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Microbicidal Activity of Octenidine Oromucosal Solution against Periodontal Bacteria and Yeast: An In Vitro Study

Andreas Wille [1], Katharina Kapoor [2], Pascal Bartling [2,*], Nadine Radischat [2], Annett Bramfeld [3], Kathrin Naujox [3] and Claudia Hildebrandt [3]

[1] Institut für Hygiene und Umwelt, Marckmannstraße 129a, 20539 Hamburg, Germany
[2] Schülke & Mayr GmbH, Robert-Koch-Straße 2, 22851 Norderstedt, Germany
[3] HygCen Germany GmbH, Bornhövedstrasse 78, 19055 Schwerin, Germany
* Correspondence: pascal.bartling@schuelke.com; Tel.: +49-162-349-4103

Abstract: Octenidine-containing antiseptic oromucosal solution has proven to be efficacious and safe for temporary bacterial reduction and plaque inhibition in the oral cavity. While its clinical outcomes are encouraging, further research is needed to assess its microbicidal activity against pathogens causing gingivitis and periodontitis. We evaluated the in vitro microbicidal activity of different dilution concentrations of a commercially-available 0.1% octenidine dihydrochloride containing oromucosal solution (OOS) against 10 periodontopathogenic bacteria and three yeast strains using the testing methodology frameworks of DIN EN 13727:2012+A2:2015 (30-s contact time) and DIN EN 13624:2022-08 (60-s contact time), respectively. The OOS showed significant bactericidal activity at 80% dilution concentration, as indicated by a log reduction factor (lg RF) ≥5 for all 10 bacteria. At 50% dilution concentration, bactericidal activity was observed against specific bacteria like *Porphyromonas gingivalis*, *Prevotella buccalis*, *Parvimonas micra*, *Eikenella corrodens*, *Dialister pneumosintes*, *Schaalia odontolytica* (*Actinomyces odontolyticus*), and *Campylobacter rectus*. Yeasticidal activity against *Candida albicans* was observed at 80% dilution concentration, whereas *Candida auris* was more susceptible to the OOS and showed lg RF ≥4 even at 50% dilution concentration. Therefore, the OOS may be an effective adjunct to periodontal therapy aimed at reducing pathogenic microbial load, inhibiting plaque formation, and thereby preventing periodontal inflammation.

Keywords: octenidine dihydrochloride; oromucosal solution; mouthrinse; periodontopathogens; bactericidal; yeasticidal; plaque inhibition

1. Introduction

Periodontal diseases, including gingivitis and periodontitis, affect nearly half the global population [1]. The onset and progression of periodontal inflammation are associated with the colonization of diverse oral microbes [2], including "red-complex" periodontopathogens, such as *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* [3]. Yeasts, such as *Candida albicans*, have also been detected in the periodontal pockets of patients with chronic periodontitis [4,5]. Interventions targeting these microbes are important to prevent the development and progression of gingivitis and periodontitis. Although mechanical plaque control is the primary treatment for periodontitis, it does not effectively inhibit plaque formation [6,7]. Hence, adjunctive use of chemical antimicrobial agents, such as mouthrinses, is necessary [8,9].

Octenidine dihydrochloride is a recognized active ingredient of oral antiseptic preparations [10–14]. It has broad-spectrum antimicrobial activity owing to its nonspecific mode of action [15], which also reduces the likelihood of bacterial resistance [16]. Clinically, octenidine mouthrinses effectively reduce salivary bacterial counts with minimal toxicity [11] and inhibit plaque formation for 5 days

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[10]. Further, in orthodontically-treated patients, rinsing with octenidine can significantly reduce the total viable oral bacteria, *Streptococcus mutans,* and *Lactobacillus* species in saliva [17]. While the clinical results of octenidine are promising, previous studies have focused chiefly on its effects against plaque formation, gingivitis, and total and cariogenic salivary bacteria. Few studies have evaluated the microbicidal activity of octenidine specifically against periodontopathogens [12].

Hence, our objective was to evaluate the antimicrobial activity of octenidine dihydrochloride against common periodontopathogens, such as the red-complex bacteria, and to broaden its recognized antimicrobial spectrum. Accordingly, we investigated the in vitro microbicidal activity of different dilution concentrations of a commercially-available 0.1% octenidine dihydrochloride oromucosal solution (OOS) against a range of periodontal bacteria and yeast strains using standard testing methods.

2. Materials and Methods

2.1. Test Product

The commercially-available product used in this study was 0.1% octenident® antiseptic (Schülke & Mayr GmbH, Norderstedt, Germany), a clear, colorless, ready-to-use OOS with mint flavour. It was stored in a dark environment at room temperature (approximately 20°C) as per the manufacturer's instructions.

2.2. Microbial Strains

Ten periodopathogenic bacteria were included (Table 1). These predominantly anaerobic or microaerophilic bacteria were cultured using appropriate nutrient media and conditions, as described in Table 1.

Table 1. Bacterial strains and culture conditions.

Bacteria	Strain	Culture conditions and media Laboratory 1	Culture conditions and media Laboratory 2
Fusobacterium nucleatum	ATCC 25586, DSM 15643	anaerobic, 37°C, 2 days, hemin- and vitamin K-enriched medium	Columbia blood agar, anaerobic, 36°C ± 1°C, 3 days
Aggregatibacter actinomycetemcomitans	ATCC 43717	10% carbon dioxide, 37°C, 2 days, 5% sheep blood agar	Brain heart infusion agar, 36° C ± 1°C, 3 days, 95% air, 5% carbon dioxide
Porphyromonas gingivalis	ATCC 33277, DSM 20709	anaerobic, 37°C, 2 days, 5% sheep blood agar	Schaedler agar, anaerobic, 36°C ± 1°C, 5 days
Prevotella buccalis	ATCC 35310, DSM 20616	anaerobic, 37°C, 2 days, 5% sheep blood agar	Schaedler agar, anaerobic, 36°C ± 1°C, 7 days
Dialister pneumosintes	DSM 11619	10% carbon dioxide, 37°C, 4 days, hemine- and vitamin K-enriched medium	Columbia blood agar, anaerobic, 36°C ± 1°C, 3 days
Campylobacter rectus	ATCC 33238, DSM 3260	10% carbon dioxide, 37°C, 2 days, 5% sheep blood agar	*Campylobacter rectus* agar, anaerobic, 36°C ± 1°C, 7 days
Capnocytophaga gingivalis	ATCC 33624, DSM 3290	10% carbon dioxide, 37°C, 3 days, 5% sheep blood agar	Capnocytophaga agar, anaerobic, 36°C ± 1°C, 3 days

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Eikenella corrodens	ATCC 23834, DSM 8340	10% carbon dioxide, 37°C, 2 days, 5% sheep blood agar	Columbia blood agar, microaerophile, 36°C ±1°C, 3 days
Parvimonas micra	ATCC 33270, DSM 20468	anaerobic, 37°C, 3 days, 5% sheep blood agar	Columbia blood agar, anaerobic, 36°C ± 1°C, 3 days
Schaalia odontolytica (Actinomyces odontolyticus)	ATCC 17982	10% carbon dioxide, 37°C, 2 days, 5% sheep blood agar	Columbia blood agar, microaerophile, 36°C ± 1°C, 2 days

The yeast strains used in the study were *C. albicans* (ATCC 10231) and two strains of *Candida auris* (DSM 21092, DSM 105986) (Table S1).

2.3. Evaluation of Bactericidal Activity

The DIN EN 13727:2012+A2:2015 standards [18] were followed with some modifications: bacteria comprising the normal oral flora were selected and obtained from DSM and ATCC cultures (Table 1), and a testing environment of 33°C, under clean conditions, was maintained using water baths and room air conditioning.

Each milliliter of octenident® antiseptic contains 1 mg of octenidine dihydrochloride. octenident® antiseptic was diluted with sterilized distilled water to obtain concentrations of 0.01%, 0.1%, 50%, and 80% (v/v). In addition, the ready-to-use product was also tested at 97% (v/v) in keeping with the DIN EN 13727:2012+A2:2015 standards [18].

The bacteria were cultured at two independent laboratories, using specific media and culture conditions (Table 1) to ensure robustness of the results. The cultures were then examined at both laboratories to identify colonies. If no colonies were visible or if they were too small, the bacteria were cultivated under the same culture conditions for 2 more days and reevaluated.

2.3.1. Quantitative Suspension Tests According to DIN EN 13727:2015

Quantitative suspension tests were performed at a temperature of 33°C according to the dilution-neutralization method or membrane filtration method of EN 13727 (5.5.2 or 5.5.3). All tests involved a 30-s contact time, and the necessary validations (control of the test conditions A, control of the inactivator B, procedure validation C) were performed in accordance with the standards.

2.3.2. Comparative Tests

Comparative tests were performed according to the EN 13727 (5.5.2.2 or 5.5.3.2) protocol under clean conditions with 0.3 g/L bovine serum albumin (bovine serum albumin fraction V). Further, control procedures were performed according to EN 13727 (5.5.2.3-5.5.2.5 or 5.5.3.3-5.5.3.5).

2.3.3. Dilution-Neutralization Method (e.g., at a Dilution Concentration of 80%)

After 2 minutes of equilibration of one part interfering substance (organic load: 0.3 g/L bovine serum albumin) and one part test organism solution, the two parts were mixed with eight parts antimicrobial solution (OOS). At the end of the contact time, an aliquot of 1 mL was transferred to 9 mL neutralizer solution. After neutralization for 10 s, a sample of 1 mL was taken in duplicate, and agar plates were inoculated.

2.3.4. Membrane Filtration Method (e.g., at a Dilution Concentration of 80%)

After 2 minutes of equilibration of one part interfering substance (organic load: 0.3 g/L bovine serum albumin) and one part test organism solution, the two parts were mixed with eight parts antimicrobial solution (OOS). At the end of the contact time, two aliquots of 0.1 mL were transferred into a separate membrane filtration apparatus. The filters were immediately rinsed with at least 150

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mL, but no more than 500 mL, rinsing liquid. The procedure was completed by filtering 50 mL of water. The membranes were then transferred to separate agar plates.

2.4. Evaluation of Yeasticidal Activity

The DIN EN 13624:2022-08 standards [19] were followed with modifications: the testing temperature was maintained at 33°C using water baths and room air conditioning.

The load substances included 0.08% mucin Type I-S, 0.25% bovine serum albumin, and 0.35% yeast extract. The yeast strains were exposed to different OOS concentrations (10%, 50%, and 80%) for 60 s. In case of ready-to-use products, the resulting concentration is 97% (v/v), and they may be tested at this concentration.

The bactericidal and yeasticidal activities of the OOS were evaluated based on the log reduction factor (lg RF). According to the DIN EN standards, the test product was considered bactericidal if the lg RF was ≥5 and yeasticidal if the lg RF was ≥4. The lowest dilution concentration demonstrating a lg RF ≥5 for each bacterial strain and lg RF ≥4 for each yeast strain was recorded.

3. Results

The microbicidal activity of the OOS against a range of periodontal bacteria and yeast strains was evaluated. The bactericidal and yeasticidal activities of the OOS at different dilution concentrations are presented as lg RF values in Tables 2, 3 and S2–S4.

3.1. Bactericidal Activity

Data from both laboratories showed that the OOS exerted strong bactericidal activity at 80% dilution concentration, with the lg RF exceeding 5 for all bacterial strains, except *A. actinomycetemcomitans.* This organism showed a lg RF ≥5 in laboratory 1 but narrowly missed the threshold with a lg RF of 4.89 in laboratory 2 (Table 2). However, it was susceptible to the bactericidal effects of the OOS at 97% dilution concentration (Table S3).

Table 2. Bactericidal activity of the octenidine oromucosal solution*.

Bacterial strain	lg RF (80% dilution concentration) Laboratory 1	lg RF (80% dilution concentration) Laboratory 2
Capnocytophaga gingivalis	>5.28	>5.06
Porphyromonas gingivalis	>5.23	>5.54
Prevotella buccalis	>5.24	5.06
Parvimonas micra	>5.41	>5.04
Eikenella corrodens	>5.26	>5.05
Aggregatibacter actinomycetemcomitans	>5.22	4.89
Fusobacterium nucleatum	5.26	>5.06
Dialister pneumosintes	>5.20	>5.49
Schaalia odontolytica (Actinomyces odontolyticus)	>5.09	>5.10
Campylobacter rectus	>5.09	>5.09

lg RF, log reduction factor. *octenident® antiseptic (Schülke & Mayr GmbH, Norderstedt, Germany).

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At 50% dilution concentration, the OOS displayed bactericidal activity against specific bacteria like *P. gingivalis*, *P. buccalis*, *P. micra*, *E. corrodens*, *D. pneumosintes*, *S. odontolytica*, and *C. rectus* (Table S2). The bactericidal effect of the OOS was lowest at 0.01% and 0.1% dilution concentrations for all bacterial strains (Tables S2 and S3).

3.2. Yeasticidal Activity

The yeasticidal activity of the OOS on *C. albicans* and *C. auris* was highest at 80% dilution concentration. However, both *C. auris* strains (DSM 21092, DSM 105986) were susceptible to the OOS even at 50% dilution concentration, whereas *C. albicans* did not pass the lg-RF ≥4 threshold at 50% dilution concentration (Tables 3 and S4).

Table 3. Yeasticidal activity of the octenidine oromucosal solution*.

Yeast strain	lg RF (80% dilution concentration)
Candida albicans ATCC 10231	>4.40
Candida auris DSM 21092	>4.74
Candida auris DSM 105986	>4.58

lg RF, log reduction factor. *octenident® antiseptic (Schülke & Mayr GmbH, Norderstedt, Germany).

4. Discussion

The management of gingivitis and periodontitis requires a comprehensive approach based on the different bacterial complexes and stages of colonization. Early colonizers facilitate biofilm formation [20] and include members of the purple complex (*S. odontolytica*) and green complex (*C. gingivalis*, *E. corrodens*, and serotype a of *A. actinomycetemcomitans*). Late colonizers, which consist of red complex bacteria (*P. gingivalis*), are strongly associated with periodontal destruction and colonize only in the presence of bridging colonizers, such as orange complex bacteria (*P. micra*, *F. nucleatum*, and *C. rectus*) [21–23].

The present in vitro study evaluated the microbicidal activity of different dilution concentrations of a commercially available 0.1% OOS against 10 periodontopathogenic bacteria and three yeast strains. At 80% dilution concentration, the OOS exhibited high bactericidal activity, with a lg RF >5 against all tested bacterial strains (Table 2, Laboratory 1 data). This finding indicates the usefulness of the OOS as an adjunct therapeutic for chemical plaque control in the management of periodontal inflammation.

In addition to bacterial complexes, the periodontal pocket also serves as a niche for yeast strains [24,25]. *C. auris*, an emerging multidrug-resistant pathogen [26], has been identified in the tracheobronchial secretions of critically ill patients with COVID-19 [27–29] as well as in other immunocompromised patients [30], suggestive of nosocomial transmission [27–33]. Notably, aspiration of microbe-laden secretions from the oropharynx is an important factor in the development of lower respiratory infection [34]. In the present study, the OOS demonstrated yeasticidal activity against *C. albicans* and *C. auris* at 80% dilution concentration, indicating that the OOS may offer a therapeutic advantage against multidrug-resistant nosocomial fungal infections, where conventional antiseptics are ineffective.

The broad-spectrum microbicidal action of octenidine dihydrochloride is attributable to its ability to disrupt the cell membrane of bacteria and yeast [35,36]. Octenidine (N, N′-(1,10 decanediyldi-1[4H]-pyridinyl-4-ylidene) bis-(1-octanamine) dihydrochloride) is a quaternary ammonium compound of the bipyridine family [16], and its amino-pyridine components contribute to mesomeric distribution of cationic charge. The two cationic pyridine components are separated by 10 methylene groups, with two terminal hydrophobic octanyl groups. Due to this structure, octenidine is amphiphilic (hydrophobic and hydrophilic domains) and resembles membrane-active antimicrobial peptides [37]. Its cationic and hydrophobic character contributes to its interaction with the bacterial cell membrane. Being positively charged, octenidine binds to the negatively-charged

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microbial cell membrane and disrupts its structural integrity [35,36]. On adhering to the bacteria, octenidine neutralizes the bacterial surface charge through electrostatic interactions and immediately penetrates the thick cell wall to reach the cell membrane. Further, the hydrophobic hydrocarbon chains of octenidine, interfere with the fatty acyl chains of the cell membrane and significantly disturb the packing order of bacterial phospholipids. As octenidine does not distinguish between these lipids, its mechanism of action lacks selectivity toward microorganisms with varying cell envelope structures and compositions. This nonspecific mode of action involving purely physical interactions may explain the strong and broad-spectrum activity of octenidine [15]. Moreover, the rapid bactericidal mechanism of octenidine targets critical membrane properties, reducing the likelihood of bacterial resistance [15,16]. Bacterial resistance to octenidine has not yet been reported. In fact, it was found to effectively destroy preformed biofilms of *S. aureus*, methicillin-resistant *S. aureus*, and vancomycin-resistant *S. aureus* in vitro [15]. The broad-spectrum activity of OOS against periodontal bacterial complexes observed in the present study is consistent with its proposed mechanism of action.

Clinical studies have reported the antiplaque efficacy of octenidine dihydrochloride. A randomized, placebo-controlled, clinical trial of 201 healthy adults demonstrated the efficacy of octenidine mouthrinse in reducing salivary bacterial counts, inhibiting plaque formation over 5 days, and reducing gingival inflammation [10]. In a randomized, placebo-controlled trial of 90 patients with gingivitis, octenidine mouthrinse was found to significantly reduce salivary bacterial counts while exerting low toxicity [11]. In addition, octenidine dihydrochloride has a sustained effect on skin and in the oral cavity [38,39]. A recent systematic review by Grover et al. revealed that rinsing with 0.1% octenidine for 30–60 s inhibited plaque formation by 38.7%–92.9% (within 4 days to 3 months of use), which was either equal to or greater than that by chlorhexidine gluconate (36.4%–68.37%). Furthermore, rinsing with 0.1% octenidine reduces microbial growth by up to 5.3 CFU/ml (log10), with superior efficacy over chlorhexidine gluconate preparations [40]. However, research on the antimicrobial activity of octenidine specifically against periodontopathogens has been limited. The results of the present study demonstrate the microbicidal activity of octenidine against periodontopathogens, indicating its potential application as an adjunct therapy in periodontitis.

As an in-vitro study, this study could not fully replicate the complexities of real-world clinical scenarios or the microbial interactions within the oral environment. Further, the microbial strains utilized were sourced from the oral flora of healthy individuals, potentially limiting the applicability of our findings to patients with gingivitis and periodontitis. Nevertheless, given the broad-spectrum activity inherent to octenidine and its mechanism of action that mitigates antimicrobial resistance, the outcomes of this study hold promise for translation into clinical practice. Future investigations should address these real-world complexities by evaluating the efficacy of octenidine in a microbial environment closely mimicking the natural periodontal ecosystem. Further research could involve use of mixed cultures comprising bacteria and yeast strains obtained from individuals with diverse periodontal statuses.

5. Conclusions

The selected OOS demonstrated in vitro microbicidal activity against common periodontopathogens at 80% dilution concentration, highlighting its potential as an adjunct to mechanical periodontal therapy. Particularly noteworthy is the yeasticidal efficacy of the OOS against *C. auris*, indicating its importance in combating multidrug-resistant yeast infections. These results emphasize the need for additional clinical studies on the application of OOS in the management of periodontal inflammation.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Table S1: Test yeast strains; Table S2: Bactericidal efficacy of the octenidine oromucosal solution* (Data from Laboratory 1); Table S3: Bactericidal efficacy of the octenidine oromucosal solution* (Data from Laboratory 2); Table S4: Yeasticidal efficacy of the octenidine oromucosal solution*

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Conflicts of Interest: A.W. is employed by the Institut für Hygiene und Umwelt, Germany, and he supervised the experimental research. A.W. has no competing interests. K.K., P.B., and N.R. are employees of Schülke & Mayr GmbH, Germany, and contributed to the planning of the experimental research and to literature review during the preparation of the manuscript. They also reviewed the manuscript. K.K., P.B., and N.R. have no competing interests. A.B., K.N., and C.H. are employees of HygCen Germany GmbH, Germany, and they supervised and performed this experimental research. A.B., K.N., and C.H. have no competing interests.

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