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Rapid detection of antibiotic resistance in *Burkholderia pseudomallei* using MALDI-TOF mass spectrometry

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Antibiotic resistance in *Burkholderia pseudomallei* (Bp) is a growing public health concern requiring urgent attention. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as a rapid bacterial identification tool with potential for future diagnostic applications. This study investigates differences in MALDI-TOF spectra of antibiotic-resistant Bp strains from northeastern Thailand without any pretreatment steps. A total of 300 Bp isolates were tested for minimum inhibitory concentration (MIC) values against five antibiotics: ceftazidime, meropenem, imipenem, co-amoxiclav, and co-trimoxazole. MALDI-TOF spectra were obtained using the direct colony method and analysed for correlations with antibiotic resistance patterns. Thirteen isolates exhibited resistance based on CLSI breakpoints, with most showing low-level MICs across all tested antibiotics. MALDI-TOF MS analysis revealed clustering patterns associated with resistance. A decision tree algorithm identified ten significant peaks that effectively distinguished resistant isolates. This study highlights MALDI-TOF MS as a rapid and reliable tool for detecting antibiotic resistance, offering timely results to support treatment decisions and streamline microbiology workflows.

Keywords MALDI-TOF MS, Machine learning, *Burkholderia pseudomallei*, Minimum inhibitory concentration, Antibiotic resistance, Geographical distribution, Peak identification

Melioidosis is caused by the environmental bacterium *Burkholderia pseudomallei* (Bp), which infects both humans and animals[1]. The disease has a high mortality rate of approximately 35%, with an estimated 2,800 deaths annually in Thailand, particularly in the northeastern region, a major endemic area[2]. Treating melioidosis is challenging due to Bp's intrinsic resistance to multiple antibiotics, including penicillin, ampicillin, first- and second-generation cephalosporins, and gentamicin[3]. Furthermore, diagnosis and management are complicated by the disease's non-specific clinical presentation.

According to the World Health Organization (WHO), antibiotic resistance is a growing global concern, exacerbated by widespread antibiotic use[4]. Melioidosis treatment follows a two-phase approach: intensive therapy with ceftazidime (CAZ) or meropenem (MERO), followed by eradication therapy with co-amoxiclav (AUG) or co-trimoxazole (SXT)[5]. The Clinical and Laboratory Standards Institute (CLSI) recommends broth microdilution as the gold standard for determining Bp resistance[6]. However, this growth-based assay takes up to 48 h, potentially delaying treatment and increasing the risk of mortality.

Antibiotic resistance in Bp remains rare. Resistance to SXT is reported in less than 1% of cases[7] CAZ resistance ranges from 0.1 to 1.5%, and AUG resistance is around 0.4% in Thailand[8–10]. Carbapenem resistance in Bp is extremely rare[9] though one case in Taiwan documented resistance to both meropenem and levofloxacin

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following treatment[11]. Despite low resistance prevalence, delays or inappropriate therapy within the first 48 h significantly increase mortality risk[12,13]. A previous study indicated that delayed administration of meropenem in melioidosis patients infected with CAZ-resistant strains can lead to fatal outcomes[13].

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has emerged as an accurate, rapid, and high-throughput technology widely used for bacterial identification[14]. With the integration of machine learning algorithms, MALDI-TOF MS holds promise for antibiotic resistance detection, marking a significant advancement in laboratory medicine by improving both efficacy assessment and prognosis prediction.

Recent studies have successfully applied MALDI-TOF MS to detect antibiotic resistance, including vancomycin-resistant *Staphylococcus aureus*[15–17] drug-resistant *Campylobacter spp.*[18] carbapenem-resistant *Klebsiella pneumoniae*[19–21] and fluconazole-resistant *Candida albicans*[22]. MALDI-TOF spectral analysis has also led to the discovery of potential biomarkers, providing new insights into bacterial resistance mechanisms[18]. While previous research explored the use of MALDI-TOF MS to detect phage proteins associated with Bp resistance, its clinical application remains impractical due to the complexity of phage-based detection[23]. To date, no studies have investigated the use of MALDI-TOF MS combined with machine learning to detect antibiotic resistance in Bp without additional treatment steps.

Rapidly identifying antibiotic resistance is essential for effective treatment and reducing mortality. MALDI-TOF MS has demonstrated promise as a rapid diagnostic tool. This study aims to analyse MALDI-TOF spectra from antibiotic-resistant Bp strains without any pretreatment steps using 300 isolates from northeastern Thailand. Identifying resistance-associated spectral patterns without requiring additional processing, such as drug co-incubation, would enable a streamlined, time-efficient approach for detecting resistance. This method has the potential to improve early diagnosis, inform treatment decisions, and reduce both mortality rates and the prevalence of antibiotic resistance.

Results

Distribution and characterization of *Burkholderia pseudomallei* phenotypic drug susceptibility

Twelve clinical resistant Bp strains were identified in 6 out of 11 studied provinces (Fig. 1, Supplementary Table 1), indicating a widespread but uneven distribution. The highest proportion of resistance was observed in Ubon Ratchathani (3/4 isolates; 75.00%), followed by Chaiyaphum (4/26 isolates; 15.38%), Buri Ram (2/19 isolates; 10.53%), Khon Kaen (2/38 isolates; 5.26%), Nakhon Ratchasima (1/47 isolates; 2.13%), and Nong Khai (1/57 isolates; 1.75%). Based on CLSI breakpoints, 12 out of 267 clinical isolates (4.49%) exhibited resistance. Resistance was observed against CAZ (2/267 isolates, 0.75%), AUG (8/267 isolates, 3.00%), and SXT (3/267 isolates, 1.12%). One isolate displayed resistance to both AUG and CAZ. Notably, AUG-resistant strains were widely distributed, particularly in Chaiyaphum, while all CAZ-resistant isolates were confined to Ubon Ratchathani. Additionally, one soil isolate (1/22; 4.55%) exhibited SXT resistance (Table 1, Supplementary Table 1). No resistance to carbapenems (imipenem and meropenem) was detected in this study.

MIC profiles for 300 Bp isolates were evaluated for five antibiotics. The MIC mode values for the antibiotics were as follows: CAZ (2 µg/mL), MERO (1 µg/mL), IMI (≤ 0.5 µg/mL), AUG (< 4/2 µg/mL), and SXT (≤ 1/19 µg/mL) (Fig. 2). While most isolates exhibited low MIC values, there was a notable trend toward higher MIC levels. The MIC variability of all antibiotics across infection sources was not statistically significant ($p > 0.05$; Supplementary Table 2). Among the tested antibiotics, IMI, AUG, and SXT were distributed mostly in the initial concentration in 97% (291 isolates), 94% (282 isolates), and 98% (293 isolates) of cases, respectively. CAZ demonstrated the highest frequency at 2 µg/mL, with 76% (229 isolates) showing an upward shift in MIC, and 18% (54 isolates) reaching 4 µg/mL. Similarly, MERO was most commonly observed at 1 µg/mL in 76% (258 isolates), with 4% (13 isolates) at 2 µg/mL (Fig. 2).

MALDI-TOF spectral analysis and antibiotic resistance correlation

Supervised cluster analysis of MALDI-TOF spectra revealed a tendency for antibiotic-resistant isolates (13/300) to group together. While AUG/CAZ and CAZ-resistant spectra showed distinct separation, AUG spectra exhibited only partial separation (Fig. 3A). SXT-resistant spectra largely overlapped with susceptible isolates but displayed a slight trend toward divergence (Fig. 3A). These findings suggest that the MALDI-TOF spectra reflect variations in protein expression associated with antibiotic resistance. A decision tree model was constructed using *m/z* ratios to differentiate resistance patterns. Among 1,184 total peaks, ten significant peaks (2250.52, 2272.25, 2362.63, 3151.96, 3977.36, 4072.81, 4605.19, 7536.54, 9732.58, and 12468.3 Da) were identified as key markers distinguishing resistant isolates. Nearly all susceptible isolates were grouped into a single node (286/287), with only one spectrum shown separately. However, none of these peaks were exclusive to resistance patterns (Fig. 3B). Spearman's rank correlation analysis showed no significant relationship between individual peak intensities and MIC levels (*rho*-squared values < 0.06) (Fig. 4).

Identification of significant biomarker peaks

The decision tree algorithm identified ten peaks as potential markers for differentiating antibiotic-resistant isolates. These peaks provided novel insights into previously unexplored spectral components in the genus *Burkholderia*. A database search in UniProt (within a ± 3 Da window) identified matches for seven peaks, while the remaining three peaks (2250.52, 2272.25, and 2362.63 Da) did not correspond to any known Bp or *Burkholderia* genus proteins (Table 2). This suggests the possibility of novel resistance-associated spectral signatures, warranting further investigation.

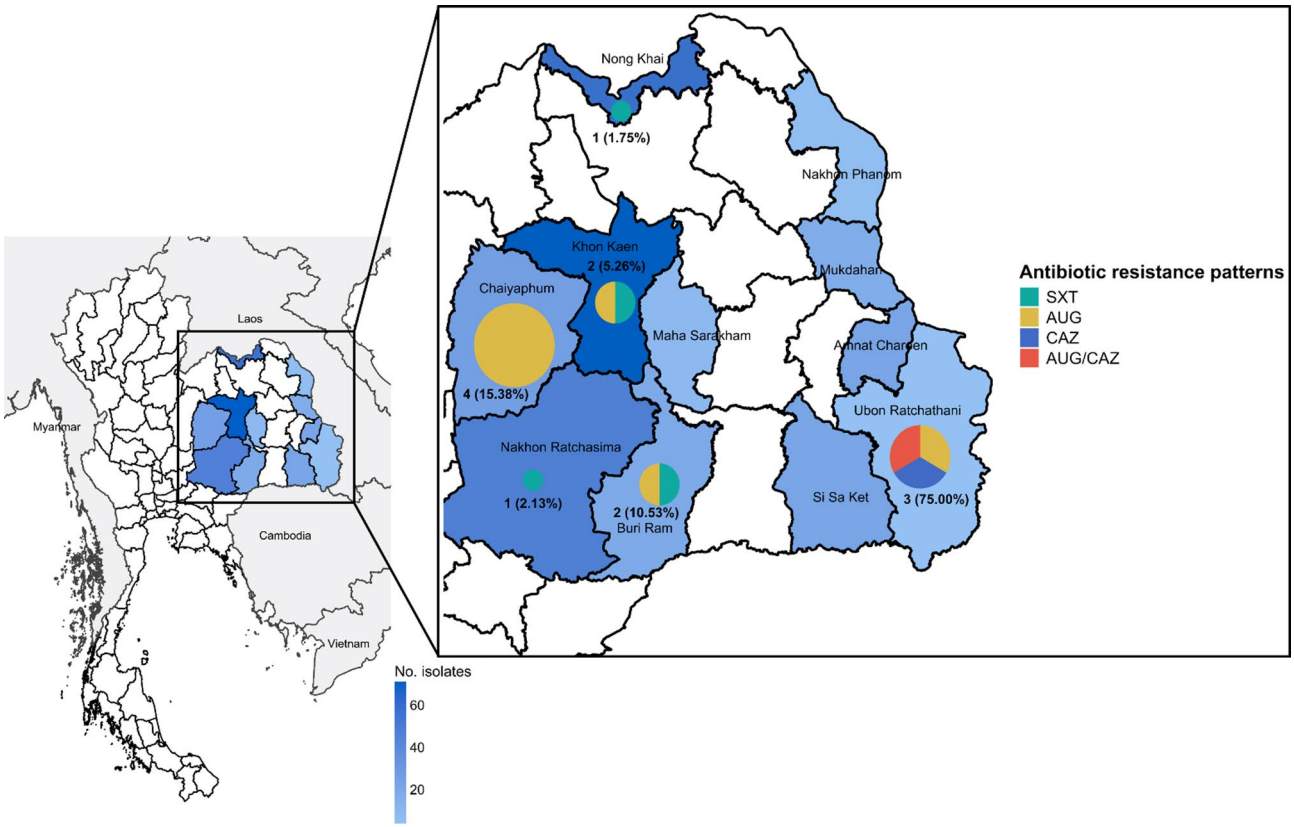

Fig. 1. Geographical distribution of 300 *Burkholderia pseudomallei* isolates sourced from humans, animals, soil and water across 11 provinces in northeastern Thailand. The blue gradient indicates the number of Bp isolates per province, while the pie charts illustrate the distribution of antibiotic resistance patterns within each province. The number displayed beneath each pie chart denotes the count and percentage of resistant isolates, which corresponds to the radius of the pie chart.

ID	Sources	Provinces	MIC values (μg/mL)					Antibiotic-resistant patterns
			CAZ	MERO	IMI	AUG	SXT	
B110	Patient	Ubon Ratchathani	16	4	4	**>16/8**	≤1/19	AUG
B130	Patient	Ubon Ratchathani	**32**	4	4	**>16/8**	≤1/19	AUG/CAZ
B230	Patient	Ubon Ratchathani	**32**	1	≤0.5	≤4/2	≤1/19	CAZ
EPBR034	Patient	Buri Ram	8	8	8	**>16/8**	≤1/19	AUG
EPBR042	Patient	Buri Ram	2	1	≤0.5	16/8	**>4/76**	SXT
EPCP121	Patient	Chaiyaphum	2	1	≤0.5	**>16/8**	≤1/19	AUG
EPCP124	Patient	Chaiyaphum	2	1	≤0.5	**>16/8**	≤1/19	AUG
EPCP131	Patient	Chaiyaphum	2	1	≤0.5	**>16/8**	≤1/19	AUG
EPCP136	Patient	Chaiyaphum	2	1	≤0.5	**>16/8**	≤1/19	AUG
EPMN102	Patient	Nakhon Ratchasima	≤1	≤0.5	≤0.5	≤4/2	**4/76**	SXT
EPNK022	Patient	Nong Khai	≤1	≤0.5	≤0.5	≤4/2	**>4/19**	SXT
SH018	Patient	Khon Kaen	8	8	4	**>16/8**	≤1/19	AUG
MBPE128	Soil	Khon Kaen	2	4	2	8/4	**4/76**	SXT

Table 1. MIC values of 13 *Burkholderia pseudomallei* isolates for five antibiotics used in melioidosis treatment. MIC; minimum inhibitory concentration, AUG; co-amoxiclav, CAZ; ceftazidime, IMI; imipenem, MERO; meropenem, SXT; co-trimoxazole. In bold indicates a resistant MIC value.

Discussion

Melioidosis remains a critical public health concern due to its high mortality rate, particularly when delays or inappropriate antibiotic treatments occur, increasing the risk of death within 48 h of symptom onset[12,13]. In this study, the proportion of CAZ- and SXT-resistant Bp isolates was consistent with previous reports in

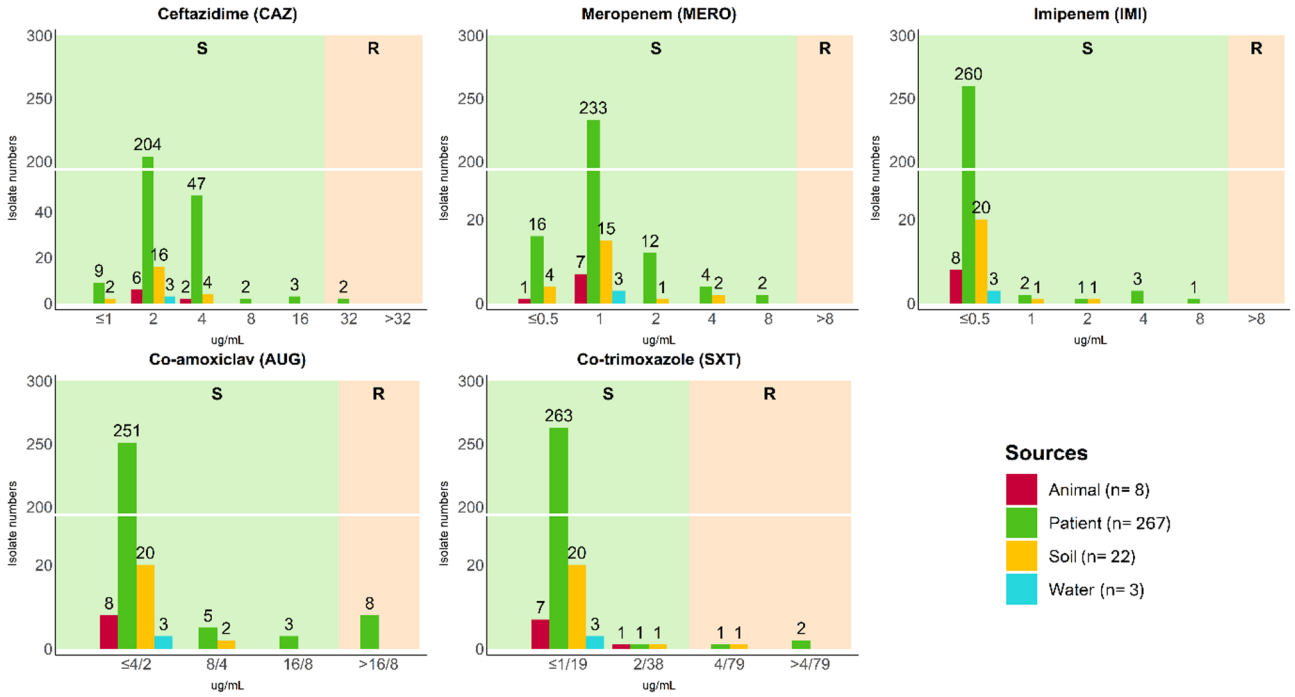

Fig. 2. MIC distribution of 300 *Burkholderia pseudomallei* isolates from various sources across five antibiotics (CAZ; ceftazidime, MERO; meropenem, IMI; imipenem, AUG; co-amoxiclav, and SXT; co-trimoxazole). S; susceptible, R; resistant.

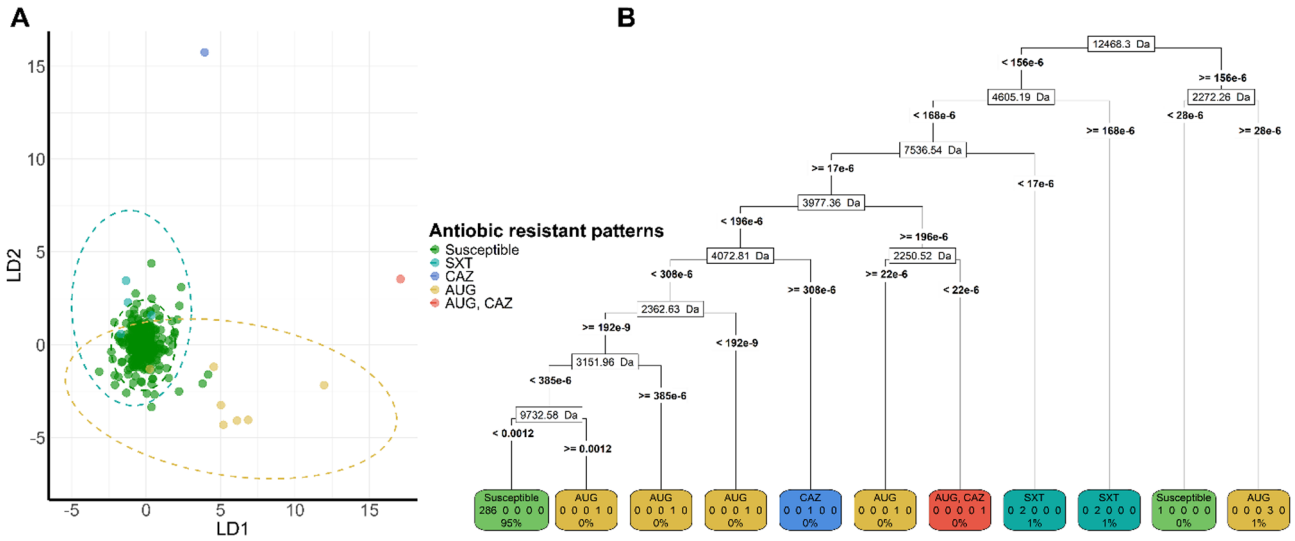

Fig. 3. Linear discriminant analysis (LDA) visualised as clusters of four antibiotic resistance patterns surrounded by ellipses indicating a 95% confidence interval (**A**). The decision tree illustrates binary decisions based on the relative intensities of the ten important peaks (2250.52, 2272.25, 2362.63, 3151.96, 3977.36, 4072.81, 4605.19, 7536.54, 9732.58 and 12468.3 Da) to differentiate between four antibiotic resistance patterns. The number inside each box represents the number of spectra. The percentage inside each box represents the proportion of spectra consecutively classified using each peak (**B**). Abbreviations: CAZ, ceftazidime; MERO, meropenem; IMI, imipenem; AUG, co-amoxiclav; SXT, co-trimoxazole.

Thailand (CAZ: 0.1–1.5%; AUG: 0.4%; SXT: <1%), while no carbapenem resistance was detected, in contrast to prior reports of MERO resistance (2%). However, AUG resistance was observed at higher levels than previously reported[7–10,13].

Resistant strains were identified in over half of the studied provinces (6/11; 54%), highlighting their widespread distribution. Ubon Ratchathani exhibited the highest proportion of drug-resistant strains (3/4;

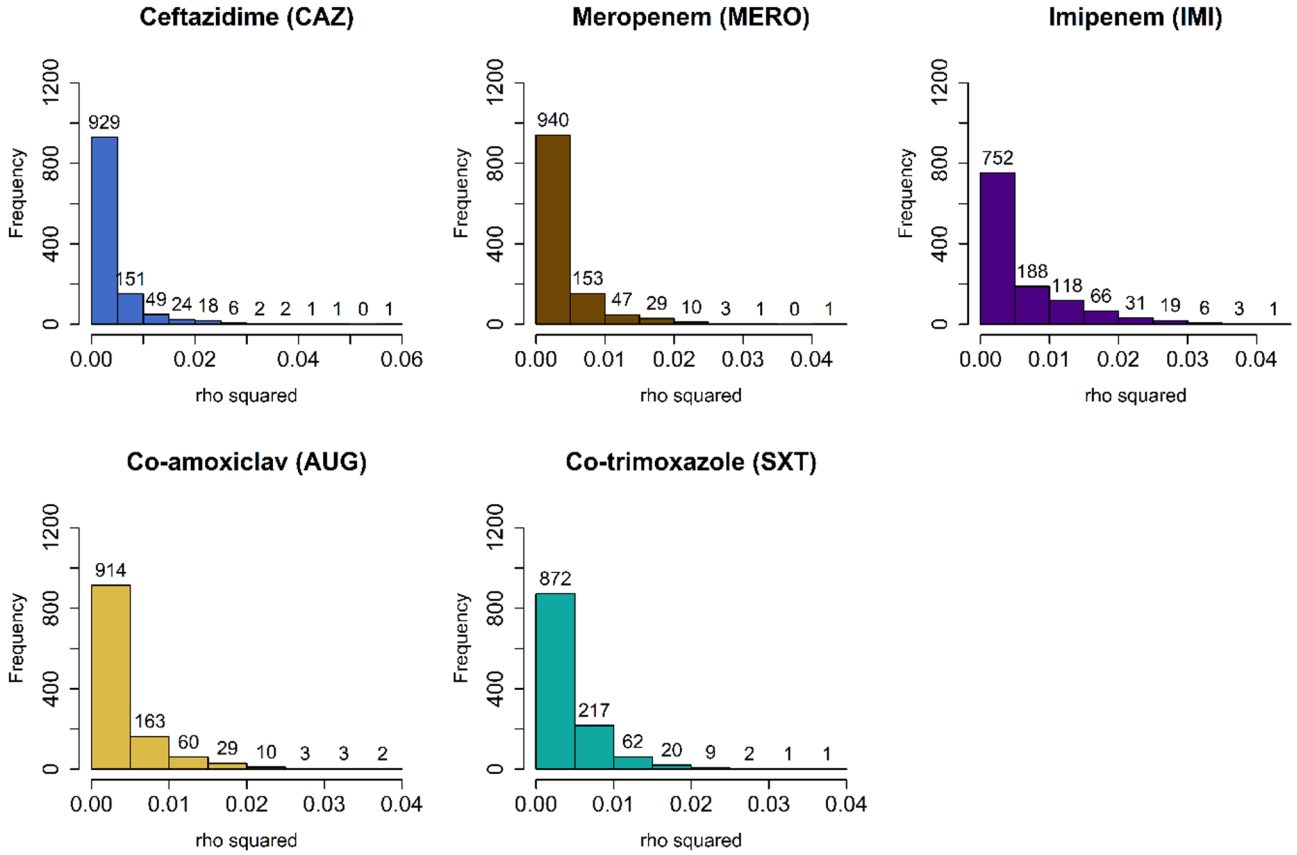

Fig. 4. The distribution of correlation coefficients for the relative intensities of each peak ($n = 1184$) against MIC levels of the five clinical antibiotics, analyzed using Spearman's rank correlation. The x-axis represents the *rho*-squared values and the y-axis represents the number of peaks. All five antibiotics show low *rho*-squared values, indicating that none of the peaks were significantly correlated with MIC levels. The maximum *rho*-squared values for CAZ, MERO, IMI, AUG, and SXT are 0.058, 0.041, 0.040, 0.037, and 0.040, respectively.

Experimental mass (Da)	Theoretical mass (Da)	Possible protein	Location	UniprotID
3151.96	3151	UDP-3-O-(3-hydroxymyristoyl)glucosamine N-acyltransferase[a]	Cytoplasmic protein	A0AAW3PYH1
3977.36	3977	Ring finger protein 208[b]	Cytoplasmic protein	B9BL45
4072.81	4070	BrnT family toxin	Cytoplasmic protein	Q6S450
4605.19	4608	Transcriptional regulator, AraC family	Cytoplasmic protein	A3P0E8
7536.54	7533	Membrane protein	Transmembrane protein	Q63TJ4
9732.58	9731	NADPH: quinone reductase	Cytoplasmic protein	A3NQX8
12468.3	12,468	Lipoprotein[c]	Outer membrane protein	A0A6P2P194

Table 2. The possible protein of the important peaks from the decision tree algorithm. [a]Identified in the closely related species *Burkholderia anthina*. [b]Identified in the closely related species *Burkholderia multivorans*. [c]Identified in the closely related species *Burkholderia lata*. Da, Dalton.

75.00%), followed by Chaiyaphum, where all isolates were AUG-resistant. Previous studies suggest that drug resistance may be linked to specific provinces, potentially influenced by clinical or agricultural practices[24]. Additionally, environmental factors such as rivers, wind direction[25] and anthropogenic activities, including human migration, may contribute to resistance dissemination[24,25]. Notably, Ubon Ratchathani has been reported to have the highest mortality rate[26,27] although this may be due to sampling bias, as most reports originate from Sunpasitthiprasong Hospital in this province[2]. The potential impact of sampling bias, particularly given the small sample size, should be considered when interpreting MIC data for resistant strains in relation to geographical distribution. Given the study's design, these findings do not provide incidence, epidemic, or prevalence data on resistant Bp in northeastern Thailand. However, they contribute to a broader understanding of resistance patterns.

All antibiotics maintained low MIC mode values, consistent with a 2018 study (CAZ: 4 µg/mL; MERO: 1 µg/mL; IMI: 0.5 µg/mL; AUG: 4 µg/mL; SXT: 1 µg/mL)[28]. However, first-line treatment drugs (CAZ, MERO) exhibited greater MIC variability compared to eradication-phase drugs (SXT, AUG). IMI, a first-line drug used less frequently in Thailand, may show a decreasing trend in susceptibility, necessitating ongoing monitoring.

Antibiotic resistance has been detected in clinical, environmental, and agricultural settings. In northeastern Thailand, an agriculture-dominant region, one soil isolate exhibited SXT resistance, suggesting an environmental reservoir of resistant strains. Natural selection, horizontal gene transfer, and antibiotic pressure may drive bacterial adaptation[29]. Commonly used antibiotics in Thai livestock, aquaculture, and agriculture include quinolones, tetracyclines, penicillins, macrolides, polymyxins, aminoglycosides, sulfonamides, amphenicols, lincosamides, and cephalosporins[30]. Many of these antibiotics are readily available without a prescription, increasing the risk of misuse and resistance development[31–34]. Cross-resistance between tetracycline and SXT in *Escherichia coli* has been reported, which is a relevant factor[35]. No antibiotic resistance was observed in isolates from zoo animals or water sources, likely due to small sample sizes, highlighting the need for further studies. Soil within zoo enclosures could act as a Bp reservoir, warranting additional investigation[36–38].

MALDI-TOF MS has advanced bacterial identification and resistance detection by analysing the 2000-20,000 Da spectral region, which includes ribosomal, nucleic acid-binding, and cold-shock proteins[39]. Supervised cluster analysis of Bp spectra successfully differentiated antibiotic resistance patterns. A decision tree model identified ten peaks associated with resistance, though none were uniquely specific. Seven proteins linked directly or indirectly to antibiotic resistance mechanisms were identified using UniProt, including those involved in lipid A biosynthesis, toxin-antitoxin systems, oxidative stress tolerance, and transcriptional regulation, similar to *E. coli*, *Pseudomonas aeruginosa*, *Brucella abortus*, and *Helicobacter pylori*[40–44]. However, the absence of matches for certain peaks suggests the presence of novel or unexplored spectral components. Resistance to CAZ and AUG in Bp is primarily mediated by *penA* mutations, promoter modifications, gene deletions, and gene amplifications[45] while SXT resistance is associated with *bpeT* and *bpeS* mutations, which regulate BpeEF-OprC efflux pump expression[45]. While the identified proteins did not directly correspond to these mechanisms, they may still be correlated. For example, in MRSA detection using MALDI-TOF MS, PSM-mec proteins were identified as correlates despite not being direct resistance determinants[46]. Further investigation into protein interactions and pathways is necessary to fully understand resistance mechanisms.

Most MALDI-TOF MS studies on antibiotic resistance focus on specific peaks, but relying on single markers may not provide sufficient discriminatory power[20,47]. MALDI-TOF MS has been shown to correlate spectral data with MIC levels in other microorganisms, such as *Fusarium* spp. treated with amphotericin B[48]. While this study found no significant correlation between individual peak intensity and MIC levels, broader spectral analysis rather than isolated peaks may be required to distinguish resistance patterns. Lack of correlation could also be influenced by the narrow MIC range, small sample size, or culture conditions. Additionally, differences in bacterial genera, sample preparation, and machine learning algorithms complicate comparisons across studies[15,20]. Lastly, our exploratory study is intended to establish a proof-of-concept and to identify promising spectral markers for resistance detection, which will require future validation.

Many limitations should be noted. The small number of resistant isolates in our study may not fully reflect the true prevalence of resistance, emphasising the need for large-scale studies. Furthermore, the limitations in sample availability and culture viability resulted in disparate sample sizes across provinces, which unfortunately deviated from the intended proportional representation of disease prevalence, particularly impacting the number of Bp isolates obtained from Ubon Ratchathani, a province with a notably high incidence rate. The limited sample size should be acknowledged, as it may result in overfitting the decision tree model. Additionally, the absence of detailed epidemiological data, such as the year of sample collection in all samples, restricts the ability to track resistance trends over time. Furthermore, the lack of detailed clinical information, including individual patient treatment history, presents a valuable opportunity for future research to investigate the evolution of Bp resistance, particularly through the study of isolates developing resistance during prolonged treatment, which may provide insight into the emergence of resistance mechanisms during treatment. Further validation using advanced techniques, such as LC-MS/MS, is necessary to confirm protein associations with resistance mechanisms. A key limitation of this study is the lack of external validation using an independent dataset to confirm the reproducibility and diagnostic accuracy of the identified MALDI-TOF MS spectral markers. This was primarily due to the limited availability of drug-resistant Bp isolates and biosafety constraints that restrict additional testing. Future studies should focus on validating these findings through larger-scale external datasets and incorporating alternative analytical approaches to enhance the robustness of MALDI-TOF MS-based resistance detection.

This study provides preliminary evidence supporting MALDI-TOF MS as a rapid tool for detecting antibiotic resistance in Bp. By integrating resistance analysis with direct colony identification, MALDI-TOF MS offers a faster and less labour-intensive alternative to molecular methods, making it highly suitable for high-throughput microbiology laboratories. With results available within minutes, this approach facilitates timely clinical decision-making, potentially improving treatment outcomes for critically ill patients with antibiotic-resistant infections.

Methods

Bacterial samples

The Biological Sample Bank of the Melioidosis Research Center, Faculty of Medicine, Khon Kaen University, Thailand, provided 268 Bp isolates in bacterial stock cultures, preserved in a 20% glycerol stock at -20˚C. These isolates were collected between 2004 and 2015 from various sources, originating from eleven northeastern Thai provinces—Amnat Charoen, Buri Ram, Chaiyaphum, Khon Kaen, Maha Sarakham, Mukdahan, Nakhon Phanom, Nakhon Ratchasima, Nong Khai, Si Sa Ket, and Ubon Ratchathani: 251 from human patients (the

source of samples includes 93 from blood, 70 from pus, 33 from sputum, 11 from urine, and 44 from unknown sources), 6 from soil, 3 from water, and 8 from dead animal tissue samples (the source of samples includes 2 from liver, 1 from the nose, 1 from the kidney, 2 from the lung, 1 from the spleen, and 1 from the heart). Additionally, 16 Bp isolates were obtained from biobank stock cultures of the clinical routine laboratory at Srinagarind Hospital (the source of samples includes 5 from blood, 4 from pus, 2 from urine, 1 from body fluid, and 4 from unknown clinical specimens, collected between 2020 and 2022), alongside 16 isolates from soil samples (the source of samples includes rice fields), using methods consistent with a previous study[49]. In total, 300 Bp isolates were analysed in this study. The selection of sampling methodologies and isolate sources was determined by the reported prevalence of Bp in each province and a prioritization of isolates demonstrating resistance to any antibiotic agent. Supplementary Table 3 provides a comprehensive breakdown of this selection. All isolates were cultured on Ashdown's agar for 48 h, followed by confirmation using a commercial latex agglutination assay (LATEX, Mahidol University, Thailand) according to the manufacturer's protocol[50].

This study used leftover specimens without any information that could lead to the identification of participants; therefore, informed consent was not required. The use of bacterial leftover samples from animals and associated data was part of a collaboration between the Melioidosis Research Center and Khon Kaen Zoo, authorized by Khon Kaen Zoo, Khon Kaen, Thailand. This research was conducted in a biosafety level 2 enhanced (BSL-2 enhanced) laboratory, in compliance with Thailand's Pathogens and Animal Toxin Act (Sects. 18, 2018). Ethical approval was granted by the Khon Kaen University Ethics Committee for Human Research (HE641201), in accordance with the Declaration of Helsinki and ICH Good Clinical Practice guidelines.

Minimum inhibitory concentration (MIC) determination

MIC determination followed the CLSI M45 3rd edition guidelines[6]. In brief, fresh Bp colonies were harvested after 24 h of incubation at 37 °C on blood agar with 5% sheep blood (Clinag Co., Limited, Thailand). Colonies were suspended in normal saline solution and adjusted to a 0.5 McFarland standard. A total of 10 µL of the McFarland suspension was added to 10 ml of cation-adjusted Mueller-Hinton broth (Clinag Co., Limited, Thailand) and mixed thoroughly. Using an automated inoculation machine (Sensititre AIM, Thermo Scientific, USA), 50 µL of the mixture was dispensed into each well of a 96-well plate (THAN1F, Sensititre™, Thermo Fisher Scientific, USA). Plates were incubated at 37 °C for 24 h in a Sensititre ARIS 2X incubator (Thermo Scientific, USA), after which MIC values were automatically recorded. The MIC distributions of five key antibiotics used for Bp treatment—ceftazidime (CAZ), meropenem (MERO), imipenem (IMI), co-amoxiclav (AUG), and co-trimoxazole (SXT) (Supplementary Table 4)—were analysed and visualised using the ggplot2 R package (version 3.4.4). CLSI breakpoints were used for all antibiotics, except for MERO, where IMI breakpoints were applied, as previously described[51]. Standardized control strains, *Escherichia coli* ATCC 25,922 and *Pseudomonas aeruginosa* ATCC 27,853, were employed for drug susceptibility testing.

Sample Preparation and MALDI-TOF MS analysis

Fresh colonies grown on blood agar were directly smeared onto a target plate. After air-drying, 1 µL of matrix solution (cyano-4-hydroxycinnamic acid, HCCA) was applied and allowed to dry at room temperature. The target plate was analysed using a MALDI-TOF MS instrument (Autoflex, Bruker Daltonics, Germany) operated with flexControl software. The instrument was set with acceleration voltages of 25.00 kV and 23.45 kV for ion sources 1 and 2, respectively, and lens voltages at 6.0 kV. For external calibration, the Bruker Daltonics Bacterial Test Standard, an extract of *Escherichia coli* DH5α, was utilised. Identification scores were calculated on a logarithmic scale (0–3) using MALDI Biotyper Compass software against an in-house database from Srinagarind Hospital, with values ≥ 2.000 for species-level identification, 1.700–1.900 for genus-level identification, and < 1.700 with no identification at the organism level. All isolates were confirmed as Bp, with an average identification score of 2.43 (range: 1.80–2.69) (Supplementary Fig. 1).

MALDI spectra (2,000–20,000 Da) were obtained and processed following a previously described pipeline[49] using the MALDIquant R package[52]. Preprocessing steps included: (i) Exclusion of 2000–2250 Da range (to remove high-intensity noise signals); (ii) Square root transformation for intensity normalisation; (iii) Savitzky-Golay filtering (half-window size: 20)[53] for intensity smoothing; (iv) Baseline correction using the SNIP algorithm (25 iterations); (v) Total ion current normalisation for spectral standardisation; (vi) Spectral alignment across all isolates; (vii) Peak detection using the MAD noise-estimation algorithm (signal-to-noise ratio: 5, half-window size: 20); (viii) Peak binning at 2000 ppm resolution. Following these steps, a peak list of 1,184 mass signals was generated for downstream analysis.

Spectral analysis of antibiotic resistance patterns and MIC correlations

Supervised clustering was performed using linear discriminant analysis (LDA) from the MASS R package (v7.3-60), which optimises the separation between resistant and susceptible isolates. Antibiotic resistance patterns were further classified using a decision tree algorithm (rpart R package, v4.1.23), which iteratively splits data based on feature values. To investigate correlations between MIC levels and spectral peak intensities, Spearman's rank correlation test was applied using the pspearman R package. The MIC distribution variability was assessed using the Brown-Forsythe test from the onewaytests R package (version 3.0).

MALDI peak identification

To investigate potential resistance mechanisms, the decision tree model was employed to identify significant spectral peaks. Peak annotation followed the approach of a previous study[54] applying a ± 3 Da window and excluding post-translational modifications. Protein identification was performed via UniProt database searches (https://www.uniprot.org/) using "*Burkholderia*" as the primary search criterion, prioritising Bp-specific entries.

If Bp-specific proteins were unavailable, the search was expanded to the *Burkholderia* genus. Identified proteins were then filtered, retaining those most likely to be functionally associated with antibiotic resistance.

Data availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Received: 12 February 2025; Accepted: 17 June 2025
Published online: 01 July 2025

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Acknowledgements

This research project was supported by the National Research Council of Thailand (NRCT) (Grant No. N41A640200), the National Science, Research, and Innovation Fund, Thailand, and the Research Fund for Supporting Lecturers in Admitting High-Potential Students to Study and Research in Their Expert Program (Grant No. 641H220). We thank the Melioidosis Research Center, Khon Kaen University, for providing most of the Bp strains used in this study.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-07687-y.

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