Synergistic effect of vancomycin combined with cefotaxime, imipenem or meropenem against *Staphylococcus aureus* with reduced susceptibility to vancomycin

Abstract

Background/aim: We investigated the synergistic effect between vancomycin and β-lactams against vancomycin-susceptible (VSSA) and non-susceptible MRSA isolates [heterogeneous vancomycin-intermediate *S. aureus* (hVISA) and VISA].

Materials and methods: A total of 29 MRSA including 6 VISA, 14 hVISA and 9 VSSA isolates were subjected to a microbroth dilution-minimum inhibitory concentration (MIC) checkerboard using vancomycin combined with cefotaxime, imipenem or meropenem. To confirm synergistic activity, the representative strains of VISA, hVISA and VSSA were then selected for the time-kill curve method.

Results: The combination of vancomycin with imipenem, meropenem and cefotaxime exhibited synergistic effects against 17 (2 VISA, 9 hVISA and 6 VSSA), 14 (3 VISA, 9 hVISA and 2 VSSA), and 5 (3 VISA and 2 hVISA) isolates respectively. Additive and indifferent effects were found in the remaining isolates but no antagonistic effect was observed. Using time-kill assay, the vancomycin combined with either imipenem or cefotaxime demonstrated synergism against both VISA and hVISA isolates, while the synergistic effect with meropenem was obtained in the VISA isolates only.

Conclusion: This study demonstrated in vitro enhanced antibacterial activity of vancomycin plus β-lactams against clinical hVISA or VISA isolates. These combinations may be an alternative treatment for MRSA infections in clinical practice.

Key words: β-Lactams**,** methicillin-resistant *Staphylococcus aureus*, synergy, vancomycin, vancomycin resistance

1. Introduction

Staphylococcus aureus is an important pathogenic bacterium which plays a significant role in human diseases especially the strain that resists to methicillin, called methicillin-resistant *S. aureus* (MRSA). Vancomycin, a glycopeptide antibiotic, discovered since 1952 has activity against a wide range of Gram positive bacteria [1]. It is often a drug of choice for the treatment of serious infections caused by MRSA. However, clinical MRSA isolates with reduced susceptibility to vancomycin, heterogeneous vancomycin-intermediate *S. aureus* (hVISA) and vancomycin-intermediate *S. aureus* (VISA), have emerged, resulting in the poor clinical outcomes [2,3]. The vancomycin monotherapy is associated with treatment failure, higher rates of hospitalization and mortality [4]. A combination of antimicrobial agents has therapeutic benefit and leads to rapid recovery of patients [5].

The concept of combination of vancomycin with β-lactams was mentioned a decade ago [6]. Vancomycin combined with β-lactams showed an additive or synergistic effect against MRSA isolates. The β-lactam drugs enhanced vancomycin surface binding, reduced cell wall thickening and acted as an inhibitor at different stages of cell wall synthesis [3,7,8]. In addition, the synergistic effect helped reducing the vancomycin dosage, resulting in reducing the risk of nephrotoxicity [9]. Therefore, clinical use of vancomycin and β-lactam combination as an alternative therapy for MRSA with reduced vancomycin susceptibility may be superior to vancomycin monotherapy. However, reports of this combination against MRSA isolates with reduced susceptibility to vancomycin are limited and the results remain inconsistent. We thus evaluated the combination of three β-lactams including cefotaxime, meropenem and imipenem with vancomycin against VISA, hVISA and vancomycin-susceptible *S. aureus* (VSSA)

isolates by using a broth microdilution checkerboard and time-kill assays. The combination therapy may provide an option for combating the critical infection caused by hVISA or VISA.

2. Materials and methods

2.1. Bacterial strains

A total of 29 clinical *S. aureus* (6 VISA, 14 hVISA and 9 VSSA) isolates collected from individual patients attending the Srinagarind Hospital, Khon Kaen University, Thailand between 2010 and 2016 were included. All isolates were identified by conventional biochemical tests such as tube coagulase, phenol red mannitol, and DNase tests and *mecA* gene was detected by PCR method [10]. The hVISA phenotype was determined by a population analysis profile with area under the curve (PAP-AUC) [2].

2.2. Antimicrobial agents

All antimicrobials used in this study were purchased from commercial sources, cefotaxime (CTX) and vancomycin (VAN) from Sigma-Aldrich, St Louis, USA, imipenem (IPM) from MSD, Whitehouse Station, NJ, USA and meropenem (MEM) from Siam Bheasach, Bangkok, Thailand.

2.3. Population analysis profile with an area under the curve ratio (PAP-AUC ratio)

For PAP of hVISA phenotype confirmation used in this study was described in a previous study [11]. Briefly, an overnight bacterial broth culture with turbidity of McFarland standard no. 0.5 was serially 10-fold diluted from 10^{0}-10^{-6}. An aliquot of 100 µL of each dilution was spread on brain heart infusion agar (BHIA) (Oxoid, Basingstoke, UK) containing various vancomycin concentrations of 0, 0.5, 1, 2, 3, 4, 5, 6, 7 and 8 µg/mL After incubation at 37°C for 48 h, bacterial colonies were counted and further converted

to a colony forming unit (CFU). The log10 number of CFU/mL were plotted against the vancomycin concentrations using Graph Pad Prism software version 5.0.1 (GraphPad Software Inc., San Diego, USA). The area under the curve (AUC) of each isolate was calculated according to the ratio of the AUC of the test strain and that of the reference hVISA strain (Mu3). PAP-AUC ratio criteria for the determination of VSSA, hVISA, and VISA strains are as described previously [11]: < 0.90 = VSSA, 0.90-1.30 = hVISA, and > 1.30 = VISA. *S. aureus* ATCC700699 (Mu50, VISA), ATCC700698 (Mu3, hVISA) and ATCC29213 (VSSA) were used as positive control strains of homogeneous, heterogeneous vancomycin resistance and negative control strains, respectively.

2.4. Susceptibility testing

The minimum inhibitory concentrations (MICs) and synergistic effect of vancomycin and β-lactam antimicrobials were tested duplicate by using a microdilution checkerboard technique, which was performed in a 96-well microtiter plates with Mueller-Hinton broth (Oxoid). The susceptibility testing by a broth microdilution method were performed and interpreted according to the CLSI guidelines (MIC breakpoint: susceptible, ≤ 2 μg/mL; intermediate, 4-8 μg/mL; and resistant, ≥ 16 μg/mL) [12-13]. The test concentrations of each β-lactam were ranging from 0.125 to 64 μg/mL and those of vancomycin were 0.125, 0.25, 0.5, 1, 2, 3, 4 μg/mL. The final bacterial inoculum was approximately 10^5 CFU/mL. The 96-well plates were incubated at 37 °C for 24 h, [14-16] and the first clear well in each row and column containing both antimicrobials was read and calculated as the fractional inhibitory concentration (FIC) index. The FIC index is the FIC of drug A (the MIC of the antimicrobial A in the combination divided by the MIC of the antimicrobial A alone) plus FIC of drug B (the MIC of the antimicrobial B in the combination divided by the MIC of the antimicrobial B alone). The FIC index value of < 0.5, 0.5-1.0, > 1-4.0

and > 4.0 were defined as synergy, additive, indifference and antagonism respectively [17]. Growth and sterility controls were tested in each test panel. In addition, *S. aureus* ATCC29213 strain were used as a control strain.

2.5. Time-kill assay

The synergy of VAN plus IPM, CTX or MEM was performed by using an inoculum of ~10^6 CFU/mL in MHB at sub-MICs (one-half of MIC) of the antimicrobials. Tubes without antimicrobial were used for growth control. Bacterial counts were taken at 0, 2, 4, 8 and 24 h. Synergy between VAN and each β-lactam was defined as a > 2 log10 CFU/mL decrease of the combination over the most active single agent after 24 h and ≥ 1 log10 CFU/mL reduction from baseline [7].

3. Results

The ranges of VAN MIC against 6 VISA, 14 hVISA, and 9 VSSA isolates were 3-> 4, 1-2 and 1-2 μg/mL respectively. The MIC ranges for CTX, IPM and MEM were 16-> 64, 4-> 64 and 4-> 64 μg/mL; 0.125-2, 0.125-64 and 0.125-64 μg/mL; and 0.25-16, 2-> 64 and 0.25-64 μg/mL, respectively. The MICs of VAN in the combination with CTX, MEM or IPM showed 1-4, 2-5, and 2-6 dilutions less than those of the VAN alone. Likewise, when CTX, MEM or IPM combined with VAN, the MICs of each agent also reduced 2-9, 2-8, and 1-9 dilutions to those of each agent alone respectively (Table 1, 2). The mean MICs of VAN when combined with IPM for the VISA, hVISA and VSSA isolates showed 91.8%, 82% and 76.2% reduction from those of the VAN alone respectively. The VAN plus either CTX or MEM also had similar activities to decrease the MICs of VAN from those using the VAN alone for VSSA group (36.1% and 63.9% decreased respectively) (Figure 1).

The VAN plus IPM showed the highest synergistic effect against 17 of the 29 isolates (58.6%; 2 VISA, 9 hVISA and 6 VSSA isolates). Similarly, the VAN plus MEM had synergistic effects against 14 isolates (48.3%; 3 VISA, 9 hVISA and 2 VSSA isolates). In contrast, the VAN plus CTX gave synergistic effect against 5 isolates only (17.2%; 3 VISA and 2 hVISA), whereas the additive results were found in most isolates (Table 1). However, a synergistic effect of VAN plus either CTX or MEM was found against a VISA isolate with high level of VAN MIC (> 4 μg/mL) (Table 2). In addition, no antagonistic result was observed in any isolates.

Among the 3 couples of antimicrobials, the VAN plus IPM had the highest inhibitory effectiveness than other two pairs (mean FIC indexes was 0.23 in the synergistic activity group). The synergistic effect (FIC indexes of ≤ 0.5) was found in most isolates with high MICs (≥ 16 μg/mL) of CTX (100%), MEM (93%) and IPM (53%) (Table 2).

Notably, the combination of VAN with 0.125 μg/mL of IPM showed indifference and synergistic effects against most of the isolates (8 and 11 isolates respectively), the cumulative percentage of synergistic effect between VAN and IPM rising to 82.4% when 0.5 mg/L of IPM was used, whereas those of the VAN plus MEM and VAN plus CTX were 42.9% and 20% when 1 μg/mL of MEM or CTX were used respectively (Figure 2). To confirm the synergistic effects determined by checkerboard method, the representative strains of VISA, hVISA and VSSA (isolate no. VI 152, hVI 300 and VS 71 respectively) were selected for the time-kill assay. The mean 24-h reductions of bacterial counts for VAN plus IPM, VAN plus MEM and VAN plus CTX were 4, 3.67 and 3 log10 CFU/mL respectively. The VAN plus IPM or CTX showed synergy against VISA (Figure 3a) and hVISA strains (Figure 3b) within 24 h of incubation whereas synergism by the VAN plus

MEM was observed in the VISA strain only. The time–kill assay of VAN plus β-lactams showed no synergistic effect for the VSSA strain (Figure 3c).

4. Discussion

Carbapenems and the 3rd generation cephalosporins have an extremely broad spectrum of antimicrobial activity to both Gram positive and Gram negative bacteria. Therefore, we tested the activity of IPM, MEM and CTX combined with VAN against MRSA isolates. The increasing use of VAN has caused a selective pressure, leading to the occurrence of vancomycin-resistant strains. This resulted in the therapeutic failure, morbidity and even death [2]. Due to limited option of therapeutic drugs, several studies have focused on the combination of antimicrobials as an alternative treatment. The appropriate antimicrobial treatments provided effective therapy, reducing antimicrobial doses and adverse effect and decrease both cost and length of hospitalization.

In this study, synergy effect of the combined drugs was found in varying number of the vancomycin-susceptible and non-susceptible MRSA isolates. Although the combination of these β-lactams and VAN were not synergistic against all isolates, no antagonistic effect was found. These results suggested that the additive and indifferent effects may have been the consequences of the method's limitation since the antimicrobials were applied in various concentrations. Therefore, the real effect may be synergistic rather than additive effects [18]. However, the checkerboard technique was mostly used as a reference method for determination of drugs synergy [16]. Our results supported that the FIC indexes of the β-lactam-VAN combination inversely correlated with the MICs of the β-lactam alone [6]. Most cases of synergistic effects (FIC indexes of < 0.5) occurred in the strains that had high MIC for CTX, MEM and IPM. Among the three β-lactams tested, IPM was considered to be the best agent to combine with VAN,

frequently showing a synergistic effect particularly against hVISA strains. In addition, the synergistic effect of VAN plus IPM can be enhanced at lower IPM concentration (0.125 μg/mL), compared with MEM (1 μg/mL), and CTX (0.5 μg/mL). The concentrations found to have a synergistic effect are clinically accessible concentrations and revealed within the range of MIC breakpoint of CLSI [13]. The vancomycin plus β-lactams demonstrated an enhanced antibacterial effect at susceptible breakpoint concentrations. Both β-lactams and VAN have activity against bacteria by preventing the biosynthesis of the bacterial cell wall. The activity of β-lactam targets at the transpeptidase enzymes, which manage the crosslink of peptidoglycan in the bacterial cell wall. In addition, the β-lactam also alters the bacterial cell surface which helps to access the specific target for the binding of VAN [19]. On the other hand; the target site of VAN is pentapeptide side chain, leading to inhibition of transglycosylation and transpeptidation. Moreover, VAN also alters the permeability of the cell membrane and selectively inhibits ribonucleic acid synthesis [20]. These activities promote the synergistic effect of their combinations.

In this study, the synergistic activity of antimicrobial combinations was confirmed by the time-kill assay. Our data supported the results of checkerboard method that VAN combined with β-lactams demonstrates synergistic activity against staphylococcal isolates with reduced susceptibility to VAN. Interestingly, the mean 24-h of bacterial reduction for VAN plus IPM was the highest compared with the other combinations.

IPM is a potent β-lactam antimicrobial that has a post antibiotic effect (PAE) against Gram-positive bacteria and resists to the hydrolysis by most β-lactamases **[**21,22**].** Although the MRSA strains are not susceptible to this agent, several studies have reported the efficacy of IPM when used in combination with other antimicrobials, including

cephalosporins and vancomycin [14,15,18,23,24], thus corresponding to this study. Therefore, the use of unconventional combinations of drugs may be an alternative for management of MRSA isolates with reduced susceptibility to VAN.

In the present study, some limitations should be noted; a few strains of VISA have been observed due to the prevalence of clinical VISA in our area thus larger samples should be evaluated in further studies. In addition, these combinations should be investigated in clinical or in vivo condition to support the recommendation of β-lactam combination therapy in routine clinical use. However, few studies have investigated in animal model for the combinations of VAN with β-lactams including nafcillin, imipenem or ceftobiprole which have found the evidence of synergy [6,25,26]. In addition, clinical studies revealed an increasing rate of microbiological eradication when using the combination of VAN with piperacillin-tazobactam or β-lactams in therapeutic groups [27-29].

In conclusion, this is an in vitro study by checkerboard and time-kill assays to determine the activity of VAN and β-lactam combinations, which demonstrated the enhanced antibacterial activity against clinical hVISA or VISA isolates, suggesting that it may be an alternative for using in clinical therapy.

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All the authors declare that they have no conflicts of interest.

References

1. Chua K, Howden BP. Treating Gram-positive infections: vancomycin update and the whys, wherefores and evidence base for continuous infusion of anti-Gram-positive antibiotics. Current Opinion in Infectious Diseases 2009; 22 (6): 525-534. doi: 10.1097/QCO.0b013e328331fbcd
2. Hiramatsu K, Hanaki H, Ino T, K Yabuta, T Oguri et al. Methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. Journal of Antimicrobial Chemotherapy 1997; 40 (1): 135-136. doi.org/10.1093/jac/40.1.135
3. Lai CC, Chen CC, Chuang YC, Chuang YC, Tang HJ. Combination of cephalosporins with vancomycin or teicoplanin enhances antibacterial effect of glycopeptides against heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) and VISA. Scientific Reports 2017; 7: 41758. doi: 10.1038/srep41758
4. Wunderink RG, Rello J, Cammarata SK, Croos-Dabrera RV, Kollef MH. Linezolid vs vancomycin: analysis of two double-blind studies of patients with methicillin-resistant *Staphylococcus aureus* nosocomial pneumonia. Chest 2003; 124 (5): 1789-1797
5. Davis JS, Van Hal S, Tong SY. Combination antibiotic treatment of serious methicillin-resistant *Staphylococcus aureus* infections. Seminar in Respiratory and Critical Care Medicine 2015; 36 (1): 3-16. doi: 10.1055/s-0034-1396906
6. Climo MW, Patron RL, Archer GL. Combinations of vancomycin and β-lactams are synergistic against staphylococci with reduced susceptibilities to vancomycin. Antimicrobial Agents and Chemotherapy 1999; 43 (7): 1747-1753. doi: /10.1128AAC.43.7.1747

7. Barber KE, Werth BJ, Ireland CE, Stone NE, Nonejuie P et al. Potent synergy of ceftobiprole plus daptomycin against multiple strains of *Staphylococcus aureus* with various resistance phenotypes. Journal of Antimicrobial Chemotherapy 2014; 69 (11): 3006-3010. doi: 10.1093/jac/dku236
8. Sieradzki K, Tomasz A. Suppression of β-lactam antibiotic resistance in a methicillin-resistant *Staphylococcus aureus* through synergic action of early cell wall inhibitors and some other antibiotics. Journal of Antimicrobial Chemotherapy 1997; 39 Suppl A: 47-51. doi: /10.1093jac/.39suppl_1.47
9. van Hal SJ, Paterson DL, Lodise TP. Systematic review and meta-analysis of vancomycin-induced nephrotoxicity associated with dosing schedules that maintain troughs between 15 and 20 milligrams per liter. Antimicrobial Agents and Chemotherapy 2013; 57 (2): 734-744. doi: 10.1128/AAC.01568-12
10. Kondo Y, Ito T, Ma XX, Watanabe S, Kreiswirth BN et al. Combination of multiplex PCRs for staphylococcal cassette chromosome *mec* type assignment: rapid identification system for *mec*, *ccr*, and major differences in junkyard regions. Antimicrobial Agents and Chemotherapy 2007; 51 (1): 264-274. doi: 10.1128/AAC.00165-06
11. Wongthong S, Dutchanutouch K, Namsaengkang V, Chanawong A, Wilailuckana C et al. Performance of vancomycin and teicoplanin disk diffusion test in isogenic vancomycin non-susceptible *Staphylococcus aureus*. The Journal of Infection in Developing Countries 2015; 9 (2): 157-164. doi: 10.3855/jidc.5059
12. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. 11th ed, CLSI document M07-A11. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2014.

13. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing. 28th ed, CLSI document M100-S28. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2018.
14. Jacqueline C, Navas D, Batard E, Miegeville AF, Le Mabecque V et al. In vitro and in vivo synergistic activities of linezolid combined with subinhibitory concentrations of imipenem against methicillin-resistant *Staphylococcus aureus*. Antimicrobial Agents and Chemotherapy 2005; 49 (1): 45-51. doi: 10.1128/AAC.49.1.45-51.2005
15. Kobayashi Y. Study of the synergism between carbapenems and vancomycin or teicoplanin against MRSA, focusing on S-4661, a carbapenem newly developed in Japan. Journal of Infection and Chemotherapy 2005; 11 (5): 259-261. doi:10.1007/s10156-005-0402-2
16. Sy CL, Huang TS, Chen CS, Chen YS, Tsai HC et al. Synergy of β-lactams with vancomycin against methicillin-resistant *Staphylococcus aureus*: correlation of disk diffusion and checkerboard methods. Journal of Clinical Microbiology 2016; 54 (3): 565-568. doi: 10.1128/JCM.01779-15
17. Doern CD. When does 2 plus 2 equal 5? A review of antimicrobial synergy testing. Journal of Clinical Microbiology 2014; 52 (12): 4124-4128. doi: 10.1128/JCM.01121-14
18. Silva LV, Araújo MT, Santos KR, Nunes AP. Evaluation of the synergistic potential of vancomycin combined with other antimicrobial agents against methicillin-resistant *Staphylococcus aureus* and coagulase-negative *Staphylococcus* spp. strains. Memorias do Instituto Oswaldo Cruz 2011; 106 (1): 44-50. doi: 10.1590/s0074-02762011000100007

19. Sieradzki K, Tomasz A. Inhibition of cell wall turnover and autolysis by vancomycin in a highly vancomycin-resistant mutant of *Staphylococcus aureus*. Journal of Bacteriology 1997; 179 (8): 2557-2566. doi:10.1128/jb.179.8.2557-2566.1997
20. Watanakunakorn C. Mode of action and in-vitro activity of vancomycin. Journal of Antimicrobial Chemotherapy 1984; 14 Suppl D: 7-18. doi: 10.1093/jac/14.suppl_d.7.
21. Baquero F, Culebras E, Patrón C, Pérez-Díaz JC, Medrano JC et al. Postantibiotic effect of imipenem on Gram-positive and Gram-negative microorganisms. Journal of Antimicrobial Chemotherapy 1986; 18 Suppl E: 47-59. doi: 10.1093/jac/18.supplement_e.47
22. Odenholt-Tornqvist I, Lowdin E, Cars O. Comparative in vitro pharmacodynamics of BO-2727, meropenem and imipenem against gram-positive and gram-negative bacteria. Clinical Microbiology and Infection 1997; 3 (1): 73-81. doi: 10.1111/j.1469-0691
23. Benquan W, Yingchun T, Kouxing Z, Tiantuo Z, Jiaxing Z et al. *Staphylococcus* heterogeneously resistant to vancomycin in China and antimicrobial activities of imipenem and vancomycin against it. Journal of Clinical Microbiology 2002; 40 (3): 1109-1112. doi: 10.1128/JCM.40.3.1109-1112.2002
24. Mohammadi-Berenjestanaki H, Khori V, Shirzad-Aski H, Ghaemi EA. In vitro synergistic effect of vancomycin and some antibacterial agents against clinical methicillin-resistant and sensitive *Staphylococcus aureus* isolates. Microbial Drug Resistance 2020; 26 (3): 218-226. doi: 10.1089/mdr.2019.0003
25. Fernandez J, Abbanat D, Shang W, He W, Amsler K et al. Synergistic activity of ceftobiprole and vancomycin in a rat model of infective endocarditis caused by methicillin-resistant and glycopeptides-intermediate *Staphylococcus aureus*.

Antimicrobial Agents and Chemotherapy 2012; 56 (3): 1476-1484. doi: 10.1128/AAC.06057-11

26. Ribes S, Pachón-Ibáñez ME, Domínguez MA, Fernández R, Tubau F et al. In vitro and in vivo activities of linezolid alone and combined with vancomycin and imipenem against *Staphylococcus aureus* with reduced susceptibility to glycopeptides. European Journal of Clinical Microbiology & Infectious Diseases 2010; 29 (11): 1361-1367. doi: 10.1007/s10096-010-1007-y
27. Dilworth TJ, Ibrahim O, Hall P, Sliwinski J, Walraven C et al. β-Lactams enhance vancomycin activity against methicillin-resistant *Staphylococcus aureus* bacteremia compared to vancomycin alone. Antimicrobial Agents and Chemotherapy 2014; 58 (1): 102-109. doi: 10.1128/AAC.01204-13
28. Dilworth TJ, Sliwinski J, Ryan K, Dodd M, Mercier RC. Evaluation of vancomycin in combination with piperacillin-tazobactam or oxacillin against clinical methicillin-resistant *Staphylococcus aureus* isolates and vancomycin-intermediate *S. aureus* isolates in vitro. Antimicrobial Agents and Chemotherapy 2014; 58 (2): 1028-1033. doi: 10.1128/AAC.01888-13
29. Gritsenko D, Fedorenko M, Ruhe JJ, Altshuler J. Combination therapy with vancomycin and ceftaroline for refractory methicillin-resistant *Staphylococcus aureus* bacteremia: A case series. Clinical Therapeutics 2017; 39 (1): 212-218. doi: 10.1016/j.clinthera.2016.12.005

Table 1. Fractional inhibitory concentration index of vancomycin plus cefotaxime, meropenem and imipenem combination against 29 *Staphylococcus aureus* using a checkerboard technique

Strains	MIC (µg/mL)			FIC index	MIC (µg/mL)		FIC index	MIC (µg/mL)		FIC index
	VAN	CTX	VAN + CTX		MEM	VAN + MEM		IPM	VAN + IPM	
VISA (6)	3-> 4	16-> 64	VAN = 0.25-2 CTX = 0.5-64	0.33-0.67 (Sy = 3, Ad = 3)	0.25-16	VAN = 0.25-2 MEM = 0.125-2	0.38-0.67 (Sy = 3, Ad = 3)	0.125-2	VAN = 0.125-1 IPM = 0.125	0.23-1.04 (Sy = 2, In = 4)
hVISA (14)	1-2	4-> 64	VAN = 0.25-1 CTX = 0.25-64	0.19-0.75 (Sy = 2, Ad = 12)	2-> 64	VAN = 0.25-1 MEM = 0.25-8	0.19-0.75 (Sy = 9, Ad = 5)	0.125-64	VAN = 0.125-0.5 IPM = 0.125-2	0.06-1.13 (Sy = 9, Ad = 2, In = 3)
VSSA (9)	1-2	4-> 64	VAN = 0.5-1 CTX = 0.25-32	0.50-1.01 (Ad = 9)	0.25-64	VAN = 0.25-1 MEM = 0.125-32	0.38-1.00 (Sy = 2, Ad = 7)	0.125-64	VAN = 0.125-0.5 IPM = 0.125-0.5	0.13-1.13 (Sy = 6, Ad = 2, In = 1)
Total (29)				Sy = 5, Ad = 24			Sy = 14, Ad = 15			Sy = 17, Ad = 4, In = 8

VISA, vancomycin-intermediate *S. aureus*; hVISA, heterogeneous vancomycin-intermediate *S. aureus*; VSSA, vancomycin-susceptible *S. aureus*; FIC, Fractional inhibitory concentration; VAN, vancomycin; CTX, cefotaxime; MEM, meropenem; IPM, imipenem

* FIC index: < 0.5: synergy (Sy); 0.5-1.0: additive (Ad); > 1-4.0: indifference (In); > 4.0: antagonism (An) [17].

Table 2. Fractional inhibitory concentration index of vancomycin plus cefotaxime, meropenem or imipenem combination against each *Staphylococcus aureus* isolates using a checkerboard technique

Strains	MIC (µg/mL)			FIC index	MIC (µg/mL)		FIC index	MIC (µg/mL)		FIC index
	VAN	CTX	VAN + CTX		MEM	VAN + MEM		IPM	VAN + IPM	
VI 123	3	16	0.25 + 4	0.33 (Sy)	0.25	0.5 + 0.125	0.67 (Ad)	0.125	0.125 + 0.125	1.04 (In)
VI 127	4	> 64	0.5 + 64	0.63 (Ad)	0.5	0.25 + 0.25	0.56 (Ad)	0.125	0.125 + 0.125	1.03 (In)
VI 152	3	> 64	2 + 0.5	0.67 (Ad)	16	1 + 1	0.39 (Sy)	2	0.5 + 0.125	0.23 (Sy)
VI 214	3	> 64	0.5 + 32	0.42 (Sy)	1	1 + 0.25	0.58 (Ad)	0.125	0.125 + 0.125	1.04 (In)
VI 7	3	64	1 + 4	0.39 (Sy)	4	1 + 0.25	0.39 (Sy)	0.125	0.125 + 0.125	1.04 (In)
VI 17	> 4	32	2 + 8	0.50 (Ad)	16	2 + 2	0.38 (Sy)	1	1 + 0.125	0.25 (Sy)
hVI 134	1	> 64	0.25 + 0.5	0.25 (Sy)	64	0.25 + 8	0.38 (Sy)	16	0.5 + 0.125	0.51 (Ad)
hVI 250	1	> 64	0.5 + 32	0.75 (Ad)	64	0.25 + 4	0.31 (Sy)	64	0.25 + 2	0.28 (Sy)
hVI 261	2	> 64	1 + 4	0.53 (Ad)	32	0.5 + 1	0.28 (Sy)	32	0.5 + 0.125	0.25 (Sy)
hVI 276	2	> 64	1 + 4	0.53 (Ad)	32	0.5 + 1	0.28 (Sy)	32	0.125 + 0.125	0.06 (Sy)
hVI 280	2	64	0.25 + 4	0.19 (Sy)	2	0.25 + 1	0.63 (Ad)	0.125	0.125 + 0.125	1.06 (In)
hVI 297	1	4	0.25 + 2	0.75 (Ad)	16	0.25 + 1	0.31 (Sy)	0.125	0.125 + 0.125	1.13 (In)
hVI 300	2	> 64	1 + 0.25	0.50 (Ad)	> 64	0.25 + 8	0.19 (Sy)	1	0.25 + 0.125	0.25 (Sy)
hVI 302	1	64	0.25 + 16	0.50 (Ad)	2	0.25 + 1	0.75 (Ad)	0.125	0.125 + 0.125	1.13 (In)
hVI 17	2	> 64	1 + 2	0.52 (Ad)	64	1 + 0.25	0.50 (Ad)	1	0.5 + 0.25	0.50 (Ad)
hVI 1	1	> 64	0.5 + 64	1.00 (Ad)	8	0.25 + 2	0.50 (Ad)	64	0.25 + 2	0.28 (Sy)
hVI 7	1	> 64	0.5 + 8	0.56 (Ad)	4	0.25 + 1	0.50 (Ad)	16	0.25 + 1	0.31 (Sy)
hVI 8	1	> 64	0.5 + 8	0.56 (Ad)	16	0.25 + 2	0.38 (Sy)	4	0.25 + 0.5	0.38 (Sy)
hVI 9	2	> 64	1 + 4	0.53 (Ad)	32	0.25 + 4	0.25 (Sy)	16	0.25 + 0.125	0.13 (Sy)
hVI 13	2	> 64	1 + 2	0.52 (Ad)	16	0.50 + 1	0.31 (Sy)	8	0.25 + 0.125	0.14 (Sy)
VS 66	1	> 64	0.5 + 32	0.75 (Ad)	32	0.5 + 4	0.63 (Ad)	32	0.5 + 0.5	0.52 (Ad)
VS 67	1	> 64	1 + 1	1.01 (Ad)	8	0.5 + 1	0.63 (Ad)	32	0.5 + 0.125	0.50 (Ad)
VS 68	1	> 64	1 + 0.25	1.00 (Ad)	16	0.25 + 8	0.75 (Ad)	8	0.25 + 0.5	0.31 (Sy)
VS 70	1	> 64	0.5 + 16	0.63 (Ad)	16	0.25 + 2	0.38 (Sy)	4	0.25 + 0.125	0.28 (Sy)
VS 71	2	> 64	1 + 0.5	0.50 (Ad)	16	0.25 + 4	0.38 (Sy)	2	0.125 + 0.125	0.13 (Sy)
VS 72	1	> 64	1 + 0.25	1.00 (Ad)	64	0.25 + 32	0.75 (Ad)	64	0.125 + 0.125	0.13 (Sy)
VS 8	1	4	0.5 + 0.5	0.63 (Ad)	0.25	0.5 + 0.125	1.00 (Ad)	0.125	0.125 + 0.125	1.13 (In)
VS 12	2	> 64	1 + 8	0.56 (Ad)	64	1 + 0.5	0.51 (Ad)	64	0.5 + 0.125	0.25 (Sy)
VS 31	1	> 64	0.5 + 32	0.75 (Ad)	16	0.5 + 2	0.63 (Ad)	16	0.25 + 0.25	0.27 (Sy)

VI, vancomycin-intermediate *S. aureus*; hVI, heterogeneous vancomycin-intermediate *S. aureus*; VS, vancomycin-susceptible *S. aureus*; FIC, Fractional inhibitory concentration; VAN, vancomycin; CTX, cefotaxime; MEM, meropenem; IPM, imipenem

* FIC index: < 0.5: synergy (Sy); 0.5-1.0: additive (Ad); > 1-4.0: indifference (In); > 4.0: antagonism (An) [17].

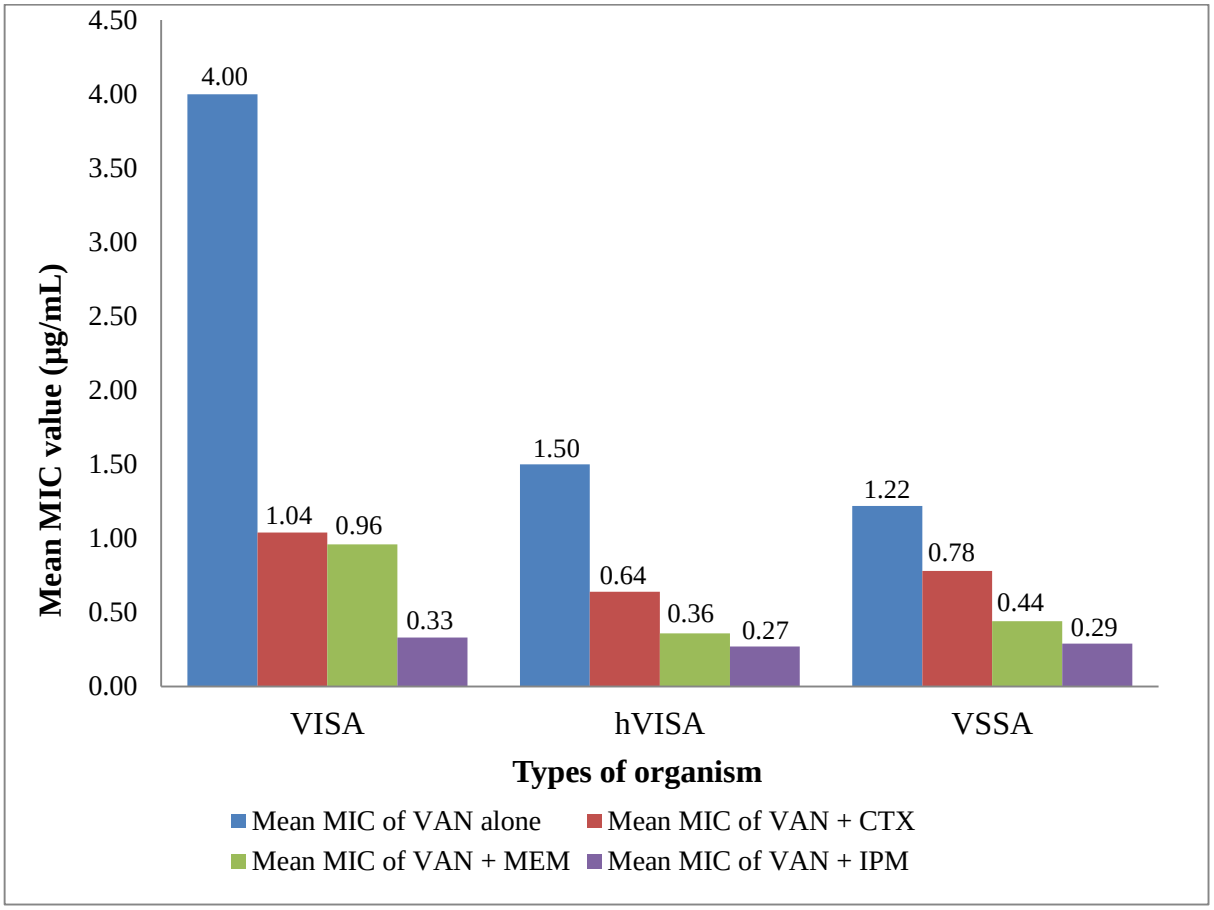

Figure 1. Comparison of the mean MIC values of vancomycin (VAN) alone and in combination with cefotaxime, CTX; meropenem, MEM; imipenem, IPM against 6 vancomycin-intermediate *S. aureus* (VISA), 14 heterogeneous VISA (hVISA) and 9 vancomycin-susceptible *S. aureus* (VSSA) isolates.

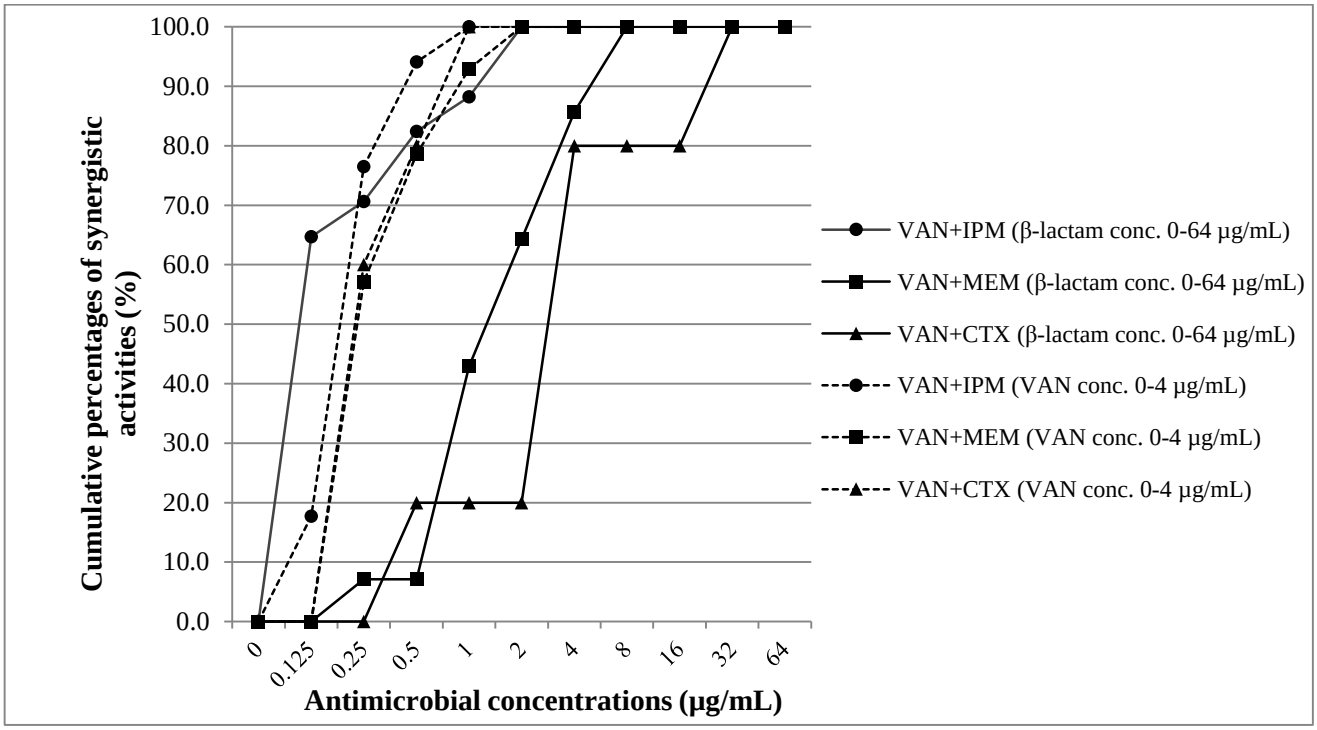

Figure 2. Cumulative percentages (%) of synergistic activities of the vancomycin (VAN) and β-lactam (imipenem, IPM; meropenem, MEM; cefotaxime, CTX) combinations affected by various concentrations of β-lactams (solid lines) and vancomycin (dashed lines) against 29 test isolates.

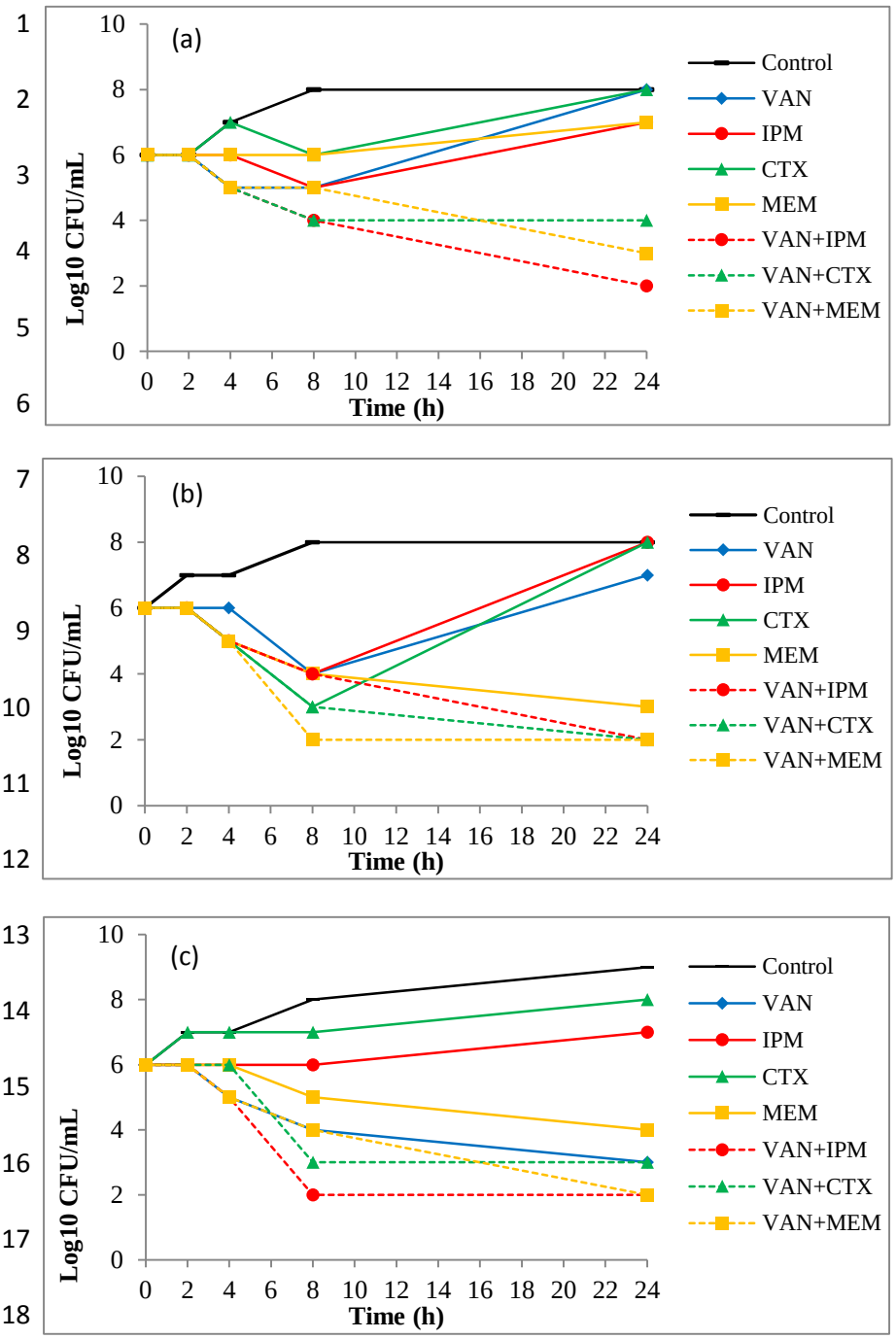

Figure 3. Time-kill curves of each antimicrobial (solid lines) and their combinations (dashed lines) against VISA (a), hVISA (b) and VSSA (c) strains.

growth controls (black lines), vancomycin (blue diamonds), imipenem (red circles), cefotaxime (green triangles) and meropenem (yellow squares).