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# Activity of Drill<sup>®</sup> lozenges on the main microorganisms responsible for upper respiratory tract infections

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## KEYWORDS

Sore throat;  
Chlorhexidine;  
H1N1 virus;  
Upper respiratory tract infections

## Summary

**Objectives:** The treatment of sore throat, considered to be essentially due to viral infection, does not require the use of antibiotics. The recommended treatment is therefore based on the use of topical anaesthetics and antiseptics. Throat lozenges play a leading role in topical treatment by allowing immediate, massive and persistent release of the active molecule at the site of infection. The objective of this study was to demonstrate the short-term and long-term in vitro antimicrobial efficacy of Drill<sup>®</sup> lozenges in relation to the main pathogens responsible for upper respiratory tract infections.

**Material and method:** The bactericidal and virucidal activity in relation to the main microorganisms responsible for upper respiratory tract infections, including the H1N1 influenza virus, was evaluated after short (5 minutes) and long (3 h) contact times, according to a methodology complying with European standards for the evaluation of chemical antiseptics and disinfectants. In parallel, the global antibacterial activity was determined on 30 strains representative of the resident flora by determination of maximum inhibitory dilutions (MID) and maximum bactericidal dilutions (MBD).

**Results:** Drill<sup>®</sup> lozenges presented an antibacterial activity inducing significant (> 90%) destruction of the main upper respiratory tract pathogens after a 5-minute contact time at high concentration and after a 3-hour contact time after dilution. Drill<sup>®</sup> lozenges also exerted an antiviral activity inducing 2 log (99%) destruction of the H1N1 virus after a 5-min contact time at high concentration, with maintenance of this activity after dilution (3 h). A homogeneous antibacterial activity was observed on the resident flora.

**Conclusion:** These in vitro tests confirm the value of chlorhexidine lozenges in the treatment of upper respiratory tract infections such as sore throat.

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## Introduction

In otorhinolaryngology, the term “sore throat” is considered to be a symptom corresponding to inflammation, fever, pain on swallowing, cough, etc. Many factors can be responsible for sore throat (allergy, smoking, etc.), but the main cause is infection. Sore throat often corresponds to the emergence of pathogenic microorganisms within a complex ecosystem [1–3]. Viral infection is predominant, justifying the current rapid diagnostic test (RDT) guidelines. Treatment of this type of disease still raises a number of problems related to control of the emergence of multiresistant bacteria [4–6], requiring limitation of the use of antibiotics, especially topical antibiotics, and the concept of microbial ecology and preservation of ecosystems. Viral infections generally resolve spontaneously after several days and the recommended treatment is essentially symptomatic to relieve pain. However, untreated bacterial infections may require medical consultation and the prescription of systemic antibiotics [7–9]. In the light of these various elements, the value of a short course (5 days) of topical antiseptics (mouthwash or lozenges) either alone or in combination with topical anaesthetics, needs to be reviewed. Three recent *in vivo* studies have demonstrated the value of antiseptic sprays, especially containing chlorhexidine, as adjuvant treatment for cough and viral or streptococcal sore throat [10–12]. The authors reported a reduction of symptoms and improvement of the patients’ quality of life. However, these studies did not provide any formal conclusions on the antimicrobial and antiviral efficacy of these treatments. The objective of the present study was therefore to complete these studies by *in vitro* evaluation of the efficacy of Drill® lozenges, containing chlorhexidine digluconate (3 mg/lozenge) and tetracaine hydrochloride (0.2 mg/lozenge), in:

short-term and long-term control of the proliferation of pathogenic microorganisms in the upper respiratory tract by determination of specific bactericidal and virucidal activities;

preservation of the upper respiratory tract ecosystem, limiting the risk of emergence of opportunistic microorganisms, by determination of the antimicrobial activity on a large spectrum of microorganisms representative of the upper respiratory tract flora.

## Material and methods

### Product and reagents

Drill® lozenges (batch A03007): 1 lozenge was diluted in 2 mL of diluent (stirring for 1.5 h at 30 °C); artificial saliva: aqueous solution of Biotrypcase (0.25 g/L), yeast extract (0.25 g/L) (Biomérieux, France) autoclaved at 121 °C for 15 min. The following compounds (Sigma Aldrich, France) were dissolved in this solution: NaCl (10.2 mM/L), KCl (10.7 mM/L), MgCl<sub>2</sub>·6H<sub>2</sub>O (0.29 mM/L), CaCl<sub>2</sub>·2H<sub>2</sub>O (1.08 mM/L), KH<sub>2</sub>PO<sub>4</sub> (2.2 mM/L), K<sub>2</sub>HPO<sub>4</sub> (4.59 mM/L), NaHCO<sub>3</sub> (0.25 mM/L). The final solution was filtered on a 0.45 µm membrane (Millipore, USA);

neutralizing solution for determination of bactericidal activity: solution of polysorbate 80 (10%), lecithin (2%), saponin (2%) sodium thiosulphate (0.5%) (Sigma Aldrich) q.s. Trypticase soy broth (Biomérieux).

### Bactericidal activity in relation to the main pathogens responsible for upper respiratory tract infections

The short-term (5 min) and long-term (3 h) bactericidal activities against the main pathogens responsible for upper respiratory tract infections were determined according to the guidelines of NF EN 1040 [13], which defines a logarithmic reduction for a defined contact time. Test product concentrations corresponded to 90%, 50%, 10% and 1% (V/V) of stock solution prepared in artificial saliva to mimic the conditions of use without the effect of the antimicrobial agents present in saliva. Nine millilitres of solution were placed in contact with 1 mL of bacterial suspension ( $2 \times 10^8$  bacteria/mL). Contact times tested at 20 °C were five minutes and three hours ± 10 seconds, depending on the concentration. Concentration/contact time pairs were selected in order to simulate immediate and long-term effects. Microorganism/test product contact was terminated by dilution-neutralization. After five minutes of neutralization, ten fold dilutions were performed and 1 mL of each dilution was inoculated onto agar (surface inoculation in two Petri dishes: *H. influenzae* and *B. catarrhalis*) for determination of the number of colony-forming units (CFU).

Reference strains corresponding to the main bacterial species responsible for upper respiratory tract infections (Institut Pasteur Collection, Paris, France) and culture conditions are indicated in Table 1.

### Virucidal activity in relation to the influenza A virus (H1N1)

The short-term (5 min) and long-term (3 h) virucidal activity against the H1N1 influenza virus was determined according to the indications of NF EN 14476 [14]. The VR-1520 viral strain was obtained from the ATCC (American Type Culture Collection, Molsheim, France) and amplified on MDCK cells (CCL-34, ATCC) in EMEM medium (Sigma Aldrich). Titration was performed on microplates using cell suspensions. The cytopathogenic effect was determined after 48 hours of culture.

Tests were performed under clean conditions (10% PBS: Sigma Aldrich) for concentrations of 80% (possible peak concentration), 50% and 10% (V/V) of stock solution prepared in water for injections (Cooper, France) for contact times of 5 min or 3 h ± 10 s at 20 °C. The action of the product was stopped by gel filtration on Sephadex LH 20 (Dutscher) of the 10<sup>-1</sup> dilution. Assays were performed on untreated cells and cells treated by disinfectant to confirm that subcytotoxic concentrations of the disinfectant did not modify cell infectivity.

**Table 1** Test strains representative of upper respiratory tract pathogens and culture conditions for determination of bactericidal activity.

Test strains	Culture medium	Incubation conditions
<i>Staphylococcus aureus</i> CIP 4.83	Trypticase soy agar <sup>a</sup>	Aerobic, 36 ± 1 °C
<i>Streptococcus pneumoniae</i> CIP 104471	Trypticase soy agar <sup>a</sup> + 5% sheep blood <sup>a</sup>	5% CO <sub>2</sub> , 36 ± 1 °C
<i>Streptococcus pyogenes</i> CIP 5641 T	Trypticase soy agar <sup>a</sup>	Aerobic, 36 ± 1 °C
<i>Fusobacterium nucleatum</i> CIP 101130	Schaedler agar <sup>a</sup>	Anaerobic, 36 ± 1 °C
<i>Haemophilus influenzae</i> CIP 102514 T	Chocolate agar <i>Haemophilus</i> <sup>a</sup>	5% CO <sub>2</sub> , 36 ± 1 °C
<i>Branhamella catarrhalis</i> CIP 73.21 T	Columbia agar + 5% sheep blood <sup>a</sup>	Aerobic, 36 ± 1 °C

<sup>a</sup> Biomérieux, Crapone, France

## Antibacterial activity in relation to the resident flora

To assess the impact of the test product on the upper respiratory tract resident flora, Maximum Inhibitory Dilutions (MID) in relation to representative strains were determined by the micromethod on liquid medium. One hundred microlitres of broth were added to each well of a sterile 96-well microplate. One hundred microlitres of test solutions (stock solution prepared in water for injections) were added to the first well of the row. Twofold serial dilutions were then performed from well 1 to well 10. Test suspensions of each microorganism were prepared extemporaneously ( $2 \times 10^8$  bacteria/mL) and the microplate was then inoculated (Denley multipoint inoculator). After incubation, the MID was defined as the highest dilution with no visible growth. Rows 11 and 12 were used for growth negative control and positive control, respectively. All tests were performed in duplicate.

Maximum Bactericidal Dilutions (MBD) were determined by subculture of MID microplates on agar medium. After incubation, the MBD was defined such as the highest dilution with no visible growth.

Bacterial strains were obtained from the Institut Pasteur Collection (Paris) or human isolates. Culture media and culture conditions are indicated in Table 2.

## Results

### Bactericidal and virucidal activity in relation to the main pathogens responsible for upper respiratory tract infections

The results, expressed as percentage reduction  $[(T - E)/T]$ , are presented in Table 3.

The results obtained in artificial saliva showed percentage reductions greater than 90% after only 5 min of contact with the highest concentration (90%) and greater than 99% for five of the six microorganisms tested when with the 50% solution. The limiting microorganism was *S. aureus*.

When the stock solution was diluted to 50% (simulation of dissolution in the mouth) and after 3 h of contact, an intense bactericidal activity ( $> 4$  log reduction) was observed for the 6 test microorganisms and the bactericidal activity was still detectable in relation to four microorganisms with the 10% solution.

The results obtained on the H1N1 strain indicate reductions greater than 99% ( $> 2$  log) in the presence of 80% and

50% solutions for a contact time of 5 min. A greater than 99.99% reduction ( $> 4$  log) was observed at the 50% concentration with a 3-hour contact time.

The antiviral activity (greater than 99% reduction) was maintained with the 10% solution by increasing the contact time to three hours.

### Antibacterial activity in relation to resident flora

Maximum values of MID and MBD (dilutions of stock solution) were 1/252 and 1/128, respectively, for all microorganisms tested.

These results indicate a significant and homogeneous antibacterial activity on all microorganisms tested, both Gram+ and Gram– species.

## Discussion

Many treatments and recommendations are considered to be effective in the treatment of sore throat and anti-inflammatory and analgesic effects play a considerable role in the treatment of these diseases, whether or not they are due to infection. For example, honey has a recognized efficacy as a systemic antitussive [15] related to its antimicrobial and healing properties. The type of antibacterial activity observed in various in vitro studies corresponds to growth inhibition [16,17] and is therefore lower than the bactericidal effect observed with an antiseptic, although this effect cannot be dissociated from the osmotic and detergent effects attributed to honey.

Several recent clinical studies have tested the value of antiseptic spray solutions or lozenges in the adjuvant treatment of viral and bacterial pharyngitis and sore throat [10–12]. In these studies, chlorhexidine, an antiseptic agent, was associated with an anti-inflammatory/analgesic agent in a spray formulation. Cingi et al. [10,11] conducted placebo-controlled studies in patients treated with penicillin for streptococcal infections or paracetamol for viral pharyngitis. In both studies, the evaluation was based on improvement of the patient's clinical signs and quality of life. After 7 days of treatment, the authors reported a significant improvement of clinical signs in both types of infections and a significant improvement of quality of life in patients with viral infections treated with the spray. The study by Schapowal et al. [12] compared two oral sprays, including one spray containing a combination of chlorhexidine and lidocaine in the treatment of cough. The authors reported a

**Table 2** Test strains representative of upper respiratory tract pathogens and culture conditions for determination of antibacterial activity.

Strains	Medium (MID)	Medium (MBD)	Incubation conditions
<i>Streptococcus milleri</i> Isolate 1	MH + 10% FCS	COS	Anaerobic 36 ± 1 °C
<i>Streptococcus milleri</i> Isolate 2	MH + 10% FCS	COS	Anaerobic 36 ± 1 °C
<i>Streptococcus milleri</i> Isolate 3	MH + 10% FCS	COS	Anaerobic 36 ± 1 °C
<i>Streptococcus mitis</i> CIP 103335 T	MH + 10% FCS	COS	5% CO <sub>2</sub> 36 ± 1 °C
<i>Streptococcus mitis</i> Isolate 1	MH + 10% FCS	COS	5% CO <sub>2</sub> 36 ± 1 °C
<i>Streptococcus mitis</i> Isolate 2	MH + 10% FCS	COS	5% CO <sub>2</sub> 36 ± 1 °C
<i>Streptococcus oralis</i> CIP 102922 T	MH	MH	5% CO <sub>2</sub> 36 ± 1 °C
<i>Streptococcus oralis</i> Isolate 1	MH	MH	5% CO <sub>2</sub> 36 ± 1 °C
<i>Streptococcus salivarius</i> CIP 102503	MH	MH	Aerobic 36 ± 1 °C
<i>Streptococcus salivarius</i> Isolate 1	MH	MH	Aerobic 36 ± 1 °C
<i>Streptococcus sanguinis</i> CIP 55128	MH + 10% FCS	COS	5% CO <sub>2</sub> 36 ± 1 °C
<i>Streptococcus sanguinis</i> Isolate 1	MH + 10% FCS	COS	5% CO <sub>2</sub> 36 ± 1 °C
<i>Streptococcus sanguinis</i> Isolate 2	MH + 10% FCS	COS	5% CO <sub>2</sub> 36 ± 1 °C
<i>Lactobacillus lactis</i> Isolate 1	MH + 10% FCS	COS	Anaerobic 36 ± 1 °C *
<i>Lactobacillus salivarius</i> CIP 103140 T	MH + 10% FCS	COS	Anaerobic 36 ± 1 °C *
<i>Lactobacillus acidophilus</i> CIP 7613 T	MH + 10% FCS	COS	Anaerobic 36 ± 1 °C *
<i>Lactobacillus acidophilus</i> Isolate 1	MH + 10% FCS	COS	Anaerobic 36 ± 1 °C *
<i>Lactobacillus acidophilus</i> Isolate 2	MH + 10% FCS	COS	Anaerobic 36 ± 1 °C *
<i>Moraxella lincolni</i> CIP 103802 T	MH + 10% FCS	COS	Aerobic 30 ± 1 °C *
<i>Staphylococcus epidermidis</i> CIP 6821	MH	MH	Aerobic 36 ± 1 °C
<i>Staphylococcus epidermidis</i> CIP 8155 T	MH	MH	Aerobic 36 ± 1 °C
<i>Staphylococcus epidermidis</i> Isolate 1	MH	MH	Aerobic 36 ± 1 °C
<i>Staphylococcus epidermidis</i> Isolate 2	MH	MH	Aerobic 36 ± 1 °C
<i>Staphylococcus epidermidis</i> Isolate 3	MH	MH	Aerobic 36 ± 1 °C
<i>Staphylococcus epidermidis</i> Isolate 4	MH	MH	Aerobic 36 ± 1 °C
<i>Staphylococcus epidermidis</i> Isolate 5	MH	MH	Aerobic 36 ± 1 °C
<i>Rothia mucilaginosa</i> CIP 7114	MH	MH	Aerobic 36 ± 1 °C *
<i>Corynebacterium imitans</i> CIP 105130	MH + 10% FCS	COS	Aerobic 36 ± 1 °C
<i>Neisseria cinerea</i> CIP 7316 T	MH + 10% FCS	COS	Aerobic 36 ± 1 °C
<i>Haemophilus parainfluenzae</i> CIP 102513	MH + 10% FCS + 1% PV	Ch. H	5% CO <sub>2</sub> 36 ± 1 °C

MH: Muller-Hinton (Biomérieux); FCS: Foetal calf serum (Lonza); COS: Columbia agar + 5% sterile sheep blood (Biomérieux); Ch. H: Chocolate agar *Haemophilus* (Biomérieux); PV: Polyvitex (Biomérieux).

Reading after 24 hours of incubation, except for (\*): reading after 48 hours of incubation.

significant improvement (greater than 50% reduction of all symptoms) in 57.8% of cases (154 patients) after three days of treatment at the dosage of two puffs 10 times daily. None of these studies evaluated the antimicrobial efficacy of the test formulations, which can be a difficult procedure in vivo, especially when based on saliva counts, due to modifications of saliva volume and flow related to the use of sprays or lozenges. The present study was therefore designed to determine the short-term (5 min) and long-term (3 h) in vitro bactericidal and virucidal efficacy of Drill® lozenges in order to confirm the potential value of the combination of an antimicrobial agent and an analgesic in the treatment of upper respiratory tract infections.

Chlorhexidine is a reference antiseptic used on the skin and mucous membranes, especially in the mouth. Recent modifications of legislation and guidelines [4–6] concerning the use of topical antimicrobial treatment now require formal demonstration of the value of chlorhexidine formulations in the treatment of upper respiratory tract infections. This study was conducted in the context of this approach. Evaluation of the bactericidal activity of Drill® on bacteria considered to be pathogenic in the upper respiratory

tract indicated a rapid action of the concentrated product (90% stock solution), even in the presence of artificial saliva that could interfere with the antiseptic activity of chlorhexidine. This activity persisted over time, even after dilution to 10%. The observed bactericidal activity corresponded to published data concerning the spectrum of chlorhexidine that includes Gram+ and Gram– bacteria [18–20]. This homogeneous antibacterial activity was associated with an antiviral activity in relation to the influenza A virus (H1N1), related to the recognized virucidal activity of chlorhexidine on enveloped viruses [21].

A global evaluation of the antibacterial activity in relation to the resident flora of the upper respiratory tract was conducted in support of the indication of Drill® in upper respiratory tract infections such as sore throat. This study confirmed the value of using an antiseptic versus antibiotics, as chlorhexidine is characterized by a non-specific mechanism of action related to a combination of cellular effects (membrane disorganization, coagulation of intracellular proteins, etc.) resulting in a broad antimicrobial spectrum [18–20]. The results of this study indicate a non-selective activity, which therefore limits the risk of

**Table 3** Percentage reduction observed on the main bacteria responsible for upper respiratory tract infections and on the H1N1 influenza virus, according to the dilution of the test product and the contact time (5 min or 3 h).

	5 minutes	3 hours
<i>S. aureus</i>	90% > 90%	—
	50% < 90%	50% > 99.999%
	—	10% < 90%
<i>S. pneumoniae</i>	90% > 99.99%	—
	50% > 99.99%	50% > 99.99%
	—	10% > 99.99%
<i>S. pyogenes</i>	90% > 99.99%	—
	50% > 99.99%	50% > 99.999%
	—	10% < 90%
<i>F. nucleatum</i>	90% > 99.999%	—
	50% > 99.99%	50% > 99.999%
	—	10% > 99.99%
<i>H. influenzae</i>	90% > 99.999%	—
	50% > 99.999%	50% > 99.999%
	—	10% > 99.9%
<i>B. catarrhalis</i>	90% > 99.99%	—
	50% > 99%	50% > 99.999%
	—	10% > 99.999%
H1N1 virus	80% > 99%	—
	50% > 99%	50% > 99.99%
	—	10% > 99%

disturbing the equilibrium of the resident flora. No resistance was observed for any of the strains tested.

## Conclusion

All of these results appear to corroborate current guidelines and emphasize the value of this approach to the treatment of upper respiratory tract infections using lozenges containing a combination of topical antiseptic and an anti-inflammatory/analgesic. This dosage form allows easy management and especially potential maintenance of effective doses at the site of the infection. Complementary in vivo trials, especially comprising treatment follow-up, should be conducted to confirm the efficacy and absence of destabilization of the endogenous flora under conditions of good use.

## Disclosure of interest

Study funded by Laboratoires Pierre Fabre - tests conducted by Fonderephar, a Cofrac-approved laboratory.

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