

In vitro activity of linezolid alone and in combination with glycopeptides against methicillin-resistant *Staphylococcus aureus*

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The emerging resistance to antibiotics and the poor pipeline of new antibacterials is creating a major health issue worldwide. Combinations of two antibiotics or antibiotics with adjuvant are emerging as a promising therapeutic approach. *Staphylococcus aureus* represents a major threat to a broad range of healthcare and community associated infections. It is imperative to develop novel antimicrobial strategies to enrich the currently shrinking therapeutic options against *Staphylococcus aureus*. In this study, we had assessed the *in vitro* activities of linezolid alone and in combination with vancomycin or teicoplanin against 54 methicillin-resistant *Staphylococcus aureus* (MRSA) strains using agar dilution method in conjunction with scanning electron microscopy. MIC₅₀ of linezolid is 2µg/ml while those of vancomycin and teicoplanin are 8µg/ml and 16µg/ml respectively. The percentage of resistant isolates to linezolid, vancomycin and teicoplanin was 5%, 9.2% and 14.8% respectively. So this study reveals the superiority of linezolid as a single agent over vancomycin and teicoplanin against MRSA isolates. The combination of linezolid/vancomycin showed better results than linezolid/teicoplanin among all concentrations. Linezolid/vancomycin and linezolid/teicoplanin acted synergistically against 78% and 50% of the MRSA isolates respectively. There was an increase in the percentage of inhibition of the tested strains due to linezolid/glycopeptide combination compared to each drug alone. There was visible cell destruction using scanning electron microscope with either vancomycin or teicoplanin combination with linezolid compared to using a single antibiotic. Synergyism may be promising for more effective chemotherapy, particularly against MRSA strains. However, testing of the combinations in animal models or in actual clinical situations is warranted.

Keywords: Oxazolidinones, linezolid, vancomycin, teicoplanin, scanning electron microscopy.

INTRODUCTION

The prevalence of bacterial pathogens resistant to the available antibiotics has increased over the past decades. The large number of infections caused by Gram-positive bacteria that are resistant to many commonly used antibiotics constitutes a major challenge for clinicians and microbiologists. *Staphylococcus aureus*

(*S. aureus*) remains a major nosocomial pathogen in human infections and the recent appearance of MRSA strains with reduced susceptibility to glycopeptides has emphasized the need for new therapeutic options for clinical practice (Sakoulas and Moellering, 2008).

The first MRSA strain was reported in 1961. Since then, both the prevalence and resistance profile of MRSA strains have increased dramatically to the point that most strains are now multi-resistant and represent a major problem for health care providers (Palazzo *et al.*, 2005).

Currently, the glycopeptide group of antimicrobials represents important therapeutic agents for the treatment

of serious MRSA sepsis. However, the acquisition of glycopeptide resistance by MRSA has been widely anticipated ever since the emergence of vancomycin-resistant strains (Venubabu *et al.*, 2011). Such infections require a bactericidal therapy, which is usually obtained with a synergistic combination of a cell wall active agent (such as vancomycin), plus an oxazolidinone (Amod *et al.*, 2005).⁽⁴⁾

Vancomycin and teicoplanin are glycopeptides antibiotics used to treat MRSA infections. Teicoplanin is a structural congener of vancomycin that has a similar activity spectrum but a longer half life (Rybak *et al.*, 1991). Glycopeptides act by binding to the terminal alanyl-d-alanine of nascent peptidoglycan chains and thus interfering with bacterial cell wall biosynthesis and resulting in cell death (Miles and Amyes, 2007).

Recent studies demonstrate increased clinical failure of vancomycin therapy in MRSA infections in which the isolates have increased MICs but are still susceptible (Sakoulas *et al.*, 2004; Moise *et al.*, 2007; Moise *et al.*, 2008). vancomycin was <10% successful in the treatment of bacteremia caused by MRSA, with vancomycin MICs of 1–2 µg/mL, compared with 56% successful when the vancomycin MIC was <0.5 µg/mL (Sakoulas *et al.*, 2004). This association of decreased *in vitro* killing with decreased clinical efficacy of vancomycin in the treatment of MRSA bacteremia has been confirmed (Moise *et al.*, 2007). Moreover, Many clinicians believe that the efficacy of vancomycin against MRSA is inferior to that of anti-staphylococcal beta-lactam antibiotics against MSSA (Falagas *et al.*, 2008, Siegman *et al.*, 2005). Furthermore, the oral absorption of vancomycin and teicoplanin is very low so they must be administered intravenously to control systemic infections, which is not the preferred route of administration for many patients (Andre *et al.*, 2010).

Oxazolidinones are a novel class of synthetic antimicrobials with potent activity on Gram-positive pathogens such as enterococci (including vancomycin-resistant enterococci (VRE)), *streptococcus pneumoniae*, coagulase-negative staphylococci and *S. aureus*, including MRSA. Linezolid, the first drug issued from this class, is active against Gram-positive bacteria and displays bacteriostatic, time-dependent activity *in vitro* on staphylococci (Moellering, 2003). Linezolid is ribosome-targeted compound. It inhibits bacterial protein synthesis through a mechanism of action different from that of other antimicrobial agents. This drug binds to a site on the bacterial 23S ribosomal RNA of the 50S subunit and prevents the formation of a functional 70S initiation complex. Linezolid is active upstream from other ribosome-targeted antibiotics at a very early stage in the bacterial translation process (Livemore, 2003). This mechanism of action is specific to this class, and no cross-resistance with other antimicrobial agents has been observed. Linezolid has been approved by US and European agencies and is indicated for the treatment of

infections caused by Gram-positive pathogens in adult patients. Since linezolid became available in 2000, clinical isolates of VRE and MRSA resistant to linezolid have been reported from treated patients (Meka and Gold, 2004, Wilson *et al.*, 2003). In clinical settings, linezolid may be combined with other antimicrobial agents in order to increase the bactericidal activity of therapy, prevent the emergence of drug-resistant subpopulations and provide a complementary antibacterial spectrum (Cedric *et al.*, 2003).

Antimicrobial combination therapy may be used to provide broad-spectrum coverage, prevent the emergence of resistant mutants and obtain a synergy between both antimicrobial agents. Because bactericidal activity is considered important in the treatment of severe infections, the use of linezolid as monotherapy appears to be problematic and the use of combinations is recommended (Tsiodras *et al.*, 2001).

Although few reports have discussed the *in vitro* activity of combinations including linezolid (Grif *et al.*, 2001, Allen *et al.*, 2002), some investigators have reported synergistic bacteriostatic and bactericidal effects of different oxazolidinones combined with glycopeptides against MRSA strains including glycopeptide-intermediate *S. aureus* (GISA) isolates (Sweeney and Zurenko, 2003; Levita *et al.*, 2006; Victor Iorian, 2005). On the other hand, some investigators have reported mainly antagonistic activity of linezolid combined with different antimicrobials tested against MRSA (Shveta *et al.*, 2009).

Further studies are needed to assess the activity of combinations including linezolid against resistant Gram-positive bacteria. The present work investigated the *in vitro* activity of linezolid alone and in combination with vancomycin or teicoplanin in order to determine the most active therapy against MRSA strains. In addition, scanning electron microscopy was performed to compare bacterial morphological alterations owing to the different combinations.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Fifty four MRSA isolates were collected from October 2010 to June 2011 from various clinical specimens including pus, urine, wound swabs, catheters, blood, sputum and CSF from patients of different inpatient and outpatient departments of different hospitals. The identification of isolates was done according to standard methods described by the Clinical Laboratory Standards Institute (CLSI, 2011). All staphylococcal isolates were inoculated onto mannitol salt agar (Oxoid) and plates were incubated at 37°C for 24–48h. Mannitol fermentation was observed and recorded. The identifi-

cation was confirmed by biochemical tests and coagulase test.

Confirmation of MRSA strains

This was carried out according to CLSI guidelines using BBL™ Oxacillin agar screen test (Müller Hinton Agar with 6 µg/mL Oxacillin and 4 % NaCl) whereby all *S. aureus* isolates were spot inoculated from a 0.5 McFarland standard suspension. The plates were incubated at 37°C for 24h. If any growth (more than one colony) was detected, the isolate was considered oxacillin or methicillin resistant (CLSI, 2011, Derek *et al.*, 2005).

Antimicrobial agents

Laboratory powders were supplied as follows: linezolid (Pharmacia Upjohn, Kalamazoo, MI, USA), vancomycin (Lilly, Saint Cloud, France), and teicoplanin (Aventis, Paris, France). Stock solutions of each antibiotic were freshly prepared at the beginning of each week and kept at 4°C.

Determination of MIC

MICs of linezolid, vancomycin and teicoplanin was performed in cation-adjusted Mueller–Hinton broth (MHB) by means of microdilution broth method (microwell method) (CLSI, 2011).

Evaluation of synergy

MICs of each antibiotic alone or in combination were determined by agar dilution technique in accordance to CLSI standards (CLSI, 2011). Inoculum of 10^6 cfu/ml was used. The initial concentrations were 16 µg/ml for linezolid, vancomycin and teicoplanin. The inoculated plates were incubated at 37 °C for 24h. The plates were visually inspected for any visible growth. The least antimicrobial concentration showing no growth was considered as the MIC of this antimicrobial. All MICs were performed in duplicate and the average was recorded.

The fractional inhibitory concentration (FIC) was calculated as follows: FIC of drug A (FIC) = MIC of drug A in combination/MIC of drug A alone, FIC of drug B (FIC) = MIC of drug B in combination/MIC of drug B alone, FIC of drug C (FIC) = MIC of drug C in combination/MIC of drug C alone. FIC Index (FIC_i), was calculated as the sum of each FIC, was interpreted as follows: FIC_i ≤ 0.5, synergism; FIC_i >0.5 < 1, partial synergism; FIC_i=1; additive; FIC_i >1 ≤ 4, indifferent reaction and FIC_i > 4.0,

antagonism (Lorenzo *et al.*, 2007).

Scanning electron microscopy

Bacteria (overnight bacterial culture diluted to obtain 10^7 cfu/ml) were cultured for 24h in MHB containing either of the three antibiotics alone (2X MIC) or linezolid in combination with vancomycin or teicoplanin (2X MIC). Primary fixation of samples was done by buffered Glutaraldehyde 2.5 % over night in refrigerator, washed by phosphate buffer (pH = 7.2) and centrifuged (3000 g, 15 min, 4 °C). Secondary fixation was done by buffered Osmium Tetroxide 1 % for one hour, then dehydration by series concentration of ethanol, embedding by resin mixture from SPI (SPI-Pon™ - Araldite® Epoxy Embedding Kit). The bacterial pellets were mounted on membrane filters (Anodisc; Whatman International Ltd, Maidstone, UK). Before examination under a scanning electron microscope (SEM) (JEOL, JSM-6060 LV), specimens were coated with 100 Å of a gold–palladium mix in an ion sputter (JEOL JFC 1100) (Stadtlander, 2006).

Spectrophotometric analysis

Spectrophotometric analysis of the antibacterial under test was done to test if there is any chemical interaction between linezolid and either of the glycopeptides tested. The spectrophotometric measures were performed using a model 320UV–Vis Perkin–Elmer double beam, double grating monochromator spectrophotometer. Samples of the drugs tested (16 µg/ml) were compared with samples of linezolid/teicoplanin or linezolid/vancomycin (16 µg/ml) and the peaks were analysed at a range of 200–400 nm.

RESULTS

The minimal concentrations at which 50 % (MIC₅₀) and 90 % (MIC₉₀) of the isolates were inhibited, MIC ranges of the three antibiotics and percentages of resistant isolates are shown in Table 1. The MIC ranges of the 54 isolates of MRSA were 2–16 µg/ml, 8–512 µg/ml and 4–512 µg/ml for linezolid, vancomycin and teicoplanin respectively. The MIC₅₀ of linezolid was 2 µg/ml less than its breakpoint (> 4 µg/ml). MIC₅₀ of vancomycin and teichoplanin were 8 µg/ml and 16 µg/ml respectively, less than their resistant breakpoints (≥ 16 µg/ml and ≥ 32 µg/ml respectively), and more than sensitive breakpoints (≤ 2 µg/ml and ≤ 8 µg/ml respectively) (Intermediate resistance). The percentage of resistant isolates against linezolid, vancomycin and teicoplanin was 5.5 %, 9.2 % and 14.8 % respectively. MIC₉₀ of all tested antibiotics were more than their break points (Rybak, 2009, CLSI,

Table 1. Biostatic activity and percentage of resistance to different antibiotics against 54 MRSA isolates

Antibiotic	MIC ($\mu\text{g/ml}$)			^c Species-related breakpoints		No. of resistant isolates (%)
	^a MIC ₅₀	^b MIC ₉₀	MIC range	R	S	
Linezolid	2	16	2-16	> 4	≤ 4	3 (5.5)
Vancomycin	8	16	8-512	≥ 16	≤ 2	5 (9.2)
Teicoplanin	16	32	4-512	≥ 32	≤ 8	8 (14.8)

^a The minimal concentration at which 50% of the isolates was inhibited^b The minimal concentration at which 90% of the isolates was inhibited^c CLSI, 2007**Table 2.** Fractional inhibitory concentration (FIC) indices of linezolid/glycopeptide combinations against MRSA isolates

Combined antibiotics tested	FIC indices				
	Synergistic reaction (FIC ≤ 0.5) (%)	Partial synergism (FIC > 0.5, < 1) (%)	Additive reaction (FIC = 1) (%)	Indifferent reaction (FIC > 1, ≤ 4) (%)	Antagonistic reaction (FIC > 4) (%)
Linezolid/Vancomycin	0.0625 -0.5 (78%)	0.5625 (6%)	— (0.00%)	— (0.00%)	4.06-8.25 (16%)
Linezolid/Teicoplanin	0.0625-0.5 (50%)	0.531-0.562 (11%)	1.0 (6%)	1.5-3 (11%)	8.5-9 (22%)

2007). Linezolid was the most active compared to the other tested antibiotics; all the isolates were inhibited by MIC value $\geq 16 \mu\text{g/ml}$. No differences were observed between assays performed on different days.

Table 2 shows fractional inhibitory concentration (FIC) indices of linezolid/glycopeptides combinations against MRSA isolates, which were determined by agar dilution assay. None of the combinations tested showed total synergy. The majority of linezolid/vancomycin combination was synergistic (78 %). Linezolid/vancomycin showed partial synergy against 6 % of the isolates (3/54) and were antagonistic against 16 % of the isolates (9/54). On the other hand, linezolid/teicoplanin combination showed synergy effect against 50 % of the isolates tested (27/54), partial synergy as well as indifferent action against 11 % of the isolates (6/54) for each, additive effect against 6 % of the isolates (3/54) and antagonistic action against 22 % of the isolates (12/54).

Figures 1 and 2 show the results of linezolid/vancomycin and linezolid/teicoplanin combinations at different concentrations of each antibiotic

respectively. The concentration of the tested antimicrobial agents were subinhibitory. Linezolid/vancomycin combination results are better than linezolid/teicoplanin combination among all concentrations tested. There was absolutely no sensitive bacteria when linezolid was used alone in a concentration range 0.25 – 1 $\mu\text{g/ml}$ against all the isolates tested While 50 % of MRSA strains were inhibited at 2 $\mu\text{g/ml}$ linezolid (Figure 1 and 2). Moreover, neither vancomycin nor teicoplanin showed any inhibitory effect when used as a single agent at a concentration range of (0.25-4 $\mu\text{g/ml}$). Only 5.6% inhibition occurred when teicoplanin was used solely in a concentration of 8 $\mu\text{g/ml}$ (Figure 2).

When low concentration (0.25 $\mu\text{g/ml}$) of vancomycin was added to 2 $\mu\text{g/ml}$ of linezolid, there was a potent synergistic effect that inhibited more than 75 % of MRSA growth. Increasing the concentration of vancomycin did not increase the sensitivity of the strains tested. On the other hand, the use of high concentration of vancomycin (2-4 $\mu\text{g/ml}$) in combination with low concentration of linezolid (0.25 $\mu\text{g/ml}$) did not reach the same potent synergistic effect since it only increased the percentage

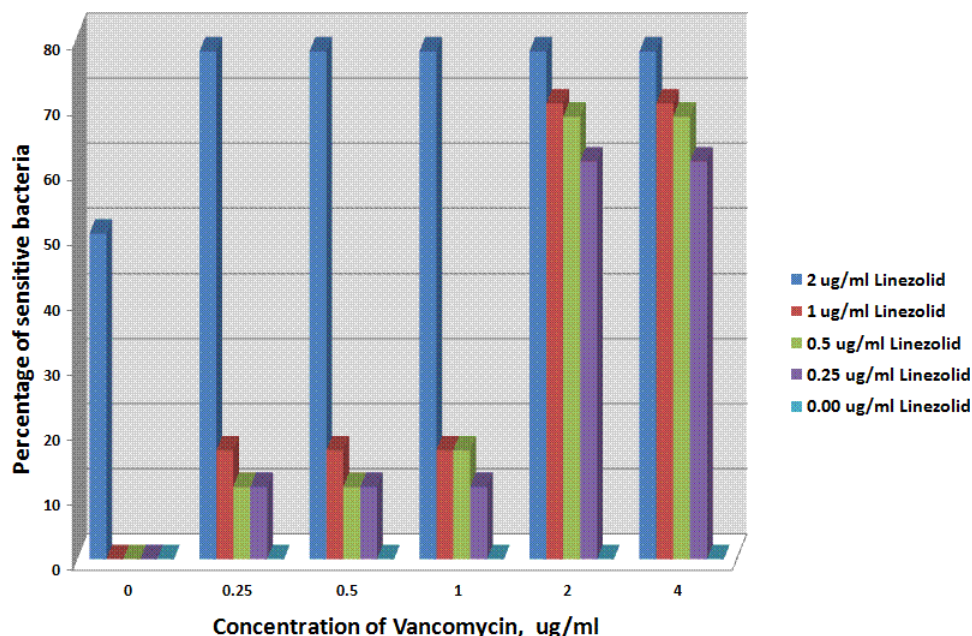


Figure 1. Percentage of inhibitory effect of linezolid/vancomycin combination at different subinhibitory concentrations of each antibiotic

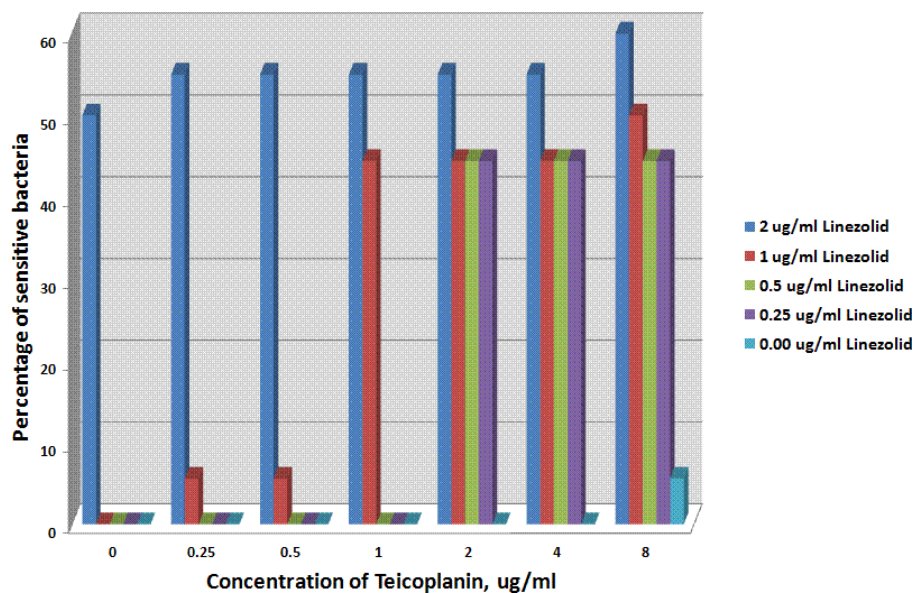


Figure 2. Percentage of inhibitory effect of linezolid/teicoplanin combination at different subinhibitory concentrations of each antibiotic.

of sensitive strains by 15% compared to using linezolid alone. Teicoplanin/linezolid combination did not show the same synergistic effect compared to linezolid/vancomycin combination (Figure 2). About 55 % of the isolates were inhibited by adding 0.25 µg/ml teicoplanin to 2 µg/ml linezolid. By combining less

concentration of linezolid (0.25-1µg/ml) to high concentration of teicoplanin (2-4 µg/ml), considerable inhibition of 45 % was observed compared to using each drug alone at this same concentrations (0% inhibition) (Figure 2).

MRSA strains with linezolid alone or combined with

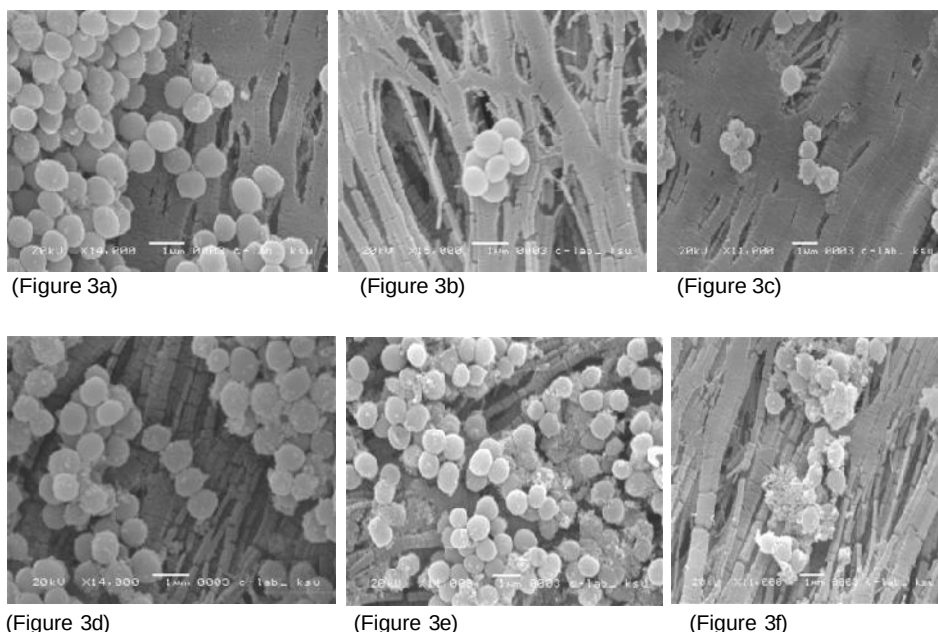


Figure 3. Scanning electron micrographs of MRSA strains. (3a) Untreated MRSA, (3b) Linezolid alone, (3c) Teicoplanin alone, (3d) Vancomycin alone, (3e) Linezolid/teicoplanin combination, (3f) Linezolid/vancomycin combination.

vancomycin or teicoplanin were photographed by electron microscopy to compare morphological alterations (Figure 3). For each combination, the most representative photograph was chosen even if morphologically normal organisms were also observed. The scanning electron microscope appearance of untreated MRSA is shown in figure 3a. The bacteria were roughly spherical and smooth. Linezolid alone (2X MIC) exhibited moderate alterations on all strains studied. No cell destruction was observed, but only abnormal forms were visible (Figure 3b). However, teicoplanin (2X MIC) had an effect on the morphological structure of the cell, probably because of alteration of the cell wall and inhibition of cell division (Figure 3c). Vancomycin (2X MIC) had a profound effect on the morphological structure of bacteria and bacterial lysis was observed for most of the cells (Figure 3d). The combination of linezolid/teicoplanin agents showed abnormal forms with separation of the central septum and bacterial lysis was also observed (Figure 3e). Similar effect but more aggressive was observed in the combination of linezolid/vancomycin agents where bacterial cells were completely independent though joined (Figure 3f).

The spectrophotometric analysis of linezolid, vancomycin and teicoplanin and their combination revealed that linezolid had two λ_{max} at 250 and 210nm, while vancomycin and teicoplanin had one λ_{max} at 210nm and 200nm respectively. The combination of

linezolid and vancomycin or teicoplanin showed no disappearance of any of the peaks. Moreover, there was almost no change in the absorbance of the peaks appeared due to the combination. This clearly indicates that there is no chemical interaction between any of the drugs tested (Figure 4).

DISCUSSION

In the face of mounting antimicrobial resistance, the need to evaluate new therapeutic options against MRSA exist (Brian and Michael, 2006). Combination therapy, with the goal to enhance the antibacterial activity of known and effective antibiotics, cannot only enhance the activity of known antibiotics but also can possibly support the clinical development of agents previously found to be very effective but too toxic for the host. Another advantage is that this approach might lead to shorter and/or lower dosing regimens, which has the potential to reduce the rate of acquirement of resistance in pathogens (Owens and Ambrose, 2005).

In the present study, we examined the potential effectiveness of two antimicrobial combinations against 54 MRSA isolates which display heterogeneous resistance to vancomycin and teicoplanin by performing agar dilution assay. Linezolid, a new drug with potent activity on Gram-positive pathogens such as MRSA, was

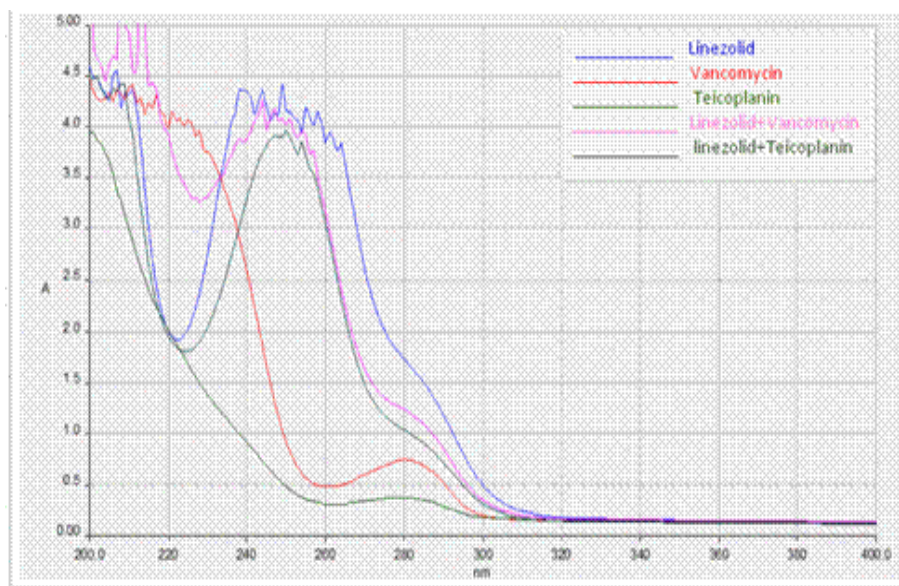


Figure 4. Spectrophotometric analysis of linezolid, vancomycin and teicoplanin and their combination

highly effective with almost 95% susceptibility in our study and was also reported in 2005 from Poland (Matynia *et al.*, 2005) and in 2007 from Japan (Kohno *et al.*, 2007) to be fully susceptible.

Our results revealed the superiority of linezolid as single agent than vancomycin and teicoplanin against MRSA isolates which agrees with Fatima *et al* findings who found that among a total of 50 MRSA isolates vancomycin showed comparatively higher MICs than linezolid (Fatima *et al.*, 2011). Almost 9% and 15% of the isolates tested were resistant to vancomycin and teicoplanin respectively and their MIC was in the range of 32-512 µg/ml and 16-512 µg/ml respectively. These values are different than Venubabu *et al* who stated that out of 285 MRSA strains obtained from ICUs of tertiary care hospitals in Hyderabad, India, 7 (2.5%) were considered resistant to vancomycin and their MIC was in the range of 16-64 mg/l (Venubabu *et al.*, 2011).

The breakpoint of Linezolid is higher than its MIC₅₀ revealing that linezolid is more active than other antibiotics tested. This high activity of linezolid against MRSA is mentioned with reports from Rybak *et al* (Rybak *et al.*, 1999), Fines and Leclercq (Fines and Leclercq, 2000) and Samra *et al.* (Samra *et al.*, 2005) who demonstrated that linezolid has an excellent *in vitro* activity against MRSA.

The combination study emphasizes the synergistic effect between linezolid and glycopeptides. It revealed that, the majority of linezolid/vancomycin combination is more synergistic (78%) than linezolid/teicoplanin combination (50%). This has proven the advantages of

combination therapy, which includes broadening of spectrum of activity and synergistic killing of infecting pathogen (Sweeney and Zurenko, 2003). This synergy between glycopeptides, which are cell-wall active agents and the oxazolidinones, which acts intracellularly, probably results from enhancement of cellular uptake of the oxazolidinone after disruption of the cell wall by the glycopeptides (Jones *et al.*, 2003). The synergy achieved was related to concentrations of the glycopeptides and linezolid and this is an important factor for *in vitro* efficacy. Our results are in agreement with Sweeney and Zurenko, who found that different combinations of linezolid with 6 drugs (amoxicillin, erythromycin, imipenem, sparfloxacin, teicoplanin, and tetracycline) were synergistic (Sweeney and Zurenko, 2003). While Levita *et al.* proved that in a subgroup of patients (n=15) who received vancomycin in combination with linezolid, daptomycin, and/or rifampin for infections caused by high-MIC strains, the end-of-treatment response appeared more favorable when compared with vancomycin alone regardless of whether the target trough was achieved (Levita *et al.*, 2006). Another agreement with our results, Victor Lorian, stated that the combination of linezolid with vancomycin resulted in improved killing of MRSA from 0.37 to 2.28 log cfu/ml greater compared with the most active single agents (Victor Lorian, 2005). The underlying hypothesis is that, treatments that inhibit multiple targets (on the same pathway or not) might delay and decrease the pathogen's ability to accumulate simultaneous mutations that affect the multiple targets (Taylor, 2002). On the other hand, Shveta *et al.* found

that, no synergistic activity was seen when the linezolid and vancomycin were combined *in vitro*. Conversely, antagonistic activity occurred in three of five strains when linezolid was added to vancomycin (Shveta *et al.*, 2009). Also similar results are revealed by Cédric *et al* (Cédric *et al.*, 2003).

Many researchers compared the effect of linezolid to teicoplanin but there was no published data showing the effect of linezolid/teicoplanin combination. Our results showed the efficacy of their combination *in vitro* even in subclinical or subinhibitory concentrations of both drugs. Although linezolid/vancomycin combination showed more potent killing effect than linezolid/teicoplanin combination but still the latter combination is more effective than using each drug alone.

In clinical practice, antagonism is the most disadvantageous interaction possible with an antimicrobial combination. However, *in vivo* antagonism has rarely been documented in the literature as compared with the large number of reports of *in vitro* antagonism (Wilson *et al.*, 2003). Our study revealed that linezolid/vancomycin antagonistic effect was against 16% of the isolates while linezolid/teicoplanin antagonistic action was against 22% of the isolates.

Many researchers hypothesize that this antagonism may be due to a reduced ability on

the part of vancomycin to bind to cells exposed to linezolid, which is bacteriostatic and decreases protein synthesis (Cédric *et al.*, 2003). Others declared that the bactericidal activity of vancomycin could be partially inhibited by linezolid in a concentration-dependent manner since vancomycin is a cell wall synthesis inhibitor, which means that bacteria must be in the growth phase to be subject to its bactericidal activity. On the other hand, linezolid is a bacteriostatic agent, and its action on the ribosome inhibits bacterial growth (Shveta *et al.*, 2009). However, since the concentration of linezolid used in our research is subinhibitory it lacked the ability to inhibit bacterial growth and still showed significant synergy when combined with both vancomycin or teicoplanin. Again there was no published data obtained in the case of linezolid and teicoplanin but it follows the same mechanism of action as vancomycin (Rybak *et al.*, 1991).

The difference in morphological alteration was observed in electron microscopy when bacteria were treated by vancomycin or teicoplanin alone or in combination with linezolid. Thus, electron microscopy observations seemed to be well correlated with the results observed in this study. So Linezolid used in combination with vancomycin or teicoplanin was more effective than linezolid used as monotherapy.

We have shown that glycopeptides are synergistic *in vitro* against MRSA combined with linezolid. Synergism may be promising for more effective chemotherapy, particularly against MRSA. However, testing of the

combinations in animal models or in actual clinical situations is warranted. These results do not account for tissue penetration and metabolism which alter the *in vivo* activity of these agents when used in combination. There needs to be more data both *in vivo* and *in vitro* to demonstrate the interaction between these two agents.

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