Oral Decontamination with Chlorhexidine Reduces the Incidence of Ventilator-associated Pneumonia

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Rationale: **Ventilator-associated pneumonia (VAP) is the most frequently occurring nosocomial infection associated with increased morbidity and mortality. Although oral decontamination with antibiotics reduces incidences of VAP, it is not recommended because of potential selection of antibiotic-resistant pathogens. We hypothesized that oral decontamination with either chlorhexidine (CHX, 2%) or CHX/colistin (CHX/COL, 2%/2%) would reduce and postpone development of VAP, and oral and endotracheal colonization.**
Objectives: **To determine the effect of oral decontamination with CHX or CHX/COL on VAP incidence and time to development of VAP.**
Methods: **Consecutive patients needing mechanical ventilation for 48 h or more were enrolled in a randomized, double-blind, placebo-controlled trial with three arms: CHX, CHX/COL, and placebo (PLAC). Trial medication was applied every 6 h into the buccal cavity. Oropharyngeal swabs were obtained daily and quantitatively analyzed for gram-positive and gram-negative microorganisms. Endotracheal colonization was monitored twice weekly.**
Results: **Of 385 patients included, 130 received PLAC, 127 CHX and 128 CHX/COL. Baseline characteristics were comparable. The daily risk of VAP was reduced in both treatment groups compared with PLAC: 65% (hazard ratio [HR] = 0.352; 95% confidence interval [CI], 0.160, 0. 791; p = 0.012) for CHX and 55% (HR = 0.454; 95% CI, 0.224, 0. 925; p = 0.030) for CHX/COL. CHX/COL provided significant reduction in oropharyngeal colonization with both gram-negative and gram-positive microorganisms, whereas CHX mostly affected gram-positive microorganisms. Endotracheal colonization was reduced for CHX/COL patients and to a lesser extent for CHX patients. No differences in duration of mechanical ventilation, intensive care unit stay, or intensive care unit survival could be demonstrated.**
Conclusions: **Topical oral decontamination with CHX or CHX/COL reduces the incidence of VAP.**

Keywords: chlorhexidine; ventilator-associated pneumonia; prevention

Ventilator-associated pneumonia (VAP) is among the most frequently occurring nosocomial infections. According to data from the National Nosocomial Infection Surveillance System, VAP is the second most common nosocomial infection, after urinary tract infection, affecting approximately 27% of all critically ill patients (1). In Europe, VAP and lower respiratory tract infections accounted for 65% of all infections in the European Prevalence of Infections in Intensive Care study (2). VAP is associated with increased morbidity and high mortality rates (3). As a result, the majority of antibiotic use in intensive care units (ICUs) is prescribed for treatment of respiratory tract infections (4, 5), and estimated costs attributable to VAP were $11,897 and $15,893 in an American and European setting, respectively (6, 7). Therefore, prevention of VAP could be a life-saving and cost-effective measure.

Oropharyngeal colonization with potentially pathogenic microorganisms, a wide range of gram-negative and gram-positive microorganisms, is pivotal in the pathogenesis of VAP (8–10). Several strategies to prevent oropharyngeal colonization have been evaluated. Application of nonabsorbable antibiotics, either in a solution or paste, to the oropharyngeal cavity was associated with significant reductions of VAP in two placebo-controlled, double-blind studies (11, 12). However, continuous prophylactic use of antibiotics enhances the risk of induction and selection of resistant pathogens, and has therefore not been recommended (13).

Antiseptics or antimicrobial peptides with limited therapeutic use, such as chlorhexidine (CHX) and colistin (COL), could be attractive alternatives for oropharyngeal decontamination. The antiseptic CHX has a broad range of activity against gram-positive microorganisms (14), including multiresistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE), although activity against gram-negative microorganisms may be less optimal (15). COL is a polymyxin with high activity against gram-positive and gram-negative microorganisms that has been extensively used in topical and nebulized applications, with remarkably little resistance development (16–18).

CHX oral rinse reduced the incidence of VAP in a low-risk population of cardiac-surgical patients (19), but has not been evaluated in long-term mechanically ventilated ICU patients. We hypothesized that oropharyngeal decontamination with CHX would reduce incidences of VAP in mechanically ventilated patients at high risk. Because of a potential lower efficacy of CHX against gram-negative microorganisms, a second study group receiving the combination of CHX/COL was included as well.

METHODS

Study Settings and Patients

The trial was conducted in two university hospitals (two mixed and two surgical ICUs) and three general hospitals (all mixed ICUs) in

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the Netherlands. Consecutive adult patients (> 18 yr of age) needing mechanical ventilation for at least 48 h were included within 24 h after intubation and start of mechanical ventilation. Exclusion criteria were a preadmission immunocompromised status (defined as leucopenia < 3.10^9/L, cumulative dose of > 750 mg corticosteroids/yr, or HIV), pregnancy, and if the physical condition did not allow oral application of study medication. All participating ICUs had standard care protocols in which a semirecumbent body position with head elevation of 30° or greater was maintained if possible. Selective decontamination of the digestive tract (SDD) and continuous aspiration of subglottal contents were not performed in any patient.

The institutional review board committee of each participating hospital approved the study protocol. Written, informed consent was obtained from all participants or a legal representative.

Study Design and Blinding

This was a randomized, double-blind, placebo-controlled trial. Eligible patients were randomly assigned to one of three study groups by a computerized randomization schedule. Randomization was stratified per hospital. Trial medication (CHX 2% in petroleum jelly [Vaseline] FNA, CHX 2% with COL 2% in Vaseline FNA, and Vaseline FNA) was produced and labeled by the Department of Clinical Pharmacy of the University Hospital Maastricht. Experimental and placebo pastes were tasteless and of comparable smell and consistency. Trial medication was administered four times daily, after removing remnants of the previous dose with a gauze moistened with saline (NaCl 0.9%). Approximately 2 cm of paste, approximately 0.5 g, was put on a gloved fingertip and administered to each side of the buccal cavity. This method was taught to each nurse on the participating wards to prevent differences in distribution/application of the trial medication. Oral swabs were obtained after clearing and before administration of new medication.

Study participation ended in case of diagnosis of VAP, death, extubation, or withdrawal of consent.

Definitions

Because bronchoscopy with quantitative cultures was not routinely performed in any of the participating ICUs (as in most Dutch ICUs), VAP was diagnosed on the clinical decision of treating physicians. In case of a clinical suspicion of VAP, bronchoscopy was advised but not mandatory. VAP diagnosis was substantiated by adjudication by three intensivists reviewing case record forms of all participating patients, while blinded for patient treatment and study center. They received patient information in sets of 10 patients and verified all diagnosis based on objective VAP criteria. These objective diagnostic criteria for VAP consisted of the presence of a new, persistent, or progressive infiltrate on chest X-ray in combination with at least three of four criteria: (*1*) rectal temperature greater than 38.0° C or less than 35.5° C, (*2*) blood leukocytosis ($> 10 \times 10^3/mm^3$) and/or left shift or leukopenia ($< 3 \times 10^3/mm^3$), (*3*) purulent aspect of tracheal aspirate, and (*4*) a positive semiquantitative culture from tracheal aspirates (cutoff $\geq 10^5$ cfu/ml) occurring after 48 h of mechanical ventilation. In case of different interpretation, consensus was reached through telephone conversations. Furthermore, clinical pulmonary infection scores (CPISs) of all patients were calculated daily.

Microbiology

Oropharyngeal swabs were collected daily, after removal of remnants and before application of new study medication. Swabs, kept in charcoal medium, were transported to a central laboratory (University Medical Centre Utrecht) and processed within 3 d after samples had been obtained. Serial dilutions of swabs were plated on McConckey and Colombia CNA agar plates for quantitative analysis of gram-positive and gram-negative microorganisms, without further determination. Oropharyngeal colonization was defined as the presence of 10^5 cfu/ml or greater, corresponding to thresholds for quantitative analysis of tracheal aspirates (20).

Endotracheal aspirates were obtained on clinical indication or twice weekly if no clinical cultures were obtained. Samples were processed according to standard microbiological procedures and analyzed semiquantitatively, using the four-quadrant method (20). A cutoff of 10^5 cfu/ml or greater was used to define positive colonization (20). To prevent sample bias, endotracheal culture results were analyzed in time windows of 4 d. For each patient, the first sample obtained per time window was used for analysis.

Clinical Measurements

Demographic and clinical data such as sex, age, origin before ICU admission, medical history, clinical admission diagnosis, APACHE II (Acute Physiology and Chronic Health Evaluation) score (21), presence of infection at time of admission, medication use, and reason for intubation were recorded. The following data were assessed daily: temperature; leukocyte counts; Pa_{O_2}/FI_{O_2} ratio; presence or absence of purulent tracheal secretions and quantity of tracheal secretions; medication use, including type and indication for antibiotic agents; chest radiograph results, and bacteriological results.

CPIS was calculated daily using the latest culture results available for each patient (22). The CPIS was assessed as an additional measure to determine objective criteria for the diagnosis VAP. Because Gram stains of endotracheal aspirates were not performed routinely in all centers, this item was left out, resulting in a maximum possible score of 11 points instead of 12.

Antibiotic use was analyzed in courses, which were defined as episodes of clinical or suspected infection for which antibiotics were prescribed. A change in antibiotics was only considered a separate course when the indication changed. Adjusting antibiotics because of microbial susceptibility results was therefore not considered a separate course.

Outcome Measures

The primary outcome measure was time to VAP. Secondary endpoints included oral colonization with gram-positive and gram-negative microorganisms, endotracheal colonization, and all-cause ICU mortality.

Number of Patients

Based on previous studies, it was expected that about 20% of the patients receiving placebo would develop VAP. Both treatments, CHX and CHX/COL, were expected to approximately halve this proportion. With time to VAP as the primary outcome variable, this corresponds to a hazard ratio (HR) of 0.444, requiring at least 81 events of VAP in at least 540 patients for the three arms of the trial (23) (one sided $\alpha = 0.025$; power = 0.80). A one-sided α was used, because placebo treatment was not considered to be able to prevent VAP. Furthermore, to adjust for the comparison of two treatment arms, each with the same placebo arm, we halved the normally used value for α of 0.05 for each comparison.

On average, a sequential analysis requires fewer patients to come to a decision than a fixed sample size analysis based on the same characteristics (HR, α, power). We designed a group sequential survival analysis using a triangular test (24). When a sequential analysis is designed, sample size is not fixed but can be estimated in the design phase of the trial. Assuming the null hypothesis ("no differences in VAP risk") is true, on average 36 events in 240 patients would be needed to conclude the trial. Assuming the alternative hypothesis ("the risk of VAP is approximately halved under real treatment") is true, on average 48 events in 321 patients would be needed to conclude the trial.

Statistical Analyses

The sequential survival analyses were stratified per center and, taking time until VAP into account, were performed by an independent statistician using the computer program PEST (Planning and Evaluation of Sequential Trials) (25). After each new group of about 10 patients, the cumulative data were analyzed. This resulted in a new point (x) in a Z/V graph (*see* Figures 2A and 2B). Z (on the vertical axis) is a measure for the effect size (i.e., the observed number of events in the placebo group minus the expected number given treatment equivalence). V is a measure for the amount of information processed thus far. In sequential survival analysis, V is approximately equal to one-quarter of the total number of VAPs observed in the data. Z is the well-known log rank test statistic and V is its variance assuming treatment equivalence. The cumulative analysis leads to one of three decisions:

1. Enough evidence is reached to stop the study and conclude that treatment significantly reduces the risk of VAP (the upper boundary of the triangle is crossed).

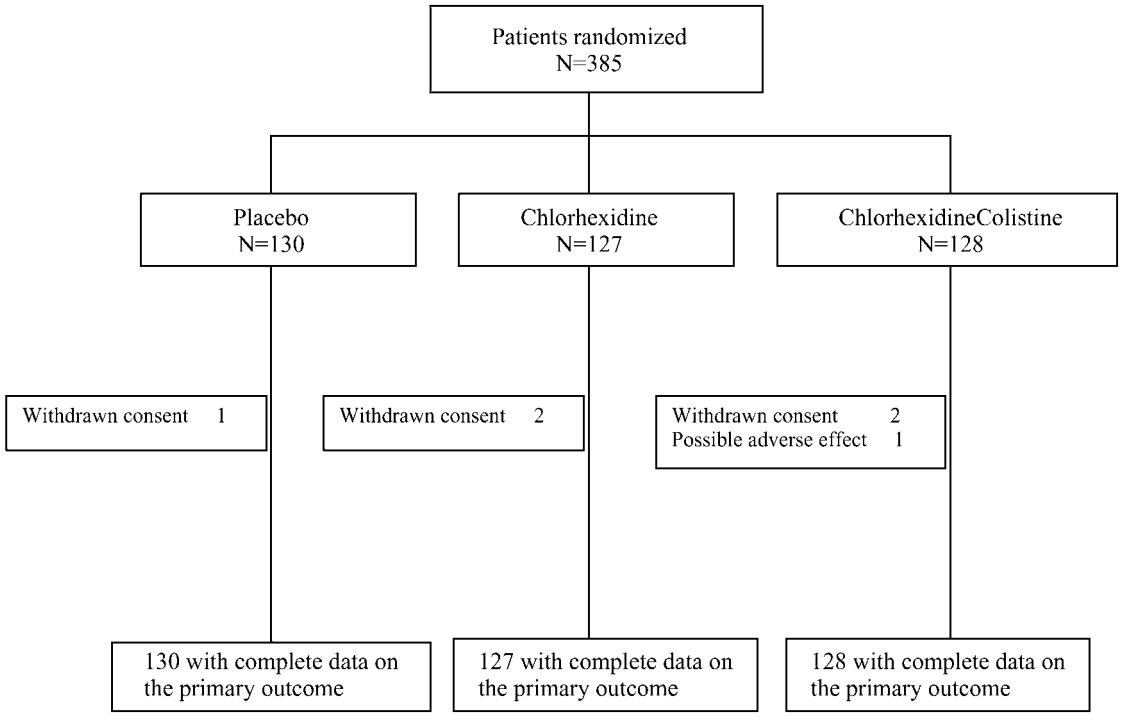

Figure 1. Disposition of patients, intention-to-treat analysis.

2. Enough evidence is reached to stop the study and conclude that treatment and placebo do not differ in their risk of VAP (the lower boundary of the triangle is crossed).
3. There is not enough evidence yet to stop the study, new data must be analyzed (the path of x's is still between the lower and upper boundary).

A sequential design guarantees the type I error (α) and the power whenever a decision to stop the study is made. The results (p values, estimates for the HR, and confidence intervals [CI]) were adjusted for the analysis of cumulative data (24).

Data are noted in absolute numbers with or without percentages, as means with standard deviations or as medians with ranges; *t* tests were used to compare continuous variables, whereas χ^2 tests were used to compare proportions. To determine the influence of baseline characteristics on VAP development, we performed a multivariate analysis in which all baseline characteristics (including oropharyngeal colonization) were used as covariates. Kaplan-Meier survival and Cox proportional hazard analyses were used to calculate the probability of remaining without VAP or death per patient, or chances of oral colonization taking time-at-risk into account. A one-sided significance level smaller than 0.025 was considered to denote statistical significance. All reported p values for the primary outcome measure are one-sided significance levels, whereas those for secondary outcome measures are two-sided. Statistical analysis was performed using SPSS (SPSS, Inc., Chicago, IL).

RESULTS

Patients

From February 2001 until March 2003, 385 patients were randomized. Baseline characteristics were comparable for all three groups (Table 1). The study was discontinued in six patients; in five cases (placebo [PLAC], 1 patient; CHX, 2 patients; CHX/COL, 2 patients) because patients refused to accept the oral paste (Figure 1). One patient (CHX/COL) was prematurely withdrawn from the trial protocol due to tongue edema on the second day of trial medication administration, which was interpreted as a possible adverse event. Based on random unobtrusive monitoring of application of trial medication, compliance was close to 100% (data not shown).

Primary Endpoint: VAP

VAP was diagnosed in 52 patients: 23 in the PLAC group (18%), 13 in the CHX-group (10%), and 16 in the combination group (CHX/COL; 13%). The independent experts, who reached consensus in each case, confirmed all VAP diagnoses established by the treating physicians. Mean CPISs were 3.8 $\pm$ 2.0 on 2,622 VAP-free patient-days and 6.2 $\pm$ 1.5 on Day 52 after VAP was diagnosed ($p < 0.001$). In those patients developing VAP, an increase in CPISs was observed on the day of diagnosis (median: CPIS, 6.0; range: 2–10) compared with the day before (median: CPIS, 4.2; range: 0–8; $p < 0.0001$ by Wilcoxon rank test).

The comparison of CHX with PLAC treatment was discontinued after analysis of 351 patients, with 115 patients under PLAC and 115 patients under CHX treatment. The upper boundary of the triangle was crossed (Figure 2A), indicating that the null hypothesis could be rejected ($p = 0.012$), with a corresponding HR equal to 0.352 (95% CI, 0.160, 0.791).

At the moment of trial discontinuation, a total of 385 patients had been included, 130 patients under PLAC and 128 under CHX/COL. At that point the upper boundary of the triangle of the comparison between CHX/COL and PLAC was approached (Figure 2B), with a corresponding HR equal to 0.454 (95% CI, 0.224, 0.925). These findings remained unchanged after multivariate analysis, with sex, pulmonary admission diagnosis, colonization status on admission, and antibiotic use on admission as covariates.

Secondary Endpoint: Colonization

For endotracheal colonization, the combination of CHX/COL was more effective than CHX, mainly because of better efficacy against gram-negative colonization. Within the first time window (Days 1–4), endotracheal cultures of 306 patients (79.5%) were analyzed (106 PLAC, 99 CHX, and 101 CHX/COL; Figure 3). There were no differences in colonization between the three groups (PLAC, 33%; CHX, 36.4%; and CHX/COL, 33.7%), with comparable colonization rates for gram-positive and gram-negative microorganisms and yeasts. In the second time window

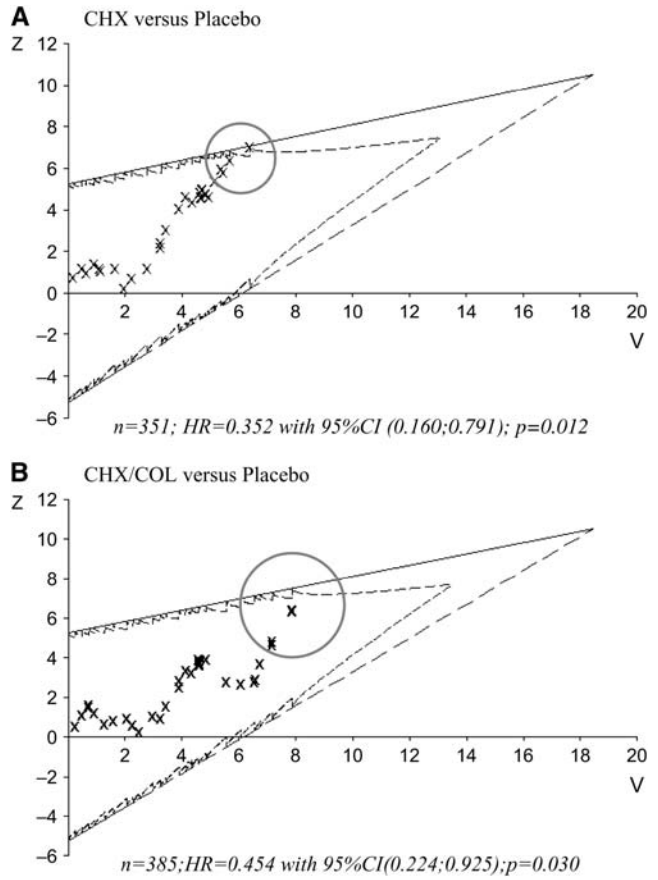

Figure 2. Results of the sequential survival analysis. The results of the comparison of chlorhexidine (CHX; *A*) and CHX + colistin (CHX/COL; *B*) treatment with placebo treatment are shown. The *horizontal axis* corresponds to the total amount of information (24). The *vertical axis* corresponds to the effect size.

TABLE 1. BASELINE CHARACTERISTICS OF ALL 385 PATIENTS

Characteristic	Placebo Group (*n = 130*)	CHX Group (*n = 127*)	CHX/COL Group (*n = 128*)
Age, yr (mean ± SD)	62.1 ± 15.9	60.9 ± 15.3	62.4 ± 19.1
Male sex, %	93 (72)	66 (52)	71 (56)
APACHE II score, mean ± SD	21.8 ± 7.43	22.2 ± 7.02	23.7 ± 7.38
Ulcer prophylaxis, %	23 (18)	34 (27)	25 (20)
Antibiotic treatment, %	41 (32)	47 (37)	46 (36)
Infection present, %	61 (47)	53 (42)	44 (34)
Medical history, %*			
Cardiovascular	69 (53)	55 (43)	52 (41)
Pulmonary	32 (25)	36 (28)	34 (27)
Head/neck surgery	5 (4)	1 (1)	5 (4)
Diabetes	12 (9)	16 (13)	15 (12)
Admission indication, %			
Cardiovascular	9 (7)	15 (12)	16 (13)
Pulmonary†	49 (38)	48 (38)	34 (27)
Neurologic	17 (13)	18 (14)	22 (17)
Gastro enteric	5 (4)	3 (2)	3 (2)
Trauma‡	12 (9)	10 (8)	16 (13)
Postoperative	29 (22)	18 (14)	22 (17)
Sepsis	6 (5)	7 (6)	10 (8)
Renal	1 (1)	2 (2)	1 (1)
Metabolic	2 (2)	5 (4)	3 (2)
Intubation indication, %			
GCS < 8	42 (32)	40 (32)	52 (41)
Respiratory insufficiency	88 (68)	87 (69)	76 (59)

Definition of abbreviations: APACHE = Acute Physiology and Chronic Health Evaluation; CHX = chlorhexidine; COL = colistin; GCS = Glasgow Coma Scale.
* More than one condition possible per patient.
† Including neurosurgery.
‡ Including neurotrauma.

(Days 5–8), 157 cultures of 198 patients in trial (79.3%) were analyzed. Endotracheal colonization was lower in CHX/COL when compared with PLAC (16 vs. 40%, p = 0.007) and to CHX (16 vs. 38%, p = 0.011), mainly due to a reduction of colonization with gram-negative bacteria. Both treatment groups had, as compared with PLAC, lower colonization rates in the third period (Figure 3), although statistical significance was not reached. Colonization with yeasts remained low in all three groups (ranging from 0 to 11.3%).

A total of 2,306 oropharyngeal swabs were obtained, representing 87% of all patient-days (placebo, 89.3%; CHX, 84.8%; and CHX/COL, 86.7%). With regard to oropharyngeal colonization, preventive effects of CHX/COL and CHX were comparable for gram-positive bacteria, but CHX/COL was more effective against gram-negative bacteria. Colonization rates with gram-negative microorganisms on admission were 52% for PLAC, 43% for CHX, and 41% for CHX/COL patients (p = 0.101 for CHX vs. PLAC, and p = 0.094 for CHX/COL vs. PLAC). As compared with placebo, application of both CHX/COL and CHX reduced oral colonization with gram-negative microorganisms, with daily hazards of 0.826 for CHX (95% CI, 0.719, 0.950; p = 0.007) and 0.44 for CHX/COL (95% CI, 0.373, 0.518; p < 0.001). The combination (CHX/COL) was more effective against gram-negative microorganisms than CHX alone, with daily hazards of 0.534 (95% CI, 0.455, 0.626; p < 0.001). Colonization with gram-positive microorganisms on admission was demonstrated in 85% in PLAC, 81% in CHX, and 78% in CHX/COL (p = 0.834 for CHX vs. PLAC, and p = 0.068 for CHX/COL vs. PLAC). CHX and CHX/COL were equally effective in reducing oral colonization with gram-positive microorganisms with HRs of 0.695 for CHX (95% CI, 0.606, 0.796; p < 0.001) and 0.732 (95% CI, 0.640, 0.838; p < 0.001) for CHX/COL (Figure 4).

Patient Outcome and Antibiotic Use

ICU mortality was comparable for all three groups: CHX compared with PLAC (HR, 1.12; 95% CI, 0.72, 1.17) and CHX/COL compared with PLAC (HR, 1.02; 95% CI, 0.66,1.59). In addition, duration of mechanical ventilation and lengths of stay in ICU or in hospital after ICU discharge were comparable in all three groups (Table 2).

Before ICU admission, systemic antibiotics had been prescribed to 41 patients in the PLAC group (32%), to 47 CHX patients (37%), and 46 patients receiving CHX/COL (36%). During the study, but before diagnosis of VAP, numbers of episodes of antibiotic use in the three study groups were comparable, with 107 episodes for PLAC patients, 113 episodes for patients receiving CHX, and 110 episodes in the CHX/COL group, yet proportions of antibiotic days were highest for patients receiving PLAC and lowest for patients in the CHX/COL group (Table 3). Treatment of VAP was not included in these antibiotic episodes. Antibiotic treatment of VAP was with appropriate antibiotics in all cases.

DISCUSSION

Oropharyngeal decontamination with either CHX or CHX/COL reduced and delayed the development of VAP in critically ill patients receiving mechanical ventilation. Daily risks of VAP decreased, with 65 and 55% for CHX and CHX/COL, respectively. The preventive effects on the occurrence of VAP were

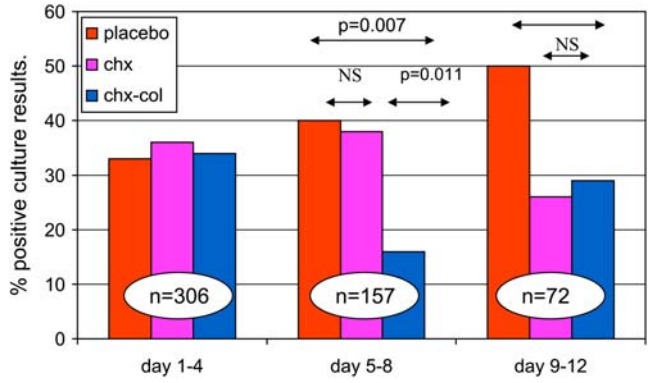

Figure 3. Endotracheal colonization. Proportion of patients colonized with either gram-positive or gram-negative microorganisms or both, in window periods of 4 d.

underscored by reduced colonization rates with gram-negative and gram-positive bacteria in oropharynx and trachea. Considering their low potential for induction and selection of antibiotic resistance and costs, oropharyngeal decontamination with CHX or CHX/COL is an attractive infection prevention measure.

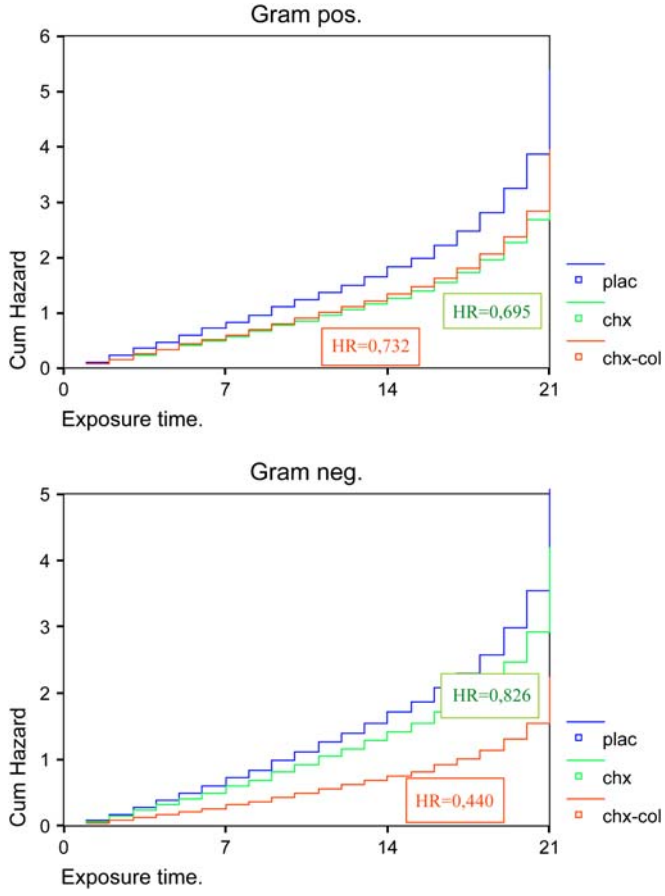

Figure 4. Hazard ratio (HR) for oral colonization with gram-positive and gram-negative microorganisms. CHX and CHX/COL were equally effective in reducing oral colonization with gram-positive microorganisms compared with placebo (PLAC; $p < 0.001$). CHX/COL was more effective against gram-negative microorganisms than CHX alone ($p < 0.001$).

There is a firm body of evidence that oropharyngeal colonization is pivotal in the pathogenesis of VAP. More than 25 yr ago, Johanson and coworkers described associations between increasing severity of illness, higher occurrence of oropharyngeal colonization, and increased risks to develop VAP (8, 9). Subsequently, cohort and sequential colonization analyses identified oropharyngeal colonization as a risk factor for VAP (26–28). Further evidence was provided by two randomized double-blind trials demonstrating reductions in oropharyngeal colonization and VAP incidence by topical application of antibiotics (11, 12). Yet, the benefits of these antibiotic-containing regimens must be balanced against the potential of increased selection of antibiotic-resistant pathogens. Ideally, oropharyngeal decontamination should be achieved with either antiseptics or antibiotic classes that are not used for patient treatment. In addition, such agents should have a low potential for induction and selection of antibiotic resistance. Both CHX and COL have excellent antibacterial effects, and resistance rates of nosocomial pathogens have remained exceptionally low, despite long-term use (16–18, 29). Up until now, COL has been used mainly as topical antibiotic in specialized patient populations. Yet, intravenous use of COL, as ultimate treatment option for multiresistant gram-negative nosocomial pathogens, has been reported with increasing frequency in recent years (30–32), reducing its attractiveness as a prophylactic agent. Our findings confirm and expand these previous observations regarding the pivotal role of oropharyngeal colonization in the pathogenesis of VAP and the potential of CHX, either alone or in combination with COL, in preventing and postponing the development of VAP. The latter is clinically relevant because it might protect patients against this nosocomial infection during the first week of ventilation when daily risks for VAP are highest (33).

The combination of COL and CHX resulted in better oropharyngeal decontamination for gram-negative microorganisms, but both regimens appeared equally effective in VAP prevention. Yet, due to the low incidence of VAP in both study groups, firm conclusions regarding any differences in efficacy cannot be reached. Possible explanations include more episodes of early-onset VAP in the combination group (7 vs. 2%), which may have been incubating before decontamination was achieved. In addition, a cutoff point of 10^5 cfu/ml may not have been sensitive enough, with persisting colonization below this threshold in the combination group and subsequent cases of VAP, despite effective oral decontamination.

The calculated costs of the interventions tested were less than $100 per patient (for 8 d, costs would include 20 min/d nursing time [± $40], medication [$11], and glove use [$3.20]). Previously, van Nieuwenhoven and coworkers determined, in a comparable patient population in the Netherlands, that prevention of VAP would be cost-saving if the relative risk for VAP due to intervention were less than 0.923, VAP incidence in the control group were more than 4%, and the costs of the intervention were less than $2,500 (7). With baseline VAP incidences of 18%, HRs for VAP of 0.454 and 0.352 for CHX/COL and CHX, respectively, and costs less than $100, the use of this strategy would be extremely cost-effective.

CHX oral rinse as a preventive measure for VAP has been evaluated before in two trials among cardiac-surgical patients (19, 34). DeRiso and coworkers found a reduction of respiratory tract infections of 69%, which included both upper and lower respiratory tract infections (19). Houston and coworkers compared CHX with a phenolic mixture in an open trial of 561 patients and reported a nonsignificant 52% reduction of nosocomial pneumonia (34). Statistical significance, however, was reached in a subgroup of 37 patients intubated for at least 24 h. Considering the specific patient population with low risks

TABLE 2. OUTCOME DATA OF THE STUDY PATIENTS FROM BOTH INTERVENTION GROUPS AND CONTROL PATIENTS

Variable	Placebo (*n* = *130*)	CHX (*n* = *127*)	p Value	CHX/COL (*n* = *128*)	p Value
Days studied, mean (SE)	5.93 (0.7)	8.44 (1.0)	0.036	7.66 (0.8)	NS
Days ventilated, mean (SD)	6.95 (8.1)	9.16 (12.0)	NS	8.52 (8.6)	NS
ICU stay, mean (SD)	12.45 (12.9)	13.77 (17.4)	NS	13.27 (18.2)	NS
Days in hospital after ICU discharge, mean (SD)	15.47 (22.7)	15.73 (37.9)	NS	13.0 (20.5)	NS
VAP	23	13		16	
Early onset* (48–96 h after intubation)	13 (0.10)	3 (0.02)	< 0.001	9 (0.07)	NS
Polymicrobial*	5 (0.04)	6 (0.05)	NS	3 (0.02)	NS
Gram-negative microorganisms*†	15 (0.12)	9 (0.07)	NS	9 (0.07)	NS
Enterobacteriaceae	8 (0.06)	7 (0.06)	NS	5 (0.04)	NS
Pseudomonas aeruginosa	4 (0.03)	0	0.046	2 (0.02)	NS
Hemophilus influenzae	4 (0.03)	2 (0.02)	NS	2 (0.02)	NS
Other	1 (0.01)	0	NS	0	NS
Gram-positive microorganisms*†	7 (0.05)	2 (0.02)	NS	5 (0.04)	NS
Staphylococcus aureus	5 (0.04)	2 (0.02)	NS	5 (0.04)	NS
Streptococcus species	2 (0.02)	1 (0.01)	NS	0	NS
Other	2 (0.02)	1 (0.01)	NS	0	NS
Gram-negative and -positive microorganisms*	1 (0.01)	1 (0.01)	NS	0	NS
Candida species*	1 (0.01)	3 (0.02)	NS	4 (0.03)	NS

Definition of abbreviations: CHX = chlorhexidine; COL = colistin; ICU = intensive care unit; NS = not significant; VAP = ventilator-associated pneumonia.

* In total numbers and percentages from total number of patients in each group.

† VAP can be caused by more species of gram-negative or gram-positive microorganisms.

for developing VAP due to short duration of intubation (in one study, 93% of the patients were detubated within 24 h [34]), these studies seemed not to be generalizable to high-risk intensive care populations. Among ventilated ICU patients, decontamination of gingival and dental plaque with a 0.2% CHX gel decreased dental plaque colonization, but failed to prevent development of VAP (35). The lower concentration of CHX used and application of gel only on teeth and gingiva instead of the buccal cavity might explain the different results as compared with our findings.

Several other preventive strategies for VAP also aimed at modulating oropharyngeal colonization or aspiration of oropharyngeal contents. Among the nonantibiotic measures, both reduction of aspiration with continuous aspiration of subglottal fluids and patient treatment in semirecumbent position appear to be effective (36, 37). Furthermore, SDD, with topical application of antibiotics in oropharynx and stomach in combination with a short course of systemic prophylaxis, is a powerful measure to reduce incidences of VAP, although its preventive effects were inversely related to the methodologic quality of studies (38). Nevertheless, because of simultaneous administration of antibiotics in the oropharynx and gastrointestinal tract, as well as systemically, the relative importance of oropharyngeal decontamination, or of any of the other components of SDD, can be determined. Yet, improved survival in patients receiving SDD was demonstrated recently (39). In a prospective trial, 943 patients were randomized to be treated in an ICU ward where all patients received SDD or in a control ward where none received SDD. ICU mortality was reduced by 35% in the SDD ward (39). Moreover, length of stay and total antibiotic costs were lower in the SDD ward and fewer pathogens were resistant for antibiotics. Although not determined, reduced infection rates seem the most logical explanation for these findings. Yet, methodologic issues, such as a preexisting survival benefit of patients treated in the SDD ward, and the exceptional high efficacy as compared with previous studies, warrant confirmation of these findings (40–42).

Conducting multicenter studies in critically ill patients remains a challenge, with a continuous conflict between the need for large patient numbers and the need for detailed data collection. Prospectively planned interim analyses may help to reduce patient numbers needed to reach sound conclusions on efficacy, as compared with the traditionally fixed sample sizes. Sequential survival analysis, as used in this trial, is a relatively new statistical technique, still infrequently used in intensive care trials. Sequential analysis resulted in a 29% reduction of patients needed in trial (1–385/540) and a 36% reduction of the needed number of events (1–52/81).

Some potential limitations of the trial need to be discussed. First, although we aimed to include all consecutive patients

TABLE 3. ANTIBIOTIC TREATMENT (ANTIBIOTIC DAYS/TOTAL NUMBER OF PATIENT-DAYS)

	Placebo (*n* = *779*)	CHX (*n* = *943*)	p Value	CHX/COL (*n* = *952*)	p Value
Penicillines	334 (0.43)	393 (0.42)	NS	413 (0.43)	NS
Cephalosporines	120 (0.15)	146 (0.15)	NS	140 (0.15)	NS
Carbapenems	50 (0.06)	50 (0.05)	NS	51 (0.05)	NS
Macrolides	75 (0.10)	73 (0.08)	NS	70 (0.07)	NS
Clindamycin	17 (0.02)	17 (0.02)	NS	11 (0.010	NS
Aminoglycosides	66 (0.08)	99 (0.10)	NS	90 (0.09)	NS
Quinolones	57 (0.07)	99 (0.10)	0.022	70 (0.07)	NS
Total antibiotic days	412 (0.53)	463 (0.49)	NS	421 (0.44)	< 0.001

Definition of abbreviations: CHX = chlorhexidine; COL = colistin; NS = not significant.

n = total number of patient days.

fulfilling the inclusion criteria, actual recruitment depended on the willingness of responsible physicians, and a formal assessment, on a daily basis, on the proportion of all eligible patients that were included was not performed. Moreover, the prediction of whether a patient was expected to remain ventilated for more than 48 h was also left to the discretion of the responsible physician. Considering the low patient numbers succumbing or being extubated within 48 h (9 among PLAC patients, 17 in the CHX group, and 16 in the combination group), physicians successfully selected these patients, but it is unknown how many patients were excluded who actually remained intubated for more than 48 h. This may, to some extent, call into question the generalizibility of our trial population.

Second, VAP was diagnosed on clinical, microbiological, and radiographic criteria. Although the combination of these criteria has a high sensitivity, specificity is low (43). As a result, incidences of VAP may have been overestimated. However, due to the double-blind trial design, all three study groups were equally affected, and random misclassification of the outcome always leads to an underestimation of the true effectiveness. Two additional scoring criteria were used to substantiate VAP diagnoses: adjudication of all daily parameters related to VAP diagnosis by three intensivists blinded for trial randomization, as well as participating ICU, and daily CPISs. Clinical judgments of treating physicians and adjudication judgments of blinded intensivists correlated well, and CPISs were significantly higher on the days of VAP diagnosis, with a substantial rise in CPIS on the day before diagnosis was established. Finally, topical antimicrobial agents applied in the oropharynx may influence culture results of endotracheal aspirates. Gastinne and colleagues (44) found measurable quantities of antibiotics in tracheal aspirates of patients receiving SDD, although Bergmans and coworkers (12) did not in patients receiving similar oropharyngeal prophylaxis. We did not determine CHX or COL levels in endotracheal aspirates.

Furthermore, our study was not designed, and thus underpowered, to determine effects on patient survival. Despite clear associations between occurrence of VAP and mortality in ICU patients (3), estimates of attributable mortality due to VAP have ranged from 0 to 50% (45–49). Inappropriate empiric antimicrobial treatment is an important determinant for attributable mortality due to VAP (50). Because all patients with VAP received appropriate treatment, attributable mortality in our population is probably low and much larger patient populations would be needed to determine differences in patient outcome.

Finally, the microbial ecology of the participating ICUs may question the generalizability of our findings. In all units, as in most Dutch ICUs, prevalence rates of MRSA and VRE were extremely low. However, considering reported antimicrobial efficacy of CHX and COL for gram-negative and gram-positive nosocomial pathogens (14), including MRSA (51), these regimens should be equally effective in settings with higher resistance levels as in our ICUs.

In conclusion, modulation of oropharyngeal colonization with CHX and CHX/COL reduced the daily probability of VAP. Considering the growing role of COL as ultimate treatment of multiresistant gram-negative bacteria, CHX seems to be preferred for preventive implications. The safety profile regarding selection and induction of antibiotic resistance and the presumed cost benefits of CHX make it highly attractive for prevention of VAP.

Conflict of Interest Statement: None of the authors have a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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References

1. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in medical intensive care units in the United States. National Nosocomial Infections Surveillance System. *Crit Care Med* 1999;27: 887–892.
2. Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, Wolff M, Spencer RC, Hemmer M. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European Prevalence of Infection in Intensive Care (EPIC) study. EPIC International Advisory Committee. *JAMA* 1995;274:639–644.
3. Chastre J, Fagon JY. Ventilator-associated pneumonia. *Am J Respir Crit Care Med* 2002;165:867–903.
4. Bergmans DCJJ, Bonten MJM, Gaillard CA, van Tiel FH, van der Geest S, de Leeuw PW, Stobberingh EE. Indications for antibiotic use in ICU patients: a one-year prospective surveillance. *J Antimicrob Chemother* 1997;39:527–535.
5. Heyland DK, Cook DJ, Griffith L, Keenan SP, Brun-Buisson C. The attributable morbidity and mortality of ventilator-associated pneumonia in the critically ill patient. The Canadian Critical Trials Group. *Am J Respir Crit Care Med* 1999;159:1249–1256.
6. Warren DK, Shukla SJ, Olsen MA, Kollef MH, Hollenbeak CS, Cox MJ, Cohen MM, Fraser VJ. Outcome and attributable cost of ventilator-associated pneumonia among intensive care unit patients in a suburban medical center. *Crit Care Med* 2003;31:1312–1317.
7. van Nieuwenhoven CA, Buskens E, Bergmans DC, van Tiel FH, Ramsay G, Bonten MJ. Oral decontamination is cost-saving in the prevention of ventilator-associated pneumonia in intensive care units. *Crit Care Med* 2004;32:126–130.
8. Johanson WG Jr, Pierce AK, Sanford JP, Thomas GD. Nosocomial respiratory infections with Gram-negative bacilli: the significance of colonization of the respiratory tract. *Ann Intern Med* 1972;77:701–706.
9. Johanson WG Jr, Pierce AK, Sanford JP. Changing pharyngeal bacterial flora of hospitalized patients: emergence of gram-negative bacilli. *N Engl J Med* 1969;281:1137–1140.
10. Bonten MJM, Gaillard CA, de Leeuw PW, Stobberingh EE. Role of colonization of the upper intestinal tract in the pathogenesis of ventilator-associated pneumonia. *Clin Infect Dis* 1997;24:309–319.
11. Pugin J, Auckenthaler R, Lew DP, Suter PM. Oropharyngeal decontamination decreases incidence of ventilator-associated pneumonia: a randomized, placebo-controlled, double-blind clinical trial. *JAMA* 1991; 265:2704–2710.
12. Bergmans DC, Bonten MJ, Gaillard CA, Paling JC, van der Geest S, van Tiel FH, Beysens AJ, de Leeuw PW, Stobberingh EE. Prevention of ventilator-associated pneumonia by oral decontamination: a prospective, randomized, double-blind, placebo-controlled study. *Am J Respir Crit Care Med* 2001;164:382–388.
13. Tablan OC, Anderson LJ, Besser R, Bridges C, Hajjeh R. Guidelines for preventing health-care–associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. *MMWR Recomm Rep* 2004;53:1–36.
14. Emilson CG. Susceptibility of various microorganisms to chlorhexidine. *Scand J Dent Res* 1977;85:255–265.
15. Hammond SA, Morgan JR, Russell AD. Comparative susceptibility of hospital isolates of gram-negative bacteria to antiseptics and disinfectants. *J Hosp Infect* 1987;9:255–264.
16. Katz SL, Ho SL, Coates AL. Nebulizer choice for inhaled colistin treatment in cystic fibrosis. *Chest* 2001;119:250–255.
17. Jensen T, Pedersen SS, Garne S, Heilmann C, Hoiby N, Koch C. Colistin inhalation therapy in cystic fibrosis patients with chronic Pseudomonas aeruginosa lung infection. *J Antimicrob Chemother* 1987;19:831–838.
18. Catchpole CR, Andrews JM, Brenwald N, Wise R. A reassessment of the in-vitro activity of colistin sulphomethate sodium. *J Antimicrob Chemother* 1997;39:255–260.
19. DeRiso AJ, Ladowski JS, Dillon TA, Justice JW, Peterson AC. Chlorhexidne gluconate 0.12% oral rinse reduces the incidence of total nosocomial respiratory infection and nonprophylactic systemic antibiotic use in patients undergoing heart surgery. *Chest* 1996;109:1556–1561.
20. Bergmans DCJJ, Bonten MJM, de Leeuw PW, Stobberingh EE. Reproducibility of quantitative cultures of endotracheal aspirates from mechanically ventilated patients. *J Clin Microbiol* 1997;35:796–798.
21. Knaus WA, Draper EA, Wagner DP, Zimmerman JE. APACHE II: a severity of disease classification system. *Crit Care Med* 1985;13:818–829.
22. Pugin J, Auckenthaler R, Mili N, Janssens JP, Lew PD, Suter PM. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of

bronchoscopic and nonbronchoscopic "blind" bronchoalveolar lavage fluid. *Am Rev Respir Dis* 1991;143:1121–1129.
23. Freedman LS. Tables of the number of patients required in clinical trials using the logrank test. *Stat Med* 1982;1:121–129.
24. Whitehead J. The design and analysis of sequential clinical trials, rev. 2nd ed. Chinchester, UK: John Wiley & Son; 1997.
25. MPS Research Unit. PEST 4: operating manual. Reading, UK: The University of Reading; 2000.
26. Bonten MJM, Bergmans DCJJ, Ambergen AW, de Leeuw PW, van der Geest S, Stobberingh EE, Gaillard CA. Risk factors for pneumonia, and colonization of respiratory tract and stomach in mechanically ventilated ICU patients. *Am J Respir Crit Care Med* 1996;154:1339–1346.
27. Garrouste-Org M, Chevret S, Arlet G, Marie O, Rouveau M, Popoff N, Schlemmer B. Oropharyngeal or gastric colonization and nosocomial pneumonia in adult intensive care unit patients: a prospective study based on genomic DNA analysis. *Am J Respir Crit Care Med* 1997; 156:1647–1655.
28. Violán JS, Fernández JA, Bordes-Benítez A, Cardenosa-Cendrero JA, de Castro FR. Impact of quantitative invasive diagnostic techniques in the management and outcome of mechancally ventilated patients with suspected pneumonia. *Crit Care Med* 2000;28:2737–2741.
29. Russell AD, Day MJ. Antibacterial activity of chlorhexidine. *J Hosp Infect* 1993;25:229–238.
30. Linden PK, Kusne S, Coley K, Fontes P, Kramer DJ, Paterson D. Use of parenteral colistin for the treatment of serious infection due to antimicrobial-resistant Pseudomonas aeruginosa. *Clin Infect Dis* 2003;37:e154–e160.
31. Stein A, Raoult D. Colistin: an antimicrobial for the 21st century? *Clin Infect Dis* 2002;35:901–902.
32. Levin AS, Barone AA, Penco J, Santos MV, Marinho IS, Arruda EA, Manrique EI, Costa SF. Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant Pseudomonas aeruginosa and Acinetobacter baumannii. *Clin Infect Dis* 1999;28:1008–1011.
33. Cook DJ, Walter SD, Cook RJ, Griffith LE, Guyatt G, Leasa D, Jaeschke RZ, Brun-Buisson C. Incidence and risk factors for ventilator-associated pneumonia in critically ill patients. *Ann Intern Med* 1998; 129:433–440.
34. Houston S, Hougland P, Anderson JJ, LaRocco M, Kennedy V, Gentry LO. Effectiveness of 0.12% chlorhexidine gluconate oral rinse in reducing prevalence of nosocomial pneumonia in patients undergoing heart surgery. *Am J Crit Care* 2002;11:567–570.
35. Fourrier F, Dubois D, Pronnier P, Herbecq P, Leroy O, Desmettre T, Pottier-Cau E, Boutigny H, Di Pompeo C, Durocher A, *et al.*; PIRAD Study Group. Effect of gingival and dental plaque antiseptic decontamination on nosocomial infections acquired in the intensive care unit: a double-blind placebo-controlled multicenter study. *Crit Care Med* 2005;33:1728–1735.
36. Collard HR, Saint S, Matthay MA. Prevention of ventilator-associated pneumonia: an evidence-based systematic review. *Ann Intern Med* 2003;138:494–501.
37. Dodek P, Keenan SP, Cook D, Heyland DK, Jacka MJ, Hand L, Muscedere J, Foster D, Mehta N, Hall R, *et al.* Evidence-based clinical practice guideline for the prevention of ventilator-associated pneumonia. *Ann Intern Med* 2004;141:303–313.
38. van Nieuwenhoven CA, Buskens E, van Tiel FH, Bonten MJM. Relationship between methodological trial quality and the effects of selective digestive decontamination on pneumonia and mortality in critically ill patients. *JAMA* 2001;286:335–340.
39. de Jonge E, Schultz MJ, Spanjaard L, Bossuyt PM, Vroom MB, Dankert J, Kesecioglu J. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. *Lancet* 2003;362:1011–1016.
40. Verbrugh A. Selective decontamination of digestive tract in intensive care. *Lancet* 2003;362:2117–2118.
41. Kim SW, Kami M, Kobayashi K, Takaue Y, Honda O. Selective decontamination of digestive tract in intensive care. *Lancet* 2003;362:2118–2120.
42. Bonten MJ, Kluytmans J, de Smet AM, Bootsma M, Hoes A. Selective decontamination of digestive tract in intensive care. *Lancet* 2003;362: 2118–2119.
43. Pingleton SK, Fagon JY, Leeper KV. Patient selection for clinical investigation of ventilator-associated pneumonia: criteria for evaluating diagnostic techniques. *Chest* 1992;102:553S–556S.
44. Gastinne H, Wolff M, Lachatre G, Boiteau P, Savy FP. Antibiotic levels in bronchialtree and serum during selective digestive decontamination. *Intensive Care Med* 1991;17:215–218.
45. Fagon JY, Chastre J, Vaugnat A, Trouillet JL, Novara A, Gibert C. Nosocomial pneumonia and mortality among patients in intensive care units. *JAMA* 1996;275:866–869.
46. Fagon JY, Chastre J, Hance AJ, Montravers P, Novara A, Gibert C. Nosocomial pneumonia in ventilated patients: a cohort study evaluating attributal mortality and hospital stay. *Am J Med* 1993;94:281–288.
47. Bregeon F, Papazian L, Visconti A, Gregoire R, Thirion X, Gouin F. Relationship of microbiologic diagnostic criteria to morbidity and mortality in patients with ventilator-associated pneumonia. *JAMA* 1997; 277:655–662.
48. Papazian L, Bregeon F, Thirion X, Gregoire R, Saux P, Denis JP, Perin G, Charrel J, Dumon JF, Affray JP, *et al.* Effect of ventilator-associated pneumonia on mortality and morbidity. *Am J Respir Crit Care Med* 1996;154:91–97.
49. Bonten MJM, Froon AHM, Gaillard CA, Greve JWM, de Leeuw PW, Drent M, Stobberingh EE, Buurman WA. The systemic inflammatory respons in the development of ventilator-associated pneumonia. *Am J Respir Crit Care Med* 1997;156:1105–1113.
50. Kollef MH, Ward S. Inadequate antimicrobial treatment: an important determinant of outcome for hospitalized patients. *Clin Infect Dis* 2000;31:S131–S138.
51. Wilcox MH, Hall J, Gill AB, Fawley WN, Parnell P, Verity P. Effectiveness of topical chlorhexidine powder as an alternative to hexachlorophane for the control of Staphylococcus aureus in neonates. *J Hosp Infect* 2004;56:156–159.