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Activity of maltodextrin and vancomycin against *staphylococcus aureus* biofilm

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1. ABSTRACT

We aimed to assess the anti-biofilm activity of vancomycin, maltodextrin, and their combination against vancomycin resistant *Staphylococcus aureus* (VRSA) and vancomycin-susceptible *S. aureus* (VSSA) strains based on an *in vitro* static model. Biofilms of 4 VSSA and 2 VRSA strains were grown in a 96-well static model. Vancomycin 2 mM, maltodextrin 10 mM, and both in combination were tested using tetrazolium salt (XTT), resazurin, and cfu/well counts. The efficacy of the antimicrobial solutions was expressed as the percentage reduction in metabolic activity with each method. Overall percentage reduction in the metabolic activity of VSSA was 79.3%, 34%, and 75.7% for vancomycin, maltodextrin, and their combination ($p<0.001$). Overall percentage reduction in metabolic activity of VRSA was 46.7%, 27.8%, and 34.6% for vancomycin, maltodextrin, and their combination ($p>0.05$). Maltodextrin did not improve the anti-biofilm efficacy of vancomycin in VSSA or in VRSA biofilms. XTT cannot replace cfu counts as a means of quantifying cell viability. Futures studies are needed to assess the synergistic effects of other non-antimicrobial molecules combined with vancomycin.

2. INTRODUCTION

Catheter-related bloodstream infection (C-RBSI) is a major cause of morbidity and mortality, which can increase to 25% in critically ill patients. C-RBSI increases the length of hospital stay and health care costs (1, 2).

C-RBSI is caused by catheter colonization of extraluminal or endoluminal routes during insertion or maintenance (3). Colonization results from the ability of microorganisms to form biofilm. The agents responsible for C-RBSI are as follows: gram-positive cocci, 70% (coagulase-negative staphylococci (4), *Staphylococcus aureus,* enterococci); gram-negative bacilli, 20% (*Escherichia coli, Klebsiella pneumoniae*); and yeast, 10% (*Candida* spp.) (5, 6).

Guidelines recommend catheter withdrawal when a C-RBSI episode is suspected, particularly when it is caused by *S. aureus* or *Candida* spp. (7, 8). However, catheter salvage is necessary in specific situations, such as absence of an alternative venous access, bleeding disorders, and specific patient conditions (9). In these situations, the main approach to an episode of C-RBSI involves the combination of systemic antimicrobial treatment with antibiotic lock therapy (ALT) (10).

Guidelines recommend vancomycin, a first-generation glycopeptide, as the main choice of treatment for staphylococcal infections (coagu-

lase-negative staphylococcus (4) or *S. aureus* infections) (11, 12). However, several studies demonstrated that the anti-biofilm activity of vancomycin was not as effective as that of other antibiotics used for multidrug-resistant staphylococci (9, 13-15). Wider spread prescription of vancomycin has led vancomycin-intermediate *S. aureus* and vancomycin-resistant *S. aureus* (VRSA) to become increasingly common throughout the world, resulting in frequent treatment failures (16-18).

In their flow biofilm-forming model, Kiamco *et al*. recently demonstrated that the addition of maltodextrin, a common polysaccharide sweetener, can enhance vancomycin activity by acting as a hyperosmotic agent, particularly in VRSA biofilm. Maltodextrin showed synergistic activity that enabled it to be used in the treatment of wound infections (19). Although VRSA will represent a global health challenge in the future, no more than 20 strains have been described worldwide (16, 20, 21). Thus, the combination of vancomycin with agents encouraging antibiotic efficacy should also target vancomycin-susceptible *S. aureus* (VSSA). Moreover, no data have been reported on the possible role of the combination of vancomycin and maltodextrin in ALT solutions.

As for available diagnostic methods to test the *in vitro* metabolic activity of *S. aureus* biofilms, we recently reported a poor correlation between tetrazolium salt (XTT) and resazurin (22). However, to the best of our knowledge, the comparison of these options in susceptibility assays and their correlation with cell viability tests, such as evaluation of colony-forming unit (cfu) counts, have only been assessed for yeasts and not for bacteria (23, 24).

Therefore, the aims of our study were to assess the anti-biofilm activity of the combination of vancomycin with maltodextrin against VSSA and VRSA strains as possible ALT and to evaluate the correlation between the 3 different diagnostic methods.

3. MATERIALS AND METHODS

The study was performed in the laboratory of the Department of Clinical Microbiology and Infectious Diseases at Gregorio Marañón Hospital, Madrid, Spain.

We designed a static *in vitro* 96-well plate model using clinical strains of *S. aureus* (4 VSSA and 2 VRSA). The VRSA strains were provided by Haluk Beyenal and Cesar A. Arias.

Vancomycin minimal inhibitory concentration value for VRSA-1, VRSA-2, and VSSA strains (mean) was, respectively: >32 mg/L, 5.8 mg/L, and 1.25 mg/L. The design was based on a 24-hour biofilm that was treated with various solutions and the results were extrapolated to the clinical setting of C-RBSI treatment with ALT.

3.1. Biofilm formation

Biofilm was formed as described by Peeters *et al*., with some modifications (25). Briefly, a loopful of 24-hour fresh culture of each strain was inoculated in 20 ml of Tryptic Soy Broth (TSB) and incubated at 37°C in an orbital shaker for 24 hours. Inoculums were then washed in 3 centrifuge-resuspension cycles with phosphate-buffered saline (PBS), and pellets were rea suspended in 10 ml of TSB. These suspensions were adjusted to 0.5 McFarland turbidity (10^8 cfu/ml) using a turbidimeter and 100 µl was inoculated onto a 96-well plate. After 24 hours of biofilm formation at 37°C, plates were washed 3 times with PBS and treatment was administered. Each strain was tested in triplicate with a positive control and with TSB as a negative control.

3.2. Vancomycin and maltodextrin solutions and treatment procedure

Vancomycin (Sigma-Aldrich Química, S.L.), maltodextrin (Sigma-Aldrich Química, S.L.), and vancomycin-maltodextrin solutions were prepared in 3% TSB in concentrations of 2 mM, 10 mM, and 2 mM-10 mM, respectively, according to the concentrations used on the paper published by Kiamco *et al* (19). After preparation, each solution was filtered using a 0.22-µm Millipore® filter. Solutions were prepared immediately before each experiment.

One hundred microliters of each solution was added to the completely dry plates, which were incubated at 37°C for 24 hours. The plates were then washed a further 3 times with PBS and dried at room temperature before the viability assays.

3.3. Quantification of metabolic activity by XTT assay

One hundred microliters of XTT (Sigma-Aldrich Química, S.L.)/menadione (0.5 mg/ml and 1.72 mg/ml) mixed at 10 ml/1 µl was inoculated in each well in darkness. The plate was then incubated at 37°C for 3 hours. Absorbance was measured at 492 nm in a spectrophotometer (Biochrom EZ Read 400), and the percentage of metabolic reduction was calculated according to equation 1.

Equation 1 $$\%\ of\ metabolic\ reduction = \left[1 - \left(\frac{Abs_{492}\ treated\ strain}{Abs_{492} positive\ control}\right)\right] * 100$$

3.4. Quantification of metabolic activity by resazurin assay

One hundred microliters of TSB 30 mg/ml and 30 µl of resazurin (Sigma-Aldrich Química, S.L.) 5 ng/

Table 1. Overall percentage reduction in metabolic activity (by XTT or resazurin) and cfu counts for *Staphylococcus aureus* biofilm strains treated with vancomycin, maltodextrin, and the combination of both

Diagnostic assay	Therapy	% Reduction	P value[1]
XTT	V	65.9	p=0.041
XTT	M	33.1	
XTT	V+M	53.4	
RZ	V	62.0	p=0.003
RZ	M	21.0	
RZ	V+M	59.3	
cfu counts	V	77.4	p<0.001
cfu counts	M	41.5	
cfu counts	V+M	73.3	

XTT, tetrazolium salt; RZ, resazurin; cfu, colony-forming unit; V, vancomycin; M, maltodextrin; V+M, vancomycin + maltodextrin.[1]Statistically significant differences were found between V and V+M compared with M alone. V and V+M were efficient against *S. aureus* biofilm using all 3 methods.

µl was added to each well of the plate in darkness and incubated at 37°C for 2 hours. Absorbance was then measured using a dual-wavelength model (570 nm measurement wavelength and 590 nm reference wavelength) in a spectrophotometer (Biochrom EZ Read 400). The percentage of metabolic reduction was calculated using equation 2.

Equation 2 $$\%Resorufin = \left(\frac{(\varepsilon OX)_{\lambda 2}\ A_{\lambda 1} - (\varepsilon OX)_{\lambda 1}\ A_{\lambda 2}}{(\varepsilon OX)_{\lambda 1}\ A°_{\lambda 2} - (\varepsilon OX)_{\lambda 2}\ A°_{\lambda 1}}\right) * 100$$

where $(\varepsilon OX)_{\lambda 2}$ is the molar extinction coefficient at 590 nm of the oxidized form, $(\varepsilon OX)_{\lambda 1}$ is the molar extinction coefficient at 570 nm of the oxidized form, $A_{\lambda 1}$ and $A_{\lambda 2}$ are the absorbances of treated wells at 570 nm and 590 nm, respectively, and $A°_{\lambda 1}$ and $A°_{\lambda 2}$ are the absorbances of positive control at 570 nm and 590 nm, respectively.

3.5. Quantification of cfu per well

The wells were vigorously scraped in 100 µl of PBS, and the triplicates of each treatment and controls were mixed separately in a pool. Four 1:100 serial dilutions were performed, and 100 µl of each dilution was streaked on blood agar plates and incubated at 37°C for 24 hours. Colonies were counted, and the reduction in $\log_{10}$ cfu/well was calculated using equation 3.

Equation 3 $$\%\ of\ viability\ reduction = \left[1 - \left(\frac{\frac{CFU}{well}\ treated\ strain}{\frac{CFU}{well}\ positive\ control}\right)\right] * 100$$

3.6. Statistical analysis

The qualitative variables are expressed with their frequency distribution. The quantitative variables are summarized as the mean (SD). Continuous variables were compared using the *t* test; non-normally distributed variables were compared using the Kruskal-Wallis test. The differences between the groups were compared using the ANOVA test with a post-hoc comparison test by Games-Howell. All statistical tests were 2-tailed.

Bland-Altman plots (95%CI) and the interclass correlation coefficient (ICC) were used to analyze the correlation between the diagnostic methods. A difference in methods of ± 10% of reduction was considered a good correlation. Consistent with Koo *et al*., ICC values were as follows: low, <0.5; moderate, 0.5<X<0.75; good, 0.75<X<0.9; and excellent, >0.9 (26).

Statistical significance was set at p<0.05 for all the tests. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Armonk, New York, USA) and XLSTAT for Windows, Version 2017.4 (Addinsoft).

4. RESULTS

The overall mean (SD) percentage reduction in metabolic activity and mean cfu in all strains when the 3 methods were assessed for vancomycin, maltodextrin, or both were, respectively, 68.4% (17.3%), 31.9% (20.5%), and 62.0% (25.0%) (p<0.05). Data regarding the overall percentage reduction for each method are shown in **Table 1**. Using the XTT assay, the mean (SD) percentage reduction in metabolic activity for vancomycin, maltodextrin, and both were 65.9% (32.7%), 33.1% (26.3%), and 53.4% (35.1%) (p=0.041), respectively. With the resazurin assay, the mean (SD) percentage reduction in metabolic activity was 62% (22.3%), 21% (8.3%), and 59.3% (21.7%)

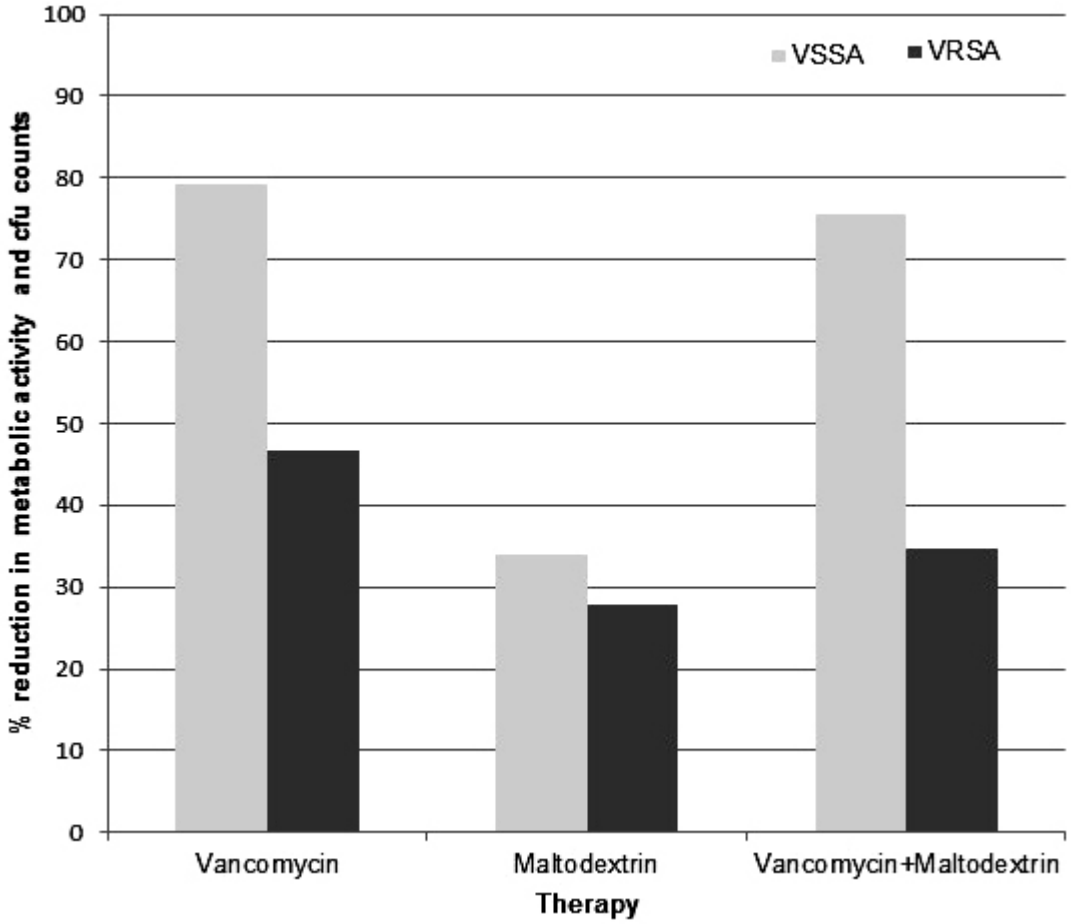

Figure 1. Overall percentage reduction in metabolic activity and cfu counts for VSSA and VRSA strains treated with vancomycin, maltodextrin, and their combination

(p=0.003) for vancomycin, maltodextrin, and both. The mean (SD) percentage reduction in cfu counts for vancomycin, maltodextrin, and both was, respectively, 77.4% (20.8%), 41.5% (22.0%), and 73.3% (23.9%) (p<0.001).

For VSSA strains, the overall mean (SD) percentage reduction in metabolic activity and cfu for vancomycin and the combination of vancomycin with maltodextrin was statistically significant compared with that of maltodextrin alone: 79.3% (9.0%) and 75.7% (15.6%) vs. 34.0% (9.3%), p<0.05 (Figure 1). In contrast, in VRSA strains, the overall mean (SD) percentage reduction in metabolic activity and cfu counts was not statistically significant between the groups: vancomycin, 46.7% (28.9%); maltodextrin, 27.8% (16.2%); and both, 34.6% (18.9%); p>0.05.

Figure 2. shows the percentage reduction for each therapy by the 3 different diagnostic methods for VSSA (2a) and VRSA (2b). No differences were found between vancomycin and its combination with maltodextrin in VSSA (p>0.05), although it was more active than maltodextrin alone (p<0.001). In VRSA, vancomycin led to a greater reduction in metabolic activity and cfu counts than the other therapies, although the differences were not statistically significant (p>0.05).

When we compared the correlation between the 3 methods used, no statistically significant correlation was found between any of the methods, either with the Bland-Altman graphs or ICCs (Figure 3). The ICC for the combinations was as follows: cfu vs. XTT, 0.53; cfu vs. resazurin, 0.69; and XTT vs. resazurin, 0.63, ie, a moderate correlation between the 3 techniques.

5. DISCUSSION

Our static *in vitro* biofilm model did not enable us to demonstrate that the combination of maltodextrin with vancomycin had a synergistic effect against VSSA and VRSA strains.

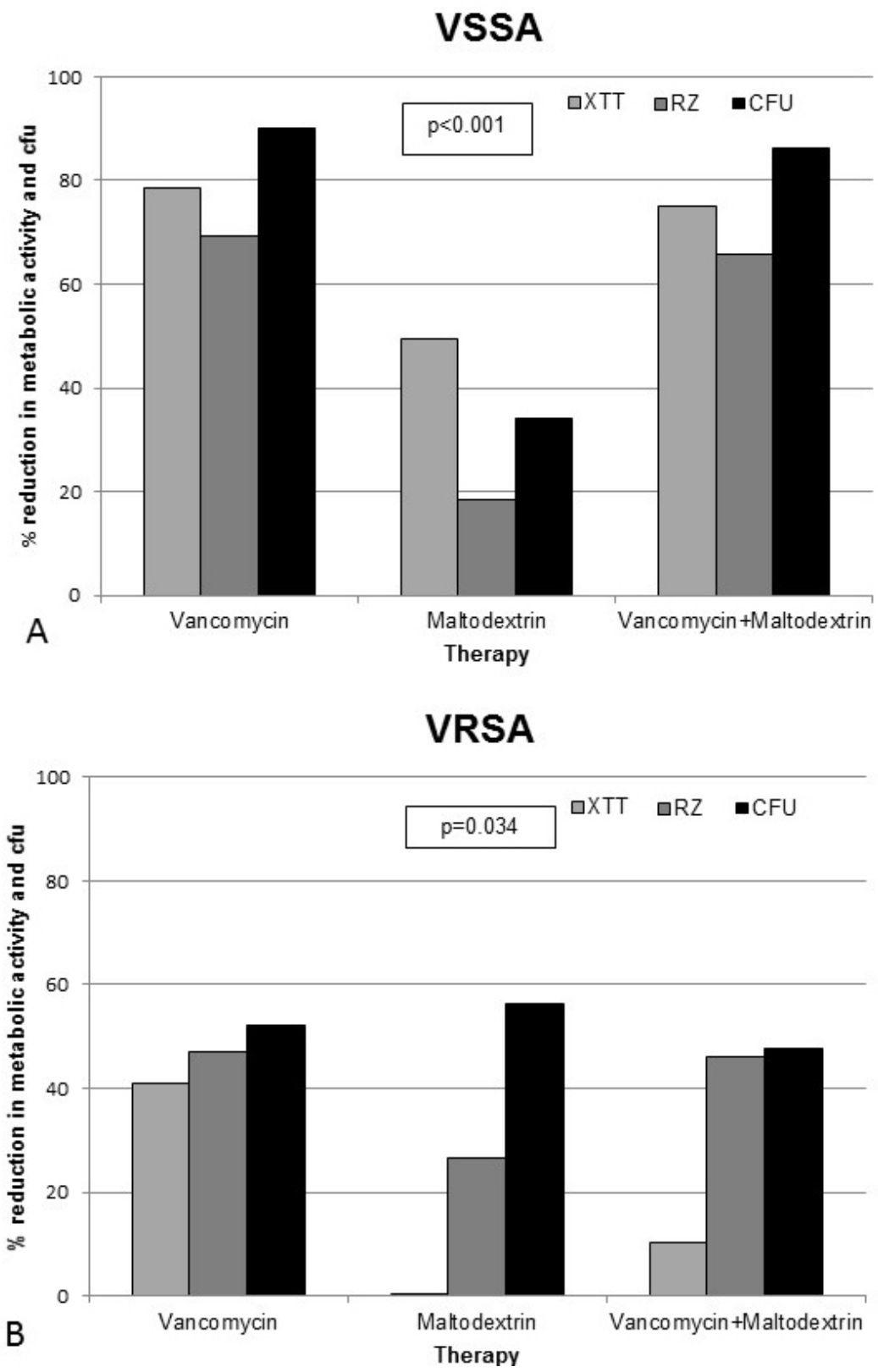

Figure 2. Percentage reduction in metabolic activity (by XTT or resazurin) and cfu counts for VSSA (A) and VRSA (B) strains treated with vancomycin, maltodextrin, and their combination. A. In VSSA, vancomycin alone and in combination with maltodextrin showed statistically significantly greater reduction in metabolic activity and cfu counts than maltodextrin alone ($p<0.001$). B. In VRSA, the only statistically significant difference was found between maltodextrin and the combination of both using the resazurin assay ($p=0.012$).

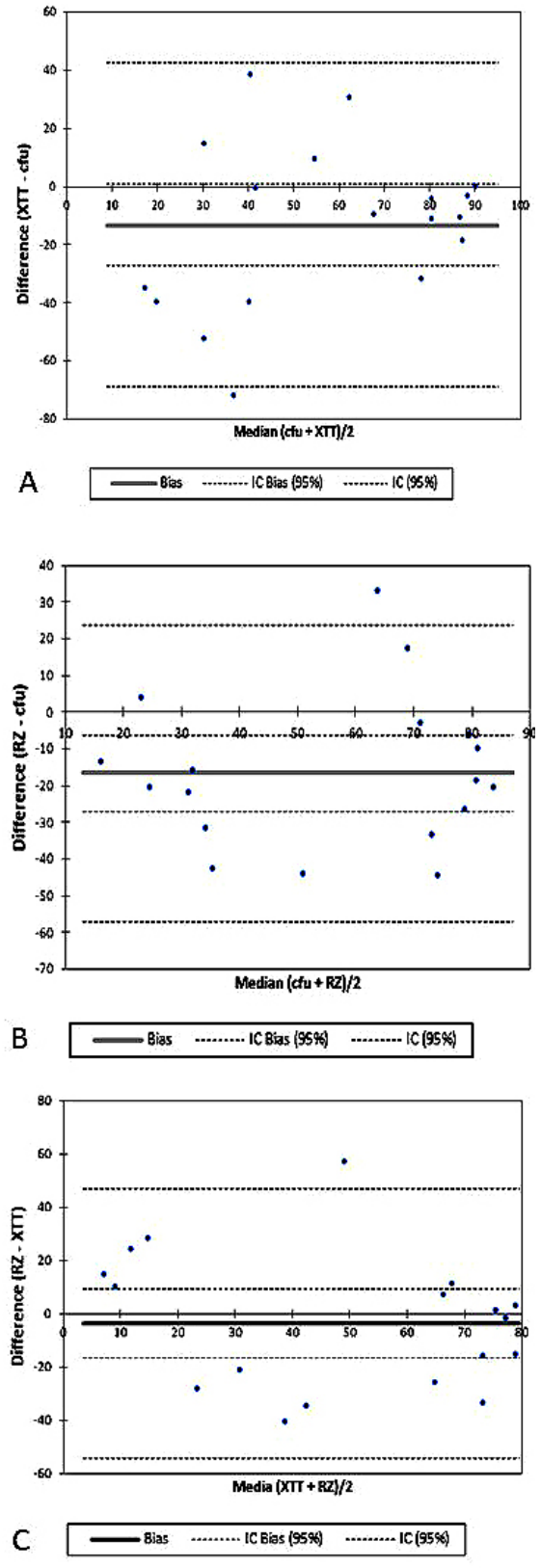

Figure 3. Bland-Altman plots for cfu counts vs. XTT (A), cfu counts vs. resazurin (B), and XTT vs. resazurin (C). XTT, tetrazolium salt; RZ, resazurin; cfu, colony forming units.

C-RSBI is a major complication in hospitals, particularly in intensive care units (3, 27). *S. aureus* C-RBSI is an issue of concern because it frequently causes long-term hospitalization, morbidity, and mortality (28). A relationship was recently reported between high minimal inhibitory concentrations (MICs) for vancomycin (MICs ≥ 1.5 µg/ml) and poor clinical outcome in patients with *S. aureus* C-RBSI (29, 30). Moreover, the anti-biofilm activity of vancomycin compared with other antibiotics in the clinical setting is also under discussion, and the use of the drug for ALT remains controversial (31). Thus, our purpose was to analyze whether the combination of vancomycin with a hyperosmotic agent increased its activity against *S. aureus* biofilm sufficiently to be used in ALT.

Kiamco *et al*. described a synergistic effect of 10 mM maltodextrin combined with 2 mM vancomycin against *S. aureus* biofilm, with a significant reduction in volumetric biofilm coverage and average diffusion distance. The authors also observed changes in biofilm morphology and in oxygen penetration, concluding that the combination of vancomycin and maltodextrin increased the efficiency of biofilm treatment in wound infections (19). In contrast, we found no statistically significant differences for the efficacy of vancomycin, whether alone or combined with maltodextrin, in reducing metabolic activity and cfu counts in VSSA or in VRSA biofilms. Although we used the same concentrations as Kiamco *et al*., the diagnostic methods for each study were different (19). While we used a static plate model and cell viability assays to analyze viability and metabolic reduction, Kiamco *et al*. used a single-pass flat plate flow reactor to assess biofilm structure, oxygen penetration, antibiotic diffusion, and cell viability. Thus, vancomycin combined with maltodextrin altered various biofilm properties but did not increase vancomycin activity when metabolic activity was being measured. We found that in VSSA, the reduction in viability measured as cfu counts was approximately 80-90% for vancomycin and for the combination of vancomycin and maltodextrin.

These findings correlated with the results of Kiamco *et al*. and indicate that both therapies are effective but that the combination of vancomycin and maltodextrin was not synergistic. In contrast, in VRSA, none of the therapies enabled a reduction greater than 52% with any of the diagnostic methods used or even showed less activity when both were combined.

Moreover, the antimicrobial susceptibility profile and the level of biofilm production can affect the efficacy of an antimicrobial treatment. A possible explanation of the differences we observed with respect to Kiamco's study in the efficacy of the combined activity of vancomycin+maltodextrin could be related to a specie-specific background. In particular, a high level of biofilm production is key in the process of the bacte-

rial tolerance. Is it possible that the absence of a statistical significance observed in our VRSA could be related to a different level of biofilm production between our strains and the one analyzed in Kiamco *et al*.

As resazurin is cheaper, less time-consuming, less toxic than XTT, and less laborious than cfu counts and the XTT assay is less time-consuming, provides faster results, and is less laborious than cfu counts, we aimed to find a correlation that could substitute cfu counts with any of the metabolic activity assays. However, as reported for yeasts (25, 32-34), we were unable to establish a correlation between cfu counts, XTT, and resazurin using either Bland-Altman plots or ICCs in bacteria.

Nevertheless, considering a ± 10% reduction in the difference between methods as a good correlaɵ tion, the correlation we obtained between the methods was moderate, suggesting that, depending on the researcher's goals, some methods are more suitable than others. However, results must be interpreted with caution.

In conclusion, based on our results in a static *in vitro* model, we could not demonstrate that maltodextrin improved the activity of vancomycin against *S. aureus* biofilm in ALT. As for diagnostic methods, neither XTT nor resazurin can replace cfu counts for the evaluation of anti-biofilm activity, as they measure different properties (metabolic activity and cell viability, respectively). Future studies are needed to find other synergistic agents to increase vancomycin anti-biofilm activity and thus optimize the conservative treatment of C-RBSI by ALT.

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7. REFERENCES

1. DS. Duzkaya, NC. Sahiner, G. Uysal, T. Yakut, A. Citak. Chlorhexidine-Impregnated Dressings and Prevention of Catheter-Associated Bloodstream Infections in a Pediatric Intensive Care Unit. *Crit Care Nurse* 36, e1-e7 (2016)
DOI: 10.4037/ccn2016561
PMid:27908954

2. A. Atilla, Z. Doganay, H. Kefeli Celik, MD. Demirag. Central line-associated blood stream infections: characteristics and risk factors for mortality over a 5.5.-year period. *Turk J Med Sci* 47, 646-652 (2017)
DOI: 10.3906/sag-1511-29
PMid:28425261

3. M. Gominet, F. Compain, C. Beloin, D. Lebeaux. Central venous catheters and biofilms: where do we stand in 2017? *Apmis* 125, 365-375 (2017)

4. JB. Dimick, RK. Pelz, R. Consunji, SM. Swoboda, CW. Hendrix, PA. Lipsett. Increased resource use associated with catheter-related bloodstream infection in the surgical intensive care unit. *Arch Surg* 136, 229-34 (2001)
DOI: 10.1001/archsurg.136.2.229
PMid:11177147

5. R. Gahlot, C. Nigam, V. Kumar, G. Yadav, S. Anupurba. Catheter-related bloodstream infections. *Int J of Crit Illness and Inj Science* 4, 162-167 (2014)

6. H. Shah, W. Bosch, KM. Thompson, WC. Hellinger. Intravascular Catheter-Related Bloodstream Infection. *The Neurohosp* 3, 144-151 (2013)
DOI: 10.1177/1941874413476043
PMid:24167648 PMCid:PMC3805442

7. LA. Mermel, M. Allon, E. Bouza, DE. Craven,P. Flynn, NP. O'Grady, Raad II, Rijnders BJ, Sherertz RJ, Warren DK. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection: 2009 Update by the Infectious Diseases Society of America. *Clin Infect Dis* 49, 1-45 (2009)
DOI: 10.1086/599376
PMid:19489710 PMCid:PMC4039170

8. NP. O'Grady, M. Alexander, LA. Burns, EP. Dellinger, J. Garland, SO. Heard, Lipsett PA, Masur H, Mermel LA, Pearson ML, Raad II, Randolph AG, Rupp ME, Saint S; Healthcare Infection Control Practices Advisory Committee (HICPAC). Guidelines for the

prevention of intravascular catheter-related infections. *Clin infect Dis* 52, e162-e193 (2011)
DOI: 10.1093/cid/cir257
DOI: 10.1093/cid/cir138

9. S. Hogan, M. Zapotoczna, NT. Stevens, H. Humphreys, JP. O'Gara, E. O'Neill. *In vitro* Approach for Identification of the Most Effective Agents for Antimicrobial Lock Therapy in the Treatment of Intravascular Catheter-Related Infections Caused by Staphylococcus aureus. *Antimicrob Agents Chemother* 60, 2923-31 (2016)
DOI: 10.1128/AAC.02885-15
PMid:26926633 PMCid:PMC4862522

10. SG. Woo, SY. Lee, SM. Lee, KH. Lim, EJ. Ha, YB. Eom. Activity of novel inhibitors of Staphylococcus aureus biofilms. *Folia Microbiol (Praha)* (2016)

11. RP. Dash, RJ. Babu, NR. Srinivas. Review of the pharmacokinetics of dalbavancin, a recently approved lipoglycopeptide antibiotic. *Infect Dis (Lond)* 49, 483-492 (2017)
DOI: 10.1080/23744235.2017.1296968
PMid:28264598

12. HP. Rang, RJ. Flower, G. Henderson, J. Ritter. Rang and Dale's pharmacology. Eighth edition ed. (Edinburgh): Elsevier Churchill Livingstone (2016)

13. W. Siala, MP. Mingeot-Leclercq, PM. Tulkens, M. Hallin, O. Denis, F. Van Bambeke. Comparison of the antibiotic activities of Daptomycin, Vancomycin, and the investigational Fluoroquinolone Delafloxacin against biofilms from *Staphylococcus aureus* clinical isolates. *Antimicrob Agents Chemother* 58, 6385-97 (2014)
DOI: 10.1128/AAC.03482-14
PMid:25114142 PMCid:PMC4249400

14. SS. Atshan, M. Nor Shamsudin, LT. Lung, Z. Sekawi, C. Pei Pei, A. Karunanidhi, J. Jeevajothi Nathan, A. Mateg Ali, E. Ghaznavi-Rad, SA. Abduljaleel, R. Awang Hamat. Genotypically different clones of *Staphylococcus aureus* are diverse in the antimicrobial susceptibility patterns and biofilm formations. *Biomed Res Int* 2013:515712 (2013)

15. K. Smith, CG. Gemmell, S. Lang. Telavancin shows superior activity to vancomycin with multidrug-resistant *Staphylococcus aureus* in a range of *in vitro* biofilm models. *Eur J Clin Microbiol Infect Dis* 32, 1327-32 (2013)
DOI: 10.1007/s10096-013-1883-z
PMid:23624635

16. IM. Gould. Treatment of bacteraemia: meticillin-resistant *Staphylococcus aureus* (MRSA) to vancomycin-resistant *S. aureus* (VRSA) *Int J Antimicrob Agents* 42, S17-21 (2013)

17. S. Gardete, A. Tomasz. Mechanisms of vancomycin resistance in *Staphylococcus aureus. J Clin Invest* 124, 2836-40 (2014)
DOI: 10.1172/JCI68834
PMid:24983424 PMCid:PMC4071404

18. B. Périchon, P. Courvalin. *Staphylococcus aureus* VRSA-11B Is a Constitutive Vancomycin-Resistant Mutant of Vancomycin-Dependent VRSA-11A. *Antimicrob Agents Chemother* 56, 4693-4696 (2012)
DOI: 10.1128/AAC.00454-12
PMid:22710116 PMCid:PMC3421854

19. MM. Kiamco, E. Atci, QF. Khan, A. Mohamed, RS. Renslow, N. Abu-Lail, BA. Fransson, DR. Call, H. Beyenal. Vancomycin and maltodextrin affect structure and activity of *Staphylococcus aureus* biofilms. *Biotechnol Bioeng* 112, 2562-70 (2015)
DOI: 10.1002/bit.25681
PMid:26084588 PMCid:PMC5096838

20. A. Moses, U. Uchenna, O. Nworie. Epidemiology of vancomycin resistant S*taphyloccus aureus* among clinical isolates in a tertiary hospital in Abakaliki, Nigeria. *Am J of Epidemiol and Infect Dis* 1, 24-26 (2013)

21. VS. Albrecht, MJ. Zervos, KS. Kaye, PK. Tosh, S. Arshad, K. Hayakawa, AJ. Kallen, LK. McDougal, BM. Limbago, AY. Guh. Prevalence of and risk factors for vancomycin-resistant *Staphylococcus aureus* precursor organisms in Southeastern Michigan. *Infect Control Hosp Epidemiol* 35, 1531-4 (2014)
DOI: 10.1086/678605
DOI: 10.1086/593316
PMid:25419776

22. B. Alonso, R. Cruces, A. Perez, C. Sanchez-Carrillo, M. Guembe. Comparison of the XTT and resazurin assays for quantification of the metabolic activity of *Staphylococcus aureus* biofilm. *J Microbiol Methods* 139, 135-137 (2017)
DOI: 10.1016/j.mimet.2017.06.004
PMid:28587857

23. PD. Khot, PA. Suci, BJ. Tyler. *Candida albicans* viability after exposure to amphotericin B: assessment using metabolic assays and colony forming units. *J Microbiol Methods* 72, 268-74 (2008)
DOI: 10.1016/j.mimet.2007.12.005
PMid:18234380

24. S. Silva, M. Henriques, R. Oliveira, D. Williams, J. Azeredo. *In vitro* biofilm activity of non-*Candida albicans Candida* species. *Curr Microbiol* 61, 534-40 (2010)
DOI: 10.1007/s00284-010-9649-7
PMid:20401483

25. E. Peeters, HJ. Nelis, T. Coenye. Comparison of multiple methods for quantification of microbial biofilms grown in microtiter plates. *J Microbiol Methods* 72, 157-65 (2008)
DOI: 10.1016/j.mimet.2007.11.010
PMid:18155789

26. TK. Koo, MY. Li. A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *J Chiropr Med* 15, 155-63 (2016)
DOI: 10.1016/j.jcm.2016.02.012
PMid:27330520 PMCid:PMC4913118

27. BR. Salonen, SL. Bonnes, N. Vallumsetla, JE. Varayil, MS. Mundi, RT. Hurt. A prospective double blind randomized controlled study on the use of ethanol locks in HPN patients. *Clin Nutr* May (2017)

28. R. San-Juan, M. Fernández-Ruiz, O. Gasch, M. Camoez, F. López-Medrano, M. Domínguez, B. Almirante, B. Padilla, M. Pujol, JM. Aguado; REIPI/GEIH Study Group. High vancomycin MICs predict the development of infective endocarditis in patients with catheter-related bacteraemia due to methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* 72, 2102-2109 (2017)
DOI: 10.1093/jac/dkx096
PMid:28379553

29. DM. Martirosov, MR. Bidell, MP. Pai, MH. Scheetz, SL. Rosenkranz, TP: Lodise. Relationship between vancomycin exposure and outcomes among patients with MRSA bloodstream infections with vancomycin Etest(R) MIC values of 1.5mg/L: A pilot study. *Diagn Microbiol Infect Dis* 88, 259-263 (2017)
DOI: 10.1016/j.diagmicrobio.2017.03.008
PMid:28449844

30. A. Soriano, F. Marco, JA. Martinez, E. Pisos, M. Almela, VP. Dimova, D. Alamo, M. Ortega, J. Lopez, J. Mensa. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. *Clin Infect Dis* 46, 193-200 (2008)
DOI: 10.1086/524667
PMid:18171250

31. S. Deresinski. Counterpoint: Vancomycin and *Staphylococcus aureus*--an antibiotic enters obsolescence. *Clin Infect Dis* 15, 1543-8 (2007)
DOI: 10.1086/518452
PMid:17516396

32. AG. Dalecki, CL. Crawford, F. Wolschendorf. Targeting Biofilm Associated *Staphylococcus aureus* Using Resazurin Based Drug-susceptibility Assay. *J Vis Exp* 5, 111 (2016)

33. MM. Tunney, G. Ramage, TR. Field, TF. Moriarty, DG. Storey. Rapid colorimetric assay for antimicrobial susceptibility testing of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 48, 1879-81 (2004)
DOI: 10.1128/AAC.48.5.1879-1881.2004
PMid:15105149 PMCid:PMC400562

34. K. Prabst, H. Engelhardt, S. Ringgeler, H. Hubner. Basic Colorimetric Proliferation Assays: MTT, WST, and Resazurin. *Methods Mol Biol* 1601, 1-17 (2017)
DOI: 10.1007/978-1-4939-6960-9_1
PMid:28470513

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