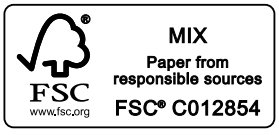
Airway management and respiratory tract colonization in intensive care patients

Irene Jongerden

2011



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Airway management and respiratory tract colonization

in intensive care patients

Luchtwegmanagement en kolonisatie van de luchtwegen
in intensive care patiënten
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 24 mei 2011 des middags te 2.30 uur

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Voor mijn ouders
En 'mijn mannen'

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CHAPTER 1

General introduction

Introduction

The majority of patients admitted to Intensive Care Units (ICUs) require an artificial airway and mechanical ventilation (MV). Reasons for this can be trauma, acute respiratory failure or the need for airway protection because of low consciousness. An important aspect of airway management in these patients is to apply oral hygiene (teeth brushing and clearing the oral cavity with a dental swab) and to remove secretions by endotracheal suctioning, one of the most common invasive procedures performed by ICU nurses [1,2]. By endotracheal suctioning, secretions are cleared from the tracheobronchial tree in order to guarantee optimal oxygenation and to prevent accumulation of secretions, tube occlusion, increased work of breathing, atelectasis and pulmonary infections [1-6]. Although necessary, the procedure is invasive, uncomfortable and potentially hazardous. It is associated with a number of complications, among them hypoxemia, nosocomial pneumonia, and microbial contamination of the patients' airway and inanimate environment [7,8]. These complications are associated with increased morbidity and mortality, an increased length of stay in hospital and increased costs.

Traditionally, ES was be performed with a single-use open suction system (OSS), which necessitated disconnection of the patient from the ventilator and introducing a single-use suction catheter into the patient's endotracheal tube. Since the late 1980s, a closed suction system (CSS) was introduced, which did not require disconnection from the ventilator and could be used multiple times for at least 24 hours, depending on CSS type and hospital protocol [9,10]. Since its introduction, CSS has become increasingly popular, mainly because of (assumed) advantages, like lower incidence of ventilator-associated pneumonia (VAP), fewer physiologic disturbances, reduced contamination of the environment, personnel and patients, and lower costs as compared to OSS.

Apart from allowing optimal oxygenation, airway management is important in preventing nosocomial infections. In mechanically ventilated ICU patients the respiratory tract can be a reservoir of antibiotic-resistant bacteria, including Gram-negative bacteria (GNB), which frequently proceeds from harmless colonization to invasive infection. Infections of the respiratory tract, especially ventilator-associated pneumonia (VAP, defined as pneumonia occurring more than 48 hours after endotracheal intubation and initiation of MV) are among the most frequently occurring ICU-acquired infections [11,12]. The occurrence of VAP has been associated with increased morbitidy, mortality and health care costs [13-15]. The detrimental effects on patient outcome and associated health care costs are even more pronounced for infections caused by antibiotic-resistant micro-organisms.

Occurrence of respiratory tract infections is almost always preceded by colonization of the oropharynx [16-18]. Acquisition of colonization can occur either endogenously or exogenously. Endogenous acquisition implies that a patient was already colonized on admission to the ICU,

but that bacterial numbers at that time were too low to be detected. Exogenous acquisition implies the occurrence of cross-transmission of micro-organisms [19]. Adherence to hand hygiene and other infection control measures is considered crucial for preventing cross-transmission. However, compliance rates among health care workers are often low [20-22] and, therefore, additional measures are urgently needed to reduce cross transmission rates. If the use of CSS indeed, as assumed, reduces contamination of the patient, health care worker and inanimate environment, it may well reduce cross-transmission, and would serve as an easy to implement and extremely feasible infection prevention measure. Currently, routine use of CSS has not been included in infection prevention guidelines due to inconclusiveness of evidence.

Despite its increasing popularity in the past decades, recommendations for the routine use of CSS have remained controversial because of inconclusive study results. Nurses in favour of OSS argued that the single use system removes secretions better, and thereby improves oxygenation. Others advocate the use of CSS for hygienic reasons (no spray of aerosols when disconnecting the patient from the ventilator) and the 'readiness-to-use' of this system. Because of the existing controversy, Dutch ICU nurses were wondering whether there was sufficient scientific evidence to prefer one ES system over the other. This question (and the results of the review that followed) initiated the studies that are described in the first part of this thesis.

Outline of this thesis

This thesis addresses scientific approaches to improve airway management, and in particular on ES system, in mechanically ventilated ICU patients. Furthermore it addresses interventions to reduce acquisition of respiratory tract colonization with GNB in this patient population.
In Chapter 2, as an introduction, studies comparing CSS and OSS have been systematically reviewed. Based on this meta-analysis a clinical trial was designed with a focus on two main assumptions concerning CSS. The objectives were to determine the (cost-) effectiveness of CSS, as compared to OSS, in reducing acquisition and cross-transmission of gram-negative bacteria in ICU (chapter 3), and to quantify changes in physiologic variables when performing endotracheal suctioning with either CSS and OSS (chapter 4).
During the trial, surveillance cultures from the respiratory tract were obtained from all ICU patients every Monday and Thursday, regardless whether they were on MV or not, during 14 months. The microbiological results from these cultures allowed a detailed analysis of risk factors for acquiring respiratory tract colonization with GNB (chapter 5) and acquisition of antibiotic resistance in two selected GNB, *Pseudomonas aeruginosa* and *Enterobacter* species (chapter 6).
In chapter 7 we have investigated the population structure of *P. aeruginosa* isolated from patients in the four ICUs that participated in the trial and compared this population structure to that of isolates from cystic fibrosis patients.

Chapter 8 is the only one not directly linked to the main trial of this thesis. In chapter 8 we investigated opinions of physicians and nurses on selective decontamination of the digestive tract (SDD) and selective oropharyngeal decontamination (SOD). Through questionnaires, expectations and assumptions on these interventions, that aim to reduce respiratory tract infections, were determined.
The results of all studies are summarized and discussed in chapter 9, together with some general directions and suggestions for clinical guidelines and future research.

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PART I

Airway Management

CHAPTER 2

Open and closed endotracheal suction systems in mechanically ventilated intensive care patients: A meta analysis

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Critical Care Medicine 2007, 35 (1), 260 – 270

Abstract

Background
Closed suction systems (CSS) are increasingly replacing open suction systems (OSS) to perform endotracheal suctioning in mechanically ventilated intensive care patients. Yet effectiveness regarding patient safety and costs of these systems has not been carefully analyzed.

Objective
To review effectiveness of CSS and OSS, with respect to patient outcome, bacterial contamination, and costs in adult intensive care patients.

Data sources
Search of MEDLINE, CINAHL, EMBASE and Cochrane databases and a manual review of article bibliographies.

Study selection
Randomised controlled trials comparing CSS and OSS in adult intensive care patients were retrieved.

Data extraction/synthesis
Assessment of abstracts and study quality was performed by two reviewers. Data were combined in meta-analyses by random effect models. Fifteen trials were identified. No significant differences were found in incidences of ventilator-associated pneumonia (eight studies, 1,272 patients) and mortality (four studies, 1,062 patients). No conclusions could be drawn with respect to arterial oxygen saturation (five studies, 109 patients), arterial oxygen tension (two studies, 19 patients), and secretion removal (two studies, 37 patients). Compared with OSS, endotracheal suctioning with CSS significantly reduced changes in heart rate (four studies, 85 patients; weighted mean difference, –6.33; 95% confidence interval, –10.80 to –1.87) and changes in mean arterial pressure (three studies, 59 patients; standardised mean difference, –0.43; 95% confidence interval, –0.87 to 0.00) but increased colonisation (two studies, 126 patients, relative risk, 1.51; 95% confidence interval, 1.12 to 2.04). CSS seems to be more expensive than OSS.

Conclusions
Based on the results of this meta-analysis, there is no evidence to prefer CSS more than OSS.

Introduction

Endotracheal suctioning (ES) is an essential and frequently performed procedure for patients requiring mechanical ventilation (MV). By ES, secretions from the tracheobronchial tree are cleared, guaranteeing optimal oxygenation and avoiding accumulation of secretions, leading to tube occlusion, increased work of breathing, atelectasis, and pulmonary infections [1-7]. Yet ES may also have adverse effects, such as disturbances in cardiac rhythm, hypoxemia (due to interruption of the mechanical ventilation and subsequently the decay of intrathoracic pressure), microbial contamination of airway and environment, and development of ventilator-associated pneumonia (VAP).

The frequency with which ES is performed differs per patient, with reported mean values varying from eight to 17 times per day [1,8-13]. Nowadays, two systems are available to perform ES: the single use, open suction system (OSS) and the multiple use, closed suction system (CSS). OSS requires disconnection from the ventilator during ES, which is not necessary when using CSS. Moreover, in contrast to OSS, the closed suction catheter can remain connected to the patient for as long as 24 hrs, according to the manufacturer, and thus can be used for multiple ES procedures [14].

CSS has become increasingly popular in the past decade. In the United States, 58% and 4% of intensive care units (ICUs) exclusively used CSS and OSS, respectively [15].

Preference of CSS more than OSS is mainly based upon assumed advantages, like lower incidence of VAP, fewer physiologic disturbances, decreased microbial contamination (and thus lower risk on cross-infections), and lower costs [8,10,16]. In a recently published international guideline for the prevention of VAP, it was suggested that cost considerations favor the use of CSS that is changed as indicated, and the system is therefore recommended. This advise, however, is based on one trial that compared costs of CSS with or without daily changes of the system; trials on cost-effectiveness of CSS compared with OSS are lacking[17].

So far, the evidence to prefer CSS more than OSS has not been systematically reviewed. Therefore, we performed a meta-analysis in which we compared the effectiveness of CSS with that of OSS with respect to infection and survival, cardio-respiratory variables, bacterial contamination, and costs.

Methods

Design

A comprehensive literature search was performed in PubMed, CINAHL, EMBASE and the Cochrane Library and by hand searching bibliographies of retrieved articles. For the Cochrane Library, the Database of Systematic Reviews, the Database of Abstracts of Reviews of Effects (DARE), and the Cochrane Central Register of Controlled Trials (CENTRAL) were searched.

The following keywords were used: *intubation, intratracheal [MeSH]; trachea [MeSH]; respiration, artificial [MeSH]; suction [MeSH]; endotracheal suction*; closed suction**. The search was confined to randomized controlled trials with human adults. The latest search was performed in May 2006.

Two reviewers independently assessed abstracts of the identified references to identify relevant studies for inclusion. Full reports were retrieved from all studies that fulfilled the inclusion criteria, that is, including adult mechanically ventilated ICU patients, comparing CSS and OSS, and measuring outcomes with respect to either infection and survival, cardio-respiratory variables, bacterial contamination, or costs. Furthermore, the randomization procedure was critically appraised. To prevent manipulation of the allocation process, the method of assigning patients to either CSS or OSS should be adequately concealed for both patient and clinician (health care worker). This method was judged by two reviewers without masking of author or source, using four ratings for quality of allocation concealment [18]:

A. Adequate concealment of the allocation
B. Uncertainty about adequate concealment of allocation
C. Allocation definitely not adequately concealed
D. Allocation concealment not used.

Discrepancies in ratings were resolved through discussion between reviewers. No additional information was sought from the original authors.

Data analysis

From each study, data were extracted on the outcomes measured. For continuous outcomes on cardio-respiratory variables, data during and after ES were extracted when provided. When data were obtained several moments after ES, the worst values were selected.

The synthesis of data was performed using random effect models. These models are preferable, since performance of ES differs between units and even nurses. For dichotomous outcomes, relative risks (RR) were calculated. For continuous outcomes, both weighted mean difference (for outcomes measured on the same scale) and standardized mean difference (outcomes measured on different scales, e.g., assessing mean arterial pressure (MAP) by using invasive or noninvasive techniques) were calculated. All effect measures were reported with 95% confidence intervals (CI).

To assess heterogeneity of treatment effects across studies, I^2 statistic was computed in Review Manager (version 4.2.8, The Cochrane Collaboration, Software Update, Oxford, UK). I^2 is derived from Cochranes Q statistic[19]. It measures the extent of inconsistency among the studies' results, and the outcome is interpreted as the percentage of total variation across studies that is due to heterogeneity rather than chance [19,20]. A value of 0% indicates that all variability in effect estimates is due to chance and that none is due to heterogeneity. Larger values show that most of the variability is due to heterogeneity rather than chance. When the I^2 was > 25% (i.e., > 25% of the variability is due to heterogeneity), no pooled effect estimates were calculated. Furthermore, when there was uncertainty about results because of differences in

criteria to measure outcomes (e.g., VAP), sensitivity analyses were performed, in which only studies with comparable criteria were included.

Results

Initially, 106 articles were identified (Fig. 1). Eighty-four studies were excluded because no comparison was made between OSS or CSS (e.g., only CSS or OSS was evaluated) or randomisation was not applied. Twenty-six articles were considered potentially relevant, of which 15 met the criteria for this review. The other 11 articles were excluded due to a) failure

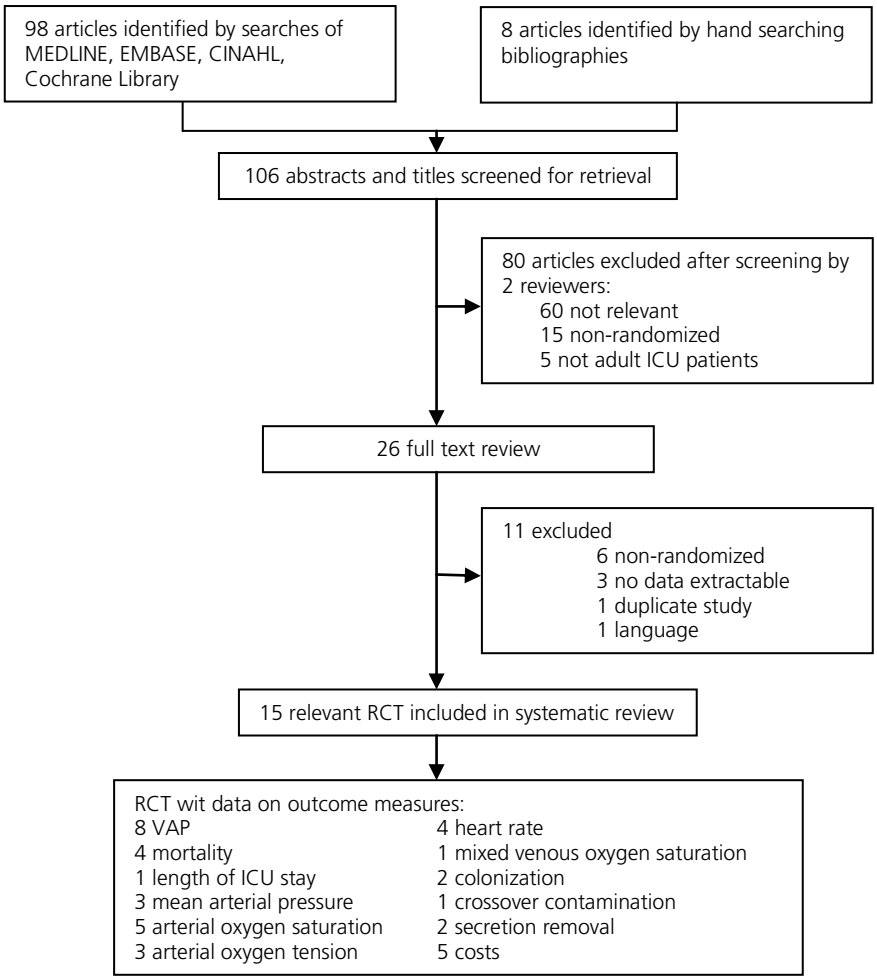

Fig. 1, Literature search flow diagram

Table 1, studies on closed suction systems and open suction systems

Study	No.	Q[a]	Category	Practice of ES	Outcomes used in review	Comment
Adams[12] (1997)	20	C		Clinical indication	Costs	Other data (on colonization and pneumonia) not available
Cereda [33] (2001)	10	C		Every 20 mins or longer for 20 secs No pre-oxygenation	MAP, HR, SaO_2, PaO_2	Crossover study, randomisation of order
Combes[30] (2000)	104	C	Neuro surgical	Every 2 hrs for ≤ 10 secs OSS: pre-oxygenation for 30 secs at FiO_2 1.0	VAP incidence	No preoxygenation when performing CSS Error in table with n, therefore values in table not used (mortality rate, ICU length of stay)
Deppe[9] (1990)	84	B	Surgical, trauma, medical	Every 3 hrs for < 10 secs Pre-oxygenation: 6-7 breaths Occasionally 5-10 mL saline	Colonization of respiratory tract, VAP incidence, ICU mortality	Differences in methods preoxygenation: ventilator (CSS) or manually (OSS)
Johnson[1] (1994)	35	C	Trauma, surgery	Clinical indication for < 15 secs Pre-oxygenation: 3-5 breaths FiO_2 1.0 Occasionally 3-5 mL saline	VAP incidence; MAP, HR, SaO_2; SvO_2; Costs	SvO_2 limited to 11 patients requiring pulmonary artery catheter
Lasocki[35] (2006)	9	C	Surgical (ALI)	Every 3 hrs for 20 secs Pre-oxygenation for 15 mins at FiO_2 1.0	PaO_2; secretion removal	Crossover study, randomization of order
Lee[34] (2001)	14	C		Every 2-4 hrs for 2 x 10 secs Hyperoxygenation for 60 secs	HR, MAP, SaO_2	Crossover study, randomization of order Patients suctioned twice (10 secs) with an interval of 30 secs
Lorente[8] (2005)	443	B	Medical surgical	No information provided	VAP incidence, ICU mortality	CSS with daily change (October 1, 2002, to December 31, 2003)
Lorente[32] (2006)	457	C	Medical surgical	No information provided	VAP incidence, ICU mortality	CSS without daily change (January 1 to September 30, 2004)

ES, endotracheal suctioning; OSS, open suction system; CSS, closed suction system; VAP, ventilator-associated pneumonia; MAP, mean arterial pressure; HR, heart rate; SaO_2, arterial oxygen saturation;

Study	No.	Q[a]	Category	Practice of ES	Outcomes used in review	Comment
Topeli[31] (2004)	78	C	Medical	Pre-oxygenation: 100% O_2 for 1 min	VAP incidence, ICU length of stay, ICU mortality, colonization of tube	OSS through swivel (T-tube), CSS no routine change catheter Colonization cultures were taken from 42 of 78 patients Patients in OSS were older ($p = .05$) and more were admitted for metabolic causes ($p < .01$). Increased age was determinant for mortality.
Rabitsch[11] (2004)	24	B	Cardio-pulmona-ry	Every 4 hrs and clinical indication Preoxyenation with 100% O_2 for 2 mins	VAP incidence, SaO_2 Crossover contamination between bronchial system and gastric juices	
Valderas (2004) [36]	26	B	Medical, surgical, trauma	Every 3h for ≤ 15 secs Pre-oxygenation: for 1 min at FiO_2 1.0	HR, SaO_2	Crossover study, randomization of order
Witmer[37] (1991)	25	C		Every 4-6 hrs during chest physiotherapy	Secretion removal	Crossover study, randomization of order Inclusion according to work assignments of physiotherapist (researcher), convenience sample 1 pass with each system during two consecutive chest physiotherapy treatments
Zeitoun[4] (2003)	47	C	Surgical, medical	No information provided	Incidence VAP	
Zielmann (1992) [13]	60	C		No information provided	Costs	Frequency ES: mean 15 (min-max 6-41) times a day

SvO_2, mixed venous oxygen saturation; ALI, acute lung injury; ICU, intensive care unit.
[a] Q, Quality: allocation concealment was adequate (A), unclear (B), inadequate (C), or not used (D).

to apply randomisation[10,14,16,21-23]; b) no relevant data being extractable[24-26]; c) duplicate publication[27]; and d) language[28].
Sample size varied from 9 to 457 patients in the included studies (Table 1). Details of randomization (such as methods or procedures) were not provided in nine studies. Although randomisation methods were provided in the remaining six studies, details about mode of concealment were not mentioned. Two studies used inadequate allocation methods, like date of intubation and bed availability [18,29]. Adequate approaches for concealment, such as a random numbers table, use of a computer system that generated a random number, and sealed envelopes were used in four studies.

Effects on infection and survival

VAP incidences were determined in eight studies (Table 2)[1,4,8,9,11,30-32], with little heterogeneity between studies (I^2 5.7%; p= .39; pooled RR, 0.96; 95% CI, 0.76-1.21)(Fig. 2).
Effects on patient survival were determined in four studies (I^2 0%; p= .86)(Fig. 3)[8,9,31,32]. No difference in ICU mortality was found (pooled RR, 1.02; 95% CI, 0.84-1.25). In only one study valid data on length of ICU stay were given, and they were in favor of OSS[31].

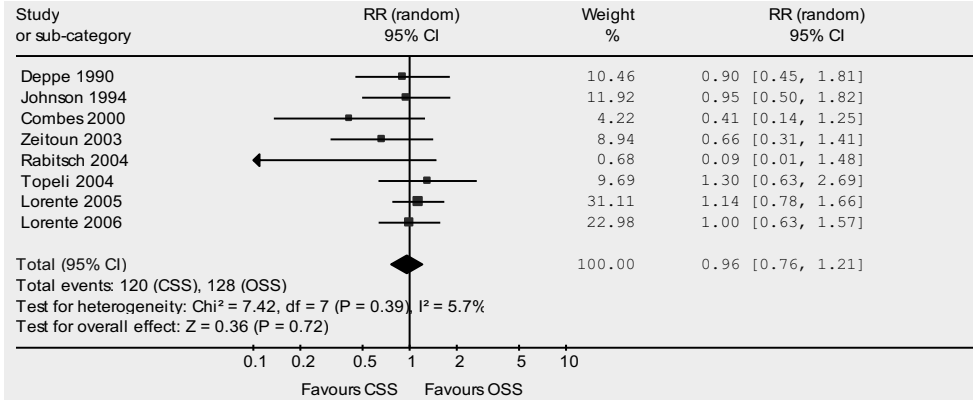

Fig. 2, Effect of suction system on incidence of ventilator-associated pneumonia

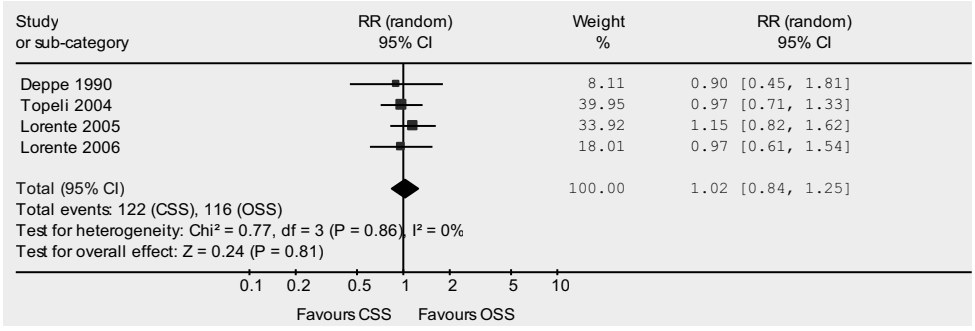

Fig. 3, Effect of suction system on mortality rate

Effects on cardio-respiratory parameters

Effects of ES system on physiologic outcomes, that is arterial oxygen saturation (SaO_2), arterial oxygen tension (PaO_2), mixed venous oxygen saturation, heart rate, and MAP, were determined in six studies (Table 3)[1,11,33-36].

Three studies evaluated changes in MAP by using either an arterial catheter or a noninvasive blood pressure cuff (I^2 0%; p= .51)(Fig. 4)[1,33,34]. A pooled standardized mean difference was calculated, since MAP was measured using different scales (invasive and noninvasive). MAP was significant higher after using OSS (pooled standardized mean difference, –0.43; 95% CI, -0.87 to 0.00). However, the absolute difference was rather small (3 to 5 mmHg difference).

Effects of ES system on SaO_2, measured by pulse oximetry, were determined in five studies, but there was substantial overall heterogeneity (I^2 86.1%; p < .00001), so pooled analyses could not be performed (Fig. 5)[1,11,33,34,36]. There was a difference in subcategories: three studies that evaluated changes in SaO_2 during ES (I^2 0%; p < .56) revealed a nonsignificant difference in weighted mean difference of 0.92% (95% CI, –0.58 to 2.41). The other five studies evaluating the changes after ES could not be pooled due to substantial heterogeneity (I^2 90.5%; p < .00001. All studies favored CSS, with slightly higher mean values and smaller standard

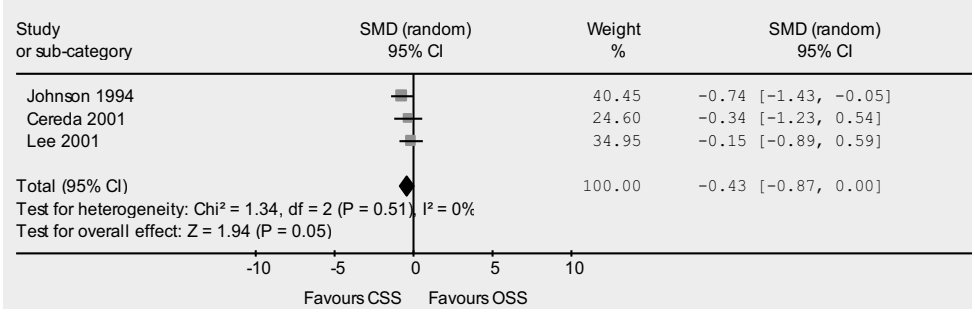

Fig. 4, Effect of suction system on Mean Arterial Pressure

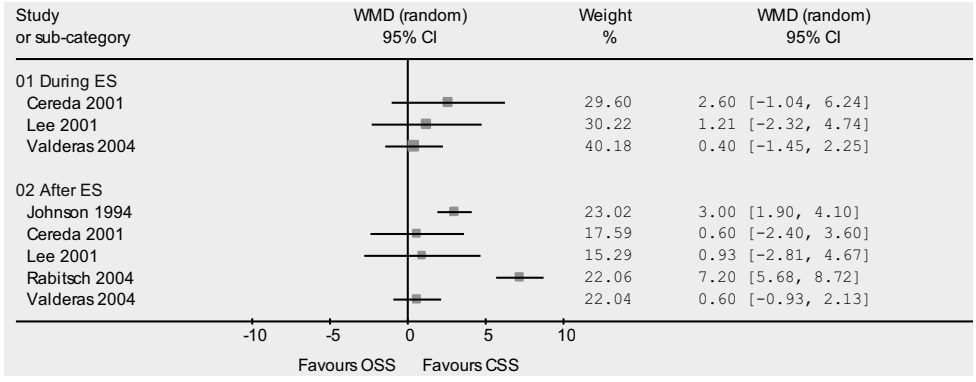

Fig. 5, Effect of suction system on arterial oxygen saturation

Table 2, Effect of suction system on intensive care outcome

Study	No.	Criteria VAP	VAP rate (%)		Mortality rate		Length of ICU stay, days (sd)	
			OSS	CSS	OSS	CSS	OSS	CSS
Combes[30] (2000)	104	Infiltrate on radiograph; Purulent secretions with positive sputum cultures; White blood cells >10,000/mm³ or <4,000/mm³ ; Rectal temperature >38°C ; MV ≥48h	9/50 (18.0)	4/54 (7.4)				
Deppe[9] (1990)	84	Infiltrate on radiograph; Purulent sputum; White blood cells >12,000/mm³ or <3000/mm³; Rectal temperature >38°C or <35.9; Length of stay ≥48h	11/38 (28.9)	12/46 (26.1)	11/38 (28.9)	12/46 (26.1)		
Johnson[1] (1994)	35	Infiltrate on radiograph and two of the following : Purulent sputum; White blood cells >12,000/mm³ ; Fever >38°C	10/19 (52.6)	8/16 (50.0)				
Lorente[8] (2005)	443	Infiltrate on radiograph; Purulent sputum; Respiratory secretions; White blood cells >10,000/mm³ or <4000/mm³; Temperature >38 or 35.5°C; Diagnosed during MV	42/233 (18.0)	43/210 (20.5)	50/233 (21.5)	52/210 (24.8)		
Lorente[32] (2006)	457	Infiltrate on radiograph; Purulent sputum; Respiratory secretions; White blood cells >10,000/mm³ or <4000/mm³; Temperature >38 or 35.5°C; Diagnosed during MV	31/221 (14.0)	33/236 (14.0)	30/221 (13.6)	31/236 (13.1)		

Rabitsch[11] (2004)	24	Infiltrate on radiograph and one of the following: Radiographic evidence of cavitation; Histological evidence of pneumonia; Positive blood culture; Purulent tracheal aspirate; Positive pleural fluid culture with fever >38°C and white blood cells >10,000/mm³ or <3,000/mm³	5/12 (41.7)	0/12 (0)[a]				
Topeli[31] (2004)	78	Infiltrate on radiograph and two of the following: Purulent secretions; White blood cells >10.000/mm³ or <3000/mm³; Temperature >38°C or <35.5°C	9/37 (24.3)	13/41 (31.7)	25/37 (67.6)	27/41 (65.9)	11.5 (1.4)	12.3 (1.1)
Zeitoun[4] (2003)	47	Infiltrate on radiograph; Purulent secretions; White blood cells ≥ 10.000/mm³; Fever (axial temperature ≥ 37.8°C)	11/24 (45.8)	7/23 (30.4)				

VAP, ventilator-associated pneumonia; OSS, Open Suction System; CSS, Closed Suction System; ICU, intensive care unit; MV, Mechanical Ventilation
[a] $p<0.05$

deviations. However, mean differences were rather small within SaO_2, varying from 96-99% after CSS to 95-98% after OSS. In one study, a larger decrease in SaO_2 was observed, from 97% after CSS to 90% after OSS [11].

Changes in heart rate, traced by electrocardiogram monitoring, were evaluated in four studies[1,33,34,36] (I^2 4.6%, p = .39)(Fig. 6). The pooled weighted mean difference was –6.33 beats / minute in favor of CSS (95% CI, –10.80 to –1.87). Although this difference was statistically significant, it is questionable whether it is clinically relevant.

Two studies determined the effect of ES system on PaO_2 by using an arterial catheter, but pooled analyses could not be performed due to substantial heterogeneity (I^2 67%; p = .08)(Fig. 7)[33,35]. In both crossover studies, a larger decrease in PaO_2 was observed after using OSS, even up to a 60% decrease in one study[35]. This drop may have been influenced by duration of ES, which was, in both studies, 20 secs instead of the recommended maximum of 15 secs. Above that, Lasocki et al. [35] performed a recruitment manoeuvre of 20 tidal volumes after CSS, which was not applied after OSS.

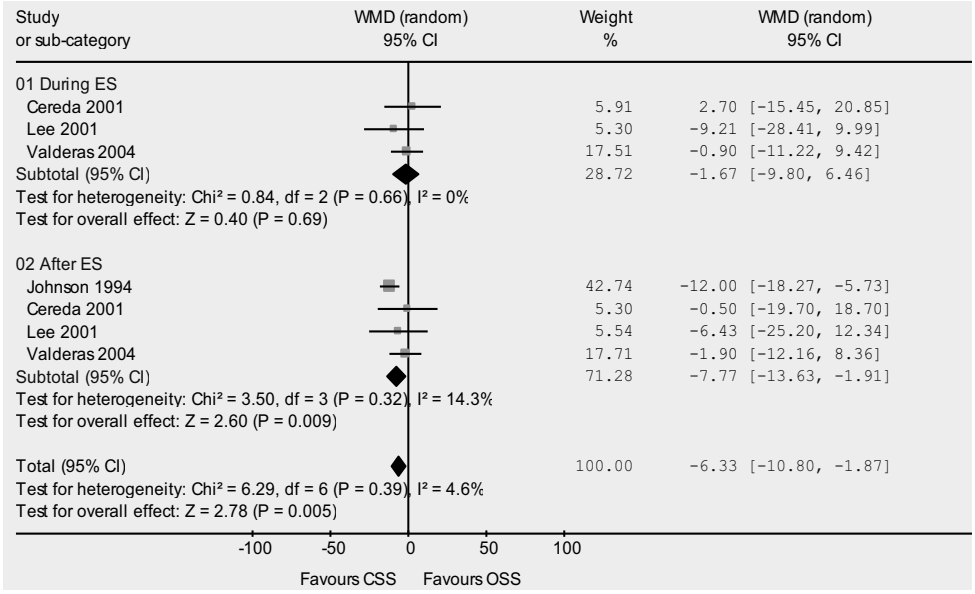

Fig. 6, Effect of suction system on heart rate

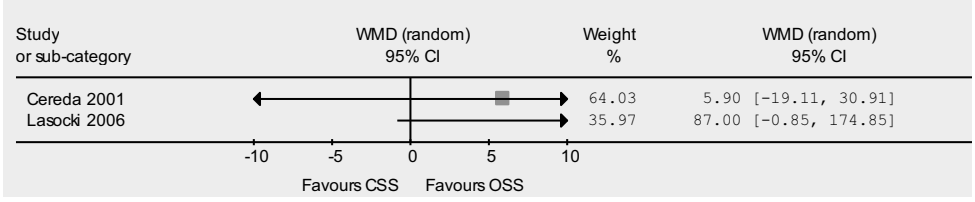

Fig. 7, Effect of suction system on arterial oxygen tension

Table 3, Effect of suctioning system on cardiorespiratory variables

Study	No	Worst mean values	PaO_2 mmHg (sd)		SaO_2 % (sd)		HR, beats/min (sd)		MAP mmHg (sd)	
			OSS	CSS	OSS	CSS	OSS	CSS	OSS	CSS
Cereda (2001)[33]	10	During ES After ES	117 (31)	123 (25)	94.6 (5.1) 97.0 (3.8)	97.2 (2.9) 97.6 (3.0)	97.5 (21.4) 98.1 (22.5)	100.2 (20.0) 97.6 (21.3)	84.5 (13.6)	80.0[a] (11.6)
Johnson[d] (1994)[1]	35	After ES			96 (1.7)	99 (0.4)[bc]	114 (7.4)	102 (6.0)	102 (7.4)	97 (5.6) [bc]
Lasocki (2006)[35]	9	After ES	171 (68)	258 (116)						
Lee (2001)[34]	14	During ES After ES			95.8 (5.7) 94.9 (6.0)	97.0 (3.6) 95.8 (3.9)	106.6 (30.9) 106.9 (29.9)	97.4 (19.8) 100.4 (19.8)	95.7 (21.7)	92.4 (21.4)
Rabitsch (2004)[11]	24	After ES			89.6 (2.5)	96.8 (1.0)[†]				
Valderas[d] (2004)[36]	26	During ES After ES			97.9 (4.1) 97.2 (3.1)	98.3 (2.6) 97.8 (2.6)	94.1 (20.5) 93.4 (18.9)	93.2 (17.3) 91.5 (18.9)		

PaO_2, arterial oxygen tension; SaO_2, arterial oxygen saturation; HR, heart rate; MAP, mean arterial pressure; ES, endotracheal suctioning; OSS, open suction system; CSS, closed suction system.

[a] $p \leq 0.05$; [b] $p \leq 0.0001$; [c] significant difference based on percent change from baseline between systems used; [d] standard errors in the original tables were converted to standard deviations by using the formula SD = SE * square root of n

Differences in mixed venous oxygen saturation were determined in only one study[1]; CSS was favored (mean mixed venous oxygen saturation 74% with CSS and 67% with OSS).

Effects on bacterial contamination and secretion volume

Bacterial contamination after ES was evaluated in one study[11]. More specifically, colonization of bronchial tree and stomach was assessed. After 3 days, simultaneous colonization with similar bacterial species in both respiratory tract and stomach was demonstrated in five of 12 patients receiving OSS and in none of 12 patients receiving CSS. Other sites of contamination, such as hands of healthcare workers and the inanimate environment of patients, were not assessed. Multiple use of CSS may lead to bacterial colonization of the endoluminal surface of the tube, and on reuse of the suction catheter these bacteria may autocontaminate the patient's respiratory tract. Two studies compared bacterial colonization of endotracheal tubes when using OSS or CSS (I^2 0%; p = .44)(Fig. 8)[9,31]. The pooled RR was 1.51 (95% CI, 1.12-2.04) for CSS, implying that colonization of endotracheal tubes occurs less frequently with OSS. Yet, in both studies colonization differences were not associated with differences in development of VAP [9,31].

Quantities of secretions removed were compared in two studies [35,37] in which OSS and CSS were used in alternating order with 3- to 6-hr intervals in the same patients. There was too much heterogeneity (I^2 40.4%; p = .20), so pooled weighted mean difference could not be calculated (Fig. 9). Both studies found that OSS was more effective in removing tracheobronchial secretions (mean weight of 2.5-3.2 g with OSS and 0.6–2.3 g with CSS).

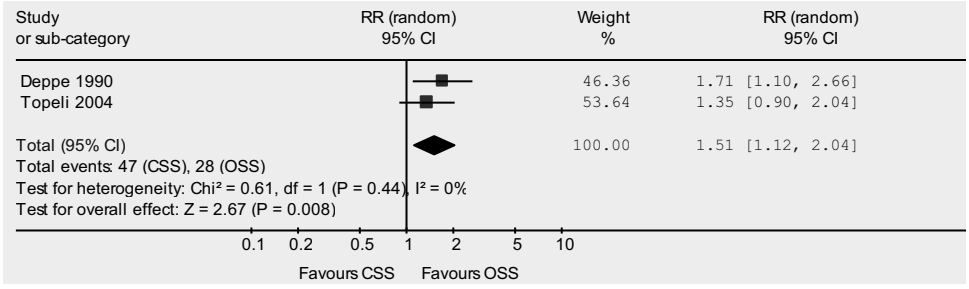

Fig. 8, Effect of suction system on colonization

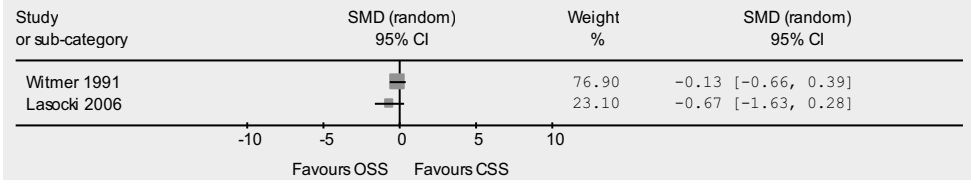

Fig. 9, Effect of suction system on secretion removal

Costs

Costs were compared in five studies [1,8,12,13,32](Table 4) and material costs of CSS were 14-100 times more expensive than OSS. When we analyzed costs per day and with number of ES procedures per day ranging from 10 to 16, CSS remained three to almost 12 times more expensive in three studies [8,12,13]. The fact that CSS reduces use of gloves, masks, and glasses during ES was taken into account in all three studies. Use of CSS appeared to be cost-effective in two studies, due to smaller price differences between both systems[1] or to extended use of CSS[32].

Discussion

The results of this meta-analysis reveal that generally assumed advantages of CSS compared with OSS, like lower incidences of VAP, lower costs, reduced bacterial contamination, and improved patient outcome, are not supported by scientific evidence. The only assumption that is supported by evidence is that CSS causes fewer physiologic disturbances, but the differences were rather small and do not seem clinically relevant.

Few studies (n = 15) have compared the effects of OSS and CSS in a randomized design. In general, the methodological quality of the included studies was not high. Although all trials used some kind of randomization, methods of concealment were provided in only six studies,

Table 4, Costs of suctioning systems

	N	Costs each		Costs per day		Conclusion
		OSS	CSS	OSS	CSS	
Adams (1997)[12]	20	£0,145	£16,89	£1,45	£16,89	CSS 11.6 times more expensive
Johnson (1994) [1]	35	£0,52	£7,29	£8,35	£7,29	CSS less expensive
Lorente (2005)[8]	443	£0,17	£5,63	£1,41	£6,25	CSS 4.4 times more expensive
Lorente (2006)[32]	457	£0,21	£6,86	£1,57	£1,64	CSS more expensive when length of MV < 4 days but less expensive when MV > 4 days
Zielmann (1992) [13]	60	£0,29	£17,03	£6,03	£18,54	CSS 3.1 times more expensive

OSS, open suction system; CSS, closed suction system; MV, mechanical ventilation. Currency: all converted to British Pounds with rate of time of study. Adams: data copied from study, already calculated in British Pounds; Johnson, January 1992; Lorente (2005), December 2003; Lorente (2006); Zielmann, January 1992 (no study period given).

of which only four were considered adequate. Inadequate or unclear allocation concealment may lead to larger estimates of effect [29]. Furthermore, performance of ES was not described accurately in most studies and differed between studies in the use of normal saline, pre-oxygenation, or duration of suctioning (10-20 secs). The latter aspects may have profound effects on physiologic variables such as oxygenation and heart rate. Finally, when we considered studies assessing the effects of different ES systems and the risk on VAP, patient categories and criteria to diagnose VAP differed somewhat. In all studies non-invasive methods were used to diagnose VAP, and main differences were in the specification of leukocytosis (<3000 or <4000/mm^3) and the necessity of all criteria to be met.

Although studies differed in methodology (design and conduct) as well as clinically (patient characteristics and performance of ES), a meta-analysis could be performed for five outcomes, since heterogeneity was low. The most frequently evaluated outcome variables was VAP, which was determined in eight studies. A significant reduction associated with the use of CSS was only found in the smallest study (n = 24)[11]. Neither in the larger studies nor in meta-analysis significant incidence reductions were found, as was also concluded in a recently published meta-analysis[38]. Because of differences in diagnosis of VAP, we performed a sensitivity analysis which included only those studies that used comparable criteria to diagnose VAP[1,4,8,9,30,32], and findings did not alter (I^2 0%; pooled RR, 0.95; 95% CI, 0.76-1.20). Therefore, it seems unlikely that subsequent and larger randomized trials will change this finding. This interpretation conflicts with recently published international guidelines in which the use of CSS is recommended as part of a VAP prevention strategy [17,39]. These recommendations are based on qualitative analyses of three[39] or four[17] similar randomized studies, which all conclude that type of suctioning system has no effect on the incidence of VAP. Despite this lack of evidence (and without performing a meta-analysis), both guidelines favor CSS.

The second largest outcome measured was mortality (four studies, 1,062 patients), and no significant differences were found in either of the studies or in meta-analysis. Statistically significant differences were found in cardiorespiratory variables: MAP and heart rate were lower after using CSS. However, the actual difference for heart rate was 6 beats/min and seems, therefore, of little clinical relevance. This also applies to MAP, in which we found a significant but clinically very small difference (3-5 mm Hg) in favor of CSS. There is no evidence that CSS is beneficial for arterial oxygen saturation. This outcome was higher after using OSS in each study, but the five studies were too heterogeneous to perform pooled analysis. Despite differences in cardiorespiratory variables, it is not possible to draw firm conclusions due to the paucity and clinical heterogeneity of data.

Available data do not support the idea that CSS is cost reducing compared with OSS. A rigorous cost-effectiveness analysis of both systems is needed and should include the societal perspective (real costs being made to perform ES, e.g., used materials and personnel time) and benefits (in terms of patient outcomes) across the health care continuum [40]. Prolongation of CSS device use, from the recommended 24 hrs to several days, will definitely influence cost efficacy. This approach has been pursued in six studies [3,32,41-44]. Prolonged use of CSS was

associated with increased microbial colonization of the device [43] without raising the incidence of VAP [3,41,44], and was considered safe and cost-effective [3,41-44]. A survey among 27 ICUs in the United States revealed that CSS devices were changed every 72 hrs, "as needed", or weekly in 37% of ICUs[45], with no negative effects mentioned.

Conceptually, prevention of bacterial transmission from patient to patient could be a beneficial, and highly relevant, effect of using CSS instead of OSS. However, up to now crosstransmission or environmental bacterial contamination has not been studied in a randomized design. Environmental contamination after ES with either OSS or CSS was compared in a non-randomized crossover study with nine patients [16]. After 144 ES procedures, both OSS and CSS were associated with significant increased colony counts measured by air sampling, but, on average, colony counts were lower after use of CSS [16]. In another small observational study (n = 14), visible droplet dissemination was detected in all OSS procedures, with bacteriologic contamination in the inanimate environment of 37% of patients [46]. There are no data on environmental contamination when changing the CSS device, a procedure that also needs tube disconnection. Interestingly, the assumed reduction in environmental contamination is a reason to use CSS, not only to minimize cross-transmission of pathogens, but also to allow performance of ES without the use of sterile gloves, which are recommended when using OSS[47]. Without scientific justification such a change in nursing practice may in fact increase hand contamination and subsequent spread of nosocomial pathogens.

This meta-analysis has some limitations. First, as in all meta-analyses, publication bias cannot be excluded. A funnel plot of the included studies on the incidence of VAP (data not shown) indeed indicated that publication bias might play a role; that is, larger studies showing beneficial effect appear to be missing. This is, however, in contrast with the concerns about publication bias, namely that positive (significant) results in favor of the newer system (CSS) are more likely to be published than negative results (type I error)[48,49].

Second, selection bias might have occurred as a consequence of our language restriction. As far as we know, we only missed one Korean study on the effects of CSS on arterial oxygen saturation and VAP in 70 patients [28]. We could not assess study quality, randomization procedures, and criteria used to diagnose VAP (incidence significantly higher in OSS group). Results on arterial oxygen saturation could, however, be read from the tables and were in agreement with our findings.

This first meta-analysis on open and closed suction systems reveals that the increased popularity of CSS is yet not sufficiently supported by scientific evidence. Randomized trials to assess one of the most pronounced assumptions, the potential benefits of CSS in reducing cross-transmission, are needed. Such trials should be specifically designed to identify the true effect measures. When randomizing individual patients, resulting in a mix of patients receiving ES with CSS and OSS, beneficial effects of CSS might be obscured by cross-transmission occurring from neighbor patients randomized to OSS. Therefore, a large multi-center crossover trial, with fixed periods in which either of both systems is used, appears to be most appropriate.

Acknowledgements

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CHAPTER 3

Effect of open and closed endotracheal suctioning on cross-transmission with Gram-negative bacteria: a prospective crossover study

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Abstract

Objective Cross-transmission of Gram-negative bacteria increases the likelihood of acquisition of infections and emergence of antibiotic resistance in intensive care units. Respiratory tracts of mechanically ventilated patients are frequently colonized with Gram-negative bacteria and endotracheal suctioning may facilitate cross-transmission. It is unknown whether closed suction systems (CSS), as compared with open suction systems (OSS), prevent cross-transmission. The objective was to determine whether CSS, as compared with OSS, reduce the incidence of cross-transmission of Gram-negative bacteria in intensive care units.

Design We performed a prospective crossover study in which both systems were tested unit-wide in 4 intensive care units.

Setting Two intensive care units from a university hospital and two from a teaching hospital participated in the trial between January 2007 and February 2008.

Patients All patients admitted to the intensive care units for > 24 hrs were included.

Intervention CSS and OSS were used for all patients requiring mechanical ventilation during 6-month clusters with the order of systems randomized per intensive care unit.

Measurements and Main Results Acquisition and cross-transmission rates of selected Gram-negative bacteria were determined through extensive microbiological surveillance and genotyping. Among 1110 patients (585 with CSS and 525 with OSS) acquisition for selected Gram-negative bacteria was 35.5 and 32.5 per 1000 patient-days at risk during CSS and OSS, respectively (adjusted hazard ratio, 1.14; 95% confidence interval, 0.9-1.4). During CSS, adjusted hazard ratios for acquisition were 0.66 (95% confidence interval, 0.45-0.97) for *Pseudomonas aeruginosa* and 2.03 (95% confidence interval, 1.15-3.57) for *Acinetobacter* species; acquisition rates of other pathogens did not differ significantly. Adjusted hazard ratios for cross-transmission during CSS were 0.9 (0.4-1.9) for *P. aeruginosa*, 6.7 (1.5-30.1) for *Acinetobacter*, and 0.3 (0.03-2.7) for *Enterobacter* species. Overall cross-transmission rates were 5.9 (CSS) and 4.7 (OSS) per 1000 patient-days at risk.

Conclusion CSS failed to reduce cross-transmission and acquisition rates of the most relevant Gram-negative bacteria in intensive care patients.

Introduction

Infections caused by Gram-negative bacteria (GNB) are associated with increased morbidity and mortality and higher healthcare costs, especially in intensive care units (ICUs) [1,2]. These infections are almost always preceded by colonization [3]. In patients not colonized with GNB at the time of ICU admission, acquisition of colonization may occur either endogenously or exogenously [4]. Endogenous "acquisition" could imply selection (for instance through antibiotic exposure) of pre-existent GNB that reach detection limits of culture methods at a certain time point. Exogenous acquisition (cross-transmission) results from lapses in infection control [5]. Mechanically ventilated patients are frequently colonized with GNB in the respiratory tract [6-8]. In these patients, endotracheal suctioning (ES) is an essential and frequently performed procedure to clear secretions from the tracheobronchial tree, to guarantee optimal oxygenation, and to avoid accumulation of secretions, tube occlusion, increased work of breathing, atelectasis and pulmonary infections [9,10]. Yet disconnection of the ventilation system and endotracheal tube during ES exposes colonized airways and contaminated material to open air with ongoing ventilation, which may facilitate airborne spread of pathogens to the patient's skin, the inanimate patient environment, and the hands of health care workers, which may facilitate cross-transmission.

Nowadays, two systems are available for ES: the single use open suction system (OSS) and the multiple-use closed suction system (CSS). The latter does not require disconnection from the ventilator and can remain connected for at least 24 hrs, depending on hospital protocol and CSS type [11]. It is widely assumed that CSS prevents contamination of the inanimate environment and subsequently reduces cross-transmission, but this has never been rigorously investigated [12-15]. The objective of our study was to determine to what extent CSS, as compared with OSS, reduces the incidence of cross-transmission of GNB in ICU-patients.

Materials and Methods

Design

Between January 2007 and February 2008, we performed a prospective crossover trial in four ICUs: two ICUs from a university hospital and two from a teaching hospital. The ICUs in the university hospital had ten beds (four single rooms, six on the ward) and eight beds (one single room, seven on the ward), and the two ICUs in the teaching hospital each had eight beds, all single rooms. Because we aimed to compare cross-transmission rates in ICUs, an individualized, randomized design would have allowed patient interaction, potentially protecting those randomized to OSS by the advantages (i.e., less cross-transmission) originating from patients randomized to CSS or vice versa (more cross-transmission in CSS because of other patients randomized to OSS). Therefore, both interventions (CSS and OSS) were implemented unit-wide

and used for all eligible patients during periods of 6 months. To control for unit-specific characteristics, a crossover of both systems was used, again for 6 months, with the order of systems randomly assigned. Randomization was performed by a mathematician neither involved in patient care, nor in the trial, and unaware of the identity of each ICU by tossing a coin for which hospital to start with what system. The first study period was preceded by a 2-week wash-in period, in which all ES procedures were already performed with the system to be used during the first study period. In between study periods there were 4 wks wash-out/wash-in, in which the system was changed (2 wks wash-out) and ES procedures were performed with the system of the second study period (2 wks wash-in). The study was designed attending the Consolidated Standards of Reporting Trials (CONSORT) statement and extended with the rationale for clustering [16-18] .

All patients admitted to the ICU for > 24 hrs were included and clinical data (Acute Physiology and Chronic Health Evaluation [APACHE] II scores, diagnosis, isolation, duration of mechanical ventilation, frequency of ES), demographic and antibiotic use data were collected through a Case Record Form. Patients in prone positioning always received CSS, because disconnection and reconnection for OSS could be difficult in some conditions. Furthermore, another ES system than the randomized one could be used on clinical indication if considered needed by the attending physician.

Both CSS and OSS were not considered experimental treatments (because they both are frequently used), and, therefore, the institutional review board of both hospitals waived the requirement for informed consent. However, all patients (or next of kin) were informed about the aim and consequences of the study with a possibility to refuse the use of patient-specific medical data for analysis.

All ES procedures were performed on indication by ICU nurses. OSS was performed through a swivel connector without disconnecting the patient from the ventilator (Utrecht) or by disconnection (Tilburg). CSS was used repeatedly and replaced every 24 hrs (Ballard Trach Care; Ballard Medical Products/ Kimberly-Clark Corporation, Draper, Utah; used in Utrecht) or every 72 hrs (Ballard Trach Care 72, Ballard Medical Products/ Kimberly-Clark Corporation, Draper, Utah; used in Tilburg). In all procedures, according to hospital protocols, use of non-sterile gloves and hand hygiene was prescribed, whereas protective masks and glasses were advised to be used on indication, as judged by attending nurses. Adherence to the study protocol was controlled daily via patient medical records and additionally four times weekly through bedside observations by research nurses. Of note, none of the patients received selective decontamination of the digestive tract or oral antiseptics like chlorhexidine.

Outcomes and definitions

The primary study outcome was the occurrence of cross-transmission with *Pseudomonas aeruginosa*, *Acinetobacter* species, and *Enterobacter* species. Secondary outcomes were acquisition rates of colonization with the individual or with any of the following Gram-negative marker pathogens: *P. aeruginosa*, *Acinetobacter* species, *Stenotrophomonas maltophilia*, and

Enterobacteriaceae (i.e., *Escherichia coli*, *Enterobacter* species, *Klebsiella* species) resistant to third-generation cephalosporins.
Cross-transmission was defined as acquired colonization with an identical genotyped pathogen and with epidemiologic linkage (i.e., overlapping time periods) to a potential source patient [19]. Colonization on admission was defined as bacterial growth from an endotracheal aspirate sample (or throat swab in the absence of endotracheal aspirate) with any of the marker pathogens in a sample obtained within 48 hrs of ICU admission. Acquired colonization with a marker pathogen was defined as a positive culture in a sample obtained at least 48 hrs after ICU admission and preceded by a negative culture for that pathogen [19]. When the first culture was taken after 48 hrs and was positive, colonization status on admission was considered unknown.
Patient-days at risk for a certain marker pathogen were defined as all days in the ICU in which the patient did not have documented colonization with that pathogen. To determine acquired colonization with any of the marker pathogens, all non-colonized patient-days were considered at risk.

Microbiological protocol

Surveillance cultures of endotracheal aspirates were obtained on admission, twice weekly thereafter (every Monday and Thursday) and on discharge. In non-ventilated patients, oropharyngeal swabs were obtained, because acquisition and cross transmission could occur in these patients as well. The samples were analyzed according to local protocol, and isolated marker pathogens were stored at -80° C. Results were communicated to the medical staff according to standard microbiological reporting practices.
From patients colonized with *P. aeruginosa*, *Acinetobacter* species, or *Enterobacter* species, the first isolate (per pathogen) was selected for genotyping. A subsequent isolate was selected in case of a change in antibiogram, morphologic differences, or when 10 or more cultures with a certain pathogen had been obtained. *P. aeruginosa*, isolates were genotyped with Multiple-Locus Variable-number tandem-repeats Analysis (MLVA) [20] , and *Acinetobacter* and *Enterobacter* species were genotyped with DiversiLab [21]. MLVA patterns were analyzed with BioNumerics software version 5.10 (Applied Maths; St-Martens-Latem, Belgium), and single locus variants (where the profile varies at one locus) were used as cutoff point for genetic relatedness. For *Acinetobacter* and *Enterobacter* species analysis was performed with DiversiLab software (version 3.4) using 95% similarity as cutoff point for genetic relatedness. Genotyping was performed after the trial; therefore medical staff was not aware of the results during the study.

Observations of hygienic precautions

Hygienic precautions were monitored by a research nurse positioned at the bedside. Nurses were told that physiological changes during ES were monitored and were not aware of the observations of hygienic precautions. Performance of hand hygiene before and after ES, use of

clean gloves (new for each procedure), apron, glasses, and mask (when indicated) were registered. Per study period and per ICU, 25 bed numbers were randomly selected by a computer program (Research Randomizer, Social Psychology Network; Middletown, CT) and a single ES procedure was monitored.

Policy for patient isolation

Both hospitals had a policy for patient isolation in ICU based upon the guidelines of the Dutch Infection Prevention Working Party [22,23]. This policy is based on different modes of spread, in which the form of isolation depends on the type of micro-organism, the site of infection or colonization, and whether or not an outbreak situation exists [23]. A sign is placed outside the room of indicated patients, indicating hand hygiene and donning of gown and gloves immediately before room entry and, depending on type of isolation, use of a mask and a cap. Forms include contact isolation (in a single room or on a ward), droplet isolation or airborne isolation (additionally requiring a mask), strict isolation (requiring use of a protective apron and a cap), and protective isolation (use of protective clothing and a mask).

Data analysis

Based on previous studies, it was conservatively estimated that at least 80% of all patients receiving mechanical ventilation would develop respiratory tract colonization with any of the marker pathogens during their ICU stay [3,24-30] and that 25% of them would acquire colonization through cross-transmission [19]. With 250 patients in each study arm, an absolute reduction of 10% (to 15%) by using CSS as compared with OSS could be demonstrated with $\alpha = 0.05$ and $\beta = 0.8$.

To determine colonization on admission and possible cross-transmission, all patients with a length of stay of >24 hrs and cultured at least once were included. For acquired colonization, patients with a length of stay of ≥48 hrs were included. We used an intention-to-treat approach and analysed data from patients according to the study period they were included in.

For univariate analysis, continuous variables were tested with Kolmogorov-Smirnov tests for normal distribution. A *t* test was used when data were normally distributed; otherwise, nonparametric Mann-Whitney tests were used. Dichotomous variables were analyzed by using chi-square tests.

Because of subtle differences in performance and materials used for ES between hospitals, analyses were stratified according to center. Differences in acquisition and cross-transmission between CSS and OSS were evaluated using Cox regression models with days in ICU until acquisition (days at risk) as time variable. The following variables were investigated as independent risk factors for acquisition: study period (CSS/OSS), glove use (mean percentage of glove use as observed per study period per unit, as a proxy for hygienic precautions), unit (1 to 4), gender (male/female), age (continuous, at time of ICU admission), APACHE II score (continuous), duration of isolation (continuous, number of days), mechanical ventilation (binominal), surgical or medical admission diagnosis, and antibiotic use on admission to ICU

(binominal). Multicollinearity between covariates was tested in advance. Because study period and use of gloves were highly correlated ($r^2 = 0.91$), glove use was excluded from regression analysis.

Imputation was used for missing data using an expectation-maximization analysis with the Impute function in SPSS software (version 15.0; Chicago, Ill), with inclusion of study period, age, gender, diagnosis, and mechanical ventilation as key variables in the imputation model. A total of 51 APACHE II scores and one diagnosis were missing (4.6% of all values). Expectation-maximization analysis revealed that data were missing at random, meaning that differences in missing data are related to the observed data, and missing values were replaced by imputed values. Apart from increasing the sample size, imputation corrects for possible bias due to selective missing values [31,32].

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, Chicago, Ill) version 15.0. A p value of <.05 was considered statistically significant.

Results

During the 12-month study period, 2070 patients were admitted to the four ICU wards and assessed for eligibility; 963 (47%) patients stayed <24 hrs, in most cases after elective surgery, and were excluded (Fig. 1). No patients declined to use their medical data. In all, 1107 admissions were included; three patients crossed over from OSS to CSS, none from CSS to OSS. As a result, 1110 patients (11,319 patient-days) were included: 585 patients (5720 patient-days) in the CSS study period and 525 patients (5599 patient-days) during OSS. Patients in the CSS group had slightly lower APACHE II scores, were more likely to be admitted for surgical reasons, and were more likely to receive mechanical ventilation (Table 1). The frequency of performing ES was comparable in both study periods (Table 2).

Adherence to using the randomized ES system was 95% of all ventilation days during CSS and 91% during OSS (Table 2). Non-adherence during OSS was most often due to prone positioning or positive end-expiratory pressure ≥12 cm H_2O (reason for using CSS) and during CSS because of weaning (reason for using OSS).

Adherence to the surveillance protocol was 97% during CSS and 96% during OSS (Table 2). Samples were not available from 113 patients (49 CSS, 64 OSS), which were mainly patients with a short ICU stay (median 3 days; interquartile range, 2-3 days), frequently admitted in-between surveillance culture days (median duration of mechanical ventilation 1 day, interquartile range, 0-2). This latter reason also applied to patients from whom an admission culture was not available.

Bedside observations for monitoring adherence to hygienic measures were performed during 94 CSS and 106 OSS procedures. Gloves were used in 91% of ES procedures performed with CSS and in all procedures with OSS ($p < .001$). However, gloves had already been used for

other procedures and were not changed during 26% of the CSS procedures and during 5% of the OSS procedures ($p < .001$).

Antibiotic use

During the CSS period, 75% of the patients received antibiotics, as compared with 73% in the OSS period. Expressed in defined daily dosages per 1000 patient-days, antimicrobial usage densities were 1280 and 1139 during CSS and OSS, respectively, with comparable densities for individual classes of antibiotics between both study groups (Table 3).

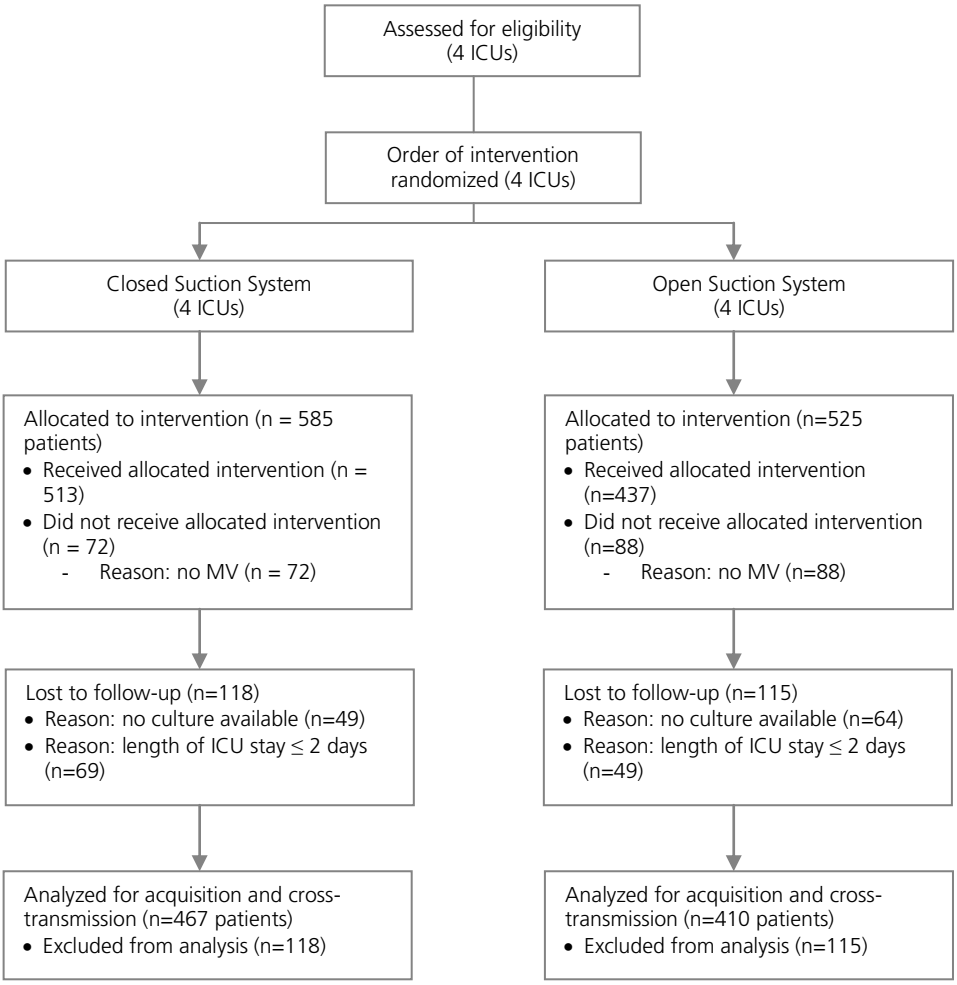

Fig 1. Flow diagram. *ICUs*, intensive care units; *MV*, mechanical ventilation

Table 1. Baseline characteristics

	CSS	OSS
No. of patients	585	525
Patient-days	5720	5599
Units, no. of patients		
Unit 1	171	138
Unit 2	151	111
Unit 3	124	148
Unit 4	139	128
Patient characteristics		
Male sex, no. (%)	368 (62.9)	362 (61.3)
Age year, median (IQR) [a]	61 (48-71)	62 (49-72)
APACHE II score, median (IQR)	19 (13-24)	20 (15-25)
APACHE II score ≥ 20, no. (%)	251 (45.7)	272 (53.3)
Diagnosis, no. (%)		
Surgical	251 (42.9)	178 (34.0)
Medical	334 (57.1)	346 (66.0)
Antibiotics on admission ICU, no. (%)	118 (20.2)	102 (19.4)
Patients on MV, no. (%)	513 (87.7)	437 (83.2)

CSS, closed system suctioning; OSS, open system suctioning; IQR, interquartile range; APACHE, Acute Physiology and Chronic Health Evaluation; ICU, intensive care unit; MV, mechanical ventilation;
[a] Age: at time of ICU admission

Colonization on admission

Of 997 patients with at least one respiratory tract culture result available, 22 (2%) had an unknown colonization status on admission (Table 4). Of the remaining 975 patients, 110 (21%) and 112 (25%) were colonized on admission with one or more (up to four) of the marker pathogens during CSS and OSS, respectively (odds ratio, 1.24; 95% confidence interval [CI] 0.92-1.68). Most patients (76% during CSS and 71% during OSS) were colonized with one pathogen, and 20% and 24% (CSS and OSS, respectively) with two pathogens. Colonization on admission with *Klebsiella* species was more frequently observed during OSS (5% during CSS and 10% during OSS, $p = .002$).

Acquired colonization

Of patients with a length of ICU stay of > 48 hrs, 173 (37%) and 152 (37%) patients acquired colonization with at least one of the marker pathogens during OSS and CSS, respectively ($p = .99$). Most patients acquired colonization with one (58% in CSS and 60% in OSS) or with two pathogens (32% in CSS and 24% in OSS). The overall acquisition rates were 35.5 and 32.5 per 1000 patient-days at risk for CSS and OSS, respectively (adjusted hazard ratio [HR] for acquisition during CSS of 1.14; 95% CI, 0.91-1.42).

Acquisition with *P. aeruginosa* occurred less frequently during CSS (10.3 and 15.7 per 1000 patient-days at risk for CSS and OSS, respectively; adjusted HR during CSS, 0.66; 95% CI, 0.45-0.97), whereas acquisition with *Acinetobacter* species occurred more frequently during CSS (7.6 and 4.0 per 1000 patient-days at risk; adjusted HR during CSS, 2.03; 95% CI, 1.15-3.57) (Table 4).

Table 2. Clinical outcome and adherence to study protocol

	CSS (n = 585; patient-days 5720)	OSS (n = 525; patient-days 5599)	*p*
ES system			
Other ES system, no. (% patients)	45 (8.8)	43 (9.8)	.560
Other ES system, % MV days	5.3	8.7	
Clinical outcome			
Duration of MV in days, median (IQR)	5 (2-10)	6 (3-13)	
Frequency ES, median per day (IQR)	6.0 (3-8)	6.0 (3-8)	
Patients in isolation, no. (%)	70 (12)	58 (11)	
Duration of isolation in days, median (IQR)[a]	6 (4-17.25)	8 (4-23.25)	
ICU LOS, median days (IQR)	6 (3-13)	6 (3-15)	
ICU mortality, no. (%)	131 (22.4)	98 (18.7)	
Microbiological cultures of respiratory tract samples			
Patients cultured, no. (%)	536 (91.6)	461 (87.8)	.036
Cultures per patient-day	0.42	0.45	
Patients cultured on admission, no. (%)	460 (78.6)	377 (71.8)	.008
Cultures on Monday/Thursday, %	96	97	NS
Patients cultured on discharge, no. (%)	209 (35.7)	189 (36.0)	NS
Hygienic precautions, %			
Hand hygiene before ES [b]	24	43	.033
Hand hygiene after ES [b]	51	54	.655
Glove use / new gloves for ES	65 / 91	95 / 100	.000
Use of mask	9	38	.000
Use of eye protection	4	17	.003
Use of gown	41	54	.070

CSS, closed system suctioning; OSS, open system suctioning; ES, endotracheal suctioning; MV, mechanical ventilation; IQR, interquartile range; ICU, intensive care unit; LOS, length of stay.
[a] Duration of isolation: calculated for isolated patients. [b] Hand hygiene could only be observed in 117 cases (before ES) and 158 cases (after ES).

Overall, antibiotic resistance levels were low and comparable in both study groups (see Table E1 in the supplemental data). For *P. aeruginosa* 19%, 9%, 8% and 8% of isolates were resistant to ceftazidime, ciprofloxacin, tobramycin and meropenem, respectively.

Cross-transmission

P. aeruginosa isolates from 158 (73 in CSS, 85 in OSS) of 182 colonized patients were available for genotyping. Isolates from four patients were nontypeable, and 224 isolates were genotyped, yielding 109 different *Pseudomonas* MLVA-types. Based on genotype and overlapping ICU stay, there were 15 events of cross-transmission during CSS and 16 during OSS, corresponding to 3.4 and 4.1 events per 1000 patient-days at risk for CSS and OSS,

Table 3. Antimicrobial density during study periods

	Total	AD CSS	OSS
No. of patients receiving antibiotics (%)	819 (74)	437 (75)	382 (73)
Antimicrobial group [a]			
Penicillin-like antibiotics [b]	537	560	514
Cephalosporins [c]			
First generation	28	33	24
Second generation	83	76	91
Third generation	124	131	117
Aminoglycosides [d]	57	56	57
Quinolones [e]	109	122	95
Carbapenems [f]	74	78	70
Glycopeptides [g]	40	35	46
Sulfamethoxazole/Trimethoprim	53	58	49
Other [h]	104	131	77

AD, antimicrobial density (defined daily doses per 1000 patient-days); CSS, closed system suctioning; OSS, open system suctioning.
[a] The antimicrobials used in the intensive care units were divided by class and group according to ATC classification defined by the World Health Organization, index 2010; [b] penicillin-like antibiotics: amoxicillin-clavulanic acid, piperacillin-tazobactam, flucloxacillin, amoxicillin, benzylpenicillin, piperacillin; [c] cephalosporins: cefazolin (first generation); cefuroxim (second generation); ceftriaxon and ceftazidime (third generation); [d] aminoglycosides: gentamycin, tobramycin; [e] quinolones: ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin; [f] carbapenems: meropenem, imipenem/cilastatin
[g] glycopeptides: vancomycin, teicoplanin; [h] other: metronidazol; clindamicin; rifampicin; erythromycin; colistin; azithromycin.

respectively (unadjusted HR, 0.88; 95% CI, 0.43-1.80) (Table 5). Extending the time window in the definition of cross-transmission to 9 days [33] between ICU discharge and admission yielded 17 and 18 cross-transmission events, corresponding to 3.8 and 4.6 events per 1000 patient-days at risk, respectively (unadjusted HR, 0.88; 95% CI, 0.45-1.72).

Acinetobacter isolates were available from 60 of 77 colonized patients and 65 isolates (40 CSS, 25 OSS) were genotyped, yielding 27 different genotypes. Incidence rates of cross-transmissions were 2.7 and 0.4 per 1000 patient-days at risk during CSS and OSS, respectively (unadjusted HR, 6.46; 95% CI, 1.46-28.63). With an extended time window of 9 days the incidence rates were 3.3 and 1.0 per 1000 patient-days at risk, respectively (unadjusted HR, 3.1; 95% CI, 1.12-8.6). Seven of 15 potential cross-transmission events with *Acinetobacter* occurred in one unit over a 2-month period (during CSS).

Enterobacter species isolates were available from 124 of 160 colonized patients and 167 isolates from 122 patients (61 in both CSS and OSS) were genotyped, yielding 82 different types. Incidence rates of cross-transmission were 0.2 and 1.0 per 1000 patient-days at risk during CSS and OSS, respectively, with a corresponding unadjusted HR of 0.24 (95% CI, 0.03-2.19). For *Enterobacter* species all cross-transmissions events (n = 5) occurred in one unit.

Table 4. Colonization status in closed and open suction periods

	Hospital 1			Hospital 2			Total		
	CSS	OSS	*p* or adjusted HR (95% CI) [a]	CSS	OSS	*p* or adjusted HR (95% CI) [a]	CSS	OSS	*p* or adjusted HR (95% CI) [a]
No. of patients with ≥ 1 culture (% of total)	296 (92)	239 (96)		240 (91)	222 (80)		536 (92)	461 (88)	
LOS 24-48 hours, no. of patients	37	23		32	28		69	51	
Admission status unknown, no. (%) of cultured patients	5 (2)	2 (1)	.39	5 (2)	10 (5)	.14	10 (2)	12 (3)	.43
Colonization on admission, no. of patients (% of cultured pt with known colonization status)	56 (19)	61 (26)	.23	54 (23)	51 (24)	.79	110 (21)	112 (25)	.14
Pseudomonas aeruginosa	17 (6)	22 (9)	.12	16 (7)	13 (6)	.74	33 (6)	35 (8)	.35
Acinetobacter species	5 (2)	2 (1)	.39	6 (3)	3 (1)	.37	11 (2)	5 (1)	.23
Stenotrophomonas Maltophilia	4 (1)	6 (3)	.32	4 (2)	2 (1)	.46	8 (2)	8 (2)	.76
Klebsiella species	9 (3)	19 (8)	.01	15 (6)	24 (11)	.07	24 (5)	43 (10)	.002
Escherichia Coli	16 (5)	13 (6)	.98	20 (8)	25 (12)	.26	36 (7)	38 (8)	.33
Enterobacter species	17 (6)	15 (6)	.80	14 (6)	6 (3)	.10	31 (6)	21 (5)	.38

Acquisition / 1000 patient-days at risk ≥ 1 selected pathogens [c]	31.5	31.3	1.01 (0.73-1.38)	40.4	33.9	1.26 (0.92-1.75)	35.5	32.5	1.14 (0.91-1.42)
Pseudomonas aeruginosa	9.2	15.3	0.65 (0.37-1.13)[b]	11.6	16.2	0.67 (0.38-1.16) [b]	10.3	15.7	0.66 (0.45-0.97) [b]
Acinetobacter species	4.0	5.9	0.66 (0.30-1.46) [b]	12.4	1.8	7.96 (2.74-23.11)[b]	7.6	4.0	2.03 (1.15-3.57) [b]
Stenotrophomonas Maltophilia	6.3	8.0	0.77 (0.39-1.51)	7.6	7.5	1.13 (0.57-2.27)	6.9	7.8	0.91 (0.56-1.47)
Klebsiella species	10.7	5.7	1.90 (0.96-3.76)	20.1	16.1	1.30 (0.78-2.16)	14.6	10.1	1.46 (0.98-2.19)
Escherichia Coli	6.8	4.9	1.29 (0.62-2.73)	15.4	11.8	1.42 (0.80-2.53)	10.6	7.8	1.34 (0.85-2.10)
Enterobacter species	13.1	17.0	0.80 (0.48-1.31)	5.6	9.5	0.63 (0.30-1.32)	9.5	13.4	0.71 (0.47-1.07)

CSS, closed system suctioning; OSS, open system suctioning; HR, hazard ratio; CI, confidence interval; MV, mechanical ventilation.
[a] *p* values were given for colonization unknown or on admission; adjusted HR were calculated for acquired colonization. Adjusted HRs for acquisition were calculated with Cox regression models. In Table 4, the adjusted HR for study period is given. Further adjusted for: unit, gender, diagnosis, days in isolation, mechanical ventilation, APACHE II score, age, antibiotics on admission. Adjusted HR for hospitals 1 and 2 without adjustment for unit; [b] *Pseudomonas* and *Acinetobacter*: all patients that acquired colonisation were on MV, and therefore MV was not included in the model. Two patients who acquired colonisation with *Enterobacter*, with *Klebsiella* or with *Stenotrophomonas* were on MV, one patient with *coli* acquisition was on MV; [c] only first acquisition per pathogen taken into account.

Table 5. Possible cross-transmission (CT) with *Pseudomonas aeruginosa*, *Acinetobacter*, or *Enterobacter* species

	CSS	OSS	Adjusted HR [a] (95% CI)
Pseudomonas aeruginosa			
No. of events (% of patients with acquired PA)	15 (33)	16 (26)	
Possible CT/1000 patient-days at risk	3.37	4.12	0.90 (0.44-1.85)
Possible CT <9 days/1000 patient-days at risk	3.82	4.63	0.90 (0.46-1.78)
Acinetobacter species			
No. of events (% of patients with acquired *Acinetobacter*)	13 (35)	2 (10)	
Possible CT/1000 patient-days at risk	2.68	0.40	6.72 (1.50-30.12)
Possible CT <9 days/1000 patient-days at risk	3.30	1.00	3.16 (1.15-8.71)
Enterobacter species			
No. of events (% of patients with acquired *Enterobacter*)	1 (2)	4 (7)	
Possible CT/1000 patient-days at risk	0.23	0.97	0.28 (0.03-2.66)
Possible CT <9 days/1000 patient-days at risk	0.23	1.46	0.14 (0.02-1.27)

CSS, closed system suctioning; OSS, open system suctioning; HR, hazard ratio; CI, confidence interval; PA, *P. aeruginosa*. [a] Adjusted for: period, hospital, gender, diagnosis, days in isolation, APACHE II score, age, antibiotics on admission.

Extending the time window to 9 days yielded one and six cross-transmission events in two units (incidence rates per 1000 patient-days at risk 0.2 and 1.5, respectively; unadjusted HR, 0.16; 95% CI, 0.02-1.31).

In 16 potential cross-transmission events there were clusters of two related isolates, and in 35 events, there were clusters of more than two related isolates. The largest cluster concerned ten potential cross-transmission events of *P. aeruginosa* during a period of 8 months (both in CSS and OSS period).

Discussion

In this prospective crossover study, unit-wide implementation of closed endotracheal suctioning failed to reduce cross-transmission and acquisition rates of the most relevant Gram-negative bacteria in ICU patients. Furthermore, based on extensive microbiological surveillance and genotyping, 80% of all acquisitions of respiratory tract colonization appeared to be from endogenous origin.

Strengths of our study include the unit-wide comparison of CSS and OSS, which excludes potential effects of patient-dependency, and with a cross-over of both systems to account for ward-specific confounding. In addition, by including all patients admitted, selection bias was avoided and by using different orders of interventions any possible carryover effects were reduced. Furthermore, extensive microbiologic surveillance and genotyping allowed accurate

quantification of cross-transmission events and structured monitoring of adherence to infection control practices allowed adjustment for differences between units.

The power calculation was based on individual patients and 250 patients per study arm were needed to demonstrate an absolute risk reduction in cross-transmission of 10-15%. Because both hospitals participated with two instead of one unit, we included more than twice the number of patients needed. With 1110 patients studied, the difference in ICU-acquired acquisition rates with any of the marker pathogens was low (adjusted HR, 1.14; 95% CI, 0.91-1.42) and it is, therefore, highly unlikely that a larger study sample size would have demonstrated a clinically relevant difference between both methods. Furthermore, there was no evidence that these acquisition rates were influenced by differences in antibiotic exposure or barrier precautions applied.

When considering *P. aeruginosa*, *Acinetobacter* species, and *Enterobacter* species, overall cross-transmission rates with any of these pathogens were 5.7 and 4.5 per 1,000 patient-days at risk during CSS and OSS, respectively, when applying the most stringent definition for such an event. Total acquisition rates for these three pathogens were fourfold higher, ranging from 21.9 (CSS) and 22.2 (OSS) per 1000 patient-days at risk. Cross-transmission rates could not be quantified for the other marker pathogens (*Escherichia coli, Klebsiella* species and *Stenotrophomonas malthophilia*), but acquisition rates varied between 23.1 (OSS) and 29.3 (CSS), fairly similar to those of *P. aeruginosa*, *Acinetobacter* species and *Enterobacter* species. If the ratio between acquisition and cross-transmission rates would also be very similar for these pathogens, the (extrapolated) total cross-acquisition rate for all six pathogens would have been approximately 12 per 1000 patient-days at risk. To the best of our knowledge, there are no such data available from other ICUs for comparison, which precludes benchmarking of our findings to those of other ICUs.

Fifty percent of all patients in our trial were colonized with at least one of the marker pathogens, either on admission or acquired. Although this overall percentage may seem lower than in other studies, in which colonization rates varied from 85% to 91% [3,24-26], these studies had smaller sample sizes (21 -123 patients) and had included patients with an expected duration of mechanical ventilation of >5 days [24,26]. Three studies have compared colonization of suction catheters [34], ventilator tubing [35] and respiratory tract [36] between CSS and OSS. Colonization rates were generally higher in both CSS (60% to 80%) and OSS (39% to 70%), as compared to our findings (48% and 51%, respectively) [34-36]. None of these studies investigated cross-transmission. Besides, they all used individual randomization of patients and had smaller sample sizes (20 - 84 patients).

In our study, approximately 80% of acquisitions were considered from endogenous origin, which is in contrast to some other results. For *P. aeruginosa*, reported proportions of acquisitions attributable to cross-transmission have ranged from 8% to 64% [37-43], while for *Acinetobacter* and *Stenotrophomonas,* these percentages have been as high as 53% and 61%, respectively [8]. Again, methodological differences between studies and the absence of

quantification of cross-transmission rates in these studies precludes a formal comparison to our findings.

The characteristics of our study population (62% male, 62 years old, median APACHE II score of 20, median length of ICU stay of 6 days and an ICU mortality rate of 20%) is comparable with populations in other large ICU trials [44,45]. Although we used imputation for missing data for APACHE II score (51 scores) and diagnosis (one missing), excluding these patients from analysis did not change outcome (adjusted HR, 1.20; 95% CI, 0.96-1.51).

The total antibiotic use in our study (i.e., 1210 defined daily dosages per 1000 patient-days) does not deviate extensively from European ICUs with reported median defined daily dosages of 1254 to 1380 per 1000 patient-days [46-48]. Furthermore, antibiotic resistance levels among the marker pathogens in our study were comparable with resistance densities in German ICUs [47], but lower than reported percentages from the United States (National Healthcare Safety Network) and from the International Nosocomial Infection Control Consortium [49,50]. Yet, it is unknown whether antibiotic resistance reduces the likelihood of transmission to other patients. For all these reasons, we think our findings are, despite a different resistance ecology, generalizible to ICUs outside The Netherlands.

Infection control measures are important in the prevention of cross-transmission and we, therefore, decided to carefully monitor the effects of OSS and CSS on hand hygiene and other practices. Although there were no protocolized differences for hygiene measures for both procedures, OSS was associated with better adherence to hand hygiene before endotracheal suctioning, glove use, and nurses more frequently used eye protection and masks. Importantly, however, adherence to hand hygiene after the procedure was comparable between both study groups. One might speculate that beneficial effects of CSS on cross-transmission were compensated by a lower adherence to infection control measures, yet both effects cannot be disentangled with the study design as used. Naturally, it would have been possible to include these hygienic aspects in our intervention, but then we would not have answered the question how unit-wide implementation of closed endotracheal suctioning in daily practice, without modification of other variables, reduced cross-transmission.

Results from some studies have suggested that mean frequency of endotracheal suctioning per patient was higher when using CSS (17 times per day) as compared with OSS (ten to 12 times per day), presumably because of easiness of CSS [9,15,34,36,51-53]. Our findings do not support this, because frequencies (both mean and median) of endotracheal suctioning were comparable in both study groups.

Like in most places in the world, OSS was less expensive than CSS (price of OSS € 0,38 per catheter and € 2.70 per swivel connecter; price per CSS € 11.20; price level The Netherlands 2009) [12,54]. Therefore, performing endotracheal suctioning with OSS, as compared with CSS, might save money without increasing incidences of cross-transmission with Gram-negative bacteria.

A limitation of our study is the exclusion of Gram-positive pathogens, such as *Staphylococcus aureus*. Yet, the prevalence of methicillin-resistant *S. aureus* (MRSA) carriage in Dutch ICU

patients is <1% and colonization with methicillin-susceptible *S. aureus* is mostly present on admission with rapid disappearance thereafter [55]. Therefore, we did not expect CSS to have discernable effects on *S. aureus* acquisition rates. Furthermore, we only investigated the practice of changing CSS every 24-72 hrs. Other investigators have evaluated the effects of extended changing of CSS (when compared with changing every 24 hrs or when compared with OSS) on the occurrence of ventilator-associated pneumonia, and this appeared not to be associated with a higher risk [56-58]. Cross-transmission, however, was not evaluated in any of these studies, and it remains, therefore, unknown if prolonged use of CSS will be associated with lower cross-transmission rates in ICUs.
In conclusion, we could not demonstrate differences in acquisition or cross-transmission with Gram-negative bacteria between CSS and OSS.

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***Supplementary Table E1**: Number of patients with antibiotic resistant pathogens*

	Pseuromonas aeruginosa		Acinetobacter species		Stenotrophomonas Maltophilia		Escherichia Coli		Enterobacter species		Klebsiella species	
	CSS	OSS	CSS	OSS	CSS	OSS	CSS	OSS	CSS	OSS	CSS	OSS
No. of patients colonized	81	101	50	27	45	45	85	77	79	81	95	95
Resistant for, no. (%) of patients colonized												
Ceftazidime	14 (17)	21 (21)	1 (2)	1 (4)	-	-	2 (2)	2 (3)	19 (24)	19 (23)	7 (7)	9 (9)
Meronem	7 (9)	8 (8)	-	-	-	-	0	0	0	0	0	0
Ciprofloxacin	8 (10)	8 (8)	0	1 (4)	-	-	10 (12)	2 (3)	12 (15)	10 (12)	7 (7)	5 (5)
Tobramycin/ Gentamycin	7 (9)	8 (8)	-	1 (4)	-	-	6 (7)	0	15 (19)	15 (19)	9 (9)	9 (9)
Cotrimoxazol	-	-	-	-	2 (4)	3 (7)	19 (22)	16 (21)	12 (15)	3 (4)	-	-

CSS, closed system suctioning; OSS, open system suctioning

CHAPTER 4

Changes in heart rate, mean arterial pressure and oxygen saturation after open and closed endotracheal suctioning: a prospective observational study

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Abstract

Purpose

To quantify changes in heart rate (HR), mean arterial pressure (MAP) and peripheral oxygen saturation (SpO_2) in mechanically ventilated ICU patients undergoing endotracheal suctioning with closed suction systems (CSS) and open suction systems (OSS).

Methods

We performed a prospective observational study nested within a cross-over trial in 4 ICUs between January 2007 and February 2008. CSS and OSS were used for all patients requiring mechanical ventilation during 6-months study periods. Per period and per unit, 25 patients were selected at random and HR, MAP and SpO_2 were measured before and after endotracheal suctioning using a standardized protocol.

Results

In total 200 ES procedures in 165 patients were monitored. Mean HR, MAP and SpO_2 changed directly after endotracheal suctioning and returned to baseline after 5 minutes. Changes in HR and MAP were comparable after using CSS and OSS, whereas in SpO_2 slightly better values were monitored 3 and 5 minutes after OSS, these differences being rather small (0.3 to 0.7%) and clinically not relevant.

Conclusions

Changes in heart rate, MAP and SpO_2 were comparable and mild during and after CSS and OSS. Both systems can be considered equally safe.

Introduction

Endotracheal suctioning (ES) is an essential and frequently performed procedure in mechanically ventilated patients in the intensive care unit (ICU). By ES, secretions are cleared from the tracheobronchial tree, guaranteeing optimal oxygenation and avoiding accumulation of secretions, tube occlusion, increased work of breathing, atelectasis and pulmonary infections[1,2]. Yet ES may also have adverse effects, such as disturbances in cardiac rhythm and hypoxemia [3]. Nowadays, two systems are available to perform ES: the single use open suction system (OSS) and the multiple-use closed suction system (CSS). The latter does not require disconnection from the ventilator and can remain connected to the patient for, at least, 24 hours, depending on hospital protocol and CSS type. In contrast, the OSS requires disconnection from the mechanical ventilator, either by complete disconnection or through opening a valve of a swivel (semi-open). It is suggested that, by interruption, the patient is predisposed to physiologic disturbances due to a decay of intrathoracic pressure, like hypoxemia, altered mean arterial pressure, and alterations in heart rate [4,5]. Several studies compared both systems with regard to physiologic disturbances, mostly with both procedures performed in a single patients [1,6-11]. Results from most studies favoured CSS, though differences between ES systems were rather small and clinically not relevant [12]. Furthermore, results are difficult to generalize, due to small sample sizes (9 to 35 patients) and differences in performance of ES (duration, use of pre-oxygenation and hyperoxygenation). It remained, therefore, inconclusive whether one system should be preferred over the other, and we aimed to determine whether CSS, as compared to OSS with or without use of a swivel connector, changes cardiorespiratory parameters after ES in mechanically ventilated ICU patients.

Methods

Design

Between January 2007 and February 2008, a prospective observational study on comparative safety of open and closed suction systems was performed with a focus on unintended side effects (physiologic disturbances) during and after endotracheal suctioning with either system. The study was nested in a larger crossover trial, in which during periods of 6 months either CSS or OSS were used for all mechanically ventilated patients [13]. Four ICUs participated in the trial: 2 ICUs from a university hospital with 10 beds (4 single rooms, 6 on the ward) and 8 beds (1 single room, 7 on the ward) and two 8-bed units from a teaching hospital (all single rooms). Since use of CSS or OSS was dictated by study protocol, it was not possible to randomize individual patients to either of both systems. Therefore, per study period and per unit, 25 bed numbers were randomly selected by a computer program (Research Randomizer) for bedside monitoring, accumulating to a total of 200 observations of ES procedures. Three times a week, from Monday to Thursday, research nurses checked whether the randomized bednumber was

occupied and whether the patient was on ventilation. If not, the research nurse verified whether the "neighbour" (higher bed number) was on ventilation. When confirmed, the attending nurse was informed about the bedside monitoring and asked to warn when ES was indicated.
Monitoring was performed whether the patient had OSS or CSS. In both study periods, non-adherence to the prescribed ES system occurred in 7% of the MV days, and was most often due to prone positioning or PEEP ≥ 12 (reason for using CSS during OSS), and because of weaning (reason for using OSS during CSS)[13].
Both CSS and OSS were not considered experimental treatments (as they both are frequently used), and, therefore, the institutional review board of both hospitals waived the requirement for informed consent. However, all patients (or next of kin) were informed about the aim and consequences of the study, with a possibility to refuse the use of patient-specific medical data for analysis.
All ES procedures were performed on indication by ICU nurses. OSS was performed through a rubber sealed swivel connector placed between the tube and the Y-piece of the ventilator circuitry (Hospital 1) or by disconnection (Hospital 2). The catheter was inserted into the endotracheal tube or tracheostomy until resistance was met and withdrawn 0,5 cm. A negative pressure of maximum 20 kPa (Hospital 2) or 30 kPa (Hospital 1) was set and the catheter was withdrawn while gently rotating. The swivel connector was closed again, the procedure during in total 10 to 15 seconds. For CSS, the procedure was similar except that the patient remained connected to the ventilator. CSS was replaced every 24 hours (Ballard* Trach Care* Double Swivel Elbow, Kimberly Clark*, Hospital 1) or every 72 hours (Ballard* Trach Care* 72 Hour, Kimberly Clark*, Hospital 2).
Pre-oxygenation and post-oxygenation was applied in both procedures when considered indicated, as judged by attending nurses, as was the use of protective masks and glasses. Non-sterile gloves were to be used during all procedures.

Outcome

The primary outcome was the change in cardiorespiratory parameters, and therefore heart rate (HR), mean arterial pressure (MAP) and peripheral oxygen saturation (SpO_2) were monitored before ES (baseline), immediately after ES and subsequently 3 and 5 minutes after ES. Data were registered by research nurses as recorded by the bedside monitor (Spacelabs Monitor 90387, Spacelabs Healthcare, Issaquah and Philips HP Merlin, Philips Healthcare, Eindhoven). Peripheral oxygen saturation (SpO_2) was measured continuously by pulse goniometry (DS-100A, Spacelabs and M1020A, Philips). The ECG tracing was continuously monitoring heart rate; mean arterial pressure was measured with an indwelling arterial catheter or, if such a catheter had not been inserted, a non invasive blood pressure cuff (Spacelabs and M1008A, Philips). Furthermore, clinical data were collected through a registration form at the time of monitoring: admission date to ICU, age, Positive End-Expiratory Pressure (PEEP), ES system, use of pre-oxygenation and post-oxygenation and whether the patient was disconnected during CSS.

Data analysis

For univariate analysis, continuous variables were tested with Kolmogorov-Smirnov tests for normal distribution. T-test were used when data were normally distributed, otherwise nonparametric Mann Whitney tests. Dichotomous variables were analyzed by using chi-square tests.

Changes in SpO_2, HR and MAP for each suction system were evaluated by using a Repeated Measures Analysis of Variance (ANOVA) including four levels of time (before ES, directly after ES, 3 and 5 minutes after ES) as within-subject variables and ES system (CSS or OSS) as between-subject factor. Additionally we added preoxygenation (yes/no), ventilation route (tube/tracheostoma), hospital (1 or 2) and PEEP (continuous) as covariates. Because of differences in performance and materials used for ES between hospitals, analyses were stratified according to center.

Imputation was used for baseline missing data using an Expectation-Maximization (EM) analysis with the Impute function in SPSS software (version 15.0, Chicago, Illinois), with inclusion of study period, age, gender, diagnosis and mechanical ventilation as key variables in the imputation model. A total of 4 APACHE II scores (2%) were missing. EM analysis revealed that data were missing at random, meaning that differences in missing data are related to the observed data, and missing values were replaced by imputed values. Apart from increasing the sample size, imputation corrects for possible bias due to selective missing values [14,15].

All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, Chicago, Illinois) version 15.0. A p-value of < 0.05 was considered statistically significant.

Results

During the 12-month study period, 200 ES procedures were monitored in 165 patients (118 males; mean age 60 ± 17 years). In 29 patients, more than one procedure was observed, all on different days. There were no significant differences in baseline characteristics of the observed patients in whom ES was performed with CSS or OSS (Table 1).

In 38 out of 200 (19%) observations the randomized bed was not occupied or the patient was not on MV at that moment, and the patient in the neighbour bed was selected for observation (Figure 1).

Bedside observations were performed during 95 CSS and 105 OSS procedures. More patients in whom ES was performed with OSS had a tracheostomy, and PEEP levels were lower as compared to CSS (Table 1). In three patients (1 CSS, 2 OSS), observational data were not complete since the patient was transported (i.e. to CT-scan) before the last observation had been performed. Furthermore, in 2 patients (1 CSS, 1 OSS), pulse oxymetry was not connected correctly, and these measurements were excluded from analysis.

Table 1: Baseline characteristics

	CSS	OSS
Patients observed - n	80	85
Patient characteristics		
Gender - % male	73	71
Age in years* – mean (sd)	59 (16.5)	61 (16.7)
APACHE II score – mean (sd)	20 (6.1)	20 (6.8)
Diagnosis - % surgical	38	35
Total duration MV – median (IQR)	19 (9-29)	17 (10-31)
Length of ICU stay – median (IQR)	17.5 (9.3-28.0)	18.0 (10.0-31.5)
ICU mortality - %	30	20
Hospital mortality - %	35	27
Number of observations	95	105
Characteristics of observations		
PEEP – median cm H_2O (IQR)	8 (5-10)	5 (5-6)
Preoxygenation - % of observations	21	27
Postoxygenation - % of observations	13	22
Duration MV before observation – median days (IQR)	6 (3-13.3)	5 (3-14)
Tube / tracheotomy - % tube	93.7	72.4

CSS Closed System Suctioning; OSS Open System Suctioning; APACHE Acute Physiology and Chronic Health Evaluation; MV Mechanical Ventilation; IQR Inter Quartile Range; sd standard deviation; PEEP Positive End Expiratory Pressure
** Age: at time of ICU admission*

Changes in heart rate

There were 103 complete measurements of OSS, and 94 for CSS. Mean HR increased with 4% directly after ES, but returned to baseline after 5 minutes, both after using CSS and OSS (Table 2). There were differences in mean HR before and after ES ($p < 0.001$), with largest differences directly after ES, as compared to baseline (median changes in HR of 4% (inter quartile range [IQR] 1%-9%) for CSS and 5% (IQR 2%-11%) for OSS). Differences were comparable for CSS and OSS (p=0.97) (Figure 2). Stratifying hospitals revealed slightly higher mean HR on all time points in hospital 2 as compared to hospital 1 (both $p < 0.001$), but again changes were not different between CSS and OSS. Restricting the repeated measures ANOVA to patients who were orally intubated (n=162) resulted in comparable changes (overall mean HR 90.7 for CSS and 89.4 for OSS, p=0.65), with higher mean values after ES ($p < 0.001$) and no difference between ES systems at these time points. Adding PEEP and preoxygenation as covariates in overall analysis did not alter overall results (p-values 0.81 and 0.93 for interaction effect of time and ES system when adding PEEP and preoxygenation as covariate, respectively).

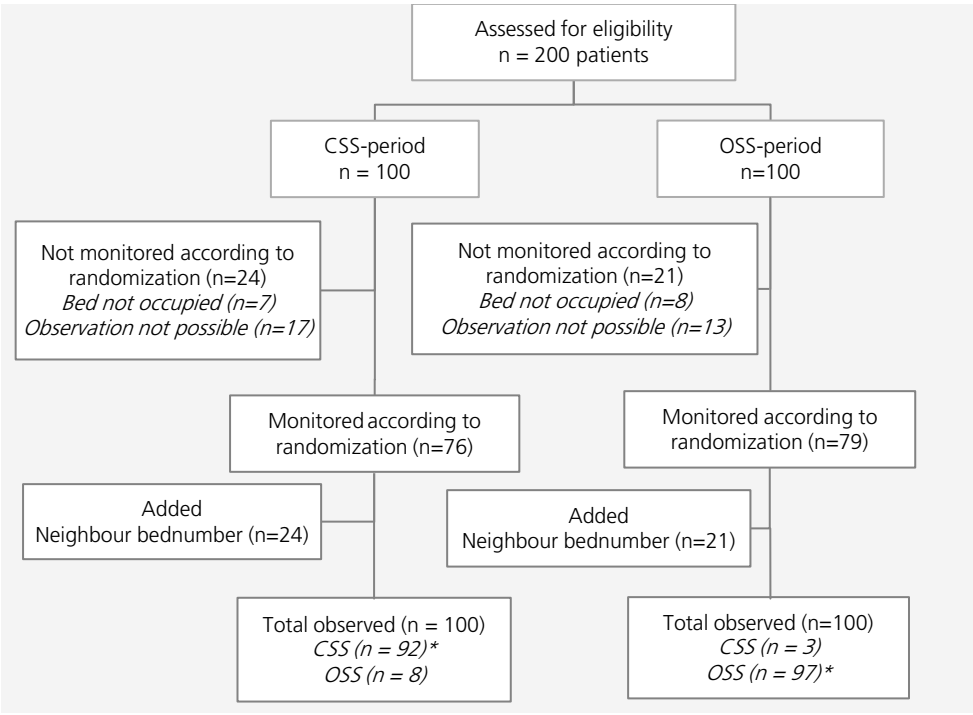

Fig. 1, Flowchart
CSS, closed suction system; OSS, open suction system
* Due to clinical indication in 7% of MV days another system instead of the prescribed ES system was used

Changes in Mean Arterial Pressure

For this analysis, there were 102 and 93 complete measurements for OSS and CSS, respectively. Mean MAP increased directly after ES, and subsequently decreased to baseline or below baseline 5 minutes after the procedure, both after using CSS and OSS (Table 2). Highest changes in MAP occurred directly after ES (median changes 5% (IQR 2%-12%) for CSS and 7% (IQR 3%-16%) for OSS). Mean MAP increased in time ($p < 0.001$), but changes were comparable after using CSS and OSS ($p = 0.34$). Mean MAP was higher in hospital 2 as compared to hospital 1 on all time points (Figure 3). In both hospitals, mean MAP increased directly after ES ($p < 0.001$ for both hospitals) and this increase did not depend on ES systems. Restricting analyses to patients who had a tube (n=160) resulted in comparable changes in MAP, with higher mean values directly after ES ($p < 0.001$) and without statistically significant differences between ES systems at these time points ($p = 0.39$). Adding PEEP and preoxygenation as covariates in overall analysis did not alter overall results (p-values 0.42 and 0.35 for interaction effect of time and ES system when adding PEEP and preoxygenation as covariate, respectively).

Table 2: Mean (sd) heart rate, MAP and arterial oxygen saturation before and after ES

	CSS	OSS	CSS	OSS	CSS	OSS	CSS	OSS	*P*
Heartrate									
Mean (sd)	89.9 (17.5)	89.3 (18.0)	93.6 (18.3)	93.3 (18.1)	90.6 (17.4)	89.8 (17.1)	89.7 (16.4)	89.3 (17.0)	.97
H1 mean (sd)	88.3 (13.3)	88.0 (15.3)	91.6 (14.1)	92.0 (15.7)	88.5 (13.4)	88.4 (14.9)	88.0 (13.4)	88.4 (15.2)	.95
H2 mean (sd)	91.8 (21.2)	90.7 (20.3)	95.8 (22.1)	94.6 (20.2)	93.0 (20.9)	91.2 (19.1)	91.7 (19.3)	90.3 (18.8)	.97
MAP									
Mean (sd)	85.5 (16.6)	87.8 (18.8)	88.8 (15.6)	92.1 (19.7)	86.4 (15.5)	89.2 (19.5)	85.8 (15.5)	87.0 (17.2)	.34
H1 mean (sd)	82.2 (18.2)	86.5 (19.1)	86.4 (18.2)	90.2 (20.5)	84.3 (17.0)	86.4 (20.0)	83.8 (17.8)	84.7 (17.9)	.17
H2 mean (sd)	89.2 (13.9)	89.1 (18.5)	91.5 (11.9)	94.0 (18.9)	88.7 (13.4)	91.8 (18.8)	88.0 (12.4)	89.2 (16.5)	.26
SpO_2									
Mean (sd)	97.2 (2.7)	97.6 (2.8)	97.7 (2.3)	97.6 (3.0)	97.6 (2.5)	97.9 (2.2)	97.5 (2.6)	98.2 (2.0)	.04
H1 mean (sd)	98.2 (2.2)	98.3 (2.1)	98.5 (1.7)	98.1 (2.6)	98.4 (2.2)	98.4 (1.8)	98.3 (2.2)	98.6 (1.7)	.21
H2 mean (sd)	96.1 (2.8)	96.9 (3.2)	96.8 (2.7)	97.0 (3.3)	96.7 (2.6)	97.5 (2.5)	96.6 (2.7)	97.9 (2.1)	.19

ES Endotracheal Suctioning; CSS Closed System Suctioning; OSS Open System Suctioning; H1 Hospital 1; H2 Hospital 2; MAP mean arterial pressure; SpO_2 arterial oxygen saturation
p-value calculated with Repeated Measures ANOVA, test of within subject effects for interaction effect

Changes in peripheral oxygen saturation

For this analysis, there were 102 and 93 compete measurements for SpO_2 after OSS and CSS, respectively. Mean SpO_2 slightly increased 5 minutes after ES, with a higher increase in mean values after using OSS as compared to CSS (p = 0.04)(Table 2). Stratified analyses revealed slightly higher mean values on all time points in hospital 1 as compared to hospital 2. In both hospitals changes after ES were not dependent on ES system (p = 0.21 and p = 0.19 for hospital 1 and 2, respectively). In patients with a tube (n=160), mean SpO_2 values were comparable with non-stratified values, with SpO_2 after CSS and OSS slightly changing over time and changing in different ways (interaction effect, p = 0.04).

When adding PEEP as a covariate in overall analysis, differences over time or between systems were not statistically significant (p = 0.07), whereas preoxygenation as covariate resulted in a significant increase over time (p < 0.001), and the increase was higher for OSS as compared to CSS (p = 0.02).

An arterial oxygen saturation of 90% or higher has been proposed as a target for adequate oxygenation during MV, i.e. to reverse hypoxemia [16,17]. In order to maintain this target level, levels measured with pulse oxymetry (SpO_2) need to be 96% [18]. In 33 (35%, CSS) and 31 (30%, OSS) of ES procedures, SpO_2 was below 96% (p=0.80) for at least one moment, and in 22 (CSS) and 19 (OSS) procedures this occurred after a baseline of 96% or higher. In three procedures (1 CSS, 2 OSS), SpO2 levels dropped below 90% after baseline levels above this threshold.

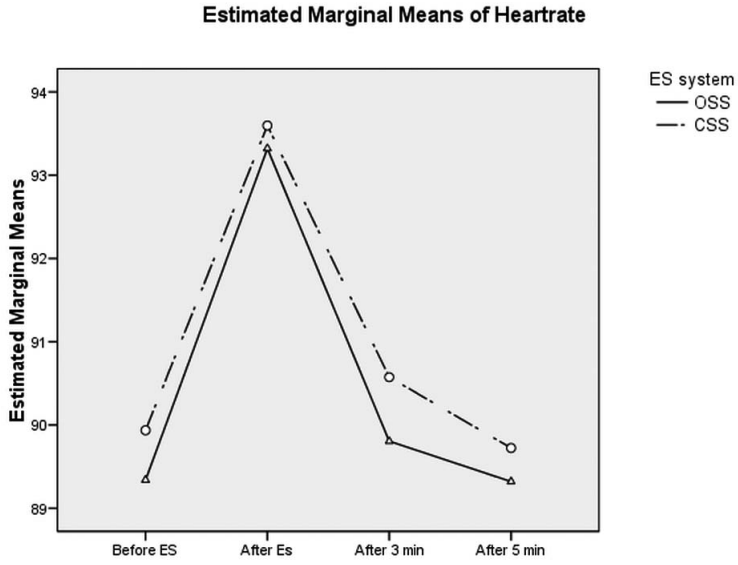

Fig 2, Mean heart rate before, during and after endotracheal suctioning

Discussion

The main feature of this study is that changes in heart rate, mean arterial pressure and peripheral oxygen saturation induced by endotracheal suctioning are minor and comparable when using either closed or open suction systems.

We performed a pragmatic study, evaluating ES as it was performed during standard care and when clinically indicated according to international guidelines [19]. Most procedures only lasted 10-15 seconds and were not associated with clinically relevant disturbances in physiologic parameters.

Although it has been suggested to apply preoxygenation before ES to minimize desaturation, especially in hypoxemic patients [19], it was applied in only 24% of ES procedures in the current study. And although preoxygenation was associated with higher SpO_2 values after OSS, as compared to CSS, differences were rather small (97.5% after CSS and 98.3% after OSS) and, therefore, not considered clinically relevant. Similarly small changes in SpO_2 after preoxygenation have been reported before [20,21].

The mean heart rate in the current study (89 and 90 beats per minute before ES in OSS and CSS, respectively) was slightly lower than reported in other studies (range 91 to 108 beats/min prior to ES) [1,5,8,10,11,20]. Yet, the mean HR only increased, on average, with four beats per minute after ES, with no difference between both suction systems, which confirms the findings of an earlier meta-analysis (6 beats/min in favour of CSS) [12].

Mean MAP values as found in our study before ES (86 to 88 mmHg in CSS and OSS,

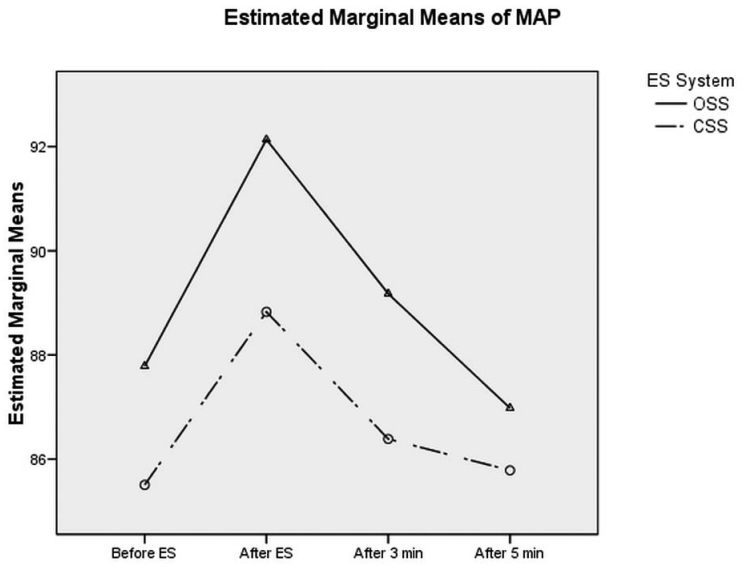

Fig 3, Mean Arterial Pressure (mean) before, during and after endotracheal suctioning
ES endotracheal suctioning; OSS open suction system; CSS closed suction system

respectively) were in the range of values reported in other studies (from 74 to 93 mmHg)[1,10,11,20,22]. In the current study, changes in MAP were not related to the suction system, which contrasts the findings of a recent meta-analysis in which the mean MAP decreased after using CSS (pooled standardized mean difference -0.43 mmHg) as compared to CSS [12].

Mean values for SpO_2 before ES in our study (97% in CSS and 98% in OSS) were comparable to those reported in other studies (ranging from 95 to 98%) [1,8-11]. However, where previous studies favoured use of CSS with slightly higher SpO_2 values, only small differences were found both in adults as in neonates [1,23-26].

CSS has been advised for adult patients needing high values of PEEP [19]. Our study protocol did not dictate this practice, but some physicians did prefer CSS in patients with PEEP values of 10 cm H_2O or higher, which contributed to the baseline difference in PEEP. In 5 out of 33 procedures in which PEEP was ≥10 cm H_2O (3 CSS, 2 OSS), SpO_2 dropped to 93-95% after ES. Including PEEP as a covariate in repeated measures analysis did not reveal differences between CSS and OSS.

In one hospital the negative pressure for ES was slightly higher (30 kPa) than recommended (20 kPa)[19]. The latter value has been recommended to prevent side effects, while still effectively removing secretions, but a clear-cut optimum value has not been defined yet. No differences in side effects were observed with a negative pressure of -400 cm H_2O as compared to -200 cm H_2O (approximately 40 to 20 kPa) in a single small-sized (n=9) study [7]. In our study, there was no evidence of any side effects of the negative pressure as used.

CSS has been advocated as a technique that may limit cardiorespiratory instability, because of pressure loss during disconnection [10,27]. However, CSS also interferes with intratracheal

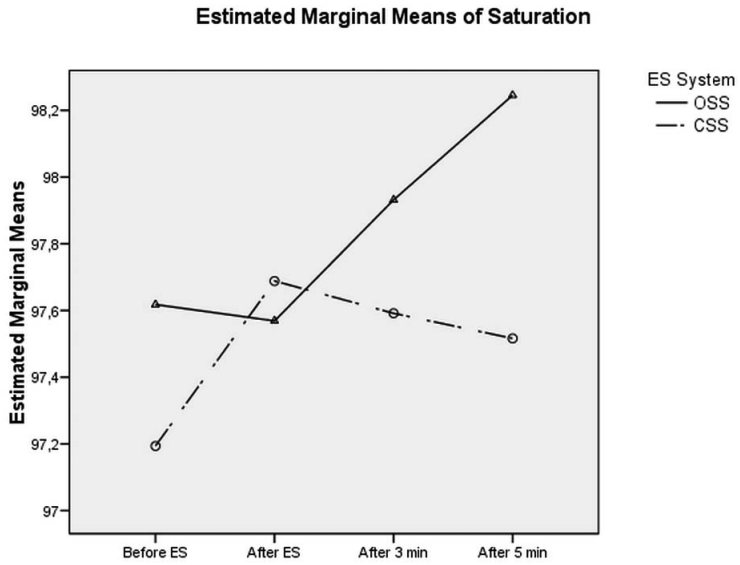

Fig 4, Mean peripheral oxygen saturation before, during and after endotracheal suctioning

pressure [28,29]. It has also been suggested that catheter size is of greater influence than suction method in this regard [30]. Yet, both aspects have not been addressed in our study.
Our study has several limitations. First, we did not randomize patients to either intervention. All patients received ES with a predefined suction system according to a study protocol. To avoid selection bias we have used a predefined schedule to include patients for this sub-study, which included the selection of another patient if the selected patient was not ventilated. There were no differences in patient characteristics between study groups, however there were differences in observations, with higher PEEP levels in CSS group and more patients orally intubated as compared to OSS.
We also did not record secretion volumes being removed after using CSS or OSS. The latter was more effective in removing secretions than CSS in animal models and in vitro [31,32]. If true in vivo, we would have expected more procedures of ES with CSS, which was not observed (both mean and median 6 times per day) [13].
In conclusion, the results of our study do not support the assumption that CSS, as compared to OSS, differently effects heart rate, MAP and peripheral oxygen saturation during and after endotracheal suctioning. Both systems can be considered equally safe in mechanically ventilated ICU patients.

Acknowledgements

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PART II

Respiratory Tract Colonization

CHAPTER 5

Risk factors for acquiring respiratory tract colonization with Gram-negative bacteria in intensive care patients

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In preparation

Abstract

Objective

To quantify acquisition of and risk factors for respiratory tract colonization with Gram-negative bacteria (GNB) in Intensive Care Unit (ICU) patients that were not colonized with GNB in the respiratory tract at the time of ICU-admission.

Methods

We performed a prospective cohort study in 4 ICUs, including patients staying > 48 hrs in ICU and without respiratory tract colonization with GNB on ICU-admission. Acquisition was determined through microbiological surveillance. GNB included *Pseudomonas aeruginosa*, *Acinetobacter* species, *Stenotrophomonas maltophilia*, *Escherichia coli*, *Enterobacter* species, *Klebsiella* species, *Citrobacter* species, *Proteus* species and *Serratia* species.

Results

In all, 250 (52%) of 481 patients acquired respiratory tract colonization with at least 1 GNB after a median of 5 days (inter quartile range 3-8 days)(acquisition rate 77.1 / 1000 patient days at risk). In Cox proportional hazard modelling, mechanical ventilation was associated with a higher risk of GNB acquisition (Hazard Ratio [HR] 2.37; 95% confidence interval [CI] 1.11 – 5.04), while admission to hospital 1 and antibiotics administered during ICU stay prior to acquisition or otherwise discharge were associated with a lower acquisition risks (HR 0.61, 95% CI 0.47-0.78 for hospital 1 and HR 0.61, 95% CI 0.44-0.82 for antibiotics). For individual pathogens, use of closed suction systems was associated with a lower risk of acquiring colonization with *P. aeruginosa*, and a higher risk of acquiring colonization with *Klebsiella* species.

Conclusions

52% Of the patients not colonized with GNB in the respiratory tract acquired colonization during ICU-stay. Risk of acquisition was higher in patients receiving mechanical ventilation and lower in the university hospital and among patients receiving antibiotics during ICU stay.

Introduction

Infections caused by Gram-negative bacteria (GNB) are associated with increased morbidity and mortality and higher health care costs, especially in intensive care units (ICUs) [1-3]. In these units, patients are at the highest risk of acquiring nosocomial infections due to their severe underlying disease and being exposed to invasive procedures like mechanical ventilation and intravenous catheters [4]. ICU acquired infections are almost always preceded by colonization, which is defined as the presence of microorganisms without generating adverse clinical effects [5,6]. Especially mechanically ventilated patients are frequently colonized with GNB in the respiratory tract, with rates varying from 50% to 91% [5,7-14]. In patients not colonized with GNB at the time of ICU-admission, acquisition of colonization may occur either endogenously or exogenously [15]. Endogenous "acquisition" could imply selection (for instance through antibiotic exposure) of pre-existent GNB that reach detection limits of culture methods at a certain time point. Exogenous acquisition (cross-transmission) results from lapses in infection control [16]. Identification of risk factors for colonization or infection with GNB has been attempted before, but mostly by addressing a single pathogen (i.e., *Pseudomonas aeruginosa*, *Acinetobacter baumannii* or *Stenotrophomonas maltophilia)*, applying a case-control study and frequently in the circumstances of a nosocomial outbreak [17-19]. Some studies focus on colonization on admission or acquired colonization, while other studies focus on prevalence of colonization without making a distinction. One study assessed risk factors for acquiring respiratory tract colonization in metallo-β-lactamase producing GNB by using univariate analysis [20], while another study used a multivariate model to assess risk factors for acquiring enteric GNB and *Pseudomonadaceae* [21]. In this study we aimed to quantify acquisition with any GNB in ICU patients, to assess the days a patient is at risk of acquiring colonization and to determine risk factors for acquiring colonization with GNB.

Materials and methods

Design

Between January 2007 and February 2008, a prospective cohort study was conducted in four ICUs: 2 ICUs from a university hospital with 10 beds (4 single rooms, 6 on the ward) and 8 beds (1 single room, 7 on the ward) and two 8-bed units from a teaching hospital (all single rooms). The cohort study was embedded in a prospective crossover trial, in which closed and open suction systems were implemented unit-wide for all eligible patients during periods of six months [22].

Patients with a length of ICU stay of > 48 hrs were eligible for study inclusion. For analysis, we included only patients with at least two microbiological cultures from respiratory tract samples and without GNB colonization on admission. Patients with unknown colonization status on admission were excluded.

Colonization on admission was defined as growth of GNB from an endotracheal aspirate sample (or throat swab in the absence of endotracheal aspirate) in a sample obtained within 48 hrs of ICU admission. Acquired colonization was defined as documentation of GNB in a respiratory tract sample obtained at least 48 hrs after ICU admission and preceded by documented absence of GNB previously [13]. When the first culture grew GNB but was taken >48 hrs after ICU admission, colonization status on admission was considered unknown.
Clinical data (APACHE II scores, main diagnosis, contact isolation, duration of mechanical ventilation, endotracheal suction system), demographic and antibiotic use data were collected through a Case Record Form. Antibiotics administered in ICU were monitored until acquisition with GNB or, if no acquisition of GNB occurred, until discharge. The study was approved by the institutional review board of both hospitals.

Outcomes

The primary outcome of our study was the first event of acquired colonization with any GNB in the respiratory tract. GNB included were *Pseudomonas aeruginosa*, *Acinetobacter* species, *Stenotrophomonas maltophilia*, *Escherichia coli*, *Enterobacter* species, *Klebsiella* species, *Citrobacter* species, *Proteus* species and *Serratia* species. Colonization was determined by surveillance cultures of endotracheal aspirates (MV patients) or oropharyngeal swabs (non-ventilated patients) that were obtained on admission and twice weekly thereafter (every Monday and Thursday) until discharge from ICU. The samples were analyzed according to local protocol, and isolated marker pathogens were stored at -80° C. Results were communicated to the medical staff according to standard microbiological reporting practices.

Policy for patient isolation

Patient isolation was based upon the guidelines of the Dutch Infection Prevention Working Party [23,24]. In case of patient isolation, a sign is placed outside the room of indicated patients, indicating hand hygiene and donning of gown and gloves immediately before room entry, and, depending on type of isolation, use of a mask and a cap.

Data analysis

Aim of our study was to determine risk factors for acquiring respiratory tract colonization with any GNB. As secondary endpoints we performed similar analyses for individual pathogens (i.e., *P. aeruginosa, Acinetobacter* species*, S. maltophilia, E. coli, Klebsiella* species and *Enterobacter* species) as well as for *Enterobacteriaceae* (i.e., *E. coli*, *Klebsiella* species, *Enterobacter* species, *Citrobacter* species, *Serratia* species and *Proteus* species).
For univariate analysis, continuous variables were tested with Kolmogorov-Smirnov tests for normal distribution. T-tests were used when data were normally distributed, otherwise nonparametric Mann Whitney tests were used. Dichotomous variables were analyzed by using Chi-square tests.

Risk factors for acquiring respiratory tract colonization were evaluated using Cox regression models, with days in ICU until first event of acquisition (days at risk) as time variable, which was defined as all days in ICU in which the patient did not have documented colonization with any GNB. Patients without acquired colonization were censored at discharge from the unit or death. Once colonized, patients were considered no longer at risk for acquisition. The following variables were designated as potential risk factors for acquisition: endotracheal suction system (open/closed system, according to study protocol in the crossover trial), hospital (H1/H2), gender (male/female), age (continuous, at time of ICU admission), Acute Physiology and Chronic Health Evaluation (APACHE II) score (continuous), duration of contact isolation (continuous, number of days until acquisition or otherwise discharge), mechanical ventilation (binominal), admission diagnosis (surgical / medical), antibiotic use on admission to ICU (binominal) and antibiotics administered during ICU stay until acquisition or discharge (binomial). All covariates were tested univariately and variables for which the p-value was <0.05 were included in a multivariate model. A backward stepwise process was used and Hazard Ratios (HR) and their corresponding 95% confidence intervals (CIs) were calculated. Multicollinearity between covariates was tested in advance and none of the variables appeared highly correlated (i.e., $r^2 > 0.80$). Therefore all covariates were included in analysis.
Imputation was used for missing data using an Expectation-Maximization (EM) analysis with the Impute function in SPSS software (version 15.0, Chicago, Illinois), with inclusion of study period, age, gender, diagnosis and mechanical ventilation as key variables in the imputation model. A total of 21 APACHE II scores were missing (4.4% of all values). EM analysis revealed that data were missing at random, meaning that differences in missing data are related to the observed data, and missing values were replaced by imputed values. Apart from increasing the sample size, imputation corrects for possible bias due to selective missing values [25,26].
All statistical analyses were performed with the Statistical Package for the Social Sciences (SPSS, Chicago, Illinois) version 15.0. A p-value of < 0.05 was considered statistically significant.

Results

From January 2007 to February 2008, 1021 patients were admitted to one of the participating ICUs for > 48 hours, of whom 540 were excluded because of colonization with GNB on admission (n = 229), unknown colonization status on admission (n = 39) or insufficient number of cultures obtained (n = 272) (Figure 1). The latter (with either one (n = 193) or no (n = 79) cultures available) were mainly patients with a short length of ICU stay (median 3 days, IQR 3-4 days), frequently admitted in-between surveillance culture days. In total 481 patients (6252 patientdays) were included, mostly male (60%) with a median age of 60 years (IQR 46 – 71 years). From these patients 2059 respiratory tract cultures were available for analysis.

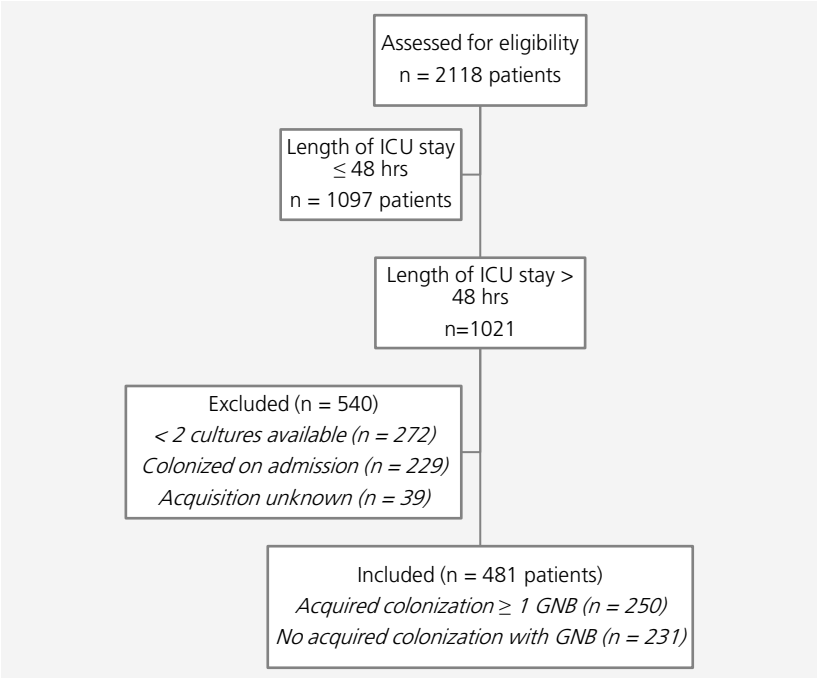

Fig 1, Flowchart
ICU intensive care unit; GNB Gram-negative bacteria

Acquired colonization

In total, 250 patients (52%) acquired colonization with ≥ 1 GNB, yielding an overall acquisition rate of 77.1/1000 patientdays at risk. Most patients acquired colonization with one (50%) or with two (29%) pathogens, mainly with *Enterobacter* spp (32%) or with *Pseudomonas aeruginosa* (30%) (Table 1). Median time until colonization with the first pathogen was 5 days (IQR 3 – 8 days), while median time before half of the study population acquired colonization was 8 days (IQR 7 – 9 days) (Figure 2).

Risk factors for acquisition

In univariate analysis, acquired GNB colonization was associated with being admitted to hospital 2, being mechanically ventilated and not receiving antibiotics on admission (Table 2). Moreover, acquired GNB colonization was also associated with a longer length of ICU stay and a higher ICU and hospital mortality rate.

In Cox proportional hazard modeling both admission to hospital 1 and antibiotic use during ICU stay were independently associated with a lower risk of acquisition of GNB, while MV was associated with a higher risk of acquisition (Table 3). As compared to hospital 2, patients admitted in hospital 1 had a 39% reduced risk for acquiring GNB colonization.

Table 1, Acquired Gram-negative bacteria (% of patients who acquired colonization)

Pathogen	N (%)	Acquisition / 1000 patient days at risk
Pseudomonas aeruginosa	74 (30)	22.7
Stenotrophomonas maltophilia	42 (17)	12.9
Acinetobacter species	34 (14)	10.4
Enterobacter species	79 (32)	24.3
Klebsiella species	71 (28)	21.8
Escherichia coli	57 (23)	17.5
Serratia species	26 (10)	8.0
Proteus species	22 (9)	6.8
Citrobacter species	13 (5)	4.0
*Other GNB **	30 (12)	9.2

* Other GNB: *Moraxella, Morganella, Achromobacter, Hafnia, Pantoea, Alcaligenes, Burkholderia, Eikenella, Leclercia, Ochrobactrum, Providencia*

Table 2, Characteristics of patients who acquired colonization with ≥ 1 GNB

	Without GNB	Acquired GNB	*P*
Patients (n = 481)	231	250	
Gender – male, n (%)	133 (58)	153 (61)	0.42*
Age – median (IQR)	59 (46-70)	61 (48-72)	0.16**
Hospital - % patients			0.00*
H1	55	45	
H2	39	61	
Diagnosis – surgical, n (%)	80 (35)	76 (30)	0.32*
APACHE II score –mean (sd)	19 (7.2)	20 (7.2)	0.15***
Antibiotics on admission – n (%)	68 (29)	52 (21)	0.03*
Antibiotics during ICU stay‡ – n (%)	167 (72)	195 (78)	0.15*
Duration AB‡ – median days (IQR)	3 (0 – 6)	4 (2 – 7)	0.18**
Isolation‡ – n patients (%)	15 (7)	20 (8)	0.52*
Isolation days – median (IQR) of patients isolated	4 (3 – 6)	6 (3 – 9)	0.37**
Mechanical ventilation – n (%)	201 (87)	243 (97)	0.00*
Duration MV– median days (IQR)	4 (2-7)	12 (7-22)	0.00**
Suction system† – CSS, n (%)	123 (53)	137 (55)	0.73*
Length of ICU stay – median days (IQR)	6 (4-10)	14 (8-25)	0.00**
Length of hospital stay – median days (IQR)	19 (11-37)	32 (19-57)	0.00**
Hospital stay before ICU admission – median days (IQR)	0 (0-3)	0 (0-3)	0.38**
Hospital stay after ICU admission – median days (IQR) of patients who survived ICU	11 (5-25)	19 (9-38)	0.002**
ICU mortality - %	26 (11)	58 (23)	0.001*
Hospital mortality - %	40 (17)	79 (32)	0.00*

GNB Gram-negative bacteria; IQR inter quartile range; sd standard deviation; APACHE Acute Physiology and Chronic Health Evaluation; MV mechanical ventilation CSS Closed Suction System; ICU intensive care unit

‡ Prior to acquisition or otherwise discharge from ICU

† Suction system: according to study protocol in the crossover trial [22]

* Chi-square test; ** Mann-Whitney U test; *** T-test

In the exploratory analyses for the individual pathogens different risk factors were identified in the different Cox proportional hazard models (Table 4). Endotracheal suctioning with closed systems (CSS) was associated with 38% lower risks of acquiring colonization with *P. aeruginosa*, but also with an almost doubled risk of acquired colonization with *Klebsiella* spp. and a tendency towards a higher risk of acquiring colonization with *Acinetobacter* spp (HR 1.95, IQR 0.95 to 4.03). Admission to hospital 1 was associated with a 55% to 63% lower risk of acquiring colonization with *E. coli* and *Klebsiella* spp, respectively, when analyzed as individual pathogens, but with a tendency towards a higher risk of acquiring colonization with *Enterobacter* spp (HR 1.60, IQR 0.996 to 2.56). For the total group of *Enterobacteriaceae* admission to hospital 1 was associated with a 45% lower risk of acquisition. Other risk factors were antibiotics on admission, which reduced the risk of acquisition with *Enterobacter* species with 50% (95% CI 0.27-0.91), but tended to increase the risk of acquisition with *S. maltophilia* (HR 1.48 (95% CI 0.77-2.85). Antibiotic use during ICU stay was associated with a 50% risk reduction for acquiring *E. coli* colonization (HR 0.15 (95% CI 0.29-0.88).

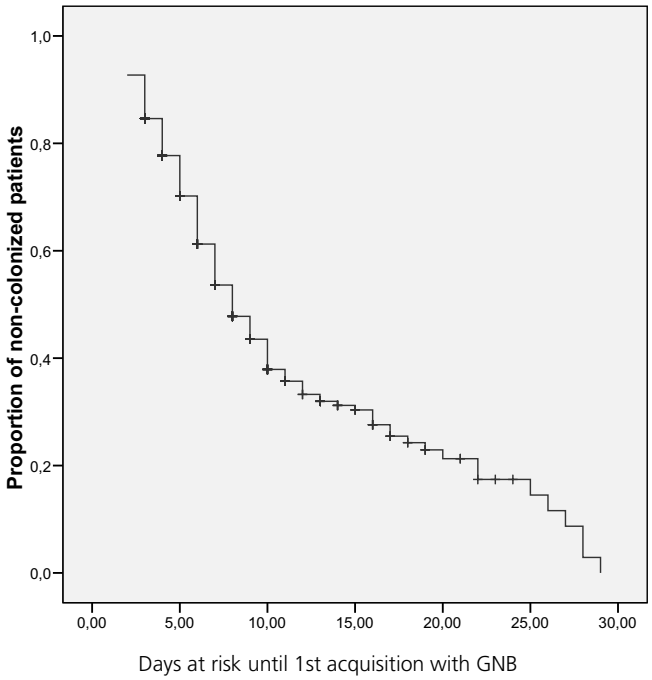

Fig 2, Kaplan-Meier survival curve with the proportion of patients not colonized until certain time points in days. Vertical lines in the curve represent censored observations

Table 3, Risk factors for acquiring colonization with ≥ 1 GNB

Risk factors	Univariate analysis HR (95% CI)	Multivariate analysis HR (95% CI)
Hospital – H1	0.59 (0.46 – 0.76)	0.61 (0.47 – 0.78)
Gender – male	1.09 (0.84 – 1.41)	
Age	1.00 (0.997 – 1.01)	
Diagnosis – surgical	0.94 (0.71 – 1.23)	
APACHE II	1.01 (0.99 – 1.02)	
Mechanical ventilation – yes	2.25 (1.06 – 4.77)	2.37 (1.11 – 5.04)
Isolation days‡	0.94 (0.89 – 0.996)	NS
Suction system - CSS	1.04 (0.80 – 1.34)	
Antibiotics on admission – yes	0.68 (0.50 – 0.93)	NS
Antibiotics during ICU stay[i] - yes‡	0.63 (0.46 – 0.86)	0.61 (0.44 – 0.82)

GNB Gram-negative bacteria; HR hazard ratio; CI confidence interval; H1 hospital 1; APACHE Acute Physiology and Chronic Health Evaluation; CSS closed suction system; ICU intensive care unit
‡ Prior to acquisition or otherwise discharge from ICU

Antibiotic use

Of all patients that acquired colonization with GNB, 78% received antibiotics prior to acquisition, as compared to 72% of patients who did not acquire GNB colonization. Expressed in Defined Daily Dosages (DDD) per 1000 patient days, antimicrobial usage densities were 1,871 and 1,365 in patients who acquired colonization with GNB and patients who did not, respectively, with marked differences for different classes of antibiotics (Table 5).

Discussion

In two Dutch ICUs the median duration until acquisition of colonization with GNB in the respiratory tract, among patients not colonized with GNB at the time of ICU admission, was five days which occurred in 52% of the patients before ICU discharge. Risk of acquisition was strongly associated with mechanical ventilation and was lower in the university hospital (as compared to the teaching hospital) and among patients that received systemic antibiotics during ICU stay. For the individual pathogens, receiving antibiotics at the time of ICU admission was associated with a lower risk to acquire colonization with *Enterobacter* species and systemic antibiotic use during ICU stay was associated with a lower risk of acquiring colonization with *E. coli*.

Our findings suggest that systemic antibiotics may offer some level of protection against respiratory tract colonization with GNB, which seems in contrast to results from other studies, in

Table 4, Hazard Ratios[†] (interquartile ranges) for *P. aeruginosa*, *Acinetobacter* spp, *S. maltophilia* and *Enterobacteriaceae**

	Suction system - CSS	Antibiotics on admission - yes	Hospital – H1	MV[#] - yes	Antibiotics during ICU stay - yes	Isolation – days
P. aeruginosa	0.62 (0.39-0.99)			N/A		
Acinetobacter spp	1.95 (0.95-4.03)			N/A		
S. maltophilia		1.48 (0.77-2.85)				
*Enterobacteriaceae**			0.55 (0.41-0.72)	2.51 (1.03-6.14)	0.56 (0.40-0.78)	
E. coli		0.45 (0.26-0.77)			0.50 (0.29-0.88)	0.66 (0.35-1.26)
Klebsiella spp	1.93 (1.18-3.16)		0.37 (0.23-0.61)			
Enterobacter spp		0.50 (0.27-0.91)	1.60 (0.996-2.56)			
Other GNB			0.43 (0.20-0.92)	N/A		N/A

CSS closed suction systems; H1 hospital 1; MV mechanical ventilation; ICU intensive care unit; N/A not applicable; GNB Gram-negative bacteria

* Enterobacteriaceae: *E. Coli*, *Klebsiella* spp, *Enterobacter* spp, *Serratia* spp, *Proteus* spp, *Citrobacter* spp

[†] Variables introduced in the model: hospital (H1/H2), gender (m/f), age, suction system (CSS/OSS), APACHE II score, diagnosis (surgical/medical), mechanical ventilation (yes/no), duration of isolation, antibiotics on admission to ICU (yes/no) or during ICU stay (yes/no)

[#] MV: All patients that acquired respiratory tract colonization with *P. aeruginosa*, *Acinetobacter* spp or other GNB were on MV, therefore MV was not included in the model. In the other pathogens 1 (*E. Coli*, *Klebsiella* spp) or 2 (*S. Maltophilia*, *Enterobacter* spp) patients that acquired colonization were on MV.

Table 5, Antimicrobial density (AD = Defined Daily Doses per 1000 patient days at risk)

		AD	
	Total	No GNB	Acquired GNB
Antimicrobial group*			
Penicillin-like antibiotics	859	687	1034
Cephalosporins			
1st generation	31	29	33
2nd generation	86	63	108
3rd generation	159	180	139
Aminoglycosides	63	39	89
Quinolones	94	94	95
Carbapenems	89	45	134
Glycopeptides	28	15	41
Sulfamethoxazole/Trimethoprim	49	78	19
Other	158	136	180
Total	1616	1365	1871

AD Antibiotic Density; GNB Gram-negative bacteria
* The antimicrobials used in the ICUs were divided by class and group according to ATC classification defined by the WHO, index 2010
Penicillin-like antibiotics: amoxicillin-clavulanic acid, piperacillin-tazobactam, flucloxacillin, amoxicillin, benzylpenicillin, piperacillin
Cephalosporins: cefazolin (1st generation); cefuroxim (2nd generation); ceftriaxon and ceftazidime (3rd generation)
Aminoglycosides: gentamycin, tobramycin
Quinolones: ciprofloxacin, levofloxacin, moxifloxacin, ofloxacin
Carbapenems: meropenem, imipenem/cilastatin
Glycopeptides: vancomycin, teicoplanin
Other: metronidazol; clindamicin; rifampicin; erythromycin; colistin; azithromycin

which previous use of antibiotics or of selected classes of antibiotics (i.e. carbapenems or 3rd generation cephalosporins) were identified as risk factors for acquiring multidrug-resistant *P. aeruginosa* [27], *Acinetobacter* spp [19,28] *S. maltophilia* [29] or GNB [20]. In these studies defined daily doses or use of antibiotics in the patient population was not quantified, which hampers a comparison with our findings. Furthermore, in our study no selection of antibiotic agents nor GNB was made, which further hampers comparison of our findings to results from other studies. However, the percentage of patients receiving antibiotics in our study (75%) is comparable to results from the Extended Prevalence of Infection in Intensive Care (EPIC II) study, in which 71% of received antimicrobial agents on the day of study [30]. In addition, overall antibiotic density in our study (1,616 DDD per 1,000 patientdays at risk) seems higher as compared to figures reported from other European ICUs, with reported medians of 1,254 to 1,380 DDD per 1000 patient days [31-33]. However, we only monitored antibiotic administration until acquisition; overall, DDDs did not deviate from other ICUs (DDD 1,165 per 1000 patient days)[22].

It seemed contradictory that, with slightly more antimicrobial agents being administered in patients that acquired GNB colonization, antibiotics were associated with a lower risk of GNB acquisition. This appeared to be related with the time variable (days at risk), since in logistic regression analysis (without the time variable included), antibiotics were not related (OR 1.08; p=0.39). Furthermore, patients who did not receive antibiotics were discharged or colonized before day 12, while in patients in whom antibiotics were administered acquisition or discharge appeared later (before day 30).

Comparison of the overall acquisition rate with GNB (52% in our study) to rates reported in previous studies is difficult, since other studies focused on selected, usually multi-resistant, GNB like metallo-β-lactamase producing GNB [20] or cephalosporin-resistant GNB [34]. The reported rate of acquired respiratory tract colonization with *P. aeruginosa* (28%) in one study [35] was in range with our results (30%), whereas for *S. maltophilia* we found a higher colonization rate as compared to the 2% reported in French ICU [29]. For the other pathogens, comparison was hampered due to incomparability of studied GNB.

The effects of using a closed suction system (CSS), as compared to open suction systems (OSS) were contradictory: CSS associated with a lower risk of acquiring *P. aeruginosa*, but with a higher risk of acquiring *Klebsiella* species. Yet, overall, acquisition rates were comparable during the study periods with CSS and OSS [22].

Strengths of our study include the detailed microbiological monitoring and the large sample size. Furthermore, our results were not confounded by other interventions that might influence acquisition of GNB, such as the use of topical antibiotics (as in selective decontamination of the digestive tract [SDD] or selective oropharyngeal decontamination [SOD]) or oropharyngeal application of chlorhexidine.

Our study has several limitations. First, we only focused on colonization with GNB, and not on actual infections. However, respiratory tract infections are almost always proceeded by respiratory tract colonization. Therefore, risk factors for colonization may well be considered risk factors for subsequent VAP [36]. Second, our study was limited to GNB.

In conclusion, of patients not colonized with GNB at the time of ICU admission, 52% acquired respiratory tract colonization before ICU discharge, which was strongly associated with mechanical ventilation, hospital and systemic antibiotic use during ICU stay.

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CHAPTER 6

Antibiotic Exposure and Resistance Development in *Pseudomonas aeruginosa* and *Enterobacter* species in Intensive Care Units

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Submitted

Abstract

Objective

We quantified the association between antibiotic exposure and acquisition of antibiotic resistance in *Pseudomonas aeruginosa* and *Enterobacter* species in Intensive Care Unit patients.

Design

Prospective cohort study.

Setting and Patients

In 1,201 patients respiratory tract colonization was determined through regular screening on admission, twice weekly and on discharge. Primary outcome was the acquisition of antibiotic resistance in prior antibiotic sensitive *P. aeruginosa* and *Enterobacter* species, with acquisition due to cross-transmission excluded based on genotyping and epidemiological linkage. Cox regression analysis, adjusted for covariates, was performed to calculate hazard ratios of antibiotic exposed compared to non-exposed patients.

Main results

In all, 194 and 171 patients were colonized with *P. aeruginosa* and *Enterobacter* species, respectively. Two or more cultures per episode were available for 126 and 108 patients. For *P. aeruginosa* ceftazidime exposure was associated with 6.3 acquired antibiotic resistance events per 100 days of exposure, whereas incidence rates were lower for ciprofloxacin, meropenem and piperacillin-tazobactam. In multivariate analysis, meropenem, ciprofloxacin and ceftazidime were significantly associated with risk of resistance development in *P. aeruginosa* (adjusted Hazard Ratio [aHR] 11.1, 95% confidence interval [CI] 2.4-51.5 for meropenem; aHR 4.1; 95% CI 1.1-16.2 for ciprofloxacin; aHR 2.5, 95% CI 1.1-5.5 for ceftazidime). For *Enterobacter*, ceftriaxone and ciprofloxacin exposure were associated with most antibiotic resistance acquisitions. No significant associations were found in multivariate analysis.

Conclusions

Meropenem exposure is associated with the highest risk of resistance development in *P. aeruginosa*. Increasing carbapenem use due to emergence of Gram-negative bacteria producing extended-spectrum beta-lactamases will enhance antibiotic resistance in *P. aeruginosa*.

Introduction

Nosocomial infections are associated with increased morbidity and mortality in patients treated in intensive care units (ICUs) [1]. To reduce these infections, many patients need antibiotic treatment, but this is also considered an important cause of emerging antibiotic resistance [2,3]. In ICUs the problem of antibiotic resistance is even more emerging, due to high vulnerability of patients, many invasive procedures, high antibiotic selective pressure and high prevalence of resistant bacteria [2]. When infections are caused by antibiotic resistant bacteria, in-hospital mortality rates and length of hospital stay are higher as compared to infections caused by antibiotic-susceptible bacteria [4].

Infections caused by antibiotic-resistant bacteria in ICU are almost always preceded by colonization, which may result from either endogenous or exogenous acquisition [5]. In case of endogenous acquisition, a patient is already colonized with – initially - undetectable bacterial numbers, which ranks increase above detection limits, for instance because of selective antibiotic pressure. Yet, it is also possible that antibiotic-susceptible bacteria acquire resistance mechanisms (or start to express resistance traits), changing their phenotype from susceptible to resistant [6]. Again, antibiotic exposure is believed to be critical for this process.

Exogenous acquisition is caused by micro-organisms from the ICU environment, either inanimate or animate. Resistant bacteria may be transferred from patient to patient, most frequently through temporarily contaminated hands of health care workers [7]. Although antibiotic selective pressure may facilitate such events of cross-transmission, lapses in adherence to basic hygiene measures must be considered crucial for this mode of transmission of antibiotic resistance.

Few studies have quantified the effects of antibiotic exposure on the endogenous selection of antibiotic resistance in *P. aeruginosa* [8-10]. However, in these studies the role of exogenous acquisition as a cause for resistance acquisition has not been ruled out, thereby obscuring direct effects of antibiotic exposure on endogenous acquisition of antibiotic resistance. Furthermore, other Gram-negative bacteria like *Enterobacter* species have not been rigorously investigated on this specific topic. In this study we, therefore, aimed to quantify the occurrence of a phenotype switch from susceptible to resistant in *P. aeruginosa* and *Enterobacter* species in colonized ICU patients.

Materials and Methods

Study design and patient population

From January 2007 through February 2008, a prospective cohort study was performed among patients admitted to the ICU for at least 48 hours and colonized with *P. aeruginosa* and *Enterobacter* species. Four ICUs participated: two units (10 and 8 beds, respectively) in the University Medical Center Utrecht and two units (each 8 beds) in St Elisabeth Hospital in Tilburg,

a large teaching hospital. Patients readmitted to ICU after initially being discharged from ICU were assigned as new patients in this study. All ICUs had a mixed population of adult patients including surgical and non-surgical patients. This cohort study was embedded within a crossover trial evaluating the effects of open and closed endotracheal suctioning on cross-transmission [11]. The institutional review board of both hospitals waived the requirement for informed consent, since cultures were part of the surveillance program.

Outcome

The primary outcome was the incidence of acquired antibiotic resistance, which was defined as the conversion from carriage with antibiotic susceptible to antibiotic resistant bacteria in subsequent respiratory tract cultures. The effects of the following antibiotics on antibiotic resistance were assessed: ciprofloxacin, ceftazidime, meropenem, piperacillin-tazobactam (for *P. aeruginosa* and *Enterobacter*); cotrimoxazol, gentamicin, ceftriaxon, tobramycin (for *Enterobacter*).

To quantify antibiotic use in our study population, the number of defined daily doses (DDDs) per 100 patient-days was calculated, according to the ATC/DDD Index 2010 from the WHO Collaborating Centre for Drug Statistics Methodology [12]. The number of acquired antibiotic resistance events was expressed per 100 days of antibiotic exposure in which patients were at risk for developing antibiotic resistance. Antibiotic treatment was only considered if it had been prescribed before the date of onset of resistance.

Bacterial sampling

All patients admitted to ICU were screened on admission, subsequent twice weekly (Monday, Thursday) and on discharge for bacterial colonization of the respiratory tract. All cultures (endotracheal aspirate in mechanically ventilated patients, oropharyngeal swabs in non-ventilated patients) were analysed according to hospital protocol. The following minimum inhibitory concentrations for determining resistant categories for the different antibiotics were used, according to the Clinical Laboratory Standards Institute: ciprofloxacin ≥4 mg/L, ceftazidime ≥32 mg/L, meropenem ≥16 mg/L; piperacillin-tazobactam ≥128/4 mg/L, cotrimoxazol ≥4/76 mg/L, gentamicin ≥16 mg/L, ceftriaxone ≥64 mg/L, tobramycin ≥16 mg/L[13]. To exclude the occurrence of possible cross-transmission, genotyping was conducted for *P. aeruginosa* and *Enterobacter* species isolates. From patients colonized with one or both species the first isolate (per pathogen) was genotyped, as were subsequent isolates in case of a change in antibiogram, morphologic differences or when ≥ 10 cultures with identical antibiograms had been obtained. Cross-transmission was defined as acquired colonization with a genetically identical pathogen and with overlapping time periods to a potential source patient. Genotyping was performed after the trial was finished, therefore medical staff was not aware of the results during the trial. *P. aeruginosa* isolates were genotyped with Multiple-Locus Variable-number tandem-repeats Analysis (MLVA) [14], and *Enterobacter* species were genotyped with DiversiLab[15]. MLVA patterns were analyzed with BioNumerics software version 5.10 (Applied

Maths), and single locus variants (where the profile varies at one locus) were used as cut-off point for genetic relatedness. For *Enterobacter* species analysis was performed with DiversiLab software (version 3.4) using 95% similarity as cut-off point for genetic relatedness.

Data analysis

To determine acquisition of antibiotic resistance, only patients of whom at least two microbial cultures were available were included in analysis; in patients with only one culture it was not retrievable whether possible antibiotic resistance was acquired.

To assess the effect of antibiotics administered during ICU admission, Cox proportional hazards models were used. The following covariates were considered for our multivariate models: age, gender, Acute Physiology and Chronic Health Evaluation (APACHE) II score, simultaneous use of other antibiotics, previous use of antibiotics before ICU admission, ICU day of first colonization, and surgical or non-surgical patient. For every multivariate model each covariate was tested for confounding by adding it to an univariate model containing the antibiotic exposure variable and examining its effect on the beta coefficient of the antibiotic exposure variable. Variables which caused substantial confounding (a change in the beta coefficient of greater than 10%) were included in the final model. The time interval between first positive culture and the occurrence of resistance acquisition was used as time variable. The date of acquisition was determined as the date on which the first resistant isolate was obtained from the patient.

Bivariate analyses with Spearman correlation coefficients (rho) were carried out to rule out multicollinearity among variables entered in multivariate analysis.

Differences in antibiotic resistance acquisitions between patients exposed to antibiotic treatment and patients not exposed to antibiotic treatment were expressed by hazard ratios with corresponding 95% confidence intervals. Data were analysed with the Statistical Package for Social Sciences version 16.0 for Mac.

Results

In all, 1,201 patients were admitted to one of the ICUs for at least 48 hours and among them 316 patients were colonized with *P. aeruginosa* or *Enterobacter* species (Figure 1). In 111 of the colonized patients, only one positive microbial culture with *P. aeruginosa* or *Enterobacter* species was acquired, leaving 205 patients with two or more isolates, corresponding to 126 and 108 patients with *P. aeruginosa* and *Enterobacter* species, respectively.

Patients in the *P. aeruginosa* group were colonized later, and had a longer length of stay as compared to those colonized with *Enterobacter* species (Table 1). Trauma was more frequently the reason for admission in patients colonized with *Enterobacter* species, whereas a respiratory cause was the most frequent reason in *P. aeruginosa*. The mortality rate in ICU was 17.5% and 16.7% for patients colonized with *P. aeruginosa* and *Enterobacter* species, respectively (Table

1). Antibiotic exposure was highest to ciprofloxacin in both groups, being 25.9 and 29.7 DDDs per 100 patient days (Table 2).

Pseudomonas aeruginosa

Of 126 patients colonized with *P. aeruginosa,* 546 cultures were available (median number of follow-up cultures 3; IQR 1-7). 189 Isolates were selected for genotyping, yielding 81 different Pseudomonas MLVA-types.

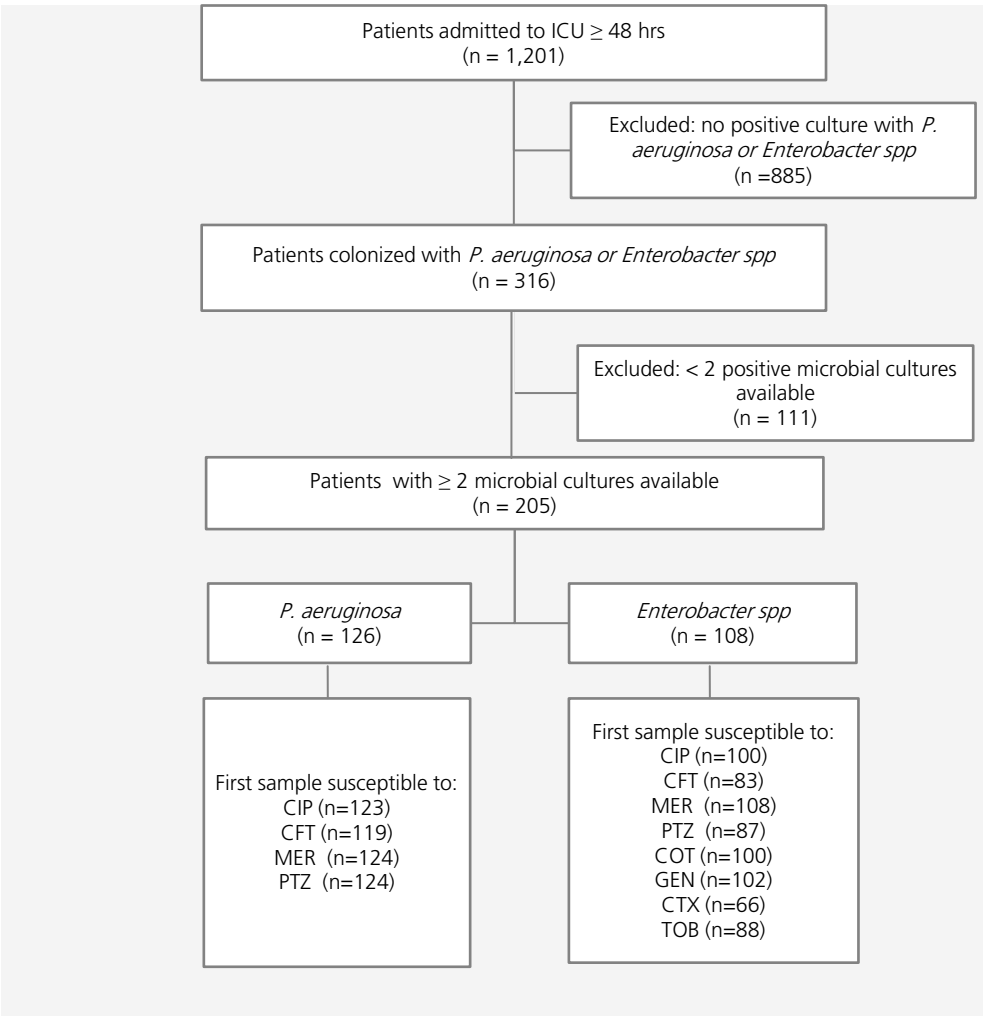

Fig. 1. Flow chart of study inclusion
n number of patients; CIP ciprofloxacin; CFT ceftazidime; MER meropenem; PTZ piperacillin-tazobactam; COT cotrimoxazol; GEN gentamicin; CTX ceftriaxone; TOB tobramycin

A phenotype switch from susceptible to resistant for one or more antibiotics occurred in 41 patients. Acquisition of resistance to ceftazidime occurred in 29 of 119 episodes (24%) of ceftazidime-susceptible *P. aeruginosa* colonization, corresponding to an acquisition rate of 2.0 (95% CI 1.3-2.8) per 100 patient days at risk. Seventeen of 29 patients had been exposed to ceftazidime for a total of 268 days, which yields an acquisition rate of 6.3 (95% CI 3.4-9.3) per 100 days of antibiotic exposure. Incidence rates were lower for ciprofloxacin, meropenem and piperacillin-tazobactam, with number of events per 100 days of antibiotic exposure ranging from 2.3 to 2.6 (Table 2).

Five patients (4.0%) had a genotypic match in MLVA type and epidemiological linkage, suggesting cross-transmission, and were, therefore, excluded in multivariate analysis. Patients who had meropenem prescribed had the highest risk of developing meropenem resistance, with an adjusted Hazard Ratio (aHR) of 11.1 (95% CI 2.4 - 51.5) (Table 3). The adjusted HRs for ciprofloxacin and ceftazidime were 4.1 (95% CI 1.1-16.2) and 2.5 (95% CI 1.1-5.5), respectively. There appeared no additional risk of piperacillin-tazobactam exposure (adjusted HR 0.8; 95% CI 0.2-3.2). Analysis of exposure to any cephalosporin (ceftazidime, cefotaxime, ceftriaxone, cefuroxime, cefazolin) and the development of ceftazidime resistance showed an adjusted HR of 5.9 (1.4 -2.5). In this analysis cross-transmission was defined as genotypical matching and overlapping time periods in ICU for presumed donor and acceptor. Expanding

Table 1. Patient characteristics

Characteristic	*Pseudomonas*	*Enterobacter*
Number of patients	126	108
Age in years - median (IQR)	59 (44-72)	62 (41 – 75)
Gender - % (female)	25%	26%
APACHE II score - mean (SD)	20.3 (6.5)	19.6 (6.8)
Mortality on ICU - %	17.5	16.7
Previous antibiotic use before ICU admission - %	19.5	17.6
Surgical versus not-surgical patient - % surgical	35	37
ICU day of first colonization - median (IQR)	5 (1-15)	4 (1-8)
Days of mechanical ventilation - median (IQR)	19 (9-29)	16 (9-28)
Length of stay - median (IQR)	26 (14-40)	20 (11-36)
Reason for ICU admission - %		
Cardial/vascular/circulatory	10.3	15.7
Gastro-intestinal	14.3	11.1
Neurologic	4.0	3.7
Neurosurgical	4.8	7.4
Pulmonary/respiratory	35.7	21.3
Sepsis	11.1	8.3
Thorax surgical	2.4	0.9
Trauma	15.1	28.7
Other	2.3	2.9

IQR inter quartile range; APACHE acute physiology and chronic health evaluation; SD standard deviation; ICU intensive care unit

Table 2. Antibiotic use and incidences of acquired antibiotic resistance in Pseudomonas aeruginosa and Enterobacter species

Antibiotic	Number of susceptible episodes [A]	Events [B] (%)	Patient days	Events [B] per 100 patient days (95% CI)	Number of antibiotic exposed episodes (days of exposure)	DDDs per 100 patient days (95% CI	Events [C] in antibiotic exposed episodes	Events [C] per 100 days of antibiotic exposure (95% CI)
Pseudomonas (n=126)								
Ciprofloxacin	123	11 (8.9)	1803	0.6 (0.3-1.0)	42 (315)	25.9 (23.8-27.9)	8	2.5 (0.8-4.3)
Ceftazidime	119	29 (24.4)	1427	2.0 (1.3-2.8)	45 (268)	19.0 (17.0-21.0)	17	6.3 (3.4-9.3)
Meropenem	124	12 (9.7)	1713	0.7 (0.3-1.1)	24 (221)	14.4 (12.8-16.1)	5	2.3 (0.3-4.2)
Piperacillin-tazobactam	124	18 (14.5)	1594	1.1 (0.6-1.7)	23 (229)	13.6 (11.9-15.3)	6	2.6 (0.6-4.7)
Enterobacter (n=108)								
Ciprofloxacin	100	13 (13.0)	1058	1.2 (0.6-1.9)	31 (220)	29.7 (27.0-32.5)	11	5.0 (2.1-7.9)
Ceftazidime	83	14 (16.9)	920	1.5 (0.7-2.3)	18 (121)	7.9 (6.1-9.6)	2	1.7 (-0.6-3.9)
Meropenem	108	0 (0.0)	1305	0	22 (262)	24.7 (22.3-27.0)	0	0
Piperacillin-tazobactam	87	11 (12.6)	1080	1.0 (0.4-1.6)	14 (89)	6.1 (4.7-7.6)	2	2.2 (-0.8-5.3)
Cotrimoxazol	100	8 (8.0)	1271	0.6 (0.2-1.1)	36 (293)	18.5 (16.4-20.6)	6	2.0 (0.4-3.7)
Gentamicin	102	9 (8.8)	1106	0.8 (0.3-1.3)	4 (11)	0.6 (0.2-1.1)	1	9.1 (-7.9-26.1)
Ceftriaxone	66	12 (18.2)	643	1.9 (0.8-2.9)	17 (104)	12.8 (10.2-15.3)	5	4.8 (0.7-8.9)
Tobramycin	88	7 (8.0)	979	0.7 (0.2-1.2)	16 (130)	6.7 (5.1-8.2)	1	0.8 (-0.7-2.3)

[A] First isolate susceptible for antibiotic
[B] Acquired resistance events in both antibiotic exposed and non-exposed episodes
[C] Acquired resistance events in antibiotic exposed episodes

Table 3. Cox regression analysis in patients with *Pseudomonas aeruginosa* and *Enterobacter* species

	Pseudomonas (n=121)*		Enterobacter (n=105)*	
	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
Ciprofloxacin (CIP) vs no CIP	2.8 (0.7-10.9)	4.1 (1.1-16.2) [A]	1.7 (0.6-4.7)	1.5 (0.5-4.3) [B]
Ceftazidime (CFT) vs no CFT	2.8 (1.3-6.1)	2.5 (1.1-5.5) [C]	1.0 (0.3-3.4)	0.8 (0.2-3.1) [D]
Meropenem (MER) vs no MER	8.7 (2.2-33.9)	11.1 (2.4-51.5) [E]	-	-
Piperacillin-tazobactam (PTZ) vs no PTZ	2.0 (0.7-5.6)	0.8 (0.2-3.2) [F]	1.1 (0.2-5.3)	1.3 (0.3-6.5) [G]
Cotrimoxazol (COT) vs no COT	n/a	n/a	3.1 (0.6-15.8)	3.1 (0.6-15.8) [H]
Gentamicin (GEN) vs no GEN	n/a	n/a	2.5 (0.3-20.0)	4.8 (0.5-45.4) [I]
Ceftriaxone (CTX) vs no CTX	n/a	n/a	1.6 (0.5-4.7)	2.4 (0.7-8.9) [J]
Tobramycin (TOB) vs no TOB	n/a	n/a	0.6 (0.1-5.4)	0.4 (0.04-4.7) [K]

HR hazard ratio; CI confidence interval; n/a not applicable
* Number of episodes, excluding episodes with possible cross-transmission
[A] Adjusted for gender, previous use of antibiotics, ICU day of first colonization; [B] Adjusted for simultaneous use of other antibiotics, previous use of antibiotics, ICU day of first colonization, surgical or not-surgical patient; [C] Adjusted for ICU day of first colonization; [D] Adjusted for age, gender, simultaneous use of other antibiotics, previous use of antibiotics, surgical or not-surgical patient; [E] Adjusted for Apache II score; [F] Adjusted for age, gender, Apache II score, simultaneous use of other antibiotics, previous use of antibiotics, ICU day of first colonization, surgical or not-surgical patient; [G] Adjusted for age, gender, simultaneous use of other antibiotics, ICU day of first colonization, surgical or not-surgical patient; [H] No adjustment required; [I] Adjusted for age, Apache II score; [J] Adjusted for age, Apache II score simultaneous use of other antibiotics, previous use of antibiotics; [K] Adjusted for age, gender, Apache II score, ICU day of first colonization, surgical or not-surgical patient

the time window to nine days in the definition of cross-transmission resulted in two additional patients with a genotypic match. Excluding these patients in multivariate analyses did not alter the results.

Enterobacter species

Of 108 patients colonized with *Enterobacter* species, 313 cultures were available (median number of follow-up cultures 2; IQR 1-4). Of these, 135 isolates were selected for genotyping, yielding 63 different types.

A phenotype switch from susceptible to resistant for one or more antibiotics occurred in 46 patients. Acquisition of resistance to ciprofloxacin occurred in 13 of 100 episodes (13%) of ciprofloxacin-susceptible *Enterobacter* colonization, corresponding to an acquisition rate of 1.2 (95% CI 0.6-1.9) per 100 patient days at risk. Eleven of 13 patients had been exposed to ciprofloxacin for a total of 220 days, which yields an acquisition rate of 5.0 (95% CI 2.1-7.9) per 100 days of antibiotic exposure. A similar incidence rate was observed for ceftriaxone (4.8 per 100 days of exposure; 95% CI 0.7 – 8.9), whereas the incidence rate for cotrimoxazol was

lower (2.0 per 100 days of exposure; 95% CI 0.4 -3.7). Incidence rates could not be reliably calculated for other antibiotics because of limited numbers of events (Table 2).
In three patients (2.8%) a genotypic match in Diversilab typing was found. These patients were excluded in multivariate analyses for the reason of possible cross-transmission. Patients with antibiotic exposure were not associated with higher risks for acquiring antibiotic resistance compared to patients without exposure (Table 3). Exposure to any cephalosporin was also not significantly associated with development of ceftazidime resistance (aHR 1.9; 95 % CI 0.4 -2.5).

Discussion

In this study a phenotypical switch from susceptible to resistant for at least one antibiotic occurred in 41 ICU patients colonized with *P. aeruginosa* and 46 colonized with *Enterobacter* species. For respiratory tract colonization with *P. aeruginosa*, exposure to meropenem was, after adjustment for covariates, associated with the highest risk of resistance development (aHR 11.1; 95% CI 2.4 -51.5). Among 124 patients colonized with meropenem-susceptible *P. aeruginosa,* meropenem exposure was 14.4 DDD/100 patient days, yielding 2.3 resistance acquisition events per 100 days of antibiotic exposure. In contrast, no single event of meropenem resistance acquisition was documented among 108 patients colonized with meropenem-susceptible *Enterobacter* species, despite meropenem exposure of 24.7 DDD/100 patient days.
Few studies have assessed the effects of individual patient antibiotic exposure on the acquisition of antibiotic resistance in *P. aeruginosa* by using time dependent variables. In a retrospective study in a single tertiary care hospital in the U.S., quinolones, third-generation cephalosporins and imipenem were all associated with acquisition of antibiotic resistance among *Enterobacteriaceae* and *P. aeruginosa*, when analysed at the individual-patient level. Among these antibiotics, imipenem was associated with the highest risk [8]. In another tertiary care U.S. hospital, emergence of resistance to imipenem and ciprofloxacin among *P. aeruginosa*, after exposure to these antibiotics, was considerably higher than the risk of ceftazidime resistance after ceftazidime exposure [9]. In a French study of ICU patients, the risk of *P. aeruginosa* resistance to imipenem (and piperacillin-tazobactam to a lesser extent) was strongly linked to imipenem exposure and no such risk could be demonstrated for ceftazidime use [10].
Our study differs from these studies in that we investigated a specific patient population (i.e., ICU patients only) instead of a hospital-wide population [8,9], that we used colonization data from protocolized surveillance instead of culture results from samples submitted to the microbiology lab for clinical indication [8-10], that we meticulously ruled out possible events of cross-transmission through genotyping and epidemiological linkage, and that we included *Enterobacter* species as a separate group in our analysis. Quantifying the occurrence of cross-transmission is important as such events may create nonlinear dynamics, obscuring the direct effects of antibiotic exposure. The standardized surveillance as used in our study minimizes the

risk of selection bias, as obtaining cultures for clinical reasons is more likely to be performed in the more severely ill patients.

Our findings, together with those from previous studies [8-10], strongly suggest that carbapenems pose a more serious risk on inducing antibiotic resistance in *P. aeruginosa* than other beta-lactam antibiotics and fluoroquinolones. Nevertheless, the confidence intervals around the risk estimates were large, which can be attributed to the small number of events. This underscores the difficulties of accurately determining the direct associations between antibiotic use and resistance. Even after inclusion of 1,201 consecutive ICU patients and analyzing 1,093 microbiological cultures in 205 patients with either *P. aeruginosa* or *Enterobacter* colonization confidence intervals of hazard ratios were overlapping and we were unable to quantify increased risks for *Enterobacter* species. Naturally, similar studies in settings with higher levels of antibiotic use and higher acquisition rates would have more power to accurately quantify risk associations. Of note, the difficulties to determine these associations on an individual patient level should not be embraced to use aggregated data instead, as this might lead to wrong interpretations [8].

Although the baseline prevalence of antibiotic resistance for *P. aeruginosa* in this study population (1-6%) was lower as compared to other ICU populations (5-37%) [9,16,17], it does not affect our findings and extrapolatibility to other ICU populations, since we here focus on the direct effect of antibiotic exposure on the process of antimicrobial resistance development in previous sensitive bacteria within a single patient.

Our study had a few limitations. First, the date of phenotype switch to antibiotic resistance was determined as the date of the first resistant isolate. Although extensive and regular culturing was conducted in this study, the exact number of days at risk would be slightly lower when the exact day of resistance switch was known, and thereby increasing the incidence rates per 100 patient days at risk or per 100 days of antibiotic exposure. However, this would not have altered our hazard ratios significantly, since both exposed and not exposed group of patients would have been equally influenced. Second, inherent to the observational design of our study, results may have been influenced by confounding variables. We attempted to minimize this by adjusting for confounding variables, such as previous and simultaneous antibiotic use, in multivariate analysis. Finally, we did not investigate the development of multiple antibiotic resistance. Combined resistance acquisition in *P. aeruginosa* was observed in half of meropenem resistance acquisitions and in half of ceftazidime acquired resistances. In ciprofloxacin and piperacillin-tazobactam about 80% to 90% concerned the development of combined resistance. It is difficult to include multiple resistances in time-dependent analyses, since resistance development for multiple antibiotics did not always occur simultaneously. Moreover, the numbers of combined resistance development were too low for statistical analysis.

Conclusion

Meropenem use in ICU patients with *P. aeruginosa* was associated with antibiotic resistance development to meropenem. The association was stronger for meropenem than for other antibiotics. These findings indicate that an increase of carbapenem use as a result of the global emergence of Gram-negative bacteria producing extended-spectrum beta-lactamases (ESBL) creates a serious risk for rapid emergence of carbapenem resistance among *P. aeruginosa*. Therefore, antibiotic stewardship to optimize carbapenem use (i.e., to minimize its unnecessary use) is recommended.

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CHAPTER 7

The population genetics of *Pseudomonas aeruginosa* isolates from different patient populations exhibits high-level host specificity

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Abstract

Objective

To determine whether highly prevalent *P. aeruginosa* sequence types (ST) in Dutch cystic fibrosis (CF) patients are specifically linked to CF patients we investigated the population structure of *P. aeruginosa* from different clinical backgrounds. We first selected the optimal genotyping method by comparing pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and multilocus variable number tandem-repeat analysis (MLVA).

Methods

Selected *P. aeruginosa* isolates (n=60) were genotyped with PFGE, MLST and MLVA to determine the diversity index (DI) and congruence (adjusted Rand and Wallace coefficients). Subsequently, isolates from patients admitted to two different ICUs (n=205), from CF patients (n=100) and from non-ICU, non-CF patients (n=58, of which 19 were community acquired) were genotyped with MLVA to determine distribution of genotypes and genetic diversity.

Results

Congruence between the typing methods was >79% and DIs were similar and all >0.963. Based on costs, ease, speed and possibilities to compare results between labs an adapted MLVA scheme called MLVA9-Utrecht was selected as the preferred typing method. In 363 clinical isolates 252 different MLVA types (MTs) were identified, indicating a highly diverse population (DI = 0.995; CI = 0.993-0.997). DI levels were similarly high in the diverse clinical sources (all >0.981) and only eight genotypes were shared. MTs were highly specific (>80%) for the different patient populations, even for similar patient groups (ICU patients) in two distinct geographic regions, with only three of 142 ICU genotypes detected in both ICUs. The two major CF clones were unique to CF patients.

Conclusion

The population structure of *P. aeruginosa* isolates is highly diverse and population specific without evidence for a core lineage in which major CF, hospital or community clones co-cluster. The two genotypes highly prevalent among Dutch CF patients appeared unique to CF patients, suggesting specific adaptation of these clones to the CF lung.

Introduction

Pseudomonas aeruginosa can cause nosocomial infections in immuno-compromised patients and patients in intensive care units (ICUs), and is a major cause of morbidity and mortality in patients with cystic fibrosis (CF) [1]. Molecular typing studies revealed the presence of so-called epidemic strains, frequently transmitted between CF patients and associated with higher morbidity and mortality [2-6]. As a consequence, many countries implemented segregation policies for CF patients [7].

In a previous cross-sectional study, investigating the population structure of respiratory *P. aeruginosa* isolates among Dutch CF patients by using multilocus sequence typing (MLST), we described two sequence types (ST), ST406 and ST497 in 15% and 5% of all patients infected with *P. aeruginosa,* respectively [8]. Both STs were not genetically linked to previously described international epidemic clones, which were not detected in this CF population.

In order to determine whether these prevalent STs are specifically linked to patients with CF, or ubiquitously present in other patient populations, we aimed to investigate the genetic relatedness and population structure of *P. aeruginosa* isolates from CF and non-CF patients. To do so, a highly discriminatory, cheap and easy to perform typing scheme, which also allows results to be easily compared with international databases, is required. Pulsed-field gel electrophoresis (PFGE) has been the most widely used typing method, but does not allow easy comparison of results of different origin because of a relatively high degree of inter-performer variation and lack of an international comparative database. MLST provides sequence-based, and thus unambiguous, results, but is rather expensive. We, therefore, first determined whether multi-locus variable number tandem-repeat analysis (MLVA) could fulfill these criteria required for library typing, by comparing a new MLVA scheme, adjusted from the published *P. aeruginosa* MLVA scheme [9], to PFGE and MLST. After identifying the optimal typing scheme, based on discriminatory power, typeability, time, ease of interpretation and of international comparability and costs, we determined the population structure of multiple *P. aeruginosa* isolates from different epidemiological backgrounds.

Materials and methods

Genotyping

To determine the optimal molecular typing method, 60 *P. aeruginosa* isolates from sputum or throat swab cultures obtained from 58 different CF patients visiting the University Medical Centre Utrecht (UMCU) in 2007, were typed by PFGE, MLST and MLVA9-Utrecht. This selection represented the genotypes and genetic diversity found in the Dutch CF patients as shown in a previous cross-sectional typing study [8]. The Discriminatory Indices (DI) and the 95% confidence intervals (CI) were calculated as described before [10,11] using Bionumerics 5.1 (Applied Maths, St-Martens-Latem, Belgium). Criteria to assign isolates to clonal clusters (CCs) were defined as

follows: PFGE types (PT) > 80% similarity in band patters, MLVA types (MTs) with identical number of repeats in 8 out of 9 loci (single locus variants) and MLST types (STs) with identical sequence in 6 out of 7 loci. CCs were named after their presumed founder MT/ST, based on eBURST criteria [12]. The quantitative level of congruence between typing methods was calculated using the adjusted Rand and Wallace coefficients, available at http://www.comparingpartitions.info/. The adjusted Rand coefficient quantifies the global agreement between two methods, whereas the Wallace coefficient indicates the probability that two isolates classified as the same type by one method are also classified as the same type by another method [13]. MLVA9-Utrecht profiles were clustered with Bionumerics software (version 5.1) by using a categorical coefficient and a graphing method called minimum spanning tree[14].

PFGE

For PFGE, 2% agarose plugs were made with equal volume bacterial suspension of 3 McFarland. Plugs were incubated overnight with 0.5 mg/ml lysozyme (Sigma-Aldrich, Zwijndrecht, Netherlands) at 37 °C. Next 1 mg/ml proteinase K (VWR, International, Amsterdam, Netherlands) was added and plugs were incubated overnight at 56 °C.
Plugs were washed for 30 min at 37°C once with 10 mM tris/1mM EDTA (TE) buffer, then 0.75 mM phenyl-methyl-sulfonyl-fluoride (PMSF) in TE buffer, and again with TE buffer. Plugs were digested with *Spe*I 5 µl (50 U) in 25µl NE buffer2 (Westburg, Leusden, Netherlands) and 220 µl water overnight at 37 °C. Electrophoresis was performed with 1% agarosegel for 20 h at 6V/cm with initial switch of 5.8 s and final switch of 38 s. *P. aeruginosa* strain ATCC 27853 was used as reference at minimal 5 lanes in each gel. The gels were stained with ethidiumbromide and bands were analysed with Bionumerics 5.1 (Applied Maths, St-Martens-Latem, Belgium). The band patterns were compared using the Dice-coefficient by using the unweighted pair group method to determine band similarity. Band patterns that were more than 80% identical were considered related conform the Tenover criteria [15,16], which state that a 2-3 band difference indicates related strains. On average we observed 16 bands in our *P. aeruginosa* PFGE gels, resulting in the 80% cut-off. Typeability was defined as all isolates that produced a band pattern divided by all isolates tested.

MLST and MLVA

Isolates were taken from the freezer and cultured on Trypticase Soy Agar II + 5% sheep blood plates (Becton, The Netherlands) overnight at 37˚C. A loop (few colonies) of bacterial cells were suspended in 20 µl lysis buffer (0.25% SDS, 0.05 M NaOH) and incubated at 95˚C for 20 min. The cell lysate was spun by short centrifugation and diluted with 180 µl buffer (10 mM Tris-HCl, pH 8.5). After thoroughly mixing, another centrifugation for 5 min at 16,000 x g was performed to remove cell debris. Supernatants were frozen at -20˚C until further use. Two and a half µl of the lysate was used in the PCR reactions for MLST and MLVA.
For MLST a touchdown PCR was performed as described before [8], adapted from the protocol by Curran *et al* [17] with HotStarTaq Mastermix (Qiagen, Valencia, CA,USA). PCR products were

sequenced (BaseClear, Leiden, the Netherlands) with the same primers as used for amplification. Sequences were analyzed using Bionumerics 5.1 (Applied Maths, St-Martens-Latem, Belgium).
Sequence types (STs) were compared to the *P. aeruginosa* Multilocus Sequence Typing website (http://pubmlst.org/paeruginosa/) developed by Keith Jolley [18] and new alleles and profiles were sent to the curator A. Baldwin.
For MLVA typing a touchdown PCR was performed adapted from the protocol by Vu-Thien *et al* [9] adding Q-buffer (Qiagen Benelux B.V., Venlo, the Netherlands) using the published primers for the following variable-number-of-tandem-repeats (VNTRs): ms77, ms127, ms142, ms211, ms213, ms215, ms216, ms217 and ms223 (called MLVA9-Utrecht). The PCR was conducted as follows: 10 min at 96˚C, then 10 cycles of 30 s at 95˚C, 30 s at 65˚C with 1˚C less every cycle and 1 min at 72˚C. This was followed by 25 cycles of 30 s at 95˚C, 30 s at 55˚C and 1 min at 72˚C, and a final incubation of 10 min at 72˚C followed. PCR products were separated on a 2% agarose gel by electrophoresis next to 100bp DNA ladder (Invitrogen, The Netherlands). The size of each amplicon was measured using Bionumerics 5.1 (Applied Maths, St-Martens-Latem, Belgium) and the number of repeats was deduced by using the MLVA alleles assignment table on the *Pseudomonas aeruginosa* genotyping site (http://minisatellites.u-psud.fr/MLVAnet/). PA01 (ATCC BAA47) was used as control for checking consistency of allele assignments. Loci that repetitively did not yield a PCR product were assigned allele "99" to be able to include these isolates in subsequent cluster analysis. MLVA9-Utrecht types (MTs) were compared with the international database "pseudomonas2007" created by Gilles (http://minisatellites.u-psud.fr/MLVAnet/). Typeability was defined as the number of isolates for which repeat numbers could be inferred for all 9 loci divided by all isolates tested.

Patients and bacterial isolates

To determine the *P. aeruginosa* population structure, 363 isolates were collected from four different patient populations: 100 respiratory isolates from 90 CF patients who either were cultured because of an exacerbation or screened for their annual check-up (group I) and 205 *P. aeruginosa* isolates from aspirate, sputum or throat swab screening cultures from patients admitted to intensive care units (ICU) (one isolate per type per patient) in two hospitals in the Netherlands (126 isolates from 97 patients in hospital 1 (group IIa) and 79 isolates from 64 patients in hospital 2 (group IIb). Screening cultures were executed on admission, twice weekly thereafter and on discharge during a period of 14 months in both hospitals. Hospital 1 is a tertiary referral (university) hospital and patients were included in two ICUs (10 and 8 beds, of which 6 and 7 beds on a ward, respectively) harboring a mixed adult patient population. Hospital 2 is non-university teaching hospital, located 80 kilometers from hospital 1, and here patients from two ICUs (8 and 8 beds, single rooms) also harboring a mixed adult patient population were included. In both ICUs, CF patients were excluded. In total, 1200 patients were admitted for more than 24 hours and screened (cultures were not available of 113 patients). Isolates of 161 of 194 colonized patients were typed and included in this study. Group III

consisted of 39 non-respiratory clinical isolates from 38 non-CF patients and non-ICU patients admitted to hospital 1. These 38 patients were mostly long-stay patients (admitted > one month) in different wards, including surgery, neurology, oncology and internal medicine. Group IV consisted of 19 isolates from 19 non-CF and non-ICU patients obtained within 48 hours after admission or during out-patient clinic visits at hospital 1. These isolates are considered "community acquired". The community acquired isolates were cultured mainly from eyes, ears, wounds and screening cultures of patients admitted for stem cell transplantation. The ethical committees (METC) of both the University Medical Center Utrecht and the St Elisabeth Hospital Tilburg approved this study and waived the requirement for informed consent (METC Utrecht protocol number: 05/311, METC Tilburg protocol number: 0655), since cultures were obtained as part of the hospital surveillance program or clinical practice.

Calculation of expected DI and MT distribution

The median value and the 95% confidence intervals for the DIs and the overlap in types between different clinical sources was calculated using Mathematica 7.0.1.0, (Wolfram Research, Champaign, Ill), by distributing the isolates 100,000 times, randomly, over the different clinical sources under the assumption that genotypes do not cluster. The number of isolates per group and the prevalence of the different MTs were considered fixed and only the distribution over the different groups was randomized.

Results

Adjusted MLVA9-Utrecht scheme

We first adjusted the published *P. aeruginosa* MLVA scheme, as originally described by Vu-Thien *et al*[9]. The original scheme contained 15 variable number tandem-repeat (VNTR) loci, of which some, due to small repeat sizes, required analysis on a DNA sequencer. To create a robust MLVA scheme that was easy to perform without the need for a DNA sequencer, we tested different combinations of the original 15 VNTR loci and calculated the DIs for the different combinations in a set of 101 *P. aeruginosa* isolates (the 100 selected CF isolates plus PA01; ATCC BAA47). VNTR loci that were not selected in the final scheme were loci with too small repeat size (< 15nt) and loci that could not be amplified in > 10% of the isolates. Based on these criteria we selected a subset of nine MLVA loci, ms77, ms127, ms142, ms211, ms213, ms215, ms216, ms217, and ms223. This scheme yielded a PCR product in 91-100% of the isolates and a high discriminatory index of 0.984 (CI 0.972-0.996).

Comparison of typing methods

Subsequently we compared the adjusted MLVA9-Utrecht scheme with PFGE and MLST by typing 60 *P. aeruginosa* isolates from CF patients with the three methods. Typeability was 100% for MLST and MLVA9-Utrecht, but only 91.7% for PFGE as 5 isolates yielded, repeatedly,

no banding patterns with this technique (Table 1). PFGE, MLVA9-Utrecht, and MLST distinguished 52, 45, and 36 types, respectively, which could be grouped in 33, 35, and 33 CCs. The DIs with 95% confidence intervals (CI) were comparable, although PFGE was slightly more discriminatory than MLST (Table 1). The three typing methods were highly congruent at the CC level with an adjusted Rand coefficient of 0.84 for PFGE vs. MLVA9-Utrecht, 0.91 for PFGE vs. MLST and 0.90 for MLST vs. MLVA9-Utrecht. Moreover, two strains that are of the same MT have a high probability of belonging to the same ST on the level of clonal clusters, as indicated by the Wallace coefficients (Table 2), which was highest between MLVA9-Utrecht and MLST (0.969).

MLST-MLVA9-Utrecht comparison revealed that the previously identified high-prevalence STs among CF-patients, ST406 and ST497 [8], were represented by MTs 27, 32, 52 and 238 and MTs 11 and 38, respectively.

Based on the high DI of MLVA9-Utrecht, the high congruence between this MLVA scheme and the other typing methods and the fact that MLVA is considerably cheaper than MLST, rapid to perform and allows data comparison with other datasets (Table 1), we selected MLVA9-Utrecht as the preferred typing method to determine the population structure of *P. aeruginosa* isolated from different epidemiological backgrounds in the Netherlands.

Population biology of P. aeruginosa clinical isolates

All 363 isolates were typed with the adjusted 9 loci-MLVA scheme and 252 different MLVA9-Utrecht types could be discerned (typing data available in supplement Data S1). Typeability was 91% and ranged from 87% to 95% in the different patient groups (Table 3). In 22 and 10 isolates one or two loci could not be amplified, respectively, and these were assigned allele "99". Of the loci that could not be amplified in all isolates, ms217, ms215 and ms77 could not

Table 1, Typing characteristics of the genotyping methods for the 60 isolates typed with all 3 methods

	PFGE	MLVA9-UTRECHT	MLST
Typeability	91.7%	100%	100%
Costs[a]	€5.78	€7.21	€121.60
Time[b]	5 days	2 days	7 days
Ease of interpretation	-	+	++
International comparison[c]	-	+	++
Discriminatory Index	0.998 [0.995 – 1.0]	0.982 [0.968 – 0.998]	0.963 [0.936 - 0.991]

[a] Cost per isolate tested, including materials, excluding labor and equipment depreciation since that is similar in all methods. MLST costs can be lower when not using outsourced sequencing
[b] Time can be shorter with MLST. In this study we outsourced sequencing that took extra time
[c] Comparison with international data in database on http://pubmlst.org/paeruginosa/ for MLST and http://minisatellites.u-psud.fr/MLVAnet/ for MLVA

Table 2, Wallace coefficients, indicating congruence between the different typing methods

	MLVA9-UTRECHT	MLST	PFGE
MLVA9-UTRECHT	NA	0.969	0.917
MLST	0.845	NA	0.918
PFGE	0.793	0.910	NA

be amplified in 12, 8 and 7 isolates, respectively. Ms127 was the only locus that could be amplified in all isolates. The genetic diversities in these four populations were similarly high, with an overall DI of 0.995 (CI 0.993-0.997) (Table 3).

The population structure of *P. aeruginosa* in this strain set based on MLVA9-Utrecht is characterized by a high level of host-specificity. Between 82% and 91% of MTs are unique for the different patient populations. Only 11 (4%) of the 252 MTs were detected in two different patient populations studied. These MTs represented 11% of the CF related types (group I), 6% of the ICU related types (group II), 9% of the non-ICU hospitalized patients (group III) and 18% of the community acquired types (group IV), respectively (table 4). The MTs found in groups III and IV (hospitalized, non-ICU patients and patients with community acquired isolates) were not found in groups I (CF patients) or II (ICU patients). When comparing MTs from the ICU populations in both hospitals, most MTs appeared to be ICU-specific (fig 1/table 4). Only three (2%) of 142 MTs were detected in samples from patients in both ICUs indicating specific clustering in both location and patient group. The DIs of CF group and ICU-1 group appeared significantly lower than what would have been expected in case of random distribution of MTs (table 5). Furthermore, calculation of expected unique and shared types between the five groups in case of random distribution revealed that the observed numbers of shared types between all the different groups was significantly lower than what would have been expected, except between group III and IV (table 4). This proves non-random clustering of MTs and the presence of patient group-specific types.

The 252 MTs could be grouped in 22 CCs, defined as clusters of three or more types that share at least 8 out of 9 loci (fig 1). The minimum spanning tree revealed that specific clustering in both location and patient group did not result in grouping of isolates from a single patient population in one genetic lineage or genetic subpopulation. In contrast, isolates belonging to a single patient population are scattered over the minimum spanning tree. In agreement with the observed host-specificity, only three CCs contained isolates from four of the five populations studied. These three CCs (CC44, CC255 and CC13) contain CF isolates that were also typed by MLST allowing comparison with other isolates in the MLST database [19]. These "mixed" CCs, detected in each patient population, are closely related to *P. aeruginosa* clones that had been detected up to 7 countries on 4 continents.

Table 3, *P. aeruginosa* MLVA9-UTRECHT typing results of four different patient populations

	Group I	Group IIa	Group IIb	Group II	Group III	Group IV	
	CF	ICU-I	ICU-II	ICU total	Hospital acquired Non-CF/non-ICU	Community acquired Non-CF/non-ICU	Total
Source	respiratory	respiratory	respiratory	respiratory	non-respiratory	non-respiratory	diverse
# isolates (from # pat)	100 (90)	126 (97)	79 (64)	205 (161)	39 (38)	19 (19)	363 (308)
typeability	88 %	93 %	100 %	95 %	87 %	89 %	91 %
# types	72	82	63	142	33	17	252
Index of diversity	.984	.981	.991	.991	.989	.988	.995
CI	(0.971-0.996)	(0.97-0.992)	(0.983-0.999)	(0.987-0.996)	(0.977-1.0)	(0.969-1.0)	(0.993-0.997)
Prevalent types (≥5%)	MT27(11%) MT11(5%)	MT44 (10%) MT68 (6%)	MT161 (8%)	NA	MT255 (5%) MT261 (5%)	MT276 (11%) MT212 (11%)	NA

CF: cystic fibrosis patients, ICU: intensive care unit patients, MT: MLVA9-UTRECHT type, #: number

Table 4, Numbers (%) of shared and unique MLVA9-UTRECHT types (MTs) in the four groups of clinical sources compared to the numbers of expected values based on 100.000 permutations (median, range and 95% confidence interval (CI)) when assuming random distribution of types

			MTs shared (percentage of total MTs) [95% CI]				
		Unique	CF	ICU-I	ICU-2	HA	CA
Source			group I	group IIa	group IIb	group III	group IV
CF	Observed	64 (89%)		6 (8%)[a]	2 (3%)[a]	0[a]	0[a]
	Expected	58 [50-66]		18 [13-23]	13 [8-18]	8 [4-12]	4 [1-7]
ICU-1	Observed	72 (88%)	6 (7%)[a]		3 (4%)[a]	0[a]	0[a]
	Expected	74 [66-82]	18 [12-23]		15 [10-20]	9 [5-13]	5 [2-8]
ICU-2	Observed	58 (92%)[b]	2 (3%)[a]	3 (5%)[a]		0[a]	0[a]
	Expected	46 [38-53]	13 [8-18]	15 [10-20]		7 [3-11]	3 [1-7]
HA	Observed	30 (91%)[b]	0[a]	0[a]	0[a]		3 (9%)
	Expected	23 [17-28]	8 [4-12]	9 [5-13]	7 [3-11]		2 [0-5]
CA	Observed	14 (82%)	0[a]	0[a]	0[a]	3 (18%)	
	Expected	10 [6-14]	4 [1-7]	5 [2-8]	3 [1-7]	2 [0-5]	

CF: cystic fibrosis patients, ICU: intensive care unit patients, HA: non-CF, non-ICU patients with hospital acquired *P. aeruginosa*, CA: non-CF, non-ICU patients with community acquired *P. aeruginosa*.
[a] : value lower than expected within 95% CI range, i.e. less overlap of types between sources than in the case of random distribution of types. [b]: more unique genotypes per source than expected, i.e. high level of source-specificity rather than random distribution.

Three CCs contained isolates from CF patients only. Two of these, CC27 and CC11, contained the two previously reported high prevalent genotypes in CF-patients, ST406 and ST497, represented in this study by MLVA9-Utrecht types MT 27, 32, 52 and 238 (CC27) and MT 11 and 38 (CC11), respectively. This means that these two high prevalent CF clones are exclusively found in CF patients (fig 1).

Discussion

Using a simplified MLVA scheme for genotyping we have demonstrated that the population structure of *P. aeruginosa* isolates is highly diverse and population specific. This implies that most clones specific for CF patients, including the highly prevalent Dutch clones MT27, 32, 52, 238 (ST406) and MT 11, 38 (ST497), are genetically distinct from clones from non-CF patients.

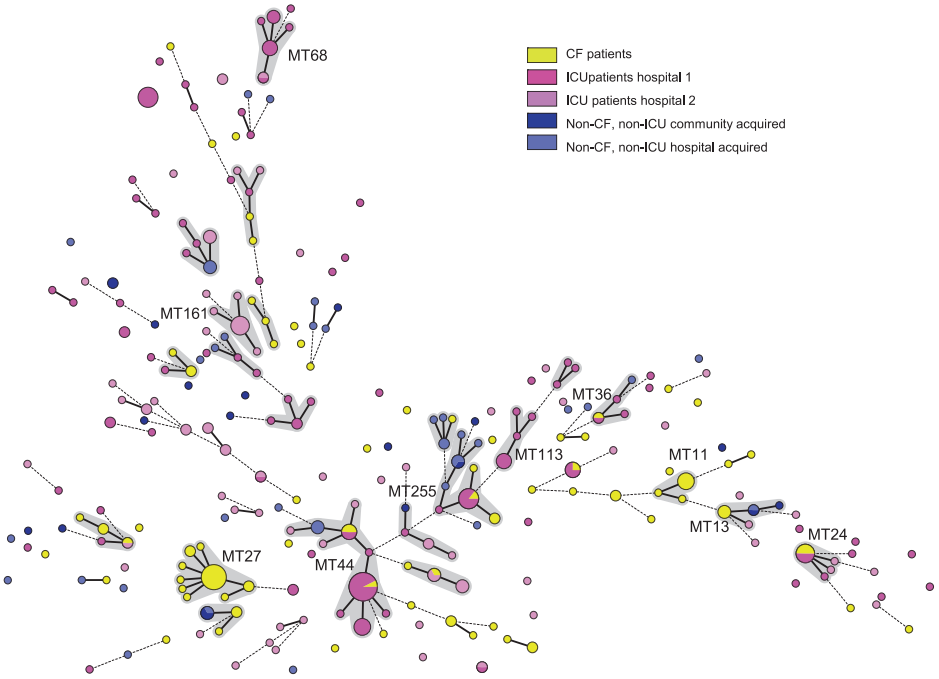

Fig 1, Minimum spanning tree of 363 P. aeruginosa isolates from different patient populations typed by MLVA9-UTRECHT
Circles represent MTs, the size of the circle is related to the number of isolates with that specific MT in this collection. Fat lines between the circles represent single locus variants (SLVs), differing only in one loci. Dotted lines represent double locus variants. Yellow color represents CF isolates, pink and purple are ICU respiratory isolates from two different hospitals, blue are non-CF non-ICU isolates (dark blue are "community acquired" isolates and light blue "hospital acquired"). Grey shading indicates clonal complexes.

The high prevalence of these clones in CF patients, therefore, is unlikely to result from transmission of particular dominant clones from the non-CF reservoir. Moreover, ICU-wards from different hospitals appeared to have location specific *P. aeruginosa* populations. MLVA9-Utrecht revealed that the *P. aeruginosa* population in the different clinical settings is highly diverse with a DI of 0.995 with no difference in diversity between hospital acquired and community acquired strains. This corroborates with previous findings in ICU patients [15,20,21]. Studies in the last decade have proposed different types of *P. aeruginosa* population structures, ranging from panmictic in the early nineties [22,23] to more clonal in 2007 [24]. The latest reports however, summarized by Pirnay in 2009, point towards a nonclonal epidemic population structure, with no distinction between clinical or environmental isolates [25]. In particular, the lack of distinction in genotype, function and chemotaxonomy between clinical and environmental *P. aeruginosa* isolates has been reported by different research groups [26,27]. Based on FAFLP, gene sequencing and virulence gene profiling Pirnay *et al* described that strains which clustered in the same clonal complexes could have been isolated from inanimate environments, animals and

Table 5, Expected Indices of Diversity (DI) and 95% confidence intervals based on 100.000 permutations based on random distribution of genotypes compared to observed DI

	CF	ICU-1	ICU-2	HA	CA
Observed DI	0.984 *	0.981*	0.991	0.989	0.988
expected DI	0.995	0.995	0.995	0.996	1.0
[95% CI]	[0.991-0.998]	[0.992-0.997]	[0.991-0.998]	[0.987-1.0]	[0.982-1.0]

* not within the 95% confidence interval (CI) range; i.e. diversity in that specific group is lower than would be expected on random distribution of types

humans, sometimes separated by thousands of miles. They concluded that there was no correlation between the clonal complexes and geographical origin or habitat. We also found that the three clonal complexes that were present in the four epidemiological backgrounds had been detected previously in up to seven other countries on four continents, indicating their global presence [28].

However, in contrast to previous research that suggested no correlation between *P. aeruginosa* clones and diseases or environmental habitats [24,26,29], we found genotypes to be highly specific for the different patient groups with only a relatively small number of clones distributed across patient population boundaries. However, since the MLVA database [28] does not provide data on the source of the isolate we cannot elaborate on the association between these types and epidemiological background. Our findings of high specificity of different sets of genotypes, not only in the various patient groups but also between ICUs in the different hospitals, are remarkable. Thus, discordant to the proposed consensus of a non-clonal epidemic population structure with some dominant clonal complexes, which are just as versatile in their habitat and geographic origin as the whole *P. aeruginosa* population [24], we found that both patient population and geographical origin appeared to be correlated to the prevalence of certain genotypes and that transmission of *P. aeruginosa* clones between ICUs, hospital wards and CF patients is rare.

The limited overlap between isolates from CF and non-CF patients also fails to support findings reported by Lanotte *et al,* who described, based on random amplification of polymorphic DNA (RAPD), a non-random distribution of isolates but with a subpopulation of isolates originating from patients with lung disease, both CF and non-CF [29]. This could result from low discriminatory power of RAPD [15].

Pirnay *et al* also concluded that, based on typing of 328 unrelated isolates including 43 CF isolates, all CF isolates clustered into a "core lineage" that is predominant in both disease and environmental habitats across the world [25]. Consequently, CF isolates belonging to the so-called "successful core lineage" are ubiquitous in the natural environment and are, therefore, more likely to infect CF patients. We failed to confirm such a level of "relatedness" in our

populations, as CF isolates, as well as the ICU isolates and other clinical isolates were dispersed over the entire minimum spanning tree. Moreover, the two most successful CF clones in our country were not detected in other patient populations and they are not genotypically closely related to non-CF isolates. This suggests no common evolutionary background of *P. aeruginosa* isolates from CF patients nor of *P. aeruginosa* isolates from the other analyzed patient groups. Our findings are more in line with the observation that the Australian Epidemic strains I and III (AESI and AESIII) could not be isolated from the environment [30,31]. These findings suggest selection of multiple specific clones with a distinct evolutionary background that are better equipped to adapt to and survive in the specific conditions in the CF lung. This also indicates that *P. aeruginosa* from many different lineages can adapt to all kinds of niches. This concurs with data from Pirnay *et al*, who found that a *P. aeruginosa* community in a Belgian river contained members of nearly all successful clonal complexes and was almost as diverse as the global population, represented by 73 clinical and environmental isolates from a previous study[26,32].
The strength of our study is the large and well-defined collection of isolates and the ability of MLVA9-Utrecht to show, highly reproducible, genotypic relatedness, with the possibility of comparing the genotypes to results contained in international databases via the internet, and that can be performed under point-of-care conditions. One should be aware that the MTs assigned in this study only refer to the MLVA9-Utrecht scheme. We did not include isolates from the environment in our study, shown to contain similar genotypes as clinical isolates in other studies [24,26,29], which may change our findings of specificity.

We conclude that the population structure of *P. aeruginosa* from different patient populations is highly diverse and characterized by high-level host-specificity and by the presence of many unique and only a limited number of more prevalent genotypes. The two genotypes (MT27/ST406 and MT11/ST497), frequently found in the Dutch CF patients, appear to be unique to CF patients and are not found in other clinical patients. Further studies are needed to elucidate the specific adaptations and survival strategies that these strains have adopted to survive in this special niche.

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PART III

Opinions

CHAPTER 8

Physicians' and nurses' opinions on selective decontamination of the digestive tract and selective oropharyngeal decontamination: a survey

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Abstract

Introduction

Use of selective decontamination of the digestive tract (SDD) and selective oropharyngeal decontamination (SOD) in intensive care patients has been controversial for years. Through regular questionnaires we determined expectations concerning SDD (effectiveness) and experience with SDD and SOD (workload and patient friendliness), as perceived by nurses and physicians.

Methods

A survey was embedded in a group-randomized, controlled, cross-over multicenter study in the Netherlands in which, during three 6-month periods, SDD, SOD or standard care was used in random order. At the end of each study period, all nurses and physicians from participating intensive care units received study questionnaires.

Results

In all, 1024 (71%) of 1450 questionnaires were returned by nurses and 253 (82%) of 307 by physicians. Expectations that SDD improved patient outcome increased from 71% and 77% of respondents after the first two study periods to 82% at the end of the study (P=0.004), with comparable trends among nurses and physicians. Nurses considered SDD to impose a higher workload (median 5.0, on a scale from 1 (low) to 10 (high)) than SOD (median 4.0) and standard care (median 2.0). Both SDD and SOD were considered less patient friendly than standard care (medians 4.0, 4.0 and 6.0, respectively). According to physicians, SDD had a higher workload (median 5.5) than SOD (median 5.0), which in turn was higher than standard care (median 2.5). Furthermore, physicians graded patient friendliness of standard care (median 8.0) higher than that of SDD and SOD (both median 6.0).

Conclusions

Although perceived effectiveness of SDD increased as the trial proceeded, both among physicians and nurses, SOD and SDD were, as compared to standard care, considered to increase workload and to reduce patient friendliness. Therefore, education about the importance of oral care and on the effects of SDD and SOD on patient outcomes will be important when implementing these strategies.

Introduction

Respiratory tract infections are a serious threat to patients in ICUs [1,2]. The incidence of these infections can be reduced by use of prophylactic antibiotic regimens, such as selective decontamination of the digestive tract (SDD) [3,4] and selective oropharyngeal decontamination (SOD) [5-7]. The concept of SDD consists of the application of topical (oropharyngeal) and enteral (nasogastric) non-absorbable antimicrobial agents, systemic administration of cephalosporins during the first four days in the ICU and maintaining the anaerobic intestinal flora with a policy favouring antibiotics without anti-anaerobic activity [8]. In SOD, only topical antibiotics in the oropharynx are applied.
The use of SDD and SOD has been the subject of intense controversy, due to methodological issues and concern about increased selection of antibiotic-resistant pathogens [3-5,7-13].
Proponents of the effectiveness of SDD point out beneficial outcomes in individual trials and meta analysis[14], whereas opponents address the lack of sound scientific evidence on patient survival and the constant threat of antimicrobial resistance [15]. Therefore, from May 2004 to July 2006, a large trial was performed in 13 ICUs in the Netherlands in which the effects of SDD and SOD on 28-day mortality were compared with standard care [16]. The trial consisted of three six-month study periods in which either SDD, SOD or standard care was used for all patients in the unit with the order of intervention randomized per centre. SDD and SOD were both effective and associated with a 13% and 11% relative reduction in 28-day mortality, respectively [16].
Bearing in mind the controversy and realizing that both the attitude towards and potential problems with new treatments might seriously affect effectiveness, we determined expectations concerning and experience with SDD as perceived by nursing and medical staff.

Materials and methods

Study protocol

Thirteen ICUs participated in the study, differing in size and teaching status and covering all levels of ICU in the Netherlands. Physicians assessed the eligibility of patients for the trial and when eligible confirmed trial medication in the patient chart. Nurses applied oral paste during SDD and SOD and administered suspension and systemic antibiotics during SDD. Furthermore, in all study periods, nurses applied oral hygiene consisting of teeth brushing and cleaning the oral cavity with a dental swab (Table 1).
Oral presentations were held at the start of every study period in each of the participating hospitals to inform nursing and medical staff about the trial and the study protocol. Furthermore, posters containing information about the study period were placed visibly in each unit. Both presentations and posters contained non-biased information about the aim of the trial and practical consequences of the next study period (oral hygiene, administration of study

Table 1, Study protocol

Study period	Oral hygiene	Oral paste[†]	Suspension[¥]	Cefotaxim[*]
SDD	+	+	+	+
SOD	+	+		
Standard care	+			

SDD, selective decontamination of the digestive tract; SOD, selective oropharyngeal decontamination.
+ applied four times a day.
[†] Oral paste consists of polymyxin, tobramycin, amphotericin B and is applied in the oropharynx.
[¥] Suspension consists of polymyxin, tobramycin, amphotericin B and is applied in the gastrointestinal tract through a feeding tube.
[*]Cefotaxim applied intravenous during first four days.

medication). Personnel from ICUs that had not used SDD before were invited to observe oral care and application of oral paste in another 'SDD-experienced' ICU.
A survey was used to determine expectations concerning and experience with SDD, and compliance to the study protocol. The survey to determine compliance to the study protocol was defined as the self-reported level at which nurses performed oral care according to the study protocol. Experience was focused on past and current experience with SDD. In the last week of each six-month study phase, all nurses and physicians working during a day (including night, day and evening shifts) received the questionnaire, which could be filled in anonymously [see Additional files 1 and 2]. With this single-day approach we expected to maximize response rates, because questionnaires could not be put aside but had to be returned the same day. In the second and third questionnaires (at the end of these study periods) it was also asked whether the nurse or physician had filled in a previous questionnaire. In the third questionnaire, nurses and physicians who participated in all three study periods were asked to grade workload, patient friendliness and effectiveness for SDD, SOD and standard care on a scale of 1 (low) to 10 (high). Patient friendliness was described as ease of application of oral hygiene and oral paste, and patient endurance of oral paste (taste, structure) to minimize additional stress in patients. Of note, nurses and physicians were not aware of the outcome results of the SDD-SOD trial at the time of the questionnaires.

Questionnaire development

A comprehensive literature search in Medline and Cumulative Index to Nursing and Allied Health Literature was performed in August 2004. The following keywords were used: questionnaires [MeSH], attitude of health personnel [MeSH], intervention studies [MeSH], and SDD [free text]. The search did not reveal questionnaires on the attitudes of nurses and physicians towards a new intervention. Therefore, qualitative techniques were used to identify items, that is, problems encountered when executing the study protocol. The questionnaires were developed on observations of oral care and semi-structured interviews with seven nurses from four different hospitals at the start of the trial: four in a SDD-period, one in a SOD and two in a standard-care period. The observations revealed that nurses did not comply entirely

with the oral hygiene protocol. During subsequent interviews the interviewer (IJ) pursued and clarified information on problems encountered during oral care and solutions to resolve reasons for non-compliance. Interviews were audio taped and transcribed verbatim. Transcripts were read and nurses' views regarding experience with SDD and problems met during oral care were identified and coded (by IJ and AS). Codes were continuously compared within and between transcripts. Agreement was reached between the researchers as to the major themes to be used in the questionnaires (concerning experience with and expectations of SDD), that is problems encountered during oral hygiene, non-compliance with the protocol, duration of oral care and expectations of SDD efficacy.

To maximize response rate, we designed a short questionnaire. For nurses, it contained four (standard care-period) to six (SDD and SOD-period) mostly closed questions, with a possibility to add comments in free text sections [see Additional file 1]. The nurses' questionnaire was pre-tested on three nurses (one research nurse and two ICU nurses), which resulted in a few linguistic changes only.

The questionnaires for physicians consisted of four closed and one open question in all study periods [see Appendix 2], addressing perceived clinical efficacy of SDD. Physicians were also asked to estimate ICU mortality rates in their standard care and SDD population, which were used to calculate the presumed relative reduction in mortality (PRRM), being the estimated mortality in SDD divided by the estimated mortality in standard care. The physicians' questionnaire was not pretested.

Analysis

Data were analyzed using SPSS15.0 (SPSS Inc, Chicago, IL, USA). Changes in opinion over time were analyzed by using chi-squared tests. Differences in time to perform oral hygiene and differences in grades were analyzed using medians (with interquartile ranges (IQR)) and non-parametric tests (Kruskal-Wallis tests, Friedman tests and Wilcoxon tests). A *P* value of less than 0.05 was considered statistically significant.

Results

A total of 1,450 questionnaires were sent to nurses and 1,024 were returned (71%): 372 after period 1, 339 after period 2 and 313 after period 3. Of 307 questionnaires sent to physicians, 253 (82%) were returned: 85 after period 1, 89 after period 2 and 79 after period 3 (Table 2). About one-quarter (27% nurses, 24% physicians) of those who received the questionnaires completed them two or three times.

Expectations on SDD efficacy

The expected effect of SDD on patient outcome, as asked after every study period, increased during the study ($P = 0.004$; Table 2). The proportion of physicians that expected SDD to have

no effects on clinical outcomes decreased from 14% after the first two periods to 4% at the end of study (P = 0.065). For nurses, these proportions were 33%, 26% and 22%, for periods 1, 2 and 3, respectively (P = 0.017). The most frequently reported expected effect of SDD was a reduction in the incidence of ventilator-associated pneumonia (VAP), and these proportions increased during the study (P = 0.001). Regarding improved ICU survival, both nurses and physicians tended to have increasing confidence in a positive effect of SDD on patient survival (P = 0.062 and P = 0.059, respectively). This corroborated the median calculated PRRM, as reported by physicians, which tended to increase from 3.0% (IQR 0 to 25) after period 1 to 16.7% (IQR 0 to 28.5) at the end of the study (P = 0.113).
The proportion of physicians that expected SDD to affect antibiotic resistance in their unit did not change significantly during the conduct of the trial. An increase in resistance was expected by 17% after period 1 and 27% at the end of study (P = 0.25) and a decrease in resistance was expected by 13% and 18% (P = 0.64) at these time points.
As we assumed that opinion on effect of SDD might be influenced by previous experience, we analyzed whether experience with SDD (either before or during the trial) was associated with expectations of SDD effects, which appeared not to be the case (chi-squared analysis, P = 0.74 and P = 0.98 for physicians and nurses respectively, data not shown). Trial results were not communicated, but neither intervention nor outcome were blinded for physicians and nurses. Data revealed that there was no correlation between the SDD-induced change in 28-day survival (observed effect in the trial) and the expected effect (by questionnaires) per hospital (r = 0.24, P = 0.43), nor between the observed effect and PRRM (r = -0.28, P = 0.36).
As additional effects of SDD, nurses mentioned better oral care, whereas physicians mostly mentioned a decrease in other infections (beside VAP), like urinary tract infections (Table 3).

Self-reported compliance to protocol

Problems during oral care, as reported by nurses, occurred frequently. It was reported that in particular non-sedated patients experienced oral care as annoying (56%), disliked the flavor of the oral paste (46%) and/or suspension (22%), refused to cooperate during oral care (36%) or were nauseous (13%) (Table 4). Despite these problems, the self-reported adherence to the study protocol was 70%. Of nurses who did not comply, an average of 8% (7% in SDD, 8% in SOD) reported to have discontinued application and 6% (8% in SDD, 5% in SOD) reported to have modified the study protocol, by using a suspension instead of oral paste for oral care. The remaining 16% forgot to apply the oral paste on one occasion or at the right time point. Most modifications of the study protocol were made in non-intubated, non-sedated patients who refused the oral paste. These modifications did not seem to be influenced by expectations of nurses: the expected effect of SDD was not associated with being fully adherent to the study protocol (P = 0.65).

Table 2. Response and expectations of the effect of SDD per study period

	Nurses				Physicians				Total			
	1st	2nd	3rd	*P*†	1st	2nd	3rd	*P*†	1st	2nd	3rd	*P*†
Response – no. (%)	372 (74)	339 (73)	313 (65)		85 (89)	89 (82)	79 (77)					
Prior experience SDD - %	53	74	87		68	85	90					
Effect SDD – no. (%)												
No effect	101 (33)	80 (26)	63 (22)	.017	12 (14)	12 (14)	3 (4)	.065	113 (29)	92 (23)	66 (18)	.004
Decrease pneumonia	135 (43)	151 (49)	165 (58)	.002	64 (75)	71 (80)	65 (84)	.354	199 (50)	222 (56)	230 (68)	.001
Increase resistance	68 (22)	68 (22)	48 (17)	.209	14 (17)	19 (21)	21 (27)	.247	82 (21)	87 (22)	69 (19)	.624
Decrease resistance	24 (8)	21 (7)	25 (9)	.672	11 (13)	13 (15)	14 (18)	.640	35 (9)	34 (9)	39 (11)	.524
Increase survival*	81 (26)	83 (27)	97 (34)	.062	36 (42)	45 (51)	47 (61)	.059				
Other	21 (7)	35 (11)	25 (9)	.129	9 (11)	11 (12)	13 (17)	.478	30 (8)	46 (12)	38 (11)	.145
Median PRRM (IQR)					3.0 (0-25)	12.9 (0-25)	16.7 (0-28.5)	.113				

IQR, interquartile range; PRRM, presumed relative reduction in mortality; SDD, selective decontamination of the digestive tract.
* Increase survival physicians based upon calculation PRRM.
† significance based upon chi-squared test (effect) or Kruskal-Wallis test (median PRRM)

Table 3. Free-text responses on additional effect of SDD – no

	Nurses	Physicians
No idea	82	1
Better oral hygiene	39	-
Increase colonization Enterococci/other bacteriae	3	6
Decrease other infections (besides VAP)	15	10
Other infection pattern	-	2
More frequent growth of yeasts	13	3
Less frequent growth of yeasts	2	-
Decrease length of stay	6	9
Increase length of stay	-	1
Better bacterial monitoring/antibiotics regimen	2	3
Increase diarrhea/change intestinal flora	3	-
Decrease multi organ failure	1	-
Decrease complications	-	1
Increase complications(wrong application)	-	1
Decrease morbidity	-	1
Decrease mechanical ventilation	-	2

SDD, selective decontamination of the digestive tract; VAP, ventilator-associated pneumonia.

Time needed for oral care

The estimated median time needed to perform oral care according to the protocol (which included applying oral paste every six hours during the SDD and SOD period) was 3.0 (IQR 0 to 5) minutes for both standard care and SOD and 5.0 (IQR 2 to 5) minutes for SDD ($P < 0.001$; Table 4). Estimated median additional times needed for oral care during SDD differed per center from 1.7 to 7.3 minutes. SDD was considered more time consuming than SOD and standard care in six centers and SOD was considered less time consuming than standard care in five.

Grades for perceived workload and patient friendliness

Both physicians and nurses graded the estimated workload lowest for standard care and highest for SDD (Table 5). Although median differences in grades for SDD and SOD were small (5 and 4 for nurses and 5.5 and 5.0 for physicians, respectively), there was a tendency both in nurses and physicians to value workload during SDD higher as compared with SOD ($P < 0.001$ for nurses and $P < 0.01$ for physicians). Free text from nurses revealed that removing rests of oral paste from the oral cavity (before applying new paste) and increased prevalence of diarrhea contributed to a perceived higher workload during SDD. There was no relation between expected effect of SDD and the grade given for workload during SDD, neither in nurses nor in physicians.

Table 4. Application of study protocol by nurses per intervention period

	SDD	SOD	Standard care	*P* ††
Extra time in minutes‡ – median (IQR)	5.0 (2-5)	3.0 (0-5)	3.0 (0-5)	0.000
Problems‡ - % of times reported	79	74		
- Patient disliked taste of oral paste - %	48	44		0.336
- Patient disliked suspension - %	22	--		--
- Patient was nauseous - %	17	9		0.003
- Patient found oral care annoying - %	54	58		0.318
- Patient did not cooperate with oral care - %	37	34		0.377
Change in application Orabase‡ - %	31	29		0.305
- once not given - %	14	12		
- given at another time - %	2	4		
- discontinued - %	7	8		
- other - %	8	5		

IQR, interquartile range; SDD, selective decontamination of the digestive tract; SOD, selective oropharyngeal decontamination.

‡ Extra time, problems and change in application as reported by nurses.

†† significance based upon chi-squared (problems, changes) or Kruskal-Wallis test (median extra time).

SDD and SOD were considered significantly less patient friendly than standard care, both by nurses and physicians, with median values for SDD and SOD of 4 in nurses (IQR 2 to 5 and 3 to 6, respectively) and 6 in physicians (IQR 4-7 and 4-6, respectively) and for standard care of 7 in nurses (IQR 3 to 9) and 8 in physicians (IQR 6 to 9). There was a difference in grade for patient friendliness given by nurses for SDD as compared with SOD (Wilcoxon test, $P < 0.001$), whereas for physicians there was no difference between the intervention periods. In free text, nurses often mentioned the taste and color of the oral paste as patient unfriendly, especially in non-ventilated and non-sedated patients. Furthermore, the suspension of SDD was considered unfriendly, especially when the nasogastric tube was removed and the patient was asked to swallow the suspension.

Discussion

The results of our study reveal that physicians and nurses considered SDD to have a higher workload and to be less patient friendly than standard care. Moreover, expectations on the effects of SDD, especially on pneumonia, changed during the study, both among physicians and nurses, independent of study order and without knowledge of trial results.

Nurses associated SOD with a lower increase of their workload than SDD. The (statistically significant) difference in perceived duration of oral care in the SDD and SOD period is remarkable, because the oral care protocol did not differ in both interventions. An explanation may be that nurses included intuitively the time needed for the preparation and administration of the gastric solution and intravenous antibiotics.

Previous studies have reported nurses' perception of oral care practices as being difficult and

Table 5. Median grades (interquartile ranges) for the three intervention periods

	N	SDD median (IQR)	SOD median (IQR)	Standard care median (IQR)	*P*††
Nurses					
Workload [a]	207	5.0 (4.0-7.0)	4.0 (3.0-6.0)	2.0 (1.0-4.0)	0.000
Patient friendliness [b]	197	4.0 (2.0-5.0)	4.0 (3.0-6.0)	7.0 (3.0-9.0)	0.000
Physicians					
Workload [a]	30	5.5 (3.8-7.0)	5.0 (3.0-6.0)	2.5 (2.0-4.0)	0.000
Patient friendliness [b]	27	6.0 (4.0-7.0)	6.0 (4.0-6.0)	8.0 (6.0-9.0)	0.003

IQR, interquartile range; N, number of responses; pt, patient; SDD, selective decontamination of the digestive tract; SOD, selective oropharyngeal decontamination.
†† significance based upon Friedman test.
[a] Workload measured on a scale from 1 (low) to 10 (high).
[b] Patient friendliness measured on a scale from 1 (poor) to 10 (excellent).

unpleasant to perform [17-19]. This was confirmed in our survey, with nurses believing that oral care, especially application of oral paste, was unpleasant and 'unfriendly' for patients. Although oral hygiene was the same in SDD and SOD, the perception of patient friendliness differed. These results suggest that introduction of SDD and SOD should be accompanied by education in which the importance of oral care is emphasized in order to reduce the perception that oral care is unpleasant [20].

Thirty percent of the nurses reported a protocol violation in the application of oropharyngeal decontamination. Nurses mostly mentioned that they failed to administer the oropharyngeal paste only once. More obvious non-adherence appeared to be associated with the sedation level and ventilation status of a patient: the self-reported discontinued application of the oropharyngeal paste occurred predominantly in non-ventilated and non-sedated, alert patients. Based on notifications on the patient record forms during the trial, we estimated that oropharyngeal decontamination had not been administered in 2.5% and 4.3% of all patient days during SDD and SOD, respectively [16]. Given these figures and the additional comments that non-compliance mainly occurred in non-ventilated, non-sedated patients, it is unlikely that these incidental failures to apply medication affected the effectiveness of the interventions.

At the start of the trial, already most nurses and physicians expected SDD to effect patient outcome and this group had a relative increase of 15% towards the end of the trial. The median PRRM tended to increase during the conduct of the trial, and came close to the 13% relative risk reduction in 28-day mortality as determined in the trial [16]. As physicians were asked to estimate this benefit after each study period, we assume that the increasing proportion of physicians that had had experience with SDD explains this gradual change.

An important objection against the widespread use of SDD or SOD has been the possibility of an increase of antibiotic resistance. This was an important reason for physicians in the UK for not using SDD [21]. Our survey revealed non-conclusive results on the physicians' expectations on the effects of SDD on antibiotic resistance. During the study increasing proportions of physicians expected that SDD would be associated with either an increase or a decrease of

antibiotic resistance. Yet, the actual observed effects revealed that carriage levels with antibiotic-resistant pathogens in the intestines and the respiratory tract reduced during SDD and SOD [16].

Strengths of our study include the high response rates for both nurses and physicians and the fact that this is, up until now, the only prospective evaluation of perceived opinions related to SDD and SOD. There are several limitations to our study. First, it was not possible to fully validate the questionnaires. No (multi-item) factor analysis was performed on the items of the questionnaire, because only one question per topic was included. On the other hand, to enhance validity, we used triangulation: a combination of, in our study, two methods (observations and subsequent interviews) to develop consistent and comprehensive questionnaires about problems and expectations [22,23]. Furthermore, the questionnaire for physicians was not pretested, unlike the questionnaire for nurses.

A second limitation is the variability in respondents, because after every study period nurses and physicians working on a selected day were invited to fill in the questionnaire. Therefore, different nurses and physicians might have filled in the first, second and third questionnaires and changes in expectations might be influenced by the different respondents. However, because of the high response rate in all participating hospitals during each of the study periods, it is unlikely that important bias has been introduced. In addition, restricting the analysis to professionals who filled in the questionnaire two or even three times revealed similar conclusions (data not shown).

Conclusions

Among multiple different interventions aiming to reduce the incidence of VAP in ICU patients, SDD and SOD are currently the only two associated with demonstrated improvements in patient survival. Yet, widespread and correct implementation of these interventions will critically depend on the acceptance by health care workers that need to perform these procedures. Therefore, we recommend education about the importance of oral care and to provide clear information about the effects of SDD and SOD on patient outcomes.

Abbreviations

IQR, interquartile range; PRRM, presumed relative reduction in mortality; SDD, selective decontamination of the digestive tract; SOD, selective oropharyngeal decontamination; VAP, ventilator-associated pneumonia.

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Key messages

- Nurses considered SDD to result in a higher workload and to be less patient friendly as compared with SOD and standard care.
- Physicians considered both SDD and SOD to result in a higher workload and be less patient friendly as compared with standard care.
- The expectations of both nurses and physicians on the effects of SDD on patient outcome, especially on pneumonia and patient survival, changed over time.
- Confidence of nurses and physicians in effects of SDD increased over time

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Appendix : Nurses' Questionnaire

(translation; original questionnaire in dutch)

Hospital:
Study period: ……
Date: ……

1. *(Question in 2nd and 3rd study period)* Did you complete this questionnaire previously after a prior study period?
   ❑ Yes ❑ No

2. Did you previously (before this trial) apply SDD?
   ❑ no
   ❑ yes, what was your experience with SDD at that time?
      ❑ good, because .....
      ❑ neutral, because .....
      ❑ not good, because .....

3. *(Question in SDD and SOD period)* Keep in mind the last patient you cared for and who was included in the SDD/SOD-trial. Was the following applicable for this patient:
   - patient disliked the flavour of the oral paste (Orabase) .....yes / no
   - patient disliked suspension.....yes / no
   - patient was nauseous .....yes / no
   - patient found oral care annoying.....yes / no
   - patient did not cooperate with oral care .....yes / no

4. *(Question in SDD and SOD period)* When at least one of the questions in 3 is answered with "yes": was this a reason to change application of oral paste (Orabase) or suspension?
   ❑ not applicable (all questions in 3 answered with "no")
   ❑ no, oral paste and suspension were applied according to protocol
   ❑ yes, application was changed, namely:
      ❑ oral paste / suspension was not applied once
      ❑ oral paste / suspension was applied at another moment
      ❑ other, namely .....

5. How many minutes do you need <u>extra</u> at a time to perform oral care due to the SDD/SOD-trial?
   ❑ no time extra
   ❑ about ..... minutes extra per time

6. What do you expect of the effectiveness of SDD?
   ❑ no effect
   ❑ indeed effect, namely (more answers possible)
      ❑ decrease in pneumonia
      ❑ increase in antibiotic resistance
      ❑ decrease in antibiotic resistance
      ❑ increase of survival of patients
      ❑ other, namely .....

7. *(Question in 3rd study period)* Did you participate in all three study periods of the SDD/SOD-trial?

❑ no, not applicable

❑ if yes, can you give a grade for each of the study periods for the following aspects?

	SDD-period	SOD-period	Standard Care
Workload (1=small, 10=high workload)	……..	……	……
Patient friendliness (1=poor, 10=excellent)	……..	……	……
Effectiveness (1=poor, 10=excellent)	……..	……	……

8. Do you have other information you like to add concerning the SDD/SOD-trial?

……

……

……

Appendix : Physicians' Questionnaire

(translation; original questionnare in dutch)

Hospital:
Study period: ……
Date:

1. *(Question in 2nd and 3rd study period)* Did you complete this questionnaire previously after a prior study period?
   ❑ Yes ❑ No

2. What is your profession in ICU?
   ❑ intensivist
   ❑ specialist not intensivist
   ❑ Resident
   ❑ Intern

3. Have you previously worked with SDD?
   ❑ yes, during this trial
   ❑ yes, in this unit before the trial
   ❑ yes, elsewhere
   ❑ no, no prior experience with SDD

4. How do you estimate current ICU mortality of the included patient group?

   About ..... %

   How do you estimate ICU mortality in this patient group after application of SDD?

   About ..... %

5. What do you expect of the effectiveness of SDD?
   ❑ no effect
   ❑ indeed effect, namely (more answers possible)
      ❑ decrease in pneumonia
      ❑ increase in antibiotic resistance
      ❑ decrease in antibiotic resistance
      ❑ other, namely .....

6. Where do you base your expectation of effectiveness and mortality upon (more answers possible):
   ❑ published trials
   ❑ own experience
   ❑ experience of others
   ❑ other, namely .....

7. *(Question added in 3rd study period)* Did you participate in all three study periods of the SDD/SOD-trial?

   ❑ no, not applicable
   ❑ if yes, can you give a grade for each of the study periods for the following aspects?

	SDD-period	SOD-period	Standard Care
Workload (1=small, 10=high workload)	……..	……	……
Patient friendliness (1=poor, 10=excellent)	……..	……	……
Effectiveness (1=poor, 10=excellent)	……	……	……

8. Do you have other information you like to add concerning the SDD/SOD-trial?

.....

.....

.....

CHAPTER 9

Summary and general discussion

Summary and general discussion

This thesis addressed approaches to improve airway management, and in particular endotracheal suctioning, in mechanically ventilated ICU patients, and acquisition of respiratory tract colonization with Gram-negative bacteria in this patient population.

Airway management

The first part of this thesis focused on endotracheal suctioning, which is an essential and frequently performed procedure in mechanically ventilated intensive care (ICU) patients. Nowadays, two systems are available for ES: the single use open suction system (OSS) and the 'newer' multiple use closed suction system (CSS). Since its introduction in the 1980s, CSS has been increasingly used because of several presumed benefits of the 'closed' nature of the system. Without the need for disconnecting the patient from the ventilator (as in OSS) spread of aerosols would be lower and this would reduce bacterial contamination of patients, health care workers and inanimate environment. Furthermore, CSS can be performed with ongoing mechanical ventilation, which would guarantee optimal oxygenation.
Conceptually, prevention of bacterial transmission from patient to patient and maintenance of optimal oxygenation in individual patients would be highly beneficial, and clinically relevant. In daily practice, CSS was often used in patients with respiratory tract infections (to avoid contamination with pathogens), in patients requiring high levels of positive end-expiratory pressure (PEEP) (to maintain optimal oxygenation) and in patients treated in prone positioning (to avoid extubation during disconnection or problems with reconnecting the patient to the ventilator). Yet, others were more skeptical about the assumed advantages of CSS over OSS, as various studies could not provide conclusive evidence to support these assumptions. Therefore, a systematic review with meta-analysis was performed (chapter 2), which included 15 studies with a randomized study design comparing effectiveness of OSS and CSS. The results of this meta-analysis revealed that generally assumed advantages of CSS compared with OSS, such as lower incidences of ventilator-associated pneumonia, reduced bacterial contamination, and improved patient outcome, were not supported by scientific evidence. Although CSS was associated with higher values for mean arterial pressure and heart rate after endotracheal suctioning, the differences were very small (for heart rate 6 beats / min, mean arterial pressure 3 - 5 mm Hg in favor of CSS) and seemed, therefore, of little clinical relevance.
Based upon the results of the systematic review, a prospective crossover study was performed in four Dutch ICUs (2 ICUs from the UMC Utrecht and two units from St Elisabeth Hospital Tilburg) (chapter 3). The primary outcome was the occurrence of cross-transmission with Gram-negative bacteria during periods in which either of both methods was used. The bacteria studied were *Pseudomonas aeruginosa*, *Acinetobacter* species, *Stenotrophomonas maltophilia*, *Klebsiella* species, *Enterobacter* species and *Escherichia coli*. The crossover design with fixed

periods in which either of both systems was used for all patients in the unit was considered most appropriate, since individual randomization would result in a mix of patients receiving ES with CSS and OSS, in which beneficial effects of CSS might be obscured by cross-transmission occurring from neighbor patients randomized to OSS. The results of this trial revealed that the routine use of CSS failed to reduce cross-transmission and acquisition rates of the most relevant Gram-negative bacteria in ICU-patients. Fifty percent of all patients in our trial were colonized with at least one of the marker pathogens, either on admission or acquired. In each of the study periods 37% of the patients acquired respiratory tract colonization with at least one of the selected Gram-negative bacteria. Overall acquisition rates were 35.5 and 32.5 per 1,000 patient days at risk during CSS and OSS, respectively. For three pathogens, *P. aeruginosa*, *Acinetobacter* species and *Enterobacter* species, cross-transmission rates were based on genotyping and epidemiological linkage of patients during their stay in ICU. Cross-transmission rates with any of the three pathogens were low: 5.7 and 4.5 per 1,000 patient days at risk during CSS and OSS, respectively, when applying the most stringent definition for such an event.

The study had a pragmatic design, in which daily clinical practice was maintained as much as possible, with a crossover design to account for differences between participating units. As a result, the participating hospitals used their own type of CSS and OSS. In the university hospital a swivel connecter was used in combination with OSS, thereby decreasing the opening to perform ES, whereas in the teaching hospital the patient was disconnected from the ventilator. For CSS, a 24- and 72-hrs system were used (same manufacturer) and changed according to these indications.
Non-adherence to the randomized ES system could have masked beneficial effects of CSS on acquisition of GNB. However, adherence was checked meticulously, and in only 7% of patient days non-adherence occurred (5% during CSS and 9% during OSS). Another aspect that could have masked outcome was disconnection of CSS, i.e., for planned change of system, when accidentally disconnected or to perform extra open suctioning. Disconnection during the CSS period was registered and occurred with a median of 1 time per day.
Other investigators have evaluated the effects of not changing the closed suction system for longer periods (when compared to changing every 24 hours or when compared to OSS). Cross-transmission, though, was not evaluated in any of these studies, and it remains, therefore, unknown if prolonged use of CSS will be associated with lower cross-transmission rates in ICUs.
Infection control measures are important to prevent cross-transmission and, therefore, adherence to hygienic precautions with CSS and OSS were monitored, without nurses being aware of this. Adherence to hand hygiene was comparable after using CSS and OSS, but OSS was associated with better adherence to hand hygiene *before* endotracheal suctioning, and gloves, eye protection and masks were more frequently used during OSS. One might speculate that the lower adherence to hygienic precautions with CSS may mask the beneficial effects of CSS on cross-transmission. Yet, both effects cannot be disentangled with the study design as

used. Naturally, it would have been possible to include these hygienic aspects in our intervention, but than we would not have answered the question whether unit-wide implementation of CSS in daily practice, without modification of other variables, reduced cross-transmission.
In addition, our study could have been underpowered. During the study preparation we calculated that 250 patients per study arm were needed to detect 10% risk reduction in cross-transmission (from 25% to 15%). Yet, since both hospitals participated with two instead of one unit, in the end more than twice the number of patients needed (total 1,110 patients) were included. The adjusted hazard ratio for ICU-acquired colonization with any of the selected pathogens during CSS was 1.14 (95% confidence interval 0.91 - 1.42) and it is, therefore, highly unlikely that a larger sample size would have demonstrated a clinically relevant difference between both methods.

The physiological consequences of CSS and OSS (i.e., oxygenation and disturbances of cardiac function) were investigated in an observational study nested within the crossover trial. In this pragmatic study, ES with either of both systems was evaluated as it was performed during standard care, i.e. when clinically indicated and performed according to hospital protocol. Changes in heart rate, mean arterial pressure and peripheral oxygen saturation (SpO_2) were monitored before and after ES with either CSS or OSS. These changes appeared minor and were comparable when using either CSS or OSS. Only mean SpO_2 values appeared higher after using OSS as compared to CSS, but differences were very small (98.2% and 97.5%) and therefore clinically not relevant.

During the study, differences in performance of ES were observed, both between hospitals and as compared to international guidelines. The latter recommend preoxygenation and postoxygenation to minimize desaturation and to use a suction pressure of less than 20 kPa. However, preoxygenation and postoxygenation were performed in 24% and 18% of all ES procedures observed, respectively, and both hospitals used different suction pressures (30 and 20 kPa, respectively). Furthermore, during the OSS study periods, some physicians preferred CSS in patients with PEEP values >10 cm H_2O, which contributed to a baseline difference in PEEP between both procedures in the observational study. These variations in performance of ES did not reveal differences between CSS and OSS. However, for patient safety, it may be important to determine the boundaries within which ES is safe to perform, that improves oxygenation and is effective in removing secretions.

Based upon the results, both CSS and OSS can be considered equally safe in mechanically ventilated ICU patients. We could not demonstrate a difference in overall cross transmission, nor in overall acquisition of respiratory tract colonization. Furthermore, no clinically relevant differences in cardiorespiratory variables were found. Finally, we have not identified characteristics and aspects of our patient population and nursing practice that would render our

findings not generalizable to other settings. Without a difference in effectiveness, a cost-effectiveness analysis becomes irrelevant, and only costs of systems remain to be analyzed. OSS is less expensive than CSS: the price of an open suction catheter is € 0,38 and of a swivel connector € 2,70, while the price of a 24 hr closed suction system is €11,20 (price level the Netherlands, 2009). For a hospital with more than 10.000 ventilation days per year (like the UMC Utrecht), and based upon a median ES frequency of 6 times per day per patient, this would save over €66,000 per year when using the swivel connector. Without this connector, savings will be even higher. Prolongation of CSS device use, from the recommended 24 hrs to several days, will reduce costs of CSS and subsequently reduce the difference between systems. The implications of the results for clinical practice are rather straightforward: the choice of the ES system to be used can be based on costs and personal preference. In our study protocol we allowed the use of CSS in patients treated in prone positioning, as disconnection (needed for OSS) could be difficult in some conditions.
During the study, most nurses preferred CSS because of its assumed reduced exposure risk to patients' secretions and its convenience (readiness-to-use). Other nurses preferred OSS for better 'feeling' of the performance of the intervention and better removal of secretions. Yet, despite these opinions, we did not observe more procedures of ES with CSS than OSS (both mean and median 6 times per day). Furthermore, preventive material as masks and glasses were used incidentally. However, we did not record secretion volumes being removed after using CSS or OSS, nor did we determine personnel contamination. The latter is closely related to compliance to hygienic precautions. Adherence to hand hygiene seldom exceeds 50%, which was also monitored in our study (chapter 3). Further improvement of adherence with infection control interventions in health care workers remains important.

Respiratory tract colonization

In the second part of this thesis the focus was on respiratory tract colonization in ICU patients. The microbiological results from the respiratory tract cultures obtained every Monday and Thursday, during 14 months, allowed a detailed analysis of risk factors for acquiring respiratory tract colonization with Gram-negative bacteria (chapter 5). Of 481 patients that were not colonized with Gram-negative bacteria in the respiratory tract at time of ICU admission, 52% acquired colonization during ICU stay. Risk of acquisition was strongly associated with mechanical ventilation and was lower in the university hospital (as compared to the teaching hospital) and among patients that received systemic antibiotics during ICU stay. For the individual pathogens, CSS was associated with a lower risk of acquiring *P. aeruginosa*, but with a higher risk of acquiring *Klebsiella* spp, and receiving antibiotics at the time of ICU admission was associated with a lower risk to acquire colonization with *Enterobacter* species and *E. coli*.
A limitation of this study is the exclusion of Gram-positive pathogens. Furthermore, episodes of colonization instead of actual infections were determined. However, respiratory tract infections

are almost always preceded by respiratory tract colonization. Therefore, risk factors for colonization may well be considered risk factors for subsequent infections like ventilator-associated pneumonia.

Furthermore, a phenotypical switch from susceptible to resistance for at least one antibiotic was determined in patients colonized with *P. aeruginosa* or *Enterobacter* species in the respiratory tract (chapter 6). In 41 out of 126 patients colonized with *P. aeruginosa*, and 46 out of 108 patients colonized with *Enterobacter* species, a phenotype switch from susceptible to resistant for one or more antibiotics occurred. Exposure to meropenem was associated with the highest risk of resistance development in patients colonized with *P. aeruginosa*. No single event of meropenem resistance acquisition was documented among 108 patients colonized with meropenem-susceptible *Enterobacter* species, despite meropenem exposure being higher in these patients (24.7 and 14.4 DDD/100 patient days for patients colonized with *Enterobacter* and *P. aeruginosa*, respectively). This suggests that carbapenems pose a more serious risk on inducing antibiotic resistance in *P. aeruginosa* than other beta-lactam antibiotics and fluoroquinolones.
As a limitation of this study, the development of resistance to multiple antibiotic was not determined. Combined resistance acquisition in *P. aeruginosa* was observed in half of meropenem resistance acquisitions and in half of ceftazidime acquired resistances. Percentages of combined resistance for ciprofloxacin and piperacillin-tazobactam acquired resistances were even higher: 80% to 90%. However, multiple resistances could not be included in time-dependent analyses, since resistance development for multiple antibiotics did not always occur simultaneously. Moreover, the numbers of combined resistance development were too low for statistical analysis.

To gain more in-depth knowledge on the ecology of *P. aeruginosa* we determined the population structure of *P. aeruginosa* in ICU patients and compared this to the population structure in cystic fibrosis patients. Strains were genotyped with a simplified Multiple-Locus Variable-number tandem-repeats Analysis (MLVA) scheme. The population structure of *P. aeruginosa* isolates appeared highly diverse and population specific, meaning that ICU clones were genetically distinct from clones specific for cystic fybrosis patients. Both patient population and geographical origin appeared to be correlated to the prevalence of certain genotypes. Resemblance of *P. aeruginosa* clones between ICUs, hospital wards and cystic fybrosis patients was documented rarely.

The final part of this thesis focused on another intervention directed towards reducing respiratory tract colonization: selective decontamination of the digestive tract (SDD) and selective oropharyngeal decontamination (SOD). Over the years, use of SDD and SOD has been controversial and ICU physicians, medical microbiologists and nurses have held strong opinions about effectiveness of SDD. Embedded in another large cluster-randomized crossover trial we investigated whether expectations about SDD among ICU-nurses and –physicians changed

during the trial, through regular questionnaires (chapter 8). Indeed, expectations of the beneficial effects of SDD increased during the study, both among ICU physicians and nurses, independent of study order and without knowledge of study results. In general SDD was considered to have a higher workload and to be less patient-friendly than standard care.

To improve quality and safety of patient care in ICUs it is important to implement evidence based and cost-effective interventions. Yet, widespread and correct implementation of these interventions will critically depend on the motivation and acceptance by health care workers that need to perform these procedures. The interventions studied in this thesis, routine use of closed suction systems and use of SDD and SOD, have been controversial since their introduction, their use often based upon assumed advantages. The rationale of use of interventions should be clearly explained through proper, recurrent education and instruction, which emphasizes the effects on patient outcomes and addresses the scientific base of widely held assumptions.

Nederlandse samenvatting

(Dutch summary)

Eenvoudig verteld

De meeste patiënten die op een intensive care afdeling worden opgenomen hebben mechanische beademing nodig, waarbij de ademhaling tijdelijk wordt ondersteund door een beademingsmachine. Het is belangrijk om bij deze patiënten regelmatig slijm weg te zuigen, omdat ze niet meer in staat zijn om dit zelf weg te slikken of weg te hoesten. Dit verwijderen van slijm is een verpleegkundige handeling die gemiddeld genomen zo'n zes maal per etmaal per patiënt plaatsvindt. Om deze handeling uit te voeren zijn twee verschillende systemen in gebruik: een open systeem en een gesloten systeem. Bij de open methode wordt de patiënt kortstondig losgekoppeld van de beademingsmachine en wordt een speciale katheter in de beademingsbuis geschoven die al zuigend wordt teruggetrokken, waarna de patiënt weer aan de beademingsmachine wordt gekoppeld. Met het gesloten systeem is loskoppelen niet nodig: slijm wordt verwijderd via een katheter die gedurende tenminste 24 uur gebruikt kan worden wanneer dat nodig is. Verwijderen van sputum bij beide methodes duurt ongeveer tien tot vijftien seconden.

In toenemende mate worden gesloten uitzuigsystemen gebruikt vanwege de verwachting dat er minder patiënten en medewerkers besmet raken met bacteriën. Bij het loskoppelen, wat nodig is voor het open systeem, kunnen sputum druppels verspreid worden. Bovendien is de verwachting dat het voor de patiënt beter is als deze niet kortstondig wordt losgekoppeld, omdat het toch een (korte) onderbreking van de beademing zou zijn.

Beide verwachtingen werden nog niet onderbouwd met resultaten uit eerder onderzoek, en zijn onderzocht in dit proefschrift. Het belangrijkste doel was om na te gaan of gesloten systemen, in vergelijking met de open systemen leiden tot minder kruisbesmetting met bacteriën. Daarvoor werd afwisselend een half jaar het ene systeem gebruikt en vervolgens een half jaar het andere systeem op 4 intensive care units. In beide groepen bleek 37% van de patiënten drager te zijn geworden van antibiotica resistente bacteriën, waarvan een vijfde door kruisbesmetting. Belangrijkste conclusie was dat er geen verband is tussen kruisbesmetting en de gebruikte systemen.

Open en gesloten uitzuigkatheters zijn even veilig om sputum te verwijderen bij patiënten die beademd worden. De keuze voor katheter kan worden gebaseerd op de kosten en op persoonlijke voorkeur. In Nederland zijn de gesloten uitzuigkatheters duurder dan de open uitzuigkatheters. Gebruik van de open uitzuigkatheters in plaats van de gesloten uitzuigkatheters zou het UMC Utrecht jaarlijks ruim € 60.000 besparen.

Nederlandse samenvatting

De meeste patiënten die op een intensive care zijn opgenomen, worden kunstmatig beademd. Hierdoor zijn ze niet meer in staat om zelf speeksel (sputum) op te hoesten, waardoor een belemmering kan ontstaan in de beademingsbuis of in de longen. Bij patiënten die beademd worden is de verzorging van de mond en het verwijderen van sputum door bronchiaal toilet van essentieel belang voor het adequaat functioneren van de ademhaling. Dit kan op verschillende manieren worden uitgevoerd. In dit proefschrift worden benaderingen beschreven om de luchtwegen te verzorgen en vrij te houden bij beademde intensive care patiënten, en in het bijzonder bronchiaal toilet. Daarnaast wordt kolonisatie van de luchtwegen met Gram-negatieve bacteriën (met name kolonisatie verworven op de intensive care) in deze patiënten populatie beschreven.

Luchtwegmanagement

Bronchiaal toilet is een essentiële en frequent uitgevoerde handeling bij beademde intensive care patiënten. Tegenwoordig zijn er twee systemen beschikbaar om deze interventie uit te voeren: het eenmalig bruikbare open uitzuigsysteem (OSS) en het 'nieuwere' meermalen bruikbare gesloten uitzuigsysteem (CSS). Dit laatste systeem is in de jaren tachtig van de vorige eeuw geïntroduceerd en wordt sindsdien in toenemende mate gebruikt vanwege de diverse veronderstelde voordelen van het 'gesloten' karakter. Zo wordt verondersteld dat gesloten systemen bacteriële besmetting van patiënten, medewerkers en directe omgeving reduceren, aangezien er geen noodzaak is om de patiënt van de beademingsmachine los te koppelen (zoals bij OSS gebeurt) waardoor minder verspreiding van sputum voorkomt. Daarnaast kan bronchiaal toilet met CSS worden uitgevoerd terwijl de beademing doorgaat, wat optimale oxygenatie van een patiënt zou garanderen.
Het voorkomen van overdracht van bacteriën tussen patiënten en het handhaven van optimale oxygenatie bij individuele patiënten zou een enorm voordeel zijn van het gesloten systeem en klinisch relevant zijn. In de dagelijkse praktijk wordt CSS veelal gebruikt bij patiënten met luchtweginfecties (om besmetting met bacteriën te voorkomen), bij patiënten die met hoge waarden van positieve eindexpiratoire druk (PEEP) beademd worden (om optimale oxygenatie te handhaven) en bij patiënten die in buikligging worden verzorgd (om detubatie tijdens ontkoppelen te voorkomen of om problemen te voorkomen met het weer aankoppelen van de patiënt aan de beademingsmachine).
Ondanks de veronderstelde voordelen zijn sommige verpleegkundigen en intensivisten meer sceptisch over de voordelen van CSS ten opzichte van OSS, omdat afzonderlijke studies geen eenduidig bewijs hebben geleverd om deze veronderstellingen te ondersteunen. Daarom is een systematisch review met meta-analyse uitgevoerd (hoofdstuk 2 van dit proefschrift), waarin 15 studies zijn geïncludeerd met een gerandomiseerde onderzoeksopzet waarin de effectiviteit van

CSS en OSS zijn vergeleken. De resultaten van deze meta-analyse tonen aan dat algemeen veronderstelde voordelen van CSS in vergelijking tot OSS, zoals een lagere incidentie van beademingspneumonieën, minder bacteriële besmettingen en betere patiënten uitkomsten, niet ondersteund worden door wetenschappelijk bewijs. Gesloten systemen bleken, in vergelijking met open systemen, wel geassocieerd met een betere hartslag en een betere gemiddelde arteriële druk na bronchiaal toilet, maar deze verschillen waren erg gering (voor hartslag 6 slagen per minuut, gemiddelde arteriële druk 3 – 5 mm Hg in het voordeel van CSS) en leken daarom van geringe klinische relevantie.
De resultaten van het systematische review lieten nog geen eenduidig bewijs zien om het ene uitzuigsysteem te prefereren boven het ander. Daarom is een prospectieve crossover studie uitgevoerd in vier Nederlandse intensive care units (twee units in het UMC Utrecht en twee units van het St Elisabeth Ziekenhuis in Tilburg). Gedurende twee perioden van elk zes maanden werd achtereenvolgens een van beide uitzuigsystemen (open of gesloten) gebruikt. Gekeken werd naar het voorkomen van kruisbesmetting en van kolonisatie met de volgende Gram-negatieve bacteriën: *Pseudomonas aeruginosa*, *Acinetobacter* species, *Stenotrophomonas maltophilia*, *Klebsiella* species, *Enterobacter* species and *Escherichia coli* (beschreven in hoofdstuk 3). De crossover studieopzet, met vaste periodes waarin een van beide typen uitzuigsystemen werd gebruikt bij alle patiënten in een unit, leek het meest geschikt om de onderzoeksvraag te beantwoorden; individuele randomisatie zou leiden tot een mix van patiënten waarbij bronchiaal toilet zou worden uitgevoerd met CSS en OSS, waarbij de voordelen van CSS teniet zouden kunnen worden gedaan door kruisbesmetting via een naastliggende patiënt die gerandomiseerd zou zijn naar OSS. De resultaten van deze studie lieten zien dat routinematig gebruik van CSS niet leidde tot een reductie in kruisbesmetting noch in het verkrijgen van kolonisatie met de meest relevante Gram-negatieve bacteriën. Vijftig procent van alle patiënten in de studie waren gekoloniseerd met tenminste een van de geselecteerde bacteriën, bij opname of verkregen tijdens het verblijf op de IC. In elke studie periode raakte 37% van de patiënten gekoloniseerd in de luchtwegen met tenminste een van de geselecteerde Gram-negatieve bacteriën (patiënten die voor IC opname niet gekoloniseerd waren). Berekend over het aantal dagen waarop patiënten nog niet gekoloniseerd waren was de verhouding 35,5 en 32,5 acquisities per 1000 dagen waarop patiënten risico liepen, gedurende respectievelijk CSS en OSS. Van drie bacteriën, te weten *P. aeruginosa*, *Acinetobacter* species en *Enterobacter* species, is vervolgens bepaald of er sprake was van kruisbesmetting, gebaseerd op genotypering en op gegevens over het verblijf op de intensive care. Kruisbesmetting met tenminste een van de drie bacteriën was laag: 5,7 en 4,5 kruisbesmettingen per 1000 risico-patiëntdagen gedurende respectievelijk CSS en OSS, als de strengste definitie voor een dergelijke situatie werd toegepast.

De studie was pragmatisch van opzet, wat betekende dat de dagelijkse klinische praktijk zoveel mogelijk in stand werd gehouden. De crossover onderzoeksopzet werd gebruikt om rekening te kunnen houden met verschillen tussen de deelnemende afdelingen, die elk hun eigen type

gesloten en open uitzuigsysteem gebruikten. In het academische ziekenhuis werd een swivel connector gebruikt in combinatie met OSS, waardoor volledig loskoppelen niet nodig was en via een dopje een kleine opening werd ontsloten waar de katheter doorheen werd gevoerd. In het opleidingsziekenhuis werden patiënten losgekoppeld van de beademingsmachine voor het uitvoeren van bronchiaal toilet. Voor het uitvoeren van bronchiaal toilet met een gesloten systeem werden een 24-uurs en een 72 uurs systeem gebruikt (van dezelfde fabrikant) en vervangen volgens de gestelde tijdsaanduiding.
Het niet opvolgen van het studie protocol, oftewel het niet gebruiken van het toegewezen uitzuigsysteem, zou de voordelen van CSS ten aanzien van kolonisatie kunnen maskeren. Het naleven van het protocol is echter uitgebreid gecontroleerd, en slechts in 7 procent van de beademingsdagen werd een ander systeem gebruikt dan was voorgeschreven (5% gedurende CSS en 9% gedurende OSS). Dit kwam bijvoorbeeld doordat een patiënt in buikligging werd verzorgd (reden om het gesloten systeem te gebruiken) of omdat de patiënt aan het ontwennen was van de beademing (reden om het open systeem te gebruiken). Een ander aspect dat de uitkomst zou kunnen vertekenen was loskoppelen van CSS, bijvoorbeeld voor geplande vervanging van het systeem, per ongeluk of om extra (open) bronchiaal toilet uit te voeren. Het loskoppelen gedurende CSS werd geregistreerd en vond plaats met een mediaan van eenmaal per dag.
Andere onderzoekers hebben geëvalueerd wat het effect is van minder frequent vervangen van CSS (in vergelijking tot iedere 24 uur vervangen of in vergelijking tot OSS). Deze studies hebben echter niet de mate van kruisbesmetting onderzocht en daarom blijft het onbekend of langer gebruik van CSS geassocieerd zal zijn met een reductie van de mate van kruisbesmetting in intensive care units.
Maatregelen ter preventie van infecties, zoals het dragen van beschermende kleding en het wassen van handen, zijn belangrijk om kruisbesmetting te voorkomen. Tijdens onze studie is daarom het opvolgen van hygiënische maatregelen tijdens CSS en OSS geobserveerd, zonder dat verpleegkundigen zich hiervan bewust waren. De mate waarin handhygiëne werd toegepast *na* gebruik van CSS en OSS was vergelijkbaar, maar OSS was geassocieerd met beter toepassen van handhygiëne *voor* bronchiaal toilet. Tevens bleken handschoenen, bril en masker vaker te worden gebruikt tijdens OSS. Dit kan suggereren dat, door minder toepassen van hygiënische maatregelen tijdens CSS, de voordelen van CSS op kruisbesmetting worden verhuld. Het effect op kruisbesmetting kan echter niet los gezien worden van de gebruikte studie opzet. Natuurlijk is het mogelijk om hygiënische aspecten in de interventie te betrekken, maar dan hadden we geen antwoord kunnen geven op de vraag in hoeverre de implementatie van CSS in de dagelijkse praktijk op een unit, zonder aanpassing van andere variabelen, tot minder kruisbesmetting zou leiden.
Verder is het mogelijk dat onze studie niet groot genoeg was om een verschil tussen beide systemen te kunnen aantonen. Tijdens de voorbereidingen van de studie hebben we berekend dat er 250 patiënten per studie arm nodig waren om 10% risico reductie op kruisbesmetting aan te tonen (van 25% naar 15%). Omdat beide ziekenhuizen met twee in plaats van de

afgesproken enkele unit deelnamen, hebben we uiteindelijk meer dan het dubbele aantal patiënten geïncludeerd (totaal 1110 patiënten). Het risico (de *Hazard Ratio)* op het verkrijgen van kolonisatie met tenminste een van de geselecteerde bacteriën tijdens IC opname gedurende CSS was 1.14 (95% betrouwbaarheidsinterval 0,91 – 1,42). Het is daarom erg onwaarschijnlijk dat een grotere steekproef een klinisch relevant verschil zou hebben aangetoond tussen de twee systemen.

De fysiologische consequenties van CSS en OSS (bijv. verstoring van hartfunctie en oxygenatie) werden bestudeerd in een observationele studie (beschreven in hoofdstuk 4). In deze pragmatische studie werd bronchiaal toilet met een van beide systemen bestudeerd zoals het werd uitgevoerd tijdens de standaard verzorging, bijv. als het klinisch nodig was. De handeling werd uitgevoerd volgens het ziekenhuis protocol. Veranderingen in hartslag, gemiddelde arteriële druk en perifere zuurstof saturatie werden geobserveerd voor en na bronchiaal toilet met CSS of OSS. Deze veranderingen bleken na gebruik van beide systemen zeer gering en de resultaten waren vergelijkbaar. Slechts de gemiddelde perifere zuurstof saturatie leek hoger nadat OSS was gebruikt in vergelijking tot CSS, maar de verschillen waren erg gering (98,2% en 97,5%) en worden daarom klinisch niet relevant bevonden.
Tijdens de studie werden verschillen in het uitvoeren van bronchiaal toilet gezien, zowel tussen de ziekenhuizen als in vergelijking tot internationale richtlijnen. Deze laatsten doen de aanbeveling om pre-oxygenatie en post-oxygenatie toe te passen (om desaturatie te beperken) en om een zuigkracht van minder dan 20 kPa te gebruiken. Pre- en postoxygenatie werden echter slechts uitgevoerd in respectievelijk 24% en 18% van de geobserveerde bronchiaal toilet procedures, en beide ziekenhuizen gebruikten verschillende zuigkracht (respectievelijk 30 en 20 kPa). Verder bleken gedurende de OSS-studieperiode sommige intensivisten de voorkeur te geven aan CSS bij patiënten met PEEP-waarden van 10 of meer. Dit droeg bij aan het verschil in PEEP tussen beide procedures bij aanvang van de observatie (baseline). De variatie in de uitvoer van bronchiaal toilet liet echter geen verschil zien tussen CSS en OSS.
In het kader van patiëntveiligheid is het echter belangrijk om de grenzen te bepalen waarbinnen bronchiaal toilet veilig uitgevoerd kan worden, terwijl de oxygenatie verbetert en sputum effectief wordt verwijderd.

Op basis van de resultaten van de crossover en de observationele studie is geconcludeerd dat zowel CSS als OSS even veilig zijn om te gebruiken bij beademde intensive care patiënten. We hebben geen verschil in algehele kruisbesmetting kunnen aantonen, noch in algeheel verwerven van kolonisatie van de luchtwegen met Gram-negatieve bacteriën bij deze patiënten. Verder zijn geen klinisch relevante verschillen in hartslag, gemiddelde arteriële druk of saturatie gevonden na bronchiaal toilet. Tot slot hebben we geen kenmerken in de patiëntenpopulatie of verpleegkundige praktijk gevonden die onze resultaten niet generaliseerbaar zouden maken naar andere intensive care units.

Zonder een verschil in effectiviteit wordt een kosten-effectiviteitsanalyse niet relevant; er kan slechts een vergelijking van de kosten worden gemaakt. OSS is goedkoper dan CSS: de prijs van een open uitzuigcatheter is € 0,38 en van een eventuele swivel € 2,70, terwijl de prijs van een 24-uurs gesloten systeem € 11,20 bedraagt (Nederlands prijsniveau 2009). Voor een ziekenhuis met meer dan 10.000 beademingsdagen per jaar (zoals het UMC Utrecht), en gebaseerd op een gemiddelde frequentie van 6 maal bronchiaal toilet per dag per patiënt, zou dit meer dan €66.000 per jaar besparen als gebruik wordt gemaakt van OSS met swivel tussenstuk. Zonder dit tussenstuk zal de besparing alleen maar groter zijn. Het langer gebruiken van CSS, van de aanbevolen 24 uur tot verscheidene dagen of slechts indien nodig (als de katheter defect of zichtbaar verontreinigd is), zal de kosten van CSS reduceren en tevens het prijsverschil tussen beide systemen geringer maken.

De betekenis van de resultaten voor de klinische praktijk is vrij eenduidig: de keuze van systeem om bronchiaal toilet uit te voeren kan worden gebaseerd op de feitelijke kosten en op persoonlijke voorkeur. In ons studie protocol hadden we een uitzondering gemaakt voor patiënten die in buikligging verzorgd werden; bij deze patiënten werd altijd CSS gebruikt, omdat loskoppelen (nodig voor OSS) de handeling moeilijk uit te voeren en daarmee onveilig kan maken.
Gedurende de studie bleek dat de meeste verpleegkundigen de voorkeur gaven aan CSS, vanwege de veronderstelde geringere blootstelling aan sputum van de patiënt en het gebruiksgemak (het systeem is klaar voor gebruik). Andere verpleegkundigen gaven de voorkeur aan OSS vanwege een beter "gevoel" tijdens de interventie en beter verwijderen van sputum. Ondanks deze meningen hebben we geen verschil in aantal malen bronchiaal toilet vastgesteld tijdens CSS en OSS (met beide systemen gemiddeld 6 maal per dag). Ter bescherming tegen sputum kan preventief materiaal gebruikt worden, zoals maskers en bril. Dit bleek zowel tijdens CSS als OSS slechts incidenteel te worden gebruikt.
Tot slot hebben we niet onderzocht in hoeverre meer sputum werd verwijderd na gebruik van CSS of OSS, noch hebben we besmetting bij medewerkers (met name verpleegkundigen) vastgesteld. Dit laatste is nauw verwant met de mate waarin medewerkers hygiënische maatregelen uitvoeren. Compliance met handhygiëne is veelal laag en komt zelden boven de 50% uit, wat ook in onze studie is vastgesteld (hoofdstuk 3). Verdere verbetering van de mate waarin medewerkers zich houden aan de maatregelen ter preventie van infecties blijft daarom belangrijk.

Kolonisatie van de luchtwegen

In het tweede deel van dit proefschrift ligt de focus op kolonisatie van de luchtwegen van IC patiënten met Gram-negatieve bacteriën. Gedurende 14 maanden zijn elke maandag en donderdag sputum- of keelkweken afgenomen. De microbiologische resultaten van deze

kweken maakten een gedetailleerde analyse van risicofactoren op het verwerven van kolonisatie van de luchtwegen mogelijk (beschreven in hoofdstuk 5). Van de 481 patiënten die niet gekoloniseerd waren met Gram-negatieve bacteriën bij opname op de intensive care unit, verkreeg 52% kolonisatie tijdens het verblijf op de IC. Risico van verkrijgen van kolonisatie was sterk geassocieerd met beademing en was lager in het academisch ziekenhuis (vergeleken met het opleidingsziekenhuis) en bij patiënten die antibiotica voorgeschreven hadden gekregen tijdens het verblijf op de IC. De analyse van risicofactoren is tevens uitgevoerd voor de afzonderlijke bacteriën. CSS bleek geassocieerd met een lager risico op het verkrijgen van kolonisatie met *Pseudomonas aeruginosa*, maar met een hoger risico op het verkrijgen van *Klebsiella* species. Antibiotica tijdens opname op de IC bleek geassocieerd met een lager risico op het verkrijgen van kolonisatie met *Enterobacter* species en met *Escherichia coli*.
Een beperking van deze studie betrof het uitsluiten van Gram-positieve bacteriën. Verder werd kolonisatie in plaats van daadwerkelijk infectie vastgesteld. Luchtweginfecties worden echter vrijwel altijd voorafgegaan door kolonisatie van de luchtwegen. Daarom kunnen risicofactoren voor kolonisatie beschouwd worden als risicofactoren voor opvolgende infecties zoals beademingspneumonie.

Van patiënten die gekoloniseerd waren met *Pseudomonas aeruginosa* of *Enterobacter* species is een fenotypische verandering van gevoelig naar resistent voor tenminste een antibioticum bepaald (beschreven in hoofdstuk 6). Bij 41 van de 126 patiënten die gekoloniseerd waren met *P. aeruginosa* trad een verandering in fenotype op van gevoelig naar resistent voor een of meer antibiotica. Bij patiënten die gekoloniseerd waren met *Enterobacter* species betrof het 46 van de 108 patiënten. Blootstelling aan meropenem was geassocieerd met het hoogste risico op resistentie ontwikkeling bij patiënten die gekoloniseerd waren met *P. aeruginosa*. Bij 108 patienten die gekoloniseerd waren met meropenem gevoelige *Enterobacter* species trad geen enkele keer het verkrijgen van meropenem resistentie op, hoewel blootstelling aan meropenem wel hoger was bij deze patiënten (24,7 en 14,4 defined daily doses per 100 patiëntdagen voor patiënten gekoloniseerd met respectievelijk *Enterobacter* en *P. aeruginosa*). Dit suggereert dat carbapenems een groter risico vormen op het ontwikkelen van antibiotica resistentie in *P. aeruginosa* dan andere betalactam antibiotica en fluorquinolonen.
Een beperking van deze studie is dat de ontwikkeling van resistentie voor meerdere antibiotica niet is bepaald. Gecombineerde resistentie bij *P. aeruginosa* kwam voor bij de helft van de verkregen meropenem resistenties en de helft van de verkregen ceftazidime resistenties. Percentages van gecombineerde resistentie voor verkregen ciprofloxacin en piperaciline-tazobactam resistenties waren zelfs hoger: 80% tot 90%. Multipele resistenties konden echter niet worden geïncludeerd in tijd-afhankelijke analyses, aangezien de ontwikkeling van resistentie voor meerdere antibiotica niet altijd gelijktijdig optrad. Bovendien was het aantal gecombineerde ontwikkelingen van resistentie te laag voor statistische analyse.

Om meer inhoudelijke kennis over de ecologie van *P. aeruginosa* te krijgen hebben we de populatie structuur van *P. aeruginosa* bij intensive care patiënten vergeleken met de populatie structuur van patiënten met cystic fibrosis (hoofdstuk 7). Stammen werden gegenotypeerd met een vereenvoudigd *Multiple-Locus Variable-number tandem repeats Analysis* schema. De populatie structuur van *P. aeruginosa* isolaten bleek zeer divers en populatie specifiek, wat inhoud dat types die bij patiënten op de IC gevonden werden genetisch anders waren dan de types die specifiek zijn voor patiënten met cystic fibrosis. Zowel patiëntenpopulatie als geografische oorsprong bleken gecorreleerd met de prevalentie van bepaalde genotypes. Vergelijking van *P. aeruginosa* stammen tussen intensive care units, ziekenhuis afdelingen en patiënten met cystic fybrosis was voorheen slechts beperkt gedocumenteerd.

Het laatste deel van dit proefschrift was gericht op een andere interventie gericht op het reduceren van kolonisatie van de luchtwegen: selectieve decontaminatie van het digestieve stelsel (SDD) en selectieve oropharyngeale decontaminatie (SOD). Gebruik van SDD en SOD is al jaren controversieel en intensivisten, medisch microbiologen en verpleegkundigen hielden er verschillende meningen op na betreffende de effectiviteit van SDD. Ingebed in een grote cluster-gerandomiseerde crossover studie zijn we nagegaan in hoeverre verwachtingen van de effectiviteit van SDD bij IC verpleegkundigen en intensivisten veranderden gedurende de studie. Hiertoe zijn na afloop van iedere studieperiode (SDD, SOD of standaard zorg) vragenlijsten verstuurd (beschreven in hoofdstuk 8). De verwachting dat SDD effectief is, met name in het voorkomen van pneumonie, nam toe gedurende de studie, zowel bij intensivisten als IC verpleegkundigen, onafhankelijk van de studie-volgorde (volgorde van periodes waarin SDD, SOD en standaard zorg werd toegepast) en zonder kennis van de studie resultaten. Daarnaast werd verondersteld dat SDD een hogere werklast gaf en minder patiëntvriendelijk was dan standaard zorg.

Om de kwaliteit en veiligheid van patiëntenzorg op intensive care afdelingen te verbeteren is het belangrijk om evidence based en kosten-effectieve interventies in te voeren. Uitgebreide en correcte invoering van deze interventies hangt echter in belangrijke mate af van de motivatie en acceptatie van medewerkers die deze procedures uit moeten voeren. De interventies die in dit proefschrift bestudeerd zijn, het routinematig gebruik van gesloten uitzuigsystemen en het gebruik van SDD en SOD, zijn controversieel sinds de invoering. Het gebruik van deze interventies is veelal gebaseerd op veronderstelde voordelen. De verantwoording voor het gebruik van interventies zou duidelijk uitgelegd moeten worden via heldere, terugkerende educatie en instructie, met nadruk op het effect op patiënten uitkomsten en met aandacht voor de wetenschappelijke basis van alomtegenwoordige veronderstellingen.

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(Acknowledgements)

Dankwoord

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[1] www.runnersweb.nl

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Halverwege was het onderzoek afgelopen en startte het completeren van de gegevens en de analyse van de resultaten. Het traject werd iets zwaarder. Ondanks het vroege tijdstip van de XEWMM stonden collega onderzoekers me bij met (epidemiologische) raad en daad. Marieke, Heidi, Marianne, Evelien, Suzanne, Lennie, dank voor alle adviezen! En ook Martin Bootsma en Carline van den Dool dank dat ik jullie lastig mocht vallen met uiteenlopende vragen.
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Een marathon wordt ook wel gezien als een duurloop over 30 km en een gevecht over 12 km[2]. Welk niveau je ook hebt, aan het eind komt bijna iedereen in de problemen. In deze virtuele marathon kwam de befaamde man met de hamer langs, die in mijn geval figuurlijk op mijn externe harde schijf sloeg. Ik kon niet meer bij mijn gegevens, waaronder bijna twee maanden analyses waarvan (beetje dom) geen backup was gemaakt. Luuk van Zutphen en Bruno

[2] Rosenmöller P. Ik loop dus ik besta. 2006 Prometheus Amsterdam

Thierbach, jullie afzonderlijke pogingen om toch nog mijn bestanden te redden mochten helaas niet baten. Maar jullie hulp heeft me goed gedaan.
In deze fase kostte mijn marathon veel tijd en uithoudingsvermogen, maar het lukte om 'op de route' te blijven en het manuscript af te ronden. Geachte leden van de beoordelingscommissie, prof. dr. M. Schuurmans, dr. J. Mintjes, prof. dr. P. Pickkers, prof. dr. A. Voss, prof. dr. W. Buhre. Het is me een eer en genoegen dat u het manuscript heeft willen beoordelen, dank voor de kritische blik en de tijd en moeite die u hiervoor heeft genomen.

Zowel in de voorbereiding als de uitvoering van de marathon zijn momenten van rust en ontspanning en afleiding belangrijk. Marianne, Fieke, Stefan, Saskia, Ilja en alle anderen van de voormalige vrouwenvleugel, we hebben diverse life events meegemaakt met elkaar, wat een stevige band heeft gecreëerd. Ik heb ontzettend veel geleerd van jullie openheid en vertrouwen, van alle serieuze en heftige momenten tot de ontspannen en humorvolle momenten. Tijdens koffie en lunch waren vooral de niet-serieuze, niet-werkgerelateerde zaken onderwerp van gesprek. Met veel plezier hebben we menig promotie of afscheid voorbereid en gevierd, en zou nu eigenlijk graag aanschuiven om herinneringen van de afgelopen jaren op te halen. Lieve Jacqueline, overbuuv bij de IC, vanuit de vrouwenvleugel kwam ik bij jou op de kamer terecht. Twee interne verhuizingen later is ons meubilair en uitzicht iets veranderd, maar wij nog niet. Ondanks onze uiteenlopende werkzaamheden zien we vooral ook raakvlakken, en ben je altijd bereid om met me mee te denken of om ergens een kritische blik op te werpen. Dank hiervoor en voor alle (niet werk gerelateerde) gezelligheid.

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De finish is in zicht, vertrouwde gezichten wachten me op. Lieve Bernadette, op gezette tijden dook je gezicht op langs de route. Je hebt de diverse "life events" met me meegemaakt, en ook dit moment past in het rijtje. Dank dat je me weer wilt bijstaan en je vaardigheden op het gebied van mental coaching op me los wilt laten.
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dat vaak was. Lieve Saadet, schone zus. Tijdens jullie Koerdische bruiloft begrepen we elkaar met gebaren, en daar is al snel de Nederlandse taal bijgekomen (helaas, ik ben zwaar achtergebleven in mijn Turks/Koerdisch). Ik heb van jou een prachtige dichtbundel, welke voor mij helaas onleesbaar is. Bij deze geef ik jou een "onleesbaar boek".

Tot slot m'n mannen. Lieve Mike, Joram en Milan. Jullie waren er overal tijdens het traject, en ik zie jullie gezichten bij de finish. Lieve Mike, mijn steun en toeverlaat. Je vroeg met de jou kenmerkende nuchterheid bij aanvang van het traject of het wel leuk was en hebt vervolgens alle hoogte- en dieptepunten ruimschoots meegekregen. En ondanks dat het voor jou niet altijd te volgen was, heb je me alle ruimte gegeven om dit project af te ronden en mijn 'marathon' te volbrengen. Als ik de finish ben gepasseerd, hoop ik meer tijd en rust te vinden voor ontspanning en voor de leuke(re) dingen in het leven.
Lieve Joram en Milan, jullie vonden het wel "cool" dat ik een boekje ging maken en dat dit jullie een vrije dag oplevert. Jullie leefden op je eigen manier met me mee. Joram, jij ontspande me met prachtige pianomuziek en verraste me met een mooie collage van tekeningen. Milan, jij keurde met veel plezier mijn promotiekleding en zocht de accessoires uit. Het is voor jullie een gebruikelijk beeld geworden dat ik achter m'n laptop zit, hetzelfde apparaat dat afgelopen maanden verboden terrein was voor jullie. Maar beloofd is beloofd: na 24 mei mogen jullie de laptop gebruiken om samen je computerspel te kunnen spelen.

Irene

Curriculum Vitae

Curriculum Vitae

Irene Paulien Jongerden was born on November 21, 1966 in Gouda and grew up in Boskoop, the Netherlands. After graduating from secondary school (Atheneum, Samenwerkingsschool voor HAVO / Atheneum in Waddinxveen), she started nursing school at the Leidse Hogeschool in Leiden, which she succesfully finished in 1989. From 1990 to 1993 she studied Nursing Science at the Faculty of Health Science, Maastricht University. She obtained her Master of Science degree in 1993. During that study, her interest in quality of health care and of nursing grew stronger, and she put this into practice in the Academic Medical Center in Amsterdam until 1999. In that year, she grabbed the opportunity to focus more on scientific research in the UMC Utrecht, Wilhelmina Children's Hospital. Under supervision of prof. dr. M. Grypdonck she started a study on compliance with contact isolation protocols, for which she received the Infection Prevention Award in 2001. In September 2002 she moved over to the department of Infectious Diseases with the assignment "to start research on infection prevention in the intensive care". Under supervision of prof. dr. M.J.M. Bonten the main objective of the project became to compare open and closed endotracheal suction systems, for which they received a grant from ZonMw in 2006. The project has resulted in the studies presented in this thesis. Currently she continues her research activities in the Department of Vital Functions.
Irene Jongerden is married with Mike Overdevest and they have two sons, Joram (1997) and Milan (2000).