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Full Length Research Paper

Prevention of emergence of fusidic acid and rifampicin resistance in *Staphylococcus aureus* using phytochemicals

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major causes of nosocomial and community infections. It has shown resistance to most of the currently available antibiotics and nicknamed 'super bug'. Anti-staphylococcal activities of tannic acid, quercetin and gallic acid ethyl ester in combination with fusidic acid and rifampicin were determined against five strains of *S. aureus*, including three clinical strains. Tannic acid and quercetin were found to be synergistic with fusidic acid and rifampicin. The effects of these combinatory pairs on the adaptive resistance of *S. aureus* were also studied. The strains were studied for ten incubation cycles under continuous influence of fusidic acid/rifampicin alone and in combination with fixed dose of phytochemicals. The minimum inhibitory concentrations (MIC) of the exposed strains were determined after every cycle to study their resistance to the antibiotic. Based on the results at the end of the tenth cycle, the fusidic acid/rifampicin exposed strains gradually selected for resistance at higher MIC values. On the other hand, the combination exposed strains demonstrated stable MIC values for the antibiotics. The results suggested prevention or delay of fusidic acid and rifampicin resistance by adding synergistic phytochemicals.

Key words: *Staphylococcus aureus,* antibiotic resistance, antibiotic combination, phytochemicals.

INTRODUCTION

Staphylococcus aureus particularly methicillin-resistant *S. aureus* or MRSA infections pose serious clinical problems in hospitals as well as community (Yu et al., 2005). It is resistant to most of the antibiotic classes currently in use (Sakoulas and Moellering, 2008). Glycopeptides have been the last in the line of antibiotics used to treat serious MRSA infections. However, there have been reports about vancomycin resistant strains in recent years (Sievert et al., 2008; Tiwari and Sen, 2006).

Although, the new drug discovery projects are currently underway and MRSA resistance problems are still common, to consider alternative drug treatments or methods to combat resistance. Phytochemicals have been long known as nutrient supplements for well being and curing diseases. Many reports about these phyto-chemicals as antibacterial, antiviral, anti-carcinogens have been published in the past (Bele et al., 2010; Cowan, 1999; Yang et al., 2006). As antibacterial agents, phytochemicals are known to disrupt or modulate the resistance mechanism of pathogenic bacteria with their multi-targeted action (Sibanda and Okoh, 2007). Therefore, the use of the phytochemicals as alternative additives for treatment of multi-drug resistant infections would help in increasing the efficacy of the treatment. In this study, selected multi-target phytochemicals were used in combination with conventional antibiotics known to

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Abbreviations: MRSA, Methicillin-resistant *Staphylococcus aureus;* **MIC,** minimum inhibitory concentrations; **CLSI,** clinical and laboratory standards institute; **MSSA,** methicillin sensitive *Staphylococcus aureus;* **NUH,** national university hospital; **IS,** iso-sensitest; **FIC and FICI,** fractional inhibitory concentration and index; **CFU,** colony forming units; **DNA,** deoxyribonucleic acid; **PAP,** population analysis profile.

Table 1. Minimum inhibitory concentrations (MICs) of fusidic acid (FA), rifampicin (Rif), tannic acid (TA), gallic acid ethyl ester (GA) and Quercetin (Quer) for 5 *S. aureus* strains.

Strain	MIC (µg/ml)				
	Rif	FA	TA	GA	Quer
Staphylococcus aureus MRSA 43300	0.016	0.0313	256	1024	512
MSSA 29213	0.004	0.0313	256	1024	512
Clinical strain C1	0.008	0.25	256	1024	512
Clinical strain C2	0.016	0.25	256	1024	512
Clinical strain C3	0.016	0.25	256	1024	512

FA: fusidic acid; Rif: rifampicin; TA: tannic acid; GA: gallic acid ethyl ester; quer: quercetin.

to induce resistance in common pathogens. Phytochemicals, tannic acid or tannin, quercetin and gallic acid ethyl ester with antibacterial properties (Cowan, 1999). These were used alone and in combination with antibiotics, fusidic acid and rifampicin. In addition, the effects of these combinatory pairs on the evolution of resistant formation against fusidic acid and rifampicin were studied. Conventional methods prescribed by Clinical and Laboratory Standards Institute (CLSI), were used to determine drug interactions in combination. Population profiling was done to analyse the resistant subpopulations in the parent strain formed under drug and drug combination influence. Dynamic and static dose responses were also studied over a period of ten days to obtain efficient outcomes.

MATERIALS AND METHODS

Microorganisms and antimicrobial agents

Five stains of *S. aureus* (MRSA 43300, methicillin sensitive *Staphylococcus aureus* (MSSA) 29213 and three MRSA clinical strains) were used in this study. Clinical strains were obtained from National University Hospital (NUH, Singapore). The microorganisms were cultured at 37°C in Iso-Sensitest (IS) broth and agar media (Oxoid, England) with aeration. All antibiotics and phytochemicals were purchased from Sigma (USA) except gallic acid ethyl ester (99% purity), that was purchased from Acros Organics (New Jersey, USA). Quercetin (98%), tannic acid (98%) and fusidic acid (98%) was used in this study.

Susceptibility testing

MICs were determined in IS broth by broth microdilution method adopted from Andrews (2001), based on CLSI guidelines (Sakharkar et al., 2009). The MIC was defined as the lowest concentration of drug that inhibited bacterial growth following incubation for 18 to 24 h at 37°C for the colony count of 10^4 to 10^5 CFU/ml (Zhao et al., 2003). Each assay was performed in triplicates.

Combination studies (checker board assay)

Fractional inhibitory concentration and index (FIC and FICI) of the drug combinations was determined by the checker board assay. Testing was performed using 96-well microtiter plates (Sakharkar et al., 2009; Tin et al., 2009). Each assay was performed in triplicates. Interpretation of results was based on standard protocols regarding FIC and FICI values (Sakharkar et al., 2009; Schwalbe et al., 2007; Tin et al., 2009; Tre-Hardy et al., 2008).

Determination of resistance

For analysis of spontaneous antibiotics (fusidic acid or rifampicin) resistance, large inocula (ca. 10^9 colony forming units (CFU) of the test strains were serially diluted and spread on agar plates with increasing concentration of antibiotics. The plates were then incubated aerobically for 48 h at 37°C before the colonies were counted. The population curves were plotted by calculating and plotting the number of cells against the concentration of the drug (Entenza et al., 2010; Pfeltz et al., 2001). Colonies isolated from antibiotics containing agar plates were re-tested for MIC values. For progressive resistance selection, bacteria were exposed in IS broth with stepwise two-fold serial increasing concentration of fusidic acid/rifampicin alone for the total of 10 consecutive days, as described previously (Entenza et al., 2010)..Briefly, a series of tubes containing two-fold increasing concentration of antibiotics were inoculated with 10^7 CFU/ml (final concentration). Following 24 h incubation at 37°C, 0.1ml samples from the tubes containing the highest antibiotic concentration and still showing turbidity were used to inoculate a new series of tubes containing similar antibiotic dilutions. To examine the potential of secondary agents (phytochemicals) in suppressing the emergence of the antibiotic resistance, experiments were performed as described above in the presence of 0.25 × MIC of the phytochemicals (quercetin, tannic acid and gallic acid ethyl ester). Sub-MIC levels were used to allow bacterial growth in the presence of the partner antibiotic. The MICs for each antibiotic were determined after each incubation cycle. The MIC for the phytochemical was also determined for the resistant isolates selected by exposure to antibiotic alone or in combinations. Each experiment was performed in duplicates. The stability of resistant isolates was accessed by serial passage of the organisms on antibiotic-free medium for 5 consecutive incubation cycles.

RESULTS

Antimicrobial susceptibilities of bacterial strains are shown in Table 1. Rifampicin MICs ranged from 0.004 to 0.016 µg/ml and fusidic acid MICs ranged from 0.031 to 0.25 µg/ml. Fusidic acids was less active against clinical strains even though all strains tested showed susceptible. Phytochemicals, tannic acid, gallic acid ethyl ester and quercetin were found to be effective against *S. aureus* with tannic acid being the most effective agent. The MIC

Table 2. Fractional inhibitory concentration and indices (FIC and FICI) of fusidic acid and rifampicin in combination with tannic acid (TA), quercetin (Quer) and gallic acid ethyl ester (GA) against 5 *S. aureus* strains.

Antimicrobial agent	MRSA 43300	MSSA 29213	C1	C2	C3	Remark
FA + TA	0.5	0.375	0.5	0.5	0.5	Synergism
FA + Quer	0.375	0.375	0.5	0.5	0.5	Synergism
FA + GA	0.75	0.75	1	1	1	Additive
Rif + TA	0.5	0.5	0.5	0.5	0.5	Synergism
Rif + Quer	0.375	0.375	0.5	0.5	0.5	Synergism
Rif + GA	1	0.75	0.75	1	1	Additive

FA: fusidic acid; Rif: rifampicin; TA: tannic acid; GA: gallic acid ethyl ester; quer: quercetin.

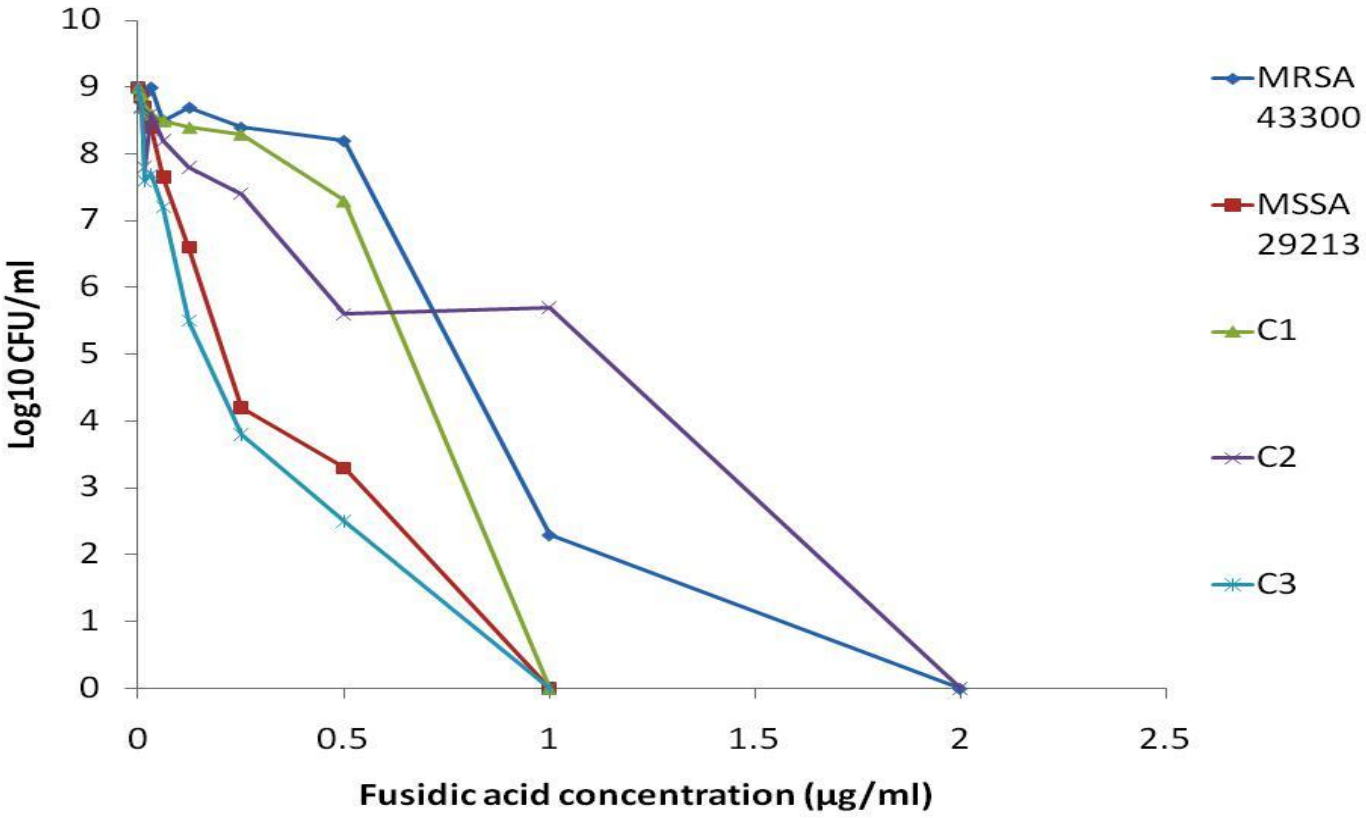

Figure 1. Population analysis profile (PAP) for fusidic acid against 5 *S. aureus* strains (MRSA 43300, MSSA 29213 and 3 MRSA clinical strains: C1, C2 and C3).

of tannic acid was much lower than gallic acid and quercetin (Table 1). For each test performed, MICs of MSSA 29213 were within the acceptable range of susceptibility. The FICI was used to qualitatively analyse the combinations of antibiotic with phytochemicals, these are tabulated in Table 2. Fusidic acid and rifampicin showed synergism with tannic acid and quercetin, and additivity with gallic acid ethyl ester against all tested strains. Resistance in the 5 strains was determined by Population analysis profile (PAP), MIC and dynamic drug response studies, by exposing parent strains to stepwise increasing drug and drug combination dilutions. To search for the presence of spontaneous antibiotics-resistant subpopulation, large bacterial inocula (10^9 CFU/ml) were spread on fusidic acid and rifampicin containing agar plates. Population analyses of fusidic acid and rifampicin were shown in Figure 1 and 2.

All *S. aureus* strains tested were susceptible to fusidic acid and rifampicin showing heterogeneous resistance, with marginal bacterial growth at concentrations greater than MICs. No growth was observed at concentration ≥ 2 µg/ml for both fusidic acid and rifampicin. The ten cycle

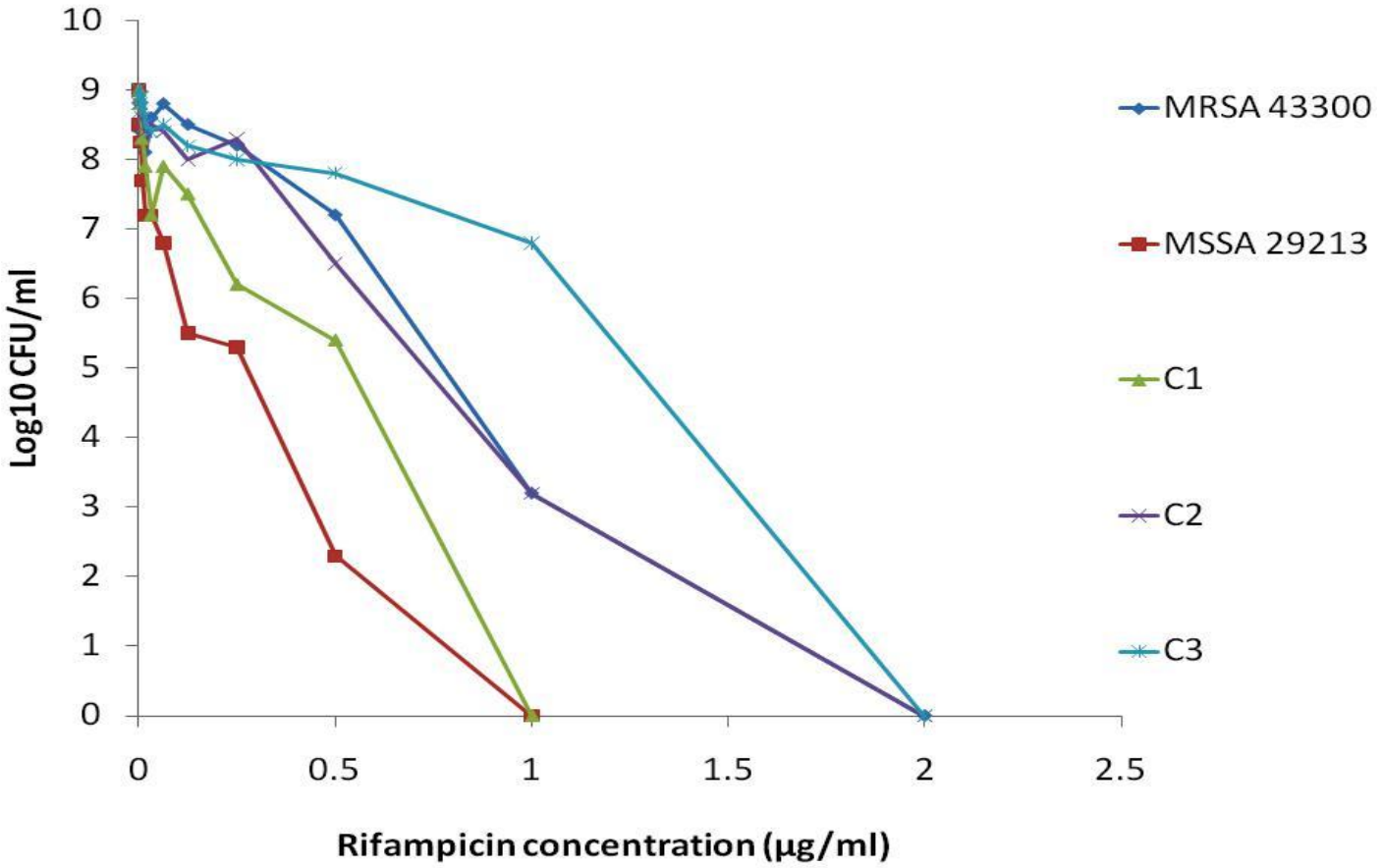

Figure 2. Population analysis profile (PAP) for rifampicin against *S. aureus* strains (MRSA 43300, MSSA 29213 and 3 MRSA clinical strains: C1, C2 and C3).

exposure of the 5 strains of *S. aureus* to fusidic acid in combination with the three phytochemicals is shown in Figure 3. The graphs show resistance selection during cyclic exposure of bacteria to two-fold stepwise increasing concentration of fusidic alone or in combination with fixed concentration (0.25×MIC) of phytochemicals (TA, Quer and GA). Serial exposure to fusidic acid alone resulted in development of resistant strains. In contrast, addition of fixed concentration of tannic acid, quercetin and gallic acid could greatly delay or prevent the occurrence of resistance, as observed in the graphs. A similar experiment with serial stepwise drug and drug combination exposure was carried out with rifampicin and the three phytochemicals at fixed dose (0.25×MIC). The graphs are shown in Figure 4. Likewise, the serial exposure of rifampicin alone could not prevent the emergence of resistance isolates. As in the case of fusidic acid and combinations, the addition of fixed concentration of the phytochemicals could greatly be delay or prevent the development of resistance. Table 3 summarizes the changes in MIC of the antibiotics (fusidic acid and rifampicin) at the end of the tenth cycle with and without the addition of phytochemicals. It can be observed from the table that fusidic acid combined with fixed concentration (0.25 × MIC) of tannic acid and quercetin resulted in no changed in MIC in all of 5 strains tested after 10^{th} cycle. Fusidic acid in combination with fixed dose gallic acid ethyl ester resulted in slightly changed MIC (up to 2 fold increase) starting from 8 to 10^{th} cycle depending on the strains. Strains exposed only to fusidic acid resulted in increase in its MIC, up to 16 folds. Fusidic acid MIC was not changed in combination with fixed concentration of tannic acid and quercetin. There was a 2 fold increased in fusidic acid MIC observed in combination with gallic acid ethyl ester in all tested strains. Similar observations were made with rifampicin, the increase observed in rifampicin MIC after the 10 cycles of exposure to the antibiotic only was up to 16 folds.

DISCUSSION

Combination therapies have been used clinically with the aim of increasing treatment efficacy and decreasing the chances of emergence of drug-resistant mutants. There

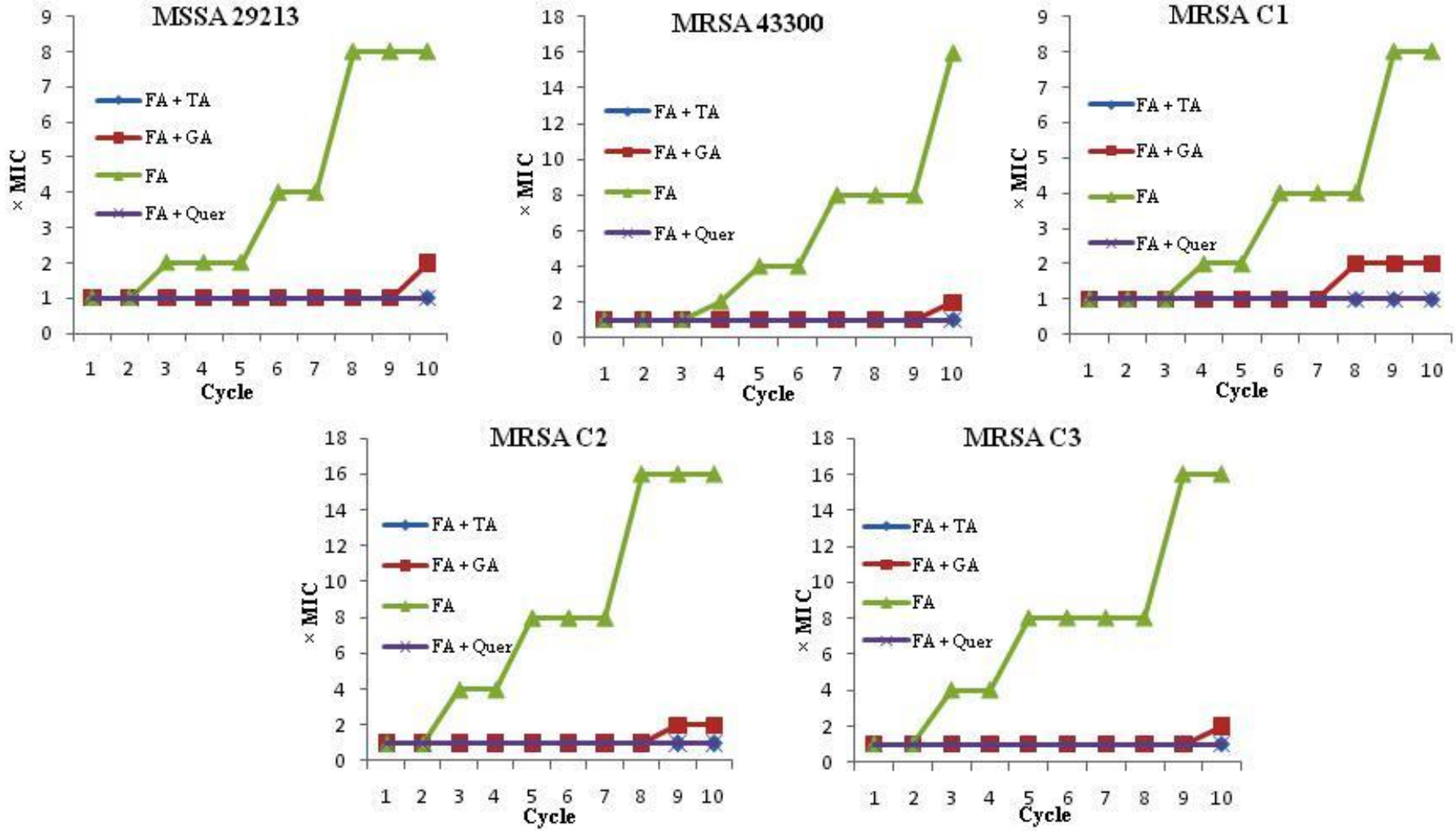

Figure 3. Selection of fusidic acid resistant *S. aureus* after serial exposure to fusidic alone or in combination with 0.25 × MIC of tannic acid, gallic acid ethyl ester and quercetin.

are no or very few reports on combination therapies using western antibiotics and phytochemicals that could delay the emergence of resistance in *S. aureus* strains (Soe et al., 2011). Entenza et al. (2010) reported that emergence of daptomycin resistance in MRSA and enterococci could be prevented or delayed by combining it with fixed concentration (sub-MIC) of ampicillin (Entenza et al., 2010). However, the benefits of using western antibiotics in combinations for resistance prevention are limited due to higher chances of resistant mutant selection. Therefore, the use of alternative antibacterial compounds that can modulate or modify resistance causing mechanisms can be more effective in combinations

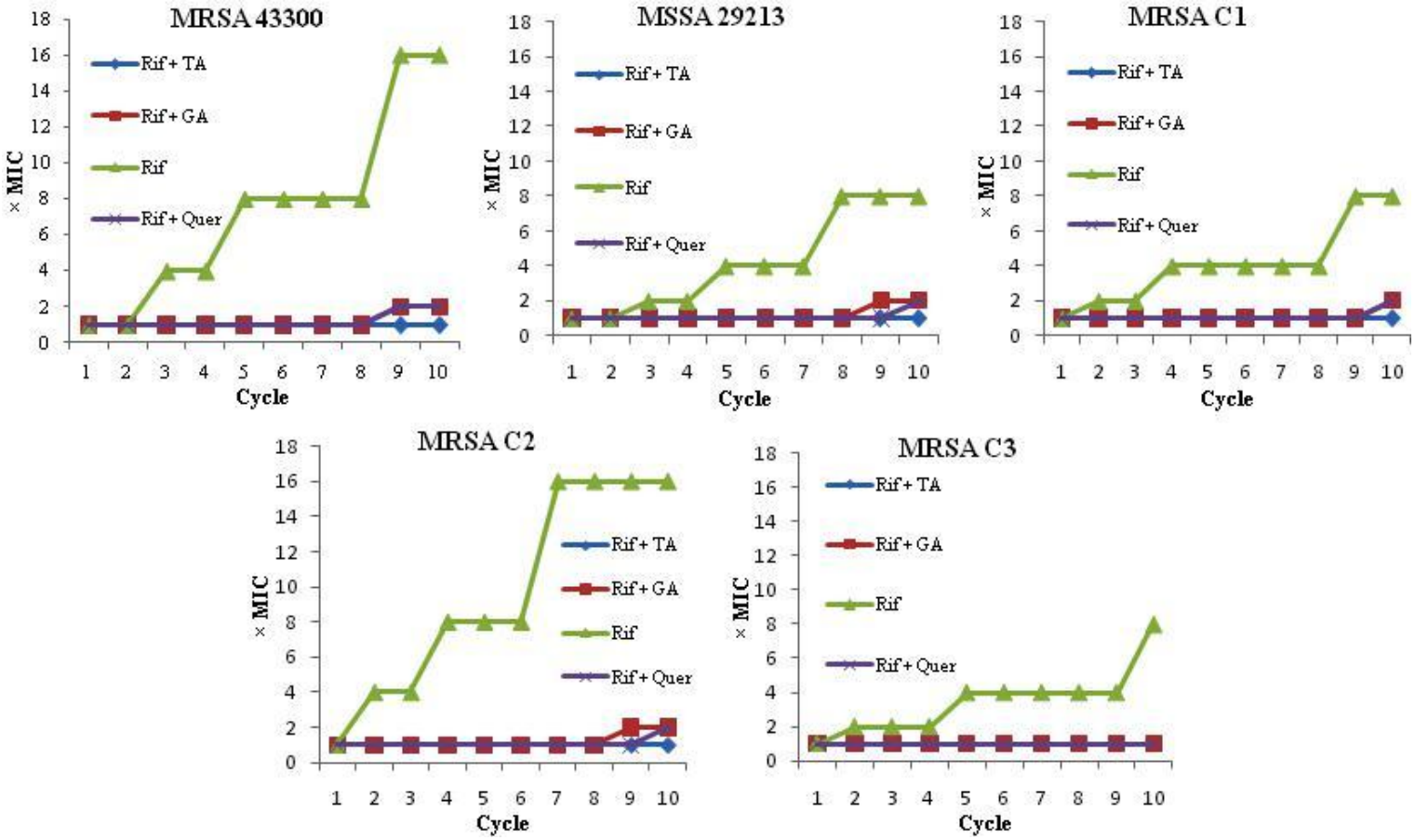

Figure 4. Selection of Rifampicin resistant *S. aureus* after serial exposure to fusidic acid alone or in combination with 0.25 × the minimum inhibitory concentration (MIC) of tannic acid, gallic acid ethyl ester and quercetin.

Table 3. Differences between MICs of western drug alone and in combination with phytochemicals after 10th continuous cycle.

Strain	Original MIC of FA (µg/ml)	MIC (×original MIC) of FA at the 10th cycle with/without phytochemicals				Original MIC of Rif (µg/ml)	MIC (×original MIC) of Rif at the 10th cycle with/without phytochemicals			
		TA	Quer	GA	None		TA	Quer	GA	None
MSSA 29213	0.0313	1	1	2	8	0.004	1	2	2	8
MRSA 43300	0.0313	1	1	2	16	0.016	1	2	2	16
C1	0.25	1	1	2	8	0.008	1	2	2	8
C2	0.25	1	1	2	16	0.016	1	2	2	16
C3	0.25	1	1	2	16	0.016	1	1	1	8

FA: fusidic acid; Rif: rifampicin; TA: tannic acid; GA: gallic acid ethyl ester; quer: quercetin.

for treatment of infections. Phytochemicals usually have broad-spectrum of functions for the well being of the plant. They possess antibacterial, anti-viral and antifungal, antioxidant and anti-carcinogenic properties, depending on the subgroups to which they belong (Cowan, 1999). Tannic acid, quercetin and gallic acid ethyl ester are phytochemicals that have broad-spectrum antibacterial action (Akiyama et al., 2001; Bele et al., 2010; Chung et al., 1998; Gatto et al., 2002; Hatano et al., 2005; Soe et al., 2010a; Soe et al., 2010b).with multi-targeted approach (Sibanda and Okoh, 2007).

As illustrated in Figure 3 and 4, the stepwise serial exposure of *S. aureus* to fusidic acid (and rifampicin, respectively) for ten days developed resistance to the drug with 8 to 32 fold increase in the MIC from the original MIC of the parent strain. However, with the fixed dose addition of phytochemicals at 0.25×MIC to the stepwise dilution array of the antibiotic, there was no or slight increase (up to 2×MIC) in MIC of the western drug (Table 3). The results clearly suggest a delay in the process of development of resistance to fusidic acid (and rifampicin) in *S. aureus* with the addition of phyto-chemicals at fixed doses. Howden and Grayson (2006) reported the MIC range of susceptibility for fusidic acid for non-resistant stains as ≤ 0.5 µg/ml and for resistant strains as ≥ 2 µg/ml (Howden and Grayson, 2006). The MIC of fusidic acid for all strains was within the susceptibility range at the start. However, at the end of the tenth cycle the MIC of fusidic acid for strains exposed to the antibiotic alone raised to ≥ 0.5 µg/ml, to the intermediate range. Although, their MICs were below the cut-off for resistance, the observed increase in MIC at the end of the tenth cycle was up to 16 folds from the original MIC (Table 3). On the other hand, there was no or 2 fold increase in MIC for strains exposed to the combination with phytochemicals at fixed dose. Therefore, the addition of phytochemicals had a potentiating effect on the activity of fusidic acid. Fusidic acid resistance is mainly caused by alterations in the elongation factor G required in protein synthesis (Jones et al., 2010; Turnidge and Collignon, 1999).

The elongation factor is associated with the translocation step of the translation process, which helps the elongation of the polypeptide chain. Chopra reported that *S. aureus* resistant mutant selected from fusidic acid containing media had altered elongation factors (Chopra, 1976). Mason et al. (2003) also reported significant association between high rates of resistance in MSSA isolates to fusidic acid with the use of topical ointments containing fusidic acid (Mason et al., 2003). The use of fusidic acid in appropriate combinations with other antibiotics may help in the reduction of rate of resistance (Howden and Grayson, 2006). Fusidic acid combined with other antibio-tics like, rifampicin, *β*-lactams and glycopeptides haven been shown to be effective against *S. aureus* that causes bacteraemia, endocarditis and osteomyelitis (Whitby, 1999a, b). However, antibiotic combinations may still have disadvantages of higher side effects and higher probability of development of resistance. The exact mechanism of potentiation of the activity of fusidic acid with the addition of phytochemicals is unknown. How-ever, the multi-targeted antibacterial compounds may have synergistic approach for disrupting major cellular functions that brings about cell lysis. The bacterial targets of tannic acid include enzyme inhibition (Brownlee et al., 1990; Cowan, 1999; Haslam, 1996), bind to specific proteins (Cowan, 1999; Stern et al., 1996), bind to adhesions, complex with cell walls, substrate deprivation, membrane deprivation and metal ion complexation (Cowan, 1999).

Quercetin acts on prokaryotic DNA gyrase that inhibits deoxyribonucleic acid (DNA) replication and eventually cell death (Plaper et al., 2003). It also affects the permeability of the cell wall, allowing easy passage to other drugs (Ramos et al., 2006). Gallic acid ethyl ester alters the liquidity of the lipid bilayer of the cell membrane due to its amphiphatic structure (Kubo et al., 2003). This alters the membrane permeability and eventually causes cell lysis. Rifampicin is the first line drug for the treatment of tuberculosis. It can be used in combination with fusidic acid (O'Neill et al., 2001), sulfamethoxazole-trimethoprim (Yamaoka, 2007), minocycline, vancomycin (Neogi et al., 2009) and daptomycin (Lefebvre et al., 2010), against Gram-positive bacterial infections especially caused by MRSA. Rifampicin is recommended to be used in combination with other drugs, as rifampicin monotherapy

is associated with rapid adaptive resistance (Howden and Grayson, 2006; Neogi et al., 2009; Neill et al., 2001; Yamaoka, 2007). Neogi et al. (2009) reported that rifampicin resistance may occur in clinical practice despite combinations with minocycline, fusidic acid or vancomycin (Neogi et al., 2009). Therefore, the development of resistance is not ruled out completely even when used in combination with other antibiotics.

Conclusion

In this study, the authors showed a delay in the development of otherwise rapid adaptive resistance in MRSA strains, with the addition of phytochemicals. The delay was about 3 to 5 times the duration of resistance adaptation otherwise induced for western antibiotics alone. These compounds with known anti-*Staphylococcal* activities could prevent or delay the emergence of resistance when used with antibiotics. Therefore, combination therapy of fusidic acid and rifampicin with tannic acid, quercetin and gallic acid ethyl ester can be potentially used as drug combinations regimes to attain higher treatment efficacy for MRSA.

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REFERENCES

Akiyama H, Fujii K, Yamasaki O, Oono T, Iwatsuki K (2001). Antibacterial action of several tannins against *Staphylococcus aureus*. J. Antimicrob. Chemother. 48(4): 487-491.

Andrews JM (2001). Determination of minimum inhibitory concentrations. J. Antimicrob. Chemother. 48(suppl 1): 5-16.

Bele AA, Jadhav VM, Kadam VJ (2010). Potential of tannnins: A review. Asian J. Plant Sci., 9(4): 209-214.

Brownlee H, McEuen A, Hedger J, Scott I (1990). Anti-fungal effects of cocoa tannin on the witches' broom pathogen *Crinipellis perniciosa*. Physiological and Molecular Plant Pathol., 36(1): 39-48.

Chopra I (1976). Mechanisms of resistance to fusidic acid in Staphylococcus aureus. J. Gen. Microbiol. 96(2): 229-238.

Chung KT, Lu Z, Chou MW (1998). Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. Food Chem. Toxicol., 36(12): 1053-1060.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12(4): 564-582.

Entenza JM, Giddey M, Vouillamoz J, Moreillon P (2010). *In vitro* prevention of the emergence of daptomycin resistance in *Staphylococcus aureus* and enterococci following combination with amoxicillin/clavulanic acid or ampicillin. Int. J. Antimicrob. Agents. 35(5): 451-456.

Gatto MT, Falcocchio S, Grippa E, Mazzanti G, Battinelli L, Nicolosi G, Lambusta D, Saso L (2002). Antimicrobial and anti-lipase activity of quercetin and its C2-C16 3-O-acyl-esters. Bioorg. Med. Chem., 10(2): 269-272.

Haslam E (1996). Natural polyphenols (vegetable tannins) as drugs: Possible modes of action. J. Nat. Prod., 59(2): 205-215.

Hatano T, Kusuda M, Inada K, Ogawa TO, Shiota S, Tsuchiya T, Yoshida T (2005). Effects of tannins and related polyphenols on methicillin-resistant *Staphylococcus aureus*. Phytochemistry. 66(17 SPEC. ISS.): 2047-2055.

Howden BP, Grayson ML (2006). Dumb and dumber - The potential waste of a useful antistaphylococcal agent: Emerging fusidic acid resistance in *Staphylococcus aureus*. Clin. Infect. Dis.,. 42(3): 394-400.

Jones RN, Biedenbach DJ, Roblin PM, Kohlhoff SA, Hammerschlag MR (2010). Update on fusidic acid (CEM-102) tested against Neisseria gonorrhoeae and Chlamydia trachomatis. Antimicrob. Agents Chemother. 54(10): 4518-4519.

Kubo I, Fujita K-I, Nihei K-I (2003). Molecular design of multifunctional antibacterial agents against methicillin resistant *Staphylococcus aureus* (MRSA). Bioorg. Med. Chem., 11(19): 4255-4262.

Lefebvre M, Jacqueline C, Amador G, Le Mabecque V, Miegeville A, Potel G, Caillon J, Asseray N (2010). Efficacy of daptomycin combined with rifampicin for the treatment of experimental meticillin-resistant Staphylococcus aureus (MRSA) acute osteomyelitis. Int. J. Antimicrob. Agents. 36(6): 542-544.

Mason BW, Howard AJ, Magee JT (2003). Fusidic acid resistance in community isolates of methicillin-susceptible Staphylococcus aureus and fusidic acid prescribing. J. Antimicrob. Chemother., 51(4): 1033-1036.

Neogi D, Yadav C, Vonnum P (2009). Rifampicin use in MRSA infections: will it add to the emergence of multidrug-resistant tuberculosis in developing countries? J. Infect. Chemother., 15(3): 203-204.

O'Neill AJ, Cove JH, Chopra I (2001). Mutation frequencies for resistance to fusidic acid and rifampicin in *Staphylococcus aureus*. J. Antimicrob. Chemother., 47(5): 647-650.

Pfeltz RF, Schmidt JL, Wilkinson BJ (2001). A microdilution plating method for population analysis of antibiotic-resistant staphylococci. Microbial. Drug Resist., 7(3): 289-295.

Plaper A, Golob M, Hafner I, Oblak M, Solmajer T, Jerala R (2003). Characterization of quercetin binding site on DNA gyrase. Biochem. Biophys. Res. Commun., 306(2): 530-536.

Ramos FA, Takaishi Y, Shirotori M, Kawaguchi Y, Tsuchiya K, Shibata H, Higuti T, Tadokoro T, Takeuchi M (2006). Antibacterial and antioxidant activities of quercetin oxidation products from yellow onion (*Allium cepa*) skin. J. Agric. Food Chem., 54(10): 3551-3557.

Sakharkar MK, Jayaraman P, Soe WM, Chow VTK, Sing LC, Sakharkar KR (2009). *In vitro* combinations of antibiotics and phytochemicals against *Pseudomonas aeruginosa*. J. Microbiol. Immunol., 42(5): 364-370.

Sakoulas G, Moellering RC (2008). Increasing Antibiotic Resistance among Methicillin-Resistant *Staphylococcus aureus* Strains. Clin. Infect. Dis. 46(Supplement 5): S360-S367.

Schwalbe R, Steele-Moore L, Goodwin A (2007). Antimicrobial susceptibility testing protocols. Boca Raton, FL, USA, CRC Press.

Sibanda T, Okoh AI (2007). The challenges of overcoming antibiotic resistance: Plant extracts as potential sources of antimicrobial and resistance modifying agents. Afr. J. Biotechnol., 6(25): 2886-2896.

Sievert DM, Rudrik JT, Patel JB, McDonald LC, Wilkins MJ, Hageman JC (2008). Vancomycin-resistant *Staphylococcus aureus* in the United States, 2002-2006. Clin. Infect. Dis., 46(5): 668-674.

Soe WM, Giridharan M, Lin RTP, Sakharkar MK, Sakharkar KR (2010a). Effect of combinations of antibiotics and gallates on biofilm formation in *Staphylococcus aureus*. Lett. Drug. Des. Discov., 7(3): 160-164.

Soe WM, Myint NL, Sing LC, Sakharkar MK, Tang TH, Sakharkar KR (2011). Ethyl gallate as a combination drug can overcome resistance in MRSA. Lett. Drug Des. Discov., 8(1): 65-68.

Soe WM, Tzer PLR, Chu SLL, Sakharkar KR, Sakharkar MK (2010b). In vitro drug interactions of gallates with antibiotics in *Staphylococcus aureus*. Front. Biosci. (Elite edition). 2: 668-672.

Stern JL, Hagerman AE, Steinberg PD, Mason PK (1996). Phlorotannin-protein interactions. J. Chem. Ecol., 22(10): 1877-1899.

Tin S, Sakharkar KR, Lim CS, Sakharkar MK (2009). Activity of Chitosans in combination with antibiotics in *Pseudomonas aeruginosa*. Int. J. Biol. Sci., 5(2): 153-160.

Tiwari H, Sen M (2006). Emergence of vancomycin resistant *Staphylococcus aureus* (VRSA) from a tertiary care hospital from northern part of India. BMC Infect. Dis., 6(1): 156.

Tre-Hardy M, Vanderbist F, Traore H, Devleeschouwer MJ (2008). *In vitro* activity of antibiotic combinations against *Pseudomonas aeruginosa* biofilm and planktonic cultures. Int. J. Antimicrob. Agents, 31(4): 329-336.

Turnidge J, Collignon P (1999). Resistance to fusidic acid. Int. J. Antimicrob. Agents. 12(SUPPL. 2).

Whitby M (1999a). Fusidic acid in septicaemia and endocarditis. Int. J. Antimicrob. Agents. 12(SUPPL. 2).

Whitby M (1999b). Fusidic acid in the treatment of methicillin-resistant *Staphylococcus aureus*. Int. J. Antimicrob. Agents, 12(SUPPL. 2).

Yamaoka T (2007). The bactericidal effects of anti-MRSA agents with rifampicin and sulfamethoxazole-trimethoprim against intracellular phagocytized MRSA. J. Infect. Chemother., 13(3): 141-146.

Yang EB, Wei L, Zhang K, Chen YZ, Chen WN (2006). Tannic acid, a potent inhibitor of epidermal growth factor receptor tyrosine kinase. J. Biochem. (Tokyo),139(3): 495-502.

Yu HH, Kim KJ, Cha JD, Kim HK, Lee YE, Choi NY, You YO (2005). Antimicrobial activity of berberine alone and in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. J. Med. Food, 8(4): 454-461.

Zhao X, Eisner W, Perl-Rosenthal N, Kreiswirth B, Drlica K (2003). Mutant Prevention Concentration of Garenoxacin (BMS-284756) for Ciprofloxacin-Susceptible or -Resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother., 47(3): 1023-1027.