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Dynamic Drug Combination Response on Pathogenic Mutations of *Staphylococcus aureus*

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Abstract. Methicillin resistant *Staphylococcus aureus* (MRSA) is a common nosocomial pathogen, causing serious complications around the world. As it is resistant to most of the contemporary antibiotics, *S. aureus* resistance is emerging as a global health concern. Therefore, there is need for new alternative compounds or strategies that are effective against MRSA infections and in preventing or delaying its resistance. In this study, combinatory effects of tannic acid (phytochemical) and fusidic acid (antibiotic) against MRSA was studied. The synergistic combinatory pair was used to understand dynamic drug combination response hypothesis on adaptive resistance of *S. aureus*. The resistance patterns (if any) were evaluated by determining minimal inhibitory concentrations and population analysis profiles of the strain under dynamic combination influence continuously for ten days. Based on the results of the initial experiments, it was found that the effectiveness of dynamic drug concentration method was largely dependent on the starting combination of phytochemical and antibiotic. A starting combination with higher concentration of phytochemical had shown higher degree of resistance reduction from the start compared to higher concentration of antibiotic.

Keywords: antibiotic resistance, *Staphylococcus aureus*, MRSA, antibiotic combinations, phytochemical, synergy, dynamic drug response

1. Introduction

Dynamic drug concentration treatment is a treatment strategy that has been used for complex diseases and conditions such as cancer and AIDS. In cancer treatments, the doses are varied based on the clinical response of patients. The application of this strategy has shown to achieve better treatment efficacies and clinical outcomes in these conditions [1, 2]. In the case of multi-drug resistant bacteria like *S. aureus,* that causes serious complications and infections, especially in hospitalized patients, dynamic drug treatment has not been tested yet. MRSA has shown resistance to various antibiotic classes across the board [3]. It has been nicknamed 'superbug' by clinicians [4] because of its rapid adaptive resistance through several mechanisms. Many strategies including antibiotic combination therapy has been applied to combat resistance in MRSA. However, resistant species are still emerging and reported around the world. For example, recently many species of vancomycin resistant MRSA have been reported from various parts of the world [5, 6].

New methods and strategies are hence, critically necessary to formulate to guard against such indiscriminate resistance in *S. aureus*. The use of dynamic drug treatment strategy incorporating resistance modulating agents such as phytochemicals [7], can be an alternative method for preventing resistance. In this study, the proposed strategy was studied on a strain of MRSA using tannic acid (phytochemical) and fusidic acid (antibiotic). The two drugs are known to interact in synergy and able to delay resistance under static drug influence.

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2. Materials and Methods

ATCC MRSA 43300 was used as the test strain. Purified powders of fusidic acid and tannic acid were purchased from Sigma, Singapore. Iso-Sensitest (IS) (Biomedia, Singapore) broth and agar was used as liquid and solid media, respectively.

The MIC of the drugs was determined by broth microdilution method described by Andrews [8]. The drug interaction was interpreted by the checkerboard assay explained elsewhere [9]. Based on the fractional inhibitory concentration index (FICI), the drug interaction was interpreted. The time-kill assay was performed on the static combination concentration of the two drugs and resistance profiles were determined by examining their MIC and population analysis profile (PAP) over time.

The dynamic drug response experiments were formulated with starting combination of 80-20%, 60-40%, 40-60% and 20-80%, of MIC of phytochemical and antibiotic established earlier for the parent strain. All combinations added up to 100% drug dosage, that quantitatively equate to MIC of the phytochemical or antibiotic that amounted to bactericidal response in the time-kill assay.

Figure 1 illustrates the four dynamic drug combination strategies used in the study, with different starting concentrations. The blue curve shows the static drug combination response for 10 cycles of incubation (1.1, 2.1, 3.1 and 4.1), where as the green and red curves show the dynamic responses (1.2, 2.2, 2.3, 3.2, 3.3 and 4.1).

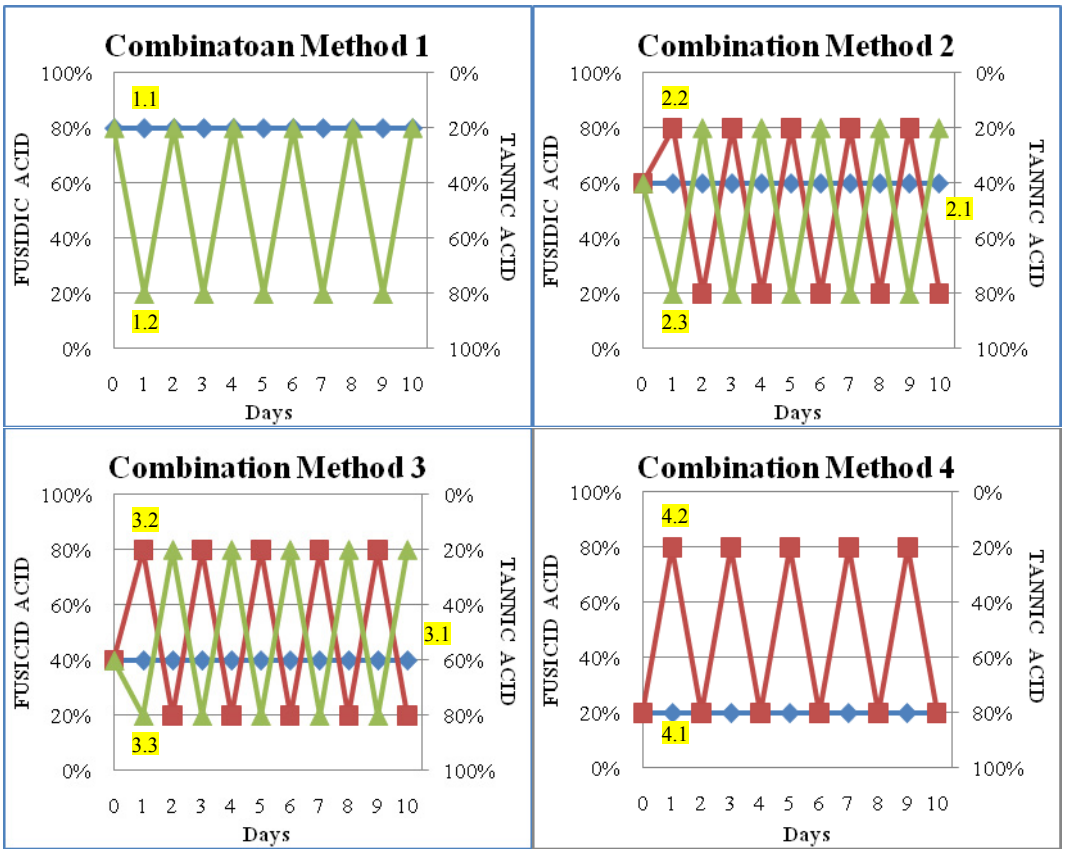

Fig. 1: Summary of the strategies tested for dynamic drug combination response. Each line graph represents the combination used in a particular cycle of incubation and each point indicates combination of fusidic acid and tannic acid. (1 incubation cycle equaled 24 hours).

3. Results

The MICs of the tannic acid and fusidic acid was 256 µg/ml and 0.03125 µg/ml, respectively. The FICI determined for the combination was 0.5, which was interpreted as synergistic. The time-kill assay also established synergy of the two drugs (results not shown).

Table 1 shows the MICs of MRSA at the end of the 10th cycle after the dynamic treatment was applied to the parent MRSA strain (see Figure 1).

Table 1. MIC of MRSA strain after 10th Cycle of each method

Methods*	**×MIC after 10th Cycle**	**Methods***	**×MIC after 10th Cycle**
Method 1.1 (s)	16	Method 3.1 (s)	2
Method 1.2 (d)	8	Method 3.2 (d)	4
Method 2.1 (s)	8	Method 3.3 (d)	2
Method 2.2 (d)	8	Method 4.1 (s)	1
Method 2.3 (d)	4	Method 4.2 (d)	1
100% FA (s)	16	100% TA	1
Positive Control (no drug)	1		
*s – static response, d – dynamic response, TA – Tannic acid, FA – Fusidic acid. (Refer Fig. 1 for methods)			

The PAP results of the strains exposed to dynamic drug combinations are shown in Figure 2.

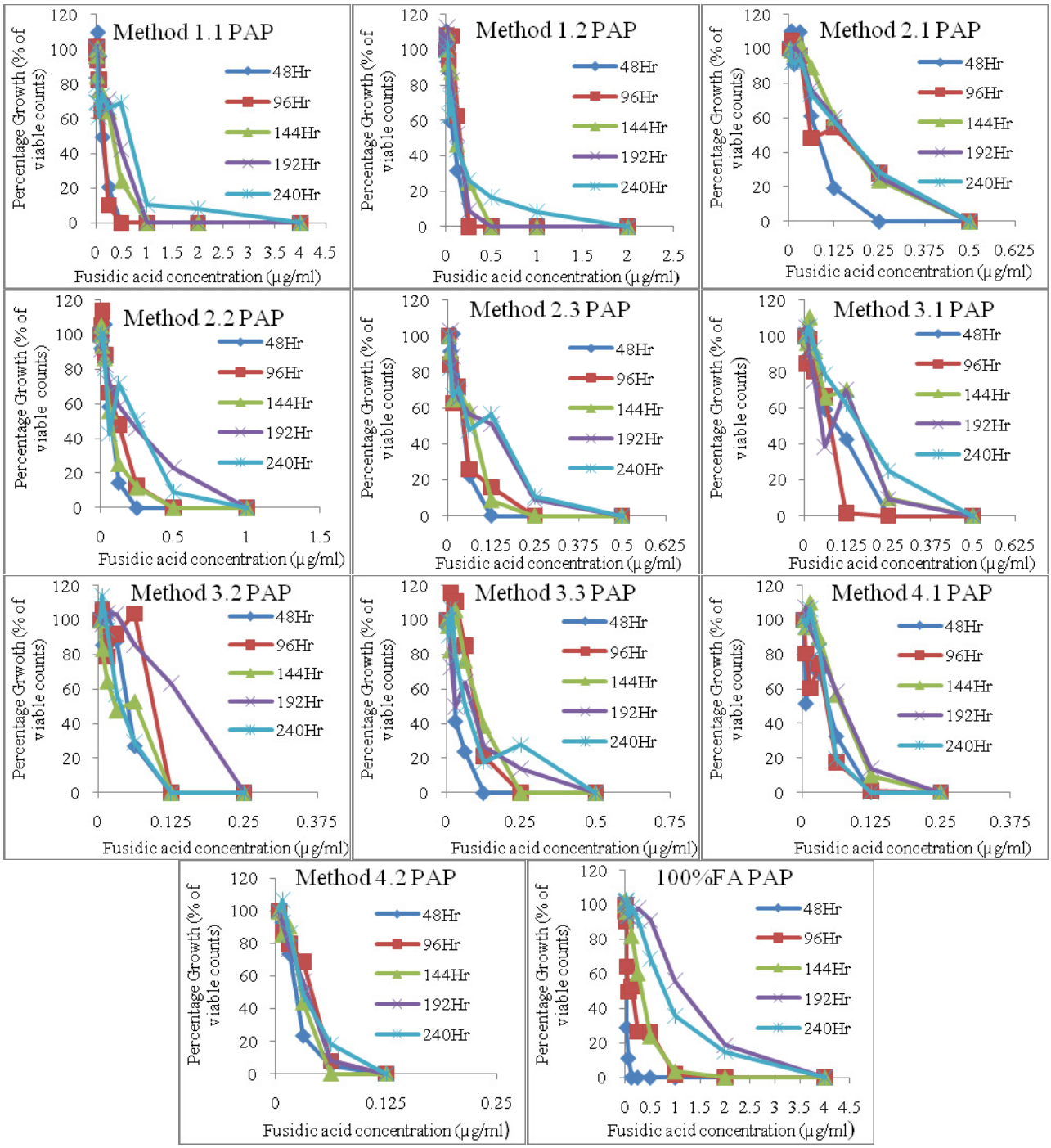

Fig. 2: PAPs of the various methods described in Figure 1 with 100% fusidic acid serving as control. (FA – Fusidic acid)

Based on the most effective method of the dynamic drug combination strategy, a semi-empirical formulation can be proposed using MatLab (R2009a) software. The formulation is explained in Figure 3 and given in Equations 1, 2 and 3.

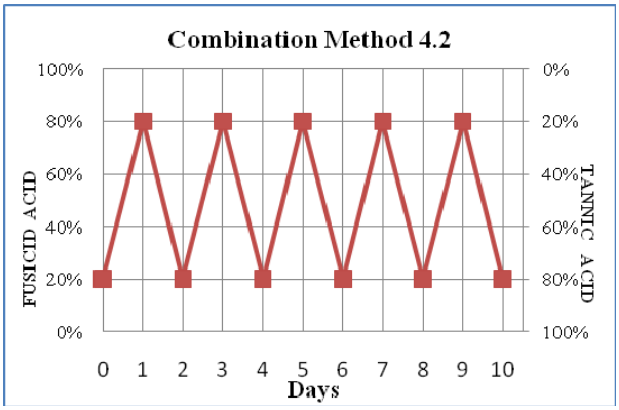

Fig. 3: Formulation of empirical model based on the most effective dynamic drug combination method

Based on the Figure 3, for Drug A (fusidic acid),

$Y = (-0085X^{10}) + (0.4233X^{9}) - (9.0794X^{8}) + (109.2063X^{7}) – (807.6444X^{6}) + (3.7849 \times 10^{3} \times X^{5}) – (1.1150 \times 10^{4} \times X^{4}) + (1.9706 \times 10^{4} \times X^{3}) - (1.8693 \times 10^{4} \times X^{2}) + (7.1192 \times 10^{3} \times X) + 20$ [Eq. 1]

Based on Figure 3, for Drug B (tannic acid),

$Y = (0085X^{10}) - (0.4233X^{9}) + (9.0794X^{8}) - (109.2063X^{7}) + (807.6444X^{6}) - (3.7849 \times 10^{3} \times X^{5}) + (1.1150 \times 10^{4} \times X^{4}) - (1.9706 \times 10^{4} \times X^{3}) + (1.8693 \times 10^{4} \times X^{2}) - (7.1192 \times 10^{3} \times X) + 80$ [Eq. 2]

Dynamic Drug Combination Model = Eq. 1 + Eq. 2 [Eq. 3]

The above formula can be used for different drug combinations of A and B as antibiotic and phytochemical, respectively.

4. Discussion

The synergistic combinatory pair of tannic acid and fusidic acid was proposed as a potential pair to reduce adaptive resistance in *S. aureus*. Different dynamic combination concentrations were used over a period of ten days and resistance profiles were determined.

MIC of the antibiotic, fusidic acid was relatively higher at the end of the 10^{th} cycle than those of the strains exposed to the other test methods. Based on the dynamic response, the methods starting with higher concentration of tannic acid showed reduced MIC values compared with those starting with higher concentration of fusidic acid. Tannic acid MIC was relatively stable and unchanged after 10 cycles, even though the MIC of fusidic acid was increased up to 16 folds except in Methods 4.1 and 4.2 where MICs of tannic acid and fusidic acid were unchanged.

It was also observed that higher proportion of tannic acid (as in Methods 4.1 and 4.2) significantly prevented resistance with higher efficacies, as seen in the results with unchanged MICs (see Table 1) and narrow PAP. Compared with other methods, combination of tannic acid and fusidic acid with different proportions could also delay resistance at similar extent compared to using single agents. Therefore, it can be suggested that dynamic combinations of phytochemical and antibiotic could help to increase the effectiveness of a treatment by delaying the process of development of resistance in MRSA. Even though the phytochemical itself could provide stable MICs throughout the 10 cycles, the higher MIC values of phytochemicals in general, make them impractical to be used alone in a clinical scenario. Using it as an additive for resistance modulation as suggested by Sibanda [7], with western antibiotics would help to increase the efficacy of a treatment using combination therapy.

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6. References

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