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Original Article

Correlation Between Bacterial Count and Duration of Tracheostomy Tubes Use

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Abstract

Background : Tracheostomy tubes (TTs) are life-sustaining medical devices widely used for patients with upper airway obstruction or requiring prolonged mechanical ventilation. However, TTs serve as an indwelling substrate that promotes bacterial colonization and biofilm formation, particularly by resistant pathogens such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Despite the well-recognized clinical risks, evidence correlating the duration of TT use with quantitative bacterial load in the Indonesian hospital setting remains limited, creating a gap in local clinical guidance for tube replacement scheduling.
Aim : To analyze the correlation between TT duration of use and bacterial colony count (CFU/mL), and to identify the predominant colonizing pathogens in order to inform evidence-based infection prevention and tube replacement timing.
Methods : This cross-sectional observational study was conducted from July 2020 to January 2021 at Dr. Sardjito General Hospital, Yogyakarta, Indonesia. A total of 20 polyvinyl chloride (PVC) Portex™ tracheostomy tubes were collected via consecutive sampling from patients undergoing decannulation procedures. Bacterial cultures were performed at the Department of Microbiology, Universitas Gadjah Mada, with bacterial load expressed as colony-forming units per milliliter (CFU/mL). Spearman's rank correlation was used for statistical analysis.
Results : Mean bacterial counts (CFU × 10^3/mL) were 48.5, 9,853.83, and 28,200.00 for TT use durations of <30 days, 30–90 days, and >90 days, respectively. Spearman's rank correlation demonstrated a strong positive association between duration and bacterial count ($r = 0.70$, $p = 0.01$). *Pseudomonas aeruginosa* was the most prevalent organism, identified in 60% of tubes.
Conclusion : Duration of TT use exceeding 30 days is significantly correlated with higher bacterial colonization burden. *Pseudomonas aeruginosa* was the predominant pathogen. These findings support the recommendation for scheduled TT replacement within 30 days as an infection control measure in tracheostomized patients.

Keywords : Bacterial count, biofilm, infection control, *Pseudomonas aeruginosa*, tracheostomy tube

INTRODUCTION

Tracheostomy is one of the most commonly performed life-saving surgical procedures in critically ill patients worldwide. It provides a reliable artificial airway in patients requiring prolonged mechanical ventilation, management of upper airway obstruction, or assistance with bronchopulmonary secretion clearance. The procedure offers several clinical advantages, including reduced respiratory effort, preservation of laryngeal structures, facilitation of oral intake, improved patient comfort, and the possibility of weaning from ventilatory support. Due to advances in critical care medicine and an increasing number of patients surviving acute respiratory failure, tracheostomy utilization rates continue to rise globally.

Despite its clinical benefits, tracheostomy is not without serious complications. The tracheostomy tube (TT) itself, as an indwelling foreign body that continuously interfaces with the warm, humid, and microorganism-rich environment of the tracheal lumen, provides an ideal substrate for bacterial adhesion and biofilm formation.[1] Biofilms are structured communities of microorganisms encased within a self-produced extracellular polymeric matrix composed of polysaccharides, proteins, and extracellular DNA.[1] This protective matrix substantially increases microbial resistance to antimicrobial agents, with minimum inhibitory concentrations for biofilm-embedded bacteria reported to be 10 to 1,000 times higher than those required to kill planktonic counterparts.[1] Furthermore, bacterial cells in the deep layers of biofilms can enter a dormant metabolic state, rendering them refractory to antibiotics that target actively replicating organisms.[2]

Biofilm formation on TTs has been documented in over 90% of tubes within the first seven days of insertion.[3] A systematic review of 20 studies encompassing 981 endotracheal tubes from ICU patients confirmed that the prevalence of biofilm-producing isolates ranged from 20% to 100% (median 72%), with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* emerging as the dominant biofilm-forming pathogens.[4] As the duration of tube use extends, biofilms progressively mature, increasing in thickness, structural complexity, and species diversity. The ICU environment constitutes a particularly permissive niche for biofilm development: concurrent use of broad-spectrum antibiotics selects for resistant biofilm phenotypes, invasive procedures breach mucosal barriers, and the high density of vulnerable immunocompromised patients facilitates cross-transmission of MDR organisms via contaminated devices.[5] Biofilm on TT surfaces constitutes an underestimated microbiological compartment in critically ill patients; confocal microscopy studies of ETTs from ICU patients have identified distinct biofilm morphological patterns corresponding to specific pathogens, underscoring the structural complexity of TT-associated biofilms that renders standard microbiological cultures insufficient for complete characterization.[6] Mature biofilms serve as chronic reservoirs for pathogenic microorganisms and facilitate the downstream contamination of the lower respiratory tract through microaspiration, resulting in ventilator-associated pneumonia (VAP), tracheobronchitis, or systemic sepsis.[7] VAP is among the most devastating hospital-acquired infections, affecting up to 40% of mechanically ventilated patients with a direct attributable mortality of approximately 10%.[8] Tracheostomy-associated infections contribute substantially to this burden, particularly in patients with prolonged intubation.

Among the pathogenic organisms colonizing TTs, *Pseudomonas aeruginosa* and *Staphylococcus aureus* emerge consistently as the most clinically relevant. *P. aeruginosa*, a Gram-negative opportunist, produces β-lactamases and efflux pumps that confer resistance to multiple antibiotic classes, and it secretes virulence factors that degrade TT biomaterials and promote persistent airway colonization.[9,10] *S. aureus*, particularly in its methicillin-resistant form (MRSA), adheres avidly to polymer surfaces, produces tissue-damaging toxins, and can achieve a persistent dormant state within biofilms.[11] A recent systematic review and meta-analysis of 49 studies confirmed that *Pseudomonas* was the most prevalent genus on both endotracheal and tracheostomy tubes across all conditions examined.[9]

METHODS

Study Design and Setting

This was a cross-sectional observational study conducted at the Department of Otorhinolaryngology Head and Neck Surgery, Dr. Sardjito General Hospital, Yogyakarta, Indonesia, in collaboration with the Department of Microbiology, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada. The study was conducted from July 23, 2020, to January 6, 2021.

Ethical Approval

The study was approved by the Medical and Health Research Ethics Committee (MHREC) of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada / Dr. Sardjito Hospital Yogyakarta. All participants or their legal guardians provided written informed consent prior to enrollment. Patient data were stored anonymously and managed in accordance with institutional data protection protocols.

Participants and Sampling

A consecutive sampling method was employed to recruit all eligible patients presenting for decannulation during the study period. Inclusion criteria were: (1) adults aged

≥18 years; (2) previous tracheostomy for any clinical indication; (3) TT in situ for at least one day; (4) willingness to provide written informed consent. Exclusion criteria were: (1) tracheostomy tube changes performed under emergency circumstances; (2) patients who refused to participate; (3) incomplete microbiological data. A total of 20 patients were enrolled during the study period.

Tracheostomy Tube Collection and Microbiological Analysis

All TTs included in this study were polyvinyl chloride (PVC) Portex™ standard tracheostomy tubes, chosen to standardize the biomaterial variable across subjects. Upon decannulation, each removed TT was immediately placed in a sterile biohazard specimen bag and transported to the Microbiology Laboratory, Universitas Gadjah Mada, within two hours of collection to prevent post-collection bacterial changes.

In the laboratory, each tube was processed using a standardized protocol. The specimen was then subjected to three cycles of vortex mixing (30 seconds each) to dislodge adherent biofilm bacteria from the tube surface. The resulting suspension was serially diluted and plated onto Blood Agar and MacConkey Agar plates, which were incubated aerobically at 37°C for 24–48 hours. Bacterial colony counts were recorded and expressed as colony-forming units per milliliter (CFU/mL). Bacterial identification was performed using standard microbiological methods including Gram staining, colony morphology, and biochemical profiling. The independent variable was the duration of TT use, defined as the number of days from the date of tracheostomy insertion to the date of decannulation, as recorded in the patient's medical chart.

Clinical Data Collection

Patient demographic and clinical data were extracted from medical records using a standardized data collection form. Variables recorded included: patient age, sex, indication for tracheostomy, duration of TT use (categorized as <30 days, 30–90 days, and >90 days), stomal condition (presence or absence of granuloma), and history of pneumonia during the tracheostomy period.

Statistical Analysis

Descriptive statistics were used to summarize continuous and categorical variables. The Spearman's rank correlation coefficient was used to assess the association between duration of TT use and bacterial colony count (CFU/mL), chosen because the data did not meet the assumptions of normality as assessed by the Shapiro-Wilk test. A p-value of <0.05 was considered statistically significant. All analyses were performed using SPSS version 25.0

RESULTS

Demographic and Clinical Characteristics

A total of 20 tracheostomized patients were enrolled in the study. Patient demographic and clinical characteristics are summarized in Table 1. The majority of participants were male (65%, n=13) with a mean age of 55.4 years (range 3275 years). The predominant indication for tracheostomy was upper airway obstruction due to malignancy (70%, n=14), followed by laryngeal or tracheal stenosis (20%, n=4), bilateral abductor paralysis of the vocal cords (5%, n=1), and prolonged use of an endotracheal tube (5%, n=1). Stomal granulation was observed in 30% of patients (n=6), and

TABLE 1
Demographic and Subject Characteristics (n=20)

Variable	n	%
Gender		
Male	13	65
Female	7	35
Tracheostomy Indication		
Upper airway obstruction (malignancy)	14	70
Bilateral abductor paralysis of vocal cord	1	5
Laryngeal/tracheal stenosis	4	20
Prolonged use of endotracheal tube	1	5
Stoma Condition		
Granuloma present	6	30
No granuloma	14	70

TABLE 1. *Continued.*

Variable	n	%
Pneumonia During Tracheostomy		
Pneumonia	5	25
No pneumonia	15	75

TABLE 2
Bacterial Count by Duration of TT Use

Sample	Duration of using	CFU (x 10 ^ 3)	Sample	Duration of using	CFU (x 10 ^ 3)
1	19	88.0	11	210	75000.0
2	14	92.0	12	48	92.0
3	34	332.0	13	3	6.0
4	20	8.0	14	63	29.0
5	242	7000.0	15	36	870.0
6	256	20000.0	16	173	28000.0
7	290	4000.0	17	270	36000.0
8	42	20000.0	18	189	15000.0
9	188	13000.0	19	623	68000.0
10	69	37800.0	20	309	16000.0

TABLE 3
Correlation Between Bacterial Count and Duration of Tracheostomy Tube Use

Correlation Test	Correlation Coefficient (r)	*p*-value	Interpretation
Spearman's Rank Correlation	0.70	0.01	Strong positive correlation

25% (n=5) had a documented episode of pneumonia during the tracheostomy period.

Bacterial Species Identified
The types of bacteria identified from TT cultures were: *Pseudomonas aeruginosa* (n=12, 60%), *Staphylococcus aureus* (n=3, 15%), a combination of *P. aeruginosa* and *S. aureus* (n=2, 10%), and other bacteria (n=3, 15%). Overall, *P. aeruginosa* was the dominant colonizing organism, present in 70% of all specimens (either alone or in combination).

Bacterial Count by Duration of TT Use
Mean bacterial counts (CFU × 10^3/mL) increased markedly with the duration of TT use. For tubes in situ for <30 days, the mean count was 48.5 × 10^3 CFU/mL; for tubes used 3090 days, the mean count was 9,853.83 × 10^3 CFU/mL; and for tubes used >90 days, the mean count was 28,200.00 × 10^3 CFU/mL. These data are illustrated in Table 2.

Correlation Analysis
Spearman's rank correlation analysis demonstrated a strong, statistically significant positive correlation between the duration of TT use and bacterial colony count (r = 0.70, p = 0.01). Results are summarized in Table 3.

DISCUSSION

The findings of this study demonstrate a strong positive correlation between the duration of TT use and the quantitative bacterial burden on TT surfaces (r = 0.70, p = 0.01), with mean bacterial counts escalating from 48.5 ×

10^3 CFU/mL in tubes used for fewer than 30 days to 28,200 × 10^3 CFU/mL in those retained for more than 90 days. This exponential increase in bacterial load reflects the natural history of biofilm maturation on polymer surfaces and has direct clinical implications for infection prevention practice.

These results are biologically plausible and consistent with the established understanding of biofilm dynamics on indwelling medical devices, and are further explicable by the specific physicochemical properties of the tube material used in this study. All 20 TTs analyzed were polyvinyl chloride (PVC) Portex™ standard tracheostomy tubes, a standardization that eliminates biomaterial variability as a confounding factor and allows the temporal colonization trend to be attributed specifically to the duration of tube use rather than material differences.[12] PVC is the most widely used polymer for TT fabrication due to its low cost, flexibility, and ease of manufacturing; however, it possesses surface characteristics that are inherently permissive to bacterial adhesion. PVC surfaces carry a net negative surface charge under physiological conditions, and their moderately hydrophobic surface energy promotes the adsorption of host proteins – including fibronectin, fibrinogen, and albumin – within seconds of contact with the humid tracheal environment. This protein conditioning film fundamentally alters the tube surface, transforming it from an abiotic polymer into a biologically receptive substrate that presents specific ligand-binding sites for bacterial surface adhesins.[7]

The initial phase of bacterial colonization on PVC involves reversible adhesion of planktonic bacteria to this conditioned surface, mediated by weak non-covalent forces including van der Waals interactions, electrostatic attractions, and hydrophobic-hydrophobic contacts between bacterial cell surface structures and the conditioned PVC film. Importantly, PVC lacks intrinsic antimicrobial properties and does not release any bacteriostatic agents, meaning that once bacteria establish initial contact with the tube surface, there is no material-derived mechanism to interrupt the colonization process. Studies specifically examining PVC TTs have shown that *Pseudomonas aeruginosa* – the dominant organism in the present study – adheres preferentially to PVC surfaces compared to silicone or polyurethane, owing to the complementarity between the bacterium's outer membrane hydrophobicity and the surface energy characteristics of PVC.[9] Over the following days, adherent bacteria begin secreting extracellular polymeric substances (EPS), a process that is further promoted by the microscale surface roughness of PVC, which provides sheltered microniches that protect early-stage bacterial clusters from shear forces generated by airflow and suctioning. This EPS secretion marks the transition from reversible to irreversible attachment, anchoring the nascent biofilm to the PVC substrate with covalent and ionic bonds that standard cleaning and suctioning cannot disrupt.[13]

As the biofilm matures on the PVC surface, it develops complex three-dimensional architectural features including fluid-filled water channels that enable nutrient transport and metabolic waste removal throughout the biofilm depth. This structural sophistication allows deeper bacterial layers to persist in a slow-metabolizing, antimicrobial-tolerant state, protected not only by the EPS matrix but also by the structural buffering effect of the PVC tube wall itself, which physically shields the inner biofilm from mechanical disruption.[13] A critical material-specific consideration is that PVC undergoes gradual surface degradation during prolonged tracheal use: plasticizers leach from the polymer matrix over time, and mechanical abrasion from suctioning catheters progressively increases the surface roughness at the micro- and nanoscale. This degradation process, documented by Backman[12] *et al*. (2009) over a six-month observation period, creates an increasingly irregular surface topography that provides more attachment points for bacteria and greater structural complexity for biofilm anchoring, amplifying colonization beyond what would occur on a chemically and physically stable surface. By 30 days, the biofilm on PVC TTs has typically achieved a mature, structured configuration that is exponentially more resistant to both antibiotic penetration and mechanical disruption than nascent biofilms.[7] This material-specific susceptibility of PVC to progressive colonization reinforces the practical recommendation arising from this study that PVC Portex™ tubes – the standard of care in most Indonesian clinical settings – should be replaced on a scheduled basis before the 30-day threshold to prevent the establishment of this mature, treatment-resistant biofilm architecture.

The predominance of *Pseudomonas aeruginosa* (60%) in the present study is a finding of substantial clinical importance. *P. aeruginosa* is recognized as one of the ESKAPE pathogens, a group responsible for a disproportionate share of nosocomial infections and antibiotic resistance globally.[14] The high prevalence of *Pseudomonas* in this study corroborates findings from multiple international studies. A recent systematic review and meta-analysis of 49 clinical microbiology studies from 2000–2024 confirmed *Pseudomonas* as the most prevalent genus on both endotracheal and tracheostomy tubes in all patient groups and sampling methods examined.[3] Similarly, Alrabiah[15] *et al*. (2021), in a single-center retrospective study in Saudi Arabia, reported *P. aeruginosa* as the predominant post-tracheostomy organism (47.7%). Ścibik[9] *et al*. (2022) reported colonization in 97% of tracheostomy tubes within the first 24 hours of insertion, with *S. aureus*, *K. pneumoniae*, and *P. aeruginosa* as the leading pathogens.

A distinctive contribution of the present study is

its prospective, quantitative approach to measuring bacterial load across three categorical duration groups, which provides actionable data for clinical decision-making on tube replacement intervals. While most published studies report qualitative microbial profiles or resistance patterns, they rarely quantify the temporal evolution of bacterial burden in terms of CFU/mL and correlate it statistically with duration of use. The data from this study demonstrate that the transition from <30 days to 30–90 days of TT use is associated with a greater than 200-fold increase in mean bacterial count, a finding that underscores the critical importance of the 30-day threshold as a clinically meaningful decision point for scheduled tube replacement. Kumarasinghe[16] *et al*. (2020) found that colonization rates increased with the frequency of tube changes but did not provide CFU-based quantification. Saravanam[17] *et al*. (2022) conducted a prospective study on microbial profiles in tracheostomy tubes and tracheostomas in South India, reporting similar predominant organisms but without a quantitative temporal correlation analysis. The present study therefore fills a specific evidence gap by providing both the species distribution and the quantitative bacterial burden across well-defined duration categories.

This study was conducted in a tertiary hospital in Indonesia, a setting with distinct epidemiological characteristics compared to high-income countries. The study population included a high proportion of head and neck malignancy patients (70%), reflecting the local disease burden. This is clinically significant because oncologic patients often receive corticosteroids, chemotherapy, or radiotherapy that impairs local immune defenses and increases susceptibility to airway colonization. Medical device-associated biofilm infections are a growing concern in Indonesian hospitals, particularly given the increasing prevalence of multidrug-resistant organisms.[14] The diversity of tracheostomy indications also means the findings are generalizable to a broader clinical spectrum than studies restricted to ICU-ventilated patients.

The clinical implications of this study's findings are multifold. First, the strong positive correlation between duration of use and bacterial count provides a scientific basis for recommending TT replacement before 30 days of use, particularly in patients who are immunocompromised, have evidence of respiratory deterioration, or harbor known drug-resistant pathogens. Second, the 60% prevalence of *Pseudomonas aeruginosa* in this population has critical implications for empirical antibiotic selection. Lepainteur[18] *et al*. (2019) cautioned that the high prevalence of *P. aeruginosa* and *Serratia marcescens* in chronically tracheostomized patients renders amoxicillin-clavulanate ineffective as empirical therapy for lower respiratory tract infections in this population. These findings collectively underscore the need for institution-specific antibiograms and individualized antibiotic stewardship protocols in tracheostomized patients.

P. aeruginosa is a notoriously difficult pathogen to treat owing to its intrinsic and acquired resistance mechanisms, including efflux pumps (MexAB-OprM, MexCD-OprJ), outer membrane impermeability, AmpC β-lactamase overexpression, and the capacity for horizontal gene transfer of resistance determinants.[19] Reynolds and Kollef (2021) provide an updated framework for understanding the epidemiology and treatment of *P. aeruginosa* infections, emphasizing that empirical antibiotic selection must be guided by local resistance patterns and the patient's prior antibiotic exposure history. For suspected tracheostomy-associated pneumonia caused by *P. aeruginosa*, first-line empirical agents with established anti-pseudomonal activity include piperacillin-tazobactam, cefepime, and carbapenems such as meropenem or imipenem-cilastatin. Among these, piperacillin-tazobactam and cefepime are generally preferred as initial agents when susceptibility data are unavailable, given their favorable safety profiles and broad Gram-negative coverage.[19] However, both agents are susceptible to hydrolysis by extended-spectrum β-lactamases (ESBLs) and AmpC enzymes frequently expressed by *P. aeruginosa* biofilm isolates, and susceptibility testing is therefore imperative before definitive therapy is initiated.[19]

For infections caused by multidrug-resistant (MDR) or difficult-to-treat (DTR) *P. aeruginosa* strains – defined as strains resistant to all first-line antipseudomonal agents – newer β-lactam/β-lactamase inhibitor combinations represent important therapeutic advances. Ceftolozane-tazobactam and ceftazidime-avibactam have demonstrated superior activity against MDR *P. aeruginosa* compared to older agents, particularly in strains overexpressing efflux pumps or producing AmpC β-lactamases.[19] The 2024 Infectious Diseases Society of America (IDSA) guidance on the treatment of antimicrobial-resistant Gram-negative infections recommends ceftolozane-tazobactam as a preferred agent for DTR *P. aeruginosa* infections, with ceftazidime-avibactam as an alternative when metallo-β-lactamase production is suspected.[20] Colistin and polymyxin B, while historically used as last-resort agents, carry significant nephrotoxicity risk and are now reserved for pan-drug-resistant strains only when no other options exist.[21]

The choice of antibiotic must also consider the pharmacokinetic/pharmacodynamic (PK/PD) challenges posed by *P. aeruginosa* biofilms on TT surfaces. Biofilm-embedded bacteria can tolerate antibiotic concentrations 10 to 1,000 times higher than planktonic minimum inhibitory concentrations (MICs), necessitating either prolonged infusion strategies to optimize time-dependent killing (particularly for β-lactams such as piperacillin-tazobactam and

meropenem) or the use of concentration-dependent agents such as aminoglycosides (amikacin, tobramycin) or fluoroquinolones (ciprofloxacin) as combination partners.[19,20] Inhaled antibiotics, including inhaled tobramycin or colistimethate, offer the advantage of delivering very high local drug concentrations directly to the airway mucosa and TT surface, potentially overcoming the concentration barrier imposed by biofilm structure.[19] For pediatric tracheostomized patients, a systematic review by Pearce[22] *et al*. (2024) similarly concluded that anti-pseudomonal agents represent the most frequently required antibiotic class and recommended routine tracheal aspirate surveillance cultures to guide targeted therapy before overt clinical infection develops. This recommendation aligns with the present study's findings and reinforces the value of microbiological monitoring in tracheostomized patients regardless of their ventilation status.

Third, the absence of a strong association between ventilator use and biofilm formation, as observed in prior studies, suggests that the physical presence of the TT itself, rather than mechanical ventilation alone, is the primary driver of colonization. This finding challenges the common assumption that infection control efforts should focus exclusively on mechanically ventilated patients and highlights the need for equally rigorous tube hygiene and surveillance protocols in non-ventilated tracheostomized patients.[23]

The presence of stomal granulation in 30% of patients in this study requires detailed discussion, as it represents a clinically significant complication of tracheostomy that is closely intertwined with microbial colonization dynamics. Granulation tissue (granuloma) at the tracheostoma is an abnormal wound-healing response characterized by the proliferation of fibroblasts, capillary buds, and inflammatory cells, primarily macrophages and neutrophils, at the tracheal mucosal interface with the TT. The fundamental stimulus for this exuberant healing response is chronic low-grade inflammation driven by the mechanical irritation of the tube against the tracheal mucosa, exacerbated by persistent bacterial colonization and biofilm formation on the tube surface.[17]

The pathophysiological relationship between biofilm and granulation formation is bidirectional and self-reinforcing. Bacterial biofilms on the TT surface continuously release lipopolysaccharides (LPS), peptidoglycans, and other pathogen-associated molecular patterns (PAMPs) that activate toll-like receptors (TLRs) on tracheal epithelial cells and resident macrophages.[7] This activation triggers a sustained pro-inflammatory cascade involving interleukins (IL-1β, IL-6, IL-8) and tumor necrosis factor-alpha (TNF-α), which recruits circulating neutrophils and monocytes to the stoma site.[24] Paradoxically, while this inflammatory response attempts to clear the biofilm, it simultaneously promotes fibroblast proliferation and collagen deposition, the cellular mechanisms underlying granulation tissue formation. Gram-negative organisms such as *Pseudomonas aeruginosa* are particularly potent drivers of this cycle because their LPS component is a powerful TLR-4 agonist, inducing a more vigorous inflammatory reaction compared to Gram-positive cell wall components.[9] This may explain the clinical observation that patients colonized by Gram-negative organisms tend to develop more exuberant granulation than those colonized by Gram-positive flora alone.

Furthermore, the granulation tissue itself creates a microenvironment that paradoxically facilitates further bacterial colonization. The richly vascularized, irregular surface of granulation tissue provides an increased surface area and altered tissue architecture that promotes bacterial adhesion. The local hypoxia characteristic of proliferating granulation tissue also favors the selection of oxygen-tolerant and anaerobic organisms and reduces the bactericidal activity of neutrophils, whose oxidative burst mechanism requires molecular oxygen.[2] Exudate from granulation tissue provides a nutrient-rich medium that sustains bacterial growth and biofilm maturation. This self-perpetuating cycle of infection, inflammation, and granulation formation represents a key mechanism by which prolonged TT retention leads to compounding clinical complications.

In clinical practice, stomal granulation carries several important consequences that extend beyond cosmetic concern. First, granulation tissue at the stoma can partially or completely obstruct the TT lumen or the stomal opening, particularly in patients with inner cannula-style tubes, leading to increased airway resistance, difficulty with tube changes, and in severe cases, acute airway compromise. Second, granulation tissue is friable and highly vascular, making it prone to contact bleeding during suctioning, tube manipulation, or speech valve attachment, which may cause patient distress and complicate nursing care. Third, exuberant granulation at the tracheal level can contribute to tracheal stenosis, a long-term complication that may require surgical or endoscopic intervention. A study by Saravanam[17] *et al*. (2022) confirmed that patients who underwent tube changes after more than one month had significantly higher grades of granulation compared to those changed within one month, directly paralleling the bacterial load escalation observed in the present study.

The 30% prevalence of granulation in this cohort highlight the clinical utility of routine stomal assessment at each tube change as an early indicator of suboptimal TT management, excessive dwell time, or uncontrolled bacterial colonization. For patients found to have Grade 2 or higher granulation, earlier TT replacement and intensified topical hygiene protocols should be strongly considered. In refractory cases, application of silver nitrate cautery, topical corticosteroids, or surgical

excision of granulation tissue may be required to restore stomal anatomy and facilitate decannulation. These considerations reinforce the practical importance of this study's recommendation that TT replacement should occur before 30 days of use, not only to limit bacterial burden but also to prevent the cascade of stomal complications driven by chronic colonization and biofilm-mediated inflammation.[24]

From a public health perspective, the increasing prevalence of multidrug-resistant (MDR) organisms in hospital environments in Indonesia and other resource-limited settings makes biofilm management a priority. The development of antimicrobial-coated TTs, incorporating materials such as silver ions, titanium dioxide, or antimicrobial peptides, offers promising future strategies to mitigate colonization.[25] Research by Ochońska[26] *et al*. (2021) further demonstrated that *K. pneumoniae* forms genetically diverse biofilms on both PVC and polyethylene TT surfaces, reinforcing the need for biomaterial-level solutions to colonization.

CONCLUSION

This study demonstrates a strong positive correlation between the duration of TT use and bacterial colonization burden ($r = 0.70$, $p = 0.01$), with mean bacterial counts increasing more than 200-fold between tubes used for fewer than 30 days and those retained for 30–90 days. *Pseudomonas aeruginosa* was the predominant colonizing pathogen, identified in 60% of specimens. These findings provide quantitative evidence supporting the recommendation for scheduled TT replacement within 30 days as a key infection control strategy in tracheostomized patients. The findings also underscore the need for empirical antibiotic regimens with anti-pseudomonal coverage when treating suspected tracheostomy-associated respiratory infections in this population. Future research should prioritize larger prospective multicenter studies with resistance profiling to refine clinical guidelines for TT management.

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CONFLICT OF INTEREST

All authors declare that they have no financial or non-financial conflicts of interest relevant to this article. The study received no external funding from pharmaceutical companies or medical device manufacturers.

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