MONO SULFAMETHOXAZOLE/TRIMETHOPRIM AND VANCOMYCIN COMBINATION ANTIMICROBIAL ACTIVITY AGAINST METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Abstract. Activity of trimethoprim/sulfamethoxazole (SXT) alone or in combination with vancomycin (VAN) was evaluated against methicillin-resistant *Staphylococcus aureus* (MRSA) from 26 clinical isolates from patients admitted to Hua Hin Hospital, Prachuap Khiri Khan Province, Thailand between January 2015 and December 2016. Antimicrobial susceptibility of SXT and VAN was determined using an E-test and of SXT-VAN combination by E-test and an checkerboard (isobologram) method. Clonal relationship among MRSA strains (n = 16) based on spa typing showed the existence of three types: spat045 (75%), spat439 (19%) and spat13880 (6%). All MRSA isolates were susceptible to VAN; STX MIC_{50} (minimum inhibitory concentration required to inhibit the growth of 50% of organisms), MIC90 and MIC range was 0.047, 0.064 and 0.032-≥32 μg/ml, respectively with one strain being resistant. STX-VAN combination demonstrated additive or indifferent effect against the majority of the isolates, except synergism (by checkerboard method) in two isolates. Pharmacodynamics of STX bactericidal activity against six representative strains exhibited concentration (up to 8-16X MIC) and time (over 24 hours) dependency. Thus, SXT-VAN combination provided a possible regimen against MRSA but its efficacy in clinical use has to be evaluated.

Keywords: *Staphylococcus aureus*, isobologram, MRSA, sulfamethoxazole/trimethoprim, vancomycin

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important pathogenic bacteria in both community and healthcare settings (Nickerson *et al*, 2009b). This organism plays a major role in skin/soft tissue, bloodstream and bone/joint infections as well as in pneumonia and endocarditis (Purrello *et al*, 2016). In Thailand, mortality rate related to MRSA bloodstream infection is approximately 50% (Nickerson *et al*, 2009a; Chaiwarith *et al*, 2014). Moreover,

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MRSA bacteremia is associated with extra hospitalization duration and increased medical expenditure (de Kraker *et al*, 2011).

Vancomycin (VAN), a glycopeptide antimicrobial, is the drug of choice for invasive MRSA infections for many decades (Purrello *et al*, 2016). The National Antimicrobial-Resistance Surveillance Center, Thailand (NARST) reported in 2017 most *S. aureus* stains are susceptible to VAN (NARST, 2017). However, Sakoulas *et al* (2004) and Lodise *et al* (2008) previously reported patients infected with S. aureus having VAN MIC (minimum inhibitory concentration) >1.0 μg/ml present with treatment failure and bacteremia compared to those infected with *S. aureus* having lower VAN MIC values, owing to presence of a sub-population of VAN-intermediate resistant *S. aureus* (hVISA) among VAN-susceptible strains (Musta *et al*, 2009). The emergence of MRSA with reduced susceptibility to VAN was reported in Asian countries (Song *et al*, 2004), and hVISA strains in Thailand was the first reported in 2009 (Lulitanond *et al*, 2009).

In clinical practice, as reduced susceptibility to glycopeptide antimicrobials might be encountered, several new antibiotics have been licensed for MRSA treatment, such as daptomycin, linezolid and lipoglycopeptides (Purrello *et al*, 2016). However, these drugs are not easily available in limited-resource countries and a re-evaluation of combinations of older generations of antibiotic agents should be evaluated to expand existing therapeutic options.

At present, sulfamethoxazole/trimethoprim (SXT, also known as cotrimoxazole) is the only fallback choice for MRSA infection recommended in clinical practice guideline (Liu *et al*, 2011). NARST reported, for the period of 2014-2016, MRSA strains exhibit 80% susceptibility to SXT. Interestingly, SXT combined with VAN shows synergistic and additive effects against MRSA (Silva *et al*, 2011). As no study, to the best of our knowledge, has been conducted in Thailand on MIC of SXT and effect of SXT-VAN combination against MRSA strains, these experiments were carried out on MRSA clinical isolates from a local hospital. The findings should be of value in evaluating the feasibility of treating MRSA with combination antimicrobial regimen.

MATERIALS AND METHODS

Bacteria strains

Clinical isolates of MRSA (n = 26) were obtained between January 2015 and December 2016 from various sites of infection of in-patients admitted to Hua Hin Hospital, Prachuap Khiri Khan Province, a 400-bed general hospital located in western Thailand. MRSA was identified using a disk diffusion test based on the Clinical and Laboratory Standards Institute guidelines (CLSI, 2018), *ie* isolate generating a clear zone $\leq$21 mm surrounding a cefoxitin (30 μg)-impregnated disc (Oxoid, Hampshire, UK) was identified as MRSA. *S. aureus* ATCC 25923 (courtesy from the Department of Medical Sciences Culture Collection, Ministry of Public Health, Bangkok Thailand) was used as a control strain.

The research protocol was approved by the Institutional Review Board, Hua Hin Hospital (COE007/2560, RECHHH043/2560).

Clonal relationship determination

Clonal relationship of MRSA isolates were evaluated based on *spa* sequence (Enright *et al*, 2000). DNA of MRSA

isolates was extracted using a commercial kit (Thermo Fisher Scientific, Waltham, MA) with inclusion of digestion by lysostaphin (Sigma-Aldrich, St Louis, MO). PCR mixture (60 µl) contained 4 µl of DNA, 1.6 µl of primer 1095F (5′-AGACGATCCTTCGGTGAGC-3′) and 1517R (5′-GCTTTTGCAATGTCATTT ACTG-3′) (20 μM), 30 µl of JumpStart Red Taq® Ready Mix (Sigma-Aldrich, St Louis, MO), and 23 μl of DNase-free water. Thermocycling was performed in a Biometra TGradient Thermocycler (Biometra, Gottingen, Germany) as follows: 95°C for 5 minutes; 30 cycles of 55°C for 60 seconds, 72°C for 60 seconds and 95°C for 60 seconds; followed by a final step of 72°C for 5 minutes. Amplicon was separated by 1% agarose gel-electrophoresis stained with ethidium bromide (Invitrogen, Carlsbad, CA), gel-purified using Gel/PCR DNA Fragments Extraction Kit (Geneaid, New Taipei City, Taiwan) and sequenced (1st Base, Selangor, Malaysia). Sequences were determined with the software Ridom StaphType (Ridom GmbH, Münster, Germany).

Antimicrobial assay

Susceptibilities of MRSA isolates to SXT and VAN were assessed using an E-test to obtain MICs (Liofilchem, Teramo, Italy). A broth micro-dilution method (CLSI, 2015) was performed to determine MICs of clindamycin (Bureau of Drug and Narcotic, Ministry of Public Health, Thailand), doxycycline (Bio Basic Canada Inc, Markham, Ontario, Canada) and fusidic acid (Sigma-Aldrich, St Louis, MO). Antimicrobial susceptibility was defined according to CLSI (2018) guidelines except for fusidic acid, which followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2018).

Antimicrobial combination evaluation

Two different tests were used to determine *in vitro* effect of SXT-VAN combination against MRSA isolates. The E-test consisted of placing two E-strips on a Mueller-Hinton agar (Difco Labs Inc, Detroit, MI) plate in a 90°-angle cross formation at the intersection between MICs of SXT and VAN and incubating at 35°C for 18 hours (Laishram *et al*, 2017). In the checkerboard (isobologram) method (Laishram *et al*, 2017), each MRSA isolate was incubated with various combinations of SXT and VAN in a cation-adjusted Mueller-Hinton broth (CAMHB) (Difco) at 35°C for 24 hours. Fractional inhibitory concentration index (FICI) is interpreted as follows: ≤0.5 = synergistic, 0.5 - ≤1.0 = additive, >1 - 4 = indifferent, and ≥4 = antagonistic.

Pharmacodynamic analysis of SXT

Pharmacodynamic study of SXT was performed using 1X, 4X, 8X, and 16X MIC value at inoculum of 10^5 CFU/ml in CAMHB medium (Difco). MRSA test strains were selected based *spa* typing, and bactericidal activity, reported as $\log_{10}$ reduction in colony count from that of initial inoculum, was determined at 0, 4, 8, and 24 hours (Jenkins and Schuetz, 2012).

RESULTS

Antibiogram profile and clonal relationship of MRSA isolates

MIC_{50}, MIC_{90} and MIC range of MRSA clinical isolates (n = 26) for SXT was 0.047, 0.064, and 0.032-≥32 μg/ml, respectively and for VAN was 1, 1.5 and 0.25-2 μg/ml, respectively (Table 1). Percent MRSA strains susceptible to clindamycin, doxycycline, fusidic acid, SXT, and VAN was at 4, 38, 58, 96, and 100%, respectively.

Three different *spa* types were

Table 1
Minimum inhibitory concentration (MIC) of trimethoprim/sulfamethoxazole (SXT) and vancomycin (VAN) and *spa* typing of methicillin-resistant *Staphylococcus aureus* clinical isolates obtained from Hua Hin Hospital, Prachuap Khiri Khan Province, Thailand (January 2015 - December 2016).

S. aureus isolate ID	MIC (μg/ml)		*spa* typing	*S. aureus* isolate ID	MIC (μg/ml)		*spa* typing
	VAN	SXT			VAN	SXT	
S1	1.5	0.094	ND	S14	0.38	0.032	ND
S2	1	0.064	ND	S15	2.0	0.047	spat045
S3	0.5	0.047	ND	S16	1.5	0.047	spat439
S4	1.0	0.047	ND	S17	0.38	0.38	spat13880
S5	1.5	0.064	ND	S18	1	0.047	spat045
S6	1.0	0.047	spat045	S19	0.38	0.064	spat045
S7	1.0	0.032	spat439	S20	0.75	0.047	spat045
S8	1.5	0.047	spat439	S21	1.0	0.032	spat045
S9	1.5	0.047	spat045	S22	0.5	0.047	spat045
S10	2.0	≥32	ND	S23	1.5	0.047	spat045
S11	1.0	0.032	ND	S24	0.75	0.047	spat045
S12	1.0	0.047	ND	S25	0.5	0.032	spat045
S13	0.75	0.047	ND	S26	0.25	0.047	spat045

ND: not determined.

identified from 16 MRSA isolates: spat045 (75%), spat439 (19%) and spat13880 (6%).

Antimicrobial effect of SXT-VAN combination

Among 25 MRSA isolates, E-test showed SXT-VAN combination exerted an additive and indifferent effect on 7 (28%) and 18 (72%) isolates respectively and checkerboard method on 3 (12%) and 20 (80%) isolates respectively, in addition to synergism in two (8%) isolates (Table 2). No antagonism was observed between SXT and VAN in the 25 MRSA isolates.

SXT pharmacodynamics

Based on *spa* typing, SXT pharmacodynamic profiles were analyzed for six MRSA isolates. Raising SXT concentrations from 1X to 16X MIC resulted in an increase in bactericidal activity (mainly in a dose-dependent manner) at 24 hours post-inoculation, but time dependency (over 24 hours) was less discernible (Fig 1).

DISCUSSION

Based on the recommendation of the Infectious Diseases Society of America for the management of VAN treatment, if reduced susceptibility to VAN is present, therapeutic options include linezolid, quinupristin/dalfopristin, telavancin, or SXT (Liu *et al*, 2011). However, quinupristin/dalfopristin and telavancin are currently unavailable in

Table 2
Effect of trimethoprim/sulfamethoxazole-vancomycin combination against methicillin-resistant *Staphylococcus aureus* clinical isolates obtained from Hua Hin Hospital, Prachuap Khiri Khan Province, Thailand (January 2015 - December 2016).

S. aureus isolate ID	E-test[a] (FICI)	Checkerboard[b] (FICI)	*S. aureus* isolate ID	E-test (FICI)	Checkerboard (FICI)
S1	Additive (0.83)	Additive (1)	S14	Indifferent (1.38)	Indifferent (1.06)
S2	Indifferent (1.5)	Indifferent (1.06)	S15	Additive (0.87)	Indifferent (1.06)
S3	Indifferent (2)	Indifferent (1.06)	S16	Indifferent (1.67)	Indifferent (1.06)
S4	Indifferent (1.24)	Indifferent (1.06)	S17	Additive (0.83)	Synergistic (0.31)
S5	Additive (0.83)	Indifferent (1.06)	S18	Indifferent (2)	Synergistic (0.38)
S6	Indifferent (1.06)	Indifferent (1.13)	S19	Additive (0.83)	Additive (1)
S7	Indifferent (2)	Indifferent (1.13)	S20	Additive (0.67)	Indifferent (1.06)
S8	Indifferent (1.35)	Indifferent (1.13)	S21	Indifferent (1.47)	Indifferent (1.06)
S9	Indifferent (1.35)	Indifferent (1.13)	S22	Indifferent (1.06)	Indifferent (1.13)
S10	ND	ND	S23	Additive (0.98)	Additive (0.75)
S11	Indifferent (1.23)	Indifferent (1.13)	S24	Indifferent (1.19)	Indifferent (1.06)
S12	Indifferent (1.18)	Indifferent (1.06)	S25	Indifferent (1.48)	Indifferent (1.06)
S13	Indifferent (1.35)	Indifferent (1.06)	S26	Indifferent (2)	Indifferent (1.06)

[a]Liofilchem, Teramo, Italy; [b]Isobologram referred to by Laishram *et al* (2017); FICI: Fractional Inhibitory Concentration Index; FICI ≤0.5 = synergistic, 0.5 - ≤1.0 = additive, >1 - 4 = indifferent, and ≥4 = antagonistic.

Thailand, while linezolid is expensive and is frequently associated with adverse drug events, particularly thrombocytopenia, in long-term use (Liu *et al*, 2011).

The study demonstrates all MRSA clinical isolates were susceptible to VAN, as previously reported by NARST in 2017 (NARST, 2017). However, up to 30% of MRSA isolates had VAN MIC of 1.5-2.0 μg/ml, values associated with high risk of treatment failure compared to MRSA with lower VAN MICs (Sakoulas *et al*,

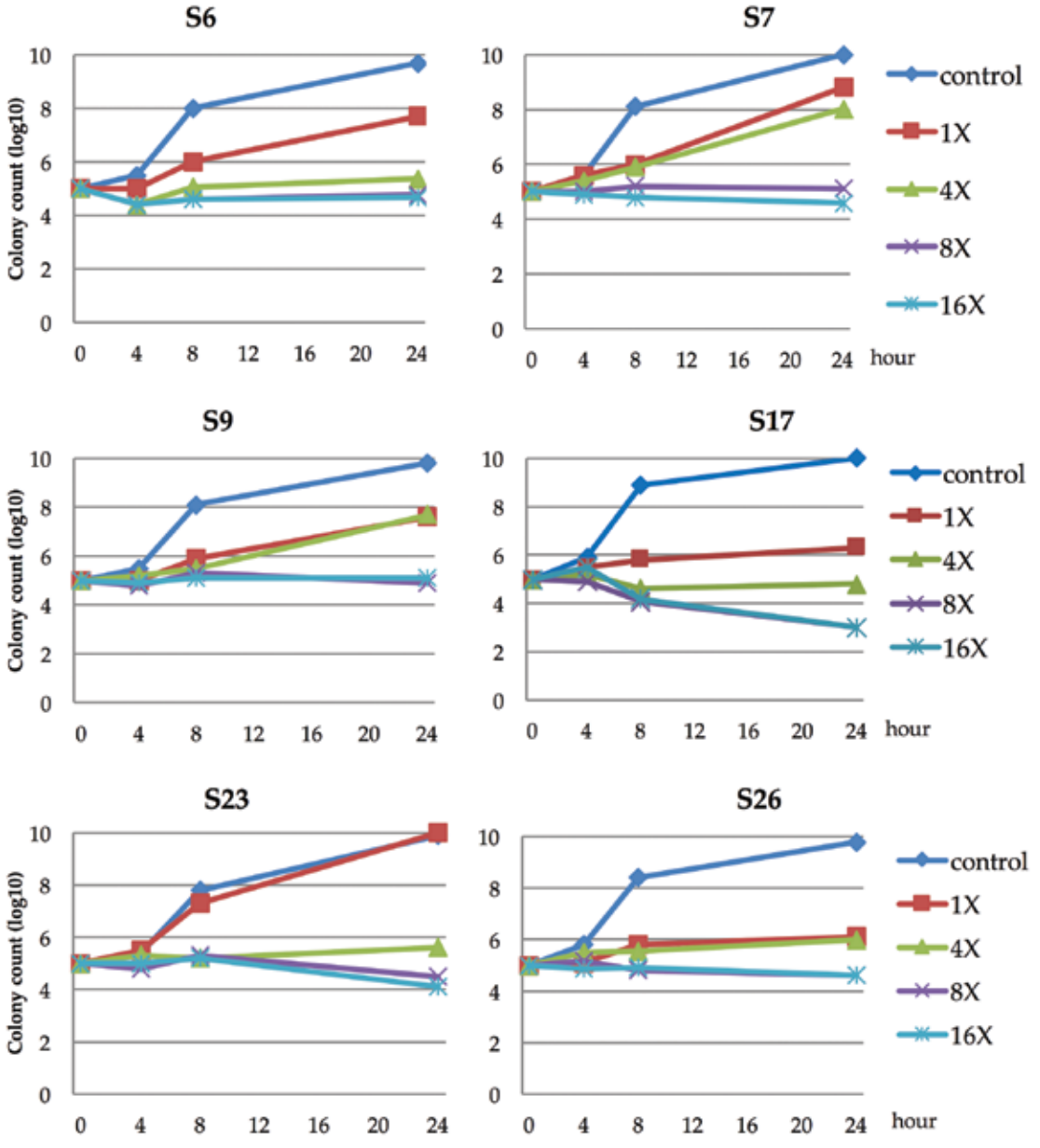

Fig 1-Pharmacodynamics of trimethoprim/sulfamethoxazole (STX) against methicillin-resistant *Staphylococcus aureus* (MRSA) clinical isolates (S6, S7, S9, S17, S23, and S26) obtained from Hua Hin Hospital, Prachuap Khiri Khan Province, Thailand (January 2015 - December 2016). MRSA isolate (10^5 CFU/ml) was incubated in a cation-adjusted Mueller-Hinton broth at 35°C in the presence of STX at 1X, 4X, 8X, and 16X minimum inhibitory concentration (MIC) and colonies counted at time indicated. Clonal types: spat045, isolate IDs S6, S9, S23, S26; spat439, isolate ID S7; spat13880, isolate ID S17; control, no STX added.

2004; Lodise *et al*, 2008). Pitaksontayothin *et al* (2017) reported the presence of 40% of MRSA strains with VAN MIC of 2 μg/ml at a provincial hospital in Thailand, requiring at least 2.5 g/day VAN to attain a cumulative fraction of 90% response in every age group; however, more than half of the patients presented trough concentration >20 μg/ml VAN, increasing risk of nephrotoxicity.

Some 96% of the MRSA isolates were susceptible to SXT, indicating its use in treating MRSA infection given its low cost, very high absorption, adequate penetration in various organs/fluids (*eg* urine, cerebrospinal fluid, lung, prostate, and skin/soft tissue), and availability in both intravenous and oral formulations (Micromedex, 2018). Nevertheless, until there is an understanding of the cause of SXT resistance in MRSA and confirmation of the findings in a larger cohort of patients from different locations in Thailand, caution should be exercised before recommending SXT for use in clinical practice.

That SXT-VAN combination exhibited additive or indifferent bactericidal effect on all MRSA test isolates is promising, allowing a lower dosage of both antimicrobials. A synergistic effect observed in a small number of MRSA isolates might be due to the checkerboard method used as the E-test indicated an additive or indifferent effect. Silva *et al* (2011) observed SXT-VAN combination with synergistic/additive effect against 90% of MRSA samples. MRSA strains with high SXT MICs benefit more from SXT-VAN combination than those with lower MICs. Nonetheless, STX-VAN combination in the treatment of MRSA infection could be an alternative therapy in a setting of MRSA with VAN MIC of 1-2 μg/ml.

Close *et al* (2002) demonstrated concentration-dependent activity of sulfamethoxazole against *S. aureus*. A more rapid bactericidal activity of SXT was observed with higher antimicrobial dosage (up to 8-16X MIC) against test MRSA isolates, and low MICs of these isolates mean that a therapeutic concentration could be attainable with a practical dosing regimen of 5 mg/kg twice daily, yielding trimethoprim and sulfamethoxazole trough plasma concentration of 2.6 and 68 μg/ml, respectively (Spicehandler *et al*, 1982)

In conclusion, the study supports sulfamethoxazole/trimethoprim as an alternative treatment option for treatment of methicillin-resistant *Staphylococcus aureus* and a combination of sulfamethoxazole/trimethoprim and vancomycin in cases where the latter minimum inhibitory concentration is the case where the latter minimum inhibitory concentration is 1-2 μg/ml. However, given the small number of bacteria isolates investigated, further studies on larger cohorts and in other regions of the country are warranted before any decisions on clinical use of the new drug regimens can be made.

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