Melioidosis: Antibiogram of cases in Brunei Darussalam

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ABSTRACT

Introduction: Appropriate choice and duration of anti-microbial therapy for the initial intensive and followed by the longer eradication phase is important in the treatment of Melioidosis. The aim of this analysis was to study the pattern of antibiotic sensitivity for *Burkholderia pseudomallei* isolated in Brunei Darussalam over a period of 10 years from 2000 to 2009. **Materials and Methods:** A retrospective analysis of data was done on patients who presented to Hospitals in Brunei Darussalam diagnosed with clinical features of *Burkholderia pseudomallei* infection. The specimens were collected and subjected to standard microbiological procedures. Identification of the bacteria was performed using API20NE system (Biomerieux, UK) and the antibiotic susceptibility was assessed using the disc diffusion method using commercially available antibiotic discs. **Results:** Over the 10-year study period, 679 isolates were processed from 623 patients. The most common sources were blood (n=368) and pus from soft tissue abscesses (n=218). Imipenem, meropenem, ceftazidime, piperacillin and chloramphenicol demonstrated the highest sensitivity rates (98%-100%). Amoxycillin/clavulanic acid was moderately sensitive with 76%-100% whilst ciprofloxacin and co-trimoxazole showed the lowest rates (6%-68%. **Conclusion:** Imipenem, meropenem, ceftazidime, piperacillin and chloramphenicol have remained to be the effective drugs for the treatment of Melioidosis over the study period. Amoxycillin/clavulanic acid was moderately sensitive whilst co-trimoxazole had a low sensitivity rate as detected using disc diffusion method.

Keywords: Melioidosis, *Burkholderia pseudomallei,* bacteriology, antibiogram, antibiotics

INTRODUCTION

Melioidosis is a disease caused by the microbe named *Burkholderia pseudomallei (B. pseudomallei)*. It was initially described in Burma, by Alfred Whitmore and his assistant, C.S Krishnaswami in 1911. Melioidosis is typically found in water and soil of countries that lie between latitude 20 degrees north and 20 degrees south, and is particularly endemic in Southeast Asia and northern Australia. [1] Melioidosis is notoriously named as the 'great mimicker' as it has a broad spectrum of clinical manifestations, from subclinical presentation to an overwhelming disease thus leading to a significant morbidity and mortality. [2-4] Thus, a high index of suspicion is crucial for detecting and treating this disease promptly.

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The mainstay of treatment for Melioidosis is antimicrobial chemotherapy such as imipenem, amoxicillin/clavulanic acid, ceftazidime, tetracycline, co-trimoxazole and chloramphenicol. According to the Brunei Darussalam's National Hospital Antibiotic Guidelines, the recommended antibiotics are amoxicillin/clavulanic acid plus ceftazidime or meropenem or imipenem for a period of six weeks followed by co-trimoxazole for the next six to 12 months. [5] There are other recommended antibiotic regimens available. [1]

The organism's intrinsic resistance to routine antibiotics impedes successful treatment and it has a tendency to recur despite prolonged courses of chemotherapy. Hence, it is essential to know its sensitivity pattern against these antibiotics over a period of time so we can ascertain their effectiveness. There are reports of antibiogram and studies using individual antibiotics from the Southeast Asian countries, however, no such data is available for Brunei Darussalam. [6-9] Moreover, Melioidosis is not uncommon in our setting. [10, 11] The aim of this study was to investigate the pattern of antibiotic sensitivity of *B. pseudomallei* isolated in Brunei Darussalam over a period of 10 years from 2000 to 2009.

MATERIALS AND METHODS

A retrospective analysis of data collected from the year 2000 until 2009 was done. The antimicrobial susceptibility testing of *B. pseudomallei* isolates from hospitals in Brunei Darussalam were identified from the records maintained in the Department of Microbiology. Further details were extracted from the computer system of the state laboratory such as type of specimen and identification of patients.

MICROBIOLOGY: Specimens were collected during the initial admission to the hospital. Smears were gram-stained to note for any organisms with particular morphology. The specimens were then inoculated on blood agar, MacConkeys agar and Cocked meat broth, which were incubated at 37 degree celcius for 24-48 hours. Subsequently, sub cultures were made from the broth culture onto another set of these plates. These were then examined for colony morphology and gram-staining.

B. pseudomallei was identified by their wrinkled nature with a metallic appearance and an earthly odour. As for gram staining, its colony showed gram-negative bacilli with bipolar staining. The cultured colonies were further identified by using the API20NE system (Biomerieux, United Kingdom). All organisms isolated were subjected for antibiotic sensitivity testing by Kirby Bauer's Disc diffusion method using Muller-Hinton agar and commercially purchased antibiotics disks and interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations.

RESULTS

The number of cases, the number of isolates and the source of samples of *B. pseudomallei* over the 10 year period are shown in Table 1. A total of 679 isolates were processed from 623 patients. The two most common sources were blood (n=368) and pus from soft tissue abscesses (n=218).

Table 2 illustrates the sensitivity pattern of antibiotics tested in percentage from the year 2000 to 2009. A graph of most routinely used antimicrobial therapy for *B. pseu-*

Table 1: The number of cases, isolates and sources of *B. pseudomallei* (2000 to 2009).

Year	Number of cases (patients)	Number of isolates	Blood	CSF	UTI	URTI	LRTI	Skin & soft tissue	Ear	GI tract
2000	25	27	16	-	-	-	-	11	-	-
2001	30	30	17	-	-	-	5	8	-	-
2002	92	92	44	-	5	1	9	32	-	1
2003	59	66	31	1	-	-	14	20	-	-
2004	39	42	20	-	-	-	6	15	-	1
2005	94	94	60	1	-	-	5	27	-	1
2006	97	97	53	-	3	-	10	30	1	-
2007	92	112	61	1	1	-	6	37	-	6
2008	57	76	49	-	-	-	7	18	-	2
2009	38	43	17	-	1	-	5	18	-	2
Total	623	679	368	3	10	1	67	218	1	11

Figures presented in absolute numbers
CSF – Cerebrospinal fluid, UTI – Urinary tract infection, URTI – Upper respiratory tract infection, LRTI – Lower respiratory tract infection, GI – Gastrointestinal

domallei is shown in figure 1.

DISCUSSION

Over the study period, we found that imipenem, meropenem, ceftazidime, piperacillin and chloramphenicol remained the most sensitive antibiotics for patients with Melioidosis in Brunei Darussalam. These antibiotics proved to be effective and correspond well with the Brunei Darussalam's National Hospital Antibiotic guidelines. Our results were also comparable with studies done in Singapore, Malaysia, Thailand, Cambodia and Australia. [6-9, 12] In Australia, the therapeutic guidelines for the treatment of melioidosis recommend using ceftazidime or meropenem or imipenem with co-trimoxazole and folic acid for a period of at least two weeks, followed by a step

Table 2: The sensitivity pattern of isolated *B. pseudomallei* (2000 to 2009).

Antibiotic/year	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
Imipenem	100	100	100	100	100	100	100	99	100	99
Meropenem	100		99	100	100	100	99	99	100	100
Ceftazidime	100	100	100	100	100	100	100	100	100	100
Piperacillin	100	100	100	100	100	100	100	100	100	100
Chloramphenicol	100	100	99	99	100	100	100	100	98	98
Ampicillin/ Sulbactam	96	98	100	92	100	100	98	98	98	97
Tetracycline	96	98	90	90	100	100	98	92	89	79
Amoxicillin/Clavulanic acid	96	98	98	76	100	97	98	95	96	94
Cefepime				95	100	97	89	92		
Cefaperazone		81	78	83	94	95	91	86	75	69
Ceftriaxone		51	54	73	83	80	71	61	66	57
Ciprofloxacin	48	48	60	63	63	48	68	41	32	39
Co-trimoxazole	54	45	27	23	27	44	50	26	32	6

Figures expressed in percentages

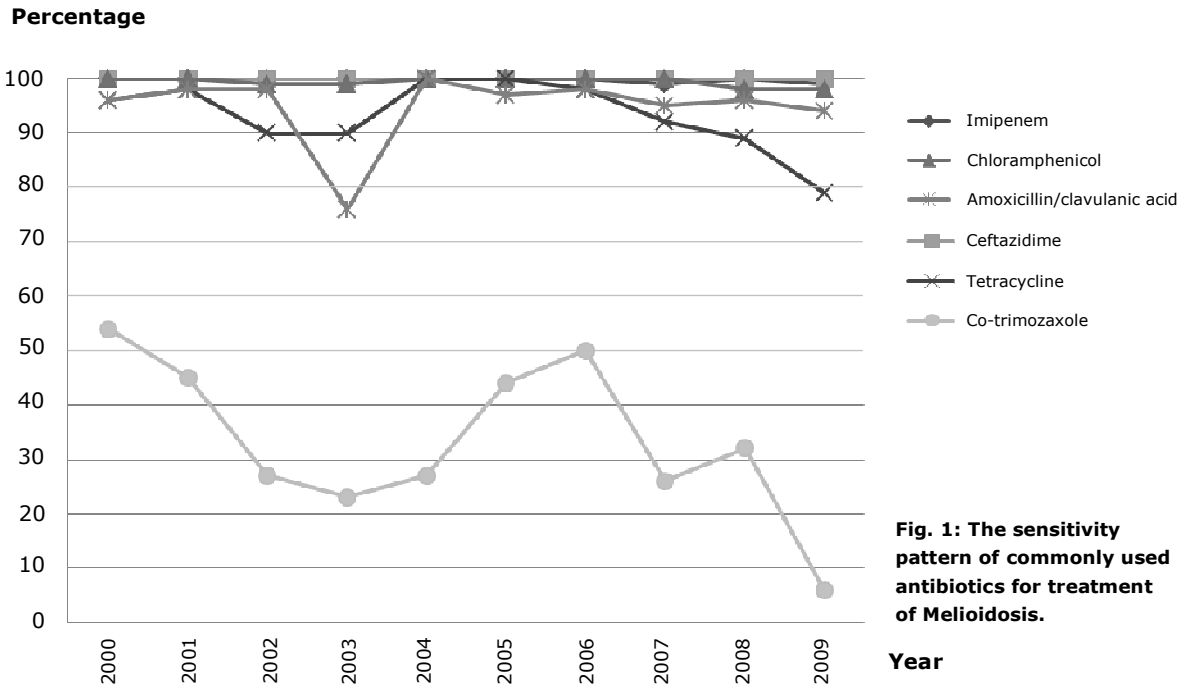

Fig. 1: The sensitivity pattern of commonly used antibiotics for treatment of Melioidosis.

down treatment with ceftazidime and co-trimoxazole for another two to four weeks. In the eradication phase, patients continue to receive co-trimoxazole and doxycycline or amoxicillin/clavulanic acid for at least another three months. [13]

Our analysis revealed that amoxicillin/ clavulanic acid was moderately sensitive against this pathogen, which is encouraging (76-100%) as this antibiotic is conventionally used worldwide as a first line treatment for any infective process. Comparatively, Sivalingam *et al.* from Singapore recorded 100% sensitivity rate whilst Raja from Malaysia reported a 95% sensitivity rate. [14, 15] Furthermore, amoxicillin/clavulanic acid is used in Thailand as one of the primary treatment options during the acute phase of Melioidosis as well as in the eradication phase for paediatric and pregnant patients. [1] On the contrary, we observed a gradual decrease in sensitivity rates from the year 2004 to 2009 (100% to 94%) with a significant dip in 2003 with 76%. A possible reason for the above could be that the pathogen is able to undergo mutation and generate a structurally altered beta lactamase enzyme leading to derepressed production. [16, 17] It is interesting to note that a higher relapse rate has been reported when using oral amoxicillin/clavulanic acid in the eradication phase compared to co-trimoxazole, doxycycline and chloramphenicol. [18]

In the present study, we found a low sensitivity rate between 6-54% for co-trimoxazole using the disc diffusion method. Wuthiekanun *et al.* from Thailand compared testing between the disc diffusion method and E test and reported an over estimated resistance rate with the disc diffusion method (71% vs. 13%). [19] Piliouras *et al.* from Australia also recorded a lower sensitivity rate when using the disc diffusion method (41%

vs. 97.5%). [20] The disc diffusion methodology is an inexpensive way of testing susceptibility pattern of microbes but inadvertently compromise their true antibiogram. Therefore, further evaluation with E test is paramount. Use of co-trimoxazole is recommended in the Brunei Darussalam National Hospital Antibiotic Guidelines and also used successfully in practice in the eradication phase for a minimum of six months.

Since Melioidosis is known to have a significant mortality and relapse rates, there is an ongoing effort to improve its treatment modalities such as the use of newer antibiotics and implementing the benefits of immunoantimicrobial therapy. Tigecycline, Ceftobiprole and BAL30072 have attributed to possess some antibiotic activity against this pathogen but they are still in the process of evaluation as well as in the early stages of clinical trials. [21-24] Granulocyte colony stimulating factor (G-CSF) also has shown to be a very good adjunctive therapy in limited studies especially during severe acute infection. It reduces the mortality rate and prolongs survival rate. [25] It has the function to reverse neutrophil deficiencies, counter inflammatory cytokines and amplify intracellular antibiotic concentration. [26-28] Unfortunately, discouraging results were shown in a randomised controlled trial that was conducted in Thailand recently. [29] The above suggests that studies are ongoing in search for more effective treatment for Melioidosis.

In conclusion, this study confirmed that over a period of 10 years, imipenem, meropenem, ceftazidime, piperacillin and chloramphenicol were the most sensitive antibiotics for patients with Melioidosis in Brunei Darussalam. Amoxicillin/clavulanic acid demonstrated a moderate sensitivity pattern whilst ciprofloxacin and co-trimoxazole were found to be the least effective antibiotics against *B. pseudomallei*. The low susceptibility rate for co-trimoxazole is most likely due to the use of the Disc Diffusion method thus, the use of E test should be considered.

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