

In vitro testing of daptomycin plus rifampin against methicillin-resistant *Staphylococcus aureus* resistant to rifampin

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ABSTRACT

الأهداف: اختبار وجود المؤازر لمقايسة الدابتومايسين (DAP) والريفامبين (RIF)، ضد البكتيريا العنقودية المقاومة للميثيسيلين (MRSA).

الطريقة: لقد تم اختبار المؤازر لمقايسة الدابتومايسين (DAP) والريفامبين (RIF) مع مرور الوقت (TKA) على ستة أنواع جديدة من البكتيريا العنقودية المقاومة للميثيسيلين، والتي لها مقاومة لعقار ريفامبين، حيث تم عزل ستة أنواع فريدة في خصائصها الإكلينيكية والجينية من أصل 489 بمعدل (1.2%)، والتي تم جمعها خلال الفترة ما بين أبريل 2003م وحتى أغسطس 2008م، بمركز أوشنر - نيواورلينز - لويزيانا - الولايات المتحدة الأمريكية.

النتائج: أظهر اختبار المؤازر لمقايسة الدابتومايسين (DAP) والريفامبين (RIF) مع مرور الوقت (TKA) أن 5 عوازل كانت حيادية، وعازلاً واحداً كان مضاداً.

خاتمة: دراستنا لم تظهر المؤازر لمقايسة الدابتومايسين (DAP) والريفامبين (RIF) مع مرور الوقت (TKA) ضد البكتيريا العنقودية المقاومة للميثيسيلين، وواحداً من العوازل كان مضاداً. وعليه، يجب أن يؤخذ هذا في عين الاعتبار عند مواجهة حالة فشل إكلينيكية للمرضى اللذين يتم علاجهم باستخدام الدابتومايسين (DAP) والريفامبين (RIF).

Objectives: To test for synergy between daptomycin (DAP) and rifampin (RIF) against RIF-resistant methicillin-resistant *Staphylococcus aureus* (MRSA) isolates.

Methods: Synergy testing using time-kill assay (TKA) was performed on 6 clinically, and genetically unique RIF-resistant MRSA isolates. The isolates were identified out of 489 (1.2%) samples collected during April 2003 to August 2006, from patients at the Ochsner Medical Center in New Orleans, Louisiana, United States of America.

Results: Synergy testing of DAP plus RIF by TKA showed that 5 isolates were indifferent, but one isolate was antagonistic.

Conclusion: Our *in vitro* study failed to demonstrate synergy between DAP plus RIF, against our RIF-resistant MRSA isolates. Clinical failure of this combination should prompt the clinician to consider antagonism, as one of the potential causes.

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Due to the resistance, or reduced susceptibility to different antimicrobials including vancomycin and daptomycin (DAP), methicillin-resistant *Staphylococcus aureus* (MRSA) infections constitute a major challenge.¹⁻⁴ Antibiotic combinations to achieve an additive or synergistic effect, may be a way to address this problem. Nevertheless, they have not been extensively studied *in vitro*, or *in vivo*. We chose to study DAP plus rifampin (RIF), due to the limited number of *in vitro* studies that have looked at this combination. Furthermore, RIF has been successfully used in animal models, as well as clinical studies, in combination with cell wall active agents, to enhance the bactericidal activity against gram-positive bacteria.⁵⁻¹⁰ Rifampin-resistant strains were selected to make it easier to demonstrate synergy, if present, and because this combination might be used to treat systemic MRSA infections empirically before susceptibilities are reported.

Methods. Standard laboratory powders of DAP (Cubist Pharmaceuticals, Inc., Lexington, Massachusetts, USA), and RIF (Sigma-Aldrich, St. Louis, Missouri, USA), and Etest® strips (AB Biodisk, Solna, Sweden) for DAP and RIF were used in the study. Six clinically and genetically unique RIF-resistant MRSA isolates were identified out of 489 (1.2%) samples collected during April 2003 to August 2006 from patients at the Ochsner Medical Center in New Orleans, Los Angeles, Louisiana, USA. As no patient data were used, the ethical approval was waived by the Institutional Review Board committee. Fingerprinting of the isolates was carried out by pulsed-field gel electrophoresis. *Staphylococcus aureus* (*S. aureus*) ATCC 29213, and *Enterococcus faecalis* ATCC 29212 were used for quality control.¹¹ Mueller-Hinton broth (Becton-Dickinson Microbiology Systems, Sparks, Maryland, USA) was prepared in the laboratory, and supplemented to the recommended 50mg/L calcium for the minimal inhibitory concentration (MIC), and time-kill assay (TKA) testing of DAP.¹² Mueller-Hinton II agar plates (Becton-Dickinson Microbiology Systems, Maryland, USA) were used for the Etest MIC determination. Trypticase soy agar with 5% sheep blood plates (Becton-Dickinson Microbiology Systems, Maryland, USA) were used for the colony counts in the TKA. Minimal inhibitory concentrations were determined by broth microdilution (BMD), and E-test method. The BMD was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.¹² The concentration range tested was 0.25-256 µ/ml for RIF, and 0.015-32 µ/ml for DAP. The Etest method was performed in triplicate following manufacturer's guidelines. The concentration range tested was 0.016-256 µ/ml for DAP, and 0.002-32 µ/ml for RIF. The CLSI 2007 interpretive standards (µ/ml) for *S. aureus* are: RIF≤1 susceptible, 2 intermediate, ≥4 resistant; DAP≤1 susceptible.¹¹ Time-kill assay was performed according to CLSI guidelines,¹³ and as described in a previous study.¹⁴ Each isolate was tested against DAP and RIF alone, and in combination, at a concentration equal to the Etest MIC for DAP and equal to 32, the highest peak serum level achievable clinically¹⁵ for RIF. The final inoculum was approximately 10⁵ CFU/ml, and was verified after plating in duplicate using a spiral plater (Spiral Biotech, Bethesda, Maryland, USA). Synergy was defined as a ≥2 log₁₀ decrease in colony count after 24 hours by the combination, compared to the most active single agent, and the number of surviving organisms in the presence of the combination was >2 log₁₀ CFU/ml below the starting inoculum.¹⁶ Indifference was defined as a <2 log₁₀ increase or decrease in colony count at 24 hours by the combination, compared with that by the most active drug alone.¹⁶ Antagonism was defined as

a ≥2 log₁₀ increase in colony count after 24 hours by the combination, compared to the most active drug alone.¹⁶

Results. The DAP plus RIF combination was indifferent by TKA against 5 isolates (Table 1). The combination was antagonistic against one isolate performed in triplicate (log₁₀ change: +2.89, +3.06, and +2.69) (Figure 1a). This isolate was also tested using 4, 8, and 12 hours as additional sampling time points. Two concentrations of RIF (16 and 32 µ/ml) were tested with a DAP concentration equal to the MIC. These isolates showed some killing up to 12 hours with the combination, but between 12-24 hours, the CFU/ml increased. The RIF 32 µ/ml combination showed antagonism, while RIF 16 µ/ml (a concentration more likely reached *in vivo*) showed indifference with the combination (Figure 1b).

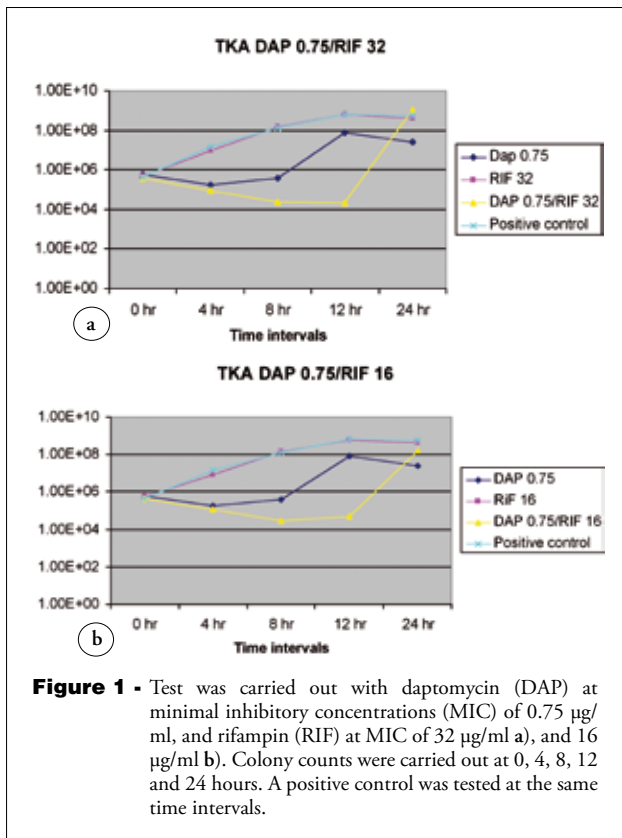
Discussion. In 1957, Jones et al¹⁷ wrote, "There is a paucity of useful data from which the therapeutic effectiveness of combinations of antibiotics can be compared to that of their individual components".¹⁷ Half a century later, this statement still holds true. The rate at which pathogenic bacteria is acquiring resistance is alarming, and there is a fear it will outpace antibiotic development rate. Studying antibiotic combinations looking for synergistic combinations that are clinically efficacious and safe, is worthwhile.

Using TKA, Credito et al⁵ studied the combination of DAP plus RIF against 50 *S. aureus* isolates (9 methicillin-susceptible, 32 methicillin-resistant, 6 vancomycin-intermediate, and 3 vancomycin-resistant strains). Only 1 strain (vancomycin-intermediate strain) showed synergy between DAP plus RIF; all

Table 1 - Daptomycin and rifampin MICs (µg/ml) by BMD and Etest (mean). Synergy testing by TKA.

MRSA isolates	RIF BMD MIC	RIF Etest MIC	DAP BMD MIC	DAP Etest MIC	TKA Log ₁₀ change (cfu/ml)
7054	>256	>32	0.5	1	+0.37 IND
7102	>256	>32	0.5	1	-1.24 IND
6429	>256	>32	0.25	0.5	+0.03 IND
6562	>256	>32	0.5	1	-1.30 IND
4959	>256	>32	0.25	0.5	-0.08 IND
5734	>256	>32	0.5	0.75	+2.89, +3.06, +2.69, +2.15 ANT

MRSA - methicillin-resistant *Staphylococcus aureus*, RIF - rifampin, BMD - broth microdilution, TKA - time-kill assay, MIC - minimal inhibitory concentrations, DAP - daptomycin, IND - indifferent, ANT - antagonistic



other combinations were “additive”.⁵ In comparison to our study, they used DAP and RIF concentrations for synergy testing at 1-2 dilutions below the MIC.

Sakoulas et al⁹ used rifampin-susceptible MRSA isolates to demonstrate that the bactericidal activity of DAP is augmented by the addition of RIF in an *in-vivo* experimental rat model of endocarditis. The combination regimen resulted in at least 3-log reduction in bacterial densities in vegetation over the treatment period, compared with bacterial densities at the start of therapy. Unlike the above study, we only used rifampin-resistant isolates. This, compartment-specific anti-microbial properties, and the immune system role in the process, make it difficult to compare those 2 studies.

Although the mechanism of antagonism between RIF and DAP in our isolates is not clear, Mwangi et al¹⁸ tracked the *in vivo* evolution of multi-drug resistance *S. aureus* in one isolate, and noticed decreased susceptibility to DAP that coincided with the development of RIF resistance. It is worth mentioning that the isolate was not exposed to DAP before. The genetic mechanisms underlying this phenomenon are unknown.¹⁸

In conclusion, our *in vitro* study failed to demonstrate synergy between DAP plus RIF, against our RIF-resistant MRSA isolates. Daptomycin plus RIF was repeatedly antagonistic by TKA for one isolate. Even

though *in vitro* antagonism, may or may not predict *in vivo* antagonism, clinical failure on this combination should prompt the clinician to consider antagonism as one of the potential causes. More RIF-resistant MRSA isolates should be tested to determine if other isolates demonstrate *in vitro* DAP plus RIF antagonism.

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References

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