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Adaptation of an *in vitro* one-compartment pharmacodynamic chemostat for testing efficacy of ceftazidime against *Burkholderia pseudomallei* outside a BSL3 facility ☆

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ABSTRACT

Non-clinical pharmacokinetic-pharmacodynamic (PKPD) models are crucial in the initial design of drug-dosage regimens and in drug development but has rarely been employed for testing high-risk organisms due to stringent handling procedures. *Burkholderia pseudomallei* is classified as a Tier 1 select agent with international guidelines recommending this organism to be handled within a biosafety level 3 (BSL3) facility. Unfortunately, BSL3 facilities are not widely available in low-resource settings. This paper describes a detailed guide for setting up an *in vitro* pharmacodynamic infection model specific for testing *B. pseudomallei* outside BSL 3 laboratory. Briefly in this study,

- All procedures involving active handling of live *B. pseudomallei* cultures were performed strictly inside a class II BSC in BSL-2 plus negative airflow laboratory.
- The model was set to simulate *B. pseudomallei*-bacteremia treated with ceftazidime, a 1st-line anti-melioidosis drug with an approximate 2-hour half-life. Model validation was performed by simulating ceftazidime half-life.
- For the pharmacodynamic study, ceftazidime was given as bolus injections at 8-hour intervals into the central culture chamber containing actively growing *B. pseudomallei*.

Specifications table

Subject area:	Pharmacology, Toxicology and Pharmaceutical Science
More specific subject area:	Pharmacokinetic and pharmacodynamic simulation of antibacterial agents against melioidosis
Name of your method:	*In vitro* one-compartment chemostat for evaluating ceftazidime PK/PD against *B. pseudomallei*
Name and reference of original method:	New *In vitro* Model to Study the Effect of Antibiotic Concentration and Rate of Elimination on Antibacterial Activity (Grasso et al., in Antimicrobial Agents and Chemotherapy, 1978)
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Background

Melioidosis, also known as Whitmore's disease, is an infection caused by the bacterium *Burkholderia pseudomallei*. It is endemic in Southeast Asia, including in Malaysia where it is considered a significant public health issue. In the absence of timely and appropriate antibiotic therapy, mortality rates for melioidosis may exceed 50% [1,2]. Current standard treatment regimens for melioidosis are formulated based on clinical observations and standard lab susceptibility methods that estimate drug minimum inhibitory concentrations (MIC) such as Etest strips and broth microdilution (BMD) assays. These approaches overlook the underlying dynamicity of both the pharmacokinetic and pharmacodynamic (PK/PD) properties, the knowledge of which is essential for determining the most appropriate anti-melioidosis treatments. *In vitro* PK/PD models provide essential data describing effects of drugs on bacterial populations, enabling optimization of multi-variated drug-dosing regimens [3–5].

Unlike static or batch cultures where bacterial nutrients are diminished over time, *in vitro* chemostat models allow for fresh media to be constantly supplied to the bacteria culture throughout antimicrobial exposure, supporting investigations of different and longer treatment durations in drug-dosing studies [6]. As insufficient nutrients affect microbial growth and activity influencing the drug-susceptibility outcomes, chemostats allow for the differences in killing behaviours of antimicrobials to be studied over time, as well as in real-time. Although PK/PD data are traditionally derived from animal models and human clinical sampling, it would be more practical, desirable, and ethical to retrieve such data from an *in vitro* model that does not necessitate use of experimental animals or human trials during the early phase of the drug's development. The PK/PD of existing and other potentially active antibiotics for melioidosis treatments remain under-investigated, limited by the risks associated with handling pathogenic *B. pseudomallei*, which CDC designates as a Tier 1 Biological Select Agent [7]. A few cases of laboratory transmitted melioidosis have been reported [8]. Thus, manipulation of Tier 1 organisms such as *B. pseudomallei* and its cultures are recommended by international guidelines to be performed in a class II biosafety cabinet (BSC) within a biosafety level three (BSL3) containment facility [9]. However, such high-level facilities are not easily available, especially in low-resource countries or settings. This protocol aims to describe in detail the set-up of a basic one-compartment *in vitro* pharmacodynamic chemostat to simulate anti-melioidosis activities of ceftazidime. Furthermore, the model described is partially set-up within a BSC class II type A2 in BSL-2 plus negative airflow laboratory for enhanced safety, as an alternative to performing laboratory activities involving high-risk pathogens within difficult-to-access BSL3 facilities.

After setting up the experiments in BSC, the safety aspects were reviewed by safety officers and the system was validated by 1) measuring the ceftazidime concentrations at the following simulated 2-hour half-lives: 0, 2, 4, and 6 h, using the highly-sensitive ultra high-performance liquid chromatography-mass spectrometry (LC-MS/MS), and 2) enumerating viable colony forming units (CFU) of the control organism not exposed to antibiotics through a series of dilution and subsequent culture on agar.

Method details

Bacterial isolate and antibiotic source

A drug-susceptible clinical isolate designated as IMR-BP4 was used in the current optimization study. Ceftazidime powder for IV injection (Cefatum, Duopharma, Malaysia) was prepared at a 10 mg/ml stock solution in water, filtered through a 0.22 μM filter membrane, and stored at −80 ˚C in aliquots if not used immediately. Minimum inhibitory concentrations were determined using Etest gradient strips (Biomeriex, France) and broth micro dilution assays in accordance with CLSI antibiotic susceptibility testing guidelines.

B. pseudomallei culture

Colonies from an overnight culture of IMR-BP4 on blood agar were suspended in normal saline (0.85%) to an approximate concentration of $\sim 1 \times 10^8$ CFU/mL bacterial cells; the CFU concentration was determined from previous growth curve experiments of IMR-BP4. This solution was then diluted 100-fold in CAMHB to achieve $\sim 1 \times 10^6$ CFU/mL cells, and was incubated with stirring on a magnetic hot plate at 37 ˚C for 30–60 min before commencing with the experiment. Two sets of the diluted cultures were prepared, one for antibiotic treatment and the other to serve as non-treated control.

Safety protocol and discard of biological waste

With limitation in accessing BSL 3 facilities, we were unable to avoid working with *B. pseudomallei* in an alternative laboratory setting. This organism is endemic in Malaysia and is commonly isolated from local clinical specimens and the environment [10]. Risk assessments had been performed and reviewed by the Institutional Biosafety and Biosecurity Committee (IBBC) together with this protocol of an *in vitro* one-compartment pharmacodynamic chemostat for antibiotic testing against *B. pseudomallei* outside a BSL 3 facility. This protocol was designed for optimal usage of a Class II Biosafety Cabinet (BSC) as a physical barrier, dependent on its superior ventilation and filtration capabilities. This hazard control strategy via engineering controls, while not eliminating the pathogen, effectively isolates and protects the operator from any such hazards [11]. Essentially, the inoculated reservoir and waste bottles, as well as any potentially aerosol-producing steps, were contained within a Class II BSC in a BSL-2 plus negative airflow laboratory for the entire experiment. The spillage kits were ready in the laboratory in the event of any accidental spillage occurrence. The biological waste was discarded according to the protocol. To assess the safety of the experiment, an open agar plate method was employed. Six plates consisted of Mueller-Hinton, Columbia Blood, and *B. pseudomallei*-selective Ashdown agar were strategically

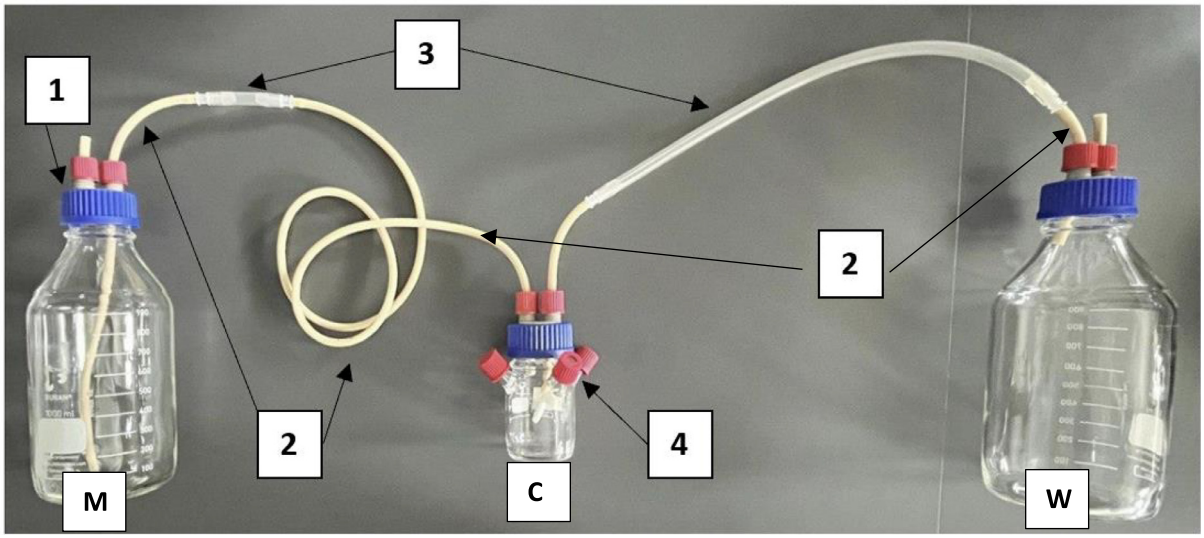

Fig. 1. Chemostat accessories and connectors. From left- M: media reservoir, C: central culture reservoir and W: waste reservoir. 1: GL45 Schott bottle caps with two GL14 thread ports, 2: Masterflex PharMed© BPT L/S 16 tubing with Luer lock connectors at each end, 3: Masterflex Pump Silicone Tubing (Platinum) L/S 17, 4: Custom Schott bottle with 3 GL14 thread glass ports containing silicone rubber seals.

placed: 1) Within the biosafety cabinet (BSC) between the work area and the posterior air circulation vent, and 2) Just outside the BSC next to the operator. This arrangement aimed to monitor for any spillage of *B. pseudomallei* during the experiment and to confirm the absence of contamination or accidental release of the pathogen in the surrounding area. Upon completion of all the necessary risk assessments, the IBBC approved the enhanced-safety protocol for working with *B. pseudomallei* in a class II BSC within an enhanced BSL 2.

One-compartment chemostat model for PK and PD studies

The *in vitro* pharmacodynamic chemostat set-up adapts a one-compartment infection model that has been described by others [6,12,13]. As shown in Fig. 1, the model consisted of a 1 L sterile media reservoir which continuously supplied the 100 mL central reservoir (culture chamber) with fresh, antibiotic free Cation-Adjusted Mueller Hinton broth (CAMHB) for the entire 24-hour run. Fig. 2 indicates chemostat set-up within a class II BSC.

A computerized peristaltic pump (MasterflexTM L/STM Digital Drive; Cole-Parmer, Vernon Hills, IL, USA) was programmed to deliver the fresh media at a rate that simulated the half-life ($t_{1/2}$) of the antibiotic being tested, which in this case was ceftazidime with a $t_{1/2}$ = 2 h. The formula used to calculate the flow-rate to achieve desired antibiotic half-lives, known as 'clearance rate' or Cl is as follows:

$$Cl = \frac{ln_2 * V(ml)}{t_{1/2}(\text{min})}$$

where V is volume of media in the central compartment in millilitres, and $t_{1/2}$ is the antibiotic's estimated half-life, in minutes. Antibiotics were administered as boluses into the culture chamber using a 3 ml syringe and 21 G needle through one of its multiple sampling ports covered with silicone rubber seals. Fresh media was pumped into the central culture chamber, continuously replacing its antibiotic-containing media, resulting in a 50% antibiotic decrease or 'elimination' after two hours. The eliminated waste was pushed out via positive pressure from the culture chamber into the waste compartment which contained a 1% solution of the disinfectant Rely+On™ Virkon™ (LanXess, US) as a decontaminating agent. Each chamber was equipped with silicone tubes and luer lock connectors to ease assembly and reduce risk of introducing contaminants into the chambers during autoclaving and setting-up of the model. The central infection chambers were all placed in a 37 °C-paraffin bath on top of a magnetic hot plate stirrer. Temperature of the paraffin bath was monitored through a K-type Thermocouple Data Logger probe placed inside a capped glass bottle filled with water and put together with the culture chambers inside the bath. All the central and waste compartments were placed and operated inside a BSC throughout the entire run.

Method validation

Pharmacokinetic studies for one-compartment chemostat validation

Prior to commencement of the *in vitro* infection experiment, the one-compartment peristaltic pump model was validated for its functionality in simulating ceftazidime half-lives of two hours. Ceftazidime for injection powder was weighed and dissolved in water

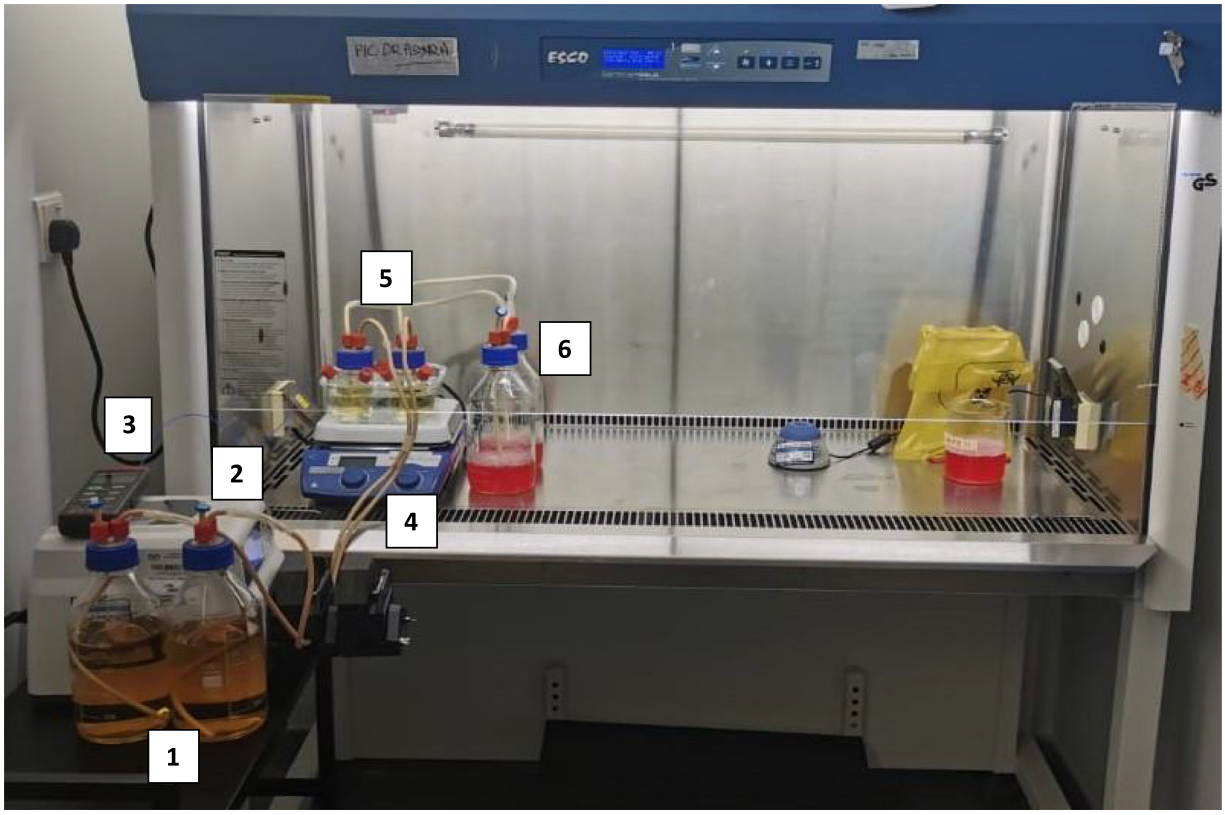

Fig. 2. Chemostat set-up within a class II BSC 1: Media reservoir containing sterile CAMHB, 2: Masterflex Peristaltic Pump set at desired flow-rate, or 'Clearence Rate', 3: Temperature monitoring probe placed inside paraffin bath, 4: Magnetic hot plate stirrer set at higher optimized temperature to ensure a maintained ~37 ˚C paraffin bath, 5: Central reservoirs containing active *B. pseudomallei* non-antibiotic control and antibiotic-exposed cultures with magnetic stir bars for constant stirring, placed in a temperature-regulated paraffin bath, and 6: Waste reservoirs containing decontaminant solution.

to a concentration of 10 mg/ml. The antibiotic solution was then passed through a 0.22 μM filter membrane before being injected into the central chamber containing sterile CAMHB via a sampling port to a final concentration of approximately 200 μg/ml. After allowing the antibiotic to mix for several minutes, 500 μl were sampled out and designated as 0-hour time-point sample. Sampling was subsequently repeated at 2, 4, and 6 h. All pharmacokinetic samples were subjected to quantification by multiple reaction monitoring (MRM) using an Acquity UPLCTM liquid chromatography system coupled to XEVO TQ-XS (Waters Corp., Milford, USA) using pure ceftazidime powder for analytics (Sigma, USA) as standard. Samples that were not used immediately were stored at −80 ˚C until further analysis.

In vitro pharmacodynamic model

A final ceftazidime concentration of 170μg/ml simulating peak serum concentrations (C_{max}) was selected based on clinically attainable concentration range [14] and was administered as multiple boluses with a time interval of 8 h, by injecting into the central infection compartment using a needle and syringe via the silicone rubber-sealed sampling ports. Samples for determining viable bacterial cells were collected at 0, 4, 8, 16, 20, and 24 h throughout the experiment and subjected to a series of 10-fold dilutions in normal saline. A hundred microlitres of the diluents were spread onto Mueller-Hinton agar and incubated at 37 ˚C for 18 to 24 h. Numbers of colony-forming units (CFU) were enumerated and multiplied with its dilution factor to determine time-specific bacterial concentration. CFU/ml time curves were prepared by plotting the sampled time points *versus* their respective log10 CFU/ml concentrations, and analysed with an emphasis on the 24-hour time point to determine the overall effects.

Results and discussion

The study aimed to optimize and adapt an *in vitro* one-compartment chemostat for pharmacodynamic experiments with the high-risk pathogen *B. pseudomallei*. The model was optimized to run in a class II biosafety cabinet (BSC) as an enhanced safety and containment alternative to the preferred settings of a biosafety level 3 (BSL3) facility, inaccessible to some researchers. Despite conducting experiments that generated bioaerosols, the risk of laboratory-acquired melioidosis was found to be low. This suggests

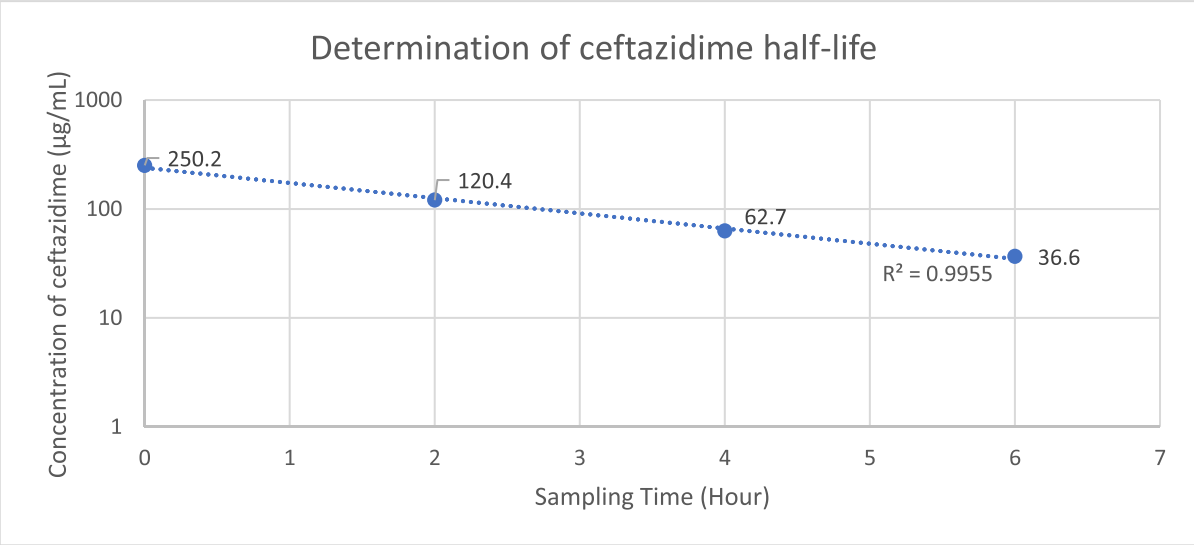

Fig. 3. LC-MS/MS measurements of two-hourly sampling intervals showing decrease in ceftazidime concentrations simulating antibiotic half-life.

that handling *B. pseudomallei* in endemic regions may be practical and safe even outside of a BSL3 (Biosafety Level 3) facility [8,9]. In the current study, experimentations involving *B. pseudomallei* were strictly handled and performed entirely in the BSC. The primary challenge was the limited space within the BSC to safely run the chemostat and process bacterial samples for viable cell counts. This issue was addressed by conducting the experiment in small batches thus facilitating the process and minimizing the risk of accidents due to overcrowding. The system complies to our safety regulations, evidenced by the randomised open plate screening showing no contamination of the pathogen inside or outside the system and its surrounding area. The screened discarded waste materials were also cleared from any viable pathogen.

B. pseudomallei IMR-BP4 minimal inhibitory concentrations

Minimum inhibitory concentrations (MIC) of ceftazidime using Etest strip and broth micro dilution (BMD) assays showed similar MIC values of <2 µg/ml, confirming susceptibility of IMR-BP4 to this antibiotic. A drug-susceptible *B. pseudomallei* strain is ideal for optimization purposes as it represents wild-type PK/PD outcomes. To our knowledge, there are currently no available publications on PK/PD activities for *B. pseudomallei* exposed to ceftazidime in an *in vitro* infection model. Results of this study using susceptible strains can be used as baseline data for further *in vitro* modelling experiments involving drug-resistant isolates or for resistance-emergence studies.

PK validation

Two-hourly ceftazidime concentrations were determined using an ultra-high-performance liquid chromatography mass-spectrometry (UPLC-MS/MS) system, which has increased-sensitivity and specificity compared to a standard HPLC. The results are plotted in a semi-log graph as shown in Fig. 3 below.

Flow-rate of the peristaltic pump was set to simulate ceftazidime's half-life. Half-life, or $t_{1/2}$ is an exponential term used to describe drug kinetics, and $t_{1/2}$ = 2 h of ceftazidime represents the time for this antibiotic to decrease by half its original concentration. As accuracy and precision in determining half-life requires a minimum of three concentration-time points obtained over an interval during which the drug concentration is decreased by at least 50% [15], we utilized four such time points: 0, 2, 4, and 6 h. The concentration slope obtained showed a coefficient of determination, R^2, of 0.9967, achieving acceptable drug-removal rates within the stipulated time. The rate of drug removal is highly influenced by stable inflow and outflow of fluids between the chemostat chambers [16], indicating pressure within the bottles were satisfactorily maintained in both the central and waste chambers of this one-compartment model. These data validate feasibility of the chemostat set up to simulate ceftazidime clearance *in vitro* even within the limiting confinements of a biosafety cabinet (BSC).

Initial antibiotic concentration at 0 h was estimated to be 200 µg/mL; our results however showed initial concentrations in excess of approximately 50 µg/ml, corresponding to a recovery rate of 125%. While minor variations in the weighing of the ceftazidime powder or dilution from the prepared stock solution may have contributed to these differences, a recovery rate greater than 120% is commonly attributed to matrix effects during the LC-MS/MS analysis [17]. Goh and colleagues [18] reported that matrix effect of CAMHB endogenous compounds were able to enhance or suppress signal intensity of target compounds, while Miao Ziao et al. [19] had to employ additional clean-up procedures in order to reduce significant interference from this culture media. Therefore, when

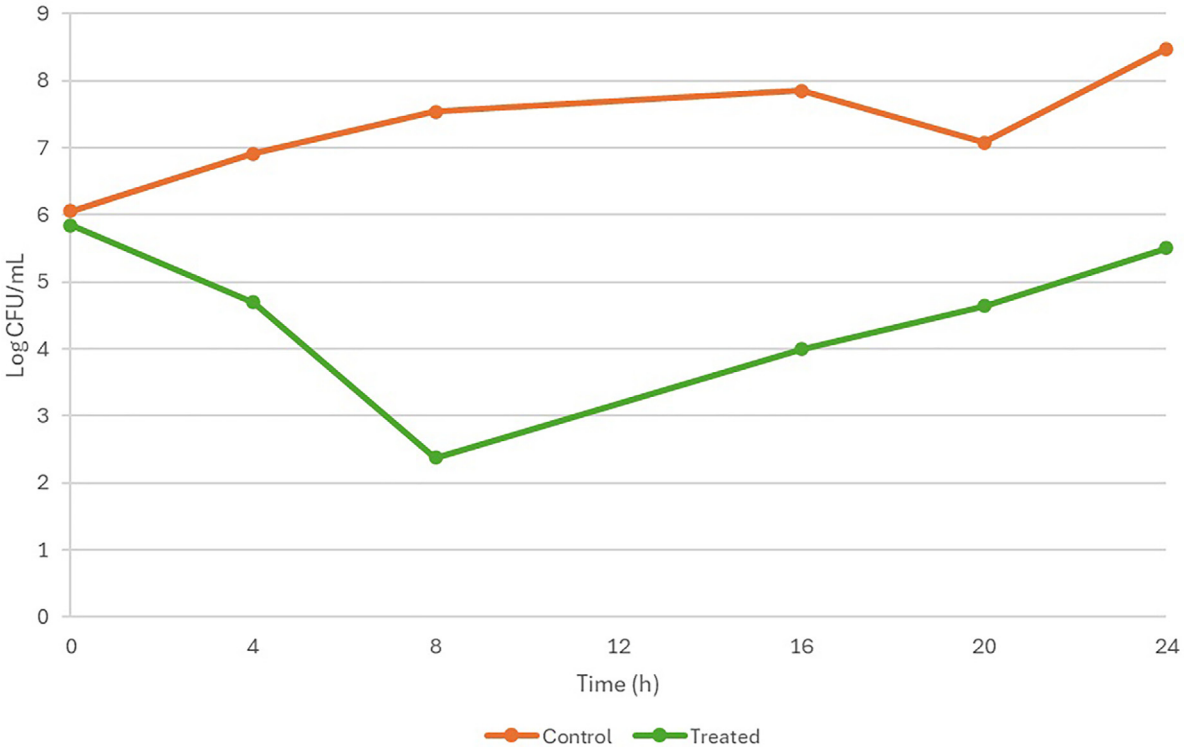

Fig. 4. The growth curve of *Burkholderia pseudomallei* IMR-BP4 in one-compartment chemostat. In treated reservoir, ceftazidime was delivered at C_{max} 170 μg/ml every 8-hour.

accurate measurements of antibiotics in CAMHB are required, for example in correlating effect of drug concentration to bacterial killing, inclusion of proper pre-treatment or sample clean-up procedures is essential.

In vitro pharmacodynamic model

The results of pilot pharmacodynamic study are shown in Fig. 4.

The control growth curve confirmed that bacterial population can thrive and are maintained under the experimental conditions offered by this one-compartment chemostat. Cultures on MH agar that were plated out at each sampling interval confirmed homogeneity of the culture. Microbiological response to the 8-hourly ceftazidime bolus injection therapy was examined through change in $\log_{10}$ CFU/ml from 0 h (CFU_0) to time t (CFU_t). A more than 3-$\log_{10}$ reduction in CFU/ml was observed after the first 8 h, indicating an initial bactericidal activity. Subsequently however, there was steady bacterial regrowth even after repeated dosing at 8 and 16 h. At 24 h, bacterial growth was almost similar to that of CFU_0 with a less than 1-$\log_{10}$ reduction in bacterial CFU/ml. The minimal bacterial killing activities in this experiment at 24 h is not entirely unexpected. This pilot experiment was using 8-hourly regimen, based on old recommendation of melioidosis therapy by Malaysian National Antibiotic Guidelines, 2014 [20]. The actual experiments in future will accommodate to the latest guideline of 6-hourly dosing regimen of ceftazidime (Malaysian National Antibiotic Guidelines, 2024 [21]). Besides, previous PK/PD studies have shown ceftazidime to be more effective when given as continuous infusion or at shorter intervals [22]. All the different parameters mentioned and extended experiment duration could be safely applied and investigated in this validated chemostat model within the BSC in future studies.

Throughout the experiment, culture levels in the central chambers were generally preserved which was indicative of a stable internal pressure leading to a satisfactory flow into and out of the chambers. However, during the optimization period, we observed occasional fluctuations in culture volume levels, presumably due to pressure instability within the chamber. These fluctuations were often noted after multiple uses of the same BPT tubing. Once the tubes were replaced, pressure was stabilized, and culture levels were maintained. Constant expanding and shrinking of inner tube diameter due to internal pressure changes, and pinching of the tubes with every tightening of the adjustable sampling ports on the bottle caps where the tubes entered through are probable factors causing pressure leakage, affecting flow rate [16]. As an alternative to periodically changing tube sets, users could consider caps with multiple built-in ports, such as Nalgene's 3 port GL45 PSF closures (supplied by Thermo Scientific) thus eliminating the risk of tube pinching. And as a side note, instead of using magnetic hot plate stirrers, a small magnetic stirring water bath could also facilitate in ensuring uniformed temperature-controlled mixing, while eliminating the use of paraffin oil.

Handling large quantities of live Tier 1 select agents necessitates strict safety protocols to minimize the risk of laboratory-acquired infections. While a BSL-3 laboratory is the ideal setting, this study demonstrates that a biosafety cabinet (BSC) Class II Type A2 within a BSL-2 laboratory with negative airflow can provide adequate safety in *B. pseudomallei* endemic regions, particularly in resource-limited settings, where a BSL-3 facility may not be readily available. This approach could also be adopted as an enhanced safety measure when working with other infectious agents of lower risk but in significant volumes or for extended periods, as commonly encountered in *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) studies.

Conclusion

This method adapts an *in vitro* one-compartment chemostat model for use in the confined, but safety-enhanced, space within a Class II BSC. This enables PK/PD experimentations with high-risk organisms such as *B. pseudomallei* which otherwise would have had to be carried out in a BSL3 laboratory or other similar containment facility. Validation of the model was achieved through ceftazidime half-life simulation pharmacokinetics prior to pharmacodynamic experimentation, and during infection simulation in which the non-treated bacterial control isolate showed steady and maintained growth throughout the 24-hour duration of the experiment. Baseline *in vitro* PK/PD data for ceftazidime exposure to *B. pseudomallei* had previously not been available. More PK/PD parameters need to be investigated to verify and support outcome of this study, and can be performed within a BSC using this study's optimized chemostat model.

Limitations

This method was designed to be used in low-resource settings in melioidosis-endemic countries only. In non-endemic countries, a fully-functioning BSL-3 facility is suggested when working with significant amounts of live *B. pseudomallei* culture.

Ethics statements

This study did not involve human subjects, animal experiments or data collected from social media platforms.

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.

CRediT authorship contribution statement

Hana Farizah Zamri: Conceptualization, Methodology, Investigation, Formal analysis, Writing – original draft. **Mohammad Ridhuan Mohd Ali:** Supervision, Validation, Writing – review & editing. **Ahmad Izzan Irfan Sha'ari:** Investigation, Data curation. **Nurul Husna Mohd Mazalan:** Methodology, Investigation. **Norazah Ahmad:** Funding acquisition, Supervision. **Nur Asyura Nor Amdan:** Funding acquisition. **Nusaibah Abdul Rahim:** Conceptualization, Formal analysis. **Zakuan Zainy Deris:** Supervision, Conceptualization, Visualization, Writing – review & editing.

Data availability

Data will be made available on request.

Acknowledgments

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References

[1] E. Birnie, H.S. Virk, J. Savelkoel, R. Spijker, E. Bertherat, D.A.B. Dance, et al., Global burden of melioidosis in 2015: a systematic review and data synthesis, Lancet Infect. Dis. 19 (2019) 892–902, doi:10.1016/S1473-3099(19)30157-4.

[2] S. Nathan, S. Chieng, P.V. Kingsley, A. Mohan, Y. Podin, M.H. Ooi, et al., Melioidosis in Malaysia: incidence, clinical challenges, and advances in understanding pathogenesis, Trop. Med. Infect. Dis. 3 (2018) 25, doi:10.3390/tropicalmed3010025.

[3] L. Zhang, H. Xie, Y. Wang, H. Wang, J. Hu, G. Zhang, Pharmacodynamic parameters of pharmacokinetic/pharmacodynamic (PK/PD) integration models, Front. Vet. Sci. 24 (2022) 860472, doi:10.3389/fvets.2022.860472.

[4] I.K. Minichmayr, V. Aranzana-Climent, L.E. Friberg, Pharmacokinetic/pharmacodynamic models for time courses of antibiotic effects, Int. J. Antimicrob. Agents 60 (2022) 106616, doi:10.1016/j.ijantimicag.2022.106616.

[5] C. Rathi, R.E. Lee, B. Meibohm, Translational PK/PD of anti-infective therapeutics, Drug. Discov. Today Technol. 22 (2016) 41–49, doi:10.1016/j.ddtec.2016.08.004.
[6] J.B. Bulitta, W.W. Hope, A.E. Eakin, T. Guina, V.H. Tam, A. Louie, et al., Generating robust and informative nonclinical *in vitro* and *in vivo* bacterial infection model efficacy data to support translation to humans, Antimicrob. Agents Chemother. 63 (2019) e02307–e02318, doi:10.1128/AAC.02307-18.
[7] CDC Health Advisory. Melioidosis locally endemic in areas of the Mississippi gulf coast [Internet]. 2022 [cited 2024 Dec 18]. Available from: https://www.cdc.gov/han/2022/han00470.html
[8] L.R. Ashdown, Melioidosis and safety in the clinical laboratory, J. Hosp. Infect. 21 (1992) 301–306, doi:10.1016/0195-6701(92)90140-h.
[9] I. Gassiep, M.J. Bauer, P.N.A. Harris, M.D. Chatfield, R. Norton, Laboratory safety: handling *Burkholderia pseudomallei* Isolates without a Biosafety Cabinet, J. Clin. Microbiol. 59 (2021) e0042421, doi:10.1128/JCM.00424-21.
[10] R. Arushothy, M.R. Mohd Ali, H.F. Zambri, V. Muthu, R. Hashim, S. Chieng, et al., Assessing the national antibiotic surveillance data to identify burden for melioidosis in Malaysia, IJID Reg. 10 (2023) 94–99, doi:10.1016/j.ijregi.2023.11.014.
[11] N.J. Sehgal, D.K. Milton, Applying the hierarchy of controls: what occupational safety can teach us about safely navigating the next phase of the global COVID-19 pandemic, Front. Public Health 9 (2021) 747894, doi:10.3389/fpubh.2021.747894.
[12] S. Grasso, G. Meinardi, I. De Carneri, V. Tamassia, New *in vitro* model to study the effect of antibiotic concentration and rate of elimination on antibacterial activity, Antimicrob. Agents Chemother. 13 (1978) 570–576, doi:10.1128/aac.13.4.570.
[13] K.L. LaPlante, S.N. Leonard, D.R. Andes, W.A. Craig, M.J. Rybak, Activities of clindamycin, daptomycin, doxycycline, linezolid, trimethoprim-sulfamethoxazole, and vancomycin against community-associated methicillin-resistant *Staphylococcus aureus* with inducible clindamycin resistance in murine thigh infection and *in vitro* pharmacodynamic models, Antimicrob. Agents Chemother. 52 (2008) 2156–2162, doi:10.1128/AAC.01046-07.
[14] B.J. Angus, M.D. Smith, Y. Suputtamongkol, H. Mattie, A.L. Walsh, V. Wuthiekanun, et al., Pharmacokinetic-pharmacodynamic evaluation of ceftazidime continuous infusion *vs* intermittent bolus injection in septicaemic melioidosis, Br. J. Clin. Pharmacol. 50 (2000) 184–191, doi:10.1111/j.1365-2125.2000.00179.x.
[15] B.J. Anderson, J. Lerman, C.J. Coté, Pharmacokinetics and pharmacology of drugs used in children, in: A Practice of Anesthesia for Infants and Children, 6th ed., Elsevier, 2018, pp. 100–176, doi:10.1016/B978-0-323-42974-0.00007-0. .e45.
[16] D. Skelding, S.F.M. Hart, T. Vidyasagar, A.E. Pozhitkov, W. Shou, Developing a low-cost milliliter-scale chemostat array for precise control of cellular growth, Quant. Biol. 6 (2018) 129–141, doi:10.1007/s40484-018-0143-8.
[17] H.A. Martins-Júnior, T.A. Kussumi, A.Y. Wang, D.T. Lebre, A rapid method to determine antibiotic residues in milk using liquid chromatography coupled to electrospray tandem mass spectrometry, J. Braz. Chem. Soc. 18 (2007) 397–405, doi:10.1590/S0103-50532007000200023.
[18] K.K.K. Goh, W.G.H. Toh, D.K.H. Hee, E.Z.W. Ting, N.G.S. Chua, F.I.B. Zulkifli, et al., Quantification of fosfomycin in combination with nine antibiotics in human plasma and cation-adjusted Mueller-Hinton II broth via LCMS, Antibiotics 11 (2022) 54 (Basel), doi:10.3390/antibiotics11010054.
[19] M. Zhao, Y.R. Cao, B.N. Guo, X.J. Wu, J. Li, J. Zhang, LC-MS/MS determination of colistin in Mueller-Hinton broth for *in vitro* pharmacodynamic studies, J. Antibiot. 67 (2014) 825–829 (Tokyo), doi:10.1038/ja.2014.73.
[20] Malaysian Ministry of HealthNational Antibiotic Guideline, Malaysian Ministry of Health, 2014.
[21] Malaysian Ministry of HealthNational Antibiotic Guideline, Malaysian Ministry of Health, 2024 Available from https://sites.google.com/moh.gov.my/nag/contents/section-a-adult/a16-tropical-infections#h.a372jnr3igkn [cited 2024 Dec 18].
[22] A. Zavrelova, M. Sima, J. Malakova, P. Rozsivalova, P. Paterova, P. Zak, et al., Superiority of ceftazidime off-label high-dose regimen in PK/PD target attainment during treatment of extensively drug-resistant *Pseudomonas aeruginosa* infections in cancer patients, Br. J. Clin. Pharmacol. 89 (2023) 1452–1461, doi:10.1111/bcp.15612.