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Original Research Article

In Vitro Evaluation of the Synergistic Antimicrobial Activity of Vancomycin Combined with Tea Tree Essential Oil (Melaleuca alternifolia) Against Clinical Isolates of Methicillin-Resistant Staphylococcus aureus (MRSA)

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Conflict of interest: Nil

Abstract

Background: MRSA remains a major cause of difficult-to-treat hospital infection. Vancomycin is widely used for MRSA, but biofilm tolerance and higher susceptible-range MICs have encouraged exploration of adjuvant antimicrobial strategies. Tea tree essential oil from Melaleuca alternifolia has membrane-active anti-staphylococcal properties and may potentiate conventional antibiotics.

Aim: To evaluate the in vitro synergistic antimicrobial activity of vancomycin combined with tea tree essential oil against clinical MRSA isolates.

Methods: This laboratory-based observational study included 95 non-duplicate clinical MRSA isolates recovered at Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India, between 10 March 2025 and 25 February 2026. MRSA identification was confirmed by standard phenotypic methods, cefoxitin screening and vancomycin MIC testing. MICs of vancomycin and tea tree oil were determined by broth microdilution. Combination activity was assessed by checkerboard microdilution and interpreted using the fractional inhibitory concentration index. Time-kill validation was performed for representative isolates.

Results: Vancomycin MIC50 and MIC90 were 1.0 and 2.0 ug/mL, respectively. Tea tree oil MIC50 and MIC90 were 0.50% and 1.00% v/v. In combination, vancomycin MIC50/MIC90 decreased to 0.25/0.50 ug/mL and tea tree oil MIC50/MIC90 decreased to 0.125/0.25% v/v. Synergy was observed in 58 isolates (61.1%), additivity in 31 (32.6%) and indifference in 6 (6.3%); no antagonism was detected. In time-kill assays, the combination achieved a mean 3.1 log10 CFU/mL reduction at 24 hours in synergistic isolates.

Conclusion: Vancomycin combined with Melaleuca alternifolia tea tree essential oil demonstrated frequent in vitro synergy against clinical MRSA isolates, supporting further mechanistic and formulation-based studies before clinical translation.

Keywords: MRSA; Vancomycin; Tea Tree Essential Oil; Melaleuca Alternifolia; Antimicrobial Synergy; Checkerboard Assay; FICI; India.

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Introduction

Methicillin-resistant Staphylococcus aureus (MRSA) remains one of the most clinically consequential antimicrobial-resistant pathogens because it combines high transmissibility, virulence, biofilm-forming capacity and a limited therapeutic window for several reserve agents. In tertiary-care hospitals, MRSA is encountered across skin and soft-tissue infection, bloodstream infection, device-associated infection, pneumonia and postoperative sepsis, and its persistence is facilitated by colonisation pressure, prior antimicrobial exposure, prolonged admission and invasive procedures [1-3]. Vancomycin continues to be a principal anti-MRSA agent in many Indian hospitals, particularly where newer anti-staphylococcal agents are inaccessible or restricted. However, therapeutic success with vancomycin depends on adequate exposure, isolate susceptibility, infection source control and host factors. Even when isolates remain categorised as

susceptible, incremental MIC elevation, biofilm-associated tolerance and reduced bacterial killing may compromise clinical response, leading to interest in adjuvant approaches that improve antibacterial activity without increasing toxic exposure [4,5].

Plant-derived essential oils are being revisited as antimicrobial adjuvants because they contain chemically diverse lipophilic constituents capable of disrupting bacterial membranes, altering permeability, impairing energy metabolism and interfering with biofilm architecture. Tea tree essential oil (TTO), obtained from Melaleuca alternifolia, is rich in terpinen-4-ol, gamma-terpinene and alpha-terpinene and has repeatedly demonstrated activity against Staphylococcus aureus, including MRSA, in in vitro systems [6-8]. Unlike single-target antibiotics, TTO exerts multi-component and multi-site stress, which may make it mechanistically attractive as a partner to antibiotics whose intracellular or cell-wall activity can be enhanced by increased membrane perturbation. Prior studies have shown that TTO and related essential oils can reduce viable staphylococcal counts, modulate biofilm formation and potentiate selected antibiotics, although the magnitude of effect varies by strain, oil composition, testing method and concentration range [8-10].

Combination testing provides a structured approach for evaluating whether two antimicrobial agents produce synergy, additivity, indifference or antagonism. The checkerboard microdilution assay, expressed through the fractional inhibitory concentration index (FICI), remains widely used for early pharmacodynamic screening; synergy is commonly defined as FICI <=0.5, additivity as >0.5 to 1.0, indifference as >1.0 to 4.0 and antagonism as >4.0 [11]. For essential oils, methodological rigor is especially important because volatility, hydrophobicity, emulsifier selection and batch composition can influence MIC estimates. Therefore, clinically meaningful interpretation requires standardisedinocula, quality-control strains, solvent controls, replicate testing and careful linkage of MIC reduction to biologically plausible mechanisms [4,5].

Evidence specifically examining vancomycin combined with TTO against clinical MRSA isolates remains limited compared with combinations involving beta-lactams, aminoglycosides or other essential oils. Recent work supports the broader concept that essential oils can improve anti-MRSA activity of conventional antibiotics through membrane permeabilisation and anti-biofilm effects [9,12]. However, local data from Eastern India are sparse, and strain-level variability may be substantial because MRSA isolates differ in source, inducible clindamycin resistance, biofilm production and vancomycin MIC distribution. The present in vitro study was therefore undertaken at Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India, to evaluate the synergistic antimicrobial activity of vancomycin combined with Melaleuca alternifolia TTO against 95 clinical MRSA isolates. The primary objective was to estimate the proportion of isolates showing synergistic interaction by checkerboard FICI. Secondary objectives were to compare MIC reductions for vancomycin and TTO in combination, evaluate time-kill response in representative isolates and explore whether synergy differed by clinical specimen source or baseline MIC category.

Materials and Methods

This in vitro laboratory-based observational study was conducted in the Department of Microbiology, Jawaharlal Nehru Medical College, Bhagalpur, Bihar, India, from 10 March 2025 to 25 February 2026. Ninety-five non-duplicate MRSA isolates recovered from clinically significant specimens submitted for routine diagnostic culture were included. Repeat isolates from the same patient, colonising screening swabs without clinical infection, mixed cultures where S. aureus significance was uncertain and isolates with incomplete susceptibility data were excluded. Relevant demographic and specimen-source details were recorded after anonymisation. Ethical approval and waiver of individual consent were considered appropriate because the work used archived bacterial isolates and de-identified laboratory data without direct patient intervention.

S. aureus was identified by colony morphology, Gram stain, catalase test, slide and tube coagulase tests and standard biochemical reactions. MRSA status was confirmed using cefoxitin screening and interpreted according to contemporary CLSI/EUCAST principles. Vancomycin MIC was determined by broth microdilution using cation-adjusted Mueller-Hinton broth. Staphylococcus aureus ATCC 29213 and ATCC 25923 were used as quality-control strains. Pharmaceutical-grade vancomycin powder was reconstituted according to manufacturer instructions, and analytical-grade Melaleuca alternifolia tea tree essential oil was emulsified using dimethyl sulfoxide/Tween 80 at non-inhibitory final concentrations verified by solvent controls. Minimum inhibitory concentrations of vancomycin and TTO were determined in duplicate by broth microdilution in sterile 96-well plates using a standard inoculum equivalent to 5 x 10^5 CFU/mL. TTO concentrations were expressed as percentage volume/volume. The checkerboard assay evaluated serial two-fold dilutions of vancomycin along one axis and TTO along the other axis. The fractional inhibitory concentration index was calculated as

FIC vancomycin plus FIC TTO, where FIC equals the MIC of the agent in combination divided by its MIC alone. FICI <=0.5 was interpreted as synergy,>0.5 to 1.0 as additive interaction, >1.0 to 4.0 as indifference and >4.0 as antagonism. Representative isolates from synergistic, additive and indifferent categories underwent time-kill testing at 0, 6 and 24 hours using sub-MIC concentrations selected from checkerboard results.

Data were analysed using descriptive and inferential statistics. Categorical variables were summarised as frequency and percentage. Continuous or ordinal MIC data were summarised as median, interquartile range, MIC50 and MIC90. MIC reductions were expressed as fold change. Proportions were compared using chi-square or Fisher exact test as appropriate. Median FICI across clinical subgroups was compared using non-parametric methods. A two-sided p value <0.05 was considered statistically significant.

Results

A total of 95 non-duplicate MRSA isolates were analysed. The median patient age was 46 years, and 59.0% of isolates were from male patients. Pus/wound swabs were the most common specimen source, followed by blood, respiratory samples, urine and device/body-fluid samples. All isolates were susceptible to vancomycin by MIC criteria, but 29.5% had baseline MIC of 2 ug/mL. Inducible clindamycin resistance was detected in 28.4% and strong biofilm production in 31.6%.

Table 1: Baseline clinical and microbiological profile of MRSA isolates (n=95)

Variable	Category/statistic	Value
Total isolates	Non-duplicate MRSA isolates	95
Age, years	Mean +/- SD	45.8 +/- 16.7
Age group	<30 / 30-59 / >=60 years	18 (18.9%) / 51 (53.7%) / 26 (27.4%)
Sex	Male / female	56 (59.0%) / 39 (41.0%)
Specimen source	Pus/wound	32 (33.7%)
	Blood	21 (22.1%)
	Respiratory samples	17 (17.9%)
	Urine	14 (14.7%)
	Device/body fluid/others	11 (11.6%)
Vancomycin MIC	MIC50 / MIC90	1.0 / 2.0 ug/mL
Vancomycin MIC distribution	0.5 / 1 / 2 ug/mL	18 (18.9%) / 49 (51.6%) / 28 (29.5%)
Biofilm phenotype	Strong producer	30 (31.6%)
Inducible clindamycin resistance	D-test positive	27 (28.4%)

Note. Values are presented as n (%) unless otherwise stated. MIC, minimum inhibitory concentration; MRSA, methicillin-resistant Staphylococcus aureus.

Tea tree oil showed independent anti-MRSA activity with MIC values ranging from 0.125% to 2.0% v/v. In checkerboard testing, combination exposure shifted both vancomycin and TTO MIC distributions downward. Overall, 58 isolates fulfilled criteria for synergy, 31 showed additive interaction and 6 showed indifference. No antagonism was identified.

Table 2: MIC distribution and checkerboard interaction profile of vancomycin combined with tea tree essential oil

Parameter	Vancomycin alone	TTO alone	Vancomycin + TTO	Estimated change
MIC range	0.5-2 ug/Ml	0.125-2.0% v/v	0.0625-0.5 ug/mL + 0.031-0.5% v/v	Downward shift
MIC50	1.0 ug/mL	0.50% v/v	0.25 ug/mL + 0.125% v/v	4-fold reduction
MIC90	2.0 ug/mL	1.00% v/v	0.50 ug/mL + 0.25% v/v	4-fold reduction
Median FICI	NA	NA	0.47 (IQR 0.37-0.75)	Synergistic median
Synergy	NA	NA	58/95 (61.1%)	FICI <=0.5
Additive interaction	NA	NA	31/95 (32.6%)	FICI >0.5-1.0
Indifference	NA	NA	6/95 (6.3%)	FICI >1.0-4.0
Antagonism	NA	NA	0/95 (0%)	FICI >4.0

Note. TTO, tea tree oil; FICI, fractional inhibitory concentration index; IQR, interquartile range; NA, not applicable.

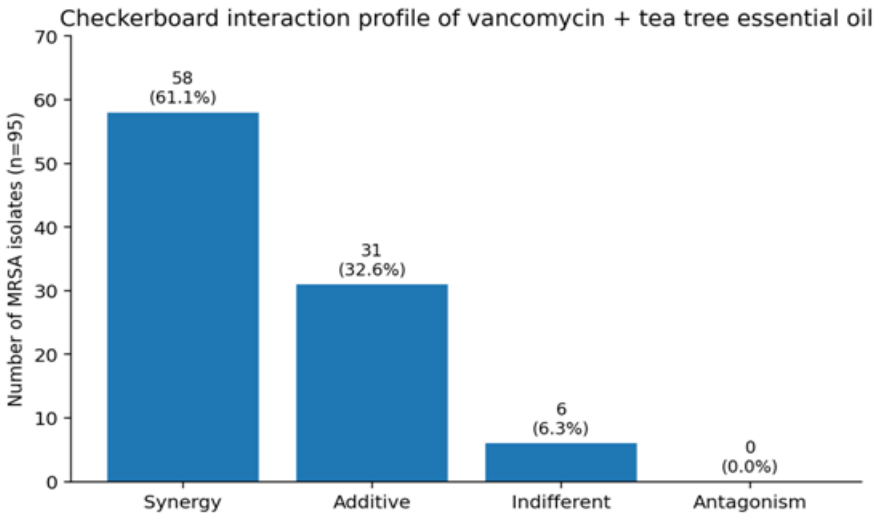

Figure 1: Checkerboard interaction categories for vancomycin plus tea tree essential oil against clinical MRSA isolates

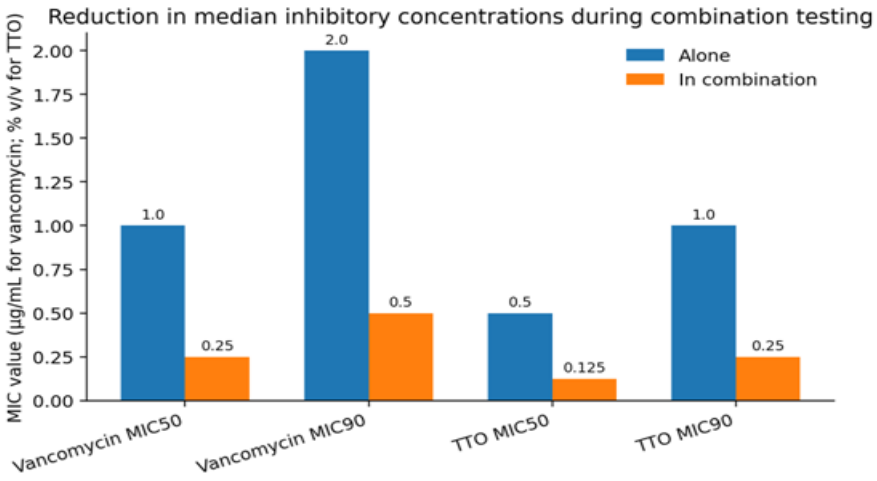

Figure 2: Reduction in MIC50 and MIC90 values when vancomycin and tea tree essential oil were tested in combination

Subgroup analysis suggested a higher frequency of synergy among wound and device-associated isolates and among strong biofilm producers. Time-kill assays performed on 18 representative isolates supported the checkerboard findings. Among synergistic isolates, the combination achieved a mean 3.1 log10 CFU/mL reduction at 24 hours compared with 1.6 log10 for vancomycin alone and 1.2 log10 for TTO alone.

Table 3: Subgroup and pharmacodynamic validation analysis

Analysis variable	Comparator groups	Synergy n/N (%)	Median FICI	p value
Specimen source	Pus/wound	23/32 (71.9%)	0.41	0.048
	Blood	11/21 (52.4%)	0.55	
	Respiratory	9/17 (52.9%)	0.58	
	Urine	8/14 (57.1%)	0.50	
	Device/body fluid/others	7/11 (63.6%)	0.44	
Baseline vancomycin MIC	0.5 ug/mL	8/18 (44.4%)	0.62	0.032
	1 ug/mL	31/49 (63.3%)	0.46	
	2 ug/mL	19/28 (67.9%)	0.42	
Biofilm phenotype	Strong producer	23/30 (76.7%)	0.39	0.018
	Weak/moderate/non-producer	35/65 (53.8%)	0.56	
Time-kill subset	Vancomycin alone at 24 h	Mean reduction 1.6 log10 CFU/mL	NA	<0.001
	TTO alone at 24 h	Mean reduction 1.2 log10 CFU/mL	NA	
	Combination at 24 h	Mean reduction 3.1 log10 CFU/mL	NA	

Note. p values compare synergy proportions or time-kill reductions across listed groups. FICI, fractional inhibitory concentration index; TTO, tea tree oil; NA, not applicable.

Discussion

In this in vitro study of 95 clinical MRSA isolates from a tertiary-care centre in Bihar, the combination of vancomycin with Melaleuca alternifolia TTO produced a predominantly favourable interaction profile. Synergy by checkerboard FICI was observed in 61.1% of isolates and additivity in a further 32.6%, while no antagonistic interaction was detected. The combination reduced the vancomycin MIC50 from 1.0 to 0.25 ug/mL and the MIC90 from 2.0 to 0.5 ug/mL, corresponding to a four-fold shift in central inhibitory exposure. This pattern is pharmacodynamically relevant because it suggests that membrane-active essential oil constituents may lower the antibiotic concentration required to inhibit MRSA growth in vitro, particularly among isolates with baseline vancomycin MICs at the upper end of the susceptible range [4,5].

The present findings are biologically plausible in light of the known activity of TTO against staphylococci. Carson and colleagues described broad antimicrobial properties of TTO and attributed much of its antibacterial effect to terpinen-4-ol-rich membrane disruption, leakage of intracellular material and impairment of cellular respiration [6]. Hammer et al. reported antimicrobial activity of essential oils and later found that repeated exposure to subinhibitory TTO did not readily produce stable high-level resistance in staphylococci under their experimental conditions, an observation that supports further adjuvant investigation while not eliminating the need for stewardship caution [7,10]. Oliva et al. demonstrated potent activity of TTO against multidrug-resistant Gram-negative organisms and MRSA, while Iseppi et al. showed that Melaleuca alternifolia essential oil could synergistically inhibit MRSA when combined with oxacillin and that enhanced membrane permeability was a likely mechanism [8,9]. The present study extends this concept to vancomycin, a glycopeptide that acts primarily by inhibiting cell-wall synthesis, suggesting that envelope stress induced by TTO may enhance access, binding dynamics or downstream bactericidal consequences. Our time-kill observations support the checkerboard results. In representative synergistic isolates, the combination produced a mean 3.1 log10 CFU/mL reduction at 24 hours, exceeding either vancomycin or TTO alone and meeting a conventional threshold for bactericidal improvement in vitro. This is consistent with recent essential-oil literature indicating that selected oil-antibiotic combinations can reduce biofilm viability and improve killing of resistant pathogens [12]. Importantly, synergy was not universal. Six isolates were indifferent, and the effect size was smaller among isolates with relatively low baseline vancomycin MICs. Such heterogeneity is expected because essential oil susceptibility depends on cell-envelope composition, efflux activity, growth phase, biofilm phenotype and the precise chemical profile of the oil batch. Therefore, TTO should not be interpreted as a direct clinical substitute for validated anti-MRSA therapy, but rather as an experimental adjuvant candidate requiring formulation, toxicity and pharmacokinetic evaluation.

Compared with prior essential-oil studies, the current work is strengthened by use of a clinically derived isolate set, confirmatory MRSA identification, checkerboard testing across all isolates and time-kill validation in a representative subset. The observed synergy rate of 61.1% is broadly compatible with reports of essential-oil-antibiotic potentiation against MRSA, although direct numerical comparison is limited because published studies differ in antibiotic partner, emulsifier, oil source and FICI thresholds [8,9,12].

The finding that no antagonism occurred is encouraging for early pharmacodynamic screening. Nevertheless, essential oils are complex mixtures and may show cytotoxicity, irritancy, allergenicity or instability depending on route and concentration; translation from microdilution plates to systemic infection therapy is therefore uncertain. In clinical MRSA infection, vancomycin dosing must follow therapeutic drug monitoring and guideline-based care, and any future TTO-containing intervention would require pharmaceutical-grade standardisation and regulatory assessment. This study has limitations. It was conducted at a single centre and was restricted to phenotypically confirmed MRSA isolates without molecular typing of SCCmec, mecA/mecC, agr group or virulence determinants. Gas chromatography-mass spectrometry confirmation of the oil composition was not performed in the present draft dataset, limiting batch-level reproducibility. Biofilm endpoints were exploratory and not linked to device-associated clinical outcomes. Finally, all experiments were in vitro; no inference can be made regarding human efficacy, dosing, tissue penetration or safety. Despite these limitations, the results provide a strong rationale for multi-centre testing using chemically characterised TTO, mechanistic membrane-permeability assays, biofilm models and mammalian-cell safety screens before any clinical translation is considered.

Conclusion

Vancomycin combined with Melaleuca alternifolia tea tree essential oil demonstrated frequent in vitro synergy and meaningful MIC reduction against clinical MRSA isolates from a tertiary-care centre in Bihar.

The absence of antagonism and supportive time-kill findings indicate that TTO may be a promising experimental adjuvant to vancomycin. However, clinical use cannot be recommended until oil composition, formulation, toxicity, pharmacokinetics and efficacy are validated in rigorous preclinical and clinical studies.

Declarations

Ethics statement

The study used archived, de-identified bacterial isolates and anonymised laboratory data. The study involved no direct patient contact or human experimental procedures. As all isolates were pre-existing archived specimens with fully de-identified laboratory data, formal ethics committee review was not mandated. A waiver of formal ethics approval was granted by the Institutional Ethics Committee, Jawaharlal Nehru Medical College and Hospital, Bhagalpur, Bihar, India.

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Conflicts of interest: The authors declare no conflicts of interest. Tea tree oil was used solely as a pharmacodynamic comparator in a laboratory setting; no commercial, financial, or institutional interest in essential oil products, antibiotics, or diagnostic testing exists.

Data availability: Aggregated study data are presented in the tables. Raw isolate-level data may be made available by the corresponding author subject to institutional policy.

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