Review Article

Oropharyngeal colonization: epidemiology, treatment and ventilator-associated pneumonia prevention

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Abstract: Oropharyngeal (OP) colonization and ventilator-associated pneumonia (VAP) mechanisms are tightly linked. A significant within-population variation in OP colonization has been described, with its composition being dependent from patients' severity. For instance, healthy subjects have a very low rate in Gram-negative bacteria (GNB) colonization, while its rate rises in comorbid patients, reaching high proportions in ICU patients. Various factors can be put forward to explain the modifications of hospital acquired OP. ICU patients might suffer from underlying diseases; the gastric reflux induced by the presence of nasogastric tubes and the patients' position influences OP colonization; salivary composition might influence OP content, as it modulates bacterial adhesion and induces reversible bacterial changes enhancing bacterial binding. The transition from OP colonization to VAP has been shown in numerous studies, with the digestive tract acting as a filter, or as a reservoir. Some therapies have been investigated to modulate OP colonization, in order to reduce the risk for VAP. Among those, mammalian antimicrobial peptides have been shown effective in reducing GNB colonization in healthy subjects, but failed in preventing VAP in ICU patients. The widely used chlorhexidine was tested in numerous trials. Data on its efficacy are conflicting, and meta-analyses yield discordant results. Above all, several drawbacks have aroused: a poor tolerance of concentrated solutions; an increased risk of death in the less severe patients; and a reduced susceptibility towards chlorhexidine of number of VAP pathogens. Proanthocyanidins, used to prevent *Escherichia coli* adhesion to the urothelium, have been tested in mice model of pneumonia with interesting results. Some complementary data are needed before moving to clinical research. Future research paths should include a reappraisal of OP colonization; finding better formulations for chlorhexidine; define the best populations to target oral decontamination and developing other strategies to prevent and treat OP colonization.

Keywords: Oropharyngeal colonization; ventilator-associated pneumonia (VAP); chlorhexidine

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Within-population variations of the oropharyngeal (OP) colonization

Fifty years ago, one of the first studies on OP colonization assessed its composition in 5 different populations (1). OP samples were studied in healthy subjects, and in 4 types of in-hospital patients with varying degrees of illness severity. In this study, Johanson *et al.* showed that the frequency of Gram-negative bacteria (GNB) colonization was linked to patients' illness severity (1). To date, results from this study stand and have not been replicated with the same completeness.

OP colonization in healthy subjects

Normal bacterial flora of the oropharynx comprises mainly anaerobes bacteria and α-haemolytic streptococci (*Streptococcus viridans and S. mitis*) (2,3). Studies have shown that the relative distribution of species may vary within the oral cavity and within individuals (2). Recent studies have also shown a clear difference in the composition between healthy subjects and individuals with periodontal diseases (4). Some pathogenic bacteria can be retrieved, such as *Staphylococcus aureus*, *S. pyogenes*, *S. pneumoniae*, *Branhamella catarrhalis*, and *Neisseria sp*. (2,3,5). Across time, various studies focused on GNB OP colonization in healthy subjects, that yielded results very similar to those initially reported (1,6,7). Johanson *et al.* (1) specifically reported GNB colonization. Healthy subjects were 82 Dallas firemen, and 47 healthcare providers who had not been exposed to antibiotics in the previous 15 days. Interestingly, when a single OP sampling was performed in these subjects, only 2% had GNB colonization. When multiple samples were performed, at least one was positive for GNB in 6% of cases. More recently, 101 US healthy soldiers were sampled at three different sites (7) (nares, oropharynx, and groin). Again, GNB OP colonization was noted in only four patients (4%). These consistent results contrast with those from another study that included 120 healthy subjects (6) and reported a much higher rate: 35.8% of the subjects had at least one sample with GNB colonization. However, the rate of GNB carriage on two consecutive samples was 6.6%. Noticeably, 2/3 of the subjects were healthcare workers (40 nurses and 40 laboratory-associated persons), which may explain the higher rate of GNB colonization in this population.

The composition of OP colonization is dependent of patients' severity of illness

Various groups of patients have been surveyed for their OP colonization, alongside healthy adults. When comparing patients from various wards Johanson *et al.* (1) evidenced a similar rate of GNB OP colonization in psychiatry patients, and in healthy subjects (6% for those who had repeated OP sampling), it reached 16% in surgical patients when they were sampled only once (and 35% when repeatedly sampled), and 57% in moribund patients (and 73% when repeatedly sampled). In a similar manner, Mackowiak *et al.* (8) were interested in studying out-patient subjects considered to be "at-risk" for aspiration. Heavy-alcohol drinkers and diabetic had close GNB colonization rates (respectively 35% and 36% of GNB colonization on a single sample). Their rate of GNB colonization rate was higher than those from epileptic patients (17%), drug-addicts (20%) or healthy subjects (18%). Other types of patients have been surveyed for their GNB OP colonization. Patients with chronic obstructive pulmonary disease (COPD) (6,9) have been shown to harbour a higher rate of GNB colonization than healthy subjects (9); and the greater the severity of their disease, the higher the prevalence. Likewise, elderly patients have been shown to have a high rate of GNB OP colonization (10,11), ranging from 20.5% to 43%. Even though these studies had similar ranges of ages, other variables were not comparable, as their dependency level or their comorbid conditions which were not equivalent.

Interesting data stem from Filius *et al.* study (12). In this prospective epidemiological survey, the authors screened the OP (and the digestive) colonization of 200 ICU and 319 general wards patients at admission, discharge from ICU and hospital, and at 1 and 3 months after discharge. If the GNB OP colonization rate was extremely low at general ward admission (1.1%), it significantly increased during hospital stay, to 12.4%, and remained high at 1 and 3 months of hospital discharge (respectively 19.4% and 20.3%). This study confirms that general wards patients are less prone to have GNB OP colonization at admission, while it colonizes the OP during hospital stay and persists after discharge. Data on ICU patients will be discussed later.

OP colonization in ICU patients

Several studies (see *Table 1*) assessed OP colonization [or dental plaque, which composition is close (3)] in ICU patients, mostly in its relationship with ventilator-associated pneumonia. Since the study of Johanson *et al.* (1), in which the most severe patients were "moribund" but not necessarily hospitalized in the ICU, OP colonization incidence with pathogenic bacteria is reported to range from 23% and 96% at ICU admission. Between 36% and 100% of ICU admitted patients acquire OP colonization during their stay (13-20). GNB represent an important part of these species (32–41%) (14,19), Enterobacteriaceae being the predominant type (20% of the samples) (15) [*Klebsiella pneumoniae*, 13–18% (13,15,16); *Citrobacter sp*. 23% (16)], followed by non-fermenting GNB (*Pseudomonas aeruginosa*, 11–32%) (13,15-17,19). But Gram-positive

Table 1 Oropharyngeal colonization in ICU patients

Study	Type of patients	Sampling technique	Proportion of patients with GNB oropharyngeal colonization	Composition of OP colonization
Johanson *et al.*, *Ann Intern Med* 1972 (13)	213 ICU patients	Posterior oropharynx gelatin sampling or swab	37% of the sampled patients, at any time during ICU stay	*Klebsiella* 22.1%; *Pseudomonas* 14.1%; *Proteus* 5.6%; *E. coli* 11.3%; *Enterobacter* 16.4%
Bonten *et al.*, *Am J Respir Crit Care Med* 1996 (14)	141 adult ventilated ICU patients	Oropharyngeal swab	36.6% enteric GNB colonization at ICU admission; 41% enteric GNB acquired colonization during ICU stay; 13% colonized with *Pseudomonas* at ICU admission; 32% of acquired colonization with *Pseudomonas*	–
Garrouste-Orgeas *et al.*, *Am J Respir Crit Care Med* 1997 (15)	86 ventilated adult ICU patients	Oropharyngeal swab	54.3% with bacterial colonization at ICU admission (Gram positive and Gram negative); 45.3% with bacterial acquisition during ICU stay (Gram positive and Gram negative)	*A. baumannii* 9.3%; *K. pneumoniae* ESBL 17.4%; Enterobacteriaceae 19.8%; Pseudomonadaceae 10.5%
Donaldson *et al.*, *Am Rev Respir Dis* 1991 (16)	34 adult ventilated Medical and Cardiac ICU patients	Posterior tonsillar fossa swab	62% of the included patients	Not detailed
Ewig *et al.*, *Am J Respir Crit Care Med* 1999 (17)	48 neuro ICU patients	Nasal or pharyngeal swab	21.7% at ICU admission; 90.7% during ICU stay	At ICU admission: *Haemophilus influenza* 4.3%; GN enteric bacilli 12.5%; *Pseudomonas* aeruginosa 4.3%;
				During ICU stay: *Haemophilus influenza* 7%; GN enteric bacilli 48.8%; *Pseudomonas aeruginosa* 34.9%
Sole *et al.*, *Am J Crit Care* 2002 (18)	18 intubated ICU patients	Oral secretions	GNB colonization in 44% of patients	*Klebsiella* 11%; *Acinetobacter* 11%; *Pseudomonas* 17%; *Proteus* 11%; *E. coli* 6%; *Enterobacter* 6%
Filius *et al.*, *Antimicrob Agents Chemother* 2005 (12)	183 surgical and neurosurgical ICU patients	Oropharyngeal swabs	Admission: 18.8% of GNB colonized patients	*E. coli*: Admission 43.1%; discharge from ICU 26.9%; discharge from hospital 13%
			During ICU stay: 43% of GNB colonized patients	*Pseudomonas aeruginosa*: admission 6%; discharge from ICU 13.2%
			At ICU discharge: 27% of GNB colonized patients; at hospital discharge	*Serratia marcescens*: discharge from ICU 1%; discharge from hospital 14.6%

ICU, intensive care unit; GNB, Gram-negative bacteria; OP, oropharyngeal; GN, Gram negative.

cocci also plays an important part in OP colonization, with *Staphylococcus aureus* reported in 15% to 78% (15,18-20), or *Streptococcus sp*. in 44% (18). The dynamics of GNB OP colonization are studied by Filius *et al.* (12). The authors report an increasing GNB OP colonization rate during ICU hospitalization (from 18% to 27%), and, again, a persistence of this colonization was evidenced at 1 and 3 months after discharge. This elegant study shows a significant decrease in *E. coli* OP colonization during ICU stay (from 43.1% at admission, to 26.9% at discharge), with a novel ascend after one month to 28.3%; and a significant increase in *P. aeruginosa* from 6% to 13.2% that persisted after hospital discharge.

These wide ranges of incidence result from heterogeneity among these studies:

- First, all these data were obtained using different sampling methods and sites: either the dental plaque (20), or OP swabbing (12-16), both (19), or sampling of salivary secretions (18). All these sites have been considered to be similar in a recent study of OP colonization sampling in five healthy subjects (3), but whether this equivalence is also true in ICU patients with GNB OP colonization has not been assessed;
- Next, one has to bear in mind that OP hygiene might not have been similar in all the patients included in these studies. We reported some years ago in a European survey that only 48% of surveyed ICU European caregivers reported using oral chlorhexidine rinses routinely (21);
- Last, microbiologic data varied among studies, some focused on GNB only (14), others added Gram-positive *cocci* (15), while others studied OP colonization epidemiology in a very comprehensive way (19,20).

To summarize, it seems clear that, although its magnitude may vary from one study to another, changes in OP colonization affect a much greater proportion of patients in the ICU than in other wards. The reason behind these differences and the pathophysiology of these changes are now to be discussed.

Why such a modification of hospital acquired OP colonization?

Various factors can be put forward to explain the modification of hospital acquired OP colonization. If they are true for general wards, we will focus on ICU conditions.

Impact of underlying disease and treatments

As discussed earlier, some comorbid conditions are associated with a higher burden of GNB OP colonization (1,6,8,9). One has to bear in mind that the presence of a comorbid condition is note rare in ICU patients (22,23), and that nearly two-thirds of ICU patients receive antimicrobials treatments (22), which might play a part in OP colonization modification.

The influence of ICU care

The frequency of gastric reflux is promoted by the presence of nasogastric tubes, suctioning material or orotracheal intubation (18,24). Aspiration of gastric content is enhanced by the supine position, may it be even semi-recumbent position. For instance, Torres *et al.* (25) showed, in 19 intubated patients, that aspiration of the gastric content existed when patients were in semi-recumbent position, although reduced compared to strictly supine position. Gastric content was labeled with technetium-99m sulphur colloids, and samples of endobronchial secretions were obtained and radioactive counts were performed, in supine or semi-recumbent positions. The study showed that radioactive patterns were higher in patents in supine position, but not null in semi-recumbent position. Interestingly, the patients in semi-recumbent position had the same micro-organism isolated in the stomach, the OP and the bronchial sample in 32%, while it accounted for 68% in patients in the supine position.

Furthermore, healthcare workers are known to be vectors of the cross-contamination and colonization (24,26).

Salivary modifications

Salivary composition might influence OP bacterial content. Various factors affect OP colonization. For instance, *K. pneumoniae* adhesion to buccal cells has been shown to be increased with a decrease in salivary pH and a decrease in salivary output (27). Likewise, Dal Nogare *et al.* performed measurements of salivary elastase, fibronectin [a protein known to inhibit GNB adhesion to epithelial cells (28)], fibronectin digestive activity, and GNB OP colonization in cardiac surgery post-operative patients (29). Fibronectin digestive activity and salivary elastase concentration were significantly increased in patients in whom GNB colonization appeared (29), in comparison to those in whom it did not.

Among mechanisms enhancing bacterial adhesion to epithelial cells, phase variation is of particular interest. It results in a reversible change in virulence factors according to the environmental situation and the needs of the bacteria (30). It involves surface antigens, such as lipopolysaccharides, capsule or glycosylated pili, with importance in colonization process, and in the binding of the bacteria to the epithelial cell.

These data concur to explain the proneness to the modification of the OP colonization in ICU patients. The link between OP colonization and VAP will now be discussed.

OP colonization and VAP pathogens are tightly linked

The first reports linking OP colonization and VAP epidemiology were made by Johanson *et al.* (13), more than 45 years ago. Since then, various reports confirmed this tight link, some including the involvement of the digestive tract, that acts either as a filter or as a reservoir (14,15). This link between the 3 sites will be detailed thereafter.

In their pioneer study, Johanson *et al.* (13) included 213 ICU patients. These were repeatedly sampled in the oropharynx and the respiratory tract. The authors therefore show that the GNB OP colonization was a risk factor for the occurrence of VAP with the same pathogen: 23% of the patients with a GNB OP colonization evolved towards a confirmed VAP, with the same pathogen; whereas a VAP occurred in only 3.3% of OP colonization-free (P<0.0001).

When focusing on gastric and OP colonization in 141 patients receiving mechanical ventilation, Bonten *et al.* (14) described a significant increase in the risk of VAP for Enterobacteriaceae and *Pseudomonas aeruginosa*. A higher risk of VAP was associated with the presence of an Enterobacteriaceae in the OP colonization either at admission, or during ICU stay (with, respectively, OR =3.41; P=0.03 and OR =3.41; P=0.04). Likewise, *P. aeruginosa* VAP was significantly associated with its ICU acquired gastric (OR =7.68; P=0.006) or OP colonization (OR =11.59; P<0.00001).

In a study focusing in 48 neuro-trauma patients, Ewig *et al.* (17) showed that prior nasal or OP colonization with *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae* was an independent risk factor for tracheal colonization, and subsequent early-onset VAP. Data of GNB colonization were less univocal as VAP risk did not significantly increase with GNB OP colonization. Nevertheless, GNB tracheal and OP colonization were low at ICU admission (respectively 10% and 16%) and significantly increased during follow-up (49% and 58%). It has to be underlined that a GNB gastric colonization existed at ICU admission for 39% of the patients, and rose to 60% during ICU stay. Finally, the presence of a GNB at any time in the gastric or OP colonization was predictive of lower respiratory tract colonization with the same pathogen.

Garrouste-Orgeas *et al.* (15) went further in identifying the genetic identities of gastric, OP colonization and VAP retrieved GNB. Eighty-six invasively ventilated patients were included. They underwent microbiological samplings from gastric and OP colonization. Pulse-field gel electrophoresis was used in order to compare the genetic identities of the collected bacteria in the different sites. Among the 36 VAP episodes, occurring in 29 patients, the OP colonization and VAP electrophoretic pattern were similar in 17 episodes; for VAP, gastric and OP colonization for 6 episodes; and for VAP and gastric colonization for one of those. Thereafter, in two-thirds of the episodes (24/36) a similar genetic pattern was evidenced between the aerodigestive colonization and VAP causative pathogen.

More recently, our group focused on *Escherichia coli* VAP and lower respiratory tract colonization (31). We prospectively sampled 132 ventilated patients at rectal, OP and respiratory sites, and studied *E. coli* isolates of the 25 who harboured *E. coli* colonization at three sites. We interestingly showed that when *E. coli* was present in a respiratory sample, *E. coli* was always present in the OP colonization sample, and that the proportion of virulent extra-intestinal isolates increased from rectal colonization to respiratory sample.

The link between digestive, OP colonization and subsequent pulmonary infection is clearly established. OP colonization is therefore a key site in order to limit and prevent VAP occurrence, with therapies aiming in modulating and reducing the OP bacterial burden. Some therapies have or are currently being investigated.

What therapies to modulate OP colonization?

Mammalian antimicrobial peptides

Among mammalian antimicrobial peptides, protegrins have been identified as having an unusually broad spectrum of antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi and some enveloped viruses (32,33). They have been found to be effective in reducing GNB OP

colonization prevalence and the density of Gram-positive bacterial load in OP colonization of healthy subjects (32). Iseganan is a synthetic protegrin analog, which was tested in immunocompromised patients, undergoing stomatotoxic chemotherapies. It significantly reduced stomatitis-associated symptoms in a phase III randomized controlled trial (34). But the hopes placed in this antimicrobial peptide to reduce the incidence of VAP in the ICU were not confirmed by the only published large randomized controlled trial (35). In this trial, Kollef *et al.* allocated 709 ventilated patients to receive either oral topical iseganan or a placebo. This study was stopped prematurely for futility, before the inclusion of the 900 planned patients: no difference in VAP occurrence, in day-14 survival or in adverse effects was evidenced. These disappointing data led to the abandon of iseganan as a preventive treatment for VAP.

Chlorhexidine

This widely used antiseptic (21,36,37) increases the bacterial cell wall permeability, leading to bacterial lysis (38). Its activity encompasses Gram-positive and -negative bacteria [with Gram positive being more susceptible (39-42)], facultative anaerobes, aerobes, yeasts and some viruses (38,40).

Data on its efficacy in preventing VAP during oral care are conflicting. Various meta-analyses were performed, with discordant results summarized below (43-48), and in *Table 2*.

No effect on VAP prevention

Pineda *et al.* (46) performed a meta-analysis of four studies, gathering the data from 1,202 ventilated patients, from 2 ICU, and 2 cardiac surgery post-operative care unit. In this meta-analysis, no significant effect of chlorhexidine oral care was evidenced in the incidence of nosocomial pneumonia, mortality rate, duration of mechanical ventilation and ICU length of stay. The trials included in this meta-analysis had several confounding factors. Among those, the heterogeneity in both control arms (indistinguishable placebos, standard oral care or Listerine), and intervention arms (0.2% or 0.12% chlorhexidine; bi- or thrice-daily; oral rinse or gel) might have blurred any effect.

Effective in VAP prevention but not on mortality

A larger meta-analysis (45) followed the latter. Seven RCTs were included, resulting in 1,650 patients. The comparators used in these seven trials were placebo for four studies; standard oral care for 2; and Listerine in one. In this second meta-analysis, a significant effect of chlorhexidine was evidenced in reducing VAP, with a relative risk (RR) of 0.74 (95% CI, 0.56–0.96; P=0.03) when using a fixed effect model. Nevertheless, the RR reduction lost significance when using a random effect model (RR 0.7; 95% CI, 0.48–1.04; P=0.08); furthermore, no effect on mortality was found, rending questionable the use of chlorhexidine to prevent a non-severe ICU adverse event.

The most recent meta-analysis, by Hua *et al.* (48) gathered 38 RCTs, 18 of those were conducted with chlorhexidine, accounting for 2,451 participants. In this meta-analysis, chlorhexidine significantly reduced the risk for VAP, with a RR of 0.74 (95% CI, 0.61–0.89; P=0.004). But again, no difference in mortality, of mechanical ventilation, or of length of ICU stay was evidenced.

Efficacy depending of chlorhexidine dosage

Labeau *et al.* (43) performed a meta-analysis of 14 studies (12 of those investigating chlorhexidine). They interestingly showed that, if the global analysis was in favour chlorhexidine in reducing VAP with a risk reduction of 0.72 (95% CI, 0.55–0.88; P=0.004), this effect was limited to chlorhexidine 2% (RR 0.53; 95% CI, 0.31–0.91), while chlorhexidine 0.12% and 0.2% did not have a protective effect.

Efficacy depending of the patients assessed

The previously cited meta-analysis, by Chlebicki *et al.* (45), Labeau *et al.* (43) and a more recent one by Klompas *et al.* (41) found a greater chlorhexidine efficacy in their subgroup analysis of cardiac surgery patients, while the effect in non-cardiosurgical populations the reduction in VAP rate was non-significant (41,43). Furthermore, in Klompas *et al.* meta-analysis, if no effect on mortality was evidenced in cardiac surgery patients, chlorhexidine increased, although non-significantly, the risk of death.

Chlorhexidine drawbacks

Several drawbacks restrain the few positive, although inconsistent, effects of chlorhexidine. The first one is its tolerance: an international randomized trial had been launched in order to evaluate, among other measures, 2% chlorhexidine efficacy (50). An unexpectedly high rate of oral mucosal lesions (29/295 patients) led to the replacement of the 2% solution by a 1% oral gel. Chlorhexidine mouthwash was however totally abandoned, again because of intolerance (50).

Table 2 Summary of the meta-analysis evaluating the effects of chlorhexidine

Meta-analysis	Number of included studies (pts)	Settings	Chlorhexidine formulation	Comparator	Main results on VAP occurrence	Main results on mortality	Main results on other outcomes
Pineda *et al.*, *Critical Care* 2006 (46)	4 (1,202 pts)	2 ICU; 2 post-cardiac surgery ICU	0.2% or 0.12%	Placebo (2 studies); standard oral care (1 study); Listerine (1 study)	No effect on pneumonia: OR 0.53; 95% CI, 0.27–1.05	No effect on mortality: OR 0.77; 95% CI, 0.28–2.11	No difference in duration of mechanical ventilation or ICU length of stay
Chlebicki *et al.*, *Crit Care Med* 2007 (45)	7 (1,650 pts)	2 cardiothoracic ICU; 4 medical or medico-surgical ICU; 1 trauma and surgical ICU	0.12%; 0.2%; 2%	Placebo (4 studies); standard oral care (2 study); Listerine (1 study)	Reduced risk of VAP: RR 0.74; 95% CI, 0.56–0.96; P=0.03 Cardiac surgery pts: reduced risk of VAP: RR 0.41; 95% CI 0.17–0.98; P=0.04	No effect on mortality: RR 1.07; 95% CI, 0.76–1.51; P=0.69	–
Hua *et al.*, *Cochrane Database Syst Rev* 2016 (48)	18 (2,451 participants)	2 cardiothoracic ICU (incl. one paediatric); 11 medical or mixed ICU (incl. 2 paediatric); 5 trauma and surgical ICU	0.12%; 0.2%; 1%; 2%; unclear	Placebo (12 studies); standard oral care (5 studies); potassium permanganate (1 study)	Reduced risk of VAP: RR 0.74; 95% CI, 0.61–0.89; P=0.004	No effect on mortality: RR 1.09; 95% CI, 0.96–1.23; P=0.20	No significant effect on duration of ventilation, duration of ICU stay
Labeau *et al.*, *Lancet* 2011 (43)	12 (2,341 participants)	2 cardiothoracic ICU; 9 medical or medico-surgical ICU; 1 trauma ICU	0.12%; 0.2%; 2%	Placebo (6 studies); standard oral care (3 study); Listerine (1 study); saline (1 study); potassium permanganate (1 study)	Global analysis: reduced risk of VAP: RR 0.72; 95% CI, 0.55–0.88; P=0.004; CHX 0.12%: RR 0.73; 95% CI, 0.51–1.05; CHX 0.2%: RR 0.79; 95% CI, 0.46–1.36; CHX 2%: RR 0.53; 95% CI 0.31–0.91	–	Considering all antiseptics tested, significant reduction of the risk of VAP in cardiac surgery pts: RR 0.41; 95% CI, 0.17–0.98 Not evidenced in mixed ICU or surgery & trauma ICU pts
Klompas *et al.*, *JAMA Intern Med* 2014 (41)	16 (3,630 pts)	3 cardiac surgery ICU; 7 medico-surgical ICU; 1 surgery ICU; 1 trauma ICU; 1 respiratory ICU; 1 neuro ICU	0.12%; 0.2%; 2%	Placebo (8 studies); Listerine (1 study); hydrogen peroxide (1 study); normal saline (3 studies); vaseline (1 study); sterile water (1 study)	Global analysis: reduced risk of VAP: RR 0.73; 95% CI, 0.58–0.92 Cardiac surgery pts: reduced risk of VAP: RR 0.56; 95% CI, 0.41–0.79 Non-cardiac surgery pts: no reduction in risk of VAP: RR 0.78; 95% CI, 0.60–1.02	No effect on mortality: RR 1.13; 95% CI, 0.99–1.28, either in cardiac surgery studies, non-cardiac surgery studies, or all studies	No effect on length of stay or length of mechanical ventilation

Table 2 (*continued*)

Table 2 (*continued*)

Meta-analysis	Number of included studies (pts)	Settings	Chlorhexidine formulation	Comparator	Main results on VAP occurrence	Main results on mortality	Main results on other outcomes
Price *et al.*, *BMJ* 2014 (49)	10	8 Mixed ICU; 1 trauma ICU; 1 neuro ICU; 1 surgical ICU	0.12%; 0.2%; 2%	6 placebo; 1 potassium permanganate; 1 normal saline; 1 bicarbonate; 1 usual care	n.a.	Chlorhexidine *vs.* control: OR 1.23; 95% CI 0.99–1.49 Selective OP decontamination *vs.* chlorhexidine: OR 0.67; 95% CI, 0.48–0.91	n.a.

VAP, ventilator associated pneumonia; ICU, intensive care unit; OR, odds ratio; RR, relative risk; CHX, chlorhexidine; pts, patients; n.a., not available.

Next, two studies reported worrisome findings on chlorhexidine effect on mortality. First, a meta-analysis performed by Price *et al.* (49) included 11 trials (2,618 critically ill patients), and found that chlorhexidine oral care was associated with an increased mortality (OR =1.25, 95% CI, 1.05–1.50) compared to control or placebo. Next, in a very recently published retrospective single-centre observational study of 82,274 all-severity patients (51), 14% of the patients received chlorhexidine oral care. The authors found that an exposure to low-doses (≤300 mg) was associated with an increased mortality (OR =2.61; 95% CI, 2.32–2.92). This association was even higher in patients with a lower risk of death, while this association was not found for patients receiving mechanical ventilation, or undergoing major cardiothoracic or vascular surgery. These two studies raise questions that still have to be answered (52).

Lastly, our group studied chlorhexidine susceptibility of 260 *E. coli* isolates responsible for VAP (53). We showed that chlorhexidine susceptibility was reduced for 26.9% of the strains, with a significant correlation between antimicrobial and chlorhexidine resistance. These findings bring another concern to chlorhexidine efficacy.

Indeed, if chlorhexidine is ineffective not only in a clinical perspective but also too on a microbiological one, and if, in addition to that inefficacy, it holds severe adverse effects, its use is highly questionable.

Some alternative therapies are therefore urgently needed. Among those, proanthocyanidins might be of special interest.

Proanthocyanidins

Cranberry proanthocyanidins have been shown to inhibit *E. coli* adhesion to the urothelium (54,55). Moreover, it has been shown to decrease *E. coli* virulence in an *in vivo* model of *Caenorhabditis elegans* (55). Our group (56) showed that different steps leading to *E. coli* pneumonia could be modulated by proanthocyanidins: bacterial growth was significantly impaired by increased concentrations of proanthocyanidins; *E. coli* adhesion to epithelial buccal cells was significantly reduced and its protective effect on mortality was assessed and confirmed in a mouse model of pneumonia, with a significant reduction in inflammatory response (56). Furthermore, unpublished preliminary data suggest a similar effect on various other pathogens. These interesting and promising studies urge to move forward this path.

What's next for OP colonization exploration?

More than 50 years after its first description, in a context of widespread antimicrobial resistance in GNB, a reappraisal of OP colonization composition is required. Meanwhile, further data to better administrate chlorhexidine, in terms of dosage, but also in defining the best population to target oral decontamination, are needed. Nevertheless, regarding the worrisome spreading of antimicrobial resistance, and given its link to chlorhexidine resistance, the development of alternatives to prevent and treat OP colonization are urgently needed.

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Footnote

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