https://doi.org/10.48048/tis.2024.8150

Cannabidiol Demonstrates Remarkable Efficacy in Treating Multidrug-Resistant *Enterococcus Faecalis* Infections *In Vitro* and *In Vivo*

Parichart Hongsing[1,2,3], Natharin Ngamwongsatit[4], Chomlak Kongart[5], Nida Nuiden[6], Krittapat Phairoh[1,2] and Dhammika Leshan Wannigama[3,6,7,8,9,10,11,*]

[1]*School of Integrative Medicine, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[2]*Mae Fah Luang University Hospital, Mae Fah Luang University, Chiang Rai 57100, Thailand*
[3]*Pathogen Hunter's Research Team, Department of Infectious Diseases and Infection Control, Yamagata Prefectural Central Hospital, Yamagata 990-2292, Japan*
[4]*Department of Clinical Sciences and Public Health, Faculty of Veterinary Science, Mahidol University, Nakhon Pathom 73170, Thailand*
[5]*Department of Thai Traditional Medicine, Ramkhamhaeng University, Bangkok 11000, Thailand*
[6]*Department of Thai Traditional Medicine, Thaksin University, Phatthalung 93210, Thailand*
[7]*Department of Infectious Diseases and Infection Control, Yamagata Prefectural Central Hospital, Yamagata 990-2292, Japan*
[8]*Department of Microbiology, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok 10330, Thailand*
[9]*Center of Excellence in Antimicrobial Resistance and Stewardship Research, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand*
[10]*School of Medicine, Faculty of Health and Medical Sciences, The University of Western Australia, Western Australia 6009, Australia*
[11]*Biofilms and Antimicrobial Resistance Consortium of ODA Receiving Countries, The University of Sheffield, Sheffield S10 2TN, United Kingdom*

([*]Corresponding author's e-mail: leshanwannigama@gmail.com)

Received: 3 March 2024, Revised: 25 March 2024, Accepted: 1 May 2024, Published: 20 August 2024

Abstract

The growing prevalence of multidrug-resistant *Enterococcus faecalis* infection has become a global concern. There is a demand for alternative antibacterial agents, such as herbal alternatives, such as Cannabidiol, that are cost-effective, non-toxic and efficient. This study investigates the Minimum Inhibitory Concentration (MIC) for planktonic cells and the Minimum Biofilm Eradication Concentration (MBEC) for biofilm formation in multidrug- resistance isolates of *E. faecalis* isolates, particularly focusing cannabidiol activity. *E. faecalis* isolates with strong biofilm presence, vancomycin, levofloxacin, and daptomycin displayed high MIC and MBEC values. Cannabidiol exhibited a significantly lower MIC (1 µg/mL) for planktonic cells and a low MBEC (2 µg/mL). Moreover, at low concentrations (2 µg/mL), cannabidiol demonstrated notable reductions in biofilm biovolume, bacterial cell viability and colony-forming unit compared to vancomycin, levofloxacin, and daptomycin. The mice treated with cannabidiol (100 mg/kg) exhibited a significant reduction in *E. faecalis* bacterial load in internal organs and increased

the survival. In conclusion, the findings underscore the promising antibiofilm properties of cannabidiol against *E. faecalis*, indicating its potential as a novel therapeutic agent.

Keywords: *Enterococcus faecalis*, *Cannabis sativa*, Antimicrobial activity, Herbal medicine, Alternative medicine, Anti-biofilm formation, Cannabidiol, Phytochemicals

Introduction

Bacteria have become increasingly resistant to antibiotics due to the overuse of existing antimicrobial drugs [1-9]. While severe antibiotic-resistant infections, such as bloodstream infections, urinary tract infections (UTIs), surgical site infections, and respiratory infections, affect hundreds of millions of children and adults globally and increase the risk of mortality and morbidity [1-9], a lack of current investment in traditional antibiotic discovery pipelines suggests an alternative approach [10].

Enterococcus faecalis is a gram-positive bacterium exhibiting facultative enteric characteristics [11]. It is a notable opportunistic pathogen that can cause a variety of infections, including endocarditis, urinary tract infections, prostatitis, intra-abdominal infection, cellulitis, and wound infection [12]. It may also lead to concurrent bacteremia, especially infective endocarditis (IE). *E. faecalis* accounts for more than 90 % of IE cases, and the standard treatment for IE involves combination therapy with either ampicillin plus gentamycin or ceftriaxone [12,13]. In addition, linezolid has historically proven effective against multidrug-resistant (MDR) Gram-positive bacteria, including *E. Faecalis* [11,13]. Nevertheless, the growing prevalence of linezolid-resistant *E. faecalis* (LZR-Efa) has become a global concern [11]. Furthermore, the challenge of treating *E. faecalis* infections effectively is compounded by the absence of new antibiotics capable of killing bacteria, the formation of biofilms and the rising rates of antibiotic resistance [11-13].

According to the World Health Organization, 80 % of the global population primarily relies on traditional medicines incorporating plant extracts or their active components [14]. Hence, there is a growing demand for alternative antibacterial agents, including herbal alternatives, characterized as cost-effective, non-toxic and efficient. Among them, Cannabidiol (CBD), the primary non-psychoactive compound with a molecular weight of 314 Da in the Cannabis plant, is a potential candidate for the development of new antimicrobial treatments [14-16]. CBD is a phyto-cannabinoid composed of a pentyl-substituted bis-phenol aromatic group (pentylresorcinol) linked to an alkyl-substituted cyclohexene terpene ring system [14-16]. It is one of over 100 cannabinoids that can be extracted from the *Cannabis sativa* L. in Cannabaceae family, many demonstrating diverse biological activities with remarkable polypharmacological properties [14-18]. CBD exhibits antimicrobial activity against Gram-positive staphylococci and streptococci with methicillin-resistant *Staphylococcus aureus* (MRSA), *Enterococcal* species [14], and Gram-negative bacteria such as *Escherichia coli*, *Salmonella typhi*, or *Proteus vulgaris* [19-20]. However, most of these studies are based on laboratory strains in *in vitro* conditions [14].

A significant research gap persists regarding the efficacy of CBD on clinical strains of *E. faecalis*, particularly those displaying multidrug resistance. Since clinical strains more accurately mimic real-world infection scenarios, addressing this gap is crucial for understanding CBD's potential effectiveness in combating such infections. Therefore, the present study has been undertaken to evaluate the antimicrobial efficacy of CBD against clinical isolates of multi-drug resistance *E. faecalis* bacterial infections *in vitro* and *in vivo.*

Materials and methods

Bacteria isolates and growth conditions

A 25 multi-drug resistance clinical isolates of *E. faecalis* were obtained from a bacteria repository held at the Pathogen Hunters Research Laboratory at Yamagata Prefectural Central Hospital, Japan**.** The bacterial isolates were cultured on Tryptic Soy Agar (TSA) with 5 % sheep blood and cryopreserved at –80 °C in tryptic soy broth with 15 % (v/v) glycerol until subsequent use in future studies.

Antimicrobial and chemical agents

Cannabidiol (Sigma-Aldrich), Vancomycin, Levofloxacin, and Daptomycin (Sigma-Aldrich) were used to prepare stock solutions < 24 h before use. All agents were dissolved in cation-adjusted Müller-Hinton II broth (MHIIB) (Sigma-Aldrich) and the solutions were sterilized through a syringe filter with a membrane nominal pore size of 0.22 μm. Serial dilutions of the antibiotic and CBD stocks were prepared in MHIIB immediately before use.

Susceptibilities to CBD, vancomycin, levofloxacin, and daptomycin

We established the susceptibility of planktonic cultures of the various strains to antibiotics using standard techniques (broth microdilution to determine minimal inhibitory concentrations, MICs) as described previously [2-5,7-9,21-23], according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST criteria for Enterobacteriaceae only [24] and U.S. Clinical and Laboratory Standards Institute (CLSI) guidelines [25]. We used *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 for quality control. Susceptibility testing was performed for all clinical isolates.

Quantification of biofilm

We quantified biofilm using a crystal violet assay as previously described [8,23,26] with all 29 isolates. In brief, we standardized an overnight culture of the strain of interest to an optical density (OD) of 0.02 at 600 nm (5×10^7 CFU/mL), added 100 μL aliquots in triplicate to flat-bottomed 96-well polystyrene microtiter plates, and incubated these cultures in the plates at 37 °C for 24 h. Subsequently, we fixed the adherent biofilms with crystal violet (0.1 %) and then released the dye with 30 % (v/v) acetic acid. We measured the resulting absorbance at 560 nm using a microtiter-plate-reading spectrophotometer. Mean absorbances and their standard deviations (SD) were calculated for all strains and negative controls tested, performed in triplicate and repeated 3 times. The cut-off value (ODβ) was defined as 3 SD above the mean OD of the negative controls: ODβ = average OD of the negative controls +3 SD of negative controls and was calculated separately for each microtiter plate. The OD of a tested strain was expressed as the mean OD of the strain minus the ODβ (OD = mean OD of a strain - ODβ). The clinical isolates were classified as described previously [21-23]. All experiments were performed in triplicate and repeated 3 times.

Minimal biofilm eradication concentrations

Minimal biofilm eradication concentrations (MBEC) were established using our previously develop fluorometric-based assay to calculate the number of viable cells within the biofilm as described previously [21-23]. In brief, MBECs were determined by adding the serially diluted antibiotics to mature biofilms and incubating at 37 °C for 24 h before staining with PrestoBlue. Before adding the antibiotics, any nonadherent cells were removed from the mature biofilms by 3 gentle washes with MHIIB. Cell viability was calculated using the following formula: Cell viability (%) = ((mean signal of corresponding well - mean signal of negative control well) / (mean signal of positive control well – mean signal of negative control well))×100.

Two cut-off values (50 and 75 % nonviable cells) were used to determine the MBEC. All experiments were performed in triplicate and repeated 3 times.

Mouse study

After the Institutional Animal Care and Use Committee (IACUC) of the Yamagata Prefectural Central Hospital, Japan, approved the procedures to be performed in the present study, we obtained specific-pathogen-free, 8-week-old, female C57BL/6 mice from SLC Japan. The mice were allowed to acclimatize for 1 week in the animal facility at a maximum of 2 mice per cage before use and were allowed food and water ad libitum. The mice were each weighed, and their health and welfare were monitored closely to determine humane clinical endpoints and for any signs of distress over the experimental period**.** For all the mouse experiments, we used 3 representatives multi-drug resistant *E. faecalis* randomly selected from the 25 isolates to represent antibiotic resistance and biofilm formation, as shown in **Table 1**. We cultured 3 representative isolates individually and then combined them into a single culture for inoculating the mice.

***In vivo* murine bacteremia model**

To establish *E. faecalis*-associated bacteremia *in vivo*, a previously described murine model of bacteremia obtained through intraperitoneal inoculation of the mixer of 3 representative isolates was used [27]. Immunocompetent mice were inoculated intravenously (with a 28-gauge, 0.5-in. Needle) into the tail vein of each mouse with 5×10^8 CFU of a bacterial suspension with 2 mL of PBS (Sigma-Aldrich) and murine *E. faecalis* associated bacteremia was allowed to develop for 6 h. To evaluate the effects of CBD, Vancomycin, Levofloxacin, and Daptomycin, infected mice were administered a single intravenously dose of PBs (control), CBD (100 mg/kg), Vancomycin (100 mg/kg), Levofloxacin (100 mg/kg) and Daptomycin (50 mg/kg), for a total of 5 groups with 10 mice in each group. For survival analysis, the same treatment was repeated once daily, and the survival of mice was monitored until a humane clinical or experimental endpoint was reached [27]. The clinical endpoint was determined using a 5-point body condition score, analyzing weight loss, decreased body temperature, respiratory distress, hampered mobility, and hunched posture. In addition, blood, heart, liver, kidneys and spleen were removed to quantify viable bacteria [27]. The experimental endpoint was defined as 10 days post-infection for mice not reaching the clinical endpoint.

Ethical approval

This study was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice guidelines, and other applicable laws and regulations, including STROBE guidelines**.** The study is part of the COVID-19 surveillance study and was reviewed and approved by the institutional review board at Yamagata Prefectural Central Hospital, Yamagata, Japan (XB/D2022). The study was carried out in compliance with the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments).

Quantification and statistical analysis

All statistical analyses were conducted using the R statistical package [28]. Data were compared by using either an unpaired 2-tailed Student t-test or unpaired 2-tailed Mann-Whitney U test. All data are presented as the mean ± S.D. Differences were considered significant when $p < 0.05$.

Results

In vitro efficacy of CBD against multi-drug resistance clinical isolates of *E. faecalis*

Table 1 reveals the MIC for planktonic cells and the MBEC for biofilm formation in different categories of *E. faecalis* isolates. Among the isolates with a strong biofilm (n = 10), Vancomycin exhibited a high MIC (> 64) for planktonic cells and an elevated MBEC (> 256), while Levofloxacin and Daptomycin also displayed resistance. Interestingly, CBD demonstrated a lower MIC of 1 µg/mL for planktonic cells and a low MBEC of 2 µg/mL, suggesting a potential effectiveness against strong biofilm formations. Antibiotic resistance persisted in cases with a moderate biofilm (n = 8), with CBD displaying a moderate MIC of 2 µg/mL for planktonic cells and a consistent MBEC of 2 µg/mL. Similar resistance patterns were observed for weak biofilm formations (n = 7), with CBD demonstrating a lower MIC and MBEC.

Table 1 Susceptibility of Cannabidiol, Vancomycin, Meropenem, and Daptomycin against *E. Faecalis* (n = 25).

	No of isolates of *E. faecalis*	**Biofilm category**	**Vancomycin (µg/mL)**	**Levofloxacin (µg/mL)**	**Daptomycin (µg/mL)**	**CBD (µg/mL)**
Planktonic cells MIC	10	Strong	> 64	> 64	16	1
	8	Moderate	> 64	> 128	32	2
	7	Weak	> 64	> 64	16	1
Biofilm MBEC	10	Strong	> 256	> 512	> 64	2
	8	Moderate	> 128	> 256	> 128	2
	7	Weak	> 256	> 512	> 64	2

Minimum inhibitory concentrations (MICs)
Minimal biofilm eradication concentrations (MBEC)
Cannabidiol (CBD)

In vitro efficacy of CBD against multi-drug resistance clinical isolates of *E. faecalis* biofilm

Moreover, low concentrations of CBD (2 µg/mL) displayed significant reductions in biofilm biovolume, with the most pronounced eradication effects observed (**Figure 1(a)**). Notably, there was a significant reduction in bacterial cell viability within the biofilm ($p < 0.001$) upon exposure, as well as inhibition of biofilm formation, when compared to standard concentrations of Vancomycin (4 µg/mL), Levofloxacin (2 µg/mL), and Daptomycin (4 µg/mL) (**Figures 1(b) - 1(c)**). Furthermore, CBD was able to significantly reduce the colony-forming unit (CFU) levels of *E. faecalis* biofilm (**Figure 1(d)**).

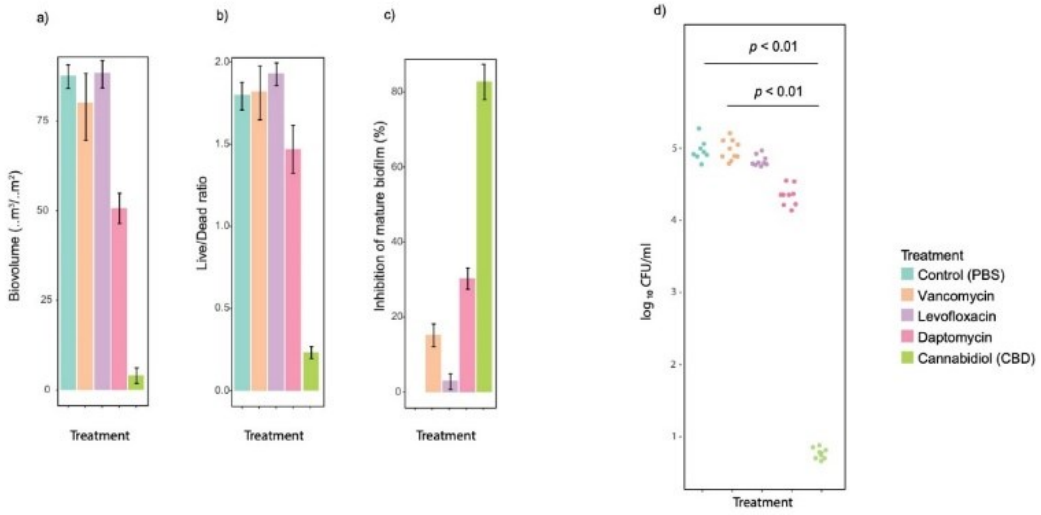

Figure 1 Effects of CBD (2 µg/mL), Vancomycin (4 µg/mL), Levofloxacin (2 µg/mL) and Daptomycin (4 µg/mL) *in vitro* on (a) biofilm biovolume and (b) biofilm cell viability Live/Dead ratio (c) biofilm formation inhibition % and (d) colony forming unit level (CFU) of *E. Faecalis* (n = 25) All experiments in a - d was performed as 3 biologically independent experiments, and the mean ± S.D. is shown. *p*-values were determined using an unpaired, 2-tailed Student's t-test.

In vivo* efficacy of CBD against multi-drug resistance clinical isolates of *E. faecalis

Throughout all tested days, mice treated with CBD (100 mg/kg) exhibited a significant reduction in *E. faecalis* bacterial load in internal organs-blood, heart, kidneys, lungs, spleen, when compared to mice treated with Vancomycin, Levofloxacin, or Daptomycin alone ($p < 0.01$) (**Figure 2(b)**). Mice treated with the CBD displayed significantly higher survival rates of 100 % until day 6 when compared to mice given Vancomycin, or Levofloxacin, or Daptomycin alone ($p < 0.01$) (**Figure 2(c)**). Interestingly, CBD-treated mice had significantly higher survival rates than the Vancomycin, Levofloxacin, or Daptomycin alone ($p < 0.01$) treated mice all through 10 days ($p < 0.0001$).

Discussion

The study's results indicate that CBD displays promising antibacterial properties, particularly in combating multidrug-resistance *E. faecalis* which has become increasingly resistant to existing antibiotics [11]. In this study, *E. faecalis* planktonic and biofilms showed significant antimicrobial tolerance against Vancomycin, Levofloxacin, and Daptomycin with an MBEC 3 times higher than their corresponding MIC values. These findings align with a previous study that indicated higher antimicrobial resistance and tolerance due to the barrier effects of biofilm matrix, which limit antibiotic penetration [29]. Notably, CBD exhibits lower MIC and MBEC values than conventional antibiotics.

Interestingly, CBD treatment not only successfully eradicated *E. faecalis* bacteremia but also decreased bacterial viabilities, demonstrating their potent efficacy in eliminating the risk of recurrence both *in vitro* and i*n vivo*. This underscores CBD's potential as an alternative treatment for multidrug-resistant *E. faecalis* infections. These findings align with previous studies demonstrating CBD's efficacy against Gram-positive bacteria and *E. faecalis* [14,15,19,20]. Interestingly, previous reports [14,18,30,31] indicated that

oral delivery appears to provide much better systemic exposure than subcutaneous dosing, but our study suggests that intravenous CBD (100 mg/kg) was able to exhibit a significant reduction of *E. faecalis* bacteria systemically and CBD administration was well-tolerated.

Moreover, our results represent the 1st documentation of the effectiveness of CBD against *E. faecalis* biofilm *in vitro*. Recent studies showed that in gram-positive bacteria, in addition to membrane depolarization, CBD is able to shut down protein, DNA, RNA and peptidoglycan synthesis pathways at low CBD concentrations (µg/mL) [14]. Gram-positive and negative bacteria biofilm also depended on DNA, RNA, and peptidoglycan synthesis pathways [7,32-34] and therefore, we believed that inhibition of all these synthesis pathways may contribute to the antibiofilm effect of CBD. The observed substantial reductions in biofilm biovolume, bacterial cell viability, and Colony Forming Units (CFU) levels further support the idea that CBD could offer a unique mechanism of action against biofilm-associated infections. Despite the exact mechanisms underlying CBD's antibiofilm effects is unknown, this study provides valuable insights into its potential application for combating multi-drug resistant *E. faecalis* biofilms. Nevertheless, the lack of *in vivo* biofilm data limited our results due to the unavailability of a reliable biofilm infection mouse model for *E. faecalis*.

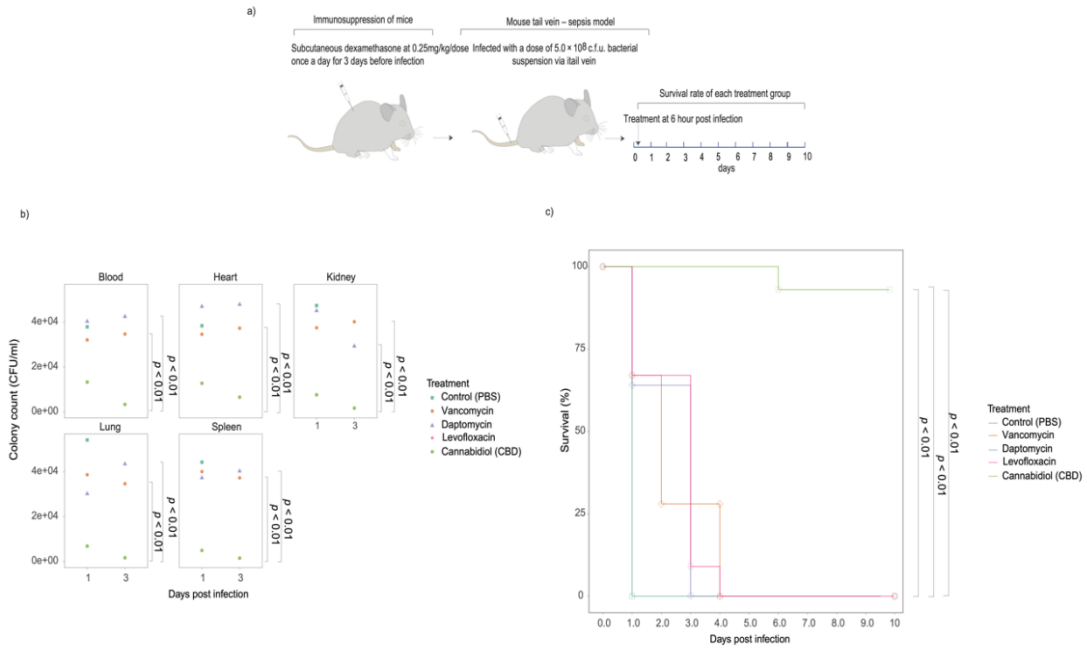

Figure 2 Effects of CBD, Vancomycin, Levofloxacin and Daptomycin on mouse bacteremia infection (a) Mouse tail vein - sepsis model (b) bacterial load in blood, heart, kidney, lung, spleen tissues and (c) survival of mice. *p*-values were determined using a 2-sided Mann-Whitney U-test. All data were presented as means ± S.D.

Conclusions

The findings of this research extend beyond the laboratory, suggesting a promising avenue for developing CBD-based treatments for multi-drug resistance *E. faecalis* infections. Future research should prioritize elucidating the molecular pathways involved in CBD's antibacterial effects and conducting clinical trials to validate its efficacy and safety as a biofilm-targeting antimicrobial agent.

Acknowledgements

We thank the staff of the Department of Infectious Diseases and Infection Control, Yamagata Prefectural Central Hospital, Yamagata, Japan, for providing bacteria isolates. Dhammika Leshan Wannigama was supported by Chulalongkorn University (2nd Century Fund-C2F Postdoctoral Fellowship), Thailand, University of Western Australia (Overseas Research Experience Fellowship,) Australia and Yamagata Prefectural Central Hospital, Yamagata, Japan (Clinical Residency Fellowship). The funder(s) had no role in study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the article for publication.

References

[1] S Na, DL Wannigama and T Saethang. Antimicrobial peptides recognition using weighted physicochemical property encoding. *J. Bioinformatics Comput. Biol.* 2023; **21**, 2350006.

[2] AMS Shein, DL Wannigama, C Hurst, PN Monk, M Amarasiri, VN Badavath, P Phattharapornjaroen, WGF Ditcham, P Ounjai, T Saethang, N Chantaravisoot, W Thuptimdang, S Luk-In, S Nilgate, U Rirerm, C Tanasatitchai, N Kueakulpattana, M Laowansiri, T Liao, R Kupwiwat, …, S Abe. Novel intranasal phage-CaEDTA-ceftazidime/avibactam triple combination therapy demonstrates remarkable efficacy in treating Pseudomonas aeruginosa lung infection. *Biomed. Pharmacother.* 2023; **168**, 115793.

[3] S Srisakul, DL Wannigama, PG Higgins, C Hurst, S Abe, P Hongsing, T Saethang, S Luk-In, T Liao, N Kueakulpattana, AMS Shein, L Gan, R Kupwiwat, C Tanasatitchai, P Wapeesittipan, P Phattharapornjaroen, VN Badavath, A Leelahavanichkul and T Chatsuwan. Overcoming addition of phosphoethanolamine to lipid A mediated colistin resistance in *Acinetobacter baumannii* clinical isolates with colistin-sulbactam combination therapy. *Sci. Rep.* 2022; **12**, 11390.

[4] AMS Shein, DL Wannigama, PG Higgins, C Hurst, S Abe, P Hongsing, N Chantaravisoot, T Saethang, S Luk-In, T Liao, S Nilgate, U Rirerm, N Kueakulpattana, S Srisakul, A Aryukarn, M Laowansiri, LY Hao, M Yonpiam, NKD Ragupathi, T Techawiwattanaboon, N Ngamwongsatit, M Amarasiri, P Ounjai, R Kupwiwat, P Phattharapornjaroen, VN Badavath, A Leelahavanichkul, A Kicic and T Chatsuwan. High prevalence of mgrB-mediated colistin resistance among carbapenem-resistant *Klebsiella pneumoniae* is associated with biofilm formation, and can be overcome by colistin-EDTA combination therapy. *Sci. Rep.* 2022; **12**, 12939.

[5] S Luk-In, T Chatsuwan, N Kueakulpattana, U Rirerm, DL Wannigama, R Plongla, R Lawung, C Pulsrikarn, S Chantaroj, P Chaichana, N Saksaengsopa, T Shanthachol, M Techapornroong, S Chayangsu and W Kulwichit. Occurrence of *mcr*-mediated colistin resistance in *Salmonella* clinical isolates in Thailand. *Sci. Rep.* 2021; **11**, 14170.

[6] U Singkham-In, PG Higgins, DL Wannigama, P Hongsing and T Chatsuwan. Rescued chlorhexidine activity by resveratrol against carbapenem-resistant *Acinetobacter baumannii* via down-regulation of AdeB efflux pump. *Plos One* 2020; **15**, e0243082.

[7] P Phuengmaung, P Somparn, W Panpetch, U Singkham-In, DL Wannigama, T Chatsuwan and A Leelahavanichkul. Coexistence of *Pseudomonas aeruginosa* with *Candida albicans* enhances biofilm thickness through alginate-related extracellular matrix but is attenuated by N-acetyl-l-cysteine. *Front. Cell. Infec. Microbiol.* 2020; **10**, 594336.

[8] DL Wannigama, C Hurst, L Pearson, T Saethang, U Singkham-In, S Luk-In, RJ Storer and T Chatsuwan. Simple fluorometric-based assay of antibiotic effectiveness for *Acinetobacter baumannii* biofilms. *Sci. Rep.* 2019; **9**, 6300.

[9] DL Wannigama, R Dwivedi and A Zahraei-Ramazani. Prevalence and antibiotic resistance of gram-negative pathogenic bacteria species isolated from *Periplaneta americana* and *Blattella germanica* in Varanasi, India. *J. Arthropod Borne Dis.* 2014; **8**, 10-20.

[10] AMS Shein, P Hongsing, S Abe, S Luk-In, NKD Ragupathi, DL Wannigama and T Chatsuwan. Will there ever be cure for chronic, life-changing Colistin-resistant *Klebsiella pneumoniae* in urinary tract infection? *Front. Med.* 2021; **8**, 806849.

[11] W Yu, J Zhang, J Tong, L Zhang, Y Zhan, Y Huang and Y Qiu. *In vitro* antimicrobial activity of fosfomycin, vancomycin and daptomycin alone, and in combination, against linezolid-resistant *Enterococcus faecalis*. *Infect. Dis. Ther.* 2020; **9**, 927-34.

[12] L Herrera-Hidalgo, B Fernández-Rubio, R Luque-Márquez, LE López-Cortés, MV Gil-Navarro and AD Alarcón. Treatment of *Enterococcus faecalis* infective endocarditis: A continuing challenge. *Antibiotics* 2023; **12**, 704.

[13] P Danneels, JF Hamel, L Picard, S Rezig, P Martinet, A Lorleac'h, JP Talarmin, R Buzelé, T Guimard, GL Moal, J Brochard-Libois, A Beaudron, J Letheulle, C Codde, R Chenouard, D Boutoille, A Lemaignen, L Bernard, V Cattoir, V Dubée and EFEMER study group. Impact of *Enterococcus faecalis* endocarditis treatment on risk of relapse. *Clin. Infect. Dis.* 2022; **76**, 281-90.

[14] MAT Blaskovich, AM Kavanagh, AG Elliott, B Zhang, S Ramu, M Amado, GJ Lowe, AO Hinton, DMT Pham, J Zuegg, N Beare, D Quach, MD Sharp, J Pogliano, AP Rogers, D Lyras, L Tan, NP West, DW Crawford, ML Peterson, M Callahan and M Thurn. The antimicrobial potential of cannabidiol. *Comm. Biol.* 2021; **4**, 7.

[15] S Burstein. Cannabidiol (CBD) and its analogs: A review of their effects on inflammation. *Bioorg. Med. Chem.* 2015; **23**, 1377-85.

[16] R Adams, M Hunt and JH Clark. Structure of cannabidiol, a product isolated from the marihuana extract of Minnesota wild hemp I. *J. Am. Chem. Soc.* 1940; **62**, 196-200.

[17] MA Elsohly and D Slade. Chemical constituents of marijuana: The complex mixture of natural cannabinoids. *Life Sci.* 2005; **78**, 539-48.

[18] JT Dearborn, HR Nelvagal, NR Rensing, K Takahashi, SM Hughes, TM Wishart, JD Cooper, M Wong and MS Sands. Effects of chronic cannabidiol in a mouse model of naturally occurring neuroinflammation, neurodegeneration, and spontaneous seizures. *Sci. Rep.* 2022; **12**, 11286.

[19] R Iseppi, V Brighenti, M Licata, A Lambertini, C Sabia, P Messi, F Pellati and S Benvenuti. Chemical characterization and evaluation of the antibacterial activity of essential oils from fibre-type *Cannabis sativa* L. (Hemp). *Molecules* 2019; **24**, 2302.

[20] MA Farha, OM El-Halfawy, RT Gale, CR MacNair, LA Carfrae, X Zhang, NG Jentsch, J Magolan and ED Brown. Uncovering the hidden antibiotic potential of *Cannabis*. *ACS Infect. Dis.* 2020; **6**, 338-46.

[21] DL Wannigama, AMS Shein, C Hurst, PN Monk, P Hongsing, P Phattharapornjaroen, WGF Ditcham, P Ounjai, T Saethang, N Chantaravisoot, P Wapeesittipan, S Luk-In, S Sae-Joo, S Nilgate, U Rirerm, C Tanasatitchai, N Kueakulpattana, M Laowansiri, T Liao, R Kupwiwat, …, S Abe. Ca-EDTA restores the activity of Ceftazidime-Avibactam or Aztreonam against carbapenemase-producing *Klebsiella pneumoniae* infections. *iScience* 2023; **26**, 107215.

[22] AMS Shein, DL Wannigama, PG Higgins, C Hurst, S Abe, P Hongsing, N Chantaravisoot, T Saethang, S Luk-in, T Liao, S Nilgate, U Rirerm, N Kueakulpattana, M Laowansiri, S Srisakul, N Muhummudaree, T Techawiwattanaboon, L Gan, C Xu, R Kupwiwat, …, T Chatsuwan. Novel colistin-EDTA combination for successful eradication of colistin-resistant *Klebsiella pneumoniae* catheter-related biofilm infections. *Sci. Rep.* 2021; **11**, 21676.

[23] DL Wannigama, C Hurst, P Hongsing, L Pearson, T Saethang, N Chantaravisoot, U Singkham-In, S Luk-In, RJ Storer and T Chatsuwan. A rapid and simple method for routine determination of antibiotic sensitivity to biofilm populations of Pseudomonas aeruginosa. *Ann. Clin. Microbiol. Antimicrobials* 2020; **19**, 8.

[24] The European Committee for Antimicrobial Susceptibility Testing, Available at: https://www.eucast.org/clinical_breakpoints, accessed March 2024.

[25] Clinical and Laboratory Standards Institute, Available at: https://clsi.org/standards/products/microbiology/documents/m100/, accessed March 2024.

[26] GA O'Toole. Microtiter dish biofilm formation assay. *J. Vis. Exp.* 2011; **47**, 2437.

[27] C Gentry-Weeks, M Estay, C Loui and D Baker. Intravenous mouse infection model for studying the pathology of *Enterococcus faecalis* infections. *Infect. Immun.* 2003; **71**, 1434-41.

[28] The R Project for Statistical Computing, Available at: https://www.r-project.org/, accessed March 2024.

[29] MA Khalil, JA Alorabi, LM Al-Otaibi, SS Ali and SE Elsilk. Antibiotic resistance and biofilm formation in *enterococcus* spp. isolated from urinary tract infections. *Pathogens* 2022; **12**, 34.

[30] S Deiana, A Watanabe, Y Yamasaki, N Amada, M Arthur, S Fleming, H Woodcock, P Dorward, B Pigliacampo, S Close, B Platt and G Riedel. Plasma and brain pharmacokinetic profile of cannabidiol (CBD), cannabidivarine (CBDV), Δº-tetrahydrocannabivarin (THCV) and cannabigerol (CBG) in rats and mice following oral and intraperitoneal administration and CBD action on obsessive-compulsive behaviour. *Psychopharmacology* 2012; **219**, 859-73.

[31] SA Varvel, JL Wiley, R Yang, DT Bridgen, K Long, AH Lichtman and BR Martin. Interactions between THC and cannabidiol in mouse models of cannabinoid activity. *Psychopharmacology* 2006; **186**, 226-34.

[32] EF Kong, C Tsui, S Kucharíková, D Andes, DP Van and MA Jabra-Rizk. Commensal protection of *Staphylococcus aureus* against antimicrobials by *Candida albicans* biofilm matrix. *mBio* 2016; **7**, e01365-16.

[33] L Tang, A Schramm, TR Neu, NP Revsbech and RL Meyer. Extracellular DNA in adhesion and biofilm formation of four environmental isolates: A quantitative study. *FEMS Microbiol. Ecol.* 2013; **86**, 394-403.

[34] BM Peters, ES Ovchinnikova, BP Krom, LM Schlecht, H Zhou, LL Hoyer, HJ Busscher, HCVD Mei, MA Jabra-Rizk and ME Shirtliff. *Staphylococcus aureus* adherence to *Candida albicans* hyphae is mediated by the hyphal adhesin Als3p. *Microbiology* 2012; **158**, 2975-86.