Imipenem reduces the efficacy of vancomycin against *Elizabethkingia* species

Ya-Sung Yang1†, Hsing-Yu Chen2†, I-Chieh Lin3, Meng-He Lin3, Wei-Yao Wang4,5, Shu-Chen Kuo6, Wen-Ting Chen3, Yun-Hsiang Cheng3,7 and Jun-Ren Sun1,3,7*

1Division of Infectious Diseases and Tropical Medicine, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan; 2Department of Medical Techniques, Taipei City Hospital Ren-Ai Branch, Taipei, Taiwan; 3National Defense Medical Center, Institute of Preventive Medicine, Taipei, Taiwan; 4School of Medicine, Chung Shan Medical University, Taichung, Taiwan; 5Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan; 6National Institute of Infectious Diseases and Vaccinology, National Health Research Institutes, Miaoli County, Taiwan; 7Department of Physiology and Biophysics, Graduate Institute of Physiology, National Defense Medical Center, Taipei, Taiwan

*Corresponding author. E-mail: tsghsun@gmail.com
†Both authors contributed equally.

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**Background:** *Elizabethkingia* spp. are emerging as nosocomial pathogens causing various infections. These pathogens express resistance to a broad range of antibiotics, thus requiring antimicrobial combinations for coverage. However, possible antagonistic interactions between antibiotics have not been thoroughly explored. This study aimed to evaluate the effectiveness of antimicrobial combinations against *Elizabethkingia* infections, focusing on their impact on pathogenicity, including biofilm production and cell adhesion.

**Methods:** Double-disc diffusion, time–kill, and chequerboard assays were used for evaluating the combination effects of antibiotics against *Elizabethkingia* spp. We further examined the antagonistic effects of antibiotic combinations on biofilm formation and adherence to A549 human respiratory epithelial cells. Further validation of the antibiotic interactions and their implications was performed using *ex vivo* hamster precision-cut lung sections (PCLSs) to mimic *in vivo* conditions.

**Results:** Antagonistic effects were observed between cefoxitin, imipenem and amoxicillin/clavulanic acid in combination with vancomycin. The antagonism of imipenem toward vancomycin was specific to its effects on the genus *Elizabethkingia*. Imipenem further hampered the bactericidal effect of vancomycin and impaired its inhibition of biofilm formation and the adhesion of *Elizabethkingia meningoseptica* ATCC 13253 to human cells. In the *ex vivo* PCLS model, vancomycin exhibited dose-dependent bactericidal effects; however, the addition of imipenem also reduced the effect of vancomycin.

**Conclusions:** Imipenem reduced the bactericidal efficacy of vancomycin against *Elizabethkingia* spp. and compromised its capacity to inhibit biofilm formation, thereby enhancing bacterial adhesion. Clinicians should be aware of the potential issues with the use of these antibiotic combinations when treating *Elizabethkingia* infections.

**Introduction**

*Elizabethkingia* spp. have emerged as important nosocomial pathogens causing outbreaks and serious infections.1 *Elizabethkingia* spp. are notorious for their extensive resistance to a broad spectrum of common antibiotics, leaving scarce therapeutic choices for clinicians and leading to attributable treatment failures and mortality.2,3 Several studies have reported favourable outcomes of neonatal and adult patients infected by *Elizabethkingia* spp. when treated with vancomycin-based antibiotic combinations, even though the rationale for vancomycin against this pathogen is still unknown.2,4,5

Our recent study showed the efficacy of minocycline-based combination therapy for *Elizabethkingia* spp.5 In the current study, we aimed to analyse the efficacy and interactions between vancomycin in combination with other antibiotics against...
Elizabethkingia spp. We used both in vitro assays to monitor potential antagonistic antibiotic effects and an ex vivo precision-cut lung slice (PCLS) model to closely mirror the actual in vivo lung infection environment.

Materials and methods

Antimicrobial susceptibility testing and combination assays

The strains used in this study and the MICs of vancomycin and imipenem against the strains are shown in Table S1 (available as Supplementary data at JAC Online). Clinical Elizabethkingia isolates were randomly selected based on our previous publication. To verify inoculum concentration, the bacterial inoculum was serially diluted 1:10 and plated on Mueller–Hinton (MH) agar for colony enumeration. MICs of antibiotics were determined using broth microdilution according to CLSI guidelines. The antibiotic discs were purchased from BD BBL Sensi-Disc (Becton, Dickinson and Company). After incubation at 35°C for 18–20 h, the inhibition zones around each antibiotic disc were examined. Synergistic effects were identified by an increase of ≥2 mm in zone diameter or bridging between zones. In contrast, antagonistic effects were characterized by a ‘D’ shaped flattening in the zone of inhibition. The pharmacodynamic interaction of ß-lactam antibiotics and vancomycin was evaluated using a microdilution chequerboard assay, as previously described. The bacterial strain was adjusted to a McFarland 1 standard and diluted to a concentration of 5 × 10^5 cfu/mL in each well of a 96-well microtitre plate with antibiotics. To visualize the contrast better, Alamar Blue reagent (BioSource International, Camarillo, CA, USA) was added to the plates. The combined antibiotic effects were quantified using the FICI index (FICI), calculated using the formula: FICI = FIC A + FIC B = (MIC of drug A in combination/MIC of drug A alone) + (MIC of drug B in combination/MIC of drug B alone). Synergism is indicated by FICI ≤ 0.5, additivity by FICI > 0.5 to < 1, indifference by FICI ≥ 1 to < 4, and antagonism by FICI ≥ 4. Time–kill assays were performed to assess the bactericidal effects of vancomycin in the presence of imipenem. Samples collected at 0, 2, 4, 8 and 24 h were plated onto agar for cfu counting after overnight incubation. Time–kill curves showing an increase of ≥1 log_{10} cfu/mL with the combination compared with the most active single agent at 8 h are indicative of antagonism.

Biofilm formation and cell adhesion assays

E. meningoseptica ATCC 13253 was adjusted to achieve a final concentration of 10^7 cfu/mL in a 96-well plate with antibiotics. Following a 48 h incubation at 35°C, adherent cells were then stained with crystal violet and dissolved in acetic acid for biofilm quantification. Human lung adenocarcinoma (A549) cells were microscopically counted to confirm cell concentration and seeded at a density of 5 × 10^3 cells/well in 96-well plates. The following day, cells were incubated for 1 h at 37°C with 5 × 10^5 cfu of bacteria under various vancomycin and imipenem conditions using DMEM (Gibco, Thermo Fisher Scientific). After incubation, the adherent cells were lysed and diluted to plate onto MH agar, and incubated at 35°C for 24 h for colony counting.

PCLS infection

The lung of the Syrian hamster was filled with 1% agarose and sectioned into 500 μm, 8 mm circular slices placed in DMEM/F-12 medium. Following overnight incubation, the slices were inoculated with 0.5 mL of E. meningoseptica ATCC 13253 (2 × 10^7 cfu/four slices) and incubated for 1 h. The slices were then treated with varying concentrations of imipenem and vancomycin. After a 24 h incubation period, slices were homogenized and used to determine bacterial load and genome amount via real-time quantitative RT–PCR (RT–qPCR). The cfu were determined after an incubation period of 24 h. Total RNA was extracted and subsequently synthesized into cDNA. The copies numbers of the E. meningoseptica rpoB and hamster GAPDH genes in the sample were assessed by comparing sample threshold cycle values to a standard curve and expressed as in a previous study.

Statistical analysis

Statistical analyses were performed using SPSS version 20.0, and statistical differences among various groups were calculated using the one-way ANOVA followed by Tukey’s test. Differences were considered statistically significant if P < 0.05.

Results

Genus-specific antagonistic effects of carbapenems with vancomycin on Elizabethkingia spp.

The double-disc diffusion test revealed antagonistic effects in five antibiotic pairs, including cefoxitin–vancomycin, imipenem–vancomycin, amoxicillin/clavulanic acid–vancomycin, cefoxitin–teicoplanin...
Vancomycin and imipenem antagonism in *Elizabethkingia*

and imipenem–teicoplanin (Figure S1). The images were shown in Figure 1(a). The FICIs of vancomycin in combination with various antibiotics are shown in Figure 1(b) and further detailed in Figure S2. Chequerboard assays revealed that each of the carbapenems (imipenem, doripenem, ertapenem and meropenem) tested exhibited similar antagonistic trends. Among these, the mean FICI for imipenem was the lowest, at 5.73. To avoid overestimating the antagonistic effects of these combinations, we selected imipenem for further investigation. Time–kill assays showed that combining 8 mg/L (1 × MIC) vancomycin with 4 mg/L imipenem (0.25 × MIC) resulted in a 6.7 log_{10} cfu/mL increase after 8 h (Figure S3a). When the vancomycin concentration was elevated to 32 mg/L (4 × MIC) alongside 4 mg/L imipenem (0.25 × MIC), an 8.2 log_{10} cfu/mL increase was observed after 24 h (Figure S3b). The antagonistic interaction between vancomycin and imipenem was demonstrated in clinically relevant *Elizabethkingia* isolates (Figures S3c–e and S4). When combined with imipenem, the vancomycin MICs for *S. aureus* ATCC 29213, hVISA Mu3 and VISA Mu50 strains decreased (Figure S5a). In contrast, the addition of imipenem resulted in increased vancomycin MICs for clinical *Elizabethkingia* isolates (Figure S5b–d).

**Imipenem antagonizes vancomycin inhibition of static biofilm formation and bacterial adhesion in vivo and ex vivo (PCLS model)**

Sub-MIC levels of vancomycin inhibited biofilm formation, but adding imipenem reduced this effect (Figure 2a). In A549 cells, 4 mg/L (0.25×MIC) vancomycin reduced adhesion by 89.8%, but when combined with 1 mg/L (0.0625×MIC) imipenem it increased adhesion to 22.1% (Figure 2b). In hamster ex vivo PCLS models, vancomycin demonstrated a dose-dependent
bactericidal effect, but adding 1 mg/L imipenem significantly increased bacterial counts (Figure 2c). Additionally, bacterial RNA copy numbers in PCLSs remained consistent (Figure 2d).

**Discussion**

To the best of our knowledge, this is the first study showing the antagonism effect in *Elizabethkingia* spp. The combination with ciprofloxacin, linezolid or levofloxacin may provide a more effective option for the treatment of these infections, and the combination of minocycline and rifampicin was found to be effective in inhibiting *Elizabethkingia* biofilms. Vancomycin targets cell wall peptidoglycans and exhibits bactericidal activity against Gram-positive bacteria. Previous studies, including ours, have shown that *Elizabethkingia* spp. generally expressed an intermediate response to vancomycin (8–16 mg/L). In this study and our previous findings, a synergistic effect of vancomycin and imipenem on HVISA Mu3 and VISA Mu50 strains was observed. However, for Mu3, an antagonistic interaction between vancomycin and imipenem was observed at low imipenem concentrations (0.002–0.5 mg/L). The reason for the observed antagonistic effect with Mu3 remains unclear, particularly as the majority of existing evidence typically suggests a synergistic effect.

Previous studies have linked *Elizabethkingia* infections, particularly from respiratory and tracheal secretions, with strong biofilm formation, impacting antibiotic effectiveness. In the current study, we found that sub-MIC levels of vancomycin effectively inhibited biofilm formation of *Elizabethkingia* spp. and reduced adherence to A549 cells, similar to the study on *Enterococcus* isolates. The ex vivo PCLS model was used to confirm these results that closely mimicked the in vivo lung environment. However, the addition of imipenem not only neutralized the inhibitory effects of vancomycin but also enhanced bacterial pathogenicity, thereby potentially leading to treatment failure.

There were limitations in the current study, despite data from our in vitro and ex vivo models. Further in vivo animal models and clinical data are needed to mimic the clinical setting of patient treatment.

In summary, we have observed that the efficacy of vancomycin may be reduced when administered in combination with carbapenems as well as other β-lactams, such as cefoxitin, and amoxicillin/clavulanic acid against *Elizabethkingia* spp. These insights could be crucial for further clinical treatments against *Elizabethkingia* infections and caution should be exercised when using vancomycin in combination with selected β-lactams, including carbapenems.

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**Transparency declarations**

All authors declare that they have no competing interests.

**Author contributions**

Yo-Sung Yang: conceptualization, formal analysis, funding acquisition, methodology, project administration; Hsing-Yu Chen: funding acquisition, methodology, writing and review & editing; I-Chieh Lin: formal analysis, methodology, resources; Wen-Ting Chen: project administration, software, validation, visualization; Meng-He Lin: project administration, validation, visualization; Yun-Hsiang Cheng; software; Wei-Yao Wang: formal analysis; Shu-Chen Kuo: methodology, validation, visualization; Jun-Ren Sun: conceptualization, formal analysis, funding acquisition, methodology, resources, supervision, validation, writing and original draft, writing and review & editing.

**Supplementary data**

Figures S1–S5 and Tables S1 and S2 are available as **Supplementary data at JAC Online**

**References**


