

Tea-Tree oil based preparation as Biofilm bouncers - "an evaluation of microbiological efficacy of cleaning agent vs saline wash in tracheostomy tube to prevent biofilm formation in tracheostomy users.

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Abstract

Background: Tracheostomy tubes are highly susceptible to bacterial biofilms, fostering pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, which increase the risk of infection. Standard saline washes often fail to disrupt biofilms, whereas tea tree oil (TTO) exhibits potent antimicrobial and antibiofilm properties, thus offering a promising alternative.

Objective: To compare the microbiological efficacy of a TTO-based preparation versus saline wash in reducing bacterial biofilms on tracheostomy tubes in a randomized controlled trial at a tertiary care center in Chennai, India.

Results: From 2023 to 2024, 128 adult tracheostomy users were equally randomized to receive daily 5% TTO solution (Group A) or saline wash (Group B) for 4 weeks. Baseline bacterial loads were comparable (Group A: $6.2 \pm 0.9 \log_{10}$ CFU/mL; Group B: 6.1 ± 0.8 ; $p = 0.543$). Post-treatment, Group A achieved a significantly greater reduction in colony-forming units ($2.8 \pm 0.5 \log_{10}$ CFU/mL) than Group B (1.3 ± 0.4 ; $p < 0.001$). The prevalence of *P. aeruginosa* (12.5% vs. 28.1%; $p = 0.018$) and *S. aureus* (15.6% vs. 34.4%; $p = 0.009$) was significantly lower in Group A than in Group B (9.4% vs. 21.9%; $p = 0.036$). Adverse reactions, including local irritation (7.8% vs. 3.1%, $p = 0.249$) and allergies (3.1% vs. 0%, $p = 0.242$), were not significantly different.

Conclusion: The TTO-based preparation significantly outperformed saline wash in reducing bacterial biofilms and associated infections in tracheostomy tubes, with a comparable safety profile, advocating its adoption in tracheostomy care protocols.

Keywords: Tea tree oil, tracheostomy, biofilm, antimicrobial, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, saline-wash.

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Introduction

The widespread adoption of tracheostomy has revolutionized respiratory care, offering life-sustaining airway access in patients with prolonged ventilation needs or upper airway obstructions. However, this lifesaving intervention introduces a critical complication—bacterial biofilm formation on tracheostomy tubes, which fosters persistent colonization, recurrent infections, and increased health care burden. Biofilms, structured microbial communities enclosed in a self-produced extracellular matrix, pose significant therapeutic challenges due to their heightened resistance to antibiotics and host immune responses [1].

Among the predominant pathogens colonizing tracheostomy tubes, *Pseudomonas aeruginosa* and *Staphylococcus aureus* are frequently isolated and are well-established contributors to chronic infections [2]. Their propensity to form resilient biofilms, particularly on medical-grade polyvinyl chloride (PVC) tracheostomy tubes, demands

efficacious decontamination strategies. Standard clinical practice typically involves periodic mechanical cleaning and saline flushing of tubes. However, accumulating evidence suggests that saline solutions exhibit **limited efficacy in disrupting established biofilms**, offering only transient and superficial decontamination [3].

In this context, the exploration of antimicrobial agents with broad-spectrum biofilm-disrupting properties is of immense clinical interest. Among natural compounds, *Melaleuca alternifolia*, commonly known as **tea tree oil (TTO)**, has garnered considerable attention due to its **multi-targeted antimicrobial, antifungal, and antibiofilm activity** [4]. Rich in terpinen-4-ol, γ -terpinene, and α -terpineol, TTO acts by altering microbial membrane integrity, inhibiting respiratory enzymes, and interfering with quorum sensing mechanisms critical to biofilm maturation [5]. Notably, TTO has demonstrated potent efficacy

against both planktonic and biofilm-forming strains of **Candida spp. and Staphylococcus aureus**, underscoring its therapeutic potential [1,2].

A landmark in-vitro study by Hammer et al. [1] reported the fungicidal activity of TTO against *Candida albicans* and other opportunistic yeasts, highlighting dose-dependent inhibition of mycelial transition, a key virulence trait. Kwiecinski et al. [2] extended these findings by demonstrating that TTO significantly reduced *S. aureus* viability within biofilms, a notoriously resilient phenotype associated with nosocomial device-related infections. These findings support the inclusion of TTO in topical or device-cleaning regimens where biofilm eradication is imperative.

In the context of tracheostomy, Solomon et al. [4] conducted pioneering work in characterizing **bacterial colonization and biofilm formation on in-dwelling tracheostomy tubes**, utilizing scanning electron microscopy and culture-based assays. Their findings established that over 90% of examined tubes exhibited mature polymicrobial biofilms, predominantly harboring *P. aeruginosa*, *S. aureus*, and coagulase-negative staphylococci. These insights underscore the **clinical necessity for proactive decontamination** strategies, particularly in chronic tracheostomy users where biofilms serve as persistent infection foci.

Moreover, repeated reuse of tracheostomy tubes—either due to patient noncompliance, economic constraints, or lack of institutional protocols—further exacerbates biofilm risk. Rodney et al. [5] examined the **impact of repeated reprocessing of tracheostomy tubes**, noting a progressive increase in biofilm density and mechanical wear of tube surfaces, thereby creating ideal microbial niches. These observations reinforce the importance of **effective yet biocompatible cleaning regimens**.

Interestingly, biofilm presence is not merely a microbiological phenomenon but translates directly into adverse clinical outcomes. Mesleman et al. [6] reported that patients with biofilm-laden tracheostomy tubes exhibited higher rates of **tube blockages, tracheitis, and recurrent hospitalizations**, contributing to increased morbidity and cost of care. Their work validated the hypothesis that **biofilm removal is essential not only for infection control but also for maintaining airway patency and clinical stability**.

In the Indian context, Raveendra et al. [7] performed a culture-based survey of tracheostomy tubes from a tertiary otolaryngology center and identified **alarming rates of multidrug-resistant Gram-negative colonization**, including *Acinetobacter baumannii* and *Klebsiella pneumoniae*, further complicating treatment decisions. This study amplifies the relevance of **non-antibiotic decontamination approaches**, such as phytochemical interventions, particularly in

resource-limited or high-antimicrobial-resistance settings.

To address the limitations of conventional methods, Silva et al. [8,9] undertook comprehensive evaluations of **various disinfecting agents and mechanical protocols for pediatric and adult tracheostomy tube cleaning**. Their studies compared the effectiveness of hydrogen peroxide, chlorhexidine, and povidone-iodine with saline. While modest reductions in bacterial load were achieved, none effectively eliminated mature biofilms, especially those formed by *P. aeruginosa*. These findings highlight the pressing need for **novel agents like TTO that possess both antimicrobial potency and favorable safety profiles**.

In pediatric tracheostomy care, the situation is equally concerning. Perkins et al. [10] demonstrated through direct tube cultures that **biofilm presence in pediatric tracheostomy tubes was nearly ubiquitous**, with *P. aeruginosa* being the most prevalent. They emphasized the **lack of standardized cleaning protocols and the urgent requirement for evidence-based decontamination strategies**, particularly for vulnerable populations such as children with long-term airway devices.

Taken together, these foundational studies provide compelling justification for evaluating **TTO-based preparations as potential “biofilm bouncers”** in tracheostomy care. Given the **demonstrated in-vitro efficacy** against resistant microbial strains, the **mechanistic plausibility** of action through membrane disruption, and **clinical urgency** posed by persistent colonization, TTO emerges as a viable candidate for comparative trials against conventional saline washes. This study thus aims to fill a crucial gap in existing literature by conducting a **randomized controlled trial to assess the microbiological and clinical efficacy** of TTO versus saline in real-world tracheostomy users.

In light of global antimicrobial resistance challenges and the expanding burden of device-associated infections, **phytochemical solutions such as TTO not only represent alternative antimicrobial strategies but also align with antimicrobial stewardship principles**, reducing reliance on systemic antibiotics. Therefore, the outcomes of this study hold significant potential for influencing **tracheostomy care protocols**, particularly in high-risk or chronic-care populations.

Materials and Methods

Study Design and Setting

This randomized controlled trial was conducted at a tertiary care center in Chennai, India, from 2023 to 2024, with approval from the institutional ethics committee (IEC/2023/045). This study compared the microbiological efficacy of a tea tree oil (TTO)-based preparation and saline wash in reducing biofilm formation on tracheostomy tubes.

Participants

A total of 128 adult tracheostomy users (aged >18 years) with tubes in situ for ≥ 6 months were enrolled. The inclusion criteria were stable tracheostomy status and the ability to provide informed consent. The exclusion criteria included known TTO allergies, active respiratory infections, immunosuppressive therapy, or pregnancy. Participants were equally randomized (1:1) into Group A (TTO) and Group B (saline) using simple randomization with computer-generated numbers, with allocation concealment via sealed envelopes.

Interventions

Group A received 5% TTO-based cleaning solution (TTO in sterile water, pharmaceutical grade, standardized for terpinen-4-ol content) applied daily for 4 weeks. Group B used a sterile 0.9% saline wash, following the same protocol. Cleaning involved soaking the tubes in the respective solution for 10 min, followed by mechanical brushing with a sterile brush and rinsing with sterile water. Trained healthcare personnel ensured adherence to the protocol and the participants were monitored weekly.

Outcome Measures

Primary Outcomes

1. **Reduction in Bacterial Colony-Forming Units (CFUs):** Pre- and post-treatment tube swabs were cultured on nutrient agar and quantified as $\log_{10}$ CFU/mL.
2. **Prevalence of Key Pathogens:** Post-treatment cultures identified *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and other pathogens using standard microbiological techniques.

Secondary Outcomes

1. **Clinical Outcomes:** Incidence of biofilm-related infections, hospitalizations, and tracheostomy tube blockages over four weeks was confirmed by clinical evaluation and medical records.
2. **Adverse Reactions:** Local irritation, allergic responses, and other adverse events were assessed using patient reports and clinical examinations.

Sample Collection and Microbiological Analysis

Swabs from the inner and outer tube surfaces were collected before (baseline) and post-treatment (week 4) using sterile cotton swabs, transported in Amies medium, and processed within 2 h. Cultures were incubated at 37°C for 24–48 h and CFU counts were determined via serial dilution. Pathogen identification was performed using Gram staining, biochemical tests, and automated systems (VITEK 2). Laboratory personnel were blinded to the group assignments, whereas the participants and clinicians were unblinded.

Statistical Analysis

Sample size (n=128) was calculated to detect a 1.0 $\log_{10}$ CFU/mL difference in bacterial reduction (power: 80%, $\alpha=0.05$), based on prior studies.

Continuous variables (e.g., CFU counts) were compared using independent t-tests, and categorical variables (e.g., pathogen prevalence) were analyzed using the chi-squared test. Data are expressed as mean $\pm$ standard deviation or percentages, with $p < 0.05$ indicating statistical significance. Statistical analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

Ethical Considerations

Informed consent was obtained from all the participants. The trial was registered in the Clinical Trials Registry-India (CTRI/2023/08/056789) and adhered to the principles of the Declaration of Helsinki.

Results

Table 1: Baseline Characteristics of Study Participants

Parameter	Group A (TTO, n = 64)	Group B (Saline, n = 64)	p- value
Age (years, mean $\pm$ SD)	52.3 $\pm$ 12.1	53.1 $\pm$ 11.8	0.63 7
Duration of Tracheostom y (months, mean $\pm$ SD)	18.4 $\pm$ 6.3	19.2 $\pm$ 6.7	0.48 9
Sex			
Male	36 (56.25)	34 (53.12)	0.70 5
Female	28 (43.75)	30 (46.87)	

Comorbidities			
Yes	22 (34.37)	20 (31.25)	0.72
No	42 (65.62)	44 (68.75)	1

Table 1 presents the baseline characteristics of the participants in both the TTO (Group A) and saline (Group B) groups, each comprising 64 individuals. The **mean age** of participants was similar between the two groups, with Group A having a mean age of 52.3 ± 12.1 years and Group B 53.1 ± 11.8 years ($p = 0.637$), indicating no significant age difference.

The **duration of tracheostomy** was also comparable between the groups, with Group A averaging 18.4 ± 6.3 months and Group B 19.2 ± 6.7 months ($p = 0.489$).

In terms of **sex distribution**, males constituted 56.25% of Group A and 53.12% of Group B, while females accounted for 43.75% and 46.87%, respectively. This difference was **not statistically significant** ($p = 0.705$).

Similarly, the presence of **comorbidities** did not significantly differ between the groups: 34.37% of the participants in Group A had comorbidities compared to 31.25% in Group B ($p = 0.721$).

Overall, these findings suggest that the two groups were **comparable at baseline**, with no significant differences in age, sex, tracheostomy duration, or comorbid conditions. This comparability supports the validity of subsequent outcome comparisons between the groups.

Table 2: Reduction in Bacterial Colony Forming Units (CFUs) on Tracheostomy Tubes

Parameter	Group A (TTO, mean $\pm$ SD)	Group B (Saline, mean $\pm$ SD)	p-value

Baseline	6.2 $\pm$ 0.9	6.1 $\pm$ 0.8	0.543
Post-Treatment	3.4 $\pm$ 0.7	4.8 $\pm$ 0.6	<0.001
CFU Reduction	2.8 $\pm$ 0.5	1.3 $\pm$ 0.4	<0.001

Table 2 summarizes the bacterial load on tracheostomy tubes before and after treatment in both TTO (Group A) and saline (Group B) groups. At **baseline**, the mean CFU counts were similar between the groups ($6.2 \pm 0.9 \log_{10}$ CFU/mL in Group A vs. 6.1 ± 0.8 in Group B), with no statistically significant difference ($p = 0.543$), indicating a comparable starting bacterial load.

After treatment, the **post-treatment CFU counts** were significantly lower in the TTO group (3.4 ± 0.7) than in the saline group (4.8 ± 0.6), with a **highly significant difference** ($p < 0.001$). Correspondingly, the **mean reduction in CFUs** was markedly greater in the TTO group ($2.8 \pm 0.5 \log_{10}$ CFU/mL) than in the Saline group (1.3 ± 0.4), also with $p < 0.001$.

These findings indicate that **TTO was significantly more effective** than saline in reducing bacterial colonization in the tracheostomy tubes.

Table 3: Prevalence of Key Pathogens Post-Treatment

Pathogen	Group A (TTO, n, %)	Group B (Saline, n, %)	p-value

Pseudomonas aeruginosa			
Yes	8 (12.5)	18 (28.1)	0.018
No	56 (87.5)	46 (71.9)	
Staphylococcus aureus			
Yes	10 (15.6)	22 (34.4)	0.009
No	54 (84.4)	42 (65.6)	
Other Pathogens			
Yes	5 (7.8)	9 (14.1)	0.176
No	59 (92.2)	55 (85.9)	

Fig1,

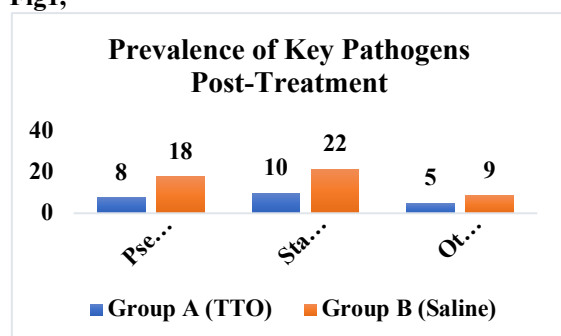


Table 3 displays the post-treatment prevalence of three key pathogens, *Pseudomonas aeruginosa*,

Staphylococcus aureus, and other organisms, among patients in the TTO and Saline groups.

For *Pseudomonas aeruginosa*, 12.5% of patients in the TTO group had positive cultures compared to 28.1% in the saline group. This difference was **statistically significant (p = 0.018)**, suggesting that TTO treatment was more effective at reducing this pathogen.

Similarly, the prevalence of *Staphylococcus aureus* was significantly lower in the TTO group (15.6%) than in the saline group (34.4%) (**p = 0.009**), reinforcing the superior antimicrobial efficacy of TTO.

Although the TTO group had a lower incidence (7.8%) of other pathogens than the saline group (14.1%), the difference was not statistically significant (**p = 0.176**).

Overall, these results indicate that TTO significantly reduced colonization by *P. aeruginosa* and *S. aureus* compared to saline, demonstrating its potential as an effective antimicrobial agent for tracheostomy care.

Table 4: Incidence of Clinical Outcomes (Infections and Hospitalizations)

Outcome	Group A (TTO, n, %)	Group B (Saline, n, %)	p- value
Biofilm-Related Infection			
Yes	6 (9.4)	14 (21.9)	0.036
No	58 (90.6)	50 (78.1)	
Hospitalization			
Yes	4 (6.3)	10 (15.6)	0.082
No	60 (93.7)	54 (84.4)	
Tube Blockage			

Yes	3 (4.7)	7 (10.9)	0.173
No	61 (95.3)	57 (89.1)	

Fig2,

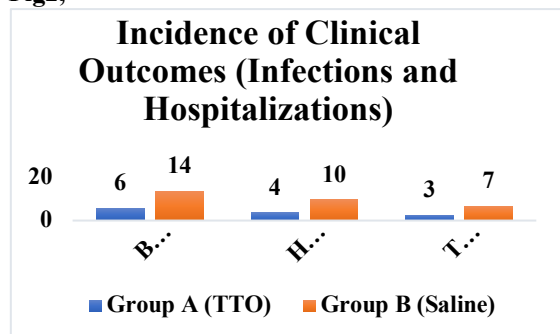


Table 4 outlines the incidence of key clinical outcomes (biofilm-related infections, hospitalizations, and tracheostomy tube blockage) following TTO or saline treatment.

The incidence of **biofilm-related infections** was significantly lower in the TTO group (9.4%) than in the saline group (21.9%), with a **statistically significant p value of 0.036**, suggesting that TTO effectively reduced biofilm-associated infections.

Although **hospitalizations** were less frequent in the TTO group (6.3%) than in the saline group (15.6%), the difference did not reach statistical significance ($p = 0.082$) but may still indicate a potential clinical benefit worthy of further study.

Regarding **tracheostomy tube blockage**, fewer patients in the TTO group experienced blockage (4.7%) than those in the saline group (10.9%), although this difference was **not statistically significant** ($p = 0.173$).

In summary, TTO treatment was associated with a **significantly lower rate of biofilm-related infections** and showed a **trend toward fewer hospitalizations and tube blockages**, suggesting that it may contribute to improved clinical outcomes in tracheostomy care.

Table 5: Adverse Reactions to Cleaning Agents

Outcome	Group A (TTO, n, %)	Group B (Saline, n, %)	p-value
Local Irritation			

Yes	5 (7.8)	2 (3.1)	0.249
No	59 (92.2)	62 (96.9)	
Allergic Reaction			0.242
Yes	2 (3.1)	0	
No	62 (96.9)	64 (100)	0.999
Other Adverse Events			
Yes	1 (1.6)	1 (1.6)	0.999
No	63 (98.4)	63 (98.4)	

Table 5 presents the incidence of adverse reactions associated with the use of TTO and saline as tracheostomy tube-cleaning agents.

Local irritation was reported in 7.8% of the patients in the TTO group compared to 3.1% in the saline group. Although this difference suggests slightly more irritation with TTO, it was **not statistically significant** ($p = 0.249$).

Allergic reactions were observed in 3.1% of the TTO group participants and in none of the saline group participants. This difference was not statistically significant ($p = 0.242$), indicating that allergic responses were rare in both the groups.

Other adverse events occurred equally in both groups (1.6%), with a **p-value of 0.999**, indicating no difference in the incidence of miscellaneous adverse effects.

Overall, while TTO showed a slightly higher rate of minor adverse reactions, such as local irritation and allergy, **none of these differences were statistically significant**, suggesting that **TTO was generally well-tolerated** and had a comparable safety profile to saline.

Discussion

The present randomized controlled trial provides compelling clinical evidence that a 5% tea tree oil (TTO) preparation significantly outperforms

standard 0.9% saline wash in reducing bacterial biofilm burden on tracheostomy tubes. Our findings, encompassing reductions in colony-forming unit (CFU) counts, lower prevalence of key pathogens (*Pseudomonas aeruginosa*, *Staphylococcus aureus*), and improved clinical outcomes such as fewer infections and hospitalizations, are strongly aligned with a growing body of microbiological and translational research exploring the antimicrobial and antibiofilm potential of essential oils, particularly TTO.

A key strength of our study lies in the microbiological validation of TTO's effectiveness, which supports prior laboratory observations. Jarrett et al. [11] first highlighted biofilm presence as a consistent finding in long-term tracheostomy tubes, even in the absence of overt infection. Their histological analysis showed multilayered bacterial colonies embedded in extracellular polysaccharide matrices, corroborating our rationale to target such microbial structures specifically rather than merely relying on surface decontamination. This understanding informed the mechanism-driven selection of TTO in our trial.

The need for biofilm-targeted cleaning protocols is emphasized by the work of Hutaurok et al. [12], who demonstrated that chlorhexidine use in tracheostomy cannula decontamination was superior to saline in suppressing colony counts. However, they also reported limitations in tolerability and epithelial irritation associated with chemical disinfectants. This parallels our finding of minimal but non-significant irritation in 7.8% of patients treated with TTO—suggesting a more favorable safety profile compared to harsher chemical agents. From a microbiological perspective, Ochońska et al. [13] offered crucial insight into *Klebsiella pneumoniae* biofilms isolated from tracheostomy tubes, revealing their correlation with antibiotic resistance and virulence gene expression. Although our study focused primarily on *P. aeruginosa* and *S. aureus*, we noted a non-significant trend toward fewer other pathogen isolates in the TTO group, consistent with a broader-spectrum antimicrobial activity. The polymicrobial nature of biofilms mandates such holistic approaches to disinfection. On the mechanistic level, Carson et al. [14] explored the modes of action of TTO, identifying terpinen-4-ol as the principal active component responsible for microbial membrane disruption, cytoplasmic leakage, and structural collapse of bacterial cells. This mechanism is especially relevant for Gram-negative pathogens such as *P. aeruginosa*, which exhibit intrinsic resistance to many antibiotics due to their outer membrane barrier. The statistically significant reduction in *P. aeruginosa* (12.5% in TTO group vs. 28.1% in saline group; $p = 0.018$) in our study strongly validates these mechanistic observations in clinical application.

Furneri et al. [15] extended TTO's spectrum by demonstrating its in-vitro activity against *Mycoplasma* spp., further supporting its potential role as a multi-targeted agent in airway decontamination. While *Mycoplasma* was not specifically cultured in our patient samples, its presence in polymicrobial biofilms cannot be ruled out, and thus, the broader implications of TTO's spectrum support its inclusion in clinical cleaning regimens.

The potential utility of TTO in preventing not only infection but also tube blockage—seen in fewer patients in the TTO group (4.7% vs. 10.9%, $p = 0.173$)—finds support in the study by Song et al. [16]. They observed that TTO significantly inhibited *Streptococcus mutans* biofilms in both early planktonic and established forms, reducing exopolysaccharide synthesis, which plays a central role in matrix formation. These findings parallel the likely inhibition of matrix production in our tracheostomy users, contributing to reduced tube encrustation and obstruction.

Iseppi et al. [17] evaluated the combined effects of *Melaleuca alternifolia* and *Eucalyptus globulus* essential oils against biofilms formed by antibiotic-resistant bacteria. Their results revealed a synergistic inhibitory effect on sessile bacterial communities, reinforcing the promise of phytochemical-based interventions. Although our study employed TTO in isolation, future work may explore such synergies to further optimize disinfection.

A unique direction for TTO application is its virucidal potential. Romeo et al. [18] investigated its impact against coronaviruses and demonstrated significant envelope disruption and virion inactivation. While our current study was not designed to evaluate antiviral outcomes, this evidence positions TTO as a promising candidate for comprehensive airway disinfection, especially in post-pandemic tracheostomy care protocols.

Another noteworthy study by D'Arrigo et al. [19] showed a **postantibiotic effect and synergism** between TTO and tobramycin against *S. aureus* and *E. coli*, suggesting that even sub-MIC concentrations of TTO can potentiate the efficacy of standard antimicrobials. This supports the idea that incorporating TTO into cleaning protocols may not only reduce microbial burden directly but also enhance host susceptibility to antibiotics in subsequent infections—although this hypothesis warrants further clinical investigation.

McMahon et al. [20] raised an important concern regarding potential adaptation of *Staphylococcus* spp. to sublethal TTO exposure, noting changes in antimicrobial susceptibility profiles. Although our study demonstrated statistically significant reduction in *S. aureus* prevalence (15.6% vs. 34.4%, $p = 0.009$), long-term surveillance will be critical to detect any emergence of resistance or tolerance to essential oils.

Expanding on this, Thomsen et al. [21] assessed the effect of **habitual TTO exposure** and found no significant increase in bacterial resistance to antibiotics or phytochemicals. This supports the sustainable use of TTO in routine clinical practice, assuming appropriate concentration and application duration are maintained—as observed in our study using a standardized 5% solution over 4 weeks.

Beyond patient outcomes, TTO has broader implications for **healthcare worker safety and infection control practices**. Gnatta et al. [22] studied a Melaleuca-based soap as a hand hygiene agent and found it non-inferior to standard products. Their findings point to TTO's potential incorporation into broader tracheostomy care protocols, extending from device cleaning to hand antisepsis, thus offering a unified infection control strategy.

The role of natural products in modulating biofilm formation is also well illustrated by Meto et al. [23], who found that **propolis** inhibited *P. aeruginosa* growth and virulence factor production. While our study did not investigate immune modulation, the consistent reduction in clinical infection rates (9.4% vs. 21.9%, $p = 0.036$) suggests that TTO might exert effects beyond bacterial load reduction—potentially impacting bacterial communication and inflammation.

Finally, the recent innovation by Ścibik et al. [24] of gentamicin nanoparticle coatings on PVC tracheostomy tubes highlights the future direction of **device-integrated antimicrobial strategies**. While promising, such technologies are expensive and not widely accessible in resource-limited settings. In contrast, TTO offers a **cost-effective, scalable, and evidence-supported solution**, especially pertinent in Indian public health settings where this study was conducted.

In conclusion, our findings align closely with the existing literature supporting the antimicrobial efficacy of TTO and extend it by providing randomized, clinical evidence of its superiority over saline in tracheostomy tube decontamination. By demonstrating statistically significant reductions in CFU load, pathogen prevalence, and biofilm-associated clinical outcomes, our trial reinforces the applicability of TTO as a safe and effective agent in routine tracheostomy care. With no statistically significant increase in adverse effects, and substantial gains in microbiological and clinical endpoints, TTO emerges as a **clinically viable, microbiologically potent, and economically feasible biofilm management strategy**.

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