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Original article

Evaluation of the *in-vitro* synergistic potential of vancomycin combined with other antimicrobial agents against methicillin-resistant *Staphylococcus aureus* isolates

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ABSTRACT

Background: Methicillin-resistant *Staphylococcus* aureus (MRSA) infection is a major public health problem, causing a wide range of infections including bacteremia. Infections caused by MRSA are associated with significant morbidity, mortality, and costs. The present study aimed to determine the frequency of MRSA isolates among bacteremic patients, to determine their antimicrobial susceptibility patterns and to evaluate the in-vitro synergy combinations of vancomycin plus imipenem, cefepime, cefazoline and piperacillin-tazobactam against these isolates. **Methods:** Fifty confirmed MRSA strains isolated from blood cultures constituted the material of this study. The BD Phoenix was used to determine the susceptibility of these isolates to different antimicrobials. The Two-agent Broth Microdilution checkerboard test was used to evaluate the effect of combinations of two antimicrobial agents on the studied isolates. **Results:** In the current study the prevalence of MRSA among bacteremic patients was 15.68%, resistance rate was as follow: Gentamicin (80%), erythromycin (68%), ciprofloxacin (64%), norfloxacin (52%), moxifloxacin (36%), trimethoprim-sulfamethoxazole (46%), doxycycline (38%), rifampin (34%), clindamycin (24%), chloramphenicol (8%), linezolid (6%), teicoplanin (2%). All isolates were fully susceptible to daptomycin and vancomycin. Synergy was seen in varying proportions of the MRSA isolates when vancomycin was combined with imipenem, piperacillin-tazobactam, cefepime and cefazolin that was 76%, 66%, 54% and 52% respectively. No antagonism was observed. The mean FIC indices for combination of Vancomycin with Imipenem were significantly inversely correlated with the vancomycin MICs of the isolates using linear regression analysis. **Conclusions:** The synergistic activity of vancomycin in combination with β- lactam antibiotics offers new insights in treatment options of serious MRSA infections.

Introduction

Staphylococcus aureus (*S. aureus*) a normal flora of skin and mucous membrane is frequently incriminated in serious infections with a high morbidity and mortality rates [1]. Of these infections the best described is bacteremia. In Europe, *S. aureus* is the second most common cause of bloodstream infection (BSI) (about 19.5%), and

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is the most important cause of BSI-related mortality [2].

Prosthetic devices are the most well-known risk factor for invasive *S. aureus* infection and *S. aureus* bacteremia **(**SAB). Prosthetic devices include; surgically implanted materials, orthopedic prostheses and central venous catheters which act as a direct channel into the blood stream. Numerous other factors predispose individuals to SAB especially; intravenous drug abuse, immunosuppressive therapy after transplantation or as cancer treatment and underlying co-morbidities like diabetes [3].

Another powerful determinant for SAB is the patient's age, where the highest incidence of SAB occurs at extremes of age [4]. Despite the clinicians and researchers' efforts in the improvement of SAB management, still it is associated with high morbidity and mortality and disposes a financial burden on health care institutes. Mortality rate in SAB patients reached up to 30% (range from 18-30%) in a recent multinational observational study [5].

Staphylococcus aureus bacteremia predominantly caused by methicillin resistant *S. aureus* (MRSA), is a grave illness with a high risk of complications namely; endocarditis, deep-seated metastatic foci, septic shock or recurrence [6,7]. All MRSA isolates harbor the mec A gene (The gene coding for methicillin resistance), which confers resistance to methicillin as well as all β-lactams, including the cephalosporins [8-10]. The Clinical and Laboratory Standards Institute (CLSI) recommended the use of cefoxitin disc diffusion method for MRSA detection [11]. Among all phenotypic methods, cefoxitin disc diffusion alone has similar sensitivity and specificity to PCR [12].

Since the emergence of MRSA strains with reduced susceptibility to vancomycin in the late 1990s [13], whether strains with decreased susceptibility [minimum inhibitory concentration (MIC) 4 – 8 μg/ml] or fully resistant strains [MIC ≥ 16 μg/ml], fears were expressed regarding the dependability of vancomycin utilization in treatment of MRSA bacteriaemia. Adding a β -lactam antibiotics to vancomycin offers a promising option as they act synergistically, this assumption was based on numerous in-vitro and animal studies [14]. Additionally, it is postulated that the rate of MRSA clearance from blood is higher if this combination is utilized [15].

Although the synergistic effect between various β- lactams and vancomycin was established in-vitro, the exact mechanism behind this synergy is yet to be revealed. Certain clarifications may include; augmentation of the host defense peptide activity against *S. aureus* by the β-lactam, and the "see-saw" effect (*S. aureus* isolates with escalating vancomycin MICs were associated with declining methicillin MICs , probably due to variations in the mec A gene or alteration in penicillin-binding proteins) whereby reduced vancomycin susceptibility results in reduced transcription of mec A and increased susceptibility to β-lactams [16].

Different methods to measure the efficacy of antimicrobial combination exist, one of the most widely exploited techniques is the checkerboard synergy test [17]. It is a technique used to evaluate interactions between biologically active agents either synergism, indifference, or antagonism [18]. Its principle is based on microdilution susceptibility testing.

Antimicrobial combination treatment is advantageous in certain situations, as when the MIC for an isolate is at or near the breakpoint for susceptibility, or when bactericidal synergy is demanded and activity against bacteria in stationary phase or in biofilm is needed. Moreover, antimicrobial combination is required to prevent the emergence of strains with reduced susceptibility to vancomycin [19].

Aim of the work was to determine the frequency of MRSA isolates among bacteremic patients in Alexandria Main University Hospital (AMUH), to determine their antimicrobial susceptibility to different antimicrobial agents, to detect emergence of intermediate susceptibility or resistance to vancomycin, teicoplanin or linezolid among MRSA isolates and to evaluate the in-vitro synergy combinations of vancomycin plus imipenem, cefepime, cefazolin and piperacillin-tazobactam against MRSA isolates.

Material

Fifty confirmed MRSA strains isolated from blood cultures, submitted to the diagnostic microbiology laboratory in AMUH constituted the material of this study.

Methods

Staphylococcus aureus isolated from blood culture specimens were identified according to the standard microbiological techniques using colonial

morphology, Gram stained film, growth on mannitol salt agar, catalase and coagulase tests (slide and tube tests). *Staphylococcus aureus* isolates were considered MRSA when the cefoxitin disc diffusion test results were resistant [11], all these isolates were further subjected to molecular detection of mec A gene using conventional PCR as confirmatory test [20].

The confirmed MRSA isolates were tested by:

I- The BD Phoenix Automated Microbiology System (Becton Dickinson Diagnostic Systems [BD], Pont de Claix, France) for determination of MIC of the following antimicrobials: cefoxitin, cefazolin, gentamicin, ciprofloxacin, clindamycin, chloramphenicol, daptomycin, erythromycin, moxifloxacin, norfloxacin, oxacillin, rifampicin, teicoplanin, trimethoprim/ sulfamethoxazole, ampicillin, penicillin G, amoxycillin-clavulanate, quinopristin-dalfopristin, vancomycin, linezolid, and doxycycline[11,21].

II- The Broth microdilution method for determination of MIC for vancomycin, Imipenem, piperacillin-tazobactam, cefepime and cefazolin**,** using the recommendations of the CLSI [11].

III- The Two-agent Broth Microdilution checkerboard test to evaluate the effect of combinations of two antimicrobial agents on the studied MRSA isolates.

The test method is based on the broth dilution susceptibility methods for evaluating the inhibitory or bactericidal activity of specific concentration in combination at a fixed time. In-vitro interaction are calculated algebraically and interpreted as synergistic, indifference, or antagonistic depending on whether the antibacterial activity of the combination is greater than, equivalent to, or less than, respectively, the activities of the individual agents [22].

(A) Preparing checkerboard antibiotic microdilution panels: i- Commonly tested therapeutic range was determined for each of the used antimicrobials (vancomycin , Imipenem , cefepime, cefazolin & piperacillin-tazobactam). ii- Concentrations ranging from four times the expected MIC to at least 1/8 times the expected MIC was included in the final panel in order to observe the occurrence and magnitude of synergism or antagonism. iii- Antimicrobial agents were dispensed into combination panel (100 µl final volume per well). Each working concentration was poured into individual reservoirs. Vancomycin was dispensed in columns (1-10) from concentration 256 to 0.5 µg/ml, so that the highest concentration was in column 1 & the least concentration was in column 10. Cefazolin , piperacillin- tazobactam and cefepime were dispensed in rows A to H from concentration 256 to 2 µg/ml. Whereas, Imipenem was dispensed in rows A to H from concentration 32 to 0.25 µg/ml. So that, the highest concentration was in row A & the least concentration was in row H. Column 11 was used for MIC of vancomycin alone, Column 12 was used for MIC of the other tested antimicrobial [22] **(Figure 1)**.

(B) preparation of inoculum; Using a sterile swab, organisms were transferred from four or five colonies of similar colony morphology to 5 ml of Cation-adjusted Mueller Hinton broth (CAMHB). Incubate at 35˚C until turbidity matched that of a Mcfarland 0.5 turbidity standard (approximately 1.5×10^8CFU/ml). The adjusted inocula was diluted in CAMHB 1:100 so that each well contained approximately $5X10^5$ CFU/ml [22].

(C) Inoculation and incubation of checkerboard panel; 100 µl of inocula was transferred to the whole plate, wells. Plates were incubated for 16 to 20h at 35 ± 2˚C in ambient air. Quality control strain *S. aureus* 29213 was included and tested with each batch of work.

(D) Reading the results; The uninoculated broth control was checked for sterility first. The MIC is the lowest concentration of antimicrobial agent(s) causing complete inhibition of growth, MIC for the used antimicrobials was determined as single agent from column 11& 12. Each combination well was examined and presence of growth or no growth was recorded.

Calculation:

For each combination interaction the fractional inhibitory concentration (FIC) of each agent was calculated as follows:

1. FIC of agent A =

$$\frac{\text{MIC of agent A in combination}}{\text{MIC of agent A alone}}$$

2. FIC of agent B =

$$\frac{\text{MIC of agent B in combination}}{\text{MIC of agent B alone}}$$

3. The summation of FIC (∑ FIC) index for each combination was calculated as follows; ∑ FIC= FIC of agent A+ FIC of agent B.
4. Providing QC was acceptable, interpretation and recording of each summation (∑ FIC) was done. Synergy is defined as ∑ FIC ≤ 0.5, indifference is defined as 0.5< ∑ FIC ≤ 4 and antagonism is defined as ∑ FIC > 4. If synergism or antagonism occurred at only one ∑ FIC within the combination, the occurrence was reported [22].

Data analysis

Data were entered and analyzed using IBM SPSS version 22. Qualitative data were presented as number and percentage. Simple correlation was used to investigate the linear relationship between MIC of vancomycin and FIC index of antibiotics combination. Pearson's correlation investigates the strength of a linear relationship between two continuous variables, it gives an estimate, the correlation coefficient (r), that give an idea about the direction and strength of association. Simple linear regression is used to estimate the nature of the linear relationship between two continuous variables where one is regarded as the outcome and the other predicts the outcome. It gives the equation of the best straight line through the observed data: y = a + b x where y is the outcome, a is the intercept, b is the regression coefficient (slope of the line) and x is the predictor variable [23].

Results

Out of 2641 blood culture bottle submitted to the Diagnostic Microbiology Laboratory, in AMUH, 338 (12.8%) were positive for growth of microorganisms.

Distribution of bacterial and fungal species in positive blood cultures (n=338) was as follow: coagulase negative *Staphylococci* represented (21.89%), *S. aureus* (20.7%), *Klebsiella spp.* (11.83%), *E.coli* (10.65%) , *Candida spp*. (6.8%), *Enterococcus spp.* (6.5%), *Pseudomonas spp*. (5.92%), *Acinetobacter spp*. (3.55%), *Strept. viridians* (3.37)%, Other *enterobacteriecea* (2.96%).

Among the 70 *S. aureus* isolated from the blood cultures, 53(75.7%) were identified as MRSA phenotypically by their resistance to cefoxitin. Out of the 53 isolates, 50 were randomly selected and subjected to detection of mecA gene genotypically by conventional PCR, thus cefoxitin disc diffusion test and PCR gave identical results.

MIC results of MRSA isolates to various antimicrobial agents tested by the Phoenix Automated Microbiology System:

Regarding the resistance rate: All the 50 MRSA isolates were fully resistant to the following antibiotics: (cefazolin, cefoxitin, ampicillin, penicillin, oxacillin, amoxycillin-clavulanate) (100%) followed by gentamicin (80%), erythromycin (68%), ciprofloxacin (64%), norfloxacin (52%), moxifloxacin (36%), trimethoprim-sulfamethoxazole (46%), doxycycline (38%), rifampin (34%), clindamycin (24%) and chloramphenicol (8%)

All the 50 MRSA isolates were fully susceptible to daptomycin and vancomycin. All isolates were susceptibile to quinupristin-dalfopristin except 2 isolates (4%) showed intermediate susceptibility. As for linezolid, (6%) of the isolates were resistant. Regarding teicoplanin, only (2%) of the isolates showed resistance and 4% intermediate susceptibility. Antibiotic susceptibility of the 50 studied isolates are shown in **table (1)**.

Results of antimicrobial combinations of the 50 MRSA isolates done by checkerboard method:

Vancomycin and imipenem: Seventy six percent (76%) of isolates showed synergism however (24%) of the isolates showed indifference results and none showed antagonism.

Vancomycin and piperacillin-tazobactam: The combination resulted in synergism in (66%) of isolates and (34%) of them showed indifference.

Vancomycin and cefepime: (54%) of MRSA isolates showed synergism and (46%) showed indifference.

Vancomycin and cefazolin: (52%) of isolates showed synergism and the others (48%) showed indifference.

Table 2 shows the results of combination of vancomycin and (imipenem, piperacillin-tazobactam, cefazolin or cefepime) as assessed by checkerboard method.

Correlation between fractional indices of vancomycin combined with various antibiotics and vancomycin MIC.

By simple linear regression analysis, the FIC index **of vancomycin combined with imipenem in MRSA isolates** =0.734 - (MIC* 0.18) (95% CI of regression coefficient of -0.349 to -0.011) and *p*=0.037.

There is no significant correlation between vancomycin MIC and fractional index of

vancomycin combined with any of the tested antibiotics except with imipenem; there was a negative correlation between them with *p*=0.037 and r= -0.296.

Table 3 and **figures 2-5** show the results of simple correlation between vancomycin MIC and fractional indices of vancomycin combined with (imipenem, piperacillin-tazobactam, cefepime and cefazolin).

Table 1. Antimicrobial susceptibility pattern of the 50 MRSA isolates by the BD Phoenix Automated Microbiology System.

Antibiotic	Resistance	Intermediate	Sensitive
	no (%)	no (%)	no (%)
Gentamicin	40 (80%)	0	10(20%)
Cefazolin	50 (100%)	0	0
Cefoxitin	50 (100%)	0	0
Ampicillin	50 (100%)	0	0
Penicillin	50 (100%)	0	0
Oxacillin	50 (100%)	0	0
Amoxycillin-Clavulanate	50 (100%)	0	0
Daptomycin	0	0	50 (100%)
Trimethoprim-Sulfamethoxazole	23 (46%)	1 (2%)	26 (52%)
Teicoplanin	1 (2%)	2 (4%)	47 (94%)
Vancomycin	0	0	50 (100%)
Clindamycin	12 (24%)	1(2%)	37(74%)
Erythromycin	34 (68%)	1(2%)	15(30%)
Quinupristin-dalfopristin	0	2(4%)	48(96%)
Chloramphenicol	4 (8%)	1(2%)	45(90%)
Linezolid	3 (6%)	0	47(94%)
Ciprofloxacin	32 (64%)	0	18(36%)
Moxifloxacin	18 (36%)	0	32(64%)
Norfloxacin	26 (52%)	3(6%)	21(42%)
Rifampin	17 (34%)	1(2%)	32(64%)
Doxycycline	19 (38%)	0	31(62%)

Table 2. Results of antimicrobial combinations of the 50 MRSA isolates done by checkerboard method.

Antibiotic combination	Synergism	Indifference	Antagonism
	no (%)	no (%)	no (%)
Vancomycin and Imipenem	38(76%)	12(24%)	0
Vancomycin and piperacillin-tazobactam	33(66%)	17(34%)	0
Vancomycin and Cefazolin	26(52%)	24(48%)	0
Vancomycin and Cefepime	27(54%)	23(46%)	0

Table 3. Correlation between fractional indices of vancomycin combined with various antibiotics and vancomycin MIC.

Antibiotic combination	Correlation coefficient# (r)	*P* value
Vancomycin and Imipenem	-0.296	0.037*
Vancomycin and piperacillin-tazobactam	-0.195	0.450
Vancomycin and Cefazolin	0.076	0.601
Vancomycin and Cefepime	-0.140	0.333

Correlation coefficient for spearman correlation
*significant at 5% level of significance

Figure 1. The checkerboard format used in the study to test in vitro synergy of vancomycin (VAN) and imipenem (IPM): VAN dilutions were distributed horizontally in rows from (1-10) while IPM dilutions were distributed vertically in columns from (A-H).

	1 256 **µg/ml**	2 128 **µg/ml**	3 64 **µg/ml**	4 32 **µg/ml**	5 16 **µg/ml**	6 8 **µg/ml**	7 4 µg/ml	8 2 **µg/ml**	9 1 **µg/ml**	10 0.5 **µg/ml**	11 VAN	12 IPM
A 32 **µg/ml**												
B 16 **µg/ml**												
C 8 **µg/ml**												
D 4 **µg/ml**												
E 2 **µg/ml**												
F 1 **µg/ml**												
G 0.5 **µg/ml**												
H 0.25 **µg/ml**											Sterility Control	Sterility Control

Figure 2. Correlation of fractional index of vancomycin combined with imipenem in MRSA isolates with vancomycin MICs by the broth microdilution method, by linear regression analysis.

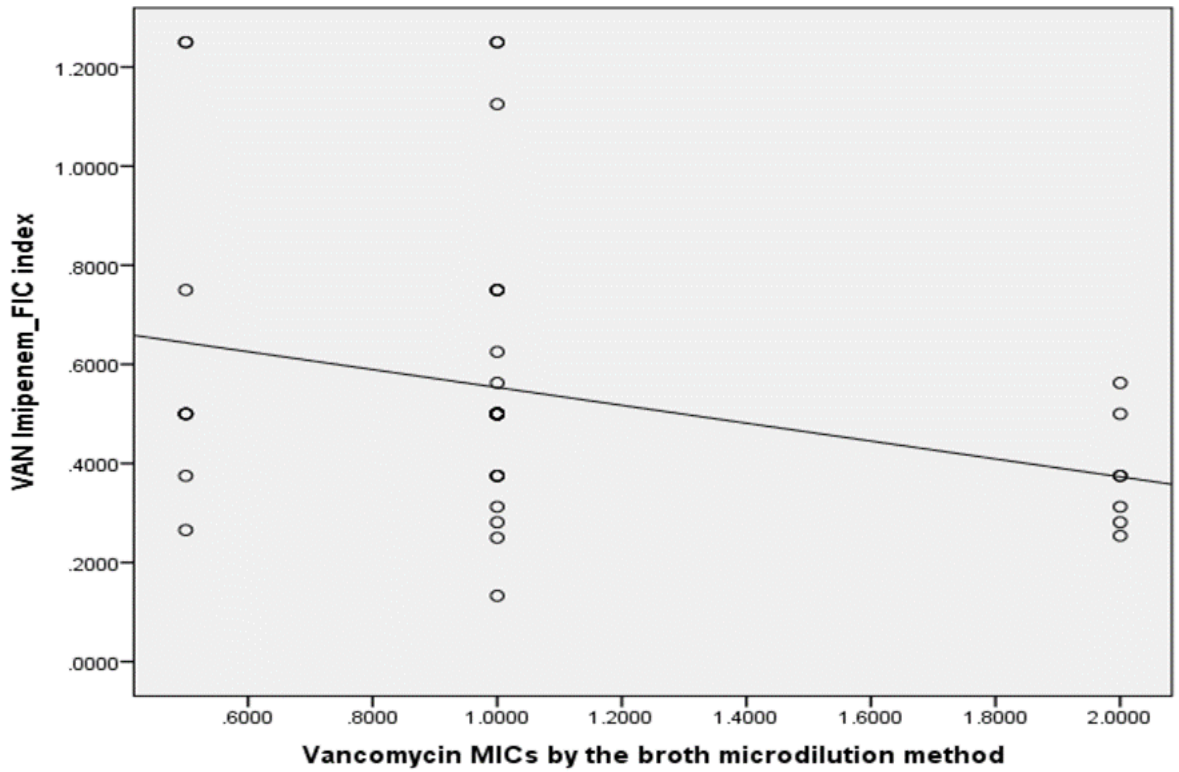


Figure 3. Correlation of fractional index of vancomycin combined with piperacillin-tazobactam in MRSA isolates with vancomycin MICs by the broth microdilution method, by linear regression analysis.

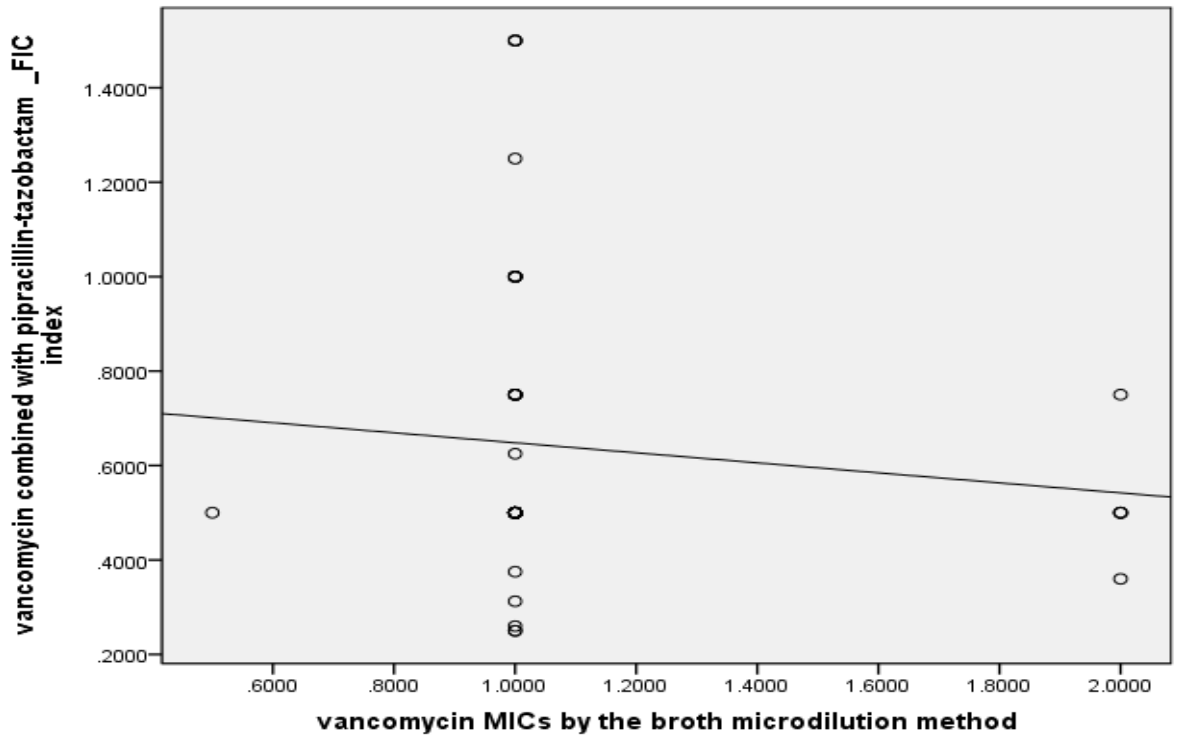


Figure 4. Correlation of fractional index of vancomycin combined with cefepime in MRSA isolates with vancomycin MICs by broth microdilution method, by linear regression analysis.

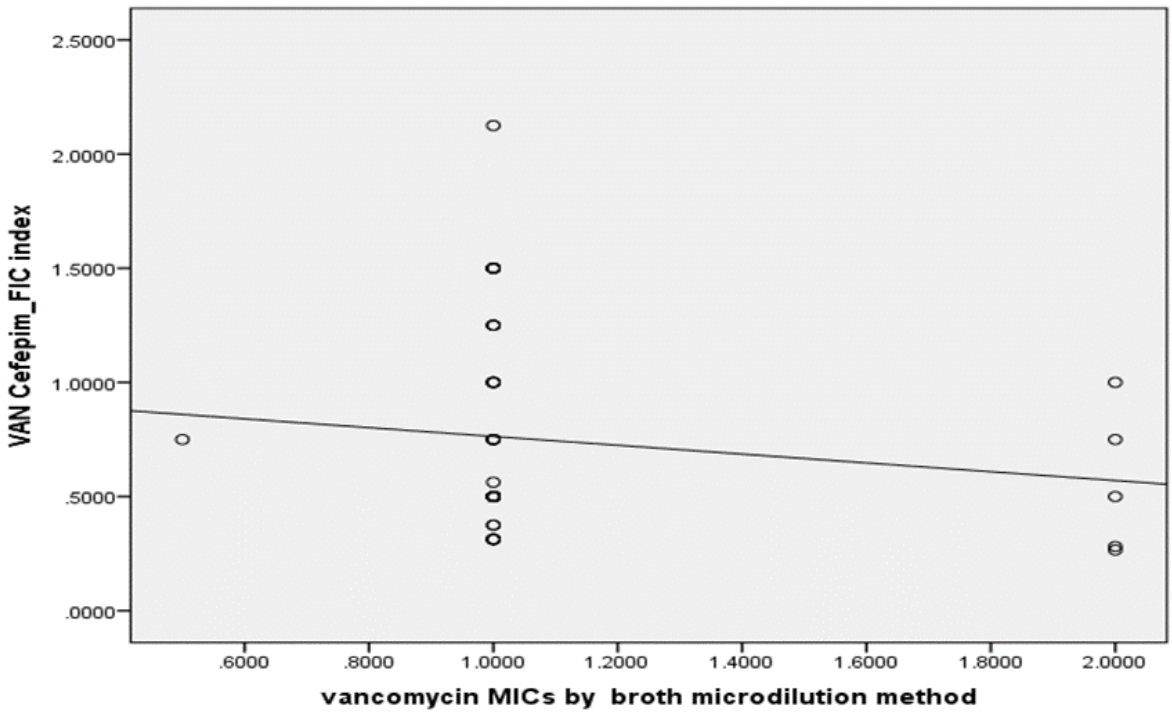
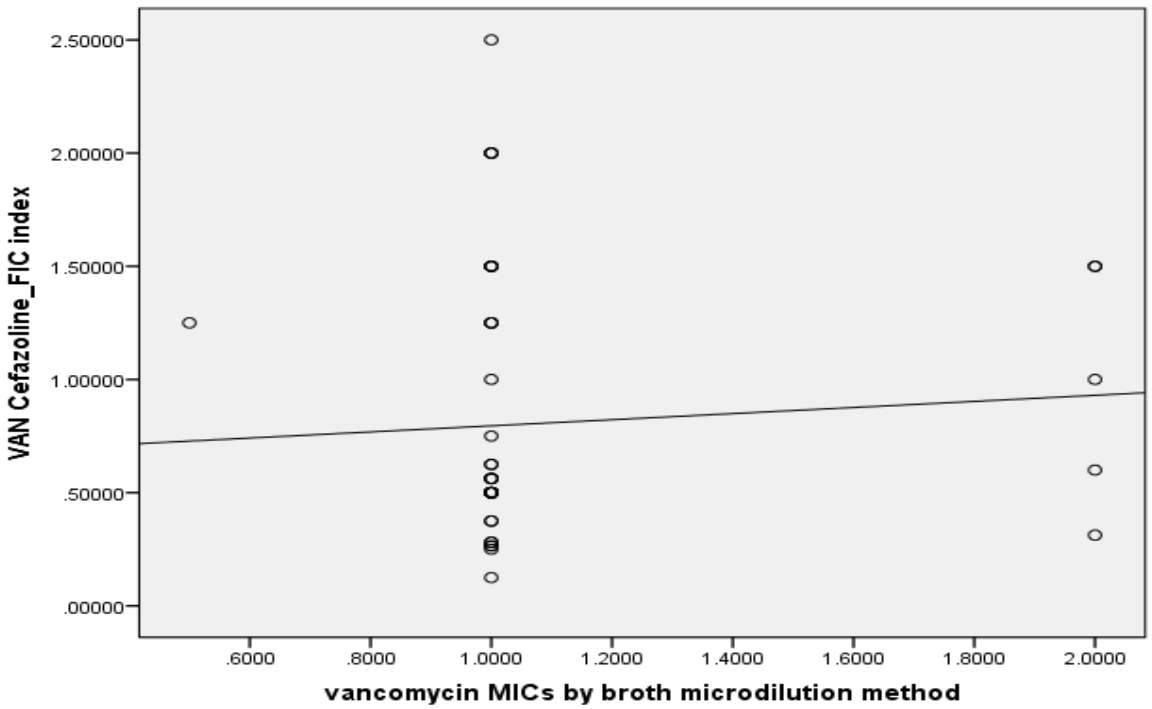


Figure 5. Correlation of fractional index of vancomycin combined with cefazolin MRSA isolates with vancomycin MICs by broth microdilution method, by linear regression analysis.



Discussion

Staphylococcus aureus is a major cause of bacteremia. Typically, the mortality of SAB increases 20% to 25% if the bacteremia was caused by MRSA rather than methicillin-susceptible *S. aureus.* Alongside mortality, MRSA bacteremia is accompanied by morbidity, increased costs in addition to inferior treatment outcomes [24].

The current standard therapy for both complicated and uncomplicated MRSA bacteremia is still vancomycin despite its numerous flaws including; deficient tissue perfusion, slow killing time, inactivity against biofilm formers, no interference with toxin production, inconvenient administration and number of side effects [19]. In recent years, there is an increasing evidence proposing the addition of a β-lactam to the standard therapy in order to improve MRSA bacteremia outcomes. This evidence is supported by in-vitro laboratory results that typically demonstrate presence of synergism between vancomycin and β-lactams against MRSA strains [24].

Since 1959, the year of introduction of methicillin to the market, there is a progressive increase in the prevalence of MRSA. In 2005, Styers et al reported the prevalence of MRSA as high up to 60% in certain centers in the United States, but great geographic variations exist worldwide [25,26].

In the present study, incidence of MRSA among *S.aureus* isolated from blood culture was (75.7%), which is near the reported from Cyprus, Italy, Portugal and Romania, which was around 60% [25,27,28]. There is a constant rise in the prevalence of MRSA globally, but the frequency of MRSA in European nations is generally lower than other regions. This can be attributed to rigid infection control practices as well as strict implementation of antibimicrobial prescribing policies in these countries [27,28].

In the present study, the BD Phoenix Automated Microbiology System was used to determine the MIC of various antimicrobial agents against the studied 50 MRSA isolates. All the tested isolates were fully resistant to cefazolin, cefoxitin , ampicillin , penicillin , oxacillin , amoxycillin-clavulanate which is a finding in all MRSA strains by definition. Very high resistance rate was detected to gentamicin (80% of isolates), followed by erythromycin (68%). Resistance to quinolone was highest for ciprofloxacin (64%) followed by norfloxacin (52%) and least resistance was against moxifloxacin (36%) which can explained by the fact that moxifloxacin is the most recent of these agents to be introduced into the market in Egypt. This resistance pattern resembles that obtained from Pakistan, India, Sri lanka and other studies from Egypt [28-31].

In this study, resistance to trimethoprim-sulphamethoxazole (SXT) was detected in (46%) of the isolates, rifampin resistance was detected in (34%), while clindamycin resistance in the current study was detected in 24% , these resistance rates are higher than Poland, Turkey, Italy and the USA [29,32]. On the contrary, only 8% of the isolates demonstrated resistance to chloramphenicol, this low resistance rate can be explained by the fact that physicians now refrain from using chloramphenicol due to its numerous side effects.

Of importance to note that the susceptibility to linezolid was observed in (94%) of the studied MRSA isolates while resistance was observed in 6%. Teicoplanin was susceptible in (94%) of our isolates, intermediate susceptibility and resistance were noticed in (4%) and (2%) respectively. These findings were in agreement with the results from India and Pakistan [25,28,30].

Empirical treatment decisions in MRSA bacteremia necessitate knowledge of the local strains and their resistance profile, , existence of co-morbidities, concomitant interventions, risk factors for a complicated clinical course and response to prior antimicrobials. In 1997 in Japan, the initial *S. aureus* isolate intermediate-resistant to vancomycin (VISA) was discovered. The VISA isolates were all MRSA [33]. While in 2002 in the United States, the first documented case of infection caused by vancomycin-resistant *S. aureus* (VRSA) (MIC > or = 32 µg/mL) [33]. Fortunately, all tested MRSA isolates in our research demonstrated no resistance to vancomycin or daptomycin. Thus, measuring the MIC for vancomycin as a routine to screen out VISA and VRSA is of utmost importance for early detection of these strains as they start to appear elsewhere [28,29,31,34].

A conflict was raised regarding failure of treatment for cases of MRSA bacteremia with vancomycin MICs >1.5 µg/mL. MRSA strains exhibiting elevated vancomycin MICs even within the susceptible range (i.e, 2.0 µg/mL) and hetero-resistant strains are considered a risk factor contributing to vancomycin treatment failure. One of the solutions to this problem can be the co-administration of anti-MRSA antimicrobials to

provide coverage for the recalcitrant mutants [1,16,19].

In the present study, all the tested MRSA isolates were proven to be fully susceptible to vancomycin with MIC value ranging from less than 0.5 to 2 µg/ml by using both, BD Phoenix automated microbiological system and broth microdilution method.

To demonstrate presence or absence of synergy between vancomycin and (imipenem, piperacillin-tazobactam, cefepime and cefazolin), we used broth microdilution checkerboard method [22]. In our study, the MIC for each tested antibiotic alone was: vancomycin ranged from 0.5 to 2 µg/ml, imipenem from 0.25 to >16 µg/ml, TZP from 8 to >64 µg/ml, cefazolin from 4 to >64 µg/ml and cefepime from 2 to >64 µg/ml.

Regarding the effect of combinations of two antimicrobial agents (vancomycin with a β-lactam), synergism was observed in all combinations but with various proportions. The highest was recorded between vancomycin and imipenem (76%), followed by piperacillin-tazobactam, cefepime and cefazolin 66%, 54% and 52% respectively. No antagonism was detected against any tested isolates, indifference was observed in 24%, 34%, 46% and 48% of the isolates to the previous combinations respectively.

In the current study, combination of vancomycin and imipenem by checkerboard method result in synergism in (76%) of MRSA isolates. This result is slightly higher than study by Rochon-Edouard et al in France (69%), but is lower than a study done in the USA (92%). The combination of vancomycin with imipenem was also evaluated by E test that showed (92%) synergistic effects against MRSA in a Brazilian study [19,35,36].

Synergy between vancomycin and piperacillin-tazobactam was observed in (66%) of the MRSA isolates in our study. In study done by Dilworth et al in 2014, synergism between vancomycin and piperacillin-tazobactam was demonstrated using time-kill studies [34].

Synergy between vancomycin with cefepime was observed in (54%) of the MRSA isolates in our study. According to **Lozniewski et al.** in France, by using also the checkerboard, synergism was recorded in (38.8%) of the studied MRSA isolates between vancomycin and cefepime. All the studied isolates in their research were fully susceptible to vancomycin with MIC values ranging from 0.5 to 2 µg/ml while cefepime MIC range was from 4 to 128 µg/ml [19,37].

In this study, combination of vancomycin and cefazolin showed synergism in (52%) of isolates which was lower than **Rochon-Edouard** study (69%), and studies from Taiwan in (60%) [18,35].

In the current study by using linear regression analysis, a significant inverse correlation was observed between; the mean FIC indices of vancomycin combined with imipenem and the MICs of vancomycin. This indicates that higher levels of vancomycin MICs were associated with increase in synergy between vancomycin and imipenem against the MRSA isolates. This observation was also noticed by other researchers who related the augmented synergy to the "seesaw effect" which is an event of increased susceptibility to beta-lactams with reduced susceptibility to vancomycin [38,39].

No significant correlation was found between MIC of vancomycin & FIC indices for vancomycin with piperacillin-tazobactam, vancomycin with cefepime and vancomycin with cefazolin.

Conclusion

Reports of multidrug-resistant MRSA from all over the globe as well as appearance of vancomycin non-susceptible isolates is an alarming trend. Thus, measuring the MIC for vancomycin as a routine to screen out VISA and VRSA is of utmost importance for early detection of these strains .

The addition of a β-lactam antibiotic alongside the standard therapy for MRSA has shown in-vitro promising outcome, in our study we demonstrated the synergistic activity of vancomycin in combination with β- lactam antibiotics which offer new insights in treatment options of serious MRSA infections. However, adequately randomized clinical trials of this intervention need to be conducted on large scale.

Conflict of interest : There is no conflict of interest.

Financial disclosure: Nothing to declare.

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