 to 2021, with the aim to increase awareness among clinicians and microbiologists in the health care community of Bangladesh. A total of 86 *B. pseudomallei*, isolated from culture of different clinical samples in 68 melioidosis cases were studied retrospectively from hospital records. Antimicrobial susceptibility result by Kirby Bauer disk diffusion method was also analyzed. The most commonly affected age group was 46-60 years and majority of them (88.2%) had diabetes mellitus. Septicaemia (51.5%) was the most common presentation followed by skin and subcutaneous tissue abscess (29.4%), septic arthritis (16.2%), UTI (13.2%), pneumonia (10.3%), visceral abscess in lung (5.9%), liver (5.9%), spleen (7.3%) etc and death was reported among 32.5% cases. One case of laboratory acquired melioidosis was diagnosed from cleaner of our Microbiology laboratory. Average isolation rate of *B. pseudomallei* was 5.6 per year in our centre. The organism was isolated most frequently from blood (40.7%) and pus/wound swab (30.2%) samples. All the isolates were highly sensitive (>95%) to ceftazidime, carbapenem, piperacillin-tazobactum, amoxicillin-clavulanic acid and tetracycline, but 53.4% and 67.2% of our strains were recorded sensitive to co-trimoxazole and ciprofloxacin by disc diffusion method. In conclusion, melioidosis might be included as notifiable disease in national surveillance system of Bangladesh to provide key part in priority-setting, planning, also monitoring and evaluation of the disease control programs. Prompt early diagnosis and appropriate antibiotic administration are the critical steps to prevent mortality and morbidity from this deadly disease.

Keywords: Melioidosis, *Burkholderia pseudomallei*, antibiotic susceptibility, Bangladesh

Introduction

Bangladesh is documented as a 'definite' endemic country for melioidosis[1], a potentially fatal infectious disease caused by *Burkholderia pseudomallei* which requires intensive antimicrobial treatment. Limmathurotsakul et al[2]. estimated total incidence of melioidosis case globally and in Bangladesh would be 1,65,000 and 16,931 using a statistical model in 2015. So far, approximate 54 culture positive cases were reported[3,4] from our country. Therefore, it is presumed that melioidosis is severely underreported in Bangladesh. Although melioidosis is known for a century years ago, most of the physicians still missed and misdiagnosed the disease as it produces protean clinical manifestation and mimic tuberculosis or other pyogenic bacterial infections. A lack of familiarity of microbiologists with the cultural characteristics of *B. pseudomallei* has also resulted in failure of recognition, identification, diagnosis and treatment. Awareness and high index of suspicion from both clinical and microbiology lab personnel are a prerequisite for correct identification of the offending organism. Laboratory diagnosis of

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melioidosis is usually made in majority of cases by culture of blood, pus or wound swab, respiratory secretions or joint fluid depending on the site of infection. The organisms grow slowly (usually 48 hrs of incubation) in ordinary media including MacConkey agar and Blood agar media. Colony morphology is variable and a single strain may display multiple colony types[5]. So, inexperienced laboratory staff frequently mistake it as contaminants. Typical characteristic features are metallic luster and wrinkling appearance of colony with prolong incubation (3-4 days) and its inherent resistance pattern for aminoglycosides and polymyxins. Definite identification of this organism (up to species level) is very much crucial as treating it with ordinary antibiotic regimen is ineffective and disease is highly fatal[6] if left untreated. Treatment of melioidosis requires prolong and definitive course of antibiotics which comprises two phases. In the first acute phase, parenteral drugs with ceftazidime (uncomplicated cases) or meropenem (in neuromelioidosis or complicated cases) for 10-14 days followed by eradication phase with oral co-trimoxazole or Amoxicillin/clavulanic acid for 12 weeks are recommended[7]. This study identifies melioidosis cases (published and unpublished) and isolation trends of *B. pseudomallei* in the microbiology laboratory of a tertiary care hospital over 21 years period with the aim to increase awareness among clinicians and microbiologists in the health care community of Bangladesh.

Materials and Methods

All *B. pseudomallei* detected from clinical samples sent to the Microbiology laboratory of BIRDEM General Hospital, Dhaka, Bangladesh over a period of 21 years were analyzed retrospectively in this study. Organism was isolated from blood, urine, respiratory secretions, pus and wound swab from in and out patients sample during 2001 to 2021. Samples were cultured in Blood agar and MacConkey agar media. *B. pseudomallei* was identified by typical colony morphology in routine laboratory and selective Ashdown agar media, Gram staining (bipolar staining), motility, biochemical tests, and inherent resistance pattern to colistin and aminoglycosides[8]. Monoclonal antibody based latex agglutination test (Melioidosis Research Center, KhonKaen, Thailand) was performed for the final identification and confirmation of *B. pseudomallei*. The isolates were tested for susceptibility to ceftazidime (30 µg), meropenem/imipenem (10 µg), piperacillin-tazobactum (30 µg), amoxicillin-clavulanic acid (30 µg), tetracycline, TMP-SMX (1.25/23.75 µg), gentamicin (10 µg) and amikacin (30 µg) by disc diffusion technique[9]. Briefly, a 0.5 McFarland suspension of each bacterial isolate was made in normal saline and inoculated onto Mueller-Hinton agar (Mast diagnostics Ltd, UK) to prepare a uniform lawn. The disc were applied at a specific distance from each other and the zone of inhibition around the antibiotic disc was measured after 18 h of incubation of plates at 37˚C. Interpretative threshold zone sizes of tested antibiotics for *B. pseudomallei* used from earlier reports[10-13] are shown in Table 1. The zone of inhibition was interpreted as sensitive, intermediate and resistant according to CLSI guideline.

Table 1: Zone diameter interpretive standards for *B. pseudomallei* used in the study

Antimicrobial agents	Disk content (µg)	Resistant	Intermediate	Susceptible
Ceftazidime	30	≤ 14	15-17	≥18
Imipenem	10	≤15	16-18	≥19
Meropenem	10	≤12	13-15	≥16
Amox-Clav	20/10	≤13	14-17	≥18
TMP-SMX	1.25/23.75	≤10	11-15	≥16
Tetracycline	30	≤12	13-15	≥16
Piperacillin-Tazobactam	100/10	≤14	15-20	≥21
Amikacin	30	≤14	15-16	≥17
Gentamicin	10	≤12	13-14	≥15

Results

A total of 86 *B. pseudomallei* isolated from culture of different clinical samples from 68 patients were analyzed. The patients were diagnosed to be suffering from melioidosis based on clinical symptoms and microbiological examination. The sociodemographic and clinical records of these patients are given in Table 2. Of the total patients, 57 were male and 11 were female. All of them were adults (mean age 47.9±12.4), except 1, who was 11 years old presented with septicaemia and neck abscess. The most commonly affected age group was that between 46-60 years. Most of them (88.2%) presented with diabetes mellitus. Occupational history revealed 17 (25%) were farmers, 7 (10.3%) overseas workes returned from Thailand, Brunei, Malaysia, Singapore and Qatar and rest were construction worker, businessman, carpenter, housewife etc. One case of laboratory acquired melioidosis was diagnosed from cleaner of our Microbiology department in the year 2020. Fever (100%) with cutaneous lesion (32.3%) was the predominant symptoms among the study population. Septicaemia (51.5%) was the most common presentation

along with skin and subcutaneous tissue abscess (29.4%), septic arthritis (16.2%), UTI (13.2%), pneumonia (10.3%), visceral abscess in lung (5.9%), liver (5.9%), spleen (7.3%) and also abscess in other sites (Table 2). Among the cases culture was positive from 51.5% of blood followed by pus/wound swab (38.2%), urine (16.2%), respiratory secretions (10.3%) and joint fluids (10.3%). Out of 68 melioid cases, 22 died, while the outcome of 11 was unknown. High number of cases were identified during 2015 (13/68, 19.1%) and 2019 (10/68, 14.7%) by culture given in Table 3. Out of 86 *B. pseudomallei* isolates, 35 (40.7%) were isolated from blood sample, 11 (12.8%) from urine, 26 (30.2%), 7 (8.1%)from pus/wound, joint fluid & sputum/tracheal aspirated sample respectively (Table 4). All (100%) the isolates were sensitive to ceftazidime and piperacillin-tazobactum. Majority of them also sensitive to imipenem (98.3%), amoxicillin-clavulanic acid (96.1%) where as 53.4% and 67.2% were found sensitive to co-trimoxazole and tetracycline by disc diffusion method (fig 1).

Table 2: Sociodemographic and clinical records of 68 culture confirmed cases of melioidosis in BIRDEM General Hospital from year 2001 to 2021

Antimicrobial agents	Disk content (µg)	Resistant	Intermediate	Susceptible
1. Mean age in years (±SD)				47.9±12.4
• <15 years				1 (1.5)
• 15-30 years				6 (8.8)
• 31-45 years				21(30.9)
• 46-60 years				31(45.6)
• >60 years				9 (13.2)
2. Sex				
• Male				57 (83.82)
• Female				11 (16.18)
3. Occupation				
• Farmer				17 (25)
• Businessman				7 (10.29)
• Housewife				9 (13.23)
• Construction worker				2 (2.94)
• Overseas worker(Thailand, Brunei, Malaysia, Singapore, Qatar)				7 (10.29)
• Others (teacher, student, political person, clerical jobs, carpenter, Microbiology lab etc)				17 (25)
• Unknown				9 (13.23)
4. Risk factors				
• DM				60 (88.23)
5. Symptoms				
• Fever				68 (100)
• Cough				10 (14.7)
• breathlessness				05 (7.35)
• Wt loss				02 (2.94)
• Dysuria/burning micturition				13 (19.11)
• Joint pain/swelling				15 (22.05)
• Abdominal pain				12 (17.64)
• Diarrhea				01 (1.47)
• Cutaneous lesion				22 (32.35)
• Headache				2 (2.94)
• Disorientation				4 (5.88)
• Orbital swelling				2 (2.94)
• Rash				1 (1.47)
• Anorexia				2 (2.94)
• Backache				2 (2.94)
6. Major clinical presentation				
• Septicaemia				35 (51.47)
• Pneumonia				7 (10.29)
• Lung abscess				4 (5.88)
• Liver abscess				4 (5.88)
• Splenic abscess				5 (7.35)
• Prostatic abscess				4 (5.88)
• UTI				9 (13.23)
• Septic arthritis				11 (16.17)
• Electrolyte imbalance				4 (5.88)
• Skin/Subcutaneous tissue abscess				20 (29.41)
• Bartholin abscess				1 (1.41)
• Septic shock				1 (1.41)
• Fascial cellulitis				1 (1.41)
• Epidural abscess				1 (1.41)
7. Culture positive samples				
• Blood				35 (51.47)
• Urine				11 (16.17)
• Pus and Wound swab				26 (38.23)
• Sputum/Tracheal aspirate				7 (10.29)
• Joint fluid				7 (10.29)
8. Outcome				
• Cured				35 (51.47)
• Died				22 (32.35)
• Lost to Follow up				11 (16.17)

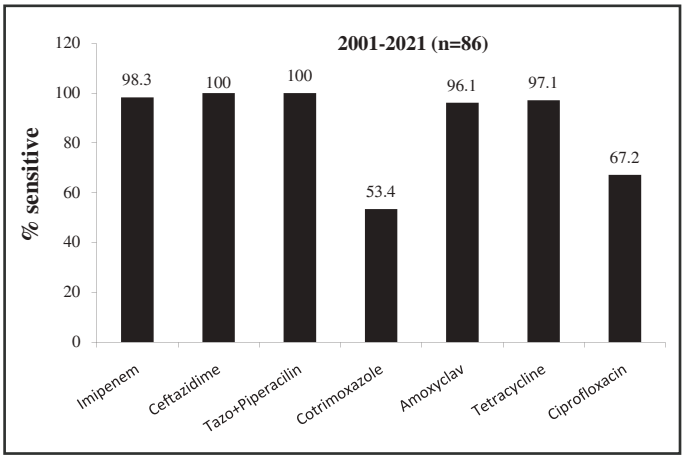
Table 3: Trends of detection of *B. pseudomallei* positive cases in Microbiology laboratory from 2001 to 2021

Year	Trends of detection positive cases	%
2001	1	1.5
2009	2	2.9
2010	2	2.9
2013	5	7.4
2014	3	4.4
2015	13	19.1
2016	8	11.8
2017	7	10.3
2018	9	13.3
2019	10	14.7
2020	2	2.9
2021	6	8.8
Total (12 years)	68	100

Table 4: Frequency of isolation of *B. pseudomallei* in different clinical sample from 2001 to 2021 (n=86)

Year	Frequency of isolation in				
	Blood	Urine	Pus/wound swab	Joint fluid	Resp Secretion
2001	-	-	1	-	-
2009	-	2	1	-	-
2010	-	1	-	-	1
2013	3	-	3	1	-
2014	1	-	1	1	1
2015	8	2	4	1	1
2016	5	-	5	1	1
2017	5	2	1	-	-
2018	6	2	1	1	-
2019	5	1	3	1	1
2020	-	-	2	-	-
2021	2	1	4	1	2
Total	**35 (40.7)**	**11 (12.8)**	**26 (30.2)**	**7 (8.1)**	**7 (8.1)**

Figure 1: Antibiotic susceptibility pattern of isolated *B. pseudomallei* from clinical samples by Kirby Bauer disc diffusion method (n=86)



Discussion

Bangladesh was considered as 'definite country' for melioidosis in 2011 when organism was recovered for the first time from soil of Gazipur district[1]. First case was reported at 1964 in a 29 year old British sailor who travelled through Chittagong[14], but melioidosis was diagnosed for the first time in a native Bangladeshi child in 1988[15]. Since then it has been sporadically reported over last several decades from Bangladeshi immigrants and also from hospitalized patients of our country[3,4,16-27]. There was no laboratory surveillance data to detect the isolation frequency and susceptibility pattern of *B. pseudomallei* in hospitals of Bangladesh. Therefore, we have reported the trends in isolation of *B. pseudomallei* from 68 cases by culture of different clinical samples during 2001 to 2021 in our Microbiology department.

In our study, most of the patients, 31(45.6%) belonged to the age group within range of 46-60 years, followed by 21 (30.9%) in the age group 31-45 years. Males were more commonly affected 83.8% (57/68). Previously Hantrakun et al[28], 2019 from Thailand had also reported the high frequency of melioidosis cases (54.4%) among the age groups of 45-64 years and a total of 67.9% infection had occurred in males.

One fourth (25%) of our patients were farmer, 13% housewife, 10% business man, 10% overseas worker returned from melioidosis endemic country (eg. Thailand, Malaysia, Singapore etc) and rest were construction workers, teacher, students and others (Table 2). Though some reports found no significant difference among different

occupational groups[1], but few reported[3] a definitive history of soil exposure and occupational activities in this group of patients. Interestingly one of our culture confirm case was diagnosed from the staff of our Microbiology lab in the year 2020 having DM. He is a 46-year-old laboratory worker who has been disinfecting and cleaning the culture tubes and plates for last 10 years. Initially, he was diagnosed clinically as rickettsial fever and treated with doxycycline for 10 days. Blood culture and triple antigen tests were found negative. Later on *B. pseudomallei* was isolated from culture of aspirated pus of subcutaneous abscess in front of anterior angle of right neck. Complete remission had occurred with definitive treatment of acute phase with injection ceftazidime for 14 days followed by oral co-trimoxazole for 3 months. This might be the first documented case of accidental occupational exposure to *B. pseudomallei* from Bangladesh. Very few cases of laboratory acquired melioidosis was reported previously[29].

Diabetes mellitus (DM) was identified as major underlying risk factor[30] (RR 5.9-13.1) for *B. pseudomallei* infection especially in South East Asia like India[31], Thailand[32], Malaysia[33] etc and Australia[34] (30% to 82% cases). In our study 88.2% of melioidosis patient have DM. As melioidosis is commonly detected in patients having uncontrolled sugar level, most of the previously reported cases[4,16-27] were detected from this tertiary care hospital which is the largest diabetes referral centre of Bangladesh.

Average annual isolation rate of *B. pseudomallei* was 5.6 (68/12 years) from different clinical samples in our center. Highest number of this offending bacteria was identified in 2015 (13 isolates) and 2019 (10 isolates). Majority of the organism were isolated from blood (40.7%) and pus/wound swab (30.2%) sample as septicaemia (51.4%) and skin/soft tissue abscess (29.4%) were the most common presentations detected among these melioidosis cases. Major obstacles in identification of *B. pseudomallei* from unsterile sample like sputum, throat swab or rectal swab/ stool is overgrowth of normal flora which may be overlooked by the laboratory personnel. Isolation frequency from these unsterile sites is perhaps further increased by the use of a selective broth preenrichment or Ashdown agar media when clinically suspected.

Detection of *B. pseudomallei* from blood culture in severe disease is associated with higher mortality[35]. In our laboratory, the blood culture was found positive 77.3% (17/22) of expired melioid patients. Some of the cases had died before the culture results were confirmed. So, for early diagnosis and prompt intervention against this deadly disease, high index of suspicion from both clinician and laboratory personnel is a prerequisite. A series of clinical trials have shown that the mortality from melioidosis can be significantly reduced (from 50% to 10%) by appropriate antibiotic treatment[36,37]. All the isolates in our study were found sensitive (>95%) to ceftazidime, carbapenem, piperacillin-tazobactum, amoxicillin-clavulanic acid and tetracycline. Though, 46.6% of our strain were found resistant to co-trimoxazole by disc diffusion method, but some of these strains were further evaluated by MIC methods. 12 were found to be sensitive. Ciprofloxacin is not recommended antibiotic for treatment of melioidosis unless there are no alternatives[8].

Therefore, identification and antibiotic sensitivity pattern of this specific organism is very much important as treating this case with ordinary antibiotic regimen is ineffective and the bacterium is intrinsically resistant to many antibiotics.

Conclusions

Melioidosis is largely underrecognized by our health care policy makers due to underreporting of fatal cases. It should be included as notifiable diseases in national surveillance system of Bangladesh. We conducted retrospective data analysis from our laboratory records to determine the trends of isolation of the causative organism, *B. pseudomallei* from different clinical samples and their susceptibility pattern. Awareness, workshop and training for laboratory personnel and clinician of possible variation in presentation and diagnosis of melioidosis cases should be provided countrywide.

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Conflict of interest

The authors have no conflict of interest to declare.

Author contributions

Lovely Barai: Writing-original draft, investigation, methodology, analysis of data (Lead)

Mili Rani Saha, Tanjila Rahman : Investigation, methodology and analysis (supporting)

Marium Sukanya, Jinat Jafrin, Jannatul Ferdous, Rokibul Hasan: analysis of data (supporting)

MSA Jilani: Edited and revised the primary draft.

All authors have gone through the draft and agreed upon the final version of the article.

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