ORAL MICROBIOME IN MECHANICALLY VENTILATED PATIENTS

Dissertação apresentada à Universidade Católica Portuguesa para obtenção do grau de Mestre em Medicina Dentária

Por:

Edoardo Calcinelli

Viseu, 2026

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Oggi non è che un giorno qualunque di tutti i giorni che verranno.
Ma quello che accadrà in tutti gli altri giorni che verranno
può dipendere da quello che farai oggi.

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ABSTRACT:

Introduction: In critically ill patients, mechanical ventilation can disrupt the ecological balance of the oral cavity, potentially promoting dysbiosis and colonization by respiratory pathogens. These alterations may contribute to the development of ventilator-associated pneumonia (VAP).

Aim of the study: This systematic review aimed to evaluate how mechanical ventilation influences oral microbiome composition, ecological balance, and clinical implications in critically ill patients. Specifically identifying ventilation-associated microbial shifts, assessing links with ventilator-associated pneumonia (VAP), and evaluating oral hygiene interventions.

Materials and Methods: A PRISMA-based systematic review was conducted using PubMed/MEDLINE, Scopus and CENTRAL. Eligible studies included randomized controlled trials and prospective cohorts analysing oral microbiota in mechanically ventilated adults using culture-based or sequencing approaches.

Results: Included studies demonstrated oral microbiome dysbiosis during mechanical ventilation, characterized by decreased commensal taxa such as *Streptococcus, Neisseria,* and *Veillonella*, alongside increased opportunistic and respiratory-associated pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa* and *Enterobacteriaceae*. *Pseudomonas aeruginosa* abundance was a potential biomarker of infection risk. Interventional studies reported that structured oral hygiene measures, including chlorhexidine protocols and toothbrushing, reduced periodontal pathogens and dysbiosis; however, associations with VAP outcomes were weak or inconsistent.

Conclusions: Mechanical ventilation promotes ecological disruption of the oral microbiome, favouring pathogen enrichment and loss of commensal diversity. Although oral hygiene interventions reduce microbial burden, evidence suggests limited effects on infection outcomes. The oral cavity remains a modifiable reservoir influencing respiratory health, highlighting the need for standardized longitudinal microbiome studies and optimized oral care strategies in ICU settings.

Keywords: Oral microbiome, Mechanical Ventilation, Ventilation Associated Pneumonia.

RESUMO:

Introdução: Em doentes em estado crítico, a ventilação mecânica (VM) pode perturbar o equilíbrio ecológico da cavidade oral, promovendo disbiose e colonização por agentes patogénicos respiratórios. Estas alterações podem contribuir para o desenvolvimento de pneumonia associada à ventilação mecânica (PAVM).

Objetivo do estudo: Avaliar a influência da VM na composição do microbioma oral, no equilíbrio ecológico e nas implicações clínicas em doentes críticos. Os objetivos incluíram identificar alterações microbianas associadas à ventilação, avaliar a sua relação com a PAVM e analisar o impacto das intervenções de higiene oral.

Materiais e métodos: Foi realizada uma revisão sistemática baseada no PRISMA nas bases PubMed/MEDLINE, Scopus e CENTRAL. Foram incluídos ensaios clínicos aleatorizados e estudos de coorte prospetivos que analisaram a microbiota oral de adultos submetidos a VM através de métodos culturais ou de sequenciação.

Resultados: Os estudos incluídos demonstraram disbiose do microbioma oral durante a VM, caracterizada pela diminuição de géneros comensais, como *Streptococcus, Neisseria* e *Veillonella*, e pelo aumento de agentes patogénicos oportunistas e respiratórios, incluindo *Staphylococcus aureus, Pseudomonas aeruginosa* e *Enterobacteriaceae*.

A abundância de *Pseudomonas aeruginosa* revelou-se potencial biomarcador de risco de infeção. Estudos intervencionais demonstraram que protocolos estruturados de higiene oral, incluindo clorexidina e escovagem dentária, reduziram patógenos periodontais e disbiose; contudo, os seus efeitos nos desfechos relacionados com PAVM foram limitados e inconsistentes.

Conclusões: A VM promove perturbações ecológicas do microbioma oral, favorecendo o enriquecimento de agentes patogénicos e a perda de diversidade comensal. Embora as intervenções de higiene oral reduzam a carga microbiana, a evidência disponível sugere benefícios limitados nos desfechos infeciosos. A cavidade oral permanece um reservatório modificável com potencial influência na saúde respiratória, reforçando a necessidade de estudos longitudinais padronizados e de estratégias otimizadas de cuidados orais em contexto de UCI.

Palavras-chave: Microbioma Oral, Ventilação Mecânica, Pneumonia Associada a Ventilação.

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List of abbreviation and acronyms

AIS Acute Ischemic Stroke

CHX Chlorhexidine

ICU Intensive Care Unit

MDROs Multidrug-Resistant Organisms

MV Mechanical Ventilation

NR Not Reported

RCT Randomized Controlled Trial

SAP Stroke-Associated Pneumonia

VAP Ventilator-associated Pneumonia

1. INTRODUCTION

1.1 Introduction to the oral microbiome

The oral microbiome is an ecosystem which provides support to mucosal integrity, local and systemic immunity, and has a role in suppressing colonization by opportunistic pathogens (1). It is composed of a heterogeneous population of microorganisms, primarily bacteria but also fungi, viruses, and archaea that continuously colonize the oral cavity, forming complex communities in several areas such as oral mucosa, the dorsal surface of the tongue, the gingival sulci, dental surfaces, and saliva (2). A balanced microbial community is considered a hallmark of oral health, whereas disruption of this equilibrium, known as dysbiosis, has been associated with oral and systemic diseases (3). Despite being continuously exposed to environmental influences such as diet, oral hygiene, salivary flow, and pH fluctuations, the oral microbiome generally maintains long-term stability through complex microbial interactions (1).

1.1.1. Structure and composition

The oral cavity hosts distinct ecological niches defined by surface anatomy, oxygen tension, nutrient gradients, and host-derived factors, leading to the coexistence of multiple microbial communities that differ in composition and function (1). The oral microbiome is predominantly composed of members of the phyla *Bacillota* (previously known as *Firmicutes*), *Actinobacteria*, *Proteobacteria, Bacteroidota, Fusobacteriota* and *Spirochaetes*, reflecting the heterogeneity of oral habitats and stages of biofilm development (2).

Supragingival plaque developing on exposed tooth surfaces is typically dominated by aerotolerant and facultative anaerobic taxa capable of persisting despite fluctuations in oxygen availability and nutrient exposure associated with diet and salivary flow (1).

Conversely, the gingival sulcus represents a relatively protected and low-oxygen microenvironment that favors strict anaerobes such as *Porphyromonas*, *Prevotella*, *Treponema*, and *Fusobacterium*, which are key members of subgingival biofilm consortia and are strongly associated with periodontal inflammation (1,3).

The ecological distinction between these niches is clinically relevant because environmental perturbations may affect them differently: reduced salivary flow and impaired clearance primarily promote supragingival biofilm accumulation and maturation, whereas altered oxygenation particularly favors subgingival anaerobic communities, ultimately shifting both niches toward dysbiotic configurations enriched in opportunistic pathogens (1).

1.1.2. Physiological functions and role in eubiosis

The oral microbiome exists in an equilibrium state in many physiological conditions essential for host health. It plays a critical role in colonization resistance, where commensal microorganisms provide protective and restricting environments and prevent the establishment/overgrowth of exogenous and opportunistic pathogens by competing for adhesion sites, nutrients, and ecological niches (2). Besides colonization resistance, the oral microbiome also involves core functions in nutrient metabolism and usage. Commensal microbial communities also actively metabolize dietary substrates, such as carbohydrates and proteins, aiding digestibility during early stages of digestion by breaking down starches and proteins and repurposing metabolic byproducts within the biofilm ecosystem along with their metabolic activities (4).

Metabolic exchanges between microbes not only contribute to microbial survival but also shape the physicochemical environment of the oral cavity by modifying local pH and nutrient availability. For example, acidogenic bacteria ferment dietary carbohydrates and release organic acids that lower pH, whereas other microorganisms produce alkaline metabolites such as ammonia that increase pH. At the same time, the breakdown of complex salivary components and dietary substrates generates smaller molecules, including amino acids, peptides, and lactate, which can be used as nutrients by neighboring species (1). Another important role of the microbiome within eubiosis is the modulation of local immunity. Ongoing close interactions of commensal microorganisms with the immune system are reported to favor immune tolerance, manipulate inflammatory signalling, and induce formation of antimicrobial peptides (AMPs) and secretory immunoglobulins (sIgA), increasing mucosal barrier function (5).

This balance in immune activity is important for preventing a pro-inflammatory state and ensuring an effective immune defence against attacks from pathogenic bacteria. Nutrient availability, oxygen tension, salivary flow, response of host-derived immune mediators, and other external factors such as orthodontic treatment or oral rehabilitation materials, continuously control microbial competition and cooperation, enabling the environment to respond to physiological instability and maintain function. Importantly, competition among microbial species helps maintain microbial diversity and

ecological balance, preventing any single taxon from dominating the community and supporting stable interactions with the host immune system. However, disruption of such interactions is likely to send the oral microbiome into a state of dysbiosis with systemic and local consequences (3).

1.1.3. Oral biofilm: structure and ecological succession

Oral biofilms are structured, three-dimensional microbial ecosystems in which microorganisms are embedded within an extracellular polymeric substance (EPS) matrix primarily composed of polysaccharides, proteins, and extracellular DNA (1). This matrix is not merely a scaffold: it provides physical cohesion, enhances adhesion to surfaces, and contributes to the formation of microenvironmental gradients (including oxygen, pH, and nutrient availability) that sustain complex multispecies communities (2).

In functional terms, biofilm architecture promotes environmental and antimicrobial protection, supports metabolic cooperation and exchange, and facilitates coordinated behaviours through intercellular signalling mechanisms such as quorum sensing (1). Within this structured matrix, microorganisms adhere to one another via species-specific co-aggregation, allowing successive waves of colonization that drive biofilm maturation (2).

Early colonizers, commonly including *Streptococcus* and *Actinomyces* species, establish initial layers and create conditions that enable the adhesion of later taxa; as oxygen becomes progressively depleted within the deepening biofilm, obligate anaerobes increasingly dominate the mature community structure (2). From a clinical perspective, this ecological succession is relevant because mature, EPS-rich biofilms are more difficult to disrupt mechanically and may more readily sustain dysbiotic configurations when host or environmental conditions change, thereby contributing to the persistence of pathogenic taxa under adverse conditions (5).

Recent studies have demonstrated the intricate 3D structure of the oral biofilm, especially the supragingival communities. Structures as the corncob formed by the association of what was thought to be the *Fusobacterium* and *Streptococci* (6) have been replaced by the discovery of *Corynebacterium matruchotii* that is covered by *Streptococci* (7).

1.2. Dysbiosis of the oral microbiome

Oral dysbiosis is defined as a disruption of the physiological equilibrium between the microbial community and the host, in which the balance between commensals and potentially pathogenic taxa becomes altered (8). Rather than reflecting the presence of a single pathogen, dysbiosis typically

involves community-level qualitative and/or quantitative shifts, including reduced beneficial commensals, expansion of pathobionts, altered metabolic outputs (e.g., increased acidogenic potential), and dysregulated host–microbe immune interactions (9).

Clinically, these ecological changes are associated with oral diseases such as caries and periodontitis and may contribute to extraoral outcomes, including respiratory and systemic inflammatory conditions, particularly in susceptible or medically compromised patients (1).

A key concept is that dysbiosis reduces the resilience of the microbial community, making it more susceptible to environmental perturbations and facilitating the overgrowth of opportunistic pathogens. Importantly, dysbiosis is not consistently associated with a reduction in microbial diversity; depending on the ecological context and disease state, diversity may either decrease or increase, while the defining feature remains a shift in community composition, functional balance, and host–microbe interactions (10)

1.2.1. Mechanisms and predisposing factors of dysbiosis

The main risk factors for dysbiosis are a diet high in sugar, smoking, alcohol consumption, reduced salivary flow, stress, antibiotic treatment, and poor oral hygiene (8). A diet rich in fermentable carbohydrates can promote the selective expansion of acidogenic and aciduric microorganisms, which may contribute to acidification of the oral environment and, over time, to shifts in microbial composition that are commonly associated with dysbiosis (1).

Similarly, chronic alcohol consumption compromises mucosal integrity and salivary function, facilitating microbial adhesion and biofilm maturation and further destabilizing microbial homeostasis (11). Saliva plays a fundamental role in maintaining oral eubiosis through its buffering capacity, antimicrobial components, and mechanical clearance of microorganisms. Reduced salivary flow, which is commonly observed in stressed individuals and critically ill patients, diminishes these protective mechanisms and promotes the accumulation of dysbiotic biofilms and opportunistic pathogens (5).

Chronic stress, through neuroendocrine, immune interactions, may further modulate oral microbial composition by reducing immune surveillance and altering the biochemical properties of saliva (1).

Antibiotic exposure, particularly to broad-spectrum agents, represents one of the most powerful drivers of oral dysbiosis. Several studies have demonstrated that even short-term or prophylactic

antibiotic use can markedly reduce microbial diversity and select for Gram-negative and multidrug-resistant organisms in the oral cavity, effectively transforming it into a reservoir of potentially pathogenic and resistant strains (12). However, although antibiotics often induce an initial decrease in oral microbial diversity and suppress susceptible commensal species, the oral biofilm appears to be relatively resilient over the medium to long term. Following antibiotic exposure, the salivary and oral microbiome frequently shows partial recovery toward its baseline composition within weeks to months. Nevertheless, this recovery is often incomplete, as the regrown biofilm may remain compositionally altered, with persistent enrichment of resistant and opportunistic taxa and a more diverse oral resistome. In addition, the close spatial organization of the biofilm may facilitate horizontal transfer of antimicrobial-resistance genes between microorganisms, allowing resistant strains to persist even after the overall community structure has apparently recovered (13).

This selective pressure not only reshapes the oral microbiome but also increases the risk of respiratory colonization and infection in vulnerable patients (9).

Finally, poor oral hygiene allows biofilm accumulation and maturation, accelerating the transition from a commensal-dominated community to a dysbiotic biofilm enriched in pathogenic species. In contrast, structured oral hygiene protocols have been shown to significantly reduce pathogenic microbial load and help preserve a more balanced oral microbiome, particularly in intensive care settings (14).

Collectively, these factors act synergistically to promote oral microbiome dysbiosis, with important implications not only for oral health but also for respiratory and systemic diseases (5).

1.2.2. Oral Conditions Related to the Oral Biofilm

The major oral diseases, particularly dental caries and periodontitis are strongly associated with microbial dysbiosis (1). In dental caries, frequent exposure to fermentable carbohydrates creates selective pressure that favors acidogenic and aciduric microorganisms, including *Streptococcus mutans* and *Lactobacillus spp*., while reducing the abundance of health-associated commensal bacteria (1,8). The resulting dysbiotic biofilm is characterized by increased acid production and enhanced extracellular polysaccharide synthesis, which contribute to biofilm maturation and the persistent acidification of the local environment. Over time, these ecological and metabolic changes promote demineralization of dental hard tissues and progression of the carious process (4,8).

Similarly, periodontitis is now regarded as a dysbiosis-driven inflammatory disease resulting from complex interactions between pathogenic microorganisms and the host immune response (3). Rather than being caused by a single pathogen, periodontal disease is associated with a shift in the subgingival microbial community, characterized by a reduction in beneficial commensals and the enrichment of anaerobic and opportunistic microorganisms with increased inflammatory potential (3,14). This dysbiotic ecosystem promotes chronic inflammation, tissue-destructive host responses, connective tissue breakdown, and alveolar bone resorption, ultimately leading to the progressive destruction of the periodontal supporting tissues (3,8).

Together, dental caries and periodontitis represent the most common clinical manifestations of oral microbial dysbiosis and illustrate how disruption of the ecological balance between the host and the oral microbiome can drive the development of chronic oral diseases.

1.2.3. Systemic impact

Oral dysbiosis may influence systemic health through multiple biological pathways, including transient bacteremia originating from inflamed periodontal tissues and the dissemination of microbial products and inflammatory mediators into the circulation (8). These mechanisms have been proposed to contribute to systemic inflammation, endothelial dysfunction, and altered immune responses beyond the oral cavity (1).

Epidemiological studies have reported associations between periodontal disease and several systemic conditions, including cardiovascular disease, diabetes mellitus, and other chronic inflammatory disorders. Although the underlying mechanisms are complex and causality remains difficult to establish, growing evidence supports the concept that the oral cavity functions as a biologically active interface capable of influencing systemic inflammatory processes when microbial homeostasis is disrupted (1,15).

These observations highlight the potential consequences of oral dysbiosis beyond local disease and reinforce the importance of maintaining a balanced oral microbiome for both oral and general health.

Among the systemic consequences associated with oral dysbiosis, respiratory diseases are of particular interest, as the oral cavity represents a major microbial reservoir directly connected to the lower respiratory tract.

1.3. Relationship between the oral microbiome and the respiratory system

Traditionally, the lower respiratory tract was considered a sterile environment in healthy individuals. However, advances in culture-independent techniques, particularly 16S rRNA sequencing and metagenomics, have demonstrated that the lungs harbor a resident, low-biomass microbial community even under physiological conditions. The pulmonary microbiome is thought to result from a dynamic balance between microbial immigration, mainly through micro

aspiration and inhalation, and microbial elimination by coughing, mucociliary clearance, and host immune defenses. In healthy subjects, this community is generally composed of microorganisms originating from the oral cavity and upper respiratory tract, including *Streptococcus, Prevotella*, and *Veillonella* species (5).

The recognition that the lungs are not sterile has important clinical implications because it complicates the distinction between normal colonization and true pulmonary infection. The detection of bacteria in respiratory samples no longer necessarily indicates disease, as some microorganisms may represent members of the physiological lung microbiome or transient colonizers derived from the oral cavity. Consequently, pulmonary infection is now understood not simply as the presence of bacteria, but as a dysbiotic shift characterized by increased bacterial burden, predominance of pathogenic taxa, loss of ecological balance, and associated host inflammatory response. This distinction is particularly challenging in mechanically ventilated patients, in whom microaspiration of oral secretions and impaired clearance mechanisms continuously modify the respiratory microbiome (5).

At the respiratory level, the oral cavity represents a dynamic microbial reservoir with a direct impact on lower airway health, particularly in susceptible populations such as elderly individuals, patients with chronic respiratory disease, and critically ill subjects (2). Under physiological conditions, the oral microbiome contributes to mucosal homeostasis and immune regulation; however, when dysbiosis occurs, the oral cavity may become enriched in opportunistic and respiratory-associated pathogens with the potential to be aspirated into the lower respiratory tract. The anatomical and functional continuity between the oral cavity, the upper respiratory tract, and the gastrointestinal system facilitates microbial translocation through multiple routes, including microaspiration of saliva, ingestion, and, in compromised mucosal conditions, hematogenous dissemination (16). These pathways are particularly relevant in hospitalized and intensive care unit (ICU) patients, in whom protective reflexes such as swallowing and coughing are often impaired and mechanical ventilation further increases the risk of aspiration.

Several studies have demonstrated that oral bacterial taxa are frequently detected in bronchial secretions and bronchoalveolar lavage samples, supporting a direct ecological link between the oral and bronchial microbiomes (5). Recent mechanistic and clinical perspectives further reinforce the concept of an oral–lung axis in which the oral microecosystem actively contributes to respiratory disease susceptibility (17). Dysbiotic oral biofilms enriched in periodontal pathogens and opportunistic microorganisms may act as reservoirs capable of seeding the lower airway through repeated microaspiration events, particularly in frail or intubated patients (18). Reviews examining the oral microecosystem highlight that microbial migration is accompanied by immune modulation and inflammatory signalling that may impair epithelial barrier integrity and promote infection persistence (17).

In this framework, impaired oral health, periodontal inflammation, and accumulation of dental plaque are increasingly recognized as key drivers in the pathogenesis of aspiration pneumonia, supporting the idea that oral disease is not merely a comorbidity but a mechanistic contributor to pulmonary infection (18).

This oral–lung axis has been implicated in the pathogenesis of aspiration pneumonia, exacerbations of chronic obstructive pulmonary disease (COPD), and alterations of the bronchial microbial community that may predispose to infection and sustained inflammation (18). In this context, the oral cavity functions not merely as a passive microbial source but as an active ecological niche capable of shaping respiratory microbial composition (16). While the oral ecosystem plays a crucial role in maintaining local physiological balance, it may also harbor inflammatory triggers, harmful microbial metabolites, and organisms with invasive potential when dysbiosis is established (5). These factors can contribute to epithelial barrier disruption, immune dysregulation, and chronic inflammatory signalling, thereby facilitating microbial persistence and dissemination to distant anatomical sites (3).

The impact of these processes is amplified in fragile patients, in whom systemic immunity is compromised and microbial translocation may result in clinically relevant respiratory and systemic infections (18). Taken together, these observations support the concept that the oral cavity represents a critical control point in the prevention of respiratory infections (5).

1.4. Mechanical Ventilation and Oral Microbiome Dysbiosis

Mechanical ventilation profoundly disrupts the oral ecosystem, particularly in critically ill patients, leading to rapid oral microbiome remodelling with important local and systemic consequences (19).

These alterations result from a combination of ventilation-related and intensive care unit (ICU)-related factors, including reduced salivary clearance, persistent oral breathing, sedation-related impairment of swallowing, antibiotic exposure, and the presence of endotracheal tubes that provide new surfaces for biofilm formation (5).

Within 24–72 hours after intubation, several longitudinal studies, including those conducted by Sands et al., Tsuda et al., Ren et al., and Song et al., have consistently reported a shift from a commensal-dominated oral microbiome toward dysbiotic communities enriched in opportunistic and respiratory-associated pathogens (20). This ecological transition is characterized by a reduction in health-associated taxa such as *Streptococcus spp., Rothia, and Actinomyces*, accompanied by the expansion of microorganisms including *Staphylococcus aureus, Enterococcus spp., Enterobacteriaceae*, and non-fermenting Gram-negative bacteria such as *Pseudomonas aeruginosa* (20).

Ventilation-associated xerostomia plays a central role in this process by disrupting the salivary film, reducing buffering capacity, and impairing mechanical microbial clearance, thereby facilitating biofilm accumulation and maturation (5). Antibiotic exposure further contributes to microbiome disruption by selecting resistant and opportunistic microorganisms within the oral cavity. Studies have demonstrated that even short-term administration of broad-spectrum antibiotics may favor the emergence of Gram-negative bacteria harbouring clinically relevant antimicrobial resistance mechanisms (12).

Current evidence indicates that mechanical ventilation is associated with rapid and reproducible changes in oral microbial composition, often detectable within the first days following intubation (21). Culture-based and molecular investigations, including 16S rRNA sequencing studies, have consistently demonstrated enrichment of respiratory-associated taxa and a concomitant reduction in commensal microorganisms, highlighting the high sensitivity of the oral ecosystem to critical illness, ventilation-related exposures, and ICU interventions (14,21).

Recent research has also demonstrated that oral care practices may influence these microbial trajectories. Structured oral hygiene interventions have been associated with reductions in oral bacterial burden and partial modulation of microbiome composition, suggesting that oral care may actively influence the progression of ventilation-associated dysbiosis rather than simply reducing superficial contamination (22). Collectively, these findings support the concept that mechanical

ventilation promotes a rapid transition from a balanced oral ecosystem toward a dysbiotic microbiome enriched in opportunistic and potentially pathogenic microorganisms.

1.5. Clinical Implication and Knowledge Gaps

The clinical relevance of ventilation-associated oral dysbiosis extends beyond local microbial alterations. The oral cavity is increasingly recognized as a major reservoir of respiratory pathogens and a key contributor to lower respiratory tract infections in mechanically ventilated patients (14). Multiple studies have demonstrated a strong concordance between microorganisms isolated from oral biofilms and those recovered from tracheal secretions or bronchoalveolar lavage samples, supporting microaspiration of colonized oral secretions along the endotracheal tube as a principal mechanism underlying ventilator-associated pneumonia (VAP) (14).

In addition to facilitating respiratory pathogen transmission, the oral cavity may function as a reservoir for multidrug-resistant microorganisms, including *Klebsiella pneumoniae, Acinetobacter spp.,* and other Gram-negative pathogens. Antibiotic-driven selective pressure may further promote the emergence and persistence of resistant strains within the oral niche, increasing the risk of lower airway colonization and infection (12). Ventilation-associated dysbiosis has also been linked to oral complications such as *Candida* colonization, mucositis, ulcerative lesions, and deterioration of oral tissue integrity, particularly in critically ill and immunocompromised individuals (23).

Given that several determinants of oral dysbiosis are potentially modifiable, preventive strategies have received increasing attention. Structured oral hygiene protocols, particularly those combining mechanical plaque removal with antiseptic agents such as chlorhexidine, have demonstrated significant reductions in oral pathogen burden and respiratory pathogen carriage when compared with chemical cleansing alone (24,25). These findings support the oral cavity as a clinically relevant and actionable target for preventing infectious complications in intensive care settings.

Despite growing evidence supporting the existence of ventilation-associated oral microbiome remodelling, important knowledge gaps remain. Available studies are highly heterogeneous regarding patient populations, sampling methods, sequencing technologies, oral hygiene protocols, antibiotic exposure, and outcome measures. Furthermore, the temporal dynamics of microbiome alterations and their direct relationship with respiratory complications have not yet been fully clarified. As a result, the extent to which specific microbiome changes contribute to respiratory outcomes remains uncertain.

A comprehensive synthesis of the available evidence is therefore necessary to better characterize oral microbiome alterations in mechanically ventilated patients and to clarify their potential clinical implications.

1.6. Objectives of the systematic review

The objective of this systematic review is to examine the current scientific evidence regarding alterations in the oral microbiome of mechanically ventilated patients and to explore the possible clinical implications of these changes for respiratory health in critically ill individuals. Mechanical ventilation can profoundly modify the oral environment through several mechanisms, including reduced salivary clearance, impaired swallowing reflexes, antibiotic exposure, and the presence of endotracheal devices that provide new surfaces for biofilm formation. These conditions may promote ecological shifts within the oral microbial community, potentially favouring the emergence of opportunistic and respiratory-associated microorganisms. Understanding how these changes occur and how consistently they are reported across the literature is essential to clarify the role of the oral cavity in the pathogenesis of respiratory complications in intensive care settings.

Specifically, this review aims to:
(i) describe the main changes reported in the composition and diversity of the oral microbiome in patients undergoing mechanical ventilation;
(ii) explore the potential role of the oral cavity as a reservoir for respiratory pathogens in mechanically ventilated patients;
(iii) summarize the clinical and environmental factors that may influence oral microbiome remodelling in intensive care units.

By bringing together findings from observational studies and interventional research conducted in intensive care environments, this review seeks to provide an integrated overview of the available evidence on oral microbiome alterations in mechanically ventilated patients. In addition, it aims to identify remaining gaps in knowledge that may guide future research in this field.

2. MATERIALS AND METHODS

This systematic review was conducted on January 2026 in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure transparency and methodological rigor in synthesizing evidence regarding oral microbiome remodelling in mechanically ventilated patients.

2.1. Databases

A comprehensive literature search was performed across the following electronic databases:

- PubMed/MEDLINE
- Scopus
- Web of Science

2.2. Search Strategy and PICO Model

The review was designed to evaluate the impact of invasive mechanical ventilation on:

- Oral microbiome composition and diversity
- Colonization of the oral cavity and lower respiratory tract by respiratory and multidrug-resistant pathogens
- Concordance between oral and lower airway microbiota
- Effects of oral hygiene interventions

To define the search criteria, the PICO model (Population, Intervention/Exposure, Comparison, Outcome) was utilized, structured as follows:

Population (P): Adult patients admitted to Intensive Care Units (ICU) undergoing invasive mechanical ventilation
Intervention/Exposure (I): Exposure to mechanical ventilation and/or implementation of structured oral hygiene protocols
Comparison (C): Non-ventilated patients, baseline pre-intubation samples, or comparison between different oral care strategies
Outcomes (O): Changes in oral microbiome composition, alpha/beta diversity, pathogen colonization, antimicrobial resistance patterns, and incidence of ventilator-associated pneumonia (VAP).

Search Strategy

The search strategy combined Medical Subject Headings (MeSH) and free-text terms related to the oral microbiome and mechanical ventilation:

("Microbiota"[Mesh] OR microbiome OR "oral microbiota" OR "oral flora" OR "oral microbiome")
AND
("Mouth/microbiology"[Mesh] OR "oral cavity " OR "oral environment ")
AND
("Ventilators, Mechanical"[Mesh] OR "mechanical ventilation) " OR "mechanically ventilated patients")
AND
("Ventilator-Associated Pneumonia"[Mesh] OR "VAP" OR "ventilator associated pneumonia")
AND
("Intensive Care Units"[Mesh] OR "ICU" OR "critical care")

Reference lists of included studies and relevant review articles were manually screened to identify additional eligible publications.

The search was limited to studies published in English between January 2015 and December 2025.

2.3. Eligibility Criteria

Inclusion Criteria:

- Studies were included if they met the following criteria:
    - Observational studies (prospective or retrospective cohorts)
    - Longitudinal microbiome studies.
    - Randomized controlled trials (RCTs)
    - English language
- Studies evaluating oral microbiota using culture-based methods, 16S rRNA sequencing, or shotgun metagenomics
- Adult ICU patients receiving invasive mechanical ventilation
- Studies reporting microbiological oral outcomes with or without respiratory correlation

Exclusion Criteria:

- Animal or *in vitro* studies
- All the gray literature including narrative reviews, editorials, conference abstracts
- Studies exclusively focused on lung microbiome without oral correlation
- Studies lacking clear description of microbial identification methodology
- Paediatric (<18yo) populations

2.4. Study Selection and Data Extraction

All retrieved records were exported to a reference management software, in this review we used Rayyan, and duplicates were removed (26).

The screening process was conducted in two phases:

1. Title and abstract screening, performed independently by three reviewers (MC, EC and PL) to assess relevance.
2. Full-text evaluation of potentially eligible articles.

Disagreements were resolved through discussion with all reviewers and keeping the classification of the majority of reviewers.

The study selection process was documented using a PRISMA flow diagram 2020.

A standardized data extraction form was developed prior to full-text analysis. For each included study, the following variables were recorded:

- First author and year of publication
- Country and clinical setting
- Study design
- Sample size and patient characteristics
- Type of mechanical ventilation and duration
- Oral sampling method (saliva, supragingival plaque, subgingival plaque, mucosal swab)
- Microbiological assessment method (culture, 16S rRNA sequencing, metagenomics)
- Reported changes in microbiota composition
- Alpha and beta diversity metrics
- Presence of respiratory pathogens or multidrug-resistant organisms (MDROs)

- Concordance between oral and respiratory isolates
- Clinical outcomes (VAP incidence, ICU length of stay, mortality)

2.5. Quality Assessment and Risk of Bias

The methodological quality of included studies was assessed using validated tools according to study design:

- Randomized Controlled Trials: Cochrane Risk of Bias 2 (RoB 2) tool
- Observational studies: Newcastle–Ottawa Scale (NOS)

Attention was given to:

- Control of confounding variables (especially systemic antibiotic exposure)
- Timing of microbiological sampling
- Blinding of outcome assessors
- Completeness of follow-up
- Studies were categorized as low, moderate, or high risk of bias.

2.6. Data Synthesis Strategy

Due to the expected heterogeneity among the included studies, particularly regarding microbiological methods, sampling sites, outcome definitions, and study design, a quantitative meta-analysis was not planned *a priori*. Instead, a qualitative synthesis of the available evidence was performed.

The findings were organized into thematic domains to facilitate interpretation of the results. These domains included:

- (i) early oral microbiome remodelling following intubation;
- (ii) the emergence of respiratory and multidrug-resistant pathogens;
- (iii) microbial concordance between oral and lower airway samples; and
- (iv) the impact of oral hygiene and decontamination protocols.

When available, reported effect sizes and statistical significance were described narratively.

The heterogeneity of study populations and microbiological assessment methods further limited the feasibility of quantitative data pooling.

3. RESULTS

3.1. Study selection

As reported in Figure 1 the initial search yielded a total of 129 articles: 5 from PubMed, 122 from Scopus, and 2 from Web of Science. After the removal of 16 duplicates, 113 records remained and were screened based on title and abstract.

During this screening phase, 92 articles were excluded for the following reasons: (1) animal or in vitro studies (n=18), (2) narrative reviews, editorials or conference abstracts (n=34), (3) studies focusing exclusively on the lung microbiome without oral correlation (n=20), (4) studies lacking a clear description of microbiological methodology (n=12), and (5) studies involving paediatric populations (n=8).

Following this process, 21 articles were selected for full-text assessment. After full-text evaluation, 9 studies were excluded because they focused exclusively on the lung microbiome without oral correlation, lacked a clear description of the microbiological identification methodology, or included populations that did not meet the predefined eligibility criteria. Consequently, 12 studies met the inclusion criteria and were included in the qualitative synthesis.

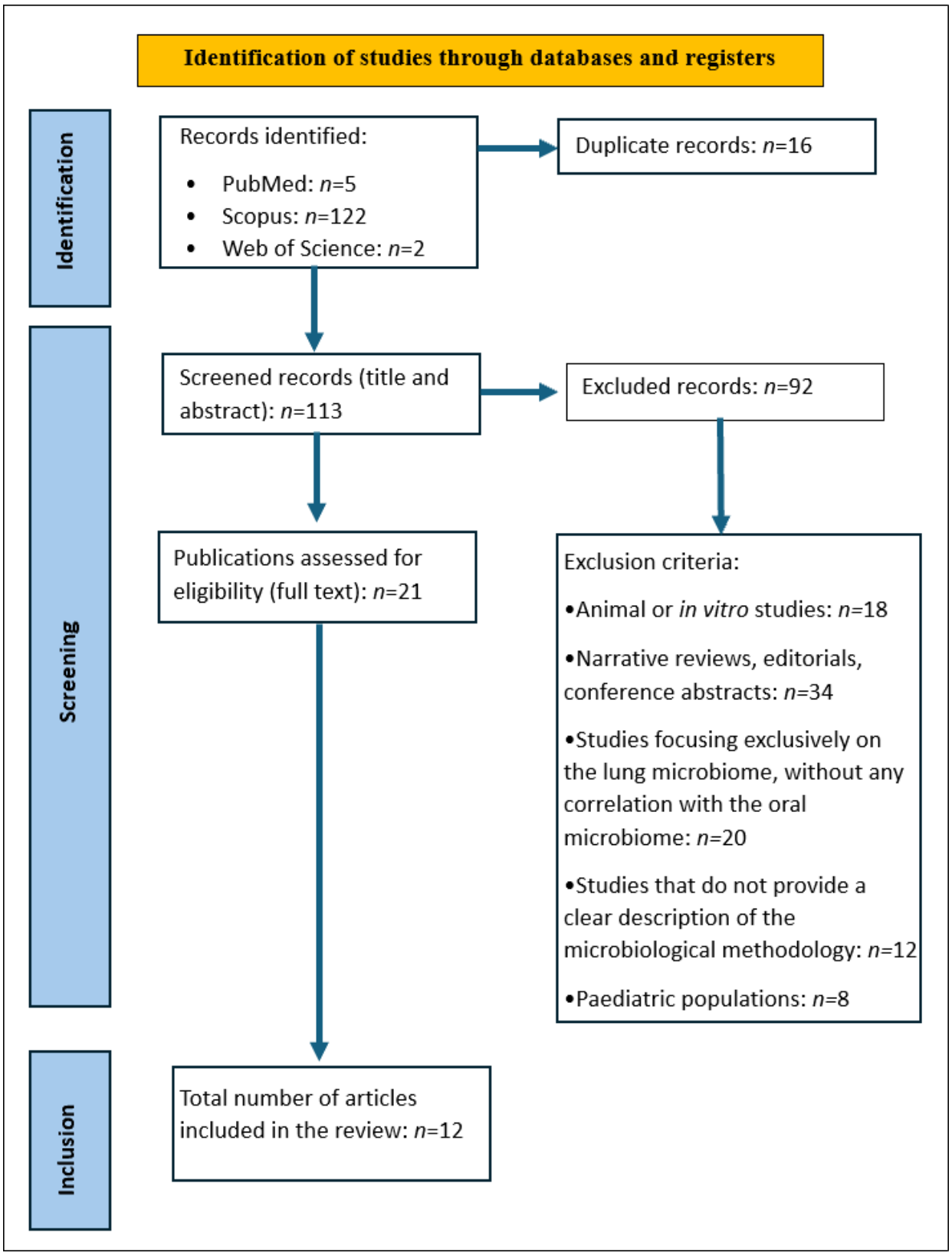

Figure 1. Overview of article selection procedure according to PRISMA guidelines. n=number of articles.

Table 1. Data extraction table from the 12 studies included in the review. Abbreviations: AIS, Acute Ischemic Stroke; CHX, Chlorhexidine; d, days; h, hours; ICU, intensive care unit; MDROs, multidrug-Resistant Organisms; MV, mechanical ventilation; NR, Not Reported; RCT, randomized controlled trial; SAP, Stroke-Associated Pneumonia; VAP, ventilator-associated pneumonia.

Author (Year)	Study Design	Population	Sample Size (n)	MV Details	Comparison Group	Oral Sampling Site	Timing of Sampling	Microbiome Analysis Method	Main Microbiome Outcomes	Key Findings	Oral Health Interventions	VAP / Infection Outcomes	Confounders
Tuon *et al.* 2017	RCT	Ventilated ICU adults	~56	MV	CHX *vs* saline	Dental plaque	ICU stay	Culture	↓ periodontal pathogens (*e.g. P. gingivalis, A. actinomycetemcomitans*)	Oral hygiene reduced infections	CHX	No link	Baseline periodontal status; oral hygiene habits (randomized allocation)
Sands *et al.* 2017	Prospective cohort study	Ventilated ICU ≥48h	~110	MV ≥48h	VAP *vs* no VAP	Oropharynx	d 1,3,5,7	Culture-based + targeted gene sequencing	Colonization by *opportunistic pathogens* (*e.g. S. aureus, P. aeruginosa, Enterobacteriaceae*)	Early taxa predicted VAP	Routine ICU oral hygiene	VAP association	Antibiotics; MV duration; illness severity
Ren *et al.* 2023	Prospective observational cohort study	AIS patients	164	MV ≤7d	No pneumonia *vs* SAP *vs* VAP	Buccal / gingiva	6h–14d	16S rRNA	Dysbiosis; ↓ *commensals (Streptococcus, Neisseria, Veillonella);* ↑ *opportunistic taxa (Pseudomonas, Acinetobacter, Enterobacteriaceae*)	Microbiota predicts SAP / VAP	None	Association with SAP / VAP	Age; comorbidities; antibiotic exposure
Tsuda *et al.* 2020	RCT	Ventilated ICU	110	MV ≥48h	VAP *vs* no VAP	Tongue / buccal / plaque	Every 48h	16S rRNA	↓ bacterial α-diversity in VAP patients (Shannon/Chao1 indices)	Microbiome predicts VAP	Routine ICU oral care	Distinct VAP-associated profiles; increase	Antibiotics; disease severity; MV duration
Gregorczyk-Maga *et al.* 2023	Prospective cohort	COVID ventilated	56	All MV	Standard *vs* brushing	Buccal / tongue	36h + 7d	MALDI-TOF	Dysbiosis; ↓ *commensals (Streptococcus, Veillonella, Neisseria);* ↑ *opportunistic pathogens (Pseudomonas, Acinetobacter, Enterobacteriaceae*)	Brushing = reduced dysbiosis	Tooth brushing	Indirect association with VAP	*Clinical status*; antibiotics; hospitalization duration

Author (Year)	Study Design	Population	Sample Size (n)	MV Details	Comparison Group	Oral Sampling Site	Timing of Sampling	Microbiome Analysis Method	Main Microbiome Outcomes	Key Findings	Oral Health Interventions	VAP / Infection Outcomes	Confounders
Song *et al.* 2023	Prospective observational study	Ventilated ICU	70	MV	With *vs* without oral infection	Oral + subglottic	Serial sampling during ICU stay	16S rRNA	Dysbiosis; enrichment of respiratory pathogens (*P. aeruginosa, A. baumannii, K. pneumoniae, S. aureus*) in VAP patients	Taxa predict VAP	Routine ICU oral hygiene	VAP association; increase	Antibiotics; MV; ICU length of stay; disease severity
Gregorczyk-Maga *et al.* 2022	Prospective cohort study	COVID ventilated	56	All MV	Tooth brushing *vs* standard oral care	Multiple oral sites	36h	MALDI-TOF	Early dysbiosis; ↓ *oral commensals (Streptococcus, Veillonella);* ↑ *opportunistic taxa (Pseudomonas, Acinetobacter, Enterobacteriaceae)*	↑ opportunistic respiratory pathogens	Routine ICU oral hygiene	Weak association with VAP	Antibiotic therapy; intubation duration; clinical severity
Choi *et al.* 2021	Prospective longitudinal observational study	Ventilated ICU	10 + 13 controls	MV	Intubated *vs* healthy	Buccal	Repeated weekly sampling	16S rRNA	Reduced bacterial alpha diversity (richness-based indices)	↑ respiratory opportunistic taxa	None	Potential VAP association; increased respiratory pathogens	Antibiotics: ICU stay; comorbidities
Marino *et al.* 2017	Prospective observational study	Ventilated ICU	~40	MV	VAP *vs* no VAP	Plaque / oropharynx	Admission + follow-up	Culture	Colonization by respiratory *opportunistic pathogens (e.g. P. aeruginosa, S. aureus, Enterobacteriaceae*)	Oral colonization predicts VAP	Routine ICU oral hygiene	Strong VAP association	Antibiotics; oral health status; MV
Souza *et al.* 2017	Prospective longitudinal observational study	Ventilated ICU	NR	MV	VAP *vs* no VAP	Tongue biofilm	Admission + 48h	Culture	Oral–tracheal overlap of respiratory opportunistic pathogens	Oral reservoir of respiratory pathogens (*e.g. P. aeruginosa, S. aureus, Enterobacteriaceae*)	Basic oral care	VAP association; weak link	Antibiotics; aspiration risk; ventilation duration
Porto *et al.* 2016	Prospective longitudinal observational study	ICU patients	NR	Mixed ICU population	No comparison group	Plaque / mucosa	Admission	Culture	↓ Periodontal pathogens (*e.g. P. gingivalis, T. forsythia, T. denticola*)	High pathogen-load	Routine oral hygiene (no targeted perio intervention)	No direct association with VAP reported	Periodontal status; oral hygiene baseline; comorbidities

Author (Year)	Study Design	Popula-tion	Sample Size (n)	MV De-tails	Compari-son Group	Oral Sampling Site	Timing of Samp-ling	Microbiome Analysis Method	Main Microbiome Outcomes	Key Findings	Oral Health Interven-tions	VAP / Infection Out-comes	Confound-ers
Sands *et al.* 2016	Prospective longitudinal observation al study	Ventilated ICU patients	~50	MV	Oral care regimens	Oropharynx	MV period	Culture/targe-ted	Changes in oral colonization by *opportunistic pathogens* (*e.g. S. aureus, P. aeruginosa*)	Oral care affects VAP	Antiseptic oral care (CHX)	Reduced VAP incidence	Antibiotic exposure; ICU stay; baseline colonization

The included studies showed considerable heterogeneity in terms of study design, patient populations, and microbiological identification methodologies. Most investigations consisted of prospective observational or longitudinal cohort studies, while only two randomized controlled trials were identified, the studies of Tuon *et al.,* 2017 and Tsuda *et al.,* 2020. The majority of participants were adult patients admitted to intensive care units receiving invasive mechanical ventilation, although some studies focused on specific clinical populations, including COVID-19 patients like the study of Gregorczyk-Maga *et al.,* 2022 or patients with acute stroke, like the study of Ren *et al.,* 2023. Sample sizes varied considerably, ranging from small exploratory cohorts such as those described by Choi *et al.* 2021 to larger longitudinal analyses including more than one hundred participants described by Ren *et al.,* 2023.
Different microbiological identification methodologies were employed across the included studies. Six studies relied primarily on conventional culture-based techniques, such as those used by Tuon *et al.,* 2017, whereas four studies employed molecular approaches based on 16S rRNA gene sequencing to characterize the oral microbiome, including the investigation by Ren *et al,* 2023. In addition, two studies used matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) for bacterial identification, like Gregorczyk-Maga *et al.,* 2022 and Gregorczyk-Maga *et al.,* 2023. Culture-based methods allow the identification of cultivable microorganisms, whereas molecular approaches such as 16S rRNA sequencing enable the detection of both cultivable and uncultivable taxa and provide a broader characterization of microbial community structure and diversity. These methodological differences influenced the depth of microbial characterization and limited direct comparability between studies.

In addition to methodological variability, differences were also observed in the oral sampling sites used for microbiological analysis. Across the included studies, samples were obtained from various oral niches, including saliva, supragingival dental plaque, subgingival plaque, and oral mucosal swabs. These sampling differences may further influence the detected microbial composition, as distinct oral habitats host microbial communities with specific ecological characteristics.

Despite this heterogeneity, several investigations, like Ren *et al.,* 2023, Marino *et al.,* 2017, Choi *et al.,* 2021 and Song *et al.,* 2023 reported consistent patterns of oral microbiome remodelling following the initiation of mechanical ventilation. Within the first 12–72 hours after intubation, several studies reported early depletion of oral taxa that are commonly abundant in eubiotic oral communities. In particular, Ren *et al.,* 2023 and Gregorczyk-Maga *et al.,* 2023 described a reduction in genera such as *Streptococcus, Neisseria* and *Veillonella*, whereas Song *et al.,* 2023 and Marino *et al.,* 2017

reported a concomitant increase in respiratory-associated pathogens, including *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Similarly, Sands *et al.,* 2016 observed progressive enrichment of taxa typically associated with the respiratory environment during the course of mechanical ventilation.

Marino *et al.,* 2017 and Ren *et al.,* 2023 reported microbial concordance between oral and lower respiratory tract samples, supporting the hypothesis that the oral cavity may act as a reservoir for respiratory colonization in mechanically ventilated patients. Similar bacterial profiles detected in oral swabs and tracheal aspirates suggest that pathogens emerging during oral dysbiosis may subsequently colonize the lower airways. A subset of studies, like Gregorczyk-Maga *et al.,* 2023 and Tsuda *et al.,* 2020 also evaluated oral hygiene interventions, including chlorhexidine-based sanitation protocols and mechanical toothbrushing. For instance, Tuon *et al.,* 2017 reported significant microbial modulation following chlorhexidine use, characterized by a reduction in commensal oral taxa such as *Streptococcus* and *Neisseria*, alongside the persistence or relative increase of opportunistic pathogens including *Staphylococcus aureus* and *Pseudomonas aeruginosa*. However, the relationship between oral hygiene strategies and clinical outcomes such as ventilator-associated pneumonia remains inconsistent across studies, as reported by Tsuda *et al.,* 2020.

Taken together, the available evidence suggests that mechanical ventilation is associated with rapid oral microbiome remodelling (20,27), characterized by reduced microbial diversity (20), depletion of commensal bacteria (20,28), and enrichment of opportunistic respiratory pathogens (21,27).

3.2 Risk of Bias Analysis

The methodological quality and risk of bias of the included studies were assessed using validated tools according to study design.

The randomized controlled trials were evaluated using the Cochrane Risk of Bias 2 (RoB 2) tool (29), which considers domains such as random sequence generation, allocation concealment, blinding of outcome assessment, incomplete outcome data, bias due to deviations from intended interventions.

Observational and cohort studies were assessed using the Newcastle–Ottawa Scale (NOS), which evaluates three main methodological domains: selection of participants, comparability of study groups, and adequacy of outcome assessment and follow-up. Particular attention was given to factors considered especially relevant in intensive care microbiome research, including control of confounding variables (particularly systemic antibiotic exposure, disease severity, duration of mechanical ventilation, and comorbidities), sample size adequacy, microbiological assessment methods, completeness of follow-up, and clarity of outcome reporting. Additional methodological limitations, such as single-center design, lack of randomization, descriptive-only analyses, and the use of culture-based methods without molecular microbiome characterization, were also considered during the assessment.

Based on the overall methodological robustness of each study, investigations were qualitatively categorized as presenting low, moderate, or high risk of bias. In observational studies, NOS scores were interpreted as follows: 7–9 points indicated low risk of bias/high methodological quality, 5–6 points indicated moderate risk, and ≤4 points indicated high risk of bias/low methodological quality.

The results of the quality assessment are summarized in figures 2-5.

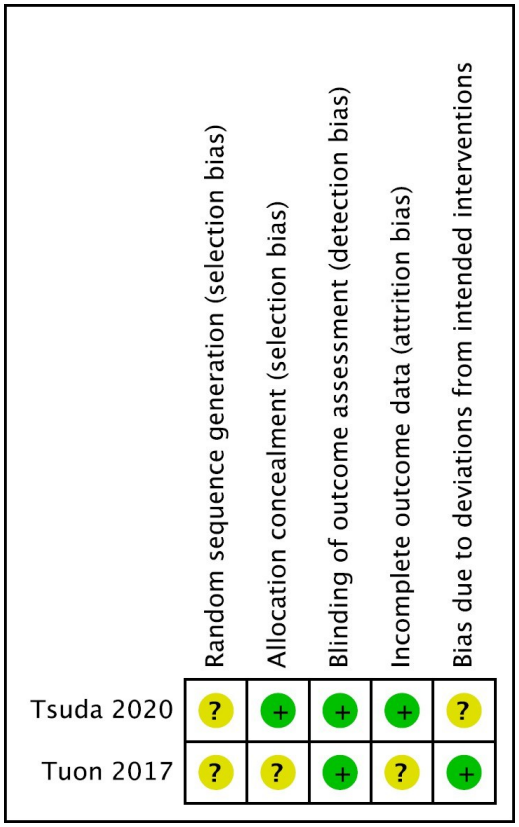

Figure 2. Risk of bias of included randomized controlled trials. Green means a low risk of bias while yellow means an unclear risk of bias.

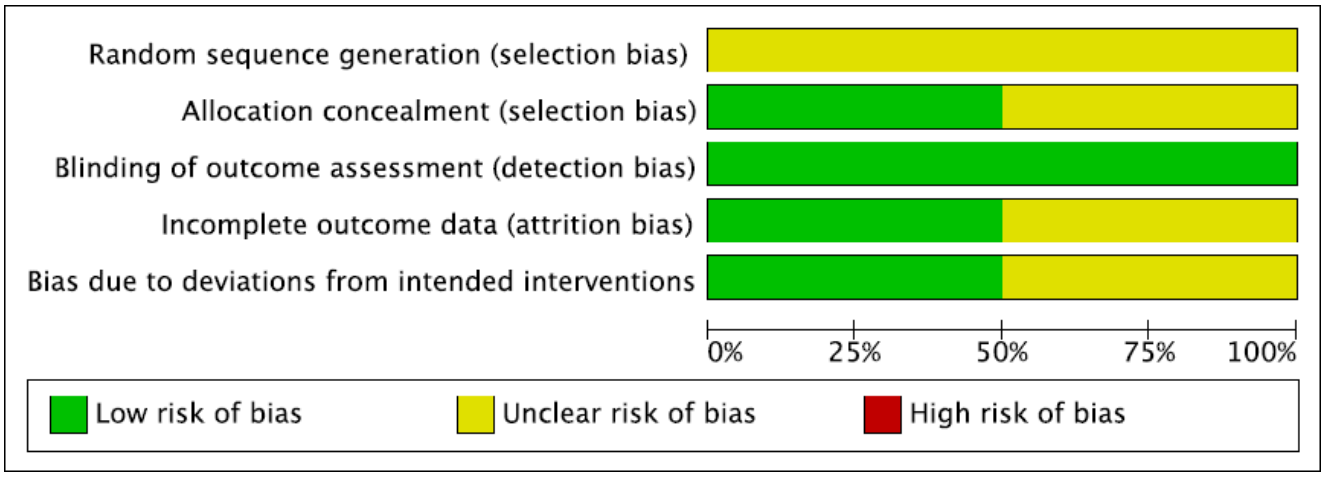

Figure 3. Risk of bias summary for randomized controlled trials.

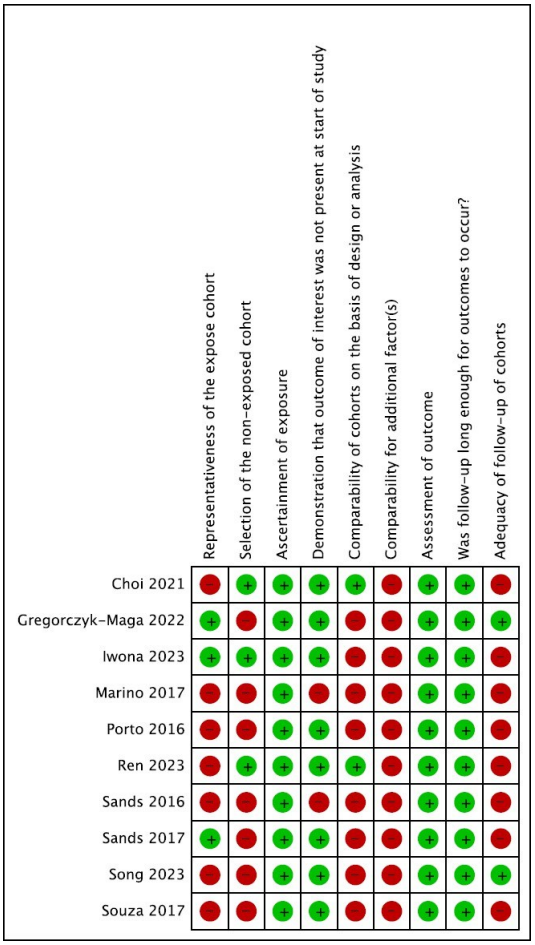

Figure 4. Risk of bias of included observational studies. Green means a low risk of bias while red means a high risk of bias.

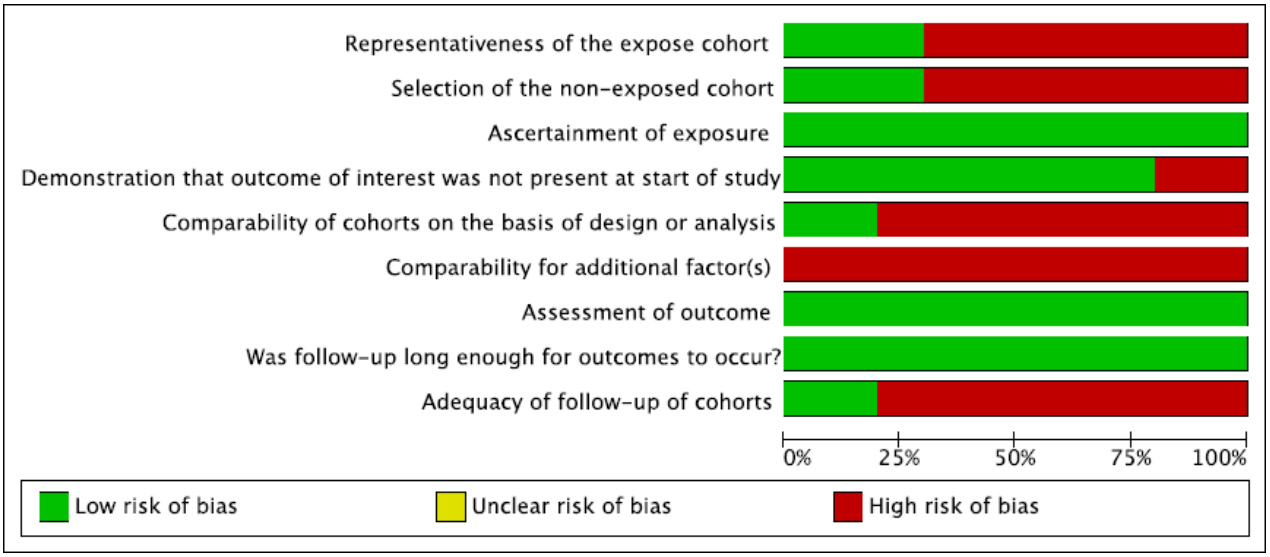

Figure 5. Risk of bias summary for observational studies.

The analysis of risk of bias revealed significant differences between the randomised controlled trials (RCTs) and the observational cohort studies included in the review.

Regarding the RCTs, the studies by Tuon *et al.,* (2017) and Tsuda *et al.,* (2020) generally demonstrate moderate methodological quality, characterised predominantly by domains with a low risk of bias and the absence of domains classified as high risk. In the study by Tuon *et al*., (2017), the risk of bias is low with regard to the assessment of outcomes ('blinding of outcome assessment') and deviations from intended interventions ('bias due to deviations from intended interventions'), suggesting that outcomes were assessed objectively and that participants adhered adequately to the trial protocol. However, random sequence generation, allocation concealment and the handling of incomplete outcome data are classified as 'unclear risk', likely due to a lack of sufficient methodological detail in the manuscript. This limits the ability to fully assess the robustness of the randomisation process and the handling of missing data.
The study by Tsuda *et al.,* (2020) exhibits slightly higher methodological quality than that of Tuon *et al.,* Indeed, in addition to a low risk of bias in outcome assessment, it also shows a low risk regarding allocation concealment and the handling of incomplete data, indicating a better description of methodological procedures and more adequate management of participant follow-up. Here too, however, the generation of the random sequence remains classified as 'unclear risk', suggesting that the randomisation methods were not described in sufficient detail. Furthermore, the domain relating to deviations from the planned interventions was assessed as not clearly definable. Overall, both RCTs provide relatively reliable evidence, although methodological quality is limited primarily by the insufficient description of certain aspects of the randomisation process.

The included cohort studies, by contrast, show more heterogeneous and generally lower methodological quality compared to the RCTs, with numerous domains classified as having a high risk of bias. The study by Choi *et al.,* (2021) presents a high risk of bias in the representativeness of the exposed cohort, in comparability for additional factors, and in the adequacy of follow-up. This suggests that the sample may not be fully representative of the target population and that the compared groups were not adequately balanced with respect to potential confounding factors. However, the study shows a low risk in the selection of the unexposed cohort, in the assessment of exposure, in the demonstration of the absence of the outcome at the start of the study, in the assessment of outcomes, and in the duration of follow-up.
The study by Gregorczyk-Maga *et al.,* (2022) demonstrates relatively better methodological quality compared to other observational studies. It presents a low risk regarding the representativeness of

the exposed cohort, the assessment of exposure, the demonstration of the absence of the outcome at the start of the study, the assessment of outcomes, the duration of follow-up and the adequacy of follow-up. However, the risk of bias remains high in the selection of the unexposed cohort and, above all, in the comparability of the groups for both primary and additional factors, indicating insufficient control of confounders.

The study by Gregorczyk-Maga *et al.,* (2023) shows an intermediate methodological profile. The representativeness of the exposed cohort, the selection of the unexposed cohort, the assessment of exposure, the demonstration of the absence of the outcome at the start of the study, the assessment of outcomes, and the duration of follow-up are all at low risk of bias. However, a high risk regarding the comparability of the groups and the adequacy of the follow-up remains, suggesting possible uncontrolled baseline differences between the groups and potential loss of participants during the study.

The study by Marino *et al.,* (2017) has one of the lowest methodological qualities among the included studies. It shows a high risk of bias in the representativeness of the exposed cohort, the selection of the unexposed cohort, the demonstration of the absence of the outcome at the start of the study, the comparability of the groups for both primary and additional factors, and the adequacy of the follow-up. The only domains with low risk concern the assessment of exposure, the evaluation of outcomes, and the duration of follow-up. These methodological limitations significantly undermine the robustness of the study's conclusions.

The study by Porto *et al.,* (2016) also highlights numerous methodological shortcomings, with a high risk of bias regarding the representativeness of the exposed cohort, the selection of the unexposed cohort, the comparability of the groups, and the adequacy of the follow-up. Conversely, the assessment of exposure, the demonstration of the absence of the initial outcome, the evaluation of outcomes and the duration of follow-up are adequately controlled.

The study by Ren *et al.,* (2023) is of moderate methodological quality. It shows a high risk of bias in the representativeness of the exposed cohort, in comparability for additional factors, and in the adequacy of follow-up, whilst the other domains are classified as low risk. This suggests a generally sound methodological design, but with limitations related to sample selection and control of confounders.

The studies by Sands *et al.,* (2016) and Sands et *al.,* (2017) show different profiles. Sands *et al.,* (2016) presents a high risk of bias in almost all domains relating to cohort selection and comparability, as well as in the adequacy of follow-up, highlighting an overall low methodological quality. Sands *et al.,* (2017), whilst showing weaknesses in the selection of the unexposed cohort and in the comparability of the groups, presents better representativeness of the exposed cohort and

more adequate follow-up, suggesting a slightly higher methodological quality compared to the 2016 study.

The study by Song *et al.,* (2023) highlights a high risk regarding the representativeness of the exposed cohort, the selection of the unexposed cohort and the comparability of the groups, whilst showing a low risk for exposure assessment, demonstration of the absence of the initial outcome, outcome assessment, duration of follow-up and adequacy of follow-up. Overall, the study appears methodologically more robust than other included observational studies.

Finally, the study by Souza *et al.,* (2017) presents numerous domains at high risk of bias, particularly regarding the representativeness of the exposed cohort, the selection of the unexposed cohort, the comparability of the groups, and the adequacy of follow-up. Although exposure assessment, demonstration of the absence of the initial outcome, outcome assessment and duration of follow-up are adequate, the presence of multiple methodological limitations reduces the overall reliability of the results.

Overall, the bias risk analysis highlights that the included RCTs provide methodologically stronger evidence than observational studies. Cohort studies offer useful data, frequently present limitations related to participant selection, group comparability and follow-up management, aspects that could influence the internal validity of the results and which must therefore be considered in the overall interpretation of the available evidence.

4. DISCUSSION

Despite the heterogeneity of the included studies and the moderate risk of bias observed in several of them, current evidence suggests that mechanical ventilation is associated with alterations in the oral microbiome in critically ill patients (14,19,20). Longitudinal studies have reported that these changes may occur early after intubation, in some cases within the first 24–72 hours (19,20). These alterations typically include shifts in microbial composition, with a reduction in health-associated oral taxa and a relative increase in opportunistic and respiratory-associated microorganisms (20,27). However, these findings should be interpreted with caution, as oral microbiome alterations in ICU patients may be influenced by multiple confounding factors, such as antibiotic exposure, disease severity, duration of mechanical ventilation, length of ICU stay, comorbidities, and baseline oral health status, which may independently contribute to both microbial dysbiosis and the development of respiratory infections (6,20).

Under physiological conditions, the oral microbiome plays a key role in maintaining mucosal homeostasis, immune regulation, and colonization resistance against opportunistic pathogens (2,11). However, the ICU environment introduces multiple disruptive factors, including reduced salivary flow, impaired mechanical clearance, antibiotic exposure, and the presence of invasive devices, that destabilize this equilibrium and favor the emergence of dysbiotic communities enriched in pathogenic taxa (5,16,23). To these unchangeable factors we can add the lack of oral hygiene, which also profoundly affects the oral microbiome and has therefore prompted the investigation of several oral care interventions, including chlorhexidine-based protocols (21), nurse-led oral hygiene programs (30) , and mechanical toothbrushing strategies (24).

A key aspect highlighted by the present review is the rapidity of oral microbiome remodelling following intubation (19,20). Several longitudinal studies demonstrated that significant compositional changes occur within the first 12–72 hours of mechanical ventilation suggesting that the oral ecosystem is highly responsive to acute environmental stressors such as reduced salivary flow, persistent oral breathing, impaired swallowing and mechanical clearance due to sedation, antibiotic exposure, altered oxygenation, and the presence of endotracheal tubes that promote biofilm formation (5).

This early shift is characterized by a reduction bacteria from the *Streptococcus* and *Neisseria* genera. These genera include species which are known to contribute to microbial homeostasis and immune modulation. Furthermore, the initial changes in the oral microbiome include an increase in

opportunistic organisms including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Enterobacteriaceae* (20,27). From an ecological perspective, this transition reflects a loss of microbial diversity and functional redundancy, which are essential features of a resilient microbiome (2). Reduced diversity has often been associated with increased susceptibility to pathogen colonization and infection, including in some studies of ventilated patients who developed VAP, although this relationship appears to be context-dependent (19,31). These findings align with broader microbiome research demonstrating that dysbiotic ecosystems are less capable of resisting external perturbations and more prone to domination by pathogenic species (1).

One of the most relevant contributions of this review is the confirmation of a microbial continuum between the oral cavity and the lower respiratory tract. Multiple studies demonstrated a significant overlap between oral and respiratory microbiota, particularly in mechanically ventilated patients (27). The detection of identical or closely related strains in dental plaque, oral mucosa, and tracheal support the hypothesis that the oral cavity may represent an important ecological niche involved in the microbial seeding of the lower respiratory tract in mechanically ventilated patients (28). This concept is further reinforced by the anatomical and physiological conditions associated with mechanical ventilation. The presence of an endotracheal tube bypasses natural defence mechanisms, including cough reflex and glottic closure, facilitating the entry of oral bacteria into the lower airways through repeated microaspiration events (18). In addition, biofilm formation on both oral surfaces and endotracheal tubes provides a stable and protected environment for microbial persistence, enhancing bacterial survival and resistance to antimicrobial agents (5).

The oral–lung axis therefore emerges as a central pathophysiological mechanism linking oral dysbiosis to respiratory infections (23). Dysbiotic oral biofilms enriched in opportunistic pathogens can act as a continuous source of microbial seeding to the lower respiratory tract, contributing to the development of ventilator-associated pneumonia (VAP) (18). This process is not limited to passive bacterial transfer but also involves immune modulation and inflammatory signalling, which may impair epithelial barrier integrity and promote infection persistence (16).

A major confounding factor in the interpretation of oral microbiome changes in mechanically ventilated patients is systemic antibiotic exposure. In the ICU setting, antibiotics are frequently initiated before or shortly after intubation, often empirically and with broad-spectrum regimens (12). This is particularly relevant because antibiotics do not simply reduce total bacterial load; rather, they may profoundly reshape community structure by suppressing susceptible commensal taxa, reducing alpha diversity, altering interspecies competition, and creating ecological niches that

favour the expansion of opportunistic and hospital-associated organisms (12).

In this context, the depletion of health-associated oral genera such as *Streptococcus*, *Neisseria*, *Veillonella*, and *Rothia* may reflect not only ventilation-related xerostomia, impaired clearance, and biofilm accumulation, but also the selective effects of systemic antimicrobial therapy (12). Antibiotic exposure may act as both a confounder and an effect modifier. As a confounder, it is strongly associated with illness severity, suspected infection, ICU length of stay, and risk of VAP, all of which are also linked to oral dysbiosis and worse clinical outcomes (27). Therefore, patients receiving broader or prolonged antibiotic regimens are often those at highest baseline risk of microbial disruption, which may lead to an overestimation of the apparent contribution of mechanical ventilation alone. At the same time, antibiotic therapy may modify the magnitude and direction of microbiome remodelling by selecting resistant or intrinsically tolerant microorganisms, including *Pseudomonas aeruginosa*, *Acinetobacter spp.*, *Klebsiella pneumoniae*, and other Enterobacteriaceae, which are repeatedly reported in ICU oral samples (27). From this perspective, the oral cavity may become not only dysbiotic but also a selective reservoir of multidrug-resistant organisms (12). This issue is further complicated by substantial inter-study variability in antibiotic-related data. Across the included studies, antibiotic exposure was not uniformly reported in terms of timing, class, spectrum, duration, indication, or cumulative burden.

In several cases, antibiotic use was acknowledged as a potential confounder but not adequately controlled for in multivariable analyses, as in the studies by Song et al., 2023, Tsuda et al., 2020, and Souza et al., 2017. In other investigations antibiotic exposure was mainly reported at baseline or as a general characteristic of the cohort, without accounting for changes in antimicrobial therapy during follow-up (14). Such heterogeneity weakens comparability between studies and limits causal inference (27). For example, when oral samples are collected after intubation in patients who have already received empirical antimicrobials, the observed reduction in commensals and enrichment of Gram-negative pathogens cannot be attributed with confidence to ventilation-associated ecological disruption alone. Moreover, antibiotic exposure may influence not only bacterial composition but also the broader ecological behaviour of the oral microbiome. By disrupting colonization resistance, antimicrobials may facilitate overgrowth of fungi such as *Candida spp.*, increase the persistence of biofilm-associated pathogens, and promote a microbial community that is less stable, less diverse, and more permissive to respiratory colonization (18). In mechanically ventilated patients, this may

have direct clinical relevance because a dysbiotic and antibiotic-selected oral biofilm can serve as a source for repeated microaspiration and lower airway seeding (18). Consequently, part of the reported association between oral dysbiosis and VAP may reflect the combined effect of ventilation-related factors and antimicrobial selection pressure rather than a direct linear pathway attributable to intubation alone.

For these reasons, antibiotic exposure should be regarded as a central methodological and biological variable in future research on ventilation-associated oral microbiome remodelling. Studies should ideally stratify patients according to antibiotic class and duration, document pre-intubation and post-intubation exposure separately and incorporate time-dependent adjustment models to better distinguish cause from co-exposure. Whenever possible, sampling should be performed before antibiotic initiation or at clearly defined intervals thereafter. Without more rigorous control of this variable, the independent effect of mechanical ventilation on oral dysbiosis will remain difficult to quantify with precision. Accordingly, the interpretation of preventive oral care strategies should also take antibiotic exposure into account since reductions in pathogen burden may depend not only on the intervention itself but also on the underlying antimicrobial regimen received by the patient (27).

In this context, the role of oral hygiene interventions becomes particularly relevant. Evidence from interventional and observational studies suggests that structured oral care protocols, including mechanical plaque removal and the use of antiseptic agents such as chlorhexidine, can reduce oral bacterial load and partially modulate microbiota composition (24). Moreover, nurse-led oral care programs have been associated with improved mucosal integrity and reduced inflammatory lesions, highlighting the importance of oral health management in ICU settings (32).

However, the impact of these interventions on clinical outcomes remains controversial. While some studies reported a reduction in respiratory pathogen colonization and VAP related infectious outcomes (24), others showed limited or inconsistent effects (19).

This discrepancy may reflect differences in study design, patient populations, intervention protocols, and timing of application, as well as the complex and multifactorial nature of VAP pathogenesis. Importantly, current strategies focusing primarily on bacterial load reduction may not fully address the ecological imbalance underlying dysbiosis. Emerging evidence suggests that preserving or restoring microbial diversity could represent a more effective approach than indiscriminate antimicrobial suppression (2). This perspective opens new avenues for research,

including the potential role of microbiome-targeted therapies aimed at maintaining ecological balance rather than simply eliminating pathogens.

4.1 Limitations

Despite the evidence presented, several limitations must be acknowledged. Some at the level of the studies available. The majority of included studies were observational and therefore subject to confounding factors. Antibiotic exposure was a potential confounder in studies such as Song et al., 2023, Tsuda et al., 2020, and Marino et al., 2017; disease severity may have influenced the findings in Sands et al., 2017, Song et al., 2023, and Gregorczyk-Maga et al., 2022; while comorbidities and specific patient populations, including acute ischemic stroke in Ren et al., 2023 and COVID-19 in Gregorczyk-Maga et al., 2022, further limited comparability across studies. Additionally, methodological heterogeneity, particularly in microbiological techniques (culture-based vs. sequencing approaches), sampling sites, and timing, limits the comparability of findings across studies (5). Furthermore, many studies were conducted in single-center settings with relatively small sample sizes, and some focused on specific populations, such as COVID-19 or stroke patients, which may reduce generalizability (20). These limitations highlight the need for well-designed, multicentre randomized controlled trials integrating advanced microbiome analysis techniques to better elucidate causal relationships.

Additional limitations related to the review itself should be acknowledged. First, only studies published in English were included, which may have resulted in language bias and the exclusion of potentially relevant evidence published in other languages. Second, although the most relevant databases for the topic were searched, only three electronic databases were included, which may have limited the comprehensiveness of the literature retrieval process. Finally, considerable heterogeneity among the included studies with respect to study design, patient populations, oral sampling sites, microbiological identification methods, and outcome measures precluded the performance of a quantitative meta-analysis. Therefore, the findings were synthesized qualitatively.

From a clinical standpoint, the findings of this review emphasize that the oral cavity should be considered a critical and modifiable target in the prevention of respiratory infections in mechanically ventilated patients (18). The early onset of dysbiosis and the demonstrated microbial overlap between oral and respiratory sites suggest that timely and targeted oral care interventions may have a meaningful impact on patient outcomes (24).

In conclusion, mechanical ventilation induces rapid and significant remodelling of the oral microbiome, promoting dysbiosis and the emergence of opportunistic pathogens. The oral cavity acts as a dynamic microbial reservoir capable of influencing lower respiratory tract colonization through microaspiration and biofilm-mediated mechanisms. Although current evidence strongly supports a role for oral microbiome alterations in the pathogenesis of ventilator-associated pneumonia, further high-quality research is required to clarify causal pathways and to optimize preventive strategies targeting the oral ecosystem in critically ill patients.

5. CONCLUSIONS

This systematic review highlights that mechanical ventilation profoundly alters the oral microbiome, promoting a rapid transition from a commensal-dominated ecosystem toward dysbiotic microbial communities enriched in opportunistic and respiratory-associated pathogens. These ecological changes appear to occur early after intubation and are influenced by multiple factors commonly present in intensive care settings, including xerostomia, impaired salivary clearance, antibiotic exposure, sedation, reduced oral hygiene, and the presence of endotracheal devices.

The available evidence consistently supports the concept that the oral cavity acts as a clinically relevant reservoir for respiratory pathogens in mechanically ventilated patients. The concordance observed between oral and lower airway microorganisms, together with the role of microaspiration in ventilator-associated pneumonia (VAP), reinforces the existence of a biologically significant oral–lung axis in critically ill individuals.

Furthermore, this review emphasizes that ventilation-associated oral dysbiosis is not limited to respiratory implications alone, but may also contribute to oral mucosal damage, periodontal inflammation, *Candida* colonization, and the emergence of multidrug-resistant organisms. These findings underline the importance of preserving oral ecological balance in ICU patients as part of a broader strategy to reduce infectious complications.

Current evidence also suggests that structured oral hygiene protocols, particularly those combining mechanical plaque removal with antiseptic agents, may significantly reduce oral pathogen burden and partially modulate microbiome remodelling during mechanical ventilation. However, despite promising microbiological findings, the impact of these interventions on major clinical outcomes such as VAP incidence, ICU length of stay, and mortality remains heterogeneous across studies.

Several limitations within the currently available literature should also be acknowledged. Considerable heterogeneity exists regarding study design, sampling methods, microbiological techniques, oral care protocols, antibiotic exposure, and outcome definitions. In addition, many studies are limited by small sample sizes, lack of longitudinal follow-up, and insufficient control of confounding variables. These limitations reduce comparability between studies and highlight the need for standardized methodologies in future research.

Future investigations should focus on longitudinal multicentre studies integrating high-resolution microbiome sequencing, resistome analysis, and clinical outcomes in order to better clarify the temporal dynamics and clinical significance of oral microbiome remodelling in mechanically ventilated patients. Further research is also needed to determine which oral care interventions most

effectively preserve microbial eubiosis while preventing pathogen overgrowth and antimicrobial resistance.

In conclusion, the oral microbiome represents a dynamic and clinically relevant component of critical care medicine. Current evidence suggests that mechanical ventilation is associated with significant alterations in oral microbial homeostasis, contributing to the development of respiratory complications and potentially systemic infections. Recognizing the oral cavity as a modifiable ecological reservoir may open new preventive and therapeutic perspectives in intensive care, reinforcing the importance of interdisciplinary collaboration between intensive care professionals, microbiologists, and oral health specialists.

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