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In Vitro Synergy of Telavancin and Rifampin Against *Enterococcus faecium* Resistant to Both Linezolid and Vancomycin

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ABSTRACT

Background: An emerging pathogen is *Enterococcus faecium* resistant to both linezolid and vancomycin (LRVRE). Antimicrobial combinations may be required for therapy and need to be evaluated. The combination of daptomycin and rifampin has demonstrated good in vitro activity against gram-positive bacteria, including *E faecium*. Telavancin, a newer lipoglycopeptide, has shown in vitro activity against *E faecium*. We evaluated the combination of telavancin and rifampin and compared the results to the combination of daptomycin and rifampin used previously on the same isolates.

Methods: Twenty-four genetically unique (by pulsed-field gel electrophoresis), clinical LRVRE isolates were collected in the United States from 2001-2004. Etest minimal inhibitory concentrations (MICs) (μg/mL) were 0.064-8 for telavancin, 1-4 for daptomycin, and 0.012 to >32 for rifampin. In vitro synergy testing was performed in triplicate by an Etest MIC:MIC ratio method, and summation fractional inhibitory concentration (ΣFIC) was calculated: synergy ≤0.5; indifference >0.5-4; and antagonism >4.

Results: The Etest method showed synergy (ΣFICs of 0.1-0.5) with telavancin + rifampin in 20/24 (83%) isolates and indifference (ΣFICs of 0.6-0.8) in 4/24 (17%) isolates. Similarly, the daptomycin + rifampin combination showed synergy (ΣFICs of 0.1-0.5) in 21/24 (88%) isolates and indifference (ΣFICs of 0.6-1.0) in 3/24 (12%) isolates by the Etest method. No antagonism was found.

Conclusions: In vitro synergy with both combinations (rifampin + telavancin or daptomycin) was 83% and 88%, respectively, by Etest against these LRVRE isolates. Although both daptomycin and telavancin in combination with rifampin showed a high incidence of synergistic activity, further in vitro synergy testing with this combination should be performed against additional *E faecium* isolates. In vitro synergy may or may not translate into in vivo effectiveness.

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INTRODUCTION

Vancomycin is one of the most widely used antibiotics in the United States for the treatment of serious gram-positive infections. Since the frequency of vancomycin-resistant organisms has increased, the use of vancomycin is being replaced by other antibiotics, such as linezolid, daptomycin, quinupristin/dalfopristin, and tigecycline.[1] One emerging pathogen is *Enterococcus faecium* resistant to both linezolid and vancomycin (LRVRE). Telavancin, a newer lipoglycopeptide, was recently approved for the treatment of complicated skin and skin structure infections caused by *Staphylococcus aureus*, streptococci, and vancomycin-susceptible *Enterococcus faecalis*.[2] In a study by Mendes et al,[3] telavancin showed good activity (minimal inhibitory concentrations [MICs] ≤1 μg/mL) against 27 (100%) strains of VanB phenotype (variable levels of inducible resistance to vancomycin only) *E faecium* but was less active (10.5% with MICs ≤1 μg/mL) against 392 VanA-type strains (inducible high-level resistance to vancomycin as well as to teicoplanin). Antimicrobial combinations may be required for therapy against these organisms and need to be evaluated. The combination of daptomycin and rifampin showed in

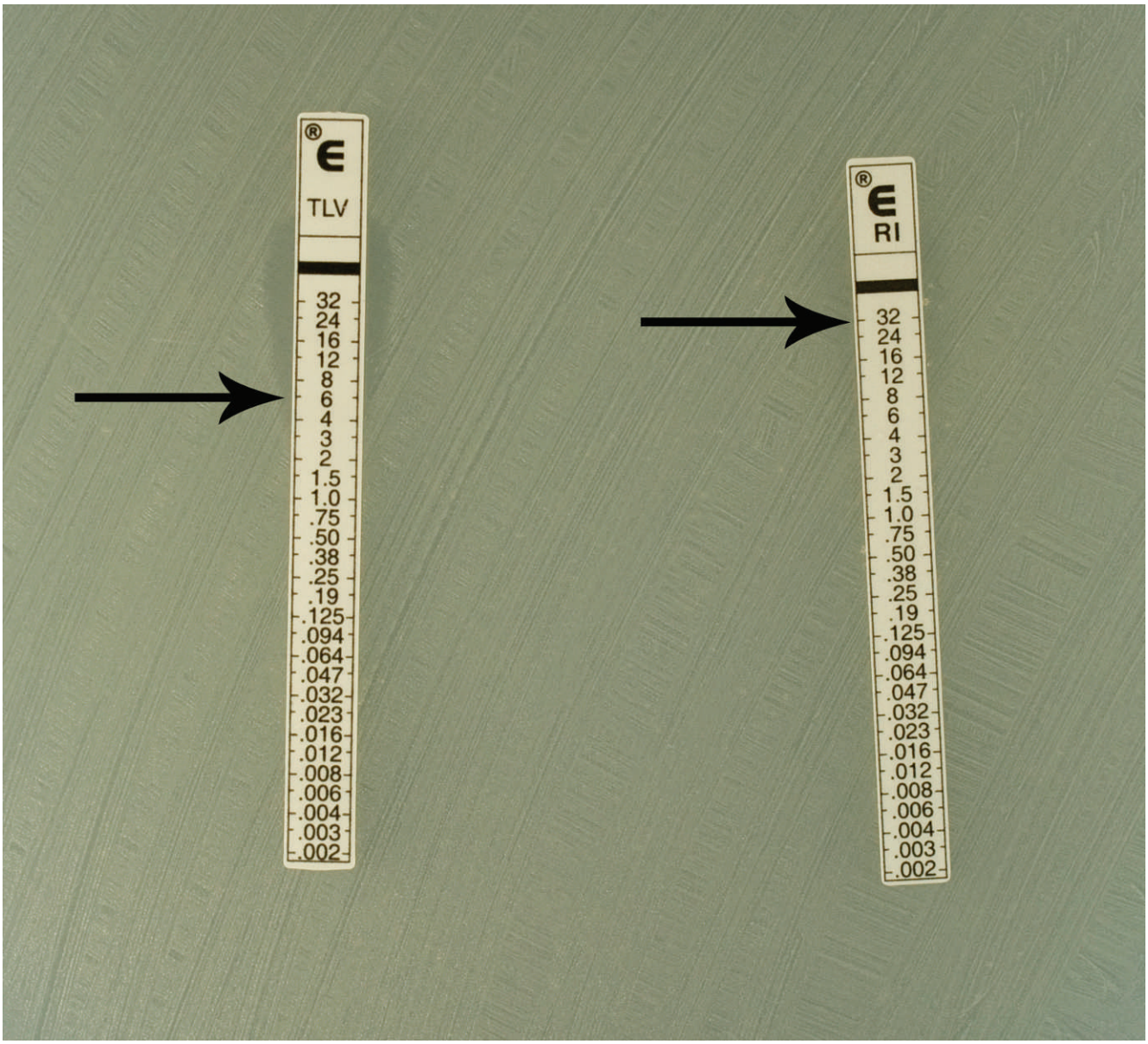

Figure 1. Isolate #12 Etest minimal inhibitory concentrations (MICs): telavancin (TLV) MIC = 6 μg/mL (left) and rifampin (RI) MIC >32 μg/mL (right).

vitro synergy by an agar diffusion Etest method (68%) against vancomycin-resistant *E faecium* (VRE),[4] by a checkerboard method (57%) also against VRE,[5] and by an Etest MIC:MIC method (88%) against linezolid-resistant VRE.[6] In another in vitro study, Lin et al[7] reported 65% synergy with the combination of telavancin and rifampin against methicillin-resistant *S aureus*.

METHODS

In our current investigation, we examined synergistic activity with telavancin when combined with rifampin against LRVRE and compared results to the combination of daptomycin + rifampin tested previously on the same isolates.

Microorganisms and Media

Twenty-four genetically unique clinical *E faecium* isolates resistant to both linezolid (MICs 8 to >256 μg/mL) and vancomycin (MICs >256 μg/mL) were collected throughout the United States from 2001-2004 and identified using the Vitek system (bioMérieux, Inc., Durham, NC). *E faecalis* ATCC 29212 was included as a quality control strain. Fingerprinting of isolates was performed by pulsed-field gel electrophoresis. Testing for VanA and VanB phenotypes was

Table. Telavancin (TLV), Daptomycin (DAP), and Rifampin (RI) Etest Minimal Inhibitory Concentrations (MICs) (μg/mL) and MIC:MIC Synergy Testing

LZD-R, VAN-R *Enterococcus faecium* (n=24)	TLV mean Etest MIC	DAP mean Etest MIC	RI mean Etest MIC	Etest synergy method TLV + RI mean ∑FIC	Etest synergy method DAP + RI mean ∑FIC
1	4	4	0.012	0.8 IND	0.6 IND
2	2	4	>32	0.2 SYN	0.1 SYN
3	8	2	0.032	0.2 SYN	0.9 IND
4	6	2	0.016	0.4 SYN	0.2 SYN
5	2	2	0.023	0.5 SYN	0.2 SYN
6	4	1	>32	0.3 SYN	0.2 SYN
7	4	2	>32	0.3 SYN	0.3 SYN
8	2	2	0.016	0.5 SYN	0.3 SYN
9	2	2	>32	0.6 IND	0.4 SYN
10	0.19	2	8	0.5 SYN	0.3 SYN
11	6	2	32	0.2 SYN	0.2 SYN
12	6	2	>32	0.1 SYN	0.5 SYN
13	6	4	16	0.2 SYN	0.2 SYN
14	8	2	0.016	0.7 IND	0.4 SYN
15	2	1	8	0.4 SYN	0.4 SYN
16	0.75	2	16	0.4 SYN	0.2 SYN
17	3	4	>32	0.1 SYN	0.2 SYN
18	8	4	0.016	0.4 SYN	1.0 IND
19	0.064	2	16	0.5 SYN	0.2 SYN
20	4	4	0.016	0.5 SYN	0.4 SYN
21	1	2	>32	0.2 SYN	0.2 SYN
22	0.19	2	6	0.5 SYN	0.4 SYN
23	3	2	>32	0.1 SYN	0.3 SYN
24	0.125	4	>32	0.7 IND	0.2 SYN

IND, indifference; LZD-R, linezolid resistant; ∑FIC, summation fractional inhibitory concentration; SYN, synergy; VAN-R, vancomycin resistant.

not performed. Media (Becton-Dickinson Microbiology Systems, Sparks, MD) included Mueller-Hinton II Broth and Mueller-Hinton II Agar (MHA) plates (for Etest MICs and synergy tests) and trypticase soy agar with 5% sheep blood plates (for subcultures of isolates).

MIC Determination

MICs were performed by Etest (bioMérieux, Inc., Durham, NC) in triplicate following manufacturer's guidelines, and the mean value was reported (Figure 1). Telavancin MICs ranged from 0.064-8 μg/mL; daptomycin MICs were 1-4 μg/mL; and rifampin MICs were 0.012 to >32 μg/mL (Table). The 2012 Clinical and Laboratory Standards Institute (CLSI) interpretive standards for enterococci are rifampin ≤1 susceptible, 2 intermediate, ≥4 resistant; daptomycin ≤4 susceptible.[8] The Food and Drug Administration breakpoint for telavancin for *E faecalis* is ≤1 susceptible. No breakpoints are available for telavancin and *E faecium*.

Synergy Testing

In vitro synergy testing was performed in triplicate by an Etest MIC:MIC ratio method.[6] All MHA plates were inoculated with a suspension of organism equivalent to a 0.5 McFarland standard. Telavancin and rifampin Etest strips were applied to different sections of an MHA plate. The agar was marked adjacent to the previously determined MIC value on each strip. The strips were removed after 1 hour of incubation at room temperature. A new telavancin strip was placed on the area of each previously removed rifampin strip so the telavancin MIC corresponded with the mark of the rifampin MIC. Rifampin strips were applied in reciprocate fashion to the area of the previous telavancin strip. The same procedure was used for the daptomycin + rifampin combination. The resulting combination ellipses were read after 24 hours of incubation at 35°C (Figure 2).

To evaluate the effect of the combination in the Etest method, the fractional inhibitory concentration

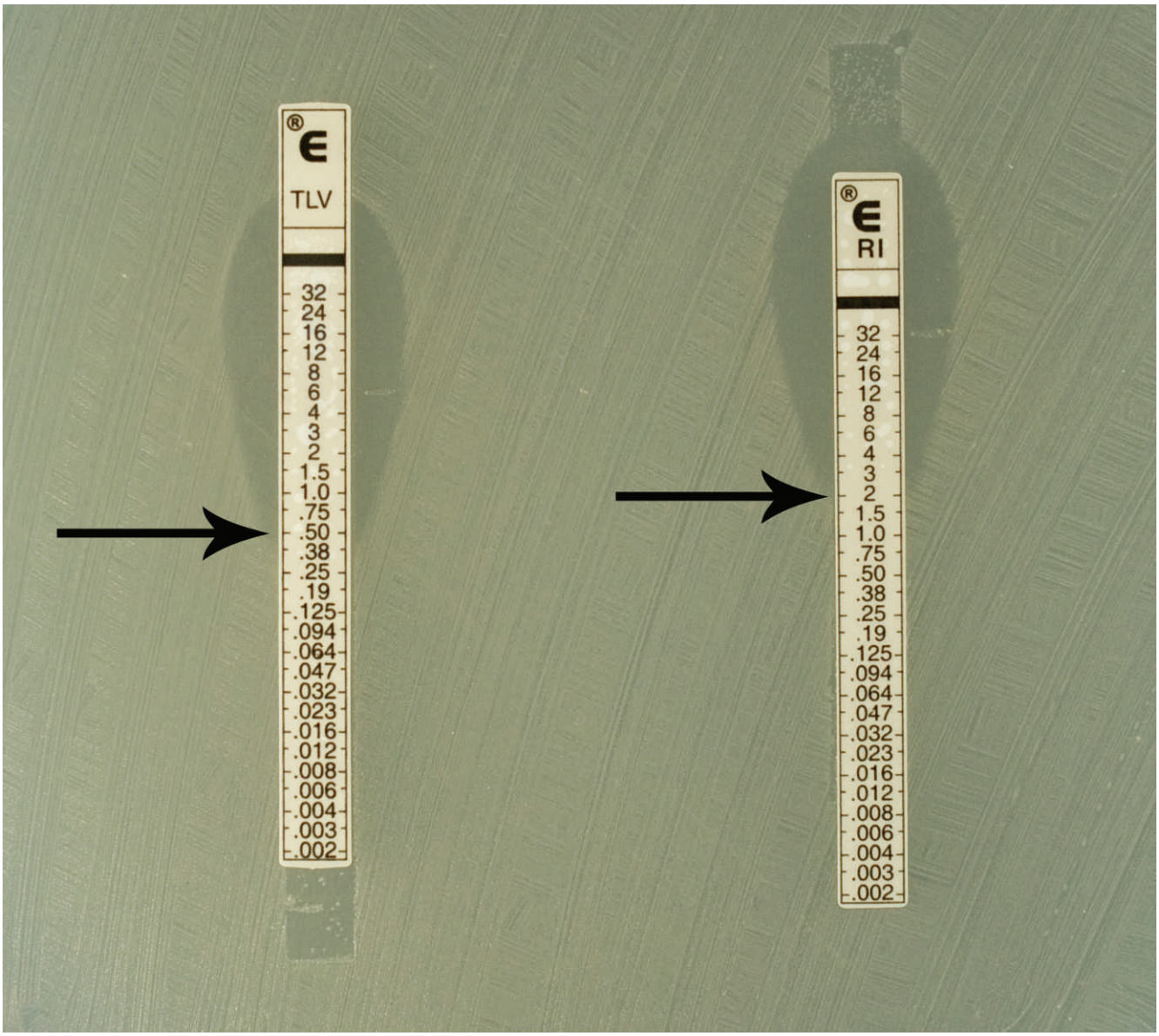

Figure 2. Isolate #12 Etest synergy test: telavancin (TLV) minimal inhibitory concentration (MIC) (after combination with rifampin) = 0.50 μg/mL (left). Rifampin (RI) MIC (after combination with telavancin) = 2 μg/mL (right). Summation fractional inhibitory concentration = 0.1 (synergy).

(FIC) was calculated for each antibiotic in each combination:

FIC of telavancin = MIC of telavancin in combination ÷ MIC of telavancin alone, and

FIC of rifampin = MIC of rifampin in combination ÷ MIC of rifampin alone.

The total (summation) fractional inhibitory concentration (ΣFIC) for each isolate was calculated according to the formula ΣFIC = FIC telavancin + FIC rifampin.

To calculate ΣFICs, high off-scale MICs (>32 μg/mL) were converted to the next 2-fold dilution (64 μg/mL), and final FIC values were rounded up to the nearest tenth (ie, 0.06 rounded to 0.1). The mean ΣFIC was used to interpret results of the Etest synergy method. Synergy was defined as ΣFIC ≤0.5, indifference as ΣFIC >0.5-4, and antagonism as ΣFIC >4.[9]

RESULTS

The Etest synergy method demonstrated synergy (ΣFICs of 0.1-0.5) with telavancin + rifampin against 20/24 (83%) of these linezolid-resistant VRE isolates and indifference (ΣFICs of 0.6-0.8) in 4/24 (17%) (Table). Similarly, the daptomycin + rifampin combination showed synergy (ΣFICs of 0.1-0.5) in 21/24 (88%) isolates and indifference (ΣFICs of 0.6-1.0) in

3/24 (12%) isolates by the Etest method (Table). Three isolates were indifferent (ΣFICs of 0.6, 0.7, 0.7) with the telavancin + rifampin combination but synergistic (ΣFICs of 0.4, 0.4, 0.2) with daptomycin + rifampin. Two isolates were synergistic (ΣFICs of 0.2, 0.4) with telavancin + rifampin but indifferent (ΣFICs of 0.9, 1.0) with daptomycin + rifampin (Table). Testing with both combinations revealed synergy in 23/24 (96%) of isolates. The one isolate interpreted as indifferent by both combinations (rifampin + telavancin or daptomycin with ΣFICs of 0.8 and 0.6) suggests a possible additive (ΣFICs of >0.5-1) effect (Table).[9] No antagonism with either combination was found.

DISCUSSION

Telavancin inhibits bacterial cell wall synthesis by interfering with the polymerization and cross-linking of peptidoglycan as well as increasing the permeability of the cell membrane. Telavancin might enhance the entry of rifampin, which specifically inhibits bacterial RNA polymerase.[10,11] Daptomycin also disrupts the bacterial cell membrane, which may also enhance the entry of rifampin.[12]

CONCLUSIONS

In vitro synergy was demonstrated with rifampin + telavancin or daptomycin against 83% and 88%, respectively, of the linezolid- and vancomycin-resistant *E faecium* isolates. Further Etest synergy testing, as well as time-kill assay with the combination of telavancin and rifampin should be performed against additional *E faecium* isolates. In vitro synergy may or may not translate into in vivo effectiveness.

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