MINISTRY OF EDUCATION AND TRAINING MINISTRY OF HEALTH
NATIONAL INSTITUTE OF MALARIOLOGY - PARASITOLOGY - ENTOMOLOGY

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LE THI YEN

CLINICAL, LABORATORY CHARACTERISTICS, ETIOLOGY AND TREATMENT OUTCOMES OF WHITMORE'S DISEASE IN CHILDREN AT THE NATIONAL CHILDREN'S HOSPITAL

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Scientific Supervisors:

Supervisor 1: Prof. PhD. Tran Thanh Duong

Supervisor 2: Ph.D. Nguyen Van Lam

Reviewer 1:

Reviewer 2:

Reviewer 3:

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3. Le Thi Yen, Nguyen Van Lam, Tran Thanh Duong, Hoang Thi Bich Ngoc, Phan Thi Thu Chung, Tran Minh Dien (2025), “Treatment results and some factors related to death and severe hospitalization of Whitmore children’s patients at National Children's Hospital from 2017 to 2023”. *Vietnam Journal of Community Medicine*, Vol. 66, Issue 2, 2025, 31-36.

INTRODUCTION

Melioidosis, also known as Whitmore's disease, is an infectious disease in humans (and animals) caused by the gram-negative bacillus *Burkholderia pseudomallei (B. pseudomallei),* which exists in the natural environment (found in soil and dirty water). The disease was first described by Whitmore and Krishnaswami in 1911. It is an infectious disease that is prevalent mainly in countries with tropical climates, especially Southeast Asia and Northern Australia.

Whitmore's disease occurs in humans at all ages, with the highest incidence occurring in adults between the ages of 40 and 60. In children, Whitmore's disease is uncommon, with a rate of 5-15% among Whitmore's patients. In Vietnam, a study found that this rate is 10% among Whitmore's patients. Clinical manifestations of parotitis or skin infection, while in adults Whitmore's disease often manifests as pneumonia. Symptoms in children are generally different from those in adults, so the time to diagnose the disease can be long and difficult due to diverse clinical manifestations, which can range from localized infections such as dermatitis, localized abscesses to sepsis.

Studies around the world have shown a particular interest in Whitmore's disease. The US Centers for Disease Control and Prevention (CDC) classified *B. pseudomallei* as a Tier 1 select biological agent in 2012. The whole genome sequencing provides an overview of the phylogeny and diversity of the bacteria as well as a better understanding of the genetic basis of resistance genes, virulence factors, and different mutations of pathogenic strains. However, the majority of B. *pseudomallei* genome sequences are from isolates in northern Australia and Thailand.

With diverse clinical characteristics, there are differences in clinical manifestations between adults and children, and high mortality rates in patients with sepsis caused by *B. pseudomallei*. Ceftazidime and Carbapenem are the first-line antibiotics in treatment, however, there have been reports of antibiotic-resistant bacteria. Given the urgent need for research on sepsis and to improve the effectiveness of diagnosing and

treating Whitmore's disease in children, we conducted the study **"Clinical and laboratory characteristics, etiology, and treatment outcomes of Whitmore's disease in children at the National Children's Hospital"** with the following three research objectives:

1. *To describe the clinical and laboratory characteristics of pediatric patients with Whitmore's disease at the National Children's Hospital from 2017 to 2023.*
2. *To identify Genome, Antimicrobial drug resistance, and virulence factors of Burkholderia pseudomallei causing Whitmore's disease in children at the National Children's Hospital from 2017 to 2023.*
3. *To evaluate treatment results of Whitmore children at the National Children's Hospital from 2017 to 2023.*

NEW CONTRIBUTIONS OF THE THESIS

This study contributes to the understanding of Whitmore's disease in children at the National Children's Hospital, highlighting that jaw angle abscess is the most common clinical manifestation, particularly in children aged ≤ 5 years old. Definitive diagnosis is based on bacterial isolation through abscess fluid culture, and treatment outcomes for children with jaw angle abscess show a better prognosis compared to those with sepsis.

Additionally, this study contributes to the whole-genome sequencing of *B. pseudomallei*. As a result, the genome sequences of 37 bacterial strains will be added to the GenBank database of Vietnam and the world, serving as a valuable reference for future studies on the genotypic characteristics of *B. pseudomallei* isolates.

THESIS STRUCTURE

The thesis consist of 133 pages, including the following sections: Introduction (2 pages), Literature review (33 pages), Research subjects and methods (28 pages), Research results (36 pages), Discussion (31 pages), Conclusion (2 pages), Recommendation (1 page). The thesis contains 25 tables, 17 figures and 133 references.

Chapter 1
LITERATURE REVIEW

1.1. Overview of Whitmore's disease

1.1.1. Epidemiology

B. pseudomallei infection in humans or animals occurs when the host comes into contact with the bacteria in soil or water.

1.1.2. Causative agent

B. pseudomallei is a Gram-negative, rod-shaped bacterium that is either straight or slightly curved. In clinical smear samples, it may exhibit a bipolar morphology**.** It is resistant to Polymyxin and Gentamicin**,** as tested by the disk diffusion method, showing no zone of growth inhibition, but is susceptible to Amoxicillin/clavulanate (zone diameter ≥18 mm).

B. pseudomallei genome consists of two circular chromosomes, chromosome 1 (4.07Mb) and chromosome 2 (3.17Mb). Chromosome 1 mainly encodes proteins involved in core housekeeping functions, such as cell wall synthesis, metabolism, and motility. Chromosome 2 mainly encodes proteins required for secondary functions related to adaptation to environmental conditions. The genetic diversity of *B. pseudomallei* demonstrates significant genetic heterogeneity among strains, largely influenced by horizontal gene transfer, recombination, and mutation**.**

1.1.3. Physiopathological Mechanism of Whitmore's disease

B. pseudomallei first invades and multiplies within epithelial cells of the mucosal surface or damaged skin, depending on the route of entry. It then spreads to various cell types. At the site of bacterial invasion, initial lesions appear as small pustules with surrounding inflammation**.** The bacteria then spread via the lymphatic system**,** infecting local lymph nodes**,** which may become suppurative**.** *B. pseudomallei* can further enter the bloodstream, reaching internal organs through tissues**,** leading to the formation of specific granulomas**.** Patients shed bacteria through sputum, feces, and urine**.** *B. pseudomallei* produces exotoxins, while its structural components generate endotoxins**,** contributing to severe clinical manifestations**.**

1.2. Clinical and laboratory characteristics, diagnosis of Whitmore's disease

1.2.1. Clinical features

Clinical manifestations are diverse, ranging from localized infections such as dermatitis, localized abscesses to sepsis.

Table 1.1: Summary of clinical manifestations of Whitmore's disease

Acute infection (85%)	The average incubation period is 9 days (ranging from 1 to 21 days), but symptoms may appear earlier in cases of inhalation exposure, especially during the rainy season.
	The average incubation period is 9 days (ranging from 1 to 21 days), but symptoms may appear earlier in cases of inhalation exposure, especially during the rainy season.
Pulmonary infection	51% in adult; 20% in children.
	Chest X-ray findings vary, showing minimal infiltration, cavitation, or diffuse parenchymal lesions.
Cutaneous infection	13% in adult; 60% in children.
	Single lesions are typically located at the inoculation site.
Pyogenic infection	Common visceral parenchymal abscesses include those in the spleen, liver, and kidneys.
	Prostatic abscess 18%; Parotitis is rare in Australia
Central nervous system infection	Intracranial abscess: thought to be secondary to bacteraemic spread
	Encephalomyelitis: presents with brainstem signs.
Bone /joint infection	Seen in 4% of cases, due to direct extension or through haematogenous spread
Other (rare)	Mycotic aneurysms, pericarditis, mediastinal masses, thyroid and scrotal abscesses

1.2.2. Laboratory characteristics

Nonspecific tests: WBC, CRP.

Specific tests: Culture and identification of *B. pseudomallei* is considered the causative agent (gold standard) for any specimen as *B. pseudomallei* is not part of the body's normal flora.

Blood culture is the most important test, as sepsis is a common condition. Other recommended culture samples include pus from abscesses and sputum from patients with pneumonia. Repeated cultures (especially of blood, sputum, urine, and pus) should be considered for patients suspected of *B. pseudomallei* infection.

1.1.3. Diagnosis of Whitmore's disease

The diagnosis of Whitmore’s disease is based on the "Guidelines for diagnosis and treatment of Whitmore’s disease", as stated in Decision No. 6101/QD-BYT issued by the Ministry of Health on December 30, 2019.

- Epidemiology: Patients have risk factors and/or a history of exposure to soil or water contaminated with bacteria.

- Clinical: Presence of one or more clinical manifestations consistent with diseases such as pneumonia, sepsis or abscesses of organs (liver, spleen, muscles, brain...). The disease has a chronic course, causing prolonged fever.

- Laboratory: *B. pseudomallei* must be isolated through culture, confirming the diagnosis.

1.3. Treatment of Whitmore's disease

1.3.1. Principles of treatment

B. pseudomalei infections require initial intravenous antibiotics phase for at least two weeks, followed by oral antibiotic maintenance therapy for at least three months.

1.3.2. Specific antibiotic treatmen

Intensive therapy: Intravenous antibiotics.

- One of the following antibiotics:

+ Ceftazidime (preferred choice): 2g slow intravenous injection, every 6 – 8 hours (children: 50mg/kg slow intravenous injection every 6 – 8 hours) maximum 8g/day or

+ Meropenem: 1g intravenously, every 8 hours (children: 25mg/kg every 8 hours), double if there is meningitis or

+ Imipenem/cilastatin: 1g intravenously, every 8 hours (children 25mg/kg every 8 hours).

- In severe cases, Carbapenem antibiotics should be chosen.

- In severe cases (sepsis, meningitis, and abscesses), Trimethoprim/Sulfamethoxazole (TMP/SMX) may be administered either orally or intravenously in combination with other antibiotics. Duration: Treatment should last for at least 2 weeks but may extend to 4–8 weeks in severe cases, including septic shock. Dose adjustment: Dosages should be adjusted for patients with renal impairment.

Eradication therapy: Use oral antibiotics to prevent recurrence. One of the following drugs:

+ TMP-SMX: 6-8 mg/kg/ (calculated based on TMP), every 12 hours.

+ Doxycillin 100mg per dose, twice daily.

+ Amoxicillin/Clavulanic: 60 mg/kg/day (based on Amoxicillin dose), maximum 1000 mg per dose, three times daily.

- Antibiotic maintenance: 3 to 6 months depending on the location of the infection.

Chapter 2
RESEARCH OBJECTS AND METHODS

2.1. Objective 1

2.1.1. Research subjects, locations and time

2.1.1.1. Research subjects

Including all pediatric patients aged 1 month to 15 years old diagnosed with Whitmore's disease at the Central Children's Hospital.

***Selection criteria*:**

Patient: A confirmed case of Whitmore's disease is diagnosed based on Decision No. 6101/QĐ-BYT, issued by the Ministry of Health on December 30, 2019, regarding the "Guidelines for Diagnosis and Treatment of Whitmore's Disease."

- Epidemiology: The pediatric patient has risk factors and/or a history of exposure to soil or water contaminated with *B. pseudomallei*.

- Clinical manifestations: Presence of one or more clinical symptoms consistent with Whitmore's disease, such as pneumonia, sepsis, or abscesses in organs (liver, spleen, muscles, brain, etc.). The disease has a chronic progression, leading to prolonged fever.

- Laboratory confirmation: *B. pseudomallei* must be isolated through culture, confirming the diagnosis.

- Consent: The pediatric patient or legal guardian agrees to participate in the study.

Medical records: The medical records must document a diagnosis of Whitmore's disease, confirmed by bacterial isolation through culture. The records must be complete, intact, and fully finalized according to hospital regulations.

Exclusion criteria:

- Suspected cases that have not been confirmed through diagnosis or are identified as hospital-acquired infections.

- Medical records of patients suspected of having Whitmore's disease but not confirmed by bacterial culture, or records that are incomplete and have not been finalized according to hospital regulations.

2.1.1.2. Research location: At the National Children's Hospital.

2.1.1.3. Research period: From January 1, 2017 to December 31, 2023. Divided into two phases: retrospective and prospective.

- From 1/2017 to 12/2021 (5 years): Retrospective review of medical records, 42 cases.

- From 1/2022 to 12/2023 (2 years): Conduct clinical prospective descriptive research, 3 pediatric patients.

2.1.2. Research method

Research design: A retrospective and prospective descriptive study.

Sampling: Convenient sampling was used, including all pediatric patients diagnosed with and treated for Whitmore's disease at the National Children's Hospital over a 7-year period (January 2017 – December 2023).

2.1.3. Research content

- General characteristics: age, gender, geography, month of admission, risk factors, and underlying conditions.

- Clinical characteristics: reason for hospital admission, duration of illness before admission, distribution by department/initial admission center, clinical manifestations, and septic shock.

- Laboratory characteristics: number of WBCs in CBC, anemia status, platelet count, CRP quantification, number of clinic specimens isolated from *B. pseudomallei* in each child, type of specimen isolated from *B. pseudomallei,* antibiotic susceptibility test.

2.2. Objective 2

2. 2.1. Objects, location , time, research

2. 2.1. 1. Research object: B. pseudomallei strains were isolated from clinical specimens of children aged 1 month to 15 years diagnosed with Whitmore disease at the Central Children's Hospital from January 2017 to the end of December 2023.

Selection criteria: *B. pseudomallei* strains were isolated from clinical samples of Whitmore patients kept in a deep freezer at the Microbiology Department, National Children's Hospital.

Exclusion criteria: *B. pseudomallei* strains failed to meet the standards during strain storage.

2. 2.1. 2. Research location: Strains kept in the microbiology department are safely transported according to regulations for bacterial genome sequencing testing at the Vietnam - Russia Tropical Center located at 63 Nguyen Van Huyen, Nghia Do Ward, Cau Giay District, Hanoi.

2. 2.1. 3. Research time: From 01/01/2017 to 31/12/2023.

2.2.2. Research method

Research design: Laboratory descriptive study.

Sampling: Convenient sampling was used, including all *B. pseudomallei strains* isolated from specimens kept in the Microbiology department of Whitmore pediatric patients at the National Children's Hospital in 7 years (1/2017 - 12/2023).

2.2.3. Research content

Description of characteristics: Genome; Genotype distribution (ST); Antibiotic resistance genes; Point mutations in PenA gene; Virulence genes of *B. pseudomallei* bacteria in the study.

Analysis of the relationship between antibiotic susceptibility patterns and antibiotic resistance genes.

2. 3. Objective 3

2.3.1. Research subjects, locations and time

2.3.1.1. Research objects: All pediatric patients participated in goal 1.

2.3.1.2. Research location: At the National Children's Hospital.

2.3.1.3. Research period: From January 1st, 2017 to December 31st, 2023.

2. 3 .2. Research methods

Study design: A retrospective and prospective descriptive study.

Sampling: Convenient sampling was used, including all 45 pediatric patients in objective 1 according to objective 3.

2.3.3. Research content

Initial antibiotic prescription: Average length of hospital stay; Surgical intervention; Children with recurrent and chronic conditions; Progression of septic shock in Whitmore patients; Treatment outcomes.

Analysis of demographic, clinical and paraclinical characteristics of fatal cases or severe cases discharged upon request.

2.4. Techniques used in research

2.4.1. Clinical examination techniques for pediatric patients (Objectives 1 and 3)

Examinations were conducted with the assistance of pediatricians treating the patients in their respective departments/wards, ensuring complete medical record documentation. Patient assessment included medical history taking, clinical examination at admission, daily monitoring during treatment, and evaluation at discharge.

2.4.2. Determination of hematological, biochemical, and imaging parameters, as well as blood culture and antibiotic susceptibility testing (Objectives 1 and 3)

Technical process: according to standard technical process of National Children's Hospital.

2.4.3. Bacterial genome analysis process, whole genome sequencing (WGS) (Objective 2)

Culturing of *B. pseudomallei* strains, followed by DNA extraction through bacterial cell inactivation. Total DNA concentration was measured, and the samples were prepared for whole-genome sequencing. Whole-genome sequencing was performed using the Illumina HiSeq X Ten 150PE platform.

2.5. Data collection process and error minimization

2.5.1. Research data collection process

Each patient has an individual medical record following the research data collection form, ensuring all required fields were completed according to the study objectives.

2.5.2. Errors minimization

To reduce potential errors in sampling, transportation, and preservation, procedures strictly followed Decision No. 57/QD-DP (March 27, 2018) issued by the Department of Preventive Medicine, Ministry of Health, regarding the "Guidelines for sampling, packaging, preservation, and transportation of infectious disease specimens**.**

2. 6. Data management and analysis

Data after being collected according to the research medical record form will be encoded, entered and analyzed using SPSS22.0 software .

2. 7. Ethics in research

The study was approved by the Ethics Council in Biomedical Research of the National Children's Hospital, No. 2068/BNVTW-HDDD September 7, 2022, and the National Institute of Malaria - Parasitology - Entomology, No. 1568/QD-VSR December 9, 2021 on the recognition of the topic in terms of Science and Ethics in research.

Chapter 3
RESEARCH RESULTS

During the study period, a total of 45 pediatric patients were diagnosed with Whitmore's disease based on the diagnostic criteria, including 42 retrospective cases and 3 prospective cases.

3.1. Clinical and laboratory characteristics of pediatric patients with Whitmore'disease at the National Children's Hospital from 2017 to 2023

3.1.1. General characteristics of the research group

Table 3.1: Distribution of pediatric patients by age group (n=45)

Age (months)	Quantity	Percentage (%)
≤ 5 years old (60 months)	*26*	*57.8*
> 5 – 10 years old (> 60 - 120 months)	*14*	*31.1*
> 10 – 15 years old (> 120 months)	*5*	*11.1*
Median	4.6 years (55.16 months)	
Min	2 months (2.17 months)	
Max	15 years old (179.65 months)	

Findings: The number of pediatric patients aged ≤ 5 years was 26 (57.8%), aged > 5 – 10 years was 14 (31.1%), and aged > 10 – 15 years was 5 (11.1%). The median age was 4.6 years, with the youngest patient being 2 months old and the oldest 15 years old.

There were 30 male patients (66.7%) and 15 female patients (33.3%), resulting in a male-to-female ratio of 2:1.

Patients were recorded from 17 northern provinces of Vietnam, with the highest numbers in Hanoi, Thai Binh, Thanh Hoa, Nghe An, and Ha Tinh.

Hospital admissions were more frequent between July and November, accounting for 24 out of 45 cases (53.3%).

Four patients (8.9%) had underlying medical conditions.

3.1.2. Clinical features

Table 3.2: Reasons for hospitalization of Whitmore children (n=45)

Reason for admission	Quantity	Rate (%)
Fever, parotid abscess	13	28.9
Parotid abscess	8	17.8
Fever	6	13.3
Fever, cough	5	11.1
Parotid abscess	4	8.9
Fever, parotid abscess - leaking fluid	2	4.4
Fever, abdominal pain	2	4.4
Cutaneous infection	1	4.4
Parotid abscess – purulent leakage	1	2.2
Fever, lethargy	1	2.2
Fever, swollen legs	1	2.2
Swollen fingers	1	2.2

Findings: Fever with jaw angle lymphadenopathy was the most common presentation, occurring in 28.9% (13/45) of cases, followed by jaw angle lymphadenopathy alone in 17.8% (8/45), and isolated fever in 13.3% (6/45).

The clinical diagnosis of pediatric Whitmore's disease in our study included six disease groups.

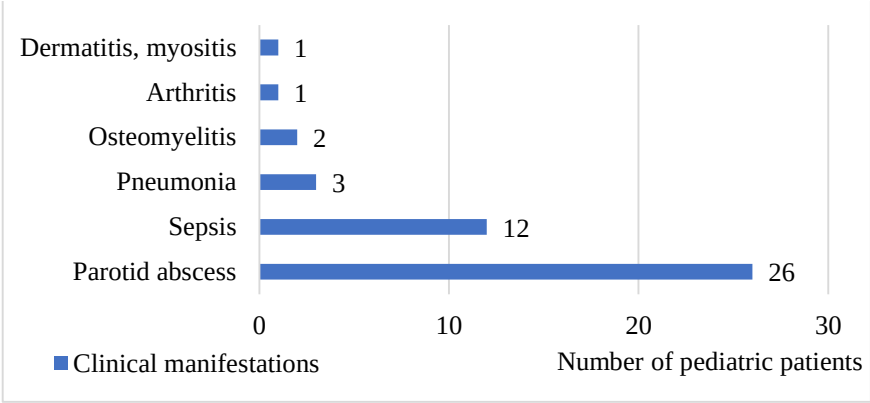

Figure 3.6: Clinical manifestations (n=45)

Findings: The most common condition was jaw angle abscess, observed in 26 out of 45 cases (57.8%), followed by sepsis in 12 cases (26.7%), pneumonia in 3 cases (6.7%), osteomyelitis in 2 cases (4.4%), arthritis in 1 case (2.2%), and dermatitis/myositis in 1 case (2.2%).

Septic shock was present in 10 out of 45 cases, accounting for 22.2%. Among them, 7 out of 10 cases developed septic shock either at the time of hospital admission or within the first 24 hours of hospitalization. Three out of 10 cases progressed to septic shock after the first 24 hours of admission. Septic shock occurred in 10 out of 12 sepsis cases (83.3%), whereas no cases of localized infection progressed to septic shock.

3.1.3. Laboratory characteristics

Table 3.6: White blood cell (WBC) count in peripheral blood (n=45)

White blood cell (G/L)	**Sepsis** Quantity, (%)	**Localized nfection** Quantity (%)	*p* - **value**
≤ 4	2 *(16.7)*	0 *(0,0)*	> *0.05*
> 4 – 12	4 *(33.3)*	13 *(39.4)*	
> 12	6 *(50.0)*	20 *(60.6)*	
Median (min – max)	15.3 (1.7 – 43.5)		

Findings: In the sepsis group, white blood cell (WBC) count was decreased (≤ 4 G/L) in 16.7% (2/12) of cases, normal (> 4 – 12 G/L) in 33.3% (4/12), and elevated (> 12 G/L) in 50% (6/12). The difference was not statistically significant ($p > 0.05$). The median WBC count was 15.3 (range: 1.7 – 43.5).

In the sepsis group, severe anemia (≤ 80 g/L) was observed in 8.3% (1/12) of cases, moderate anemia (> 80 – 110 g/L) in 25% (3/12), and no anemia (> 110 g/L) in 66.7% (8/12). The difference was not statistically significant (p > 0.05). The median hemoglobin (Hb) concentration was 120 (range: 77 – 136).

In the sepsis group, thrombocytopenia (≤ 150 G/L) was found in 50% (6/12) of cases, normal platelet counts (> 150 – 500 G/L) in 41.7% (5/12), and thrombocytosis (> 500 G/L) in 8.3% (1/12). The difference was statistically significant (p < 0.01). The median platelet count was 362 (range: 9 – 809).

Table 3.9: CRP quantitative test results (n=45)

CRP (mg/L)	**Sepsis** Quantity (%)	**Localized infection** Quantity (%)	**p-value**
≤ 6	0	11 *(33.3)*	*< 0.05*
> 6	12 *(100)*	22 *(66.7)*	
Median (min – max)	98.4 (0.18 – 403.25)		

Findings: In the sepsis group, C-reactive protein (CRP) levels were elevated (> 6 mg/L) in 100% (12/12) of cases. The difference was statistically significant (p < 0.05).

The number of clinical specimens from which *B. pseudomallei* was isolated per patient: 1 specimen in 37 cases (82.3%); 2 specimens in 6 cases (13.3%); 3 specimens in 1 case (2.2%); 4 specimens in 1 case (2.2%)

Table 3.11: Types of cultured specimens that isolated bacteria B. pseudomallei (n=56)

Specimen type	**Quantity**	**Rate (%)**
Plague	29	51.7
Blood	12	21.4
Endotracheal	5	8.9
Cerebrospinal fluid	3	5.4
Pleural effusion	2	3.6
Bone fluid	2	3.6
Joint fluid	1	1.8
Blister fluid	1	1.8
Monkey King	1	1.8

Findings: The highest isolation rate of *B. pseudomallei* from clinical specimens in pediatric patients was from lymph node pus, accounting for 51.7% (29/56), followed by blood samples at 21.4% (12/56).

Table 3.12: Antibiogram of B. pseudomallei

Antibiotic type	**Sensitive** Quantity, (%)	**Intermediary** Quantity, (%)	**Resistance** Quantity, (%)	**Total** Quantity (%)
Ceftazidime (CAZ)	28 *(70.0)*	11 *(27.5)*	1 *(2.5)*	40 *(100)*
Meropenem (MEM)	17 *(100)*	0	0	17 *(100)*
Imipenem (IPM)	42 *(100)*	0	0	42 *(100)*
Amoxicillin – clavulanic acid (AMC)	25 *(62.5)*	13 *(32.5)*	2 *(5.0)*	40 *(100)*
Trimethoprim – sulfamethoxazole (SXT)	9 *(53.0)*	4 *(23.5)*	4 *(23.5)*	17 *(100)*

Findings: The antibiotic susceptibility rates of *B. pseudomallei* were as follows: Meropenem: 100% (17/17); Imipenem: 100% (42/42); Ceftazidime: 70% (28/40); Amoxicillin-clavulanic acid: 62.5% (25/40); Trimethoprim-sulfamethoxazole: 53% (9/17)

3.2. General characteristics of the genome, antimicrobial drug resistance, and virulence factor of *B. pseudomallei* causing Whitmore's disease in children at the National Children's Hospital from 2017 to 2023

In this study, 56 bacterial strains were isolated from 45 pediatric patient samples. However, the Department of Microbiology, National Children's Hospital still preserved 37 bacterial strains, isolated from 29 patients. We conducted DNA extraction and whole genome sequencing (WGS) of *B. pseudomallei*.

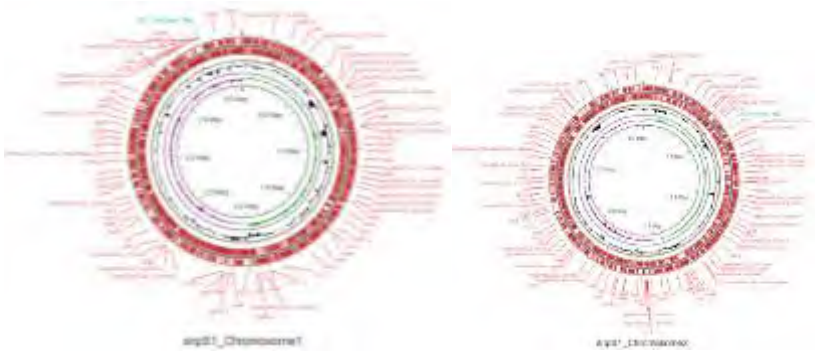

Figure 3.9: Genome sequences of chromosome 1 and chromosome 2 of *B. pseudomallei* (Strain code S1, research code STT 47)

Figure 3.10: Whole genome sequence of *B. pseudomallei* *(Strain code S1, research code STT 47)*

Findings: We performed whole-genome sequencing of 37 *B. pseudomallei* strains using the Illumina technique. The sequencing data were used to analyze the genotypic distribution and antibiotic resistance characteristics of the bacteria based on the MLST database.

Among the 37 bacterial strains, 13 sequence types (ST) were identified, including one novel ST group (S10, S12, S13) that has not been previously reported. The characteristics and distribution of ST genotypes are presented in Table 3.14.

Table 3.14: Genotypes (ST) of B. pseudomallei strains

Number of strains	Strain name	ST	ace	gltB	gmhD	lepA	lipA	narK	ndh
1	*S20*	68	3	4	11	1	5	4	6
1	*S11*	544	1	3	2	3	8	4	3
1	*S26*	507	3	1	11	3	5	4	6
1	*S33*	500	1	4	13	1	1	4	3
1	*S19*	50	3	1	2	1	1	4	3
1	*S32*	46	3	1	2	1	1	3	3
2	*S1, S6*	392	1	2	6	1	1	4	1
2	*S24, S25*	169	1	1	2	3	8	4	
3	*S10, S12, S13*	-	1	2	2	2	1	4	1
4	*S14, S7, S8, S9*	545	1	12	2	3	5	22	1
5	*S2, S23, S3, S31, S34*	541	3	4	2	3	5	4	1
5	*S15, S16, S21, S4, S5*	221	1	12	2	3	5	29	1
10	*S17, S18, S22, S27, S28, S29, S30, S35, S36, S37*	67	3	4	3	4	1	4	6

Findings: MLST analysis based on seven core genes (*ace*, *gltB*, *gmhD*, *lepA*, *lipA*, *narK*, and *ndh*) identified 12 sequence types (ST) among the studied strains.

Among these 12 ST groups, ST67 was the most common, accounting for 10 out of 37 strains (27.0%), followed by ST221 and ST541, each representing 5 out of 37 strains (13.5%), and ST545, which accounted for 3 out of 37 strains (8.1%). Other ST groups, including ST46, ST50, ST68, ST500, ST507, and ST544, were each detected in a single case. Notably, this study identified three strains, designated S10, S12, and S13, belonging to a previously unreported ST group, which has not yet been listed in the PubMLST database.

Table 3.17: Rate of antibiotic resistance genes in strains B. pseudomallei (n=37)

Drug resistance genes	***Quantity***	***Rate (%)***
amrB	37	100
CEOA	37	100
CEOB	37	100
opcM	37	100
BpsOmp38	36	97.3
amrA	35	94.6
OXA-59	34	91.1
PenA	26	70.3
OXA-57	4	10.8
QnrD1	2	5.4

Findings: Four genes, *amrB, ceoA, ceoB,* and *opcM*, associated with resistance to aminoglycosides and fluoroquinolones, were detected in 37 out of 37 strains (100%). Genes related to beta-lactam resistance included *PenA* in 26 out of 37 strains (70.3%), *OXA-59* in 91.9%, and *OXA-57* in 10.8% of strains. Notably, *BpsOmp38*, a gene responsible for multidrug resistance, was found in 36 out of 37 strains (97.3%), while *QnrD1* had the lowest prevalence at 5.4%.

A total of 147 virulence genes were identified, with the *tssB-5 (bimA)* gene, which facilitates phagosome evasion and actin-tail formation, present in all 37 analyzed strains.

Antibiotic susceptibility testing for Ceftazidime (CAZ), Amoxicillin-Clavulanate (AMC), and Trimethoprim-Sulfamethoxazole (SXT) in bacterial strains carrying *PenA*, *OXA-59*, and *OXA-57* resistance genes showed that resistance rates were lower than susceptibility rates. However, the difference was not statistically significant ($p > 0.05$).

The *BpsOmp38* gene was present in 100% of antibiotic-resistant groups.

3.3. Treatment results of Whitmore children at the National Children's Hospital from 2017 to 2023

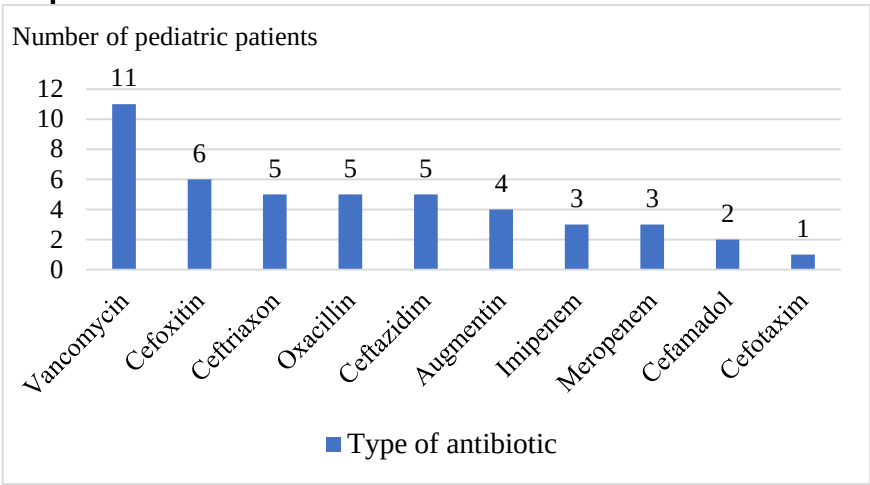

Figure 3.12: Initial empirical antibiotic prescription

Findings: The initial antibiotics used were Vancomycin and Oxacillin in 16 out of 45 cases (35.6%). Empirical antibiotic treatment with Ceftazidime, Imipenem, or Meropenem was administered in 11 out of 45 cases (24.4%).

In this study, 45 Whitmore's disease cases were recorded, including 42 inpatient cases and 3 outpatient cases treated at the clinic with prescribed oral antibiotics. The average duration of hospitalization was 21 ± 16 days (range: 2 - 61 days), with a median duration of 19 days, approximately 3 weeks per hospital treatment course.

Surgical intervention was performed in 29 out of 45 cases (64.4%). The most common surgical procedure was abscess drainage of the jaw angle, performed in 24 out of 29 cases (82.8%). Other interventions included lymph node abscess drainage or surgical placement of drainage catheters, performed in 2 out of 29 cases (6.9%) for pleural effusion drainage, 2 out of 29 cases (6.9%) for subperiosteal abscess drainage, and 1 out of 29 cases (3.4%) for joint abscess drainage.

Two out of 45 cases (4.4%) had recurrent or chronic disease. Among these, one patient had an underlying condition, while the other had no prior health issues.

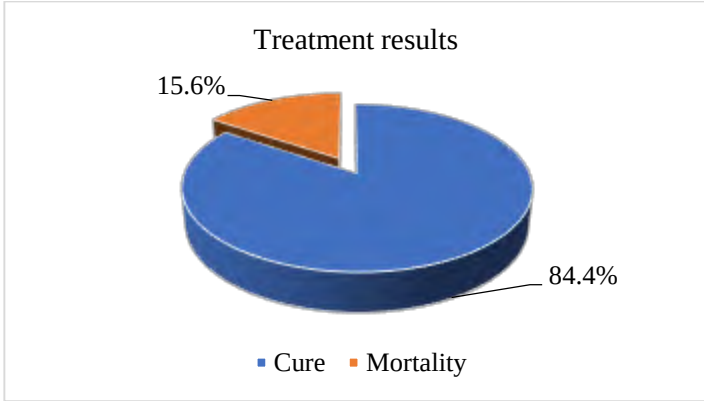

Figure 3.17: Treatment results

Findings: The cure rate was 84.4% (38/45); the mortality rate was 15.6% (7/45) and all 7 children had sepsis.

Chapter 4
DISCUSSION

4.1. Clinical and laboratory characteristics of pediatric patients with Whitmore'disease at the National Children's Hospital from 2017 to 2023

The majority of patients in the study were aged ≤ 5 years, accounting for 57.8%, with a median age of 4.6 years. A study conducted in Malaysia reported a median age of 4.7 years, while a study in Cambodia found a median age of 5.7 years. The similarity in median ages among these three studies may be attributed to their location within Southeast Asia, a tropical region where Whitmore's disease is endemic.

Males made up 66.7% of the cases, which is consistent with a 24-year study in Australia, where 53% of pediatric Whitmore's disease cases were male. This could be explained by the higher levels of outdoor activity and environmental exposure among boys, increasing their risk of contact with *B. pseudomallei*, which is commonly found in soil and contaminated water.

The most common reason for hospital admission was fever with jaw angle lymphadenopathy at 28.9% (13/45), followed by isolated jaw angle lymphadenopathy at 17.8% (8/45), and isolated fever at 13.3% (6/45). Cases presenting with only jaw angle lymphadenopathy had a longer disease progression before hospital admission, whereas those with high fever were admitted sooner. These initial symptoms help guide clinicians

in diagnosing the disease and selecting appropriate laboratory tests for confirmation.

The study identified six clinical groups, with jaw angle abscess being the most common at 57.8% (27/45). This result is consistent with a study in Cambodia, where parotid abscess was the most frequently observed condition, accounting for 27.3% of cases. Similarly, a study in Thailand found parotid gland infection in 38% of pediatric Whitmore's disease cases. This suggests that parotid gland infections are the most common presentation of pediatric Whitmore's disease in Southeast Asia. However, studies in Australia reported that skin and soft tissue abscesses were the most frequent clinical manifestation in children (60%), with no cases of parotid abscess or jaw angle abscess. The differences in clinical presentation between Southeast Asian countries and Australia may be due to variations in geographical conditions and the presence of different bacterial genotypes, leading to distinct disease manifestations.

Sepsis and pneumonia are the most common clinical presentations of Whitmore's disease in adults. A 20-year study by J. Currie found that pneumonia was the leading cause of hospital admission in 51% of cases, followed by sepsis in 55%, with 21% of patients developing septic shock. However, a 24-year study in Australia reported that children were at a lower risk of developing sepsis compared to adults, with a 15% incidence in children compared to 58% in adults. In this study, sepsis was observed in 25.5% of pediatric cases, which is consistent with findings from Cambodia, where 22.5% of cases presented with sepsis, ranking second after soft tissue infections and parotid abscesses.

Neutrophils play a critical role in host defense, eliminating up to 90% of intracellular *B. pseudomallei*. Reduced neutrophil and macrophage counts have been shown to increase infection rates and mortality in animal models. In this study, two patients (4.4%) had leukopenia ($\leq$ 4 G/L), both of whom had sepsis and refractory septic shock.

A study by Cheng identified CRP as a useful inflammatory marker in Whitmore's disease diagnosis. However, CRP levels at hospital admission are not highly sensitive for detecting Whitmore's disease, and normal CRP levels cannot exclude acute, chronic, or recurrent infections in febrile patients from endemic regions. In this study, the median CRP level at admission was 98.4 mg/L (range: 0.18 – 403.25), whereas a study by Son reported a median CRP level of 32 mg/L (range: 22 – 92.7). This suggests that while CRP is commonly used to diagnose and monitor infections, it does not rule out severe infections.

Proper sample collection is crucial for bacterial culture and accurate pathogen identification. The primary specimen types for *B. pseudomallei* culture include blood, pus, nasopharyngeal secretions, cerebrospinal fluid (CSF), and other fluids, depending on the patient's clinical presentation at the time of examination. In this study, *B. pseudomallei* was most frequently isolated from lymph node pus (53.6%, 31/58), followed by blood samples (20.7%, 12/58), with other fluids such as tracheal secretions, CSF, and pleural fluid accounting for the remaining cases.

B. pseudomallei is intrinsically resistant to Penicillin, Ampicillin, first- and second-generation Cephalosporins, Gentamicin, Tobramycin, and Streptomycin. Initial treatment involves intravenous antibiotics (Ceftazidime - CAZ, Meropenem - MEM, Imipenem - IPM) for at least two weeks, followed by oral maintenance therapy (Amoxicillin/Clavulanic acid - AMC, Trimethoprim/Sulfamethoxazole - SXT) for a minimum of three months.

Primary resistance to Ceftazidime (CAZ) has been reported at rates of 0.1 – 1.5% in Thailand and 0.6 – 2.4% in Malaysia. In this study, the resistance rate for CAZ was 2.4% (1/42), AMC was 4.8% (2/42), and SXT was 22.2% (4/18).

The intermediate resistance rates were 28.6% (12/42) for CAZ, 33.3% (14/42) for AMC, and 27.8% (5/18) for SXT. High intermediate resistance can reduce antibiotic susceptibility, but intermediate resistance does not necessarily indicate treatment failure. These antibiotics may still be clinically effective at standard doses if they achieve higher local concentrations at the infection site (e.g., in urine) or if they can be safely administered at higher-than-standard doses.

No resistance to carbapenem antibiotics was detected in this study. The susceptibility rates for Meropenem (MEM) and Imipenem (IPM) were both 100%, with MEM (18/18) and IPM (44/44) showing full efficacy.

4.2. General characteristics of the genome, antimicrobial drug resistance, and virulence factor of *B. pseudomallei* causing Whitmore's disease in children at the National Children's Hospital from 2017 to 2023

In 2004, the genome sequence of *B. pseudomallei* was first analyzed and published for the *B. pseudomallei* K96243 strain. It consists of two circular chromosomes with sizes of 4.07 Mbp and 3.17 Mbp, placing it among the top 5% of microorganisms with the largest sequenced genomes. The genome sequencing results of the bacterial strains in this study were fully consistent with the size of the previously published

reference strain *B. pseudomallei* K96243. All sequenced strains were successfully compared to existing *B. pseudomallei* sequences in global databases, confirming with 99% accuracy that they belong to the *B. pseudomallei* species. This further reinforces the reliability and accuracy of our study.

Multilocus Sequence Typing (MLST) is a genetic classification method based on variations in the sequences of seven housekeeping genes. The slow mutation rate of these alleles and the absence of significant recombination make MLST an ideal method for comparing genotypes across strains and laboratories. MLST is widely used to determine relationships between bacterial strains, track global transmission, and study antibiotic resistance patterns. Additionally, it allows the identification of genetic variation trends among bacterial populations.

Based on the whole-genome sequencing of 37 *B. pseudomallei* strains, we analyzed subtypes (STs) of *B. pseudomallei* strains circulating in Vietnam and their relationship with strains found in other regions of the world. Our MLST analysis identified 12 ST groups, indicating significant genetic diversity and distribution among *B. pseudomallei* strains in northern Vietnam. A phylogenetic analysis comparing our isolates with reference strains from different countries confirmed previous reports suggesting that *B. pseudomallei* originated in Australia before spreading to Asia. The strains found in Vietnam are closely related to those circulating in China, Thailand, Laos, and Myanmar.

ST67 was identified as the most common *B. pseudomallei* genotype in Vietnam, accounting for 27% of isolates, with distribution across multiple northern provinces. This ST67 subtype has also been previously reported as a dominant strain in Singapore. ST50, detected in our study, was first identified in Vietnam and India in the 1990s, suggesting ongoing bacterial migration. Additionally, ST46 was found in one strain in our study and is known to be widely distributed across Asia, including Malaysia, China, Vietnam, Thailand, and Cambodia.

ST67 was the most frequently identified ST in our study, accounting for 27% (10/37) of isolates. We attempted to identify a correlation between this dominant genotype and clinical manifestations in pediatric patients. However, no significant association was observed. This finding aligns with previous studies conducted in China and other countries, where researchers also failed to establish a clear relationship between common ST subtypes and clinical symptoms. One possible explanation for this lack of association is the small sample size.

Whole-genome analysis revealed three bacterial strains (S10, S12, S13) that could not be classified into any known ST. The genetic profile of these strains has not been recorded in the *B. pseudomallei* GenBank database. These may represent a newly identified ST, potentially arising from an allelic mutation, detected for the first time in Vietnam and not previously reported in any other country. Our data show that these three strains were isolated from a single patient and share identical genetic and allelic profiles. This suggests that these three isolates likely represent a single *B. pseudomallei* strain from the same patient, which is consistent with our study findings.

4.3. Treatment results of Whitmore children at the National Children's Hospital from 2017 to 2023

The initial antibiotic treatment included Vancomycin and Oxacillin in 35.6% (16/45) of cases, while empirical treatment with Ceftazidime (CAZ), Imipenem (IPM), and Meropenem (MEM) was administered in 24.4% (11/45) of cases. Since the primary clinical presentation was jaw angle lymphadenopathy, clinicians often suspected Staphylococcus and other Gram-positive bacteria, leading to the use of Vancomycin and Oxacillin.

A total of 42 patients received inpatient treatment, with an average hospital stay of 21 days (range: 2 – 61 days). A typical inpatient treatment course lasted approximately 2 to 3 weeks. In an Australian study, the average duration of the intensive treatment phase was 26 days (range: 14 – 34 days). The prolonged intensive and eradication phases (each averaging 26 days) significantly reduced the relapse rate from 5.2% to 0.5%, regardless of adherence to oral maintenance therapy. These findings suggest that a prolonged intensive treatment phase plays a crucial role in reducing the risk of relapse in Whitmore’s disease.

Surgical drainage is often required for large, isolated abscesses in the liver, muscles, and prostate, but it is unnecessary or impractical for multiple small abscesses in the spleen, liver, and kidneys. In this study, surgical intervention was primarily performed for jaw angle abscesses, accounting for 24 out of 29 cases (82.8%). Additional procedures included lymph node abscess drainage, soft tissue abscess incision, and surgical drainage catheter placement. Among the cases: 2/29 (6.9%) underwent pleural effusion drainage; 2/29 (6.9%) underwent subperiosteal abscess drainage; 1/29 (3.4%) underwent joint abscess drainage. Surgical intervention not only aids in confirming the diagnosis but also significantly improves treatment outcomes.

With advancements in treatment strategies, relapse rates have declined from approximately 10% to below 5%. Among survivors of Whitmore's disease, new infections are now more common than relapses. In this study, chronic or recurrent infections accounted for 4.4% (2/45) of cases, which is comparable to an Australian 30-year study reporting a 5% relapse rate (60 patients), including 44 relapse cases and 20 new infections.

Early diagnosis and initiation of targeted antibiotic therapy for *B. pseudomallei* are critical for successful treatment of Whitmore's disease. In pediatric retrospective studies, in-hospital mortality rates ranged from 7% in Australia to 16.8% in Cambodia. A study in Malaysia found septic shock to be closely linked to mortality, with all fatal cases experiencing septic shock. Among 32 recovered patients, only three survived septic shock. The mortality rate for septic shock cases was 77% (10/13).

In this study, the overall mortality rate was 15.6% (7/45), with the primary cause of death being irreversible septic shock. All fatal cases had sepsis, with a mortality rate of 58.3% (7/12) in septic patients.

Whitmore's disease is recognized as a leading cause of bacterial sepsis in children in parts of Thailand. Compared to adults, pediatric cases of Whitmore's disease have lower mortality rates and are less frequently associated with immunosuppression.

CONCLUSION

The study analyzed 45 pediatric Whitmore's disease cases at the National Children's Hospital and performed whole-genome sequencing on 37 isolated *B. pseudomallei* strains. The key findings are as follows:

1. Clinical and laboratory characteristics of pediatric patients with Whitmore'disease at the National Children's Hospital (2017 - 2023)

The most affected age group was children ≤ 5 years, accounting for 57.8% (26/45). The male-to-female ratio was 2:1 (30/15). Four cases (4/45) had underlying conditions.

The duration of illness before hospital admission was predominantly 6 - ≤ 14 days, accounting for 48.9%. The most common clinical presentation was jaw angle abscess (57.8%, 26/45), followed by sepsis (26.7%, 12/45). Septic shock occurred in 22.2% (10/45) of cases.

In 17.7% (8/45) of cases, *B. pseudomallei* was isolated from two or more clinical specimens. The most commonly identified specimen was abscess pus (51.7%, 29/56), followed by blood (21.4%, 12/56). Antibiotic susceptibility rates for *B. pseudomallei* were as follows:

Meropenem 100% (17/17); Imipenem: 100% (42/42); Ceftazidime: 70% (28/40); Amoxicillin-clavulanic acid: 62.5% (25/40); Trimethoprim-sulfamethoxazole: 53% (9/17).

2. General characteristics of the genome, antimicrobial drug resistance, and virulence factor of *B. pseudomallei* causing Whitmore's disease in children at the National Children's Hospital from 2017 to 2023

The 37 *B. pseudomallei* strains analyzed were fully consistent in genome size with the reference strain K96243. Twelve sequence types (STs) were identified, with the most common being ST67 (10/37, 27.0%), followed by ST221 and ST541 (5/37, 13.5%), and ST545 (3/37, 8.1%). Other STs (ST46, ST50, ST68, ST500, ST507, ST544) were each identified in one strain. A previously unreported ST group was also discovered.

Beta-lactam resistance genes such as BpsOmp38 were 97.3%, OXA-59 was 91.9%, PenA was 70.3% and OXA-57 was 10.8%. Resistance genes to 2 groups of antibiotics Aminoglycoside and Fluoroquinolone had amrB, ceoA, ceoB, opcM were 100% and QnrD1 was 5.4% (2/37).

A total of 147 virulence genes were identified, with the tssB-5 (bimA) gene, responsible for phagosome evasion and actin-tail formation, present in all 37 strains.

3. Treatment results of Whitmore children at the National Children's Hospital (2017 – 2023)

Initial empiric antibiotic use was 24.4% (11/45). The average hospital stay was 21 ± 16 days (range 2 - 61 days). 64.4% (29/45) received surgical intervention, of which 82.8% (24/29) had abscess drainage or surgical placement. Recurrence and chronic infection were 4.4% (2/45). The cure rate was 84.4% (38/45); mortality rate was 15.6% (7/45) with all fatal cases due to sepsis.

RECOMMENDATIONS

In pediatric patients presenting with jaw angle lymphadenopathy and abscess, clinicians should not overlook Whitmore’s disease. Aspiration of abscess fluid should be performed for culture and bacterial identification, followed by targeted antibiotic therapy.

Due to differences in clinical presentation between children and adults, further nationwide studies are needed in Vietnam. Collaboration with the Whitmore’s Disease Network is recommended to analyze antibiotic resistance genes of *B. pseudomallei* strains in Vietnam.