Anaerobic bacteria commonly colonize the lower airways of intubated ICU patients

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Objectives To investigate respiratory tract colonization by aerobic and anaerobic bacteria in mechanically ventilated patients.

Methods Bacterial colonization of the stomach and the respiratory tract was qualitatively and quantitatively analyzed over time in 41 consecutive mechanically ventilated patients in a Swedish intensive care unit (ICU), with special emphasis on elucidation of the role of anaerobic bacteria in the lower respiratory tract. Samples were taken from the oropharynx, gastric juice, subglottic space and trachea within 24 h (median 14 h) of intubation, and then every third day until day 18 and every fifth day until day 33.

Results The patients were often heavily colonized with microorganisms not considered to belong to a healthy normal oropharyngeal and gastric flora on admission to the ICU. A majority harbored enterococci, coagulase-negative staphylococci and *Candida* spp. in at least one site on day 1. Anaerobic bacteria, mainly peptostreptococci and *Prevotella* spp., were isolated from subglottic and/or tracheal secretions in 59% of the patients. Different routes of tracheal colonization for different groups of microorganisms were found. Primary or concomitant colonization of the oropharynx with staphylococci, enterococci, enterobacteria and *Candida* was often seen, while *Pseudomonas* spp., other non-fermenting Gram-negative rods and several anaerobic species often primarily colonized the trachea, indicating exogenous or direct gastrointestinal routes of colonization.

Conclusions Mechanically ventilated patients were heavily colonized in their lower airways by potential pathogenic microorganisms, including a high load of anaerobic bacteria. Different routes of colonization were shown for different species.

Keywords Intubation, tracheal colonization, anaerobic bacteria, ventilator-associated pneumonia, intensive care unit

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INTRODUCTION

Ventilator-associated pneumonia (VAP) is a serious complication in patients treated in intensive care units (ICUs) and is reported to occur in 9–24% of patients intubated for longer than 48 h [1]. In a single-day point prevalence study of 1417 European ICUs, pneumonia and VAP represented 39% of all ICU-acquired infections [2]. The risk factors for developing VAP, besides those that lead to admission to the ICU, such as septic shock, earlier transplantation, or advanced chronic heart or lung failure, also include those occurring during the ICU stay. The former cannot be influenced, but, regarding the latter factors, there are possibilities for preventing, or at least lowering, the incidence rate of infection [1]. In healthy persons, the trachea is usually sterile [3]. The pathogenesis of VAP is considered to start with colonization of the trachea due to the risk factors listed above; this may proceed to a VAP [4–6]. Several endogenous and exogenous reservoirs for tracheal colonization have been suggested, including aspiration of colonized gastric contents, aspiration of oropharyngeal

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secretions, and spread of microorganisms from other patients or personnel in the ward, although the role of the stomach as a major reservoir has been questioned [4,7]. Also, it has been proposed that intestinal microorganisms may disseminate to the airways via duodenal reflux to the stomach, via the skin of the patients, or via the hands of personnel [7]. However, only a few studies have conducted serial sampling of the different sites, and no conclusive results on main colonization routes have been obtained. Continuous subglottic drainage of intubated patients is reported to decrease the incidence of early-onset VAP, suggesting that microaspiration bypassing the cuff may lead to tracheal colonization and eventually to lung infection [8,9]. Though microaspiration is a plausible theory, more descriptive studies of colonization of the airways of mechanically ventilated patients are needed. The literature shows large discrepancies between countries, as well as between different hospitals in the same country, in terms of antibiotic resistance and microbial colonization of the patients [9–11]. The presence and role of anaerobes in the upper and lower airways in intubated patients are still unclear. Contradictory results have been reported, and the levels of anaerobes found in patients with VAP vary between 0% and 23% [12,13].

The aim of the present study was to assess the impact of oropharyngeal and gastric colonization on the emergence of tracheal colonization over time in mechanically ventilated patients in a Swedish multidisciplinary ICU. Special emphasis was placed on elucidating the role of anaerobic bacteria in the lower respiratory tract.

MATERIALS AND METHODS

Huddinge University Hospital, Karolinska Institutet, Stockholm, Sweden is a 1000-bed hospital with all specialities except neurosurgery and burns. The multidisciplinary ICU has nine to 12 beds and 1000–1200 admissions every year, the majority of patients being adults.

Patients

Forty-eight consecutive patients, expected to need assisted ventilation for at least 3 days, during two periods (April to August 1998, and September to December 1999), were considered for the study. The only formal exclusion criterion was age less than 18 years. In addition, seven patients were excluded due to lack of informed consent ($n = 1$), extubation before day three ($n = 3$), death before day three ($n = 2$), and transfer to another hospital before day three ($n = 1$). In total, 41 patients were included in the study. The majority of the patients were extubated in the ICU ($n = 35$), but withdrawn consent ($n = 1$) or transfer to other hospitals ($n = 5$) were also reasons for discontinuation of sampling. Two of the patients were ventilated for more than 33 days, when sampling was discontinued according to the protocol. Fifteen of the patients were females, and the median age of the 41 patients was 59 years (range 20–82 years). The median APACHE-II score during the 24 h after admission was 19 (range 0–44), and the ICU mortality rate was 10/41. Ten of the patients (24%) had undergone transplantation of liver ($n = 5$), bone marrow ($n = 2$), kidney ($n = 2$), or liver and bone marrow ($n = 1$). The patients were mechanically ventilated for 3 to >33 days (median 6 days). Twelve of the patients were ventilated for 12 days or more. One patient had a permanent tracheostomy at admission, and 16 of the patients were exposed to at least one reintubation or tracheostomy operation. Twelve of the patients had clinical pneumonia at the time of admission. Nine of the patients had not received any antibiotic treatment at the time of admission. During the ICU stay, 39 of the 41 patients were treated with at least two antibacterial agents, often as combination therapy, and 13 received five to seven different antimicrobial agents, including antiviral and antifungal therapy. The most frequently used antibiotics were cefuroxime ($n = 17$), imipenem ($n = 15$), metronidazole ($n = 14$), ceftazidime ($n = 13$), and cloxacillin ($n = 9$). Only two patients were treated with vancomycin, while one patient was not given any antimicrobial treatment. Noteworthy points about the general medication were that, during their stay in the ICU, 15 patients were treated with corticosteroids, 10 had received other immunosuppressive therapy, and none was given any muscle-relaxant drug.

Sampling procedures

The first sampling was performed within 24 h (median 14) from intubation; sampling was then done every third day until day 18, and every fifth day until day 33. Samples were taken from the oropharynx, gastric channel, subglottic space and

trachea. The oropharyngeal samples were taken from both the tonsils and the pharynx wall, avoiding contamination from the tongue with a depressor. A sterile swab and a charcoal transport medium were used. Samples from the subglottic space were aspirated with a sterile syringe from a channel of the tube (EVAC Hi Lo; Mallinckrodt, Athlone, Ireland) after disinfection of the outer surface of the channel with 70% isopropanol, and put into a sterile tube with equal volumes of transport medium. From the stomach and the trachea, the secretions were aspirated directly into a sterile tube after disinfection of the outer surface, and equal volumes of sterile transport medium were added. The gastric samples were taken in the mornings 3 h after the enteral nutrition was discontinued. In the transplanted patients, it was often difficult to obtain gastric samples, due to the immunosuppressive therapy, which was partly administered through the gastric tube. All samples were taken by either of two investigators, transported to the laboratory within 30 min, and frozen at −70 °C until analyzed.

Microbiological analyses

Samples from the oropharynx, gastric juice, subglottis and tracheal secretions were diluted in 10-fold serial dilutions to 10^{-6} in pre-reduced media. If needed, subglottic and tracheal secretions were lysed with an equal amount of sputolysin. The suspensions were inoculated on selective and non-selective agar media as previously described [14]. Aerobic agar plates were incubated for 24 h at 37 °C, while agar plates for anaerobic microorganisms were incubated for 48 h at 37 °C in anaerobic jars (GasPak; BBL, Cockeysville, MD, USA). After incubation, different colony types were counted and isolated in pure cultures for identification to species or genus level by morphologic, biochemical and serologic tests, and also for anaerobic microorganisms by gas–liquid chromatography [15,16]. The lower limits of detection were 100 CFU/mL of oropharyngeal secretion, 20 CFU/mL of gastric juice, and 20–40 CFU/mL of subglottic and tracheal secretions, respectively.

RESULTS AND DISCUSSION

Clinical findings

None of the patients developed VAP according to the criteria of the protocol, which were: (1) new and persistent infiltrate on chest X-ray; and (2) three of the following clinical signs: (a) temperature >38.0 °C or <36.0 °C, (b) leukocytosis (> 10 000/μL) or leukopenia (< 4000/μL), (c) purulent tracheal secretion, (d) C-reactive protein (CRP) > five times the upper normal value, or (e) positive tracheal culture [8]. It was not an aim of the study to demonstrate a low incidence of VAP, but the finding of 0/41 in a consecutive series of ventilated patients is nevertheless different from a 20% incidence ($P < 0.05$ with 80% statistical power). This outcome may have several explanations. Every patient is nursed by 1.5–2 nurses; during the daytime each doctor is responsible for only three or four patients, and during nights and at weekends there is an intensive care specialist in the unit, which makes continuous evaluation of the patients possible for 24 h/day. Sedation can be limited, due to short intubation periods, early tracheotomy is performed if prolonged ventilator treatment is expected, nasotracheal intubation is only used in small children, and no muscle-relaxant drugs are used, which means that the patients can cough and cooperate better in the daily physiotherapy sessions. Furthermore, altered antibiotic treatment, based on knowledge of the local resistance pattern, is initiated on early suspicion of VAP, e.g. fever, increasing CRP, purulent and/or increasing volume of secretion and increasing oxygen demand in the ventilator, and it is directed towards the microorganisms colonizing the trachea.

Microbiological findings

The microbial colonization patterns of the oropharynx, stomach, subglottic region and trachea of the 41 patients intubated for three to 33 days are shown in Figures 1–7.

Initial colonization

The patients were often heavily colonized with microorganisms not considered to belong to a healthy normal oropharyngeal or gastric flora on admission to the ICU. A majority of them harbored enterococci, coagulase-negative staphylococci (CNS) and *Candida* spp. in the subglottis and/or trachea, and nine of the patients were colonized by anaerobic bacteria in their lower airways during the first 24 h of intubation. Tracheal colonization by potential pathogens has previously been shown to be already common at the

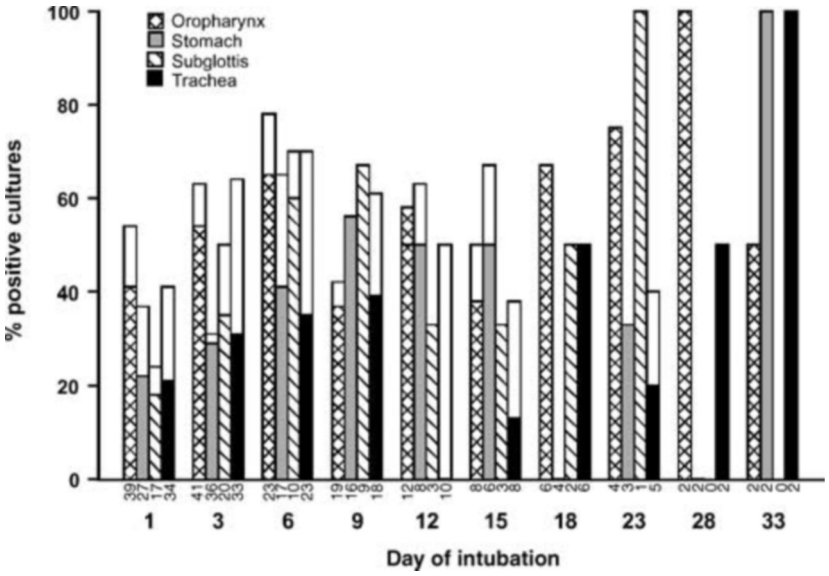

Figure 1 Frequency of cultures positive for coagulase-negative staphylococci in samples taken from the oropharynx, stomach, subglottis and trachea in mechanically ventilated patients collected sequentially during up to 33 days of intubation. The number of cultures taken on each sampling occasion is depicted immediately below the *x*-axis. Open bars represent findings above the detection limit; filled or hatched bars represent findings of $>10^4$ CFU/mL.

first day of intubation in severely ill patients, and previous hospitalization for more than 48 h has been shown to be a risk factor for tracheal colonization with hospital pathogens [17].

Progression of colonization

The majority of the patients were colonized with rather constant levels of dominating microorganisms over time, while in four of 41 a more than 100-fold increased concentration and in nine of 41 a markedly decreased concentration over time were found. An increasing load over time was associated with enterococci, Enterobacteriaceae, non-fermenting Gram-negative rods, and Gram-negative anaerobes. Decreased levels occurred mainly with *Candida* spp. Progression of colonization of a specific species from the oropharynx to the subglottis and the trachea over time was seen in 15 of 41 patients. In most of the other patients, specific species were concomitantly cultured from the upper and the lower airways, while some species were mainly isolated from the lower airways without previously colonizing the oropharynx and/or the stomach. Less than 4% of subglottic and/or

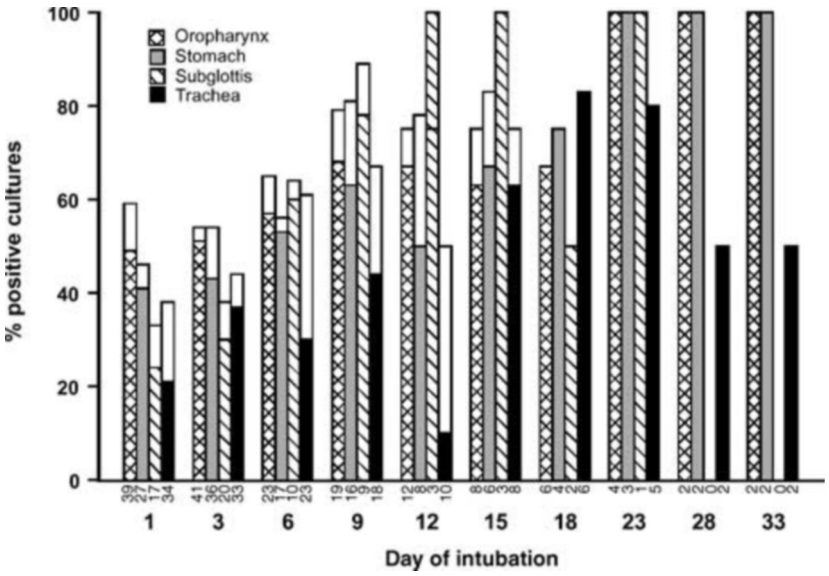

Figure 2 Frequency of cultures positive for *Enterococcus* spp. in samples taken from the oropharynx, stomach, subglottis and trachea in mechanically ventilated patients collected during up to 33 days of intubation. The number of cultures taken on each sampling occasion is depicted immediately below the *x*-axis. Open bars represent findings above the detection limit; filled or hatched bars represent findings of $>10^4$ CFU/mL.

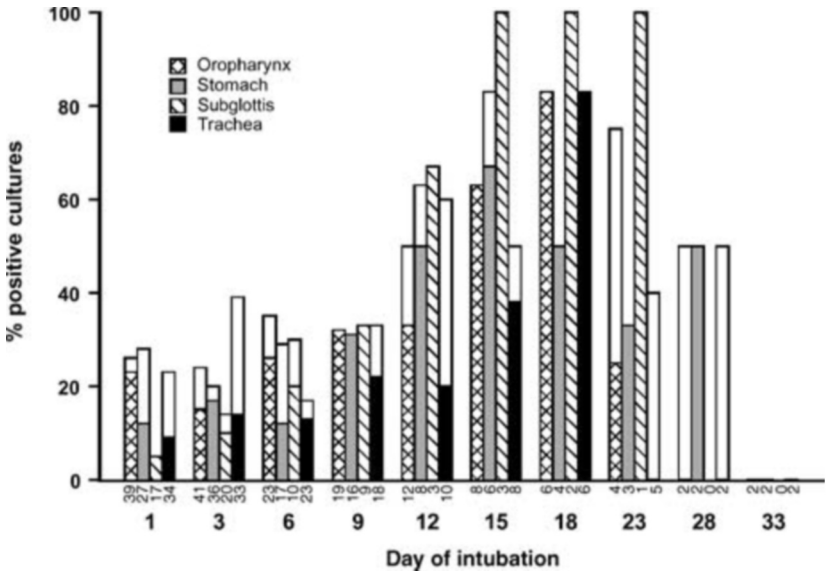

Figure 3 Frequency of cultures positive for Enterobacteriaceae in samples taken from the oropharynx, stomach, subglottis and trachea in mechanically ventilated patients collected during up to 33 days of intubation. The number of cultures taken on each sampling occasion is depicted immediately below the *x*-axis. Open bars represent findings above the detection limit; filled or hatched bars represent findings of $>10^4$ CFU/mL.

tracheal strains were first isolated from the stomach as a sole source, although candidas were often simultaneously isolated in both the oropharynx and the stomach before disseminating to the lower airways. These findings are in accordance with previous reports [5,7]. The majority of strains isolated from the subglottic and tracheal regions were concomitantly isolated from the upper respiratory tract. Interestingly, a high proportion of *Pseudomonas* spp., other non-fermentative Gram-negative rods and some anaerobic species were primary colonizers of the lower airways before, or without, appearing in other sites. The two former are well known to exist in hospital wards with high antimicrobial pressure, and may easily spread between patients, causing nosocomial infections; that is, an exogenous route is probable. This finding is in accordance with the results of Mahul et al. [9] and Vallés et al. [8], who found that hourly or continuous aspiration of subglottic secretions postponed VAP and reduced the colonization of Gram-positive cocci and *Haemophilus influenzae*, while non-fermentative Gram-negative rods such as *Pseudomonas* and *Acinetobacter* spp. were commonly isolated from tracheal aspirates independently of subglottic secretion

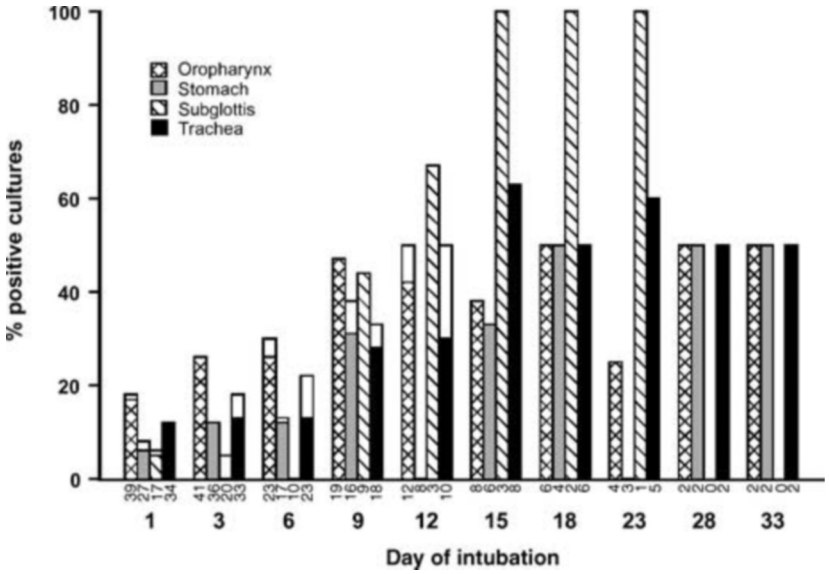

Figure 4 Frequency of cultures positive for non-fermentative Gram-negative rods in samples taken from the oropharynx, stomach, subglottis and trachea in mechanically ventilated patients collected during up to 33 days of intubation. The number of cultures taken on each sampling occasion is depicted immediately below the *x*-axis. Open bars represent findings above the detection limit; filled or hatched bars represent findings of $>10^4$ CFU/mL.

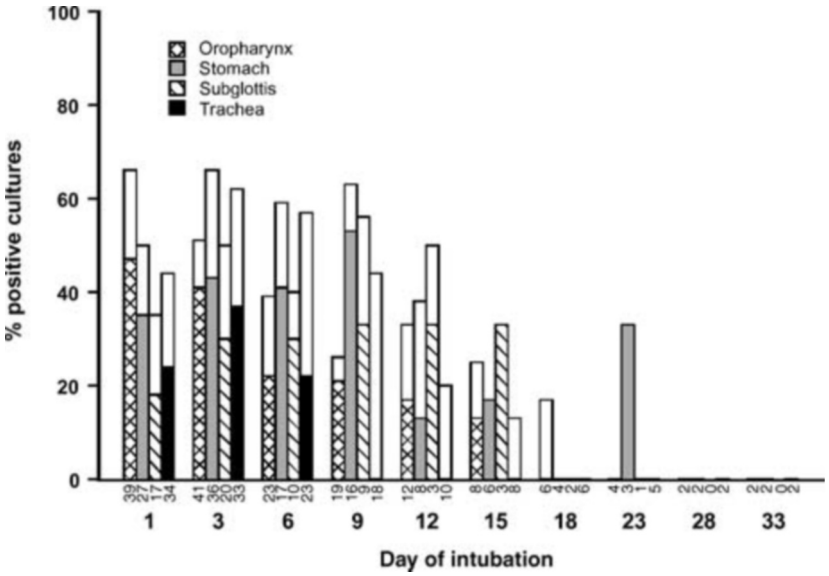

Figure 5 Frequency of cultures positive for *Candida* spp. in samples taken from the oropharynx, stomach, subglottis and trachea in mechanically ventilated patients collected during up to 33 days of intubation. The number of cultures taken on each sampling occasion is depicted immediately below the *x*-axis. Open bars represent findings above the detection limit; filled or hatched bars represent findings of $>10^4$ CFU/mL.

drainage and without concomitant isolation from the subglottis. Primary tracheal colonization with these species may be explained by a preferential tropism to tracheal epithelial cells [18]. Also, it has been previously shown that microbial biofilms in the endotracheal tube may act as a reservoir of potentially pathogenic microorganisms [19].

Colonization with aerobic microorganisms

Staphylococci were isolated from 38 of the patients (93%), and always from the trachea in patients intubated for at least 6 days. All of these 38 patients harbored CNS (Figure 1). *Staphylococcus aureus* was isolated from eight patients; in six of these, it was isolated from the lower airways at levels of 10^4–10^7 CFU/mL. Thirty-four of the patients (83%) were colonized with *Enterococcus* spp. during the study period; 29 of these had harbored *Enterococcus faecalis* and 19 *Enterococcus faecium* (Figure 2). *Streptococcus pneumonie* and *Moraxella catarrhalis* were isolated from the trachea in only one patient each. Among Enterobacteriaceae, *Klebsiella pneumoniae* ($n = 8$ patients), *Enterobacter cloa-*

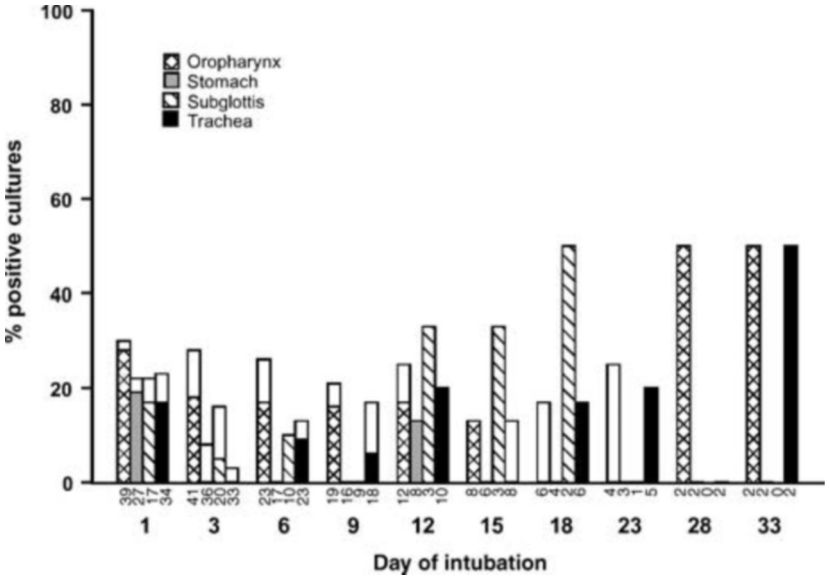

Figure 6 Frequency of cultures positive for *Peptostreptococcus* spp. in samples taken from the oropharynx, stomach, subglottis and trachea in mechanically ventilated patients collected during up to 33 days of intubation. The number of cultures taken on each sampling occasion is depicted immediately below the *x*-axis. Open bars represent findings above the detection limit; filled or hatched bars represent findings of $>10^4$ CFU/mL.

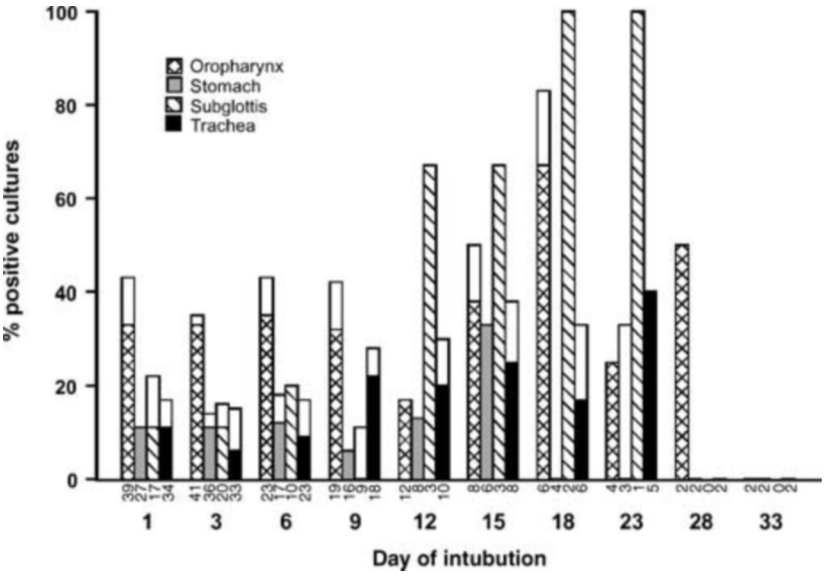

Figure 7 Frequency of cultures positive for anaerobic Gram-negative bacteria (mainly *Prevotella* spp.) in samples taken from the oropharynx, stomach, subglottis and trachea in mechanically ventilated patients collected during up to 33 days of intubation. The number of cultures taken on each sampling occasion is depicted immediately below the *x*-axis. Open bars represent findings above the detection limit; filled or hatched bars represent findings of $>10^4$ CFU/mL.

cae ($n=5$), *Escherichia coli* ($n=4$) and *Proteus mirabilis* ($n=3$) dominated, but *Morganella morganii*, *Klebsiella oxytoca*, *Citrobacter freundii* and *Enterobacter aerogenes* were also found (Figure 3). A difference was noticed between transplanted and non-transplanted patients: in the first group, only one of 11 patients was colonized with Enterobacteriaceae, while in the latter group, enterobacteria were isolated from at least one site in 16 of 30 patients ($P<0.05$, chi-square test). Non-fermentative Gram-negative rods were often isolated in high numbers from the lower airways ($n=16$ patients), mainly *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*, but also *Burkholderia cepacia*, *Pseudomonas fluorescens/putida*, and *Shewanella putrefaciens*. *Acinetobacter* spp. were isolated from six patients, while *Flavobacterium* spp., *Aeromonas hydrophila* and an unidentified non-fermentative rod were found in one patient each (Figure 4). *H. influenzae* was isolated from the oropharynx in only two patients, while a majority of the patients were colonized by one or several α-hemolytic streptococci, micrococci, *Neisseria* spp. and *H. parainfluenzae*, mainly in the oropharynx but also in the lower airways. Eighty per cent of the patients were colonized by *Candida* spp. (*Candida albicans*, *Candida glabrata*, and/or *Candida kruseii*) in any of the sites, and 73% in the lower airways (Figure 5). All the transplanted patients were colonized by *Candida* spp. in the subglottic and/or tracheal secretions, while 20 of the 31 non-transplanted patients harbored *Candida* in those sites (non-significant, chi-square test). High concentrations, $>10^4$ CFU/mL, of *Candida* spp. were found in six of the 10 transplanted patients and in 13 of the 31 non-transplanted patients.

Colonization with anaerobic bacteria

In contrast to the results of other investigators, anaerobic bacteria were isolated from the subglottic and/or tracheal secretions in the majority (59%) of patients, always in mixed cultures with either aerobic bacteria or yeasts. From day 12 to day 23, between 40% and 100% of all samples from the lower airways yielded anaerobes. The most frequently isolated anaerobic bacteria were *Peptostreptococcus* ($n=16$ patients) (Figure 6) and *Prevotella* ($n=12$) (Figure 7). Others were *Veillonella* ($n=7$), *Fusobacterium* ($n=4$), *Actinomyces* ($n=9$), *Bifidobacterium* ($n=4$), *Clostridium* ($n=3$), and other anaerobic Gram-positive rods, mainly *Eubacterium* spp. ($n=12$). Lactobacilli were isolated from almost all patients at any site, and from the lower airways in 26 patients. Since all patients in the ward were given *Lactobacillus*-containing probiotics (4×50 mL daily), lactobacilli are not included in any analyses of the bacteriologic findings. Notably, *Clostridium* species were isolated from seven patients. Anaerobic species were often present in all four loci at the same time. Clostridia are spore-forming organisms that may easily be spread in the ward. An exogenous or rectal–tracheal route was plausible in two patients who were exclusively colonized in the trachea by

Clostridium difficile, an intestinal pathogen which also has been reported to cause bacteremia [20].

Previous studies on colonization of the respiratory tract in mechanically intubated patients have been mainly focused on aerobic bacteria, especially on Enterobacteriaceae, *Pseudomonas* spp. and *S. aureus*, which are considered to be the main pathogens in VAP [8,9,21,22]. The importance of anaerobic bacteria in VAP is still unclear and little studied. Doré et al. recovered anaerobic bacteria, mainly *Prevotella* spp., by using protected specimen brushes, from 23% of 130 patients with a first episode of VAP. Pneumonia with specimens yielding anaerobes was significantly more frequent in orotracheally intubated patients (which was the case for all but one of the patients in the present study) than in nasotracheally intubated patients and in patients with a tracheostomy [13]. In this study, single specimens were collected, and no colonization route was studied. Peptostreptococci, *Prevotella* and *Fusobacterium* spp. are all common inhabitants of the oral cavity, suggesting an oral route of colonization. Surprisingly, more than one-third of these strains (38%) were primary colonizers of the lower airways without previous isolation from the oropharynx. Among the 24 patients colonized with anaerobic bacteria in the subglottic and/or tracheal secretions, seven died during the study period, compared to three of 17 who were not colonized by anaerobes (non-significant). Besides prolonged incubation, no risk factor for anaerobic colonization of the lower respiratory tract could be found, considering age, APACHE score, reintubation or tracheostomy, antimicrobial treatment, or underlying disease. Information on the role of anaerobic bacteria in VAP is currently sparse in the literature. The importance of anaerobic bacteria as specific pathogens was elucidated in 53 mechanically ventilated patients with VAP, from whom anaerobic bacteria, mainly *Prevotella* spp., were cultured. Patients who were treated with antibiotics active against anaerobes had a significantly better outcome than those who did not receive any treatment active against anaerobes [23]. Since several of the anaerobic species isolated in the present study exhibit various virulence factors and can cause serious infections, the finding of anaerobes in the lower respiratory tract should not be overlooked [24].

In conclusion, the results of the present study indicate heavy colonization of the lower airways in mechanically ventilated patients, including potentially pathogenic anaerobic bacteria such as peptostreptococci, prevotellas, clostridia and fusobacteria. Different colonization patterns for different groups of microorganisms were found, indicating primary or concomitant colonization of the oropharynx for staphylococci, enterococci, enterobacteria and candidas, while *Pseudomonas* spp. and other non-fermenting Gram-negative rods and several anaerobic species often primarily colonized the trachea, indicating exogenous routes of colonization. Since VAP is preceded by colonization of the lower airways, knowledge of colonization routes is necessary in order to develop strategies for preventing or interrupting colonization by potential pathogens.

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