Infectious Medicine 3 (2024) 100150

Contents lists available at ScienceDirect

Infectious Medicine

journal homepage: www.elsevier.com/locate/imj

Original Article

Molecular epidemiology of *Burkholderia pseudomallei* in Hainan Province of China based on O-antigen

Jinzhu Huang[a,1], Shiwei Wang[a,1], Xiaoxue Lu[a], Liangpeng Suo[a], Minyang Wang[a], Juanjuan Yue[a], Rong Lin[b], Xuhu Mao[a], Qian Li[a,*], Jingmin Yan[a,*]

[a] *Department of Clinical Microbiology and Immunology, College of Pharmacy and Medical Laboratory, Army Medical University (Third Military Medical University), Chongqing 400038, China*

[b] *Department of Pneumology, People's Hospital of Sanya, Sanya 572022, China*

ARTICLE INFO

Keywords:
Burkholderia pseudomallei
LPS genotype
Melioidosis
Molecular epidemiology
O-antigen

ABSTRACT

Background: *Burkholderia pseudomallei* is a gram-negative bacterium widely found in Southeast Asia and northern Australia. This bacterium, which lacks an available vaccine, is the causative agent of melioidosis and has properties that potentially enable its exploitation as a bioweapon.

Methods: Polymerase chain reaction assays targeting each of the lipopolysaccharide (LPS) genetic types were used to investigate genotype frequencies in *B. pseudomallei* populations. Silver staining, gas chromatography-mass spectrometry (GC-MS), and immunofluorescence were used to characterize LPS.

Results: In our study, a total of 169 clinical *B. pseudomallei* isolates were collected from Hainan Province, China between 2004 and 2016. The results showed that LPS genotype A was the predominant type, comprising 91.1% of the samples, compared with only 8.9% of LPS genotype B. The majority of patients were male and were diagnosed with sepsis or pneumonia. Silver staining and GC-MS demonstrated that LPS genotypes A and B exhibited distinct phenotypes and molecular structures. Immunofluorescence tests showed there was no cross-reaction between LPS genotypes A and B.

Conclusions: This is the first report on the molecular epidemiology of *B. pseudomallei* based on O-antigen in China. Tracking the regional distribution of different LPS genotypes offers significant insights relevant to the development and administration of LPS-based vaccines.

1. Introduction

Melioidosis is a lethal infectious disease endemic to tropical regions. The causative agent of melioidosis, *Burkholderia pseudomallei*, exhibits significant antibiotic resistance [1]. In some cases, the reported fatality rates reach up to 40%, with relapse rates around 20% [2,3]. Infections with *B. pseudomallei* are most common in Southeast Asia and northern Australia; however, China, particularly Hainan Province, is also a melioidosis endemic area [4–6]. As a result of its high pathogenicity and lethality rate, extensive antibiotic resistance, and historical use of its closely related strain, *Burkholderia mallei*, in warfare [1,7,8], *B. pseudomallei* poses a potential threat as a biological weapon. Currently, no vaccine is available to protect against *B. pseudomallei*, and the virulence mechanisms of this pathogen are poorly understood. Melioidosis is a significant public health issue that severely impacts impoverished and rural communities [9].

Lipopolysaccharide (LPS), an essential component of the outer membrane of gram-negative bacteria, is vital for maintaining cell integrity and for triggering the host's innate immune response [10]. LPS generated protective immunity in a mouse model of melioidosis, exhibiting promise for vaccine research [11]. LPS comprises 3 structural elements, namely lipid A, a core oligosaccharide,

Abbreviations: PCR, polymerase chain reaction; LPS, lipopolysaccharide; GC-MS, gas chromatography-mass spectrometry; OD, optical density.

* Corresponding authors:
E-mail addresses: liqianjane@tmmu.edu.cn (Q. Li), yanjm@tmmu.edu.cn (J. Yan).

[1] These authors contributed equally to this article.

https://doi.org/10.1016/j.imj.2024.100150
Received 30 August 2024; Received in revised form 26 October 2024; Accepted 29 October 2024

and O-antigen [12]. Generally, the lipid A structure is conserved among bacteria, whereas the O-antigen structure varies between species and is, therefore, the basis for serotype classification [10]. *Burkholederia pseudomallei* LPS has been classified into A, B, and B2 genotypes. LPS genotype A is the most prevalent in both Australia (85%) and Southeast Asia (97.7%). LPS genotype B is more common in Australian (13.8%) than in Southeast Asian isolates (2.3%), and Papua New Guinea and Australia are the only countries to have recorded cases of LPS genotype B2 [13]. Although understanding the diversity of LPS genotypes is crucial for serological diagnosis and vaccine development, such information is lacking for *B. pseudomallei* in China. In this study, we investigated LPS molecular epidemiology, based on O-antigen, in a large population of *B. pseudomallei* isolates in China.

2. Materials and methods

2.1. Preparation of mouse polyclonal immune sera and determination of antibody titers

Mouse immune serum samples were produced according to a previously reported protocol [14]. Briefly, complete Freund's adjuvant was first emulsified in an equal volume of PBS containing LPS genotype A or B (0.5 mg/mL). BALB/c female mice (6–8 weeks old) received 50 μg of pure LPS subcutaneously on days 0, 14, and 35, with PBS serving as the control. Seven days after the final vaccination, terminal bleeds ($n = 5$ mice/group) were carried out. ELISA was employed to determine the antibody response to LPS genotype A or B, in accordance with a previous report [15]. The 96-well Costar plates were coated with LPS (5 μg/mL, 100 μL/well) and incubated at 4°C overnight. After blocking for 2 hours at room temperature with PBST (containing 1% BSA), the plates were incubated with serially diluted mouse serum samples (1:1000, 1:2000, 1:4000, 1:8000, 1:16,000, 1:32,000, 1:64,000, and 1:128,000, 100 μL/well) for 40 min at 37 °C. The plates were then incubated for an additional 40 min at 37 °C with anti-mouse IgG horseradish peroxidase-conjugated antibodies (CST; 1:10,000 dilution). The plates were developed with tetramethylbenzidine (TMB, BBI Life Science, China), and then examined at 450 nm with a microplate reader (Bio-Rad, America). To calculate the endpoint titers for individual mice, the precise value of P/N ($OD_{sample} - OD_{blank}/OD_{negative\ control} - OD_{blank}$) more than 2.1 was taken into account.

2.2. Isolate collection and extraction of LPS

The bacteria used in this study were *B. pseudomallei* strains collected from Hainan Province, China, according to the following criteria. Inclusion criterion: isolates that tested culture-positive for *B. pseudomallei* at the laboratory of Sanya People's Hospital and Haikou People's Hospital (Hainan Province, China). Exclusion criterion: repeated isolates from the same site of the same patient. A prior study confirmed all clinical isolates through the VITEK-2 system (BioMerieux, Marcy-l'Etoile, France) and 16S ribosomal DNA gene sequencing [16]. LPS extraction was conducted using the heated aqueous-phenol method [17]. Briefly, *B. pseudomallei* isolates were cultivated in Luria–Bertani–Lennox (LB) broth at 37°C for 14 h with agitation at 200 rpm. Then, the cultured bacteria were centrifuged and the bacterial pellet was treated with phenol (final concentration of 2%) for 30 min. An equal volume of 90% (w/v) phenol preheated to 65°C was added, and the resultant mixture was stirred for 15 minutes. After centrifuging at 18,000 × *g* for 15 min, the collected water phase was dialyzed against ddH_2O, and then further purified by enzymatic digestion (RNase and DNase, 37°C for 2 hours; proteinase K, 60°C for 3 hours). Following further centrifugation, the supernatant was harvested and the crude lipopolysaccharide was obtained through freeze drying. The lyophilized LPS fractions were dissolved in distilled water and then fractionated via gel permeation chromatography on a column (1.5 × 100 cm) of Sephadex G-100, with 0.15 M NaCl buffer at a flow rate of 0.15 mL/min. The purified LPS was subjected to further analysis.

2.3. LPS genotyping and analysis of clinical case information

Based on a previous report [13], polymerase chain reaction (PCR) was employed to detect genotypes A, B, and B2. The primers used in the PCR were as follows: genotype A primers, wbiE-F: TCAAACCTATCCGCGTGTCGAAGT and wbiE-R: TCGTCGTCAAGAAATCCCAGCCAT; genotype B primers, BUC_3396-F: GACCGCCCGAATCTTTTTCTGATTCCGTC and BUC_3396-R: TCGACCAGAAGACAAGGAGAAAGGCCAC; genotype B2 primers: BURP840_LPSb16-F: GATTCACGCGATACGCCGGTGTAGAACAG and BURP840_LPSb16-R: CGTCTACAACCGGGTAGTTCGCGATTACAAC. For statistical analyses, clinical cases were classified according to LPS genotype.

2.4. SDS-PAGE and chemical identification of LPS

Lyophilized LPS fractions, dissolved in sample buffer (0.1 M Tris/HCl [pH 6.8], 0.1% bromophenol blue, 1% DTT, 2% SDS, and 20% sucrose), were separated by 12% SDS-PAGE. The profile was then visualized using silver nitrate staining postoxidation with periodate [18]. The 3-deoxy-D-manno-2-octulosonic acid (Kdo) content of LPS was determined using the modified thiobarbituric acid (TBA) assay, as described previously [14]. The monosaccharide composition was determined using gas chromatography-mass spectrometry (GC-MS) with slight modifications, as described in our previous work [14].

2.5. Immunofluorescence assay

B. pseudomallei isolates BPC006 and BPC010 represented LPS genotype A strains and isolates BPC001, BPC004, and BPC007 represented LPS genotype B strains. Immunofluorescence experiments to identify different LPS genotypes were performed according to previous methods [14]. Briefly, the bacteria (10^7 CFU/mL) were first incubated with anti-LPS genotype A or B mouse serum (1:400), using *E. coli* as a control. Bacteria were then incubated with goat anti-mouse IgG-Alexa Fluor 488 (Invitrogen, America, A32723; 1:1000), followed by further incubation with rabbit anti-bacterial serum (1:400). Finally, bacteria were incubated with donkey anti-rabbit IgG-Alexa Fluor 555 (Invitrogen, America, A32794; 1:1000). Fluorescence on bacterial cells was visualized using a TCS SP5 confocal microscope (Leica SP8, Germany), and analyzed using ImageJ software.

3. Results

3.1. LPS genotypes and phenotypes

The 169 *B. pseudomallei* isolates were obtained at Sanya People's Hospital and Haikou People's Hospital, Hainan Province, China. BPC006 and BPC004 were selected as representative LPS genotype A and B isolates, respectively (Fig. 1A). LPS genotype A was found to be the most common genotype (91.1%), whereas LPS genotype B was less prevalent (8.9%). LPS genotype B2 was not identified. Melting curves (Fig. 1B) were used to differentiate the LPS genotypes.

The silver-stained SDS-PAGE electropherogram showed that the BPC004 strain (LPS genotype B) exhibited a distinctive ladder pattern and a wider molecular weight spectrum (40–100 kD) than the BPC006 strain (LPS genotype A) (55–70 kD). Monosaccharide composition analysis by GC-MS (Fig. 2B) demonstrated that LPS from BPC004 primarily consisted of Rha, Gal, Xyl, and Glc in a molar ratio of 4.4:1.0:0.9:0.9, confirming it as LPS genotype B from our prior structural characterization study [18]. The monosaccharide composition of LPS from the BPC006 strain was composed of 6-deoxy-Tal and Glc in a molar ratio of 1.87:1.0, matching the LPS genotype A reported in a previous study [19].

3.2. Serological specificity of LPS

Fig. 3 illustrates the antibody titers, determined by plotting the ELISA optical density (OD) values against the number of serum dilutions. The results indicated that the immunized mice exhibited a robust antibody response against LPS genotype A or B, with endpoint titers exceeding 3.2×10^4. The serological specificity of *B. pseudomallei* LPS was further assessed through immunofluorescence and visualized using confocal laser scanning microscopy. As shown in Fig. 3B, the co-localization of red and green fluorescence in *B. pseudomallei* strain BPC006 incubated with anti-genotype A mouse serum, and BPC004 incubated with anti-genotype B mouse serum, suggested successful attachment of serum antibodies to the bacterial surface LPS. The absence of green fluorescence co-localization in strains BPC001 and BPC007 with anti-genotype A mouse serum, and BPC006 and BPC010 with anti-genotype B mouse serum, implied the failure of serum antibodies to bind to the bacterial surface LPS. Strains BPC006 and BPC010 (LPS genotype A) were seroreactive with the antibody from mice immunized with genotype A. By contrast, strains BPC001, BPC004, and BPC007 (LPS genotype B) were seroreactive with the antibody from mice immunized with genotype B.

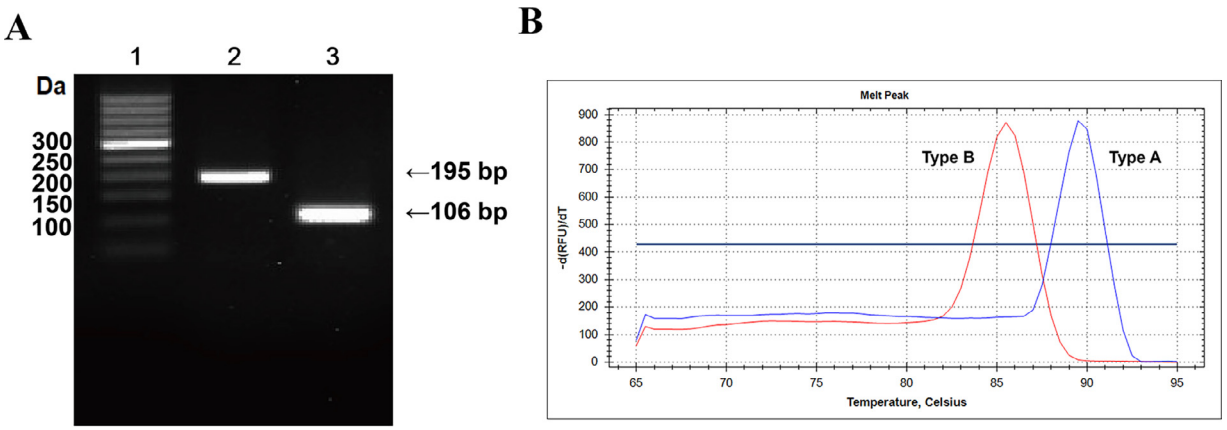

Fig. 1. Differences in the LPS genotypes of *B. pseudomallei* isolates. (A) Agarose gel electrophoresis of the serotyping PCR products. Lanes: 1, molecular size standard (DL500 marker); 2, LPS genotype A (BPC006); 3, LPS genotype B (BPC004). (B) PCR amplicons from O-antigen biosynthesis gene targets were differentiated by melting dissociation.
Abbreviations: LPS, lipopolysaccharide; PCR, polymerase chain reaction.

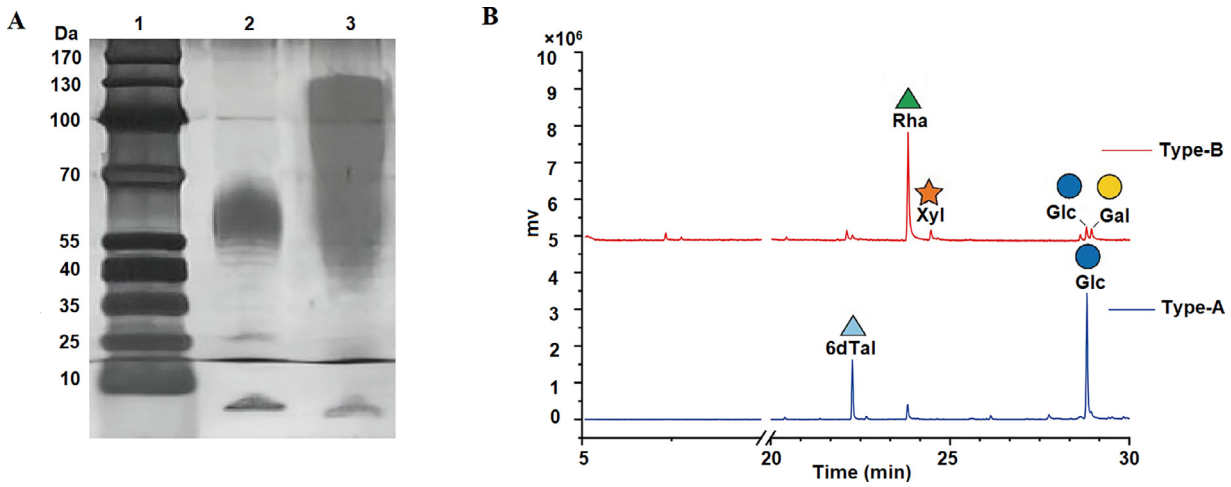

Fig. 2. Differences in the LPS phenotypes of *B. pseudomallei* isolates. (A) Silver-stained SDS-PAGE of different LPS phenotypes. Lanes: 1, molecular size standard; 2, LPS genotype A; 3, LPS genotype B. (B) Gas chromatography-mass spectrometry (GC-MS) of derivatives treated with acetic anhydride and pyridine from LPS genotype A (BPC006) and genotype B (BPC004) *B. pseudomallei* isolates.
Abbreviations: LPS, lipopolysaccharide; GC-MS, gas chromatography-mass spectrometry.

3.3. Geographic distribution and clinical records of LPS genotyping

The 169 *B. pseudomallei* isolates were isolated from 19 urban regions of Hainan Province, China, as depicted in Table 1. There were 2 exceptions, one isolate originating from Russia and another from Fujian Province, China. Both LPS genotypes A and B were evenly dispersed in coastal areas. Patients infected with *B. pseudomallei* LPS genotype A and B were predominantly male, with all patients carrying genotype B being male (Table 2). The most common types of infections were sepsis and pulmonary infections. Notably, diabetes, a primary underlying condition of infectious melioidosis, was frequently reported as a significant risk factor [20]. Among the collected samples, blood was the most prevalent sample type, outnumbering the cases of septicemia, followed by sputum. Urine was the sample type for the genotype A specimen but did not constitute any of the genotype B specimens. Patient outcomes showed that all fatalities involving genotype A were male patients with septicemia, whereas deaths involving genotype B were males with pneumonia.

Table 1
Geographic distribution of *B. pseudomallei* isolates based on the LPS genotypes in China.

District/LPS type	Genotype A	Genotype B
Sanya	31	0
Haikou	21	2
Dongfang	18	0
Danzhou	13	1
Vanning	10	2
Wenchang	11	0
Ledong	10	1
Lingshui	7	0
Qionghai	5	1
Baoting	4	0
Chengmai	3	2
Changjiang	3	5
Anding	2	0
Baisha	2	0
Qiongshan	1	0
Hunan	1	0
Yangpu	1	0
Neimonggu	1	0
Russia	1	0
Fujian	0	1
Not known	9	0
Total	154	15

Table 2
Clinical records of different LPS genotypes among the 169 *B. pseudomallei* isolates.

Variables/LPS genotype[a]		Genotype A	Genotype B
Total		154	15
Sex	Male	123	15
	Female	28	0
Infection type	Sepsis	47	6
	Lung infection	49	5
Diabetes		35	9
Specimen type	Blood	93	11
	Sputum	20	3
	Pus	24	0
	Urine	5	0
Outcome	Cured	35	5
	Died	4	2

[a] Some information about patients was missing.

4. Discussion

The closely related bacterium *B. mallei*, which causes glanders, is considered to be a clonal derivative of *B. pseudomallei*. In the past, *B. mallei* was detectable globally and was used as a biological weapon in the World Wars with serious consequences. The former Soviet Union reportedly weaponized *B. mallei* and deployed it in Afghanistan in the 1980s. However, since aggressive control measures were introduced in the early 20th century, glanders has been eradicated in most countries, except in some parts

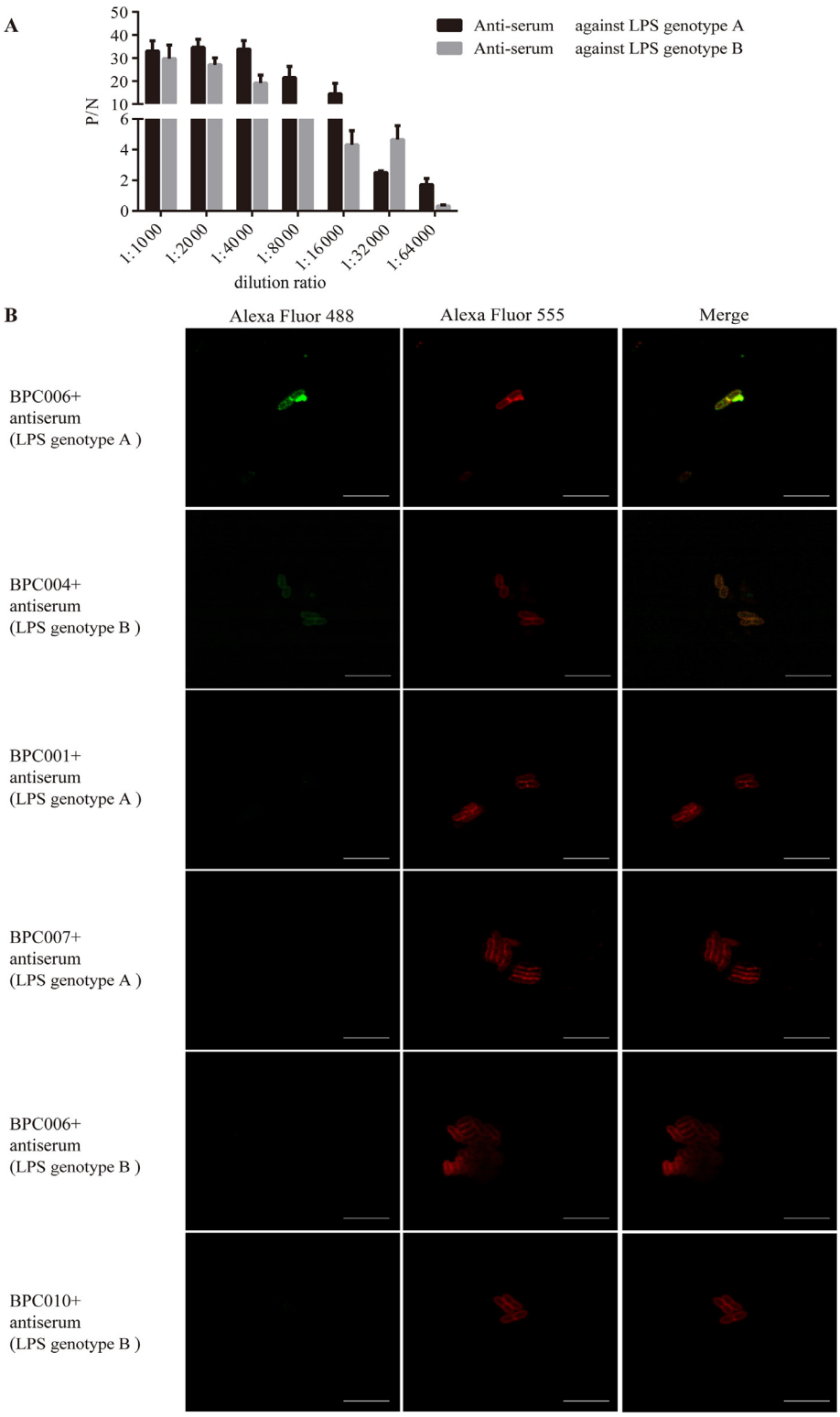

Fig. 3. Specificity of sera antibodies against LPS genotype A and B strains. (A) Groups of 5 BALB/c female mice were immunized subcutaneously with 50 μg of LPS genotype A or B at the indicated time points, with PBS being used as a control. The serum at week 6 was subjected to an ELISA. Antibody titers were calculated according to the ELISA binding signals of 5 mice per group. Bars show the mean ± SEM. (B) Immunofluorescence of inactivated bacteria by specific polyclonal antibodies. Scale bar, 7.4 μm. *Abbreviations*: LPS, lipopolysaccharide.

of North Africa, Asia, and Eastern Europe [21]. Both *B. mallei* and *B. pseudomallei* are designated as Tier 1 Select Agents by the Centers for Disease Control based on their high mortality rates, previous use as bioweapons, illness severity via respiratory exposure, intrinsic antibiotic resistance, and the lack of a current vaccine. Disease symptoms range from acute septicemia to chronic infection. The facultative intracellular lifestyle of these organisms facilitates persistence among a broad variety of hosts [22].

The regional distribution of melioidosis in China not uniform, with the majority of recent cases reported in hospitals in Hainan Province, the country's most southerly, tropical region [6]. The recent surge in Hainan's sta-

tus as a global tourist destination has heightened the attention of local government and epidemiologists to the risk of *B. pseudomallei* infection [6]. The geographic location of Hainan Province accounts for its extreme weather variations, and melioidosis outbreaks have been correlated with extreme weather events and seasonal transitions [23]. Additionally, reports of travelers contracting melioidosis while visiting endemic regions outside Hainan Province have garnered more focus on this disease, and the discovery of melioidosis cases outside of tropical zones has triggered further concern [24]. The growing incidence of melioidosis highlights the urgent need to improve surveillance and prevention strategies. O-antigen-based molecular epidemiological studies provide a vital theoretical basis for future vaccine development and use. We have previously reported on the risk factors and antibiotic resistance of *B. pseudomallei* in Hainan Province [6,16]. The current study is the first report of molecular epidemiology based on the O-antigen of *B. pseudomallei* in China, detailing an 11-year analysis of 169 *B. pseudomallei* isolates in Hainan Province.

Our findings revealed complete consistency between the LPS genotypes and phenotypes of the 169 *B. pseudomallei* isolates through PCR and SDS-PAGE. Serological specificity also demonstrated that there was no cross-reactivity between the 2 genotypes, A and B. In Southeast Asia and Australia, regional variations exist in the prevalence of LPS genotypes, with LPS genotype A being the most common, followed by LPS genotype B, whereas LPS genotype B2 is rare among Australian strains [13]. Consistent with prior reports, LPS genotype A was also prevalent in Hainan Province, China (LPS A: 91.1%, LPS B: 8.9%). In Australia, Darwin and its surrounding areas reported the highest prevalence of LPS genotype A ($n = 660$; 96% LPS A and 4% LPS B), while Katherine, Katherine remote, and East Arnhem had the most cases of LPS genotype B ($n = 79$; 60% LPS A and 40% LPS B). Additionally, 6 *B. pseudomallei* isolates of LPS genotype B2 were discovered in Australia. Phylogenomics revealed that 5 of the 6 LPS B2 genomes of "Top End" origin (i.e., the uppermost region of the Northern Territory of Australia) clustered together, suggesting that LPS B2 is geographically limited [25]. The high prevalence of LPS genotype A is also reflected in its widespread presence across many closely related species, suggesting the possibility of horizontal gene transfer among species [26]. However, it is worth noting that the relationship between LPS genotype and clinical outcome remains unclear. Previous studies have shown that mortality, bacteremia, and septic shock did not differ significantly between LPS genotypes A and B. Host risk factors and strain virulence differences may be more important determinants of disease severity and outcome [27]. Furthermore, in bacterial infections, the host bacterial response to LPS involves different signaling pathways. Previous studies from our group showed that infection with an O-antigen-deficient mutant strain, Δ*wbiI*, resulted in rapid onset of endotoxic shock and significant proinflammatory cytokine release in mice [28]. The mechanism of interaction between LPS and *B. pseudomallei* will be the subject of further investigation.

LPS has proven to be a safe and effective antigen candidate for vaccine development, as evidenced by the licensed glycoconjugate vaccines for *N. meningitidis, S. pneumoniae*, and *H. influenzae* [29]. There are significant differences in the molecular structures of LPS O-antigen among the 3 serotypes of *B. pseudomallei* [13]. The structure of LPS genotype A was identical or highly similar to that of the reference strain 1026b [30], whereas that of LPS genotype B was distinct and complex. Our prior research provided new information on the molecular structure of the type B O-antigen of *B. pseudomallei* [31]. Importantly, understanding the molecular structures of diverse O-antigen types allows us to assess the phenotypic effects of this genetic diversity. Further investigations are needed for structural analysis of the O-antigen types B and B2. Owing to structural differences in the O-antigen, antibodies from various LPS genotypes (genotypes A and B) do not cross-react with each other. This has implications for developing an LPS-based vaccine. In regions where both LPS genotype A and B strains are present, a vaccine strategy based solely on LPS genotype A is unlikely to provide adequate protection. Therefore, the genetic fingerprints of the LPS genotypes must be determined before developing LPS vaccines [25].

In our study, the majority of the melioidosis cases were in Sanya and Haikou cities, 2 well-known tourist destinations in the north and south of Hainan Province. As coastal cities, they are more vulnerable to unpredictable weather changes, which can contribute to the spread of pathogens through water and air [32]. Our patient population comprised significantly more men than women, consistent with the characteristics of melioidosis. Men who work outdoors or with soil or water have previously been identified as high risk for melioidosis [33]. According to our findings, pneumonia and septicemia were the most common manifestations of the disease, as described previously [1]. Furthermore, diabetes mellitus was identified as the most prevalent preexisting condition among melioidosis patients [34].

The limitations of our study were as follows. Our single-center epidemiological survey of *B. pseudomallei* cannot accurately represent the prevalence in Hainan Province. In addition, the small number of LPS genotype B isolates may generate statistical deviation. Similarly, the lack of clinical information on outpatients may have impacted statistical accuracy. Nonetheless, our work provides insight into the molecular characteristics correlating with the O-antigen of *B. pseudomallei* and may contribute to the prevention and control of *B. pseudomallei* in an important endemic area of China.

5. Conclusion

This is the first report on the molecular epidemiology of *B. pseudomallei* based on O-antigen in China. A total of 169 isolates were collected from Hainan Province, China during 2004 and 2016, with 91.1% being of LPS genotype A and 8.9% being of LPS genotype B. We further confirmed the discrepancies in molecular structure and serological specificity of *B. pseudomallei* between the different LPS genotypes, offering a theoretical basis for the further development of different types of LPS-based vaccines. Given the recent increase in cases of melioidosis, the study of molecular epidemiology-based O-antigen is of upmost importance for the development and application of future vaccines.

Funding

This work was supported by the National Natural Science Foundation of China (No. 81901681 and No. 82272350) and the Science and Technology Innovation Ability Enhancement Special Project of Army Medical University (No. 2019XQN04).

CRediT authorship contribution statement

Jinzhu Huang: Writing – original draft, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Shiwei Wang:** Investigation, Formal analysis, Data curation. **Xiaoxue Lu:** Software, Resources, Methodology, Investigation. **Liangpeng Suo:** Validation, Resources, Methodology, Data curation. **Minyang Wang:** Visualization, Validation, Resources, Methodology, Investigation. **Juanjuan Yue:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Rong Lin:** Visualization, Resources, Methodology, Investigation. **Xuhu Mao:** Writing – review & editing, Visualization, Project administration, Funding acquisition, Conceptualization. **Qian Li:** Writing – review & editing, Validation, Supervision, Software, Methodology, Formal analysis, Conceptualization. **Jingmin Yan:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization.

Acknowledgments

None.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data available statement

Not applicable.

Ethics statement

The study obtained approval from the Army Medical University's Human Research Ethics Committee and Laboratory Animal Welfare (approval number AMUWEC20223354), and the Medical Ethics Committee of Sanya People's Hospital (approval number 202125) with all clinical samples being made anonymous.

Informed consent

Not applicable.

References

[1] W.J. Wiersinga, H.S. Virk, A.G. Torres, et al., Melioidosis, Nat Rev Dis Primers 4 (2018) 17107, doi:10.1038/nrdp.2017.107.
[2] D. Limmathurotsakul, S. Wongratanacheewin, N. Teerawattanasook, et al., Increasing incidence of human melioidosis in Northeast Thailand, Am J Trop Med Hyg 82 (6) (2010) 1113–1117, doi:10.4269/ajtmh.2010.10-0038.
[3] K.B. Gibney, A.C. Cheng, Reducing the melioidosis burden: public health, chronic disease prevention, or improved case management? Lancet Infect Dis 19 (8) (2019) 800–802, doi:10.1016/s1473-3099(19)30303-2.
[4] A.L. Baker, T. Pearson, J.W. Sahl, et al., *Burkholderia pseudomallei* distribution in Australasia is linked to paleogeographic and anthropogenic history, PLoS One 13 (11) (2018) e0206845, doi:10.1371/journal.pone.0206845.
[5] C. Mukhopadhyay, T. Shaw, G.M. Varghese, et al., Melioidosis in South Asia (india, Nepal, Pakistan, Bhutan and Afghanistan), Trop Med Infect Dis 3 (2) (2018) 51, doi:10.3390/tropicalmed3020051.
[6] Y. Fang, H. Chen, Y.L. Li, et al., Melioidosis in Hainan, China: a restrospective study, Trans R Soc Trop Med Hyg 109 (10) (2015) 636–642, doi:10.1093/trstmh/trv065.
[7] D.A. Dance, Melioidosis: the tip of the iceberg? Clin Microbiol Rev 4 (1) (1991) 52–60, doi:10.1128/cmr.4.1.52.
[8] R.W. Titball, M.N. Burtnick, G.J. Bancroft, et al., *Burkholderia pseudomallei* and *Burkholderia mallei* vaccines: are we close to clinical trials? Vaccine 35 (44) (2017) 5981–5989, doi:10.1016/j.vaccine.2017.03.022.
[9] E. McRobb, M. Kaestli, E.P. Price, et al., Distribution of *Burkholderia pseudomallei* in northern Australia, a land of diversity, Appl Environ Microbiol 80 (11) (2014) 3463–3468, doi:10.1128/aem.00128-14.
[10] T.J.J. Inglis, A.Q. Sousa, The public health implications of melioidosis, Braz J Infect Dis 13 (1) (2009) 59–66, doi:10.1590/s1413-86702009000100013.
[11] M. Caroff, D. Karibian, Structure of bacterial lipopolysaccharides, Carbohydr Res 338 (23) (2003) 2431–2447, doi:10.1016/j.carres.2003.07.010.
[12] M. Ho, T. Schollaardt, M.D. Smith, et al., Specificity and functional activity of anti-*Burkholderia pseudomallei* polysaccharide antibodies, Infect Immun 65 (9) (1997) 3648–3653, doi:10.1128/iai.65.9.3648-3653.1997.
[13] A. Tuanyok, J.K. Stone, M. Mayo, et al., The genetic and molecular basis of O-antigenic diversity in *Burkholderia pseudomallei* lipopolysaccharide, PLoS Neglected Trop Dis 6 (1) (2012) e1453, doi:10.1371/journal.pntd.0001453.
[14] M. Zhang, X. Li, W. Yang, et al., Structural characterization of a novel pentasaccharide repeating unit from *Burkholderia pseudomallei* strain BPC006 and its role in diagnosis and immunogenicity, J Pharm Biomed Anal 205 (2021) 114340, doi:10.1016/j.jpba.2021.114340.
[15] M.N. Burtnick, T.L. Shaffer, B.N. Ross, et al., Development of subunit vaccines that provide high-level protection and sterilizing immunity against acute inhalational melioidosis, Infect Immun 86 (1) (2018) e00724 -e00717, doi:10.1128/IAI.00724-17.
[16] C. Rao, Z. Hu, J. Chen, et al., Molecular epidemiology and antibiotic resistance of *Burkholderia pseudomallei* isolates from Hainan, China: a STROBE compliant observational study, Medicine 98 (9) (2019) e14461, doi:10.1097/MD.0000000000014461.
[17] I. Steinmetz, M. Rohde, B. Brenneke, Purification and characterization of an exopolysaccharide of *Burkholderia* (*Pseudomonas*) pseudomallei, Infect Immun 63 (10) (1995) 3959–3965, doi:10.1128/iai.63.10.3959-3965.1995.
[18] C.M. Tsai, C.E. Frasch, A sensitive silver stain for detecting lipopolysaccharides in polyacrylamide gels, Anal Biochem 119 (1) (1982) 115–119, doi:10.1016/0003-2697(82)90673-x.
[19] M.H. Norris, H.P. Schweizer, A. Tuanyok, Structural diversity of *Burkholderia pseudomallei* lipopolysaccharides affects innate immune signaling, PLoS Negl Trop Dis 11 (4) (2017) e0005571, doi:10.1371/journal.pntd.0005571.
[20] A.J.H. Simpson, P.N. Newton, W. Chierakul, et al., Diabetes mellitus, insulin, and melioidosis in Thailand, Clin Infect Dis 36 (5) (2003) e71–e72, doi:10.1086/367861.
[21] G.D. Dvorak, A.R. Spickler, Glanders, J Am Vet Med Assoc 233 (4) (2008) 570–587, doi:10.2460/javma.233.4.570.

[22] S.A. Aschenbroich, E.R. Lafontaine, R.J. Hogan, Melioidosis and glanders modulation of the innate immune system: barriers to current and future vaccine approaches, Expert Rev Vaccines 15 (9) (2016) 1163–1181, doi:10.1586/14760584.2016.1170598.
[23] U. Parameswaran, R.W. Baird, L.M. Ward, et al., Melioidosis at Royal Darwin Hospital in the big 2009-2010 wet season: comparison with the preceding 20 years, Med J Aust 196 (5) (2012) 345–348, doi:10.5694/mja11.11170.
[24] B.J. Currie, D.A.B. Dance, A.C. Cheng, The global distribution of *Burkholderia pseudomallei* and melioidosis: an update, Trans R Soc Trop Med Hyg 102 (2008) S1–S4, doi:10.1016/s0035-9203(08)70002-6.
[25] J.R. Webb, A. Rachlin, V. Rigas, et al., Tracing the environmental footprint of the *Burkholderia pseudomallei* lipopolysaccharide genotypes in the tropical "Top End" of the Northern Territory, Australia, PLoS Neglected Trop Dis 13 (7) (2019) e0007369, doi:10.1371/journal.pntd.0007369.
[26] J.K. Stone, M. Mayo, S.A. Grasso, et al., Detection of *Burkholderia pseudomallei* O-antigen serotypes in near-neighbor species, BMC Microbiol 12 (2012) 250, doi:10.1186/1471-2180-12-250.
[27] J.R. Webb, D.S. Sarovich, E.P. Price, et al., *Burkholderia pseudomallei* lipopolysaccharide genotype does not correlate with severity or outcome in melioidosis: host risk factors remain the critical determinant, Open Forum Infect Dis 6 (4) (2019) ofz091, doi:10.1093/ofid/ofz091.
[28] X. Li, S. Wang, P. Wu, et al., Effect of O antigen glycosyl isomerase gene mutation on biological property and pathogenicity of *Burkholderia pseudomallei* strain BPC006, Int J Biol Macromol 258 (2024) 128922, doi:10.1016/j.ijbiomac.2023.128922.
[29] F. Micoli, L. Del Bino, R. Alfini, et al., Glycoconjugate vaccines: current approaches towards faster vaccine design, Expert Rev Vaccines 18 (9) (2019) 881–895, doi:10.1080/14760584.2019.1657012.
[30] D. DeShazer, P.J. Brett, D.E. Woods, The type II O-antigenic polysaccharide moiety of *Burkholderia pseudomallei* lipopolysaccharide is required for serum resistance and virulence, Mol Microbiol 30 (5) (1998) 1081–1100, doi:10.1046/j.1365-2958.1998.01139.x.
[31] S. Huo, X. Li, S. Wang, et al., Characterization of *Burkholderia pseudomallei* O antigens in different clinical strains, Int J Biol Macromol 225 (2023) 795–808, doi:10.1016/j.ijbiomac.2022.11.143.
[32] A.C. Cheng, S.P. Jacups, D. Gal, et al., Extreme weather events and environmental contamination are associated with case-clusters of melioidosis in the Northern Territory of Australia, Int J Epidemiol 35 (2) (2006) 323–329, doi:10.1093/ije/dyi271.
[33] E.M. Meumann, A.C. Cheng, L. Ward, et al., Clinical features and epidemiology of melioidosis pneumonia: results from a 21-year study and review of the literature, Clin Infect Dis 54 (3) (2012) 362–369, doi:10.1093/cid/cir808.
[34] S. Chowdhury, L. Barai, S.R. Afroze, et al., The epidemiology of melioidosis and its association with diabetes mellitus: a systematic review and meta-analysis, Pathogens 11 (2) (2022) 149, doi:10.3390/pathogens11020149.