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Antiseptic mouthwashes as a potential strategy for controlling oral dysbiosis: Antimicrobial effect against opportunistic pathogens

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ABSTRACT

Objectives: Oral dysbiosis is increasingly recognized as a clinically relevant condition associated with oral diseases, systemic complications, and the emergence of antimicrobial-resistant microorganisms. Antiseptic-containing mouthwashes are widely used as adjunctive, non-antibiotic tools in oral hygiene; however, comparative data on their activity against dysbiosis-associated microorganisms and their clinical efficiency at formulation-relevant concentrations remain limited.

Methods: In this study, the antimicrobial activity of commercially available mouthwashes containing octenidine dihydrochloride (OCT), chlorhexidine digluconate (CHX), polyhexamethylene biguanide (PHMB), and cetylpyridinium chloride (CPC) was evaluated against Gram-positive and Gram-negative bacteria and *Candida* spp. associated with oral dysbiosis. Minimum inhibitory concentrations (MICs) were determined by broth microdilution and expressed both as product dilutions and active compound concentrations. Clinical relevance was assessed using the Clinical Efficiency of MIC (CEMIC) index.

Results: OCT-containing mouthwashes demonstrated the lowest MIC and CEMIC values and the most consistent antimicrobial activity against all tested microorganisms. CHX-based formulations showed high activity against Gram-positive bacteria and *Candida* spp. but were markedly less effective against Gram-negative pathogens. PHMB exhibited lower antimicrobial potency; however, its CEMIC values indicated clinical efficiency at formulation-relevant concentrations.

Conclusions: The results demonstrate *in vitro* antimicrobial activity of antiseptic mouthwashes against microorganisms associated with oral dysbiosis and suggest that they may have potential as non-antibiotic adjuncts, although this requires clinical validation.

Clinical Significance: This study demonstrates that antiseptic mouthwashes, particularly those containing octenidine, chlorhexidine and polyhexanide, effectively control oral dysbiosis at commercial concentrations. By using the CEMIC index, our findings provide evidence-based guidance for non-antibiotic oral care, reduce antimicrobial resistance risk, and support improved oral health.

1. Introduction

The oral cavity harbors a complex and dynamic microbiome that plays a pivotal role in maintaining oral and systemic homeostasis. Under physiological conditions, the core oral microbiota is dominated by commensal microorganisms, primarily belonging to the genera *Streptococcus, Prevotella, Veillonella, Neisseria, Gemella, Rothia, Granulicatella*, and *Haemophilus* [1–3]. These microorganisms contribute to colonization resistance, modulate local immune responses, and maintain the integrity of the epithelial barrier. Disruption of this balanced ecosystem, however, may result in oral dysbiosis, a clinically relevant condition characterized by qualitative and quantitative alterations in the oral microbiota [4].

Oral dysbiosis should be regarded not merely as a microbiological imbalance but as a distinct clinical entity associated with a wide spectrum of pathological consequences. It is characterized by a reduction in

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beneficial commensal bacteria and an overrepresentation of opportunistic and pathogenic microorganisms, particularly Gram-negative bacteria and yeast-like fungi of the genus *Candida* [5]. Dysbiotic oral microbiota is frequently observed in individuals with poor oral hygiene, systemic diseases, immunosuppression, advanced age, polypharmacy, and following antibiotic or anticancer therapies [6]. Importantly, oral dysbiosis often develops silently, without overt inflammatory symptoms, which may delay its recognition and management [7].

The clinical consequences of oral dysbiosis extend far beyond local oral pathology. Locally, dysbiosis is associated with increased incidence and severity of gingivitis, periodontitis, peri-implantitis, denture stomatitis, oral candidiasis, halitosis, and impaired mucosal healing [8]. From a systemic perspective, the dysbiotic oral cavity may act as a reservoir for opportunistic and multidrug-resistant microorganisms, including *Staphylococcus aureus, Enterococcus* spp., *Enterobacterales*, and *Candida* spp. These pathogens may disseminate to distant body sites via aspiration or hematogenous spread, contributing to severe infections such as aspiration pneumonia, ventilator-associated pneumonia, infective endocarditis, and bloodstream infections, particularly in patients hospitalized or receiving long-term care [6,9–14]. Moreover, growing evidence suggests that chronic oral dysbiosis may contribute to systemic inflammation and has been implicated in the progression of metabolic, cardiovascular, and neurodegenerative disorders [15,16]. In the context of the present study, oral dysbiosis is operationally defined as the overrepresentation of opportunistic and potentially pathogenic microorganisms, rather than a comprehensive alteration of the entire oral microbiome.

A particularly alarming aspect of oral dysbiosis is its role in the emergence and persistence of antimicrobial-resistant microorganisms [17]. The oral cavity provides favorable conditions for biofilm formation, horizontal gene transfer, and long-term colonization by resistant strains [18]. In this context, oral dysbiosis represents not only a local health problem but also a public health concern, aligning with the One Health concept of interconnected human, animal, and environmental health [19].

Effective prevention of oral dysbiosis requires a multifaceted approach aimed at preserving or restoring microbial balance rather than indiscriminate microbial eradication [20]. Key preventive strategies include proper mechanical plaque control, maintaining adequate oral hygiene, regular dental care, optimizing the hygiene of removable prostheses, and minimizing unnecessary systemic antibiotic use [21]. In patients at increased risk of dysbiosis, such as the elderly, immunocompromised individuals, oncological patients, and those in long-term care facilities, adjunctive chemical plaque control plays a particularly important role. In this regard, topical antiseptics delivered via mouthwash represent a valuable preventive and supportive measure, allowing targeted reduction of pathogenic microorganisms and limiting microbial overgrowth while minimizing systemic adverse effects [22].

Among the chemical agents used in mouthwash formulations for the prevention and management of oral dysbiosis, several antiseptic compounds have gained clinical relevance due to their broad-spectrum antimicrobial activity and favorable safety profiles. Chlorhexidine digluconate (CHX) remains one of the most extensively studied oral antiseptics and is considered a reference compound in chemical plaque control, owing to its strong bactericidal and bacteriostatic effects against Gram-positive and Gram-negative bacteria, as well as yeasts [23]. Octenidine dihydrochloride (OCT) has emerged as an effective alternative, exhibiting rapid and potent antimicrobial activity, including against multidrug-resistant microorganisms, with limited systemic absorption and a lower propensity for inducing resistance [24]. Polyhexamethylene biguanide (polyhexanide, PHMB) is another antiseptic increasingly used in oral care products, valued for its membrane-disrupting mechanism of action [25]. Additionally, quaternary ammonium compounds, such as cetylpyridinium chloride, are often incorporated into mouthwashes due to their ability to reduce microbial adhesion [26]. Importantly, these antiseptics differ in their antimicrobial spectra, mechanisms of action, substantivity, and potential impact on the oral microbiota, highlighting the need for comparative studies assessing their effectiveness specifically against microorganisms associated with oral dysbiosis.

Given the global rise in antimicrobial resistance and the principles of antibiotic stewardship, antiseptic-containing mouthwashes are increasingly recognized as a rational, non-antibiotic strategy for managing oral dysbiosis [27]. However, despite their widespread use, comparative data regarding their effectiveness against dysbiosis-associated microorganisms remain limited. Moreover, most available studies focus on single antiseptic agents or model oral pathogens, without addressing the complex spectrum of opportunistic bacteria and yeasts characteristic of dysbiotic oral microbiota. In addition, the clinical relevance of *in vitro* antimicrobial activity is rarely assessed relative to the concentrations used in commercial formulations.

The novelty of the present study lies in the comprehensive, head-to-head comparison of multiple commercially available antiseptic-containing mouthwashes representing different chemical classes of antiseptics, tested against a broad panel of clinically relevant bacterial and fungal species with documented potential to colonize the dysbiotic oral cavity. Furthermore, this study integrates classical MIC determination with the calculation of the Clinical Efficiency of MIC (CEMIC) index, which provides a complementary, translational interpretation of antimicrobial activity relative to clinically relevant concentrations. By combining microbiological susceptibility testing with a clinically oriented efficiency metric, this approach provides a more realistic assessment of the therapeutic potential of antiseptic mouthwashes than MIC analysis alone. Therefore, the aim of this study was to evaluate the antimicrobial activity of commercially available mouthwashes containing different antiseptic agents against pathogenic bacteria and fungi with the potential to colonize the dysbiotic oral cavity, with particular emphasis on their comparative effectiveness, clinical efficiency at formulation-relevant concentrations, and applicability as non-antibiotic tools in the prevention and management of oral dysbiosis.

2. Materials and methods

2.1. Studied strains

The study included clinical strains obtained from the oral cavity or throat, derived from the collection of the Chair and Department of Medical Microbiology. The tested microorganisms comprised Gram-positive bacteria: *Staphylococcus aureus* (n = 8) and *Enterococcus faecalis* (n = 6); Gram-negative bacteria: *Enterobacter cloacae* (n = 4), *Klebsiella pneumoniae* (n = 5), and *Proteus mirabilis* (n = 5); and yeast species: *Candida albicans* (n = 7) and *Candida glabrata* (n = 3). In addition, reference strains were included: *S. aureus* ATCC 29,213, *E. faecalis* ATCC 29,212, *E. cloacae* ATCC BAA-2341, *K. pneumoniae* ATCC BAA-2146, *P. mirabilis* ATCC 12,453, *C. albicans* ATCC 10,231, and *C. glabrata* ATCC 15,126. Bacterial isolates were cultured on Tryptic Soy Broth and Agar, whereas fungal strains were maintained on Sabouraud Dextrose Broth and Agar (Graso Biotech, Starogard Gdański, Poland). All cultures were incubated at 36 °C for 24 h under standard aerobic conditions.

The microorganisms selected for this study represent opportunistic bacterial and fungal species frequently associated with oral dysbiosis, particularly in immunocompromised, elderly, and hospitalized patients. The selection was based on their documented ability to colonize the oral cavity and contribute to local and systemic infections.

2.2. Studied mouthwashes

The study design included two experimental arms: [1] pure antimicrobial compounds and [2] commercially available mouthwash formulations. These arms were not intended to represent direct one-to-one comparisons, but to provide complementary information on intrinsic antimicrobial activity and formulation-based effectiveness.

Seven pure chemical compounds, used either alone or in combination with other substances in oral mouthwashes, were investigated. In addition to widely used antiseptics (OCT, CHX, PHMB), other compounds such as hydrogen peroxide, potassium permanganate, ethacridine lactate, and boric acid were included as reference antimicrobial agents due to their historical or occasional clinical use in oral or mucosal antisepsis. Benzydamine hydrochloride (BNZ) is a non-steroidal anti-inflammatory agent with primarily anti-inflammatory and analgesic properties and limited direct antimicrobial activity; therefore, it was included as a non-antiseptic comparator. The following compounds were included:

- Octenidine dihydrochloride (OCT) (Schülke & Mayr GmbH, Germany); 500 μg/mL,
- Chlorhexidine digluconate (CHX) (Sigma-Aldrich, Poland); 1000 μg/mL,
- Polyaminopropyl biguanide (Polihexanide, PHMB) (Arxada AG, Switzerland); 1000 μg/mL,
- Hydrogen peroxide (H_2O_2) (Hasco-Lek S.A., Poland); 30,000 μg/mL,
- Potassium permanganate ($KMnO_4$) (Hasco-Lek S.A., Poland); 10,000 μg/mL,
- Ethacridine lactate (ET) (Herbapol Poznań, Poland); 1000 μg/mL,
- Boric acid (BA) (Herbapol Poznań, Poland); 30,000 μg/mL
- Benzydamine hydrochloride (BNZ) (Chemat, Poland); 1500 μg/mL.

Seven commercially available mouthwashes containing various active antimicrobial agents were evaluated in this study. The active ingredients included octenidine dihydrochloride (OCT), polyaminopropyl biguanide (polyhexanide, PHMB), chlorhexidine digluconate (CHX), cetylpyridinium chloride (CPC), and benzydamine hydrochloride (BNZ). The tested products were as follows:

- Octenident ® (Schülke & Mayr GmbH); OCT 800 μg/mL,
- Octenisept Oral Mono ® (Schülke & Mayr GmbH, Germany); OCT 1000 μg/mL,
- ProntOral ® (B Braun, Germany); PHMB 1500 μg/mL,
- Eludril Classic ® (Pierre Fabre, France); CHX 1000 μg/mL,
- Eludril Extra ® (Pierre Fabre, France); CHX 2000 μg/mL,
- SeptOral Med ® (Avec Pharma sp.z o.o., Poland); CHX 2000,
- Perio Aid Intensive Care ® (Dentaid, Spain); CHX 1200 μg/mL + CPC 500 μg/mL,
- Hascosept ® (HASCO - Lek S.A., Poland); BNZ 1500 μg/mL.

2.3. Minimum inhibitory concentrations (MIC)

The minimum inhibitory concentrations (MICs) of the tested mouthwashes were determined using the broth microdilution method in sterile 96-well microtiter plates (Nest Scientific Biotechnology, Wuxi, China). The procedure was carried out according to the methodology described in our previous work [28]. In brief, two-fold serial dilutions of each mouthwash were prepared in cation-adjusted Mueller-Hinton Broth (Graso Biotech, Starogard Gdański, Poland), resulting in final concentrations ranging from 100 % to 0.1 %, along with an untreated control. The plates were incubated at 36 °C for 24 h. After incubation, MIC values were determined visually or, if necessary, by colorimetric reaction after adding 10 μL of a 1 % aqueous solution of thiazolyl blue tetrazolium bromide (MTT) (Sigma-Aldrich, Poznań, Poland). All MIC determinations were performed in three independent replicates.

2.4. Clinical efficiency of MIC (CEMIC)

To evaluate the potential clinical relevance of the obtained MIC values, the Clinical Efficiency of MIC (CEMIC) index was calculated. The CEMIC index was designed as a translational parameter linking *in vitro* antimicrobial activity (MIC) with clinically applied (formulation-relevant) concentrations of antiseptic agents. This parameter represents the ratio between the experimentally determined MIC and the standard therapeutic concentration of the tested antimicrobial agent, as expressed by the Equation [29]:

$$\text{Clinical Efficiency of MIC (CEMIC)} = \frac{\text{MIC}}{\text{Clinical concentration}}$$

This approach allows estimation of whether the observed inhibitory effect is achievable under real-use conditions, particularly for topical formulations such as mouthwashes.

Interpretation of CEMIC values was based on the following criteria:

- CEMIC < 0.1 - high clinical efficiency,
- $0.1 \leq$ CEMIC ≤ 0.9 - moderate clinical efficiency,
- CEMIC > 0.9 - low clinical usefulness at the tested concentration.

The proposed threshold values (<0.1, 0.1–0.9, >0.9) are based on a pragmatic interpretation of the ratio between MIC and clinical (therapeutic) concentration. A CEMIC value <0.1 indicates that the MIC is at least tenfold lower than the applied concentration, suggesting a substantial therapeutic margin, whereas values approaching or exceeding 1 indicate limited or no margin of effectiveness.

2.5. Antibiofilm activity

The antibiofilm effect was assessed using the crystal violet assay. The methodology was described previously [30]. Briefly, mature biofilms were formed in 96-well plates using Tryptic Soy Broth and bacterial suspensions adjusted to 0.5 McFarland, and incubated at 36 °C for 72 h. The wells were then treated with 100 μL of antiseptics at commercial concentrations or mouthwashes for 5 or 30 min (to provide a partial approximation of shorter contact conditions). After treatment, the biofilms were fixed with methanol, stained with 1 % crystal violet, and the bound dye was subsequently solubilized with 96 % ethanol. Biofilm biomass was quantified by measuring the optical density at 630 nm using a microplate reader (ELISA 250, bioMérieux, Warsaw, Poland).

2.6. Statistics

One-way ANOVA with Tukey post-tests was used to assess the statistical significance of differences in the biofilm mass. Results were considered significant at a p-value of less than 0.05. Data analysis was performed using InStat 3.10 software (GraphPad Software, Boston, MA, USA).

3. Results

3.1. Antimicrobial activity (MIC)

The antimicrobial activity of the tested mouthwashes varied depending on the antiseptic agent and the group of microorganisms examined (Tables 1–4; Fig. 1). Overall, formulations containing octenidine dihydrochloride (OCT) demonstrated the highest antimicrobial activity against all tested microorganisms, including Gram-positive bacteria, Gram-negative bacteria, and *Candida* spp.

For Gram-positive bacteria, OCT-based mouthwashes exhibited the lowest MIC values, ranging from 0.024 % to 0.098 % of the product concentration, corresponding to 0.195–0.78 μg/mL of the active compound. Chlorhexidine (CHX)-containing formulations also showed high activity against this group, with MIC values generally within a similar range, although slightly higher for certain strains. In contrast, the PHMB-based mouthwash required higher concentrations to inhibit bacterial growth, with MIC values ranging from 0.195 % to 1.56 % of the product concentration (2.9–23.4 μg/mL of the active compound), indicating lower antimicrobial potency against Gram-positive pathogens.

Gram-negative bacteria were markedly less susceptible to all tested

Table 1
Minimum Inhibitory Concentration (MIC) values against oral dysbiotic microorganisms expressed as a percentage of the pure compound's commercial concentration.

Pathogens	MICs of pure compounds in commercial concentrations (%)							
	OCT	CHX	PHMB	H_2O_2	$KMnO_4$	ET	BA	BZ
Staphylococcus aureus	0.098–0.195	0.098–0.195	0.39–0.78	0.39–0.78	100	3.125–6.25	12.5	12.5
Enterococcus faecalis	0.049–0.098	0.049–0.098	0.195–0.78	0.098–0.39	50–100	1.56–6.25	6.25–12.5	12.5
Enterobacter cloacae	0.39	3.125	1.56–6.25	0.78–1.56	>100	12.5	12.5	25
Klebsiella pneumoniae	0.39–0.78	0.78–1.56	6.25	0.78–3.125	100 and >100	12.5–50	25	12.5–25
Proteus mirabilis	0.39–0.78	1.56	3.125–6.25	0.78–1.56	100 and >100	12.5	12.5	12.5–25
Candida albicans	0.098–0.195	0.195–0.39	0.39–0.78	0.39–0.78	100 and >100	1.56–6.25	1.56–6.25	6.25–25
C. glabrata	0.098–0.195	0.195–0.39	0.39–0.78	0.39–0.78	100	1.56–6.25	3.125–6.25	6.25–25

Table 2
Minimum Inhibitory Concentration (MIC) values against oral dysbiotic microorganisms expressed as the active antiseptic compound concentration (μg/mL).

Pathogens	MICs of pure compounds (μg/mL)							
	OCT	CHX	PHMB	H_2O_2	$KMnO_4$	ET	BA	BZ
Staphylococcus aureus	0.49–0.98	0.98–1.95	3.91–7.8	117–234	10,000	31.25–62.5	3750	187.5
Enterococcus faecalis	0.24–0.49	0.49–0.98	1.95–7.8	29.3–117	5000–10,000	15.6–62.5	1875–3750	187.5
Enterobacter cloacae	1.95	31.25	15.6–62.5	234–469	>10,000	125	3750	375
Klebsiella pneumoniae	1.95–3.91	7.8–15.6	62.5	234–938	10,000 and >10,000	125–500	7500	187.5–375
Proteus mirabilis	1.95–3.91	15.6	31.25–62.5	234–469	10,000 and >10,000	125	3750	187.5–375
Candida albicans	0.49–0.98	1.95–3.91	3.91–7.8	117–234	10,000 and >10,000	15.6–62.5	469–1875	93.8–375
C. glabrata	0.49–0.98	1.95–3.91	3.91–7.8	117–234	10,000	15.6–62.5	938–1875	93.8–375

Table 3
Minimum Inhibitory Concentration (MIC) values against oral dysbiotic microorganisms expressed as a percentage of the mouthwash concentration.

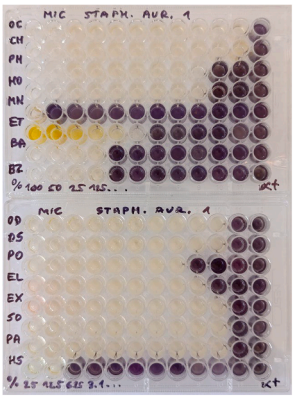
Pathogens	MICs of mouthwashes (%)							
	Octenident	Octenisept Oral Mono	ProntOral	Eludril Classic	Eludril Extra	SeptOral Med	Perio Aid Intensive Care	Hascosept
Staphylococcus aureus	0.049–0.098	0.049–0.098	0.195–0.78	0.098–0.39	0.049–0.098	0.049–0.098	0.049	12.5
Enterococcus faecalis	0.024–0.049	0.024	0.39–1.56	0.195–0.39	0.098–0.195	0.098–0.195	0.098–0.195	12.5
Enterobacter cloacae	0.39	0.39	1.56–3.125	3.125–6.25	1.56	1.56	1.56	25
Klebsiella pneumoniae	0.39–0.78	0.39	1.56–3.125	3.125–12.5	0.78–1.56	0.78–1.56	0.78–1.56	12.5–25
Proteus mirabilis	0.39	0.195–0.39	1.56–3.125	1.56–3.125	1.56	1.56	0.78–1.56	25–50
Candida albicans	0.098–0.78	0.098–0.195	0.195–0.78	0.195–0.78	0.195–0.39	0.195–0.39	0.098–0.39	12.5–25
C. glabrata	0.098–0.195	0.098	0.39–0.78	0.195–0.78	0.39	0.39	0.195–0.39	12.5–25

Table 4
Minimum Inhibitory Concentration (MIC) values against oral dysbiotic microorganisms expressed as the active antiseptic compound concentration (μg/mL) for the tested mouthwashes.

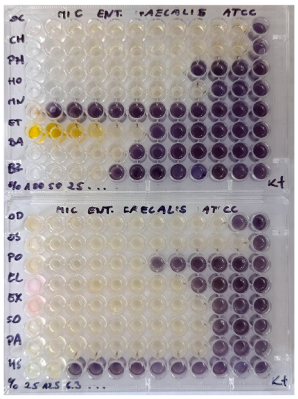
Pathogens	MICs of active compounds of mouthwashes (μg/mL)							
	Octenident (OCT)	Octenisept Oral Mono (OCT)	ProntOral (PHMB)	Eludril Classic (CHX)	Eludril Extra (CHX)	SeptOral Med (CHX)	Perio Aid Intensive Care (CHX + CPC)	Hascosept (BNZ)
Staphylococcus aureus	0.39–0.78	0.49–0.98	2.9–11.7	0.98–3.9	0.98–1.95	0.98–1.95	0.6 + 0.24	187.5
Enterococcus faecalis	0.195–0.39	0.24	5.9–23.4	1.95–3.9	1.95–3.9	1.95–3.9	1.2–2.3 + 0.49–0.98	187.5
Enterobacter cloacae	3.125	3.9	23.4–46.9	31.25–62.5	31.25	31.25	18.8 + 7.8	375
Klebsiella pneumoniae	3.125–6.25	3.9	23.4–46.9	31.25–125	15.6–31.25	15.6–31.25	9.4–18.8 + 3.9–7.8	187.5–375
Proteus mirabilis	3.125	1.95–3.9	23.4–46.9	15.6–31.25	31.25	31.25	9.4–18.8 + 3.9–7.8	375–750
Candida albicans	0.78–6.25	0.98–1.95	2.9–11.7	1.95–7.8	3.9–7.8	3.9–7.8	1.2–4.7 + 0.49–9.4	187.5–375
C. glabrata	0.78–1.56	0.98	5.9–11.7	1.95–7.8	7.8	7.8	2.3–4.7 + 0.98–9.4	187.5–375

antiseptics compared with Gram-positive bacteria. Nevertheless, OCT-containing formulations remained the most effective agents against this group, with MIC values ranging from 0.39 % to 0.78 % of the product concentration, corresponding to 3.125–7.8 μg/mL of OCT. CHX-based mouthwashes showed substantially higher MIC values against Gram-negative bacteria, ranging from 9.4 to 125 μg/mL for the active compound. PHMB-containing preparations exhibited the weakest activity against Gram-negative bacteria, with MIC values ranging from 23.4 to 46.9 μg/mL.

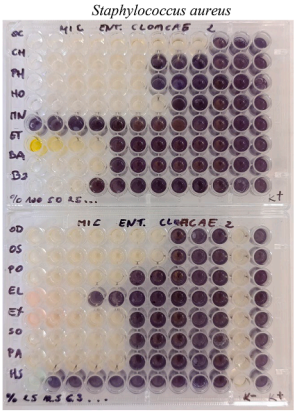
All tested mouthwashes demonstrated antifungal activity against *Candida* spp.; however, species-dependent differences were observed. The lowest MIC values were consistently recorded for OCT-containing formulations (0.098–0.78 % of the product concentration; 0.78–6.25 μg/mL of the active compound). CHX-based mouthwashes displayed comparable antifungal activity, with MIC values ranging from 1.2 to 7.8 μg/mL of the active compound. PHMB-based preparations required higher concentrations to inhibit fungal growth (2.9–11.7 μg/mL). Among the tested yeasts, *Candida albicans* were generally more susceptible, whereas *C. glabrata* exhibited higher MIC values, indicating reduced susceptibility.



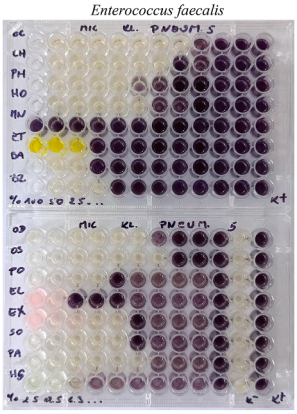
Staphylococcus aureus



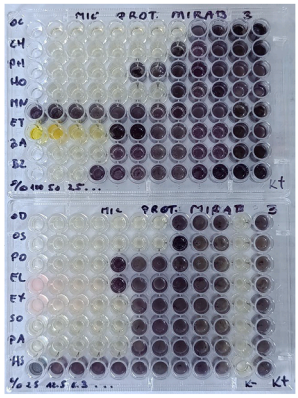
Enterococcus faecalis



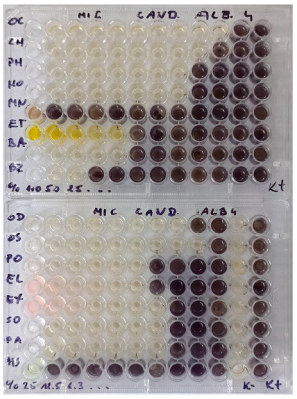
Enterobacter cloacae



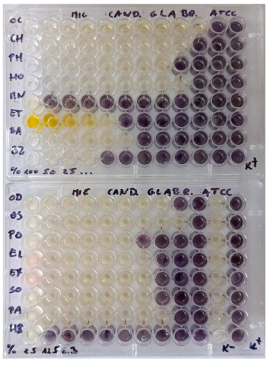
Klebsiella pneumoniae



Proteus mirabilis



Candida albicans



C. glabrata

(caption on next column)

Fig. 1. Sample photos of minimal inhibitory concentrations (MICs) studies. The purple-brown coloration observed after MTT addition indicates viable, metabolically active bacterial cells capable of reducing MTT to formazan, whereas wells lacking this coloration indicate bacterial cell death. Abbreviations: OC - octenidine dihydrochloride; CH - chlorhexidine digluconate; pH - polyhexamethylene biguanide; HO - hydrogen peroxide; MN - potassium permanganate; ET - ethacridine lactate; BA - boric acid; BZ - benzydamine hydrochloride; OD - Octenident; OS - Octenisept Oral Mono; PO - ProntOral; EL - Eludril Classic; EX - Eludril Extra; SO - SeptOral Med; PA - Perio Aid Intensive Care; HS - Hascosept; K- – negative control; K+ - positive control.

It should be emphasized that the comparison between pure compounds and commercial formulations has inherent limitations. Pure substances reflect intrinsic antimicrobial activity, whereas commercial mouthwashes are complex formulations. The inclusion of additional reference compounds provides contextual benchmarking of antimicrobial potency across a broader spectrum of agents.

The clinical relevance of the obtained MIC values was further evaluated using the Clinical Efficiency of MIC (CEMIC) index (Table 5). For OCT, CEMIC values ranged from 0.00024 to 0.0078 across all microorganism groups, indicating high clinical efficiency of MIC. CHX and PHMB also demonstrated high efficiency (CEMIC < 0.1 for most strains), although elevated CEMIC ranges were observed for Gram-negative bacteria. As expected, the BNZ-containing formulation exhibited substantially higher MIC values, consistent with its non-antiseptic mechanism of action. Preparations containing BNZ, ET and BA showed CEMIC values exceeding 0.1, indicating moderate clinical usefulness at the tested concentrations. However, the highest CEMIC levels ranging from 0.5 to >1.0 were for $KMnO_4$.

3.2. Antibiofilm activity

No antibiofilm activity of the pure compounds or mouthwashes was observed after 5 min of exposure in the crystal violet assay against *S. aureus, Escherichia coli*, and *C. albicans*, with the exception of the Octenisept Oral Mono mouthwash (Table 6). After 30 min of exposure, antibiofilm activity against all three pathogens was demonstrated by products containing OCT, CHX, PHMB, H_2O_2, ET, and BA. No antibiofilm effect was observed for products containing $KMnO_4$ or BNZ (Table 7).

4. Discussion

Oral dysbiosis is increasingly recognized as a clinically relevant condition associated with both local oral pathology and systemic complications [6]. In this context, effective control of dysbiosis-associated microorganisms represents a major challenge in contemporary oral healthcare. The present study provides a comparative evaluation of the antimicrobial activity of commercially available antiseptic-containing mouthwashes against microorganisms commonly associated with dysbiotic oral microbiota.

The MIC assays revealed substantial differences in antimicrobial efficacy among the tested antiseptic agents. Preparations containing octenidine dihydrochloride consistently demonstrated the highest antimicrobial activity against all examined groups of microorganisms, including Gram-positive bacteria, Gram-negative bacteria, and *Candida* spp. These findings are consistent with previous reports describing the broad-spectrum activity of octenidine and its effectiveness against both susceptible and multidrug-resistant pathogens, including non-fermenting Gram-negative bacteria [24]. A relatively wide MIC range for *Candida albicans* observed with the Octenident ® formulation likely reflects strain-specific differences in susceptibility, associated with variations in cell wall structure, membrane composition, and stress responses. The very low MIC and CEMIC values observed for octenidine indicate high *in vitro* antimicrobial potency, highlighting its strong therapeutic potential for controlling dysbiosis-associated

Table 5
Clinical Efficiency of Minimum Inhibitory Concentrations (CEMIC) of pure compounds and mouthwashes against oral dysbiotic microorganisms.

Pure compounds or mouthwashes with below antiseptic	CEMIC values for pathogens			Clinical Efficiency of MIC
	Gram-positive bacteria	Gram-negative bacteria	*Candida* yeasts	
OCT	0.00024–0.002	0.002–0.0078	0.00098–0.0078	High
CHX or CHX+CPC	0.00049–0.0098	0.0078–0.125	0.001–0.0078	High-Moderate
PHMB	0.0019–0.0156	0.0156–0.0625	0.0019–0.0078	High
H_2O_2	0.00098–0.0078	0.0078–0.031	0.0039–0.0078	High
$KMnO_4$	0.5–1.0	1.0 to >1.0	1.0 to >1.0	Moderate-Low
ET	0.0156–0.0625	0.125–0.5	0.0078–0.031	High-Moderate
BA	0.0625–0.125	0.125–0.25	0.0156–0.0625	High-Moderate
BNZ	0.125	0.125–0.5	0.125–0.25	Moderate

Table 6
Absorbance values (OD at 630 nm) corresponding to biofilm reduction in the crystal violet test after 5 min of exposure.

Pure compound or mouthwash	Tested microorganisms		
	S. aureus	*E. coli*	*C. albicans*
Positive control	0.425 ± 0.038	0.507 ± 0.033	0.465 ± 0.035
OCT	0.336 ± 0.035 ^{ns}	0.385 ± 0.034 ^{ns}	0.356 ± 0.048 ^{ns}
CHX	0.356 ± 0.008 ^{ns}	0.358 ± 0.043 ^{ns}	0.364 ± 0.049 ^{ns}
PHMB	0.361 ±0.03 ^{ns}	0.411 ± 0.015 ^{ns}	0.378 ± 0.021 ^{ns}
H_2O_2	0.355 ± 0.011 ^{ns}	0.378 ± 0.045 ^{ns}	0.361 ± 0.04 ^{ns}
$KMnO_4$	0.383 ± 0.032 ^{ns}	0.375 ± 0.045 ^{ns}	0.403 ± 0.035 ^{ns}
ET	0.296 ± 0.022 ^{ns}	0.382 ± 0.026 ^{ns}	0.367 ± 0.058 ^{ns}
BA	0.343 ± 0.071 ^{ns}	0.366 ± 0.043 ^{ns}	0.402 ± 0.022 ^{ns}
BNZ	0.361 ± 0.048 ^{ns}	0.362 ± 0.032 ^{ns}	0.393 ± 0.02 ^{ns}
Octenident (OCT)	0.35 ± 0.048 ^{ns}	0.43 ± 0.053 ^{ns}	0.374 ± 0.032 ^{ns}
Octenisept Oral Mono (OCT)	0.34 ± 0.021 *	0.417 ± 0.079 ^{ns}	0.395 ± 0.043 ^{ns}
ProntOral (PHMB)	0.417 ± 0.05 ^{ns}	0.471 ± 0.015 ^{ns}	0.469 ± 0.037 ^{ns}
Eludril Classic (CHX)	0.344 ± 0.014 ^{ns}	0.391 ± 0.032 ^{ns}	0.384 ± 0.044 ^{ns}
Eludril Extra (CHX)	0.35 ± 0.016 ^{ns}	0.426 ± 0.065 ^{ns}	0.364 ± 0.035 ^{ns}
SeptOral Med (CHX)	0.4 ± 0.024 ^{ns}	0.469 ± 0.05 ^{ns}	0.422 ± 0.049 ^{ns}
Perio Aid Intensive Care (CHX + CPC)	0.376 ± 0.025 ^{ns}	0.386 ± 0.031 ^{ns}	0.4 ± 0.01 ^{ns}
Hascosept (BNZ)	0.455 ± 0.103 ^{ns}	0.541 ± 0.028 ^{ns}	0.481 ± 0.45 ^{ns}

Legends: ns - no statistical significance ($p > 0.05$); * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

Table 7
Absorbance values (OD at 630 nm) corresponding to biofilm reduction in the crystal violet test after 30 min of exposure.

Pure compound or mouthwash	Tested microorganisms		
	S. aureus	*E. coli*	*C. albicans*
Positive control	0.489 ± 0.045	0.547 ± 0.037	0.522 ± 0.04
OCT	0.33 ± 0.021 ***	0.344 ± 0.016 ***	0.318 ± 0.027 ***
CHX	0.316 ± 0.016 ***	0.33 ± 0.032 ***	0.36 ± 0.04 *
PHMB	0.394 ± 0.03 *	0.414 ± 0.053 *	0.366 ± 0.038 **
H_2O_2	0.337 ± 0.029 ***	0.418 ± 0.04 *	0.376 ± 0.046 *
$KMnO_4$	0.437 ± 0.028 ^{ns}	0.513 ± 0.031 ^{ns}	0.464 ± 0.053 ^{ns}
ET	0.334 ± 0.023 ***	0.389 ± 0.029 **	0.385 ± 0.051 *
BA	0.303 ± 0.014 ***	0.365 ± 0.029 ***	0.332 ± 0.044 ***
BNZ	0.435 ± 0.029 ^{ns}	0.155 ± 0.029 ^{ns}	0.476 ± 0.051 ^{ns}
Octenident (OCT)	0.307 ± 0.018 ***	0.307 ± 0.053 ***	0.304 ± 0.03 ***
Octenisept Oral Mono (OCT)	0.299 ± 0.014 ***	0.305 ± 0.025 ***	0.308 ± 0.035 ***
ProntOral (PHMB)	0.395 ± 0.033 *	0.419 ± 0.042 *	0.37 ± 0.026 *
Eludril Classic (CHX)	0.318 ± 0.023 ***	0.359 ± 0.022 ***	0.379 ± 0.061 *
Eludril Extra (CHX)	0.343 ± 0.066 ***	0.347 ± 0.021 ***	0.382 ± 0.052 *
SeptOral Med (CHX)	0.276 ± 0.02 ***	0.353 ± 0.04 ***	0.317 ± 0.056 ***
Perio Aid Intensive Care (CHX + CPC)	0.255 ± 0.007 ***	0.321 ± 0.072 ***	0.305 ± 0.043 ***
Hascosept (BNZ)	0.434 ± 0.038 ^{ns}	0.435 ± 0.023 ^{ns}	0.406 ± 0.036 ^{ns}

Legends: ns - no statistical significance ($p > 0.05$); * - $p < 0.05$; ** - $p < 0.01$; *** - $p < 0.001$.

microorganisms.

CHX-based formulations also exhibited high antimicrobial activity, particularly against Gram-positive bacteria and *Candida* spp., confirming its long-established role as a reference oral antiseptic [23]. However, the markedly higher MIC values observed for Gram-negative bacteria indicate important limitations of CHX in the context of dysbiotic oral microbiota enriched with opportunistic species. Reduced susceptibility of Gram-negative bacteria to CHX has been reported previously and is commonly attributed to differences in outer membrane permeability and efflux mechanisms [17,29]. In the present study, the addition of cetylpyridinium chloride to chlorhexidine did not result in a clear improvement in antimicrobial efficacy. This observation may be explained by several factors. Both CHX and CPC are cationic agents that exert their antimicrobial effect primarily through disruption of microbial cell membranes. Due to their similar mechanisms of action, their combined use may not produce a synergistic effect, but rather a non-additive interaction. In addition, potential physicochemical interactions between these compounds cannot be excluded. As cationic surfactants, CPC and CHX may compete for binding sites on bacterial cell surfaces or interact with each other in solution, potentially reducing the

effective availability of the active forms.

PHMB exhibited lower antimicrobial potency in MIC assays compared with octenidine and chlorhexidine, particularly against Gram-negative bacteria. Nevertheless, the calculated CEMIC values remained below the threshold, reflecting high clinical efficiency at formulation-relevant concentrations. These findings are consistent with previous studies demonstrating moderate *in vitro* antimicrobial activity of PHMB, combined with a good tolerability and safety profile, which may support its use in long-term oral hygiene regimens where reduced toxicity and mucosal compatibility are required [25].

All tested mouthwashes showed antifungal activity against *Candida* spp. The lowest MIC values against all tested yeasts were observed for OCT-containing formulations, followed by CHX-based products, while PHMB required higher concentrations to inhibit fungal growth. These results suggest that antiseptic mouthwashes may represent a useful adjunctive strategy in the prevention and management of oral candidiasis associated with dysbiosis, particularly in immunocompromised and elderly patients.

Benzydamine hydrochloride differs fundamentally from the tested antiseptic agents, as its primary mechanism of action is anti-inflammatory rather than antimicrobial. Therefore, it should not be interpreted as a direct comparator to antiseptics such as OCT, CHX, or PHMB. The high MIC values observed for BNZ are consistent with its pharmacological profile. Its inclusion in this study was intended to provide clinical context, as benzydamine-containing formulations are commonly used in oral care despite lacking primary antiseptic activity.

It should be noted that the antimicrobial activity of commercial mouthwash formulations is influenced not only by the active compounds but also by the excipients present. Components such as surfactants, pH modifiers, stabilizers, and solvents may affect antimicrobial performance by altering membrane permeability, improving penetration, or modifying the chemical stability and bioavailability of the active agents. Therefore, the observed activity of mouthwashes reflects the combined effect of active ingredients and formulation components rather than the intrinsic potency of the active compound alone. Consequently, direct comparison between pure compounds and their corresponding commercial formulations has inherent limitations. Differences in antimicrobial activity may result not only from the concentration of the active substance but also from formulation-dependent factors.

From a clinical perspective, the present findings underscore the importance of selecting antiseptic agents not only for their general antimicrobial properties but also for their activity against microorganisms specifically associated with oral dysbiosis. The consistently lower MIC values observed for OCT suggest that it may be of potential interest in the context of high-risk populations, including elderly individuals, immunocompromised patients, and residents of long-term care facilities, in whom colonization by opportunistic Gram-negative bacteria and *Candida* spp. is frequent. Moreover, the consistently low CEMIC values observed for most antiseptics may be relevant to antimicrobial stewardship strategies, although this requires clinical validation. Further clinical studies are required to confirm these observations under real-use conditions.

Several limitations of this study should be acknowledged. First, the *in vitro* design does not fully reflect the complexity of the oral environment, where antimicrobial activity is influenced by saliva, host factors, and multispecies biofilms. Importantly, the study primarily assessed planktonic microorganisms, whereas *in vivo* oral microbiota exists predominantly in biofilm form, which exhibits substantially higher tolerance to antiseptics. Although antibiofilm activity was evaluated, the crystal violet assay used to assess it reflects only total biomass and does not provide information on biofilm viability or parameters such as MBEC or MBIC. Second, the discrepancy between the prolonged incubation time used for MIC determination (24 h) and the short contact time of mouthwashes in clinical use (30–60 s) limits direct translational interpretation. MIC values should therefore be considered indicators of intrinsic antimicrobial potency rather than predictors of clinical effectiveness. Third, the relatively limited number of isolates per species does not allow for comprehensive MIC distribution analysis or calculation of epidemiological parameters such as MIC50 and MIC90, and the results should be interpreted as indicative rather than population-level data. Additionally, antimicrobial resistance profiles of the tested isolates were not systematically characterized, which limits conclusions regarding activity against resistant strains. The CEMIC index applied in this study provides a translational perspective; however, it remains an exploratory parameter that has not yet undergone independent validation. Finally, the study does not address the impact of antiseptics on commensal microbiota or overall microbial community structure. Therefore, the findings cannot be interpreted as evidence of restoring ecological balance or directly “controlling” oral dysbiosis.

5. Conclusions

This study demonstrates that antiseptic-containing mouthwashes exhibit substantial antimicrobial activity against microorganisms associated with oral dysbiosis, including opportunistic and rtally relevant bacterial and fungal pathogens. Among the tested agents, octenidine dihydrochloride showed the most potent and consistent antimicrobial effects in this *in vitro* model, followed by chlorhexidine digluconate and polyhexamethylene biguanide. Importantly, low CEMIC values indicate clinical efficiency at formulation-relevant concentrations, supporting the potential of their use as non-antibiotic adjuncts for the prevention and management of oral diseases.

The observed differences in antimicrobial activity among agents suggest the importance of rational product selection based on susceptibility profiles, although this requires confirmation in clinical studies. Future research should focus on longitudinal clinical studies and biofilm-based models to evaluate the long-term impact of antiseptic mouthwash use on oral microbiota balance, and host–microbe interactions. Integrating antiseptic-based oral care into antimicrobial stewardship and One Health frameworks may help reduce the need for systemic antibiotics and limit antimicrobial resistance.

CRediT authorship contribution statement

Marzena Korbecka-Paczkowska: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization. **Magdalena Paczkowska-Walendowska:** Writing – original draft, Visualization. **Judyta Cielecka-Piontek:** Supervision, Data curation. **Tomasz M. Karpiński:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Author Marzena Korbecka-Paczkowska was employed by the company Medi-Pharm. The remaining authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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