

Multilocus Sequence Typing of Two Strains of *Burkholderia pseudomallei* from Melioidosis Patients in the Philippines

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INTRODUCTION

Melioidosis is an infection caused by gram-negative bacillus, *Burkholderia pseudomallei*, that is common in Thailand and Northern Australia. However, the prevalence in the Philippines is still unknown.

To understand the epidemiology of melioidosis, it is imperative to know the dynamics of its infectious agent. One of the widely used methods to establish *B. pseudomallei* endemicity is through a genotyping technique called multilocus sequence typing (MLST).

MLST harnesses the discriminating power of multiple housekeeping genes or markers to differentiate bacterial isolates.

METHODOLOGY

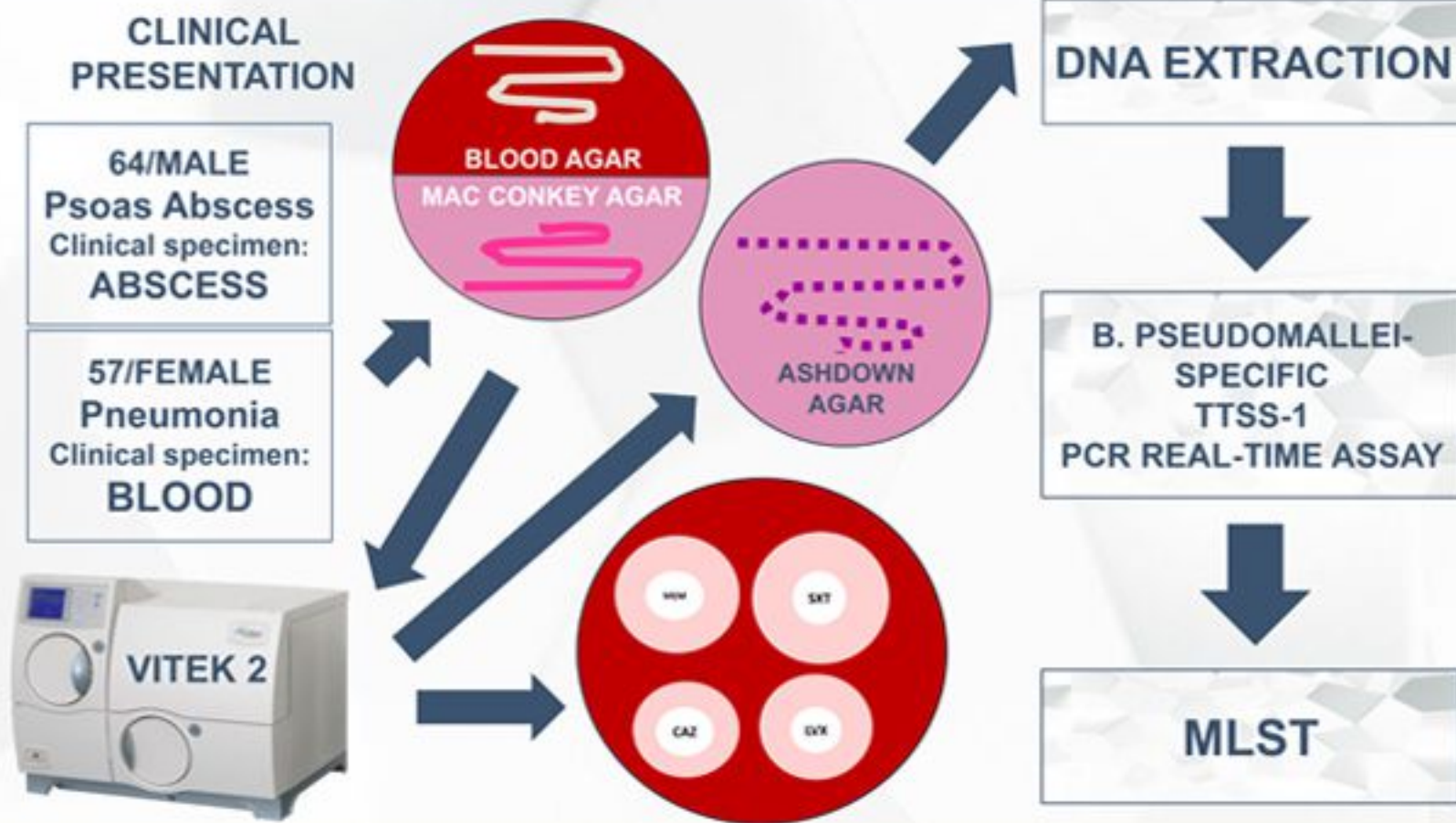


Figure 1. Bp MLST group 1

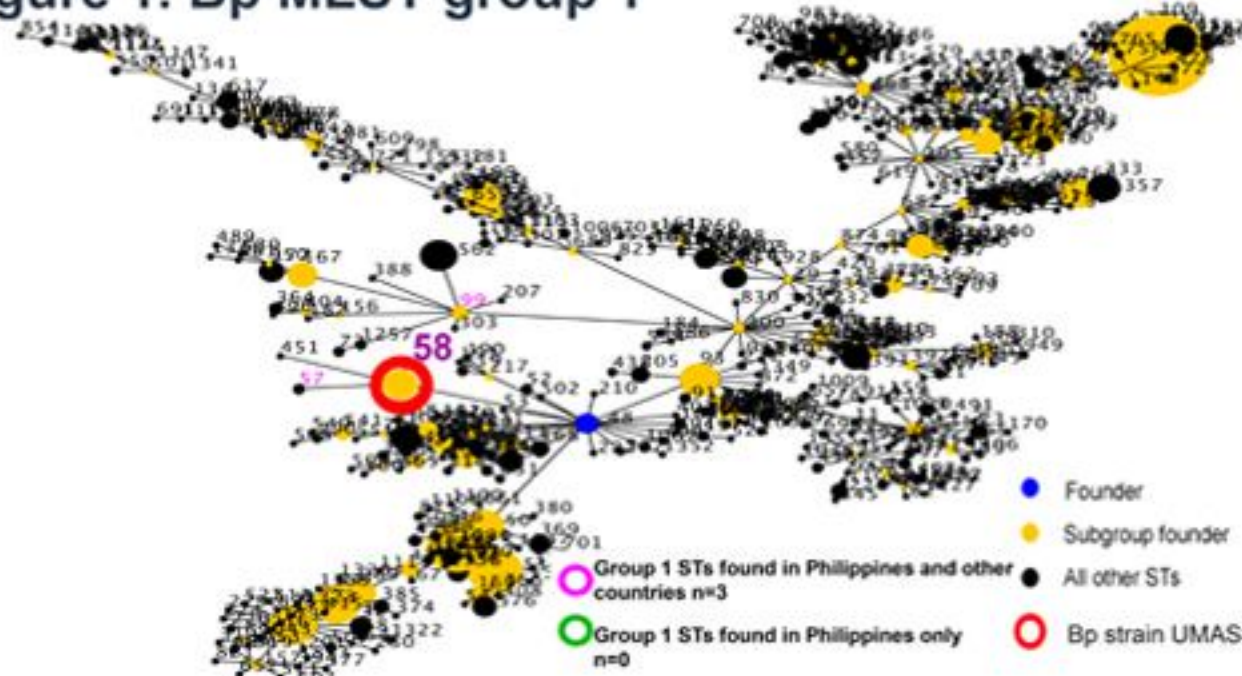
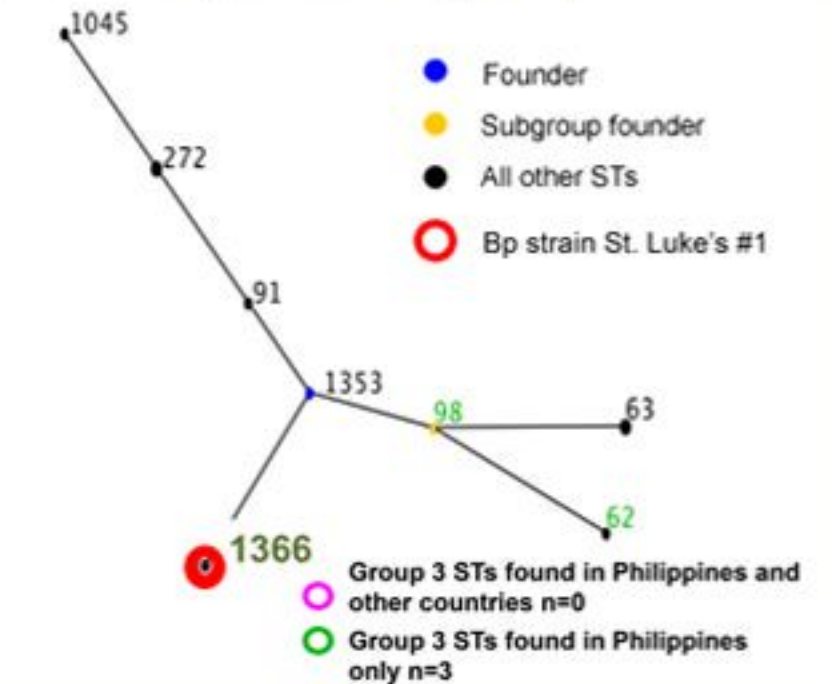


Figure 2. Bp MLST group 3



Once the allelic profile was created, the database of *B. pseudomallei* (Bp) STs was searched. Each isolate was assigned a unique name for proper identification and are as follows: **UMASS strain** and **St. Luke's strain**. The **UMASS strain** was identified as **ST 58** but the **St. Luke's strain** was a newly identified ST. The allelic profile was submitted to the database and assigned the **new ST 1366**. eBurst analysis was used to group all STs in the database by relatedness for visualization (Figure 1 & 2).

Bp **UMASS strain** was seen to have clustered to the largest group of known Bps, **Group 1** (Figure 1). Group 1 includes hundreds of STs with the founder located in Thailand (Figure 1, blue dot). **ST 58** (yellow dot with red ring), identified to be the ST of UMASS strain, is related to the founder ST and clusters with other environmental and clinical strains from China, Thailand, and a single strain from Malaysia. This is interesting to note because it corroborates with the clinical origin of the strain. In addition, the UMASS strain was the first Bp to be classified as ST 58 in the Philippines. This may be due to the fact that there is not much effort being exerted in characterizing Bp strains in the Philippines and is therefore a very big opportunity for further studies.

St. Luke's strain, was found to belong to Bp **Group 3** (Figure 2). This group, compared to Group 1, is an extremely small group of Bps originally containing 7 STs, now 8 with the recent addition of **St. Luke's strain (ST 1366)**. Characteristically, this group contains strains isolated from humans and monkeys in Southeast Asia and Micronesia, which somehow contradicts the clinical origins of St. Luke's strain. Furthermore, Group 3 is interesting in that the founder, ST 1353, was isolated in the USA from an individual who traveled to the Philippines. Another strain, ST 91, was also isolated in the USA and is believed to be an exported case from Asia. ST 272 includes two strains from Malaysia and ST 1045 includes one strain from the Micronesian island of Yap. ST 63 contains 2 strains isolated from monkeys in Indonesia. The remaining STs 62, 98, and 1366 are unique to the Philippines. The single strains included in each of ST 62 and 98 were isolated in 1990 from monkeys in the Philippines. Bp St. Luke's strain of the newly assigned ST 1366 represents a new ST in the small-interrelated group 3 (Figure 2). **Group 3 Bp represent a potential source for domestic and exported melioidosis as well as animal and human cases in the islands of the Pacific and far South East Asia.**

RESULTS

Table 1. Summary of Phenotypic Characterization, Susceptibility Pattern, and Molecular Characterization

SPECIMEN	PHENOTYPIC						SUSCEPTIBILITY PATTERN				STRAIN NAME	SEQUENCE TYPE (ST)
	GRAM STAIN	BLOOD AGAR	MAC CONKEY AGAR	ASHDOWN AGAR	VITEK 2 IDENTIFICATION	VITEK 2 DETECTION	CAZ	MEM	SXT	LVX		
Psoas Abscess	Negative	White Colonies	Wrinkled Light Pink Colonies	Wrinkled Purple Colonies	<i>B. pseudomallei</i>	Excellent	S	S	S	S	UMASS	58
Blood	Negative	White Colonies	Dry Light Pink Colonies	Wrinkled Purple Colonies	<i>B. pseudomallei</i>	Excellent	I	R	R	S	St. Luke's	1366

CAZ - Ceftazidime, LVX - Levofloxacin, MEM - Meropenem, SXT - Trimethoprim-Sulfamethoxazole, S - Sensitive, I - Intermediate, R - Resistant

CONCLUSION

This paper presented current data showing both phenotypic and molecular features of two (2) clinically-isolated strains of *B. pseudomallei*. Phenotypic data showed white colonies and light pink colonies on Blood Agar and MacConkey Agar respectively and wrinkled purple colonies on Ashdown's agar. On the other hand, amplification of housekeeping genes or molecular markers and its subsequent MLST showed us that one of the isolates (UMASS strain) is a part of an already established group of Bp strains. In contrast, the other isolate (St. Luke's strain) is a new Bp ST that is a part of a rather interesting group of Bp STs, which has a relatively diverse host interaction and seems to share a common place of origin.

Although this paper reports typing of only two Bp strains, the findings here suggest that more MLST should be done on more Bp clinical samples to better understand the distribution and epidemiology of melioidosis in the Philippines.

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