Oropharyngeal Bacterial Colonization after Chlorhexidine Mouthwash in Mechanically Ventilated Critically Ill Patients

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ABSTRACT

Background: Oropharyngeal care with chlorhexidine to prevent ventilator-associated pneumonia is currently questioned, and exhaustive microbiologic data assessing its efficacy are lacking. The authors therefore aimed to study the effect of chlorhexidine mouthwash on oropharyngeal bacterial growth, to determine chlorhexidine susceptibility of these bacteria, and to measure chlorhexidine salivary concentration after an oropharyngeal care.

Methods: This observational, prospective, single-center study enrolled 30 critically ill patients under mechanical ventilation for over 48 h. Oropharyngeal contamination was assessed by swabbing the gingivobuccal sulcus immediately before applying 0.12% chlorhexidine with soaked swabs, and subsequently at 15, 60, 120, 240, and 360 min after. Bacterial growth and identification were performed, and chlorhexidine minimal inhibitory concentration of recovered pathogens was determined. Saliva was collected in 10 patients, at every timepoint, with an additional timepoint after 30 min, to measure chlorhexidine concentration.

Results: Two hundred fifty bacterial samples were analyzed and identified 48 pathogens including *Streptococci* (27.1%) and Enterobacteriaceae (20.8%). Oropharyngeal contamination before chlorhexidine mouthwash ranged from 10^3 to 10^7 colony-forming units (CFU)/ml in the 30 patients (median contamination level: $2.5{\cdot}10^6$ CFU/ml), and remained between $8{\cdot}10^5$ (lowest) and $3{\cdot}10^6$ CFU/ml (highest count) after chlorhexidine exposure. These bacterial counts did not decrease overtime after chlorhexidine mouthwash (each minute increase in time resulted in a multiplication of bacterial count by a coefficient of 1.001, $P = 0.83$). Viridans group streptococci isolates had the lowest chlorhexidine minimal inhibitory concentration (4 [4 to 8] mg/l); Enterobacteriaceae isolates had the highest ones (32 [16 to 32] mg/l). Chlorhexidine salivary concentration rapidly decreased, reaching 7.6 [1.8 to 31] mg/l as early as 60 min after mouthwash.

Conclusions: Chlorhexidine oropharyngeal care does not seem to reduce bacterial oropharyngeal colonization in critically ill ventilated patients. Variable chlorhexidine minimal inhibitory concentrations along with low chlorhexidine salivary concentrations after mouthwash could explain this ineffectiveness, and thus question the use of chlorhexidine for ventilator-associated pneumonia prevention. **(ANESTHESIOLOGY 2018; 129:1140-8)**

MILLIONS of patients undergo mechanical ventilation in intensive care units throughout the world yearly. Recent estimates suggest that these numbers will only increase.[1] These patients are exposed, among other risks, to the one of ventilator-associated pneumonia, the most frequent life-threatening nosocomial infection.[2,3] Bacterial oropharyngeal colonization is the first recognized step toward tracheal colonization, which subsequently leads to ventilator-associated pneumonia. This has stemmed from many studies evidencing the temporal and microbiologic relationship between oropharyngeal and tracheal colonization and ventilator-associated pneumonia.[4–6]

Editor's Perspective

What We Already Know about This Topic

- Chlorhexidine is frequently used to reduce oropharyngeal bacterial colonization in mechanically ventilated patients. How effective the drug is remains unclear.

What This Article Tells Us That Is New

- Bacterial colonization was evaluated in 30 mechanically ventilated patients before and after application of 0.12% chlorhexidine.
- Chlorhexidine did not reduce colonization and may, therefore, be less effective than previously assumed.

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This universal understanding of the pathophysiology of ventilator-associated pneumonia has formed the basis of oropharyngeal decontamination. Three distinct classes of agents including nonabsorbable antibiotics,[7–10] antiseptics (mainly chlorhexidine),[11–20] and natural antimicrobial peptides[21] have been evaluated in several studies, providing very heterogeneous results. Factors that explain this variability include patient case mix (with a greater efficacy of oropharyngeal decontamination in surgical patients[14,17]), differences in classes of agents, and for each class, parameters such as concentration (for chlorhexidine), frequency and method of administration, and the potential combination with systemic antibiotics (for selective oropharyngeal decontamination). Although oropharyngeal decontamination with antibiotics seems more effective than with antiseptics, the development of antibiotic resistance has limited its widespread use.[8] Hence, in a majority of countries, chlorhexidine is the most commonly used agent,[22,23] and its effect on ventilator-associated pneumonia prevention has been evaluated in many studies.[11–20] Several meta-analyses of these studies have been published with conflicting results. Some recent ones[14,17] indicate that chlorhexidine reduces incidence of nosocomial pneumonia in cardiac surgery patients, but does not in others. This has led some to question the use of chlorhexidine in this patient population.[17] Paradoxically, direct microbiologic assessment of chlorhexidine on oropharyngeal bacterial colonization, at the patient's bedside, is lacking.[24] Thus, we aimed to study chlorhexidine oral care effects on oropharyngeal bacterial microbiota, as well as the susceptibility of oropharyngeal strains to chlorhexidine, and measure residual chlorhexidine salivary concentration in a subset of patients. We hypothesized that oropharyngeal bacterial inoculums might not be affected by chlorhexidine exposure, and that chlorhexidine salivary concentration would rapidly decrease after its administration.

Materials and Methods

Study Design

This observational, single-center study was conducted in a 12-bed university hospital, medicosurgical intensive care unit. Consecutive critically ill patients admitted to the intensive care unit, receiving invasive mechanical ventilation for more than 48 h were included. For technical and organizational reasons, screening was only possible during weekdays. Noninclusion criteria were the following: cervical or mouth surgery in the last 15 days; history of oropharyngeal neoplasm, or of cervical or oropharyngeal radiation therapy; tracheotomy; and age less than 18 yr. In order to be able to detect a significant decrease in bacterial growth, patients whose samples retrieved less than 10^3 CFU/ml bacteria before chlorhexidine care were secondarily excluded, as were those who had two or more missing microbiologic samples. Demographic and clinical data were recorded.

Chlorhexidine Oral Care

All patients under invasive mechanical ventilation had protocol-driven full oral care with 0.12% chlorhexidine solution (Paroex, chlorhexidine digluconate 0.12%; Lichtenheldt GMBH, Germany) every 6 h.[15,25] The procedure included a first oropharyngeal swab (dry swab; DaklaPack, the Netherlands) in the lower gingivobuccal sulcus, to detect and quantify initial bacterial inoculum. This first swab was always performed after the night shift's last oral care and just before the day shift performed its first oral care so as to assess the maximal level of bacterial colonization. Once the swabbing was completed and immediately delivered to the microbiology laboratory for analysis, the oral care consisted of applying 15 ml chlorhexidine with soaked compresses on the teeth, gums, gingival mucosa, palate, and tongue, with a movement from back to front. No rinsing of the mouth was performed after the oral care.

Subsequent swabs were sampled immediately, and at 15, 60, 120, 240, and 360 min after the oral care. For the last 10 patients, 0.5 ml of saliva was collected with a syringe in the lower gingivobuccal sulcus, at 15, 30, 60, 120, 240, and 360 min after the oral care. These samples were then stored at –20°C in conical centrifuge tubes (Nunc; Thermo Scientific, France) for subsequent chlorhexidine-concentration measurement. Every single oral care was reported on the daily patient chart.

Microbiologic Study

Upon reception, swabs were discharged into 0.5 ml of sterile water. Then, samples were diluted to 1/1,000, and 100 µl of this dilution was plated with a rake onto three agar plates: chromogenic agar (UriSelect; Biorad, France), Drigalski agar (Sigma-Aldrich, France), and chocolate agar + PolyViteX (BioMérieux, France). All plates were incubated aerobically at 37°C, with an additional 5% CO_2 for the chocolate agar. After 24 h of incubation, the total bacterial count of a sample was counted from the nonselective chocolate agar plate. Quantification of Gram-negative and Gram-positive bacteria was also performed from the three agar plates. Only the two dominant pathogens were stored at –80°C in glycerol media.

Chlorhexidine minimal inhibitory concentrations of dominant pathogens of each patient were determined using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (Wayne, Pennsylvania).[26] Strains were cultured in 10 ml of brain heart infusion broth (Sigma-Aldrich, France) in conical centrifuge tubes (Nunc;

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Thermo Scientific, France), and incubated for 18 h at 37°C under agitation (200 rotations per min). *Streptococcus* and *Haemophilus* strains were cultured using Haemophilus Test Medium supplement (Oxoid S.A., France) in a carbon dioxide humidified incubator. After 18 h incubation, each culture was diluted to 1/1,000 in blood heart infusion broth (with addition of Haemophilus Test Medium supplement for *Streptococcus* and *Haemophilus* strains). Then, 90 µl of each diluted culture was added to 10 µl of chlorhexidine solution, at different concentrations (0.25 to 256 mg/l), in 96-well microplates (Corning Inc., USA). The microplates were incubated at 37°C aerobically (in a carbon dioxide humidified incubator for *Streptococcus* strains). Minimal inhibitory concentration was read at 24 h. The experiment was repeated three times.

Chlorhexidine Salivary Concentration Study

We determined salivary chlorhexidine concentration for the last 10 patients (February to April 2016). The samples were analyzed by high-pressure liquid chromatography.[27] Sputasol (Oxoid S.A.) was used at the extraction phase to optimize the saliva fluidization: 100 µl of Sputasol was added to 200 µl of saliva. Then, 300 µl of 4.5 M sodium hydroxide and 400 µl of acetonitrile were added. The obtained sample was vortex-mixed and centrifuged for 1 min at 14,000 rpm. Then, 200 µl of the organic phase was transferred into a dry tube and mixed with 370 µl of the mobile phase buffer component. A 20-µl aliquot was injected into the high-pressure liquid chromatography system. A Nova-Pak C18 column (4 µm, 3.9 mm × 150 mm; Waters, France) was used, with a flow rate of 0.8 ml/min. Chlorhexidine was detected at 260 nm. The chromatographic chain was piloted and the peaks determined using the Empower 2 software (Waters). Calibration range and quality controls were prepared in saliva (Saliva, Artificial Oral Fluid, OraFlex; LGC, England). The range was between 0.5 and 50 mg/l (0.5, 1, 2, 5, 10, 20, 30, 50). Any sample concentration greater than the range was diluted in order to allow for chlorhexidine concentration measurement.

Ethics

The Ethics Committee of the French Intensive Care Society (Paris, France) approved the study (n°13-41). Informed consent was not requested due to the purely observational design of our study leading to a waiver of informed consent. Patients and/or family were, however, informed of the study, its purpose and objectives. The study was registered at clinicaltrial.gov (NCT03290105).

Statistical Analysis

The prespecified and *a priori* defined primary outcome was the reduction in total colony-forming units (CFU) over time after chlorhexidine exposure. An *a priori* effect size was difficult to define due to lack of sufficiently precise previous data in the literature on which to base the calculation. We had no same or largely overlapping data sets previously examined for similar outcome measures by our group. Descriptive statistics were analyzed with GraphPad Prism 7 (GraphPad Software, USA), and the mixed model analysis was carried out using SAS version 9.3 (SAS Institute, USA). Results are presented as the median and range for quantitative variables, or frequency and proportion for categorical variables. We investigated temporal changes in total colony-forming unit per milliliter values, using a linear mixed model to take into account that multiple samples came from individual patients.[28] As colony-forming unit per milliliter data were not normally distributed, they were transformed using the natural logarithmic transformation model. A model with time (baseline, 15, 30, 60, 120, 240, and 360 min), as the repeated-measures factor was constructed. Subjects' identification was included as a random effect to account for the variability due to individual differences between subjects. The interaction of time with (1) mono- or polymicrobial status and (2) isolates' genus was also assessed to test whether time courses of the CFU differed between mono- and polymicrobial samples and between types of isolates, respectively. We selected the unstructured covariance based on the Akaike information criterion. Normality and homoscedasticity of the residuals were examined using graphical methods. Secondary outcomes were the microbiologic analysis of patients' oropharyngeal colonization, minimal inhibitory concentrations of oropharyngeal bacteria to chlorhexidine, and chlorhexidine salivary concentration. Hypothesis testing was two-tailed. There was no *post hoc* testing. A P value < 0.05 was considered statistically significant.

Results

Patients

One hundred sixty-eight consecutive patients were admitted to our intensive care unit during the 16-week study period (January to March 2014, and February to April 2016; see patient flow chart, fig. 1 in the Supplemental Digital Content, http://links.lww.com/ALN/B787). Of these, 44 were

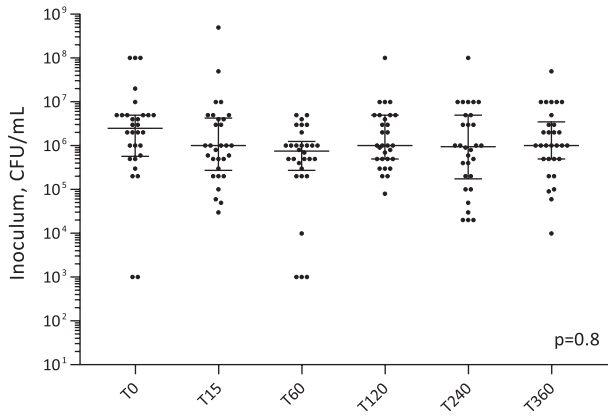

Fig. 1. Evolution of the total inoculum of oropharyngeal isolates, for each patient, at the different timepoints. Results are expressed as means and range. There was no significant change in the total inoculum of oropharyngeal isolates over time. CFU, colony-forming units; T, timepoints, followed by the elapsed minutes since the beginning of the oropharyngeal care.

Table 1. Patient Characteristics

Characteristics	Total, n = 30
Age (yr)	63 [52–71]
Male sex, n (%)	23 (76.7)
Comorbid conditions, n (%)	
Neoplastic disease	4 (13.3)
Cirrhosis	2 (6.7)
Chronic kidney disease	4 (13.3)
Dialysis	1 (3.3)
COPD	6 (20)
HIV	2 (6.7)
Chronic heart failure	7 (23.3)
Chronic alcohol consumption	9 (30)
Reason for ICU admission, n (%)	
Acute respiratory failure	15 (50)
Coma	6 (20)
Septic shock	7 (23.3)
Cardiogenic shock	2 (6.7)
SAPSII	52 [45–73]
Ongoing exposure to antibiotic therapy, n (%)	26 (86.7)
Amoxicillin, n	5
Amoxicillin-clavulanate, n	3
Piperacillin, n	2
Piperacillin-tazobactam, n	2
Third generation cephalosporin, n	9
Azole, n	3
Aminoglycoside, n	6
Carbapenem, n	3
Median time between intubation and inclusion, days	4 [3–7]
Median duration of ventilation, days	11 [8–20]
Median length of ICU stay, days	12 [9–23]
ICU mortality, n (%)	5 (16.7)

Data are presented as n (%) or median [interquartile range], unless otherwise stated.

COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; ICU, intensive care unit; SAPSII, Simplified Acute Physiology Score II.

ventilated for more than 48 h, and 34 patients were included. Four patients had at least one exclusion criterion. Characteristics of the remaining 30 patients are displayed in table 1. Median age was 63 yr [52 to 71], with a median Simplified Acute Physiology Score II of 52 [45 to 73]. Twenty-six patients (86.7%) had antibiotics at time of inclusion. These mainly included a third-generation cephalosporin (nine patients), or amoxicillin (either alone [five patients], or in combination with clavulanic acid [three patients]). Eight patients ultimately developed ventilator-associated pneumonia (including five with diverse Enterobacteriaceae and three with *Pseudomonas aeruginosa* pneumonia). For each patient, bacteria responsible for ventilator-associated pneumonia were those documented in the oropharyngeal samples.

Microbiology of Oropharyngeal Colonization

Two hundred fifty samples were collected from the 30 patients. Forty-eight oropharyngeal isolates were identified. These were mainly streptococci (27.1%) and Enterobacteriaceae (20.8%; table 2). Twelve oropharyngeal samples were monomicrobial

Table 2. Oropharyngeal Isolates Characteristics

Oropharyngeal Isolates	Isolates, n = *48*	CHX MIC (mg/l)
Viridans group streptococci	13 (27.1)	4 [4–8]
Staphylococci	8 (16.7)	24 [14–32]
Staphylococcus haemolyticus	7	32 [12–32]
Staphylococcus aureus	1	16
Enterococci	8 (16.7)	16 [7–20]
Enterococcus faecalis	7	16 [12–24]
Enterococcoccus faecium	1	4
Enterobacteriaceae	10 (20.8)	32 [16–32]
Escherichia coli	4	24 [12–32]
Enterobacter cloacae	2	16 [16-16]
Proteus mirabilis	2	48 [40–56]
Proteus vulgaris	1	32
Hafnia alvei	1	32
Nonfermenting Gram-negative pathogens	7 (14.6)	16 [12–24]
Pseudomonas aeruginosa	6	16 [10–16]
Achromobacter xylosoxidans	1	32
Haemophilus influenzae	1 (2.1)	16
Branhamella catarrhalis	1 (2.1)	16

Data regarding isolates are presented as n (%) and data regarding CHX MIC as median [interquartile range].

CHX, chlorhexidine; MIC, minimal inhibitory concentration.

(six viridans group streptococci, three *P. aeruginosa*, one *Staphylococcus haemolyticus*, one *Escherichia coli*, one *Achromobacter xylosoxidans*), and 18 were polymicrobial.

Changes over Time of Oropharyngeal Bacterial Growth before and after Chlorhexidine Exposure

There were no significant differences in bacterial inoculum per patient over time (fig. 1). Indeed, bacterial counts before chlorhexidine mouthwash did not decrease over time (each minute increase in time resulted in a multiplication of bacterial count by a coefficient of 1.001, $P = 0.83$). Median count before chlorhexidine exposure was $2.5 \cdot 10^6$ CFU/ml and remained between $8 \cdot 10^5$ and $3 \cdot 10^6$ CFU/ml after chlorhexidine exposure (figs. 1 and 2 in the Supplemental Digital Content, http://links.lww.com/ALN/B787). The median inoculum of the 12 monomicrobial samples ($2 \cdot 10^6$ to $5 \cdot 10^5$ CFU/ml, with a nadir of $4 \cdot 10^5$ CFU/ml 240 min after oral care) showed no significant variations *versus* that of the 18 polymicrobial ones ($2 \cdot 10^6$ to $1 \cdot 10^6$ CFU/ml with a nadir of $1 \cdot 10^6$ CFU/ml 60 min after oral care, $P = 0.7$). No significant changes in bacterial growth were observed for any of the different genera of isolated strains (fig. 2). Regarding the species or strains for which oral care led to an initial bacterial count (albeit statistically nonsignificant) decrease, this decrease did not exceed one log, and bacterial regrowth was observed very rapidly afterward.

Minimal Inhibitory Concentrations of Oropharyngeal Bacterial to Chlorhexidine

Enterobacteriaceae had the highest chlorhexidine minimal inhibitory concentration (32 [16 to 32] mg/l, table 2). Of note, even the bacteria exhibiting the lowest minimal

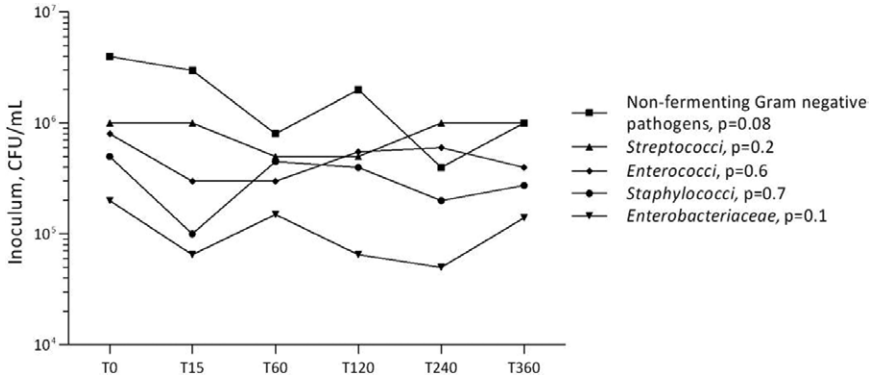

Fig. 2. Bacterial growth of the different genus of isolated strains, at the different timepoints. Timepoints are expressed as T, followed by the elapsed minutes since the beginning of the oropharyngeal care. Results are expressed as means and range. There was no significant change in bacterial growth of the different genera of isolated strains over time. CFU, colony-forming units.

inhibitory concentration to chlorhexidine (4 [4 to 8] mg/l) were not affected by chlorhexidine exposure: inoculum of viridans group streptococci isolates varied from $1{\cdot}10^6$ to $5{\cdot}10^5$ CFU/ml at the minimum and again reached $1{\cdot}10^6$ CFU/ml 240 min after the oral care.

Chlorhexidine Salivary Concentration

For the 10 patients whose salivary chlorhexidine concentration were measured, the median salivary chlorhexidine concentration reached a maximum of 47 [19 to 61] mg/l, 15 min after administration, and then dropped to 7.6 [1.8 to 31] mg/l as early as 60 min after the oropharyngeal chlorhexidine care. It gradually decreased thereafter, reaching 2.95 mg/l 360 min after the mouth rinse (fig. 3, $P < 0.0001$). This was associated with the persistence of a strong oropharyngeal bacterial inoculum, between 10^5 and 10^7 CFU/ml.

Discussion

Although a few studies have dealt with chlorhexidine effect on oropharyngeal colonization,[29–34] this study included an evaluation of the kinetics of oropharyngeal bacterial colonization minutes and hours following chlorhexidine administration, and measuring chlorhexidine oral concentration in critically ill patients. More precisely, most of the studies dealing with chlorhexidine effect on oropharyngeal colonization did not quantify oropharyngeal inoculums,[30,33,34] or controlled them only once, several hours or days after the oral care.[29,31,32]

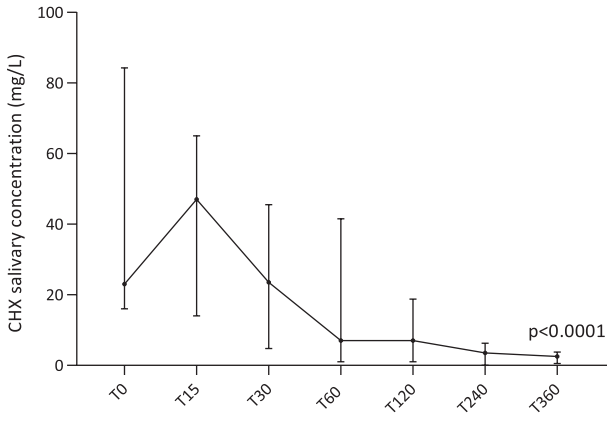

Fig. 3. Chlorhexidine (CHX) salivary concentration at the different timepoints. Timepoints are expressed as T, followed by the elapsed minutes after the oropharyngeal care. Results are expressed as means and range. There was a significant change in chlorhexidine over time.

Results can be summarized as follows: (1) there was no significant change in median bacterial counts after a standard 0.12% chlorhexidine oropharyngeal care; (2) this result was found irrespective of the bacterial genus involved; (3) even strains with a low minimal inhibitory concentration to chlorhexidine, such as viridans group streptococci, were not affected by 0.12% chlorhexidine; and (4) the chlorhexidine salivary concentration rapidly decreased after its administration during oropharyngeal care. Taken together, these results suggest that 0.12% chlorhexidine may have almost no efficacy *in vivo* on oropharyngeal colonization. These results question the use of chlorhexidine to prevent ventilator-associated pneumonia and provide some explanation for the negative results of chlorhexidine on ventilator-associated pneumonia prevention.[14,17]

Chlorhexidine oral care is widely used to prevent ventilator-associated pneumonia.[22] Yet meta-analyses have yielded discordant results on its effectiveness.[11–20] The major problem of these analyses is that studies included heterogeneous categories of patients and very heterogeneous practices in terms of frequencies of oral care, chlorhexidine concentrations (from 0.12 to 2%), and modes of antiseptic administration (mouthwash, dental paste, swabbing of the mucous membranes), that together question the reliability of the findings. Moreover, chlorhexidine seems to be effective to prevent nosocomial pneumonia only among cardiac surgery patients.[17] In addition, the vast majority of patients included in the three studies after cardiac surgery were intubated less than 48 h. Therefore, one cannot make conclusions about the long-term effect of chlorhexidine in patients ventilated for longer periods. Interestingly, the analysis of

the 13 studies focusing on medical patients only did not find any effect of chlorhexidine on prevention of ventilator-associated pneumonia.[17] This suggests that the positive effect of chlorhexidine reported by some studies is biased by the short duration of ventilation. Importantly, a nonsignificant trend toward an increased mortality in patients randomized to chlorhexidine use was noted (risk ratio, 1.13 [95% CI, 0.99 to 1.28]).[17] Another meta-analysis reported a significant increased mortality in patients randomized to chlorhexidine use (odds ratio, 1.25 [95% CI, 1.05 to 1.50]), possibly related to microaspirations of small amounts of chlorhexidine, leading to acute lung injury.[16] Finally, a very recent study also found that exposure to chlorhexidine oral care was associated with increased risk of death (odds ratio, 2.61 [95% CI, 2.32 to 2.92]).[35] Thus, the use of chlorhexidine remains debated, with some societies having withdrawn chlorhexidine use from their recommendations,[36–38] while others have funded a large international multicenter study to evaluate the benefits of chlorhexidine 2% oral care.[39]

Surprisingly, chlorhexidine has been broadly used for decades in the intensive care unit without prior evaluation of its antibacterial efficacy and its persistence in significant concentrations in the oropharynx of critically ill patients. Most of the only available data can be found for odontological outpatients,[40] who are obviously very different from mechanically ventilated intensive care unit patients.[41] Our results clearly indicate the persistence of a high oropharyngeal bacterial inoculum in intubated patients, despite well-conducted chlorhexidine oral care. This raises the question: Why could chlorhexidine be ineffective? Reported minimal inhibitory concentration levels of chlorhexidine for Enterobacteriaceae and staphylococci were respectively around 4 and 1 to 2 mg/l.[42,43] These figures are considerably lower than those measured in our study (respectively, 32 [16 to 32] and 24 [14 to 32] mg/l). Two non–mutually exclusive explanations may be brought forward for our observations: decreased bacterial susceptibility to chlorhexidine and insufficient concentrations at the site of interest. At the individual level, oropharyngeal isolates, repetitively exposed to chlorhexidine, develop resistance to chlorhexidine. This phenomenon has been suggested to occur at least *in vitro*: Kitagawa *et al.* described an increase of *Enterococcus faecalis* chlorhexidine minimal inhibitory concentration after repeated exposure to chlorhexidine, due to a change in protein expression profiles.[44] After repeated passages in media containing increasing chlorhexidine concentrations, Braoudaki and Hilton observed an increase of *E. coli* O157's minimal inhibitory concentration from 4 to 512 μg/ml.[45] At the population level, one may hypothesize that over the years, *E. coli*'s susceptibility to chlorhexidine has changed, with bacteria becoming more resistant. We indeed have recently described very different chlorhexidine susceptibility patterns in *E. coli* isolates responsible for pneumonia in ventilated patients.[46] Decreasing chlorhexidine susceptibility has also been described for *Staphylococcus aureus* isolates, after an increase in the use of chlorhexidine in oncology and cardiac surgery pediatric patients between 2001 and 2011.[47,48]

The conflicting results on chlorhexidine efficacy reported in the different meta-analyses obviously question the salivary availability of chlorhexidine. Surprisingly, we found no data reporting values for chlorhexidine salivary concentrations in critically ill ventilated patients. To address this point, we measured salivary chlorhexidine concentration in the last 10 patients. The reason chlorhexidine concentrations were not measured in all patients relates to the delay in establishing the appropriate high-pressure liquid chromatography setup. Our measurements are consistent with those performed in healthy volunteers and nonventilated patients, which reported very low chlorhexidine concentrations early after chlorhexidine oral care.[49,50] Our results indicate a very rapid drop in chlorhexidine salivary concentration as early as 60 min after the oral care (reaching a low of 7.6 mg/l), which is lower than most of the bacteria minimal inhibitory concentration we found. A possible explanation for the rapid decrease could be chlorhexidine absorption to mucin, and partly to albumin in saliva.[51] Hence, a too rapid a drop in chlorhexidine oropharyngeal concentrations may explain its antimicrobial ineffectiveness. Moreover, as previously discussed, subinhibitory chlorhexidine concentrations may contribute to the development of chlorhexidine resistance in oropharyngeal pathogens.

We recognize our study has a few limitations. First, it was not controlled. However, the main objective was to assess chlorhexidine oral care effects on oropharyngeal bacterial microbiota, and to try to unravel its reported ineffectiveness rather than to compare it to another agent. Second, the number of included patients could be regarded as small, in a single-center study. Thus, results might not be generalizable, reflecting the habits and bacterial ecology of this intensive care unit. The results do, however, represent 250 bacterial samples that consistently showed high persistent oropharyngeal bacterial inoculum, despite well-conducted chlorhexidine oral care. It is thus highly unlikely that a larger number of patients would have yielded very different results. We deliberately chose to include only those patients ventilated for more than 48 h because we wished to assess chlorhexidine efficacy in established oropharyngeal colonization. Whether or not oral care with chlorhexidine prevents oropharyngeal colonization from occurring was not directly assessed in the present study. It could be hypothesized that initial bacterial inoculums were much higher than those we measured, and that chlorhexidine just maintained the level of bacteria. However, all our patients received chlorhexidine oral care from the beginning of their intensive care unit admission. The fact that all 30 patients had a very high level of oropharyngeal colonization by day 3 suggests that chlorhexidine was indeed not able to prevent colonization from occurring. Moreover, had chlorhexidine been effective, one

would have expected significant changes in bacterial levels, in parallel with variations in chlorhexidine concentrations. We believe that the stability of bacterial counts suggests that chlorhexidine exposure was ineffective. Third, we used 0.12% chlorhexidine, which is not the highest chlorhexidine concentration available, but the highest in France at the time of the study.[15,17] One might conclude that our results might not be generalizable for other chlorhexidine concentrations. However, stronger solutions of chlorhexidine are not available worldwide, and they are known to be poorly tolerated, causing oral mucosa lesions, because of cytotoxicity.[39,52,53]

Providing exhaustive, longitudinal, fully quantitative (and not semiquantitative as in most studies) bacterial cultures in parallel with assays of chlorhexidine salivary concentration is a definite strength of our study. What alternatives can be proposed to clinicians that would envisage abandoning chlorhexidine? Unfortunately, evidence regarding the efficacy of existing alternatives to chlorhexidine mouth rinse is insufficient.[20] Hence, new approaches need to be developed. We have recently shown that proanthocyanidins extracted from cranberry had the ability to decrease bacterial adhesion to fresh human buccal epithelial cells and that in an animal model, they decreased the virulence of pathogens responsible for ventilator-associated pneumonia.[54] They may be an interesting alternative that obviously requires clinical demonstration of their potential benefit.

Conclusions

To summarize, we showed that, despite its broad use, 0.12% chlorhexidine has almost no effect on oropharyngeal bacterial microbiota in patients requiring invasive mechanical ventilation for more than 48 h, even on strains exhibiting low minimal inhibitory concentrations. High oropharyngeal bacterial inoculums persist, and chlorhexidine salivary concentration rapidly decreases below bacteria minimal inhibitory concentrations to chlorhexidine. These results may partly explain why ventilator-associated pneumonia rates remain above 10 to 15% in intensive care unit patients, despite application of dedicated bundles.[25,55] Given the number of patients that routinely receive oral care with chlorhexidine, our results have major and immediate clinical and economical repercussions since they directly question the pertinence of using chlorhexidine in this indication, and provide some explanations for the divergent results of studies on ventilator-associated pneumonia prevention with chlorhexidine.

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Competing Interests

The authors declare no competing interests.

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