Ventilator-associated pneumonia: studies on pathogenesis, diagnosis and prevention

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**Ventilator-associated pneumonia:
studies on pathogenesis,
diagnosis and prevention**

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Ventilator-associated pneumonia: studies on pathogenesis, diagnosis and prevention

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Universiteit Maastricht,
op gezag van de Rector Magnificus,
Prof Dr AC Nieuwenhuijzen Kruseman,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op vrijdag 5 februari 1999 om 14.00 uur

door

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Aan mijn moeder

Ter herinnering aan mijn vader

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1

Introduction

Introduction

The present thesis deals with ventilator-associated pneumonia (VAP) which is a frequent complication in mechanically ventilated patients admitted to intensive care. The incidence rates of VAP may vary due to factors like patient population, type of intensive care unit (ICU) and criteria used to diagnose this infection. Ventilator-associated pneumonia is preceded by colonization of the upper respiratory tract, and these pathogens are derived from endogenous or exogenous sources. Several routes of colonization by which these pathogens are transported from their source to the upper respiratory tract have been hypothesized and studied. The epidemiology, routes of colonization and their role in the pathogenesis of VAP will be described in **chapter 2** of this thesis. Since prevention of VAP is an important goal for intensive care medicine, the results from studies which evaluate the strategies currently in use or suggested for the prevention of VAP are critically reviewed as well and compared with the recommendations for prevention of hospital-acquired or nosocomial pneumonia as formulated by the American Thoracic Society and the Centers for Disease Control and Prevention.

Critically ill patients frequently have an infection on admission to ICU and are prone to develop new infections. The majority of these infections involve the respiratory tract. As a corollary, the total antibiotic consumption is approximately 10 times greater in ICU than in general hospital wards. In addition to the associated costs, antibiotic consumption potentially causes selection and induction of antibiotic resistance. Surveillance studies on the incidence of infections, antibiotic prescription policies and knowledge about antimicrobial susceptibilities of causative pathogens are the first step in managing this problem. In **chapter 3** the results of a one-year prospective analysis of the incidence of infections, their etiologic pathogens, antibiotic use and related costs in our general ICU ward are described. Special emphasis is given to the indications for antibiotic use, which are divided into those related to bacteriologically proven and non-bacteriologically proven infections, and those related to prophylactic use. Another approach to anticipate on the high consumption of antibiotics is to optimize the criteria used for the diagnosis of infection. Ventilator-associated pneumonia is usually diagnosed on a combination of clinical, radiographic and microbiological criteria which have a high sensitivity, but low specificity for VAP. As a result, patients may receive antibiotics unnecessarily. Specificity can be increased by the addition of quantitative cultures of samples of protected specimen brush and bronchoalveolar lavage to the diagnostic criteria. In **chapter 4** a prospective study is described which evaluated whether adding the results of protected specimen brush and bronchoalveolar lavage to the generally used criteria for diagnosing VAP influenced antibiotic prescription by intensive care physicians. In addition, we analyzed some costs associated with diagnosis and antimicrobial therapy of VAP.

In the pathogenesis of VAP, gastric colonization with potentially pathogenic

microorganisms is assumed to be important. In this hypothesis, microorganisms initially colonize the stomach, subsequently colonize the upper respiratory tract, and finally are aspirated into the lungs. Gastric colonization may be stimulated by decreased gastric acidity, secondary to stress-ulcer prophylaxis or gastric nutrition. Several methods have been studied to reduce gastric colonization by attempting to maintain intragastric acidity. However, in several studies by others and previous research in our group, the use of sucralfate for stress-ulcer prophylaxis and modulation of enteral feeding failed to influence the incidence of infection, colonization of stomach and respiratory tract, and levels of intragastric acidity. Moreover, sequential analysis of colonization strongly suggested that the stomach was not an important source of bacteria causing VAP, and that the gastro-pulmonary route of colonization may not be as important in the pathogenesis of VAP as generally suggested.

Many risk factor analyses for the development of VAP in critically ill patients have been performed. Well known risk factors that have been identified are severity of illness, intubation, duration of mechanical ventilation and a history of chronic obstructive pulmonary disease. Unfortunately, preceding colonization at different body sites has not been included as a risk factor in any of these analyses. In order to further elucidate the pathogenesis of colonization and infection of the respiratory tract in mechanically ventilated patients, we studied risk factors for the development of colonization of stomach, oropharynx and trachea, and the development of VAP with enteric Gram-negative bacteria (EGB) and *Pseudomonadaceae*. The results of this multivariate analysis are described in **chapter 5**.

Early-onset and late-onset VAP each have a different etiologic spectrum. Early-onset VAP is mainly caused by *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae*, while late-onset VAP is caused by nosocomial pathogens such as *Enterobacteriaceae*, *S. aureus* and *Pseudomonas aeruginosa*. Together these nosocomial pathogens are responsible for the majority of serious infections among critically ill patients and, therefore, often grouped and labelled as potentially pathogenic microorganisms. Although potentially pathogenic microorganisms are regarded as a single group, it should be kept in mind that each species has its own characteristics with regard to preferred site of colonization, routes and vectors of transmission, and clinical spectrum. For instance, quite often, *S. aureus* is the cause of VAP. In contrast to community-acquired pneumonia caused by *S. aureus*, the clinical presentation of staphylococcal pneumonia in mechanically ventilated patients has not been described in great detail. In **chapter 6** the incidence of colonization of the upper respiratory tract by methicillin-sensitive *S. aureus* and its association with the development of VAP caused by this species is described. In addition, the clinical spectrum, microbiological results and outcome of patients with VAP caused by *S. aureus* are discussed.

In many other ICUs, as well as in ours, *P. aeruginosa* is the most prevalent microorganism causing VAP. A number of microbiological and clinical aspects of this pathogen are highlighted in **chapters 7, 8 and 9**. Colonization with *P. aeruginosa* may originate from exogenous or endogenous sources. The intestine, the oropharynx and the stomach could serve as endogenous sources, whilst the inanimate environment and other patients are potential exogenous sources. Although the sources and reservoirs of *P. aeruginosa* are largely known, the various routes of colonization that lead to VAP have not been established. Moreover, when studying routes of colonization with *P. aeruginosa*, determination of similarity of isolates is crucial, especially since colonization may occur with several different isolates, both at one particular body site and at different body sites. A comparison of phenotypes of isolates of *P. aeruginosa* is relatively easy to perform, but lacks specificity. Genotyping techniques have a higher specificity and discriminatory power and are considered the method of choice to compare bacterial isolates when studying nosocomial outbreaks. However, these techniques are laborious and expensive, and may not be feasible for all laboratories. In **chapter 7** the usefulness of four methods of phenotyping, as a method of initial screening for investigating colonization patterns of *P. aeruginosa*, were determined. The results of the phenotyping methods were compared with those of pulsed-field gel electrophoresis of enzyme restricted chromosomal DNA. The results of these phenotyping and genotyping methods were used to study routes of colonization with *P. aeruginosa* leading to VAP. Chronological analysis of colonization in the rectum, stomach, oropharynx and trachea, enables determination of initial sites of colonization and the spread of microorganisms to other body sites. In addition, the biotyping techniques can determine whether a patient is colonized with a single or multiple genotypes. These analyses are described in **chapter 8**. *Pseudomonas aeruginosa* may be derived from exogenous sources. Sinks, distilled water systems, faucets and ventilator equipment are among the many potential sources that have been reported to be the cause of nosocomial outbreaks of colonization or infection. Other patients colonized with *P. aeruginosa* present in the same ICU are also important exogenous sources. Transfer of these pathogens, from source to patient (cross-colonization), most probably occurs via hands of nursing and medical staff or contaminated equipment. Understanding the relative importance of this route of colonization, in epidemic and endemic situations, is crucial in order to develop effective preventive measures against infection. In **chapter 9** an analysis of colonization and infection with *P. aeruginosa* following the clinical observation of three episodes of VAP due to *P. aeruginosa* in a short period of time was performed. Since these infections were assumed to be the result of cross-colonization of microorganisms, the importance of this route of colonization for *P. aeruginosa* was determined using pulsed-field gel electrophoresis for analysis of similarity of isolates.

As stated before, the bacteria causing VAP may also be transmitted from

endogenous sources. Since the gastropulmonary route of colonization has been considered to be important in the pathogenesis of late-onset VAP, modulation of gastric colonization has been attempted as a measure to prevent VAP. However, none of these interventions have proven unequivocally to reduce the incidence of VAP. Furthermore, sequential analyses of colonization failed to demonstrate an important role of the gastropulmonary route. Selective decontamination of the digestive tract (SDD) decreases the incidence of VAP. The concept of SDD aims to eradicate microorganisms from the intestine, the stomach and the oropharynx by nonabsorbable antibiotics, which are combined with systemic antibiotic prophylaxis during the first days of ICU admission. Resistance concerns, lack of cost-benefit analyses and absence of beneficial effects on mortality rates have limited the widespread use of SDD. From a conceptual point of view, it has remained unclear which part or parts of SDD prevents VAP. Since the importance of gastric and intestinal colonization has been questioned, and systemic antibiotics during the first days of intubation appear to prevent early-onset but not late-onset VAP, we hypothesized that decontamination of the oropharynx, without modulating gastric and intestinal colonization, and without systemic antibiotic prophylaxis, would reduce the incidence of VAP in critically ill intensive care patients. In **chapter 10**, the last study of the thesis, this hypothesis was tested in a prospective, randomized, double-blind, placebo-controlled study in 226 patients receiving either topical antimicrobial prophylaxis in the oropharynx or placebo.

In summary, the main questions addressed in this thesis are:

1. What is the incidence of infections in critically ill patients admitted in our ICU? **Chapter 3.**
2. What are the indications for antibiotic use and the related costs in a general ICU ward? **Chapter 3.**
3. Does addition of quantitative culture results of samples obtained by bronchoscopic techniques to the generally used criteria for diagnosing VAP influence antibiotic prescription? **Chapter 4.**
4. What are the risk factors for the development of colonization of stomach, oropharynx, and trachea with EGB and *Pseudomonadaceae*? **Chapter 5.**
5. What are the risk factors for the development of VAP caused by EGB and *Pseudomonadaceae*? **Chapter 5.**
6. Does the clinical spectrum and outcome of staphylococcal VAP differ from that of staphylococcal community-acquired pneumonia? **Chapter 6.**
7. Can phenotyping techniques be used as an initial method of screening for *P. aeruginosa* in order to reduce the number of isolates that need to be genotyped? **Chapter 7.**
8. Is the respiratory tract the most important initial site of colonization with *P. aeruginosa* or is colonization with this pathogen not restricted to the respiratory tract? **Chapter 8.**
9. Are patients admitted to ICU colonized with a single or with multiple genotypes of *P. aeruginosa*? **Chapter 8.**

10. Does cross-colonization influence the rates of colonization and infection with *P. aeruginosa* in an ICU where colonization with this species is endemic? **Chapter 9.**
11. Can VAP be prevented by modulation of oropharyngeal colonization, without influencing gastric and intestinal colonization and without systemic prophylaxis? **Chapter 10.**

2

The pathogenesis and prevention of ventilator- associated pneumonia: A review

Parts of this review will be published in chapters in the Yearbook of Intensive Care and Emergency Medicine, 1999 (Vincent JL, editor) and Hospital Epidemiology and Infection Control, 2nd ed. (Mayhall CG, editor)

Introduction

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection among mechanically ventilated patients¹, and has been associated with increased morbidity, longer hospital stay, increased health care costs and higher mortality rates^{2,3}. Prevention of this infection is, therefore, a major challenge for intensive care medicine. Pneumonia is defined as VAP when diagnosed in an intubated, mechanically ventilated patient after more than 48 h of ventilation. On the basis of time of diagnosis, two types of VAP are distinguished: early-onset VAP, occurring within the first 4 d of mechanical ventilation, and late-onset VAP, occurring thereafter⁴.

Incidence

Incidence rates of VAP among intensive care unit (ICU) patients depend on the type of ICU, the severity of illness of patients studied and the criteria for diagnosis. Several methods are used to diagnose pneumonia in clinical and research situations. Often, one out of three combinations of criteria is used in clinical studies; the modified criteria of the Centers for Disease Control and Prevention (CDC)⁵, the Clinical Pulmonary Infection Score (CPIS)⁶, or the criteria of the Memphis Consensus Conference⁷. The first two methods include only clinical criteria and chest radiograph results (Table 2.1). The Memphis criteria include results of quantitative cultures of samples of secretions from the lower respiratory tract obtained by protected specimen brush, bronchoalveolar lavage or protected bronchoalveolar lavage in addition to the clinical and radiographic criteria (Table 2.1). In a recent study, all three methods were used to assess the incidence of VAP, overall incidences were 21.7% for CDC, 19.9% for CPIS and 9.3% probable and 0.4% definite pneumonia as defined by the Memphis criteria⁸. Obviously, the overall incidence of pneumonia decreases when the definition of pneumonia becomes more strict. Therefore, whether investigators used bronchoscopic techniques in their diagnosis of VAP or just clinical and radiographic parameters is important with regard to incidence rates of VAP. At present, in the absence of a 'gold standard' for the diagnosis of VAP, the combination of clinical parameters, chest radiograph and quantitative cultures of protected specimen brush and/or bronchoalveolar lavage obtained by bronchoscopy appears to have highest sensitivity and specificity^{7,9,10}. In a number of studies aiming to ascertain incidences of VAP, or to evaluate modalities to diagnose VAP, or studies in which risk factors for VAP were assessed, the incidences of VAP range from 8.6 to 64.7% (Table 2.2). Moreover, in studies on the effect of preventive measures on the occurrence of VAP, incidences of up to 78% have been reported in the control groups²⁸.

Table 2.1 Diagnostic criteria of pneumonia used in clinical studies

Criteria	CDC*	CPIS†	MCC‡	
			probable pneumonia	definite pneumonia
Rales or dullness to percussion on physical examination	+I	-	-	-
Radiographic infiltrate (new, progressive, persistent)	+II	+	+	+
Temperature	-	+	(+)	(+)
Blood leukocytosis/leukopenia	-	+	(+)	(+)
Purulent sputum	+ ^{a,b}	+	+	+
On Gram's stain >25 granulocytes and <10 squamous epithelial cells§	-	-	-	-
Positive culture of transtracheal aspirate (or bronchial brushing, biopsy)	+ ^{a,b}	+	+	-
Number of tracheal secretions	-	+	-	-
Oxygenation (PaO ₂ /FIO ₂)	-	+	(+)	(+)
Quantitative culture of PSB or BAL	-	-	+ ^c	-
Positive blood culture	+ ^{a,b}	-	+ ^c	-
Pleural fluid culture	-	-	+ ^c	-
Isolation of virus or detection of virus antigen in respiratory secretions	+ ^b	-	-	-
Diagnostic single antibody titer (IgM) or fourfold increase in paired serum samples (IgG) for pathogen	+ ^b	-	-	-
Radiographic pulmonary abscess and positive needle aspirate culture	-	-	-	+ ^d
Open-lung biopsy or post-mortem lung histologic examination and negative lung parenchyma culture (<10 ⁴ cfu/g)	-	-	+ ^c	-
Open-lung biopsy or post-mortem lung histologic examination and positive lung parenchyma culture (>10 ⁴ cfu/g)	+ ^b	-	-	+ ^d

PSB, protected specimen brush; BAL, bronchoalveolar lavage.

* CDC, modified criteria of the Centers for Disease Control and Prevention⁶. Pneumonia must meet the criterium I and any of the criteria ^a, or criterium II and any of the criteria ^b.

† CPIS, Clinical Pulmonary Infection Score (CPIS)⁶. Scoring system with 1 or 2 points awarded for presence of criteria, range 0-12, with pneumonia defined by a score of 7 or higher. Additional point score for purulent sputum when number of tracheal secretions is ≥ 14 .

‡ MCC, Memphis Consensus Conference⁷. The criteria denoted with (+) are not necessary for the diagnosis but enhance the clinical suspicion of pneumonia. Pneumonia must meet the criterium clinical suspicion of pneumonia and any of the criteria ^c for probable and any of the criteria ^d for definite pneumonia.

§ The number of granulocytes and squamous epithelial cells per low-power field on microscopic examination of the Gram stain is used in some studies to confirm the macroscopic diagnosis of purulent sputum.

Table 2.2 Studies on the incidence of VAP in various patient populations using different diagnostic criteria

Author	Population	No	Diagnosis*	No VAP (%)
Apte ¹¹	Medical	34	R/CI/Sp/TA/M/P	22 (64.7)
Kingston ¹²	Mixed	24	R/CI/Sp/TA/M	10 (41.7)
Craven ¹³	Mixed	233	R/CI/Sp/TA/M	49 (21.0)
Reusser ¹⁴	Neurologic	40	R/CI/TA/M	15 (37.5)
Rodriguez ¹⁵	Trauma	294	R/CI/TA/M	130 (44.2)
Rello ¹⁶	Trauma	161	R/CI/Sp	38 (23.6)
de Latorre ¹⁷	Med/Surg	80	R/CI/Sp	12 (15.0)
Fiddian-Green ¹⁸	Mixed	62	Clinical†	8 (12.9)
Vincent ¹	Mixed	10038	CDC	967 (46.9)
Beck-Sague ¹⁹	Surgical	145	CDC	15 (10.3)
Fagon ²⁰	Mixed	567	R/Sp/PSB	49 (8.6)
Rello ²¹	Med/Surg	264	R/CI/Sp/PSB	58 (21.9)
Papazian ²²	Med/Surg	586	R/CI/Sp/PSB	97 (16.6)
Garrouste-Orgeas ²³	Med/Surg	86	R/CI/Sp/PSB	31 (35.6)
Fagon ²⁴	Mixed	1978	R/Sp/PSB/BAL	328 (16.6)
Torres ²⁵	Mixed	322	MCC	78 (24.0)
Bonten ²⁶	Mixed	64	MCC	11 (17.2)
Kollef ²⁷	Mixed	521	MCC‡	77 (14.8)

Med, Medical; CDC, modified criteria of the Centers for Disease Control and Prevention⁵ (see table 2.1); Surg, Surgical; MCC, criteria from the Memphis Consensus Conference⁷ (see table 2.1).

* The abbreviations used in the description of the diagnostic criteria are: R, new, progressive or persistent radiographic infiltrate; CI, clinical parameters (fever, leukocytosis or leukopenia, oxygenation); Sp, purulent sputum; TA, positive culture of tracheal aspirate; M, microscopic assessment of Gram stain (number of granulocytes and squamous epithelial cells); P, rales or dullness to percussion on physical examination; PSB, protected specimen brush; BAL, bronchoalveolar lavage.

† Endpoint of analysis in this study was: 'a clinical diagnosis of nosocomial pneumonia'. No specific criteria were described.

‡ Bronchoscopy with PSB and/or BAL was not performed in all patients with a clinical suspicion of pneumonia.

Etiology

Besides the difference in duration of mechanical ventilation at time of diagnosis, early-onset and late-onset VAP also have a different etiologic spectrum. Early-onset VAP is mainly caused by *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae*, pathogens that presumably already colonize the respiratory tract at the time of intubation. Late-onset VAP is caused by nosocomial pathogens such as Enterobacteriaceae, *S. aureus* and *Pseudomonas aeruginosa*. Because these nosocomial pathogens are known to cause serious infections under certain circumstances they are usually grouped and labelled as potentially pathogenic microorganisms (PPMO). In many studies, colonization and infection with PPMO is analyzed instead of analysis of the separate species. Although PPMO are regarded as a single group, it should be kept in mind that

each species has its own characteristics with regard to preferred site of colonization, routes and vectors of transmission, and clinical spectrum.

Studies using quantitative cultures of bronchoalveolar lavage and/or protected specimen brush demonstrate that approximately 60% of all cases of VAP are associated with Gram-negative bacteria, mainly *P. aeruginosa* (20%), and 35% with Gram-positive bacteria^{20,21,29,30}. *S. aureus* is the most frequent Gram-positive pathogen causing VAP (20%). In comatous multiple trauma patients, incidences of *S. aureus* VAP as high as 56% have been reported^{16,31-33}. VAP is often polymicrobial with incidences ranging from 20 to 60% of all episodes of VAP^{29,34}. The importance of anaerobic bacteria in the pathogenesis of VAP has not been studied extensively. Isolation of anaerobic bacteria requires specific transport conditions and culture media, which usually are not systematically achieved during bacteriologic investigation of respiratory tract samples. The incidence of VAP in which anaerobic bacteria are involved, therefore, is probably underestimated. In a prospective study of 130 episodes of VAP, aerobic and anaerobic bacteria were isolated from protected specimen brush ($\geq 10^3$ cfu/ml) in 26 (20%) patients, and anaerobic bacteria only were isolated in four (3%) patients³⁰.

The proportional distribution of the species causing VAP within the etiologic spectrum, as well as their antibiotic susceptibility, may vary considerable between hospital settings, patient populations and countries.

Risk factors

The risk factors associated with the development of VAP have been determined in studies using multivariate analysis techniques and case-control designs or have been suggested on the basis of reviews. Determination of risk factors for VAP has several clinical implications. They offer prognostic information about the probability of developing VAP, they help to reveal the pathogenesis of VAP, and they may provide possible targets for preventive strategies. By risk stratification, one can determine which patients may benefit most from pneumonia prophylaxis³⁵. Risk factor analyses for ICU-acquired pneumonia (i.e., pneumonia diagnosed in ICU patients with or without mechanical ventilation) have clearly identified mechanical ventilation to be the most important risk factor³⁶⁻⁴⁰. Moreover, Langer and colleagues⁴¹ reported on the relation between duration of ventilation and incidence of VAP, which rises from 5% in patients receiving one day of assisted ventilation to 68.8% in patients who are ventilated for >30 d. In general, the risk factors that have been identified can be divided into three groups (Table 2.3). Firstly, risk factors that are well known (intubation, duration of mechanical ventilation etc.) but are very difficult to modify and that offer no or only limited possibilities for prevention. Secondly, risk factors which seem to play a role in the pathogenesis of VAP and which have stimulated the development of a number of preventive strategies. Finally, those risk factors which have been identified only incidentally or need further investigations to assess their significance and the possibility for prevention.

Table 2.3 Risk factors for ICU-acquired and ventilator-associated pneumonia**1. Identified risk factors with no or only limited possibilities for prevention**

ICU-acquired pneumonia

- naso/orotracheal intubation^{37,39}
- duration of MV^{36,40}
- severity of illness^{36,40,42}
- history of COPD³⁷
- reason for admission:
 - trauma^{36,38}
 - neurologic disease^{39,42}
 - thoraco/abdominal surgery^{37,43}
 - coma^{31,36}
- age^{37,38}

Ventilator-associated pneumonia

- emergent intubation¹⁶
- duration of MV^{25,44,45}
- severity of illness^{15,46}
- history of COPD^{25,44,47}
- reason for admission:
 - headtrauma^{13,15,48}
 - hypotension¹⁵
 - blunt trauma¹⁵
 - coma⁴⁹
 - neurosurgery⁴⁸
 - ARDS⁴⁸
- age⁴⁶

2. Identified risk factors that offer possibilities for prevention

ICU-acquired pneumonia

- antacids⁴²
- large volume aspiration³⁷
- presence of nasogastric tube⁴³
- impaired airway reflexes^{36,39}
- depressed consciousness³⁷

Ventilator-associated pneumonia

- antacids or H₂-antagonists^{13,19}
- large volume aspiration^{25,48}
- enteral nutrition⁴⁹
- contaminated ventilator circuits^{13,50}
- reintubation^{25,27,49,51}
- previous antibiotic use^{19,44-47,52,53}
- nonelevated head position⁴⁶

3. Risk factors identified incidentally or needing further investigations to assess their influence on infection and possibilities for prevention

ICU-acquired pneumonia

- male gender⁴²
- recent bronchoscopy⁴³
- thoracic drainage⁴²
- coagulation products⁴²

Ventilator-associated pneumonia

- male gender²⁷
- fall-winter season¹³
- failure of CASS⁵²
- inadequate intracuff pressure⁵²
- administration of aerosols²⁷
- presence of a tracheostomy²⁷
- transport out of the ICU²⁷
- sinusitis^{54,55}

MV, mechanical ventilation; COPD, chronic obstructive pulmonary disease; CASS, continuous aspiration of subglottic secretions.

Recently, several risk factor analyses identified previous antibiotic use to be significantly associated with the development of VAP^{19,46,52}. Rello and coworkers⁴⁴ reported that prior use of antibiotics was a risk factor for *P. aeruginosa* VAP. The same conclusion was reached by Talon and coworkers⁴⁷ in patients who received metronidazole. Other investigators concluded that prior broadspectrum antibiotic use was a risk factor for VAP caused by 'potentially drug-resistant bacteria' (methicillin-resistant *S. aureus*, *P. aeruginosa*, *Acinetobacter baumannii*, and *Stenotrophomonas maltophilia*)⁴⁵. On the other hand, the absence of prior antibiotic treatment was a risk factor for VAP caused by *H. influenzae*⁵³. Lately, attention has been drawn to the association between the mode of mechanical ventilation, ventilator-induced lung injury and inflammation or infection^{56,57}.

Colonization

The relation between colonization of the upper respiratory tract and the development of VAP was established by Johanson and coworkers in 1972⁵⁸. Nosocomial respiratory infections developed in 23% of ICU patients with upper respiratory tract colonization but in only 3% of noncolonized patients⁵⁸. Moreover, upper respiratory tract colonization increased with severity of illness. Repeated oropharyngeal cultures obtained from 33 normal subjects revealed Gram-negative bacteria in 6% of subjects, while these pathogens were cultured from 35% of moderately ill hospitalized patients and even 73% of moribund patients⁵⁹. Since then, many variables have been determined that enhance colonization and infection of the respiratory tract in ICU patients⁶⁰. Although increased exposure to pathogens may play a role, this cannot exclusively explain increased colonization rates. Nursing and medical staff have similar colonization rates as normal subjects not working in a hospital setting⁵⁹. A reduced capacity to clear pathogens and/or increased adherence of microorganisms are more likely mechanisms to account for the higher colonization rates in critically ill patients. The latter mechanisms can be the result of decreased immunologic host function, destruction of epithelial surfaces^{61,62}, impaired mucociliary clearance, pro-inflammatory enzymes and fibronectin reducing proteases⁶³. Furthermore, during ICU stay, approximately 60% of patients receive systemic antibiotics. Antibiotic therapy can rapidly change the commensal oropharyngeal flora, resulting in an increase in oropharyngeal and upper respiratory tract colonization with aerobic Gram-negative or Gram-positive bacilli and yeasts, possibly due to loss of the normal bacterial flora^{60,64} and to selection of pathogens which are resistant to the antibiotics used⁶⁵⁻⁶⁹.

Routes of colonization

Microorganisms reach the lungs after aspiration of colonized oropharyngeal fluid. *Microaspiration* occurs frequently, both in healthy people as well as in critically ill patients^{70,71}. Pathogens colonizing the respiratory tract and causing VAP are derived from either endogenous or exogenous sources. The stomach and intestine are the most important endogenous sources. In addition, pathogens colonizing the upper respiratory tract (oropharynx, sinus cavities, the nares and those on dental plaque) may be aspirated. Contaminated environment (sinks, faucets, sheets etc.), contaminated equipment (mechanical ventilation devices, ventilator circuits, radiographic equipment etc.), contaminated enteral feeding and other colonized patients in ICU are potential exogenous sources.

Several routes of colonization by which pathogens are transported from their endogenous or exogenous sources to the upper respiratory tract of the patient are possible. In the gastropulmonary route of colonization^{72,73}, endogenous bacteria reach the upper respiratory tract via the stomach and subsequently colonize oropharynx and trachea after which the bacteria are aspirated in the lower respiratory tract. This route of colonization has been propagated as important in the pathogenesis of VAP for many years. The rectopulmonary route of colonization

has attracted less attention. In this route, intestinal microorganisms spread from the rectal area via the patient's skin or the hands of health care personnel to the upper respiratory tract. Finally, transfer of pathogens from exogenous sources most probably occurs via hands of nursing and medical staff, which enables direct inoculation of microorganisms into the tracheobronchial tree during manipulation of ventilator circuits or tubes⁷⁴⁻⁷⁶. This is called the exogenous route of colonization or cross-colonization when another patient is the exogenous source.

Essential conditions to study the pathogenesis of VAP

Since colonization is not always followed by infection, infection rates with a certain pathogen form only the tip of the iceberg of the complete epidemiology^{75,77}. When studying the epidemiology of microorganisms in ICU, surveillance of colonization is indispensable. In clinical practice, surveillance is advised only for high-risk patients in specific clinical settings⁷⁸. However, when determining the epidemiology of ICU pathogens in detail, surveillance should include all patients within the ICU, as well as equipment and environmental surfaces for certain pathogens. Surveillance cultures from patients should be taken on admission and subsequently with a frequency high enough to study sequences of colonization from initial body sites to other body sites. Moreover, patients may be colonized or infected with multiple genotypes of the same species, both at one particular body site and at different body sites⁷⁴. Therefore, analysis of a single isolate may not accurately represent the bacterial flora. Analysis of several isolates and determination of similarity of isolates is crucial⁷⁹.

Comparison of bacterial phenotypes, such as antibiotic susceptibility patterns, serotypes, phage types and outer membrane protein types, is relatively easy to perform, but lacks specificity⁷⁹. Genomic DNA fingerprinting techniques, such as pulsed-field gel electrophoresis (PFGE), random amplification of polymorphic DNA and arbitrarily primed polymerase chain reaction, have a higher specificity and discriminatory power while maintaining epidemiological linkage. These techniques are, therefore, considered the methods of choice to determine identity of bacterial isolates in the epidemiology of nosocomial outbreaks⁸⁰⁻⁸². However, the techniques are often cumbersome and expensive, and therefore not always feasible in routine practice.

In summary, to be able to study routes of colonization that may lead to VAP it is necessary to:

1. determine the incidence of VAP, as diagnosed by bronchoscopic techniques;
2. perform surveillance cultures of all patients present in ICU, and possibly environment and equipment;
3. culture several body sites on admission and with a sufficient frequency thereafter;
4. analyze several isolates of each species, cultured from each site;
5. determine similarity of isolates of a certain pathogen by genotyping techniques.

Routes of colonization

Endogenous

Gastric colonization and the gastropulmonary route

In critically ill patients, gastric acidity may be decreased (i.e., pH value higher) due to decreased acid production, because of the application of enteral feeding or stress-ulcer prophylaxis (antacids, H_2 -antagonists, $H^+K^+ATPase$ inhibitors). If the gastric environment favours bacterial growth, bacteria may multiply, hence colonization with Gram-negative bacteria occurs frequently at this site^{34,72,73}. Because of the simultaneous occurrence of gastric colonization and the development of VAP, a causal relationship has been assumed. In the so-called gastropulmonary route of colonization, bacteria presumably reach the upper respiratory tract retrogradely via the colonized stomach, and bacteria are aspirated in the lower respiratory tract. Based on studies reporting correlations between development of VAP and concurrent or preceding gastric colonization with the same species, a central role in the pathogenesis of VAP was assigned to gastric colonization^{72,83,84}. However, in five recent studies, the gastropulmonary route of colonization was found to be unimportant with respect to colonization or infection of the upper respiratory tract^{14,17,26,34,85}, while another four studies did support a role for gastric colonization^{83,86-88}. In these studies the percentage of patients in whom the stomach served as a source of colonization or infection of the respiratory tract ranged from 4% to 24% for colonization and from zero to 15% for the development of VAP⁸⁹. Thus, the role of the stomach and gastropulmonary route of colonization remains a subject of debate⁹⁰. Nevertheless, based on the alleged importance of gastric colonization, modulation of colonization at this site is still being used as a measure to prevent VAP.

Oropharyngeal colonization

The results of studies performed by Johanson and coworkers^{58,59} in the early seventies, pointed towards an association between colonization of the upper respiratory tract and the development of VAP. However, at that time, VAP could not be diagnosed with bronchoscopy and the diagnosis relied on relatively nonspecific clinical, radiographic and microbiological criteria. Moreover, only antibiotic susceptibility patterns and serotyping were employed to determine similarity of isolates, because molecular genotyping techniques were not yet available. Remarkably, in the following years research on the pathogenesis of VAP almost exclusively focused on the role of gastric colonization and the gastropulmonary route of colonization. Approximately 20 years later, new studies on sequences of colonization in patients who developed VAP provided additional evidence in support of Johanson's earlier findings. The upper respiratory tract was the site of first colonization in 14 out of 15 cases of VAP, in a study in which qualitative endotracheal aspirate cultures were used for the diagnosis of pneumonia¹⁴. Torres et al.⁷² compared pharyngeal, gastric and respiratory tract colonization in 63 patients with and without VAP, as diagnosed by bronchoscopic

techniques. They found a strong association between upper respiratory tract colonization and VAP and concluded that both pharyngeal and gastric colonization were important reservoirs for bacteria causing VAP. However, in both studies colonization was not assessed serially, so no conclusions with respect to the actual sequences of colonization could be drawn. In a number of other studies serial cultures of multiple body sites were obtained in order to determine sequences of colonization leading to VAP. In doing so, de Latorre and coworkers¹⁷ concluded that tracheal colonization precedes VAP in most patients and that pharyngeal colonization, rather than gastric colonization, is the main source of microorganisms found in patients with VAP. Cade and coworkers⁸⁵ studied 100 consecutive patients, of whom 49 had positive throat cultures which were preceded by gastric colonization in 15 (25%) patients. There was no association between the presence of a positive gastric culture and the development of infection. Two studies from our group showed that 85% and 96% of the pathogens causing VAP had been isolated previously from tracheal aspirates, and 50% and 75% from the oropharynx^{26,34}. Gastric colonization preceded VAP in only 30% and 31% of the cases^{26,34}. Garrouste-Orgeas and coworkers²³ analyzed oropharyngeal and gastric colonization prior to VAP in 86 patients. VAP was diagnosed by bronchoscopic techniques and identity of strains was based on results of PFGE. Oropharyngeal colonization (either on admission or acquired) correlated better with VAP than gastric colonization. Moreover, PFGE demonstrated that identical strains were isolated from both oropharyngeal and bronchial samples in 28 out of the 31 cases of pneumonia.

In conclusion, the role of oropharyngeal colonization as an antecedent to VAP^{60,91} has regained attention after having been partially neglected for years. The evidence at hand strongly suggests an important role for the oropharynx in the pathogenesis of VAP and offers a potential target for preventive strategies.

Intestinal colonization and the rectopulmonary route

The intestines are a large endogenous source of Gram-negative bacteria, which may spread to the upper respiratory tract via the patients' skin or hands of health care personnel. This so-called rectopulmonary route of colonization, in reality, is an 'exogenous' route for endogenous microorganisms. Still, due to decreased intestinal peristalsis and gastric emptying in critically ill patients, bacteria can colonize the proximal small intestine and subsequently migrate to the stomach via duodenogastric reflux^{88,92}. The rectopulmonary route of colonization has attracted only little attention, especially when compared with other routes of colonization. To our knowledge only four studies have been performed with special attention to the rectopulmonary route. In one of these, the rectum was the most common primary site of colonization with *P. aeruginosa* among 153 patients admitted to a surgical ICU⁹³. In that study, 10 (6.5%) patients acquired colonization with *P. aeruginosa* (seven from rectal swab cultures, three from nasal cultures, and three from tracheal secretions cultures), of whom one patient developed pneumonia⁹³. In another study, the rectum was the most commonly observed colonization site for

P. aeruginosa among 186 admissions to three hospital wards. In all, 20 patients acquired colonization with *P. aeruginosa* in the rectum or oropharynx or both; only two acquired oropharyngeal colonization alone. Unfortunately, data on gastric and oropharyngeal colonization were not reported in the first study⁹³ and data on gastric and tracheal colonization⁹⁴ are lacking in the latter study which precludes conclusions regarding routes of colonization. Noone et al.⁷⁴ studied 27 intubated ICU patients of whom 15 were colonized with *P. aeruginosa* (rectum, pharynx, trachea, groin, toe web, or ear). Among 12 patients with rectal colonization, tracheal colonization occurred in 5, but in only one case with the same serotype at both sites; tracheal colonization preceded isolation from rectal swabs by 2 d⁷⁴. Among cardiac surgery patients receiving cefazolin prophylaxis, 58 of 87 became colonized or infected with *Enterobacter* spp. At least 48% of these patients were already colonized before admission to ICU (mainly in the rectum). Moreover, the typing data strongly suggested that the *Enterobacter* strains came from endogenous sources⁹⁵.

In summary, available data suggest that rectal colonization with *Enterobacter* spp. and *P. aeruginosa* frequently occurs in critically ill patients, but secondary colonization of the upper respiratory tract seems infrequent. The relevance of rectal colonization and the rectopulmonary route of colonization in the pathogenesis of VAP remains largely undetermined.

Exogenous

Data on the role of exogenous sources in colonization and infection of ICU patients are derived mainly from case-reports and descriptions of outbreaks. Sinks⁹⁶⁻⁹⁹, distilled water systems¹⁰⁰, faucets¹⁰¹, tube-feeding formulas^{102,103} and ventilator circuits¹⁰⁴ have been reported as exogenous sources of PPMO, causing outbreaks of nosocomial infections. Especially *P. aeruginosa* possess the ability to proliferate in aqueous sources throughout the hospital. In addition, patients themselves are major reservoirs of nosocomial pathogens¹⁰⁵. It is unlikely that airborne transmission contributes to the spread of staphylococci and Gram-negative bacilli^{91,105}. Therefore, transfer of these pathogens most probably occurs via hands of nursing and medical staff or equipment (stethoscopes, blood pressure meters etc.)^{74-76,99}. Direct inoculation of pathogens into the tracheobronchial tree from contaminated hands is possible during manipulation of ventilator circuits or tubes⁹¹. If the tracheobronchial epithelium is able to bind pathogens, colonization and subsequent pneumonia may occur. This hypothesis is supported by studies reporting lower incidences of nosocomial infections after increasing handwashing frequency, use of gloves or antiseptic handwashing products¹⁰⁶⁻¹⁰⁸.

The importance of cross-colonization in nonepidemic situations has rarely been studied. Olson and coworkers⁷⁵ addressed this issue in a study of 270 patients admitted to a medical-surgical ICU ward; 63 (23%) patients were colonized with *P. aeruginosa* on admission, 33 (16%) acquired colonization and twelve (36%) of 33 acquisitions resulted from cross-colonization. Similar results were reported by Allen and colleagues¹⁰⁹. In contrast, Chetchotisakd et al.¹¹⁰ did not find an

important role of cross-colonization in ICU. In a prospective surveillance in five ICUs during six months, they only found 14 (10%) of 137 isolates of bacteria (*P. aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae* and enterococci) cultured from patients with suspected infection to be acquired by cross-colonization. Although molecular biotyping methods were used to determine identity of isolates, it is very likely that the true incidence of cross-colonization has been underestimated. Only a single isolate from a suspected site of infection was analyzed (the tip of the iceberg), without including isolates from colonized patients. Our group studied the influence of nonabsorbable antimicrobial prophylaxis of the stomach and oropharynx on respiratory colonization among patients in two identical ICUs. Prophylaxis was given to half of the patients in one of the ICUs, while the other half served as a control group. Patients treated in the other ICU, where no prophylaxis was given, formed the second control group. Colonization rates were lowest among patients who received prophylaxis. However, control patients who were treated in the same ward as patients receiving prophylaxis were found to be colonized less frequently and, if anything, later than controls in the ward where no prophylaxis was given. These findings strongly suggest that cross-colonization occurs frequently¹¹¹.

In summary, the few data available do suggest that cross-colonization, mainly from patient to patient via hands of health care workers or equipment, may be an important route of colonization, even in endemic situations.

Prevention of VAP

In recent years, guidelines for the prevention of hospital-acquired or nosocomial pneumonia have been formulated by the American Thoracic Society (ATS)¹¹² and by the Centers for Disease Control and Prevention (CDC) with the consensus recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC)⁷⁸. (The original texts of both guidelines, concerning the prevention of bacterial pneumonia, are added as addendum 1 and 2 at the end of this chapter.) Both guidelines incorporate general recommendations regarding infection control practices that undoubtedly decrease incidences of many infections, including VAP. These recommendations, however, are not specifically directed against VAP. For example, pneumococcal and influenza vaccination of at risk populations, handwashing protocols and isolation of patients with multiple resistant respiratory tract pathogens are regarded as: 'currently available preventive strategies with probable efficacy', in the ATS guidelines¹¹². Similarly, the CDC guidelines strongly recommend staff education, surveillance for bacterial pneumonia in high risk ICU patients and vaccination as general measures. Furthermore, an extensive list of recommendations regarding interruption of transmission of microorganisms from human or inanimate sources to patients is described. These recommendations include sterilization, disinfection and maintenance instructions of ventilator systems and circuits, and handwashing and barrier precautions⁷⁸. Moreover, a number of

recommendations have been added that are strongly advocated for all hospitals and that are viewed as effective on the basis of strong suggestive evidence, even though comparative studies have not been done. These recommendations include: discontinuation of enteral-tube feeding and removal of endotracheal and/or enteral tubes as soon as possible, routine verification of appropriate placement of the feeding tube, and routine assessment of the patient's intestinal motility, clearing of secretions above the inflated tube cuff prior to tube removal or moving of the tube⁷⁸.

In table 2.4 the recommendations aiming specifically to prevent VAP from both guidelines are listed. The value categorization for each recommendation of both guidelines are included as are the results of the relevant studies evaluating the beneficial effects of these recommendations. Only studies with the occurrence of VAP as endpoint of intervention are considered. The number of patients and episodes of VAP reported by the studies on a certain recommendation have been grouped, and from these data the relative risk reduction (RRR) with 95% confidence intervals (95% CI) were calculated. The RRR is the reduction of adverse events achieved by a treatment, expressed as a proportion of the control rate. In other words, it is the difference in event rates between the control and treatment groups, divided by the event rate in the control group. A RRR of 0 means that the incidence of VAP is equal in control and treatment group, while a RRR of 1.00 means that no VAP occurred in the treatment group. So, the closer the RRR approaches 1.00, the more effective the treatment. The use of RRR is sometimes criticized because it does not reflect the magnitude of the risk without therapy (i.e., baseline risk)^{184,185}. However, because the incidences of VAP in the control groups of the analyzed studies vary considerably due to differences in the diagnostic criteria, the use of a relative risk measure is desirable. From the incidence rates, also the number of patients needed to treat (NNT) in order to prevent one VAP were calculated and presented (Table 2.4). The NNT is a measure that incorporates both the baseline risk and the magnitude of the risk reduction¹⁸⁴. The preventive strategies most frequently studied and their contribution to the understanding of the pathogenesis of VAP are described in the following section.

Selective decontamination of the digestive tract

In 1971 the concept of colonization resistance was proposed by van der Waaij, who suggested a beneficial effect of the anaerobic flora in resisting colonization by aerobic Gram-negative bacilli in the digestive tract¹⁸⁶. Many infections are caused by these enteric bacilli. Selective decontamination of the digestive tract (SDD) was developed to selectively eliminate the aerobic Gram-negative bacilli and yeasts from the digestive tract, leaving the anaerobic flora unaffected. The first clinical studies with this technique were performed in granulocytopenic patients and showed favourable results¹⁸⁷. In the early 80's, Stoutenbeek and coworkers adapted the technique for ICU patients¹¹³.

Table 2.4 Strategies to prevent ventilator-associated pneumonia

Preventive strategy	ATs*	CDCT	No	VAP/Study	VAP/Control	RRR	(95% CI)	NNT
Preventive strategy								
- Study group / Control group								
Selective Digestive Decontamination								
- ORO + GAS + SYS / - ¹¹³⁻¹²⁷	3	UI	14	103/1248	286/1098	0.68	(0.61-0.74)	6
- ORO + GAS / - ^{57,68,128-131}	3	UI	6	70/459	117/464	0.40	(0.22-0.54)	10
- ORO + GAS + SYS / SYS ^{69,132-136}	3	UI	5	33/276	66/298	0.46	(0.21-0.63)	10
- GAS / - ^{136,137}	3	UI	2	3/133	14/134	0.78	(0.25-0.94)	12
- ORO / - ^{28,138}	3	UI	2	4/38	32/42	0.86	(0.64-0.95)	2
- ORO + SYS / - ¹³⁹	3	UI	1	13/58	23/30	0.71	(0.51-0.83)	2
- SYS / - ¹⁴⁰	3	lat	1	12/50	25/50	0.52	(0.15-0.73)	4
Stress-ulcer prophylaxis								
- sucralfate / acid neutralizing [§]	2	II	14	231/1318	343/1531	0.22	(0.08-0.33)	20
- sucralfate / H ₂ -antagonists ^{§, 86, 141, 145}	2	II	10	187/1092	256/1172	0.22	(0.08-0.34)	21
- sucralfate / antacids ^{§, 86, 145, 146}	2	II	4	38/230	59/232	0.35	(0.16-0.55)	11
- sucralfate / antacids and/or H ₂ -antagonists ^{148, 150}	2	II	2	24/109	25/117	-0.03	(-0.69-0.37)	-167
Enteral Feeding (EF)								
- TEN / TPN ¹⁵¹	2	-	1	0/29	6/30	1.00		5
- postpyloric feeding / gastric feeding ¹⁵²	2	UI	1	6/19	4/19	-0.50	(-3.48-0.50)	-10
- orogastric tube / nasogastric tube	2	UI	-	na	na	na		na
- small-bore tube / large-bore tube ¹⁵³	2	UI	1	na	na	na		na
- intermittent EF / continuous EF ¹⁵⁴⁻¹⁵⁶	2	UI	3	8/65	18/61	0.58	(0.11-0.80)	6
- acidified EF / nonacidified EF ¹⁵⁷	-	UI	1	na	na	na		na
- semirecumbent / supine body position ^{70, 71, 158}	2	lb	3	na	na	na		na
Subglottic Secretions Drainage (SSD)								
- SSD / no SSD ^{52, 159-160}	2	UI	3	34/190	54/163	0.46	(0.21-0.63)	7
Continuous Lateral Rotational Therapy (CLRT)								
- CLRT / no CLRT ¹⁶¹⁻¹⁶⁶	2	UI	6	36/230	71/230	0.49	(0.27-0.64)	7
Ventilator Circuits								
- HME / HUM ^{167, 172}	2	UI	6	42/538	65/492	0.41	(0.15-0.59)	19
- infrequent change / frequent change ^{173, 174}	2	la	2	34/302	33/274	0.07	(-0.46-0.41)	143
- no change / change ¹⁷⁵⁻¹⁷⁷	2	UI	3	82/433	94/451	0.09	(-0.19-0.30)	53
- closed / open suction catheter system ¹⁷⁸⁻¹⁸⁰	2	UI	3	26/78	27/71	0.12	(-0.36-0.43)	21
- orotracheal / nasotracheal intubation ¹⁸¹	-	UI	1	9/151	17/149	0.48	(-0.13-0.76)	19
Topical Tracheobronchial Antibiotics (TTA)								
- TTA / no TTA ^{182, 183}	3	-	2	23/417	47/412	0.52	(0.22-0.70)	17

ATS, American Thoracic Society; CDC, Centers for Disease Control and Prevention; No, number of studies; VAP, number of Ventilator-associated Pneumonias; Study, number of study patients; Control, number of control patients; RRR, Relative Risk Reduction; 95% CI, 95% confidence interval; NNT, Number Needed to Treat; ORO, Oropharyngeal decontamination; GAS, Gastrointestinal decontamination; SYS, Systemic antibiotics; TEN, Total Enteral Nutrition; TPN, Total Parenteral Nutrition; na, incidence of VAP not analyzed; HME, Heat and Moisture Exchangers; HUM, Heated Humidifiers.

- * Categorization of preventive strategies by the ATS statement: (1) currently available and probably effective for specific populations and indications; (2) currently available, promising in efficacy, and being used by some hospitals on a regular basis; (3) currently available but of unproven value, being used in investigational studies, or on limited clinical basis; and (4) unproven regimens, still being evaluated. (See Addendum 1).
- † Categorization of CDC recommendations: Ia, strongly recommended for all hospitals and strongly supported by well-designed experimental or epidemiologic studies; Ib, strongly recommended for all hospitals and viewed as effective by experts in the field and a consensus of HICPAC based on strong rationale and suggestive evidence, even though definitive scientific studies may not have been done; II, suggested for implementation in many hospitals, recommendations may be supported by suggestive clinical or epidemiologic studies, strong theoretical rationale, or definitive studies applicable to some but not all hospitals; UI (UNRESOLVED ISSUE), no recommendation, practices for which insufficient evidence or consensus regarding efficacy exists. (See Addendum 2).
- ‡ The recommendation NOT to administer systemic antimicrobial agents to prevent nosocomial pneumonia was categorized Ia.
- § The results of studies comparing sucralfate to acid neutralizing prophylaxis have all been combined. Data on the comparisons with antacids and H₂-antagonists have also been analyzed separately for which the data of two studies^{86,146} comparing sucralfate with antacids and with H₂-antagonists were split up. In two other studies^{148,150} this was not possible because control patients received antacids and/or H₂-antagonists, and separate analyses were not performed or reported.

The full concept of SDD aims to eradicate microorganisms from the intestine, the stomach and the oropharynx by nonabsorbable antibiotics, which are combined with systemic antibiotic prophylaxis during the first days of ICU admission. In the SDD regimen the combination of colistin and an aminoglycoside are generally used both of which are effective against Gram-negative bacilli and *S. aureus*. Moreover, both agents are nonabsorbable and do not affect the anaerobic intestinal flora. Amphotericin B was added to prevent overgrowth with yeasts and systemic prophylaxis to prevent early infections. Since the introduction of this preventive strategy, dozens of studies in a variety of ICU populations have been performed^{28,67-69,113-122,125-140,188}. However, SDD has some potential drawbacks such as antibiotic resistance of Gram-negative bacteria and the occurrence or selection of resistant Gram-positive microorganisms. Although a number of studies reported no increased incidences of resistant bacteria^{114,118,120,125,126,130-132,138,139}, overgrowth and even infections with Gram-positive bacteria, resistant to the antibiotics used for SDD, have been reported in several trials^{65-69,119,121,122,128,134,137,189-191}, as were increased colonization and infection rates due to Gram-negative resistant bacteria^{115,119,121,122,191}. Moreover, the lack of cost-benefit analyses and of beneficial effects on mortality rates have further limited the widespread use of SDD¹¹².

Meta-analyses of SDD

Up till now six meta-analyses of SDD studies have been published, all six with comparable results. They all conclude that SDD decreases the incidence of VAP caused by aerobic Gram-negative bacteria with RRRs ranging from 0.40 to 0.78. However, mortality rates are largely unaffected (RRR ranging from 0.06 to 0.13)¹⁹²⁻¹⁹⁷. The authors of four of the six meta-analyses, therefore, state that the routine use of SDD cannot be recommended^{192,194-196}. The Selective Deconta-

mination of the Digestive Tract Trialists' Collaborative Group have performed two meta-analyses. After the first one they concluded that: '...definite conclusions about the effect of prophylaxis on mortality cannot be drawn despite the large number of trials available...'¹⁹³. In contrast, after the second meta-analysis they recommend the use of a combination of topical and systemic antibiotic prophylaxis to reduce overall mortality in critically ill patients. The RRR of all regimens of SDD together was 0.06, for the studies using topical and systemic antibiotics it amounted to 0.15¹⁹⁷.

Although meta-analyses are attractive in many ways, there appear to be major problems with interpretation. Due to publication bias and many other biases that may be introduced in the process of locating, selecting, and combining studies, the findings of meta-analyses may be misleading. For instance, should meta-analyses include unpublished studies; is the literature search to be restricted to the English language; should trialists be contacted for individual patient data?. Specifically with regard to SDD, one wonders whether the results of studies with different SDD regimens, different definitions of the diagnosis of VAP and various study designs can be pooled. Therefore, the findings of the meta-analyses on SDD need to be interpreted with caution.

The full regimen of SDD

Unfortunately, the full regimen of SDD does not help to elucidate the pathogenesis of VAP. Since SDD aims to modulate colonization at three sites: the oropharynx, stomach and intestine in combination with systemic prophylaxis, it has remained unclear which part of SDD prevents VAP. The first study of Stoutenbeek and coworkers addressed this topic. In a prospective open trial they consecutively compared three prophylactic antibiotic regimens (intestinal decontamination $n=17$, oropharyngeal and intestinal decontamination $n=25$, oropharyngeal and intestinal decontamination combined with systemic antibiotics $n=63$) with an historical control group who did not receive any prophylaxis ($n=59$). They concluded that intestinal decontamination alone had no effect on infection rates. The addition of oropharyngeal decontamination effectively prevented secondary pneumonia. Finally, the additive effect of systemic prophylaxis (cefotaxime 50-100 mg/kg/day iv, from arrival to ICU until no more pathogens were isolated from oropharynx or respiratory tract), prevented primary pneumonia^{113,198}. However, this was a nonrandomized, open study with relatively few patients per group and the diagnosis of pneumonia was not established by bronchoscopic techniques. Nevertheless, the full regimen of SDD was propagated as the method of choice for infection prevention, and since then only a few studies analyzed the relevance of the various individual parts of SDD.

The full regimen of SDD (including oropharyngeal and intestinal decontamination and systemic prophylaxis) was studied in at least 14 trials^{113-122,125-127,188} with VAP or respiratory tract infections as endpoint (Table 2.4). Only two of these had a double-blind placebo-controlled design^{117,121}. Nine reported beneficial effects on incidences of VAP^{114,116,118,121,125} or all respiratory tract infections^{113,115,119,127}. Both

double-blind studies, however, did not. The overall RRR of VAP in these 14 trials was 0.68 (95% CI: 0.61-0.74) with a NNT of 6.

SDD without systemic prophylaxis

The effect of selective decontamination of oropharynx, stomach and intestines per se has been determined in 11 studies. In six of these no systemic prophylaxis was employed^{67,68,126-131}, while in the other five studies all patients (study and control) received systemic prophylaxis^{69,132-135}.

Half of the six studies on the effect of SDD without systemic prophylaxis reported beneficial effects on incidences of VAP. For instance, Korinec et al.¹³¹ studied 123 neurosurgical patients in a randomized, double-blind, placebo-controlled design and found incidences of VAP, diagnosed by bronchoscopy, of 24% in SDD and 42% in control patients ($p < 0.04$). With a similar study design, comparable results were found by Quinio and coworkers¹²⁸ in 148 multiple trauma patients on mechanical ventilation with incidences of VAP of 25% in SDD treated patients and 51% in controls ($p = 0.01$). Furthermore, Unertl et al.¹³⁰ in a randomized study among 39 neurologic patients reported incidences of VAP of 5% in SDD and 45% in control patients ($p < 0.01$). In contrast, no differences in the incidences of VAP, diagnosed by 'blind' protected specimen brush, between study groups (27% in SDD group vs. 26% in control group) were found in 61 patients in a medical-surgical ICU⁶⁷. Gastinne and coworkers⁶⁸ also failed to find beneficial effects of SDD in 445 mechanically ventilated patients admitted to medical ICUs. Pneumonia was diagnosed in 26 (12%) of 220 SDD treated patients and in 33 (15%) of 225 control patients. Finally, in addition to these two randomized, double-blind, placebo-controlled trials, Flaherty and coworkers¹²⁹ studied 107 cardiac surgery patients. Randomization was performed by alternating treatment in 2 wk periods to either oropharyngeal and gastrointestinal application of polymyxin, gentamicin and nystatin or sucralfate for stress-ulcer prophylaxis. One out of 51 patients treated with SDD and five out of 56 patients receiving sucralfate developed pneumonia.

Among the five trials in which all patients received systemic prophylaxis, no beneficial effects were reported by the four with a double-blind and placebo-controlled design^{69,133-135}. In the study by Hartenauer and coworkers¹³² who included 200 consecutive patients in a cross-over controlled trial, the incidence of VAP was 10% in study and 46% in control patients.

In summary, the effects of oropharyngeal and gastrointestinal decontamination per se are doubtful since half of the studies report beneficial effects on incidences of VAP, while no difference in incidence rates were found in the other three. Moreover, the five studies in which all patients (study and control) received systemic prophylaxis suggest that oropharyngeal and gastrointestinal decontamination have only limited additional effect to systemic antibiotics. The RRR of VAP, as calculated from the combined results of these 11 studies, is 0.42 (95% CI: 0.28-0.53) with a NNT of 10.

Gastrointestinal decontamination

The effects of gastrointestinal decontamination on incidences of VAP have been evaluated in only three studies^{136,137,189}. Brun-Buisson et al.¹³⁷ administered neomycin, polymyxin E, and nalidixic acid in liquid form through the nasogastric tube without systemic prophylaxis or oropharyngeal paste to control a nosocomial outbreak of intestinal colonization and infection with multiresistant *Enterobacteriaceae*. Although the outbreak was controlled successfully, intestinal decontamination did not reduce the number of nosocomial infections: the incidence of pneumonia was 8.3% in the treated group and 14.4% in the control group. In another study, none of 97 patients receiving intestinal decontamination developed VAP (diagnosed with quantitative cultures of protected specimen brush), compared with eight of 84 control patients ($p<0.05$)¹³⁶. Finally, Cerra and coworkers¹⁸⁹ reported a decrease in the overall incidence of infections which almost reached statistical significance ($p=0.08$). Unfortunately, the incidence of VAP was not reported.

In summary, gastrointestinal decontamination alone seems to have a beneficial effect on overall infection rates, but the influence on incidences of VAP remains unclear with one positive and one negative study. Combination of the results of the studies by Brun-Buisson and Godard yields a RRR of 0.78 (95% CI: 0.25-0.94) with a NNT of 12 (95% CI: 7-43). The width of the 95% confidence intervals of RRR and NNT is indicative for the necessity to carry out more studies.

Oropharyngeal decontamination

The effects of oropharyngeal decontamination alone have been assessed in two studies. In a double-blind trial Pugin et al.²⁸ randomized 52 patients to receive either a solution of polymyxin B, neomycin and vancomycin or placebo in the retropharynx. Colonization with aerobic Gram-negative bacteria was significantly reduced in oropharynx and stomach, resulting in a relative risk reduction for VAP of 0.79. Rodriguez-Roldan et al.¹³⁸ used an oropharyngeal paste containing tobramycin, amphotericin B and polymyxin E. Decontamination of oropharynx and trachea was established in 10 of 13 patients receiving active medication, none of whom developed pneumonia. In contrast, 11 (73%) of 15 patients receiving placebo medication developed pneumonia. Attention must be drawn to the high incidences of VAP in the control groups of both studies (78% and 73% respectively), which is probably due to the use of clinical and microbiological criteria, rather than bronchoscopic techniques, to diagnose VAP. Unfortunately, since gastric colonization was significantly decreased in the study by Pugin et al.²⁸, their findings do not elucidate the relative importance of gastric and oropharyngeal colonization in the pathogenesis of VAP.

Oropharyngeal decontamination in combination with systemic prophylaxis was studied by Abele-Horn and colleagues¹³⁹ in a randomized, controlled trial. The incidences of both primary (0% vs. 33%; $p<0.001$) and secondary pneumonia (22% vs. 47%; $p<0.001$), were reduced significantly in study patients. Pneumonia was diagnosed by quantitative cultures of tracheal aspirates ($>10^4$ cfu/ml). Again,

the total incidence of pneumonia (primary and secondary) in the control group was very high (77%).

Another way to achieve oropharyngeal decontamination, not using antibiotics, is the use of chlorhexidine. For instance, an oral rinse of 0.12% chlorhexidine reduced the incidence of respiratory tract infections among 353 cardio-surgical patients from 9% in control patients to 3% in patients receiving oropharyngeal decontamination with chlorhexidine¹⁹⁹. This difference was mainly due to a reduction of infections with Gram-negative pathogens. However, it is not known to what extent prolonged application of chlorhexidine will affect oral, oesophageal and gastric mucosa in critically ill ICU patients, and neither is the risk of chlorhexidine resistance after long-term application²⁰⁰⁻²⁰².

Systemic prophylaxis

Prevention of pneumonia with systemic antibiotics was attempted first by Lepper and coworkers in 1954²⁰³. No reduction of tracheal colonization or infection was achieved in 72 tracheotomized poliomyelitis patients. Two other studies from that time also failed to modify the incidence of infection by antibiotic prophylaxis in unconscious patients²⁰⁴ and patients with acute heart failure²⁰⁵. Moreover, the latter two studies reported selection of resistant pathogens. It was not until 1989 that another attempt was made to prevent pneumonia with systemic antibiotics. Then, Mandelli and coworkers randomized 570 ICU patients, half of which were intubated, to receive either 24 h of cefoxitin, penicillin G or no prophylaxis. The incidence of early-onset pneumonia (primary outcome measure) was 6.1% in patients receiving antibiotics and 7.2% for controls²⁰⁶. More recently, in a prospective, randomized, but not double-blind or placebo-controlled, trial in 100 mechanically ventilated patients with coma, systemic antibiotic prophylaxis with cefuroxime (two 1,500 mg doses) was studied. Prophylaxis resulted in a lower incidence of VAP compared with the control group (12/50 (24%) vs. 25/50 (50%); $p=0.007$; RRR 0.52). This difference was due to a reduction in the episodes of early-onset VAP in the cefuroxime group (8/50 (16%) vs. 18/50 (36%); $p=0.022$; RRR 0.56), while the incidences of late-onset VAP were comparable (8% vs. 14%)¹⁴⁰. The impact of this regimen on antibiotic susceptibility of pathogens causing VAP remains to be established.

The results of all the trials with various SDD regimens and of the meta-analyses indicate that SDD reduces the incidence of VAP, leaves mortality rates unaffected and increases the occurrence of multiple resistant pathogens. Several controversies remain. For instance, although never proven, the beneficial effects of SDD appear to be related to the criteria used to diagnose pneumonia. Incidences of VAP in control groups range from 18.5%¹²⁵ to 85.1%¹¹⁸ when the SDD regimen significantly prevented VAP, and ranged from 5.3%¹²⁰ to 25.8%⁶⁷ when no significant effects were found. A considerable part of the studies reporting favourable results of SDD are poorly designed (nonrandomized, unblinded, historical control groups, nonbronchoscopic diagnosis of VAP etc.). Moreover, the

preventive effects of the individual parts of SDD are not fully elucidated. The systemic antibiotics within the regimen seem to prevent early-onset, but not late-onset VAP. The effects of gastrointestinal decontamination are scarcely investigated and remain unclear with one positive and one negative trial. Since the role of gastric colonization and the gastropulmonary route of colonization is regarded unimportant based on the evidence from studies on the pathogenesis of VAP, the additive effect of gastrointestinal decontamination may be considered limited. Although the influence of oropharyngeal decontamination also was studied infrequently, the results at hand are very promising, especially with regard to late-onset VAP. Oropharyngeal decontamination alone or in combination with 24 h of systemic antibiotic prophylaxis has RRRs that are even higher than those of the full regimen of SDD, although only a fraction of the antibiotics used in the full regimen are needed. Moreover, the data from the sequence studies strongly point towards an important role for oropharyngeal colonization in the pathogenesis of VAP.

Stress-ulcer prophylaxis

Since critically ill patients on mechanical ventilation are prone to develop gastritis and/or gastric ulcers^{141,142,207}, stress-ulcer prophylaxis is routinely provided. In this respect, gastric acidity may be reduced by neutralization of gastric acid (antacids) or by inhibition of acid production (H_2 -antagonists, $H^+K^+ATPase$ inhibitors), both of which will decrease the natural protection against bacterial overgrowth. In contrast to these agents, sucralfate has been claimed to prevent stress-ulcers without influencing gastric acidity²⁰⁸. Theoretically, patients who receive sucralfate should maintain lower intragastric pH values compared with patients receiving antacids or H_2 -antagonists. Moreover, *in vitro* studies demonstrated bactericidal and bacteriostatic effects of sucralfate, mainly at high pH values²⁰⁹⁻²¹¹. As a result, sucralfate should prevent bacterial overgrowth in the stomach and, according to the alleged importance of the gastropulmonary route of colonization, also reduce the incidence of VAP. Administration of sucralfate for stress-ulcer prophylaxis has, indeed, shown favourable effects on the incidence of VAP in two studies^{86,148}. However, there are 12 studies showing no significant effect on the incidence of VAP^{8,34,141-147,149,150,212}. In a prospective, randomized, double-blind study by our group in which patients were stratified on the basis of their initial gastric pH, sucralfate was compared with a fixed dosage of antacids³⁴. Intragastric acidity was determined by means of a computerized intragastric pH monitoring. No significant differences in median pH values were observed. Colonization rates in the stomach, the oropharynx and the trachea and the incidence of VAP were comparable in both treatment groups. However, patients colonized with PPMO in the stomach had higher median pH values compared with noncolonized patients. This underscores the relation between intragastric acidity and gastric colonization³⁴. The observation that sucralfate does not prevent VAP could be due to the fact that sucralfate did not decrease intragastric pH levels below 3.5 in most of the studies⁸⁹. Our findings were confirmed by two other double-blind studies

testing the preventive effects of sucralfate. A study by Artigas and coworkers¹⁴³, comparing sucralfate and ranitidine, was terminated prematurely after inclusion of 146 patients because no difference in incidence of VAP between both study groups was observed (published only in abstract form). In addition, similar data were reported in a prospective, double-blind, placebo-controlled study in which 1200 patients had been randomized to either sucralfate or ranitidine⁸.

Modulation of enteral feeding

In addition to stress-ulcer prophylaxis, enteral feeding, which usually has a pH of 6, may reduce intragastric acidity due to dilutional alkalization. If enteral feeding would be a risk factor for the development of VAP, this would support the role of the gastropulmonary route of colonization in the pathogenesis of VAP. Several studies showed high rates of gastric colonization in patients receiving enteral feeding^{213,214}. Hence, modulation of enteral feeding has been used as a possible approach to interrupt the gastropulmonary route of colonization and to reduce the incidence of VAP. In this regard, intermittent enteral feeding would be expected to be superior to continuous enteral feeding, as gastric acidity increases during the periods that feeding is discontinued. Three studies have been performed with conflicting results. Lee et al.¹⁵⁴ reported lower intragastric pH values and incidence rates of VAP in patients receiving intermittent enteral feeding compared with a historical control group who received continuous enteral feeding. Skiost and coworkers¹⁵⁵ randomized 16 patients to either intermittent enteral feeding or continuous enteral feeding for a five day period. They concluded that intermittent enteral feeding resulted in lower postfasting gastric pH and lower rates of gastric colonization with pathogenic organisms. No patients developed nosocomial pneumonia during the five-day study period. Our group¹⁵⁶ failed to find a beneficial effect of intermittent enteral feeding on intragastric acidity, gastric colonization and incidence of VAP in a prospective randomized trial in which intermittent enteral feeding and continuous enteral feeding were compared in 60 patients.

The effects of acidified (pH 3.5) enteral feeding¹⁵⁷ and postpyloric feeding¹⁵² were determined in one study each. In both studies favourable results with respect to intragastric acidity and gastric colonization were found. However, respiratory tract colonization was not studied and the numbers of patients included were too small to evaluate incidences of VAP. Large, prospective randomized and controlled trials are needed to confirm the effects of modulation of enteral feeding as a means to decrease the incidence of VAP²¹⁵.

Subglottic secretions drainage

During mechanical ventilation subglottic secretions and oropharyngeal fluids may accumulate above the inflated endotracheal cuff. This fluid will contain large amounts of microorganisms. Microaspiration of these secretions along the tracheal cuff results in colonization and possibly infection of the lower respiratory tract. Drainage of subglottic secretions with specifically designed devices may, therefore,

prevent VAP²¹⁶. This has been evaluated in two studies in which bronchoscopic techniques were applied for the diagnosis of VAP. In a prospective randomized study, manual intermittent subglottic secretion drainage was associated with a lower incidence of nosocomial pneumonia (13%) compared with control patients (26%)¹⁵⁹. In another prospective randomized study, continuous subglottic suctioning was studied in 76 patients of whom 18% developed VAP, compared with a 33% incidence of VAP in control patients ($n=77$). Interestingly, the reduction was caused by a lower incidence of VAP in the first week of ventilation (early-onset VAP), but not of late-onset VAP¹⁶⁰. In a recent risk factor analysis among 83 patients, failure of the continuous aspiration of subglottic secretions technique did not influence the development of pneumonia among patients undergoing antibiotic treatment (33.0% vs. 38.5%; $p>0.20$), but was strongly associated with pneumonia (42.1% vs. 8.3%; $p<0.01$) among intubated patients not receiving antibiotics⁵². In contrast, Girou and colleagues²¹⁷ concluded from a bacteriological evaluation in 14 patients that the use of continuous subglottic aspiration associated with semirecumbent position does not modify tracheal colonization.

Body position

Enteral feeding increases gastric volume, especially in critically ill patients who often have reduced gastric motility and delayed gastric emptying due to the underlying disease or as a result of medication²¹⁸. In these patients the risk of aspiration of gastric contents is enhanced. Torres and coworkers⁷¹ analyzed gastroesophageal reflux in ventilated patients on enteral feeding, using radioactive labelled gastric nutrition. They found that patients in supine position had higher counts of radioactivity in endobronchial secretions compared with patients treated in a semirecumbent position. Moreover, the length of time in supine position appeared to be a risk factor for aspiration of gastric contents. In a follow-up study in 15 patients by the same group, it was concluded that gastroesophageal reflux occurs irrespective of body position, and that a semirecumbent position does not protect completely from gastroesophageal reflux¹⁵⁸. These data confirmed the results reported by Ibáñez et al.⁷⁰ in a similar study. In addition to the patients position, gastroesophageal reflux may be influenced by the presence and even size of the nasogastric tube^{70,153,158}. In none of these studies were the effects on incidences of VAP evaluated.

Continuous lateral rotational therapy

Another potentially effective approach is continuous lateral rotational therapy. Continuous rotation is thought to stimulate mobilization of respiratory tract secretions, thereby preventing the development of atelectasis and pneumonia. Six studies compared continuous lateral rotational therapy with turning by nursing staff every 2 h in conventional beds. All studies reported reduced incidences of pneumonia in patients on rotation¹⁶¹⁻¹⁶⁶, the difference being statistically significant in only one of these¹⁶³.

Other preventive strategies

The use of topical tracheobronchial antibiotics, administered either as an aerosol (polymyxin B, gentamicin) or in a solution (gentamicin, colistin), has been attempted to reduce the incidence of pneumonia caused by Gram-negative bacteria, especially *P. aeruginosa*. Four studies, performed between 1973 and 1975, of which two had a controlled design^{182,183}, reported significantly decreased incidences of upper airway colonization and/or pneumonia caused by these microorganisms^{182,183,219,220}. However, the study by Klastersky and colleagues¹⁸³, using endotracheal administration of gentamicin, reported that bacteria isolated from treated patients were slightly more resistant to gentamicin than microorganisms recovered from respiratory tract of controls. Feeley and coworkers²²⁰ used an aerosol with polymyxin B which successfully prevented *P. aeruginosa* pneumonia. However, the incidence of pneumonia caused by polymyxin-resistant microorganisms (*Pseudomonas* spp. and *Serratia* spp.) and intrinsically resistant pathogens (*Proteus* spp., *Flavobacterium* spp. and *Streptococcus faecalis*) was high, and the overall mortality rate for patients with acquired pneumonia was 64%²²⁰. Furthermore, a randomized study in 30 thermally injured patients with inhalation injury, using aerosolized gentamicin, reported no differences in the number of patients with infiltrates on chest radiographs, necessity of mechanical ventilation and pulmonary complications between study and control patients²²¹. Moreover, continued use of gentamicin in this study resulted in resistant strains of *Klebsiella* and *Pseudomonas* emerging in burn wounds and sputum²²¹. Because of the frequent occurrence of infection with highly resistant bacteria and the lack of (or even negative) effect on mortality, the use of topical tracheobronchial antibiotics was not recommended. More recently, Rouby and coworkers²²² performed a nonrandomized study in which 347 consecutive patients receiving intratracheal colistin were compared with a historical control group of 251 patients. The incidences of pneumonia caused by Gram-negative bacteria and polymicrobial pneumonia were significantly reduced in the group receiving colistin. Mortality was comparable in both groups and no increase in the number of VAP caused by colistin-resistant microorganisms was observed.

Bacterial contamination of the ventilator tubing circuit may predispose to the development of VAP¹⁰⁴. Frequent changing of these circuits (including in-line suction catheters, heat and moisture exchangers and heated humidifiers) may be beneficial to decrease the bacterial burden. On the other hand, frequent manipulation of ventilator tubing circuits may lead to introduction of nosocomial pathogens. Five studies have addressed the effects of lengthening intervals between circuit changes on colonization of the patient and circuits, and incidence of VAP¹⁷³⁻¹⁷⁷. They all concluded that decreasing the frequency of ventilator circuit changes did not increase incidences of VAP or patient and circuit colonization. Therefore, substantial reductions in the costs of mechanical ventilation can be obtained without apparent adverse effect. Studies using a closed-suction catheter system for endotracheal suctioning found similar incidences of pneumonia compared with the open-suction system with single-use sterile suction

catheters¹⁷⁸⁻¹⁸⁰. Finally, during mechanical ventilation heating and humidifying inspired gases is necessary. Heated humidifiers are used most frequently, but these cause accumulation of water in the circuit that may become colonized with bacteria¹⁰⁴. Heat and moisture exchangers are a possible alternative in which the formation of condensate in ventilation circuits is avoided due to the combination of humidification with antimicrobial filtering properties. However, heat and moisture exchangers failed to reduce the incidence of pneumonia in five studies comparing both methods¹⁶⁷⁻¹⁷¹. Kirton and coworkers¹⁷² are the only group to report a significant reduction in late-onset VAP, but not early-onset VAP, with the use of heat and moisture exchangers. In general, the influence of the ventilator circuit and secretion management strategies on VAP rates is limited²²³.

Conclusions

Critical assessment of the various studies on the prevention of VAP is very difficult. Due to the small numbers of patients included in the studies, heterogeneous ICU populations, and differences in diagnostic criteria and quality of the studies a comparison of the studies is hampered. Moreover, some strategies have only been studied scarcely. Ideally, preventive strategies are studied in well designed multicenter trials, including large numbers of comparable patients. However, for mechanically ventilated patients this is next to impossible in view of the numbers of patients, differences in severity of illness, causes for ICU admission and differences in treatment.

Nevertheless, two guidelines have been formulated for the prevention of VAP. The validation of the preventive strategies as described in the guidelines for the prevention of hospital-acquired or nosocomial pneumonia by ATS¹¹² and CDC⁷⁸ are to some extent comparable although different ways of categorization have been used (Table 2.4). Still, some remarkable differences exist. The validations of the use of SDD for prevention of VAP are comparable. The ATS considers SDD of unproven value (category 3) while the CDC regards SDD as a practice for which insufficient evidence is available (category UI). However, according to the principles of Evidence Based Medicine, the application of topical antibiotic prophylaxis is the only strategy proven to be effective for prevention of VAP and has been studied most frequently. The validations of both guidelines most likely account for all types of SDD since no information is provided regarding the various individual parts of SDD, with the exception of the administration of systemic antimicrobial agents, a strategy which was NOT recommended in the CDC guidelines (category Ia).

The use of sucralfate for stress-ulcer prophylaxis is considered promising by ATS (category 2) and is suggested for implementation in many hospitals (category II) by CDC. However, the majority of clinical trials and all randomized, double-blind, placebo-controlled trials, have failed to show favourable effects of sucralfate on the incidence of VAP. Moreover, sucralfate was found to be inferior to ranitidine with regard to the prevention of clinically important gastrointestinal bleeding. It must be pointed out that the guidelines under analysis date back to the time

before the results of the randomized, double-blind, placebo-controlled trials were available. In the revisions of these guidelines the recommendations regarding the use of sucralfate undoubtedly will be adjusted.

The various preventive strategies associated with enteral feeding and (prevention of) gastric aspiration are thought to be efficacious by ATS (category 2), although very few studies on their efficacy have been performed, the results of which are controversial. The CDC considers these regimens to be an unresolved issue (category UI), although a semirecumbent body position during mechanical ventilation is strongly recommended (category Ib) by this institution even though definitive scientific evidence for this recommendation is lacking.

Subglottic secretions drainage and continuous lateral rotational therapy both are regarded as: 'promising in efficacy and being used by some hospitals on a regular basis' by ATS (category 2), which is in line with the scientific evidence. Presently, a number of studies are being executed on subglottic secretions drainage which will provide additional information on its efficacy in the near future. Continuous lateral rotational therapy has only been studied in selected patient populations and favourable results on incidence rates of VAP have been reported. The mechanism for this beneficial effect remains to be established. Whether this technique is applicable to large numbers of patients is also unclear, especially since the associated costs of this technique are considerable. The CDC considers both these strategies to be 'practices for which insufficient evidence or consensus regarding efficacy exists (category UI).

Although preventive strategies aimed at ventilator circuits are categorized as promising in efficacy (category 2) by the ATS, the accessory guidelines in fact indicate that the proposed measures (i.e., heat and moisture exchangers, frequent, infrequent or no change of circuits and closed-suction systems) do not seem to add to the risk of developing VAP. This is congruent with the categorization of the CDC (category UI).

Topical tracheobronchial antibiotics are not included in the guidelines of the CDC and considered to be of unproven value by ATS.

In conclusion, the strategies which have proven to be the most effective for preventing VAP are not part of both guidelines due to side-effects and a lack of effect on secondary outcome parameters. The use of sucralfate is the only measure that is recommended in both guidelines although scientific evidence indicates that this method is ineffective. The CDC classifies modulation of enteral feeding, subglottic secretions drainage and continuous lateral rotational therapy for the prevention of VAP as unresolved issues. The ATS does recommend enteral feeding associated strategies although only few and inconclusive studies have been performed, while subglottic secretions drainage and continuous lateral rotational therapy are recommended which is in line with the promising results of the first clinical trials. The guidelines concerning ventilator circuit associated strategies from both ATS and CDC are in line although the categorizations are different.

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Addendum 1

Copied in part from the official statement of the American Thoracic Society, prepared by an ad hoc Committee of the Scientific Assembly on Microbiology, Tuberculosis and Pulmonary Infections, which was adopted by the American Thoracic Society board of directors, november 1995.

The entire statement is published as: Hospital-acquired Pneumonia in Adults: Diagnosis, Assessment of Severity, Initial Antimicrobial Therapy, and Preventive Strategies. A Consensus Statement. *American Journal of Respiratory and Critical Care Medicine* 1995; 153: 1711-1725.

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Prevention of hospital-acquired pneumonia

Although there is widespread interest in the prevention of hospital-acquired pneumonia (HAP), there are no regimens that are proven to be effective for a wide range of hospitalized patients. A number of preventive strategies have been applied and fall into one of four categories: (1) currently available and probably effective for specific populations and indications; (2) currently available, promising in efficacy, and being used by some hospitals on a regular basis; (3) currently available but of unproven value, being used in investigational studies, or on limited clinical basis; and (4) unproven regimens, still being evaluated.

Regimens with Probable Efficacy for Specific Indications

In this category are pneumococcal and influenza vaccination, handwashing, and isolation of patients with multiply resistant respiratory tract pathogens. The efficacy of vaccinations against *S. pneumoniae* and influenza virus in preventing HAP is unknown, but they are effective for preventing respiratory infection and hospitalization in specific at risk populations. Indirectly, by preventing respiratory infection and hospitalization, these vaccines may reduce the incidence of HAP, and they represent sound medical care for patients qualifying for the vaccines. Handwashing between patient contact is a basic and often neglected behaviour by medical personnel. This simple manoeuvre can prevent the transfer of pathogens from patient to patient and can keep the hands of medical personnel free of potentially pathogenic bacteria. Such 'exogenous sources' of organisms can lead to the direct inoculation of organisms into the tracheobronchial tree, and if the tracheobronchial epithelium is able to bind such organisms, colonization and subsequent pneumonia may occur. Similarly, the transfer of resistant pathogens from patient to patient may be prevented by isolating patients with highly resistant organisms such as MRSA. Unfortunately, such 'barrier methods' will not be effective in preventing infection with organisms that are part of the critically ill patient's endogenous flora; thus, most Gram-negative pneumonias cannot be avoided by isolation methods.

Regimens with Probable Effectiveness Used Widely in Some Clinical Settings

The approaches that fit into this descriptive category include nutritional support, attention to the size and nature of the gastrointestinal reservoir of microorganisms, careful handling of ventilator tubing and associated equipment, subglottic secretion drainage, and lateral rotational bed therapy.

Nutritional support is commonly used in hospitalized patients because of the observation that malnutrition can increase the risk of pneumonia. Nonetheless, there is no clear-cut evidence that nutritional support can reduce the risk of HAP. If nutritional support is given, careful attention to the route and volume of feeding, as well as to a number of other methodologic factors, is necessary and may influence the incidence of HAP. When enteral nutrition, delivered by a feeding jejunostomy, has been compared with total parenteral nutrition (TPN), it has been associated with a lower incidence of HAP. Although the mechanism for this occurrence is unknown, it has been speculated that enteral nutrition stimulates the intestinal mucosa, thereby preventing bacterial translocation (a possible mechanism for pneumonia), and that enteral nutrition compared with TPN, changes the synthesis of inflammatory mediators by the liver to a favourable balance for host defense function.

The superiority of enteral nutrition has been demonstrated with distal enteral feeding, while feedings delivered to the stomach may not have the same effect. If feedings are delivered to the stomach, a number of methodologic issues must be considered. These include the effect of feedings on gastric volume and pH, because elevation of either of these variables can expand the size of the gastric bacterial reservoir, which can

potentially yield an inoculum of bacteria that could reach the lower respiratory tract. In addition, if patients being fed into the stomach are kept supine rather than semierect, pneumonia is more likely. With the use of large-bore feeding tubes and bolus feedings, aspiration may also occur more often than with the use of smaller tubes and continuous feeding methods. One other consideration is the site of feeding tube insertion. One study has shown that the use of orogastric tubes, rather than nasogastric tubes, can reduce the incidence of nosocomial sinusitis, an infection that may lead to subsequent HAP.

Although there are no FDA approved regimens for intestinal bleeding prophylaxis, many critically ill patients receive this intervention, and debate continues about its effect on the incidence of HAP. It is probably too simplistic to say that any intervention that raises gastric pH is undesirable, because gastric volume as well as pH must be considered, along with some of the other factors already mentioned. In three meta-analyses, the use of sucralfate (which does not increase gastric pH) was associated with a reduced incidence of pneumonia, when compared with the use of either antacids alone (which increase both gastric pH and volume) or in combination with H_2 -antagonists. However, there is disagreement about whether the use of H_2 -antagonists alone will lead to a higher rate of pneumonia when compared with sucralfate or placebo. In some recent studies, H_2 -blockers did not increase the risk of pneumonia when compared with placebo, although the use of sucralfate may reduce the incidence of pneumonia when compared with H_2 -antagonists. If sucralfate is protective, it may work best for late-onset HAP rather than for early forms of infection. The conflicting results with H_2 -antagonists may reflect the fact that these agents elevate gastric pH without increasing gastric volume. In addition, many of the analyses have not considered the impact of concomitantly administered gastric feedings. If the goal of nutritional support is to deliver enteral feeding into the distal intestine, it may be unnecessary to place a second tube into the stomach just to give sucralfate, and the use of H_2 -blockers in patients with small-bowel feeding tubes may be safe. However, antacids should be avoided in patients who are at risk for HAP.

Bacteria can proliferate in respiratory therapy equipment, and the incidence of pneumonia is increased if the tubing is manipulated frequently rather than infrequently. Recently, it has been shown that even if ventilator tubing is never changed during the course of mechanical ventilation, the risk of pneumonia is not increased. Most hospitals have a policy to change ventilator tubing every 48-72 h, but there are no data to indicate that any particular schedule to tubing changes is likely to reduce the incidence of pneumonia. When tubing is handled, the condensate should always be drained away from the patient because it can contain large concentrations of bacteria. The use of a heat and moisture exchanger (HME) avoids such condensate and does not seem to add to the risk of pneumonia. In-line medication nebulizers should be washed and cleaned after each use to avoid contamination with high levels of bacteria. Closed suction catheters do not appear to alter the incidence of pneumonia but may increase the rate of airway colonization.

In a few medical centers, subglottic secretion aspiration is achieved with the use of a special endotracheal tube, designed with a suction port above the endotracheal tube cuff, in the subglottic area. By removing contaminated respiratory secretions before they enter the lung, subglottic secretion aspiration has been reported to reduce the incidence of some types of HAP in intubated patients. The suctioning can be done intermittently or continuously, and continuous aspiration appears to delay the onset of HAP, preventing early forms of infection. This approach cannot prevent infection with organisms that have the capacity to colonize the lung after direct inoculation, and thus, it has not effectively prevented pneumonia caused by *P. aeruginosa* and other forms of late onset HAP. Once endotracheal tubes capable of allowing subglottic secretion drainage are more widely available, this approach may be used more regularly.

Lateral rotational bed therapy has been used in both medical and surgical patients and can reduce the incidence of pneumonia in certain populations. These include surgical trauma patients and some medical patients, particularly those with sepsis. The mechanism for this beneficial effect is unknown but probably relates to mobilization of tracheobronchial secretions. The cost association with this intervention has limited its widespread use and emphasizes the need to apply it to carefully selected patients.

Regimens of Unproven Value Used on Limited Investigational or Clinical Basis

Included in this category is the selective digestive decontamination (SDD) regimen of topical and systemic antibiotic prophylaxis and topical tracheobronchial antibiotics.

SDD has been studied for many years and involves the use of topical oral and intestinal antibiotics, often with a systemic antibiotic added for the first few days of the regimen, with the goal being elimination of all potential pathogens from the gastrointestinal tract. With sterilization of 'endogenous' bacterial sources, infection may be avoided. In the many SDD studies there is a general trend towards reducing the incidence of pneumonia, but this has usually occurred in unblinded studies. In blinded studies, the incidence of pneumonia

has not always been reduced, particularly if invasive methods were used to define the presence of HAP. In general the efficacy of the regimen has been questioned because mortality reduction is often not seen, although one recent analysis suggested a modest reduction in mortality with the use of SDD, provided that systemic antibiotics were included in the regimen. Widespread use of SDD has not been adopted because of concerns about the emergence of antibiotic-resistant organisms, the cost of the regimen, and the limited benefit of SDD on mortality. SDD may be effective for specific populations, particularly surgical patients rather than medical patients. More data are needed to define the patients who may benefit from this intervention.

Topical antibiotics delivered to the lower respiratory tract alone have been studied and were shown to have no effect on mortality. Although this approach was able to reduce the incidence of pneumonia in some studies, it frequently led to infection with highly resistant pathogens, and when patients developed pneumonia on this regimen, it was often a fatal event. At the present time this approach cannot be recommended.

Unproven Regimens Still Being Evaluated

This category includes the use of biologic response modifiers, monoclonal antibodies to specific bacterial antigens, and manipulations of endogenous sources of bacteria by mechanical means. In this latter category is the potential to prevent the accumulation of a bacterial biofilm on the endotracheal tube. The endotracheal tube can harbour the growth of large numbers of bacteria along its inner surface, and bacteria at this site will persist in the airway, free from the effect of antibiotics and host defense. The development of new biomaterials for endotracheal tubes could lead to the elimination of a tracheal tube biofilm and the eradication of a reservoir of large numbers of bacteria in the airway.

Biologic response modifiers that hold promise for reducing the incidence of HAP are being investigated. These substances are immunomodulators and have the ability to either upregulate or downregulate host defense mechanisms, which in the appropriate setting may have beneficial effects on pulmonary host defenses against invading pathogens. Because extrapulmonary infections have been associated with abnormalities in pulmonary defense mechanisms, strategies that effectively reduce the severity of systemic infection (such as sepsis) might be beneficial in lowering rates of secondary HAP. To date, there are no proven interventions that accomplish this goal. Efforts to intervene in the cytokine/mediator cascade initiated by Gram-negative bacteria and/or lipopolysaccharide, which is thought to be responsible for the deleterious consequences of the sepsis syndrome, are ongoing. Antilipopolysaccharide antibodies (E5 and HA-1A) have not proved efficacious in improving outcome in patients with Gram-negative bacterial sepsis. Ongoing studies of other biologic response modifiers include antibodies to the cytokine tumor necrosis factor, interleukin-1 receptor antagonist, and the cyclooxygenase inhibitor, ibuprofen. Other potential modulators include platelet-activating factor antagonists.

Other strategies have been investigated to accelerate the resolution of pneumonia after it is acquired and to prevent the development of pneumonia. Passive immunization with hyperimmune anti-pseudomonas immune globulin for patients with documented *P. aeruginosa* pneumonia has failed to result in any benefit in mortality rates or length of hospital stay. The cytokine granulocyte colony-stimulating factor (G-CSF) is a hematopoietic cytokine that enhances both the number and function of circulating neutrophils. Preclinical evaluations in models of infection and results of trials in neutropenic oncologic patients show beneficial effects of G-CSF in hastening the resolution and preventing the acquisition of infections. A recent trial has shown that the administration of G-CSF is safe in patients with community-acquired pneumonia.

Another cytokine of potential utility in preventing pneumonia or accelerating its resolution is interferon-gamma (IFN). While systemically administered IFN is not always effective in models of sepsis or in patients at risk for sepsis, local administration into the lung appears effective in decreasing the burden of infecting organisms and augmenting local host defenses in several animal models of pneumonia. Aerosole administration of IFN to normal humans results in activation of the alveolar macrophage, without concurrent activation of systemic mononuclear cells. IFN remains a potential therapeutic modality for bacterial and nonbacterial pathogens in the lung, and clinical investigations will likely proceed in the near future.

Thus, while there are no proven modalities for immunoprophylaxis of HAP, investigations are ongoing with biologic response modifiers as both preventive and therapeutic strategies in this disease setting. It is possible that single agents or combinations thereof, alone or in conjunction with available antibiotics, may prove effective in reducing the mortality or morbidity of HAP.

Addendum 2

Copied in part from the Centers for Disease Control and Prevention Guideline for Prevention of Nosocomial Pneumonia.

The entire guideline is published as: Tablan OC, Anderson LJ, Arden NH, Breiman RF, Butler JC, McNeil MM, and The Hospital Infection Control Practices Advisory Committee. Guideline for Prevention of Nosocomial Pneumonia. *Infection Control and Hospital Epidemiology* 1994; 15 (9):588-627.

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PART II. RECOMMENDATIONS FOR PREVENTION OF NOSOCOMIAL PNEUMONIA

The Hospital Infection Control Practices Advisory Committee

Introduction

The recommendations are presented according to the etiology of the infection, in the following order: bacterial pneumonia, including Legionnaires' disease; fungal pneumonia (aspergillosis); and virus-associated pneumonia (respiratory syncytial virus (RSV) and influenza infections). Each topic is subdivided according to the following general approaches for nosocomial infection control, as applicable to the infection:

1. staff education and infection surveillance
2. interruption of transmission of microorganisms by eradicating infecting microorganisms from their epidemiologically important reservoirs, and/or preventing person-to-person transmission
3. modifying host risk for infection.

As in previous CDC guidelines, each recommendation is categorized on the basis of existing scientific evidence, theoretical rationale, applicability, and economic impact. However, the previous CDC system of categorizing recommendations has been modified as follows:

CATEGORY IA. Strongly recommended for all hospitals and strongly supported by well-designed experimental or epidemiologic studies.

CATEGORY IB. Strongly recommended for all hospitals and viewed as effective by experts in the field and a consensus of HICPAC based on strong rationale and suggestive evidence, even though definitive scientific studies may not have been done.

CATEGORY II. Suggested for implementation in many hospitals. Recommendations may be supported by suggestive clinical or epidemiologic studies, a strong theoretical rationale, or definitive studies applicable to some but not all hospitals.

NO RECOMMENDATION; UNRESOLVED ISSUE. Practices for which insufficient evidence or consensus regarding efficacy exists.

Bacterial pneumonia

I. Staff Education and Infection Surveillance

A. Staff Education

Educate health care workers regarding nosocomial bacterial pneumonias and infection control procedures to prevent their occurrence.

CATEGORY IA

B. Surveillance

1. Conduct surveillance for bacterial pneumonia in ICU patients at high risk for nosocomial bacterial pneumonia (eg, patients with mechanically assisted ventilation, selected postoperative patients) to determine trends and identify potential problems. Include data regarding the causative microorganisms and their antimicrobial susceptibility patterns. Express data as rates (eg, number of infected patients or infections per 100 ICU days or per 1,000 ventilator days) to facilitate intra-hospital comparisons and determine trends.

CATEGORY IA

2. Do not routinely perform surveillance cultures of patients or of equipment or devices used for respiratory therapy, pulmonary-function testing, or delivery of inhalation anesthesia.

CATEGORY IA

II. Interruption of Transmission of Microorganisms

A. Sterilization or Disinfection, and Maintenance of Equipment and Devices

1. General Measures

- a. Thoroughly clean all equipment and devices to be sterilized or disinfected.

CATEGORY IA

b. Sterilize or use high-level disinfection for semicritical equipment or devices, ie, items that come into direct or indirect contact with mucous membranes of the lower respiratory tract. High-level disinfection can be achieved either by wet heat pasteurization at 76°C for 30 minutes or by using liquid chemical disinfectants approved as sterilants/disinfectants by the Environmental Protection Agency and cleared for marketing for use on medical instruments by the Office of Device Evaluation, Center for Devices and Radiologic Health, Food and Drug Administration. Follow disinfection with appropriate rinsing, drying, and packaging, taking care not to contaminate the items in the process.

CATEGORY IB

- c. 1) Use sterile (not distilled, nonsterile) water for rinsing reusable semicritical equipment and devices used on the respiratory tract after they have been chemically disinfected.

CATEGORY IB

2) No recommendation for using tap water (as an alternative to sterile water) to rinse reusable semicritical equipment and devices used on the respiratory tract after they have been subjected to high-level disinfection, whether or not rinsing is followed by drying with or without the use of alcohol.

UNRESOLVED ISSUE

- d. Do not reprocess an equipment or device that is manufactured for single use only, unless data show that reprocessing the equipment or device poses no threat to the patient, is cost-effective, and does not change the structural integrity or function of the equipment or device.

CATEGORY IB

2. Mechanical Ventilators, Breathing Circuits, Humidifiers and Heat-Moisture Exchangers

a. Mechanical Ventilators

Do not routinely sterilize or disinfect the internal machinery of mechanical ventilators.

CATEGORY IA

b. Ventilator Circuits with Humidifiers

- 1) Do not routinely change more frequently than every 48 h the breathing circuit, including tubing and exhalation valve, and the attached bubbling or wick humidifier of a ventilator that is in use on an individual patient.

CATEGORY IA

- 2) No recommendation for the maximum length of time after which the breathing circuit and the attached bubbling or wick humidifier of a ventilator in use on a patient should be changed.

UNRESOLVED ISSUE

- 3) Sterilize reusable breathing circuits and bubbling or wick humidifiers, or subject them to high-level disinfection between their uses on different patients.

CATEGORY IB

- 4) Periodically drain and discard any condensate that collects in the tubing of a mechanical ventilator, taking precautions not to allow condensate to drain towards the patient. Wash hands after performing the procedure or handling fluid.

CATEGORY IB

- 5) No recommendation for placing a filter or trap at the distal end of the expiratory-phase tubing of the breathing circuit to collect condensate.

UNRESOLVED ISSUE

- 6) Do not place bacterial filters between the humidifier reservoir and the inspiratory-phase tubing of the breathing circuit of a mechanical ventilator.

CATEGORY IB

7) Humidifier fluids

- a) Use sterile water to fill bubbling humidifiers.

CATEGORY II

- b) Use sterile, distilled, or tap water to fill wick humidifiers.

CATEGORY II

- c) No recommendation for the preferential use of a closed, continuous-feed humidification system.

UNRESOLVED ISSUE

c. Ventilator Breathing Circuits with Hygroscopic Condenser-Humidifiers or Heat-Moisture Exchangers

1) No recommendation for the preferential use of hygroscopic condenser-humidifier or heat-moisture exchanger rather than a heated humidifier to prevent nosocomial pneumonia.

UNRESOLVED ISSUE

2) Change the hygroscopic condenser-humidifier or heat-moisture exchanger according to manufacturer's recommendation and/or when evidence of gross contamination or mechanical dysfunction of the device is present.

CATEGORY IB

3) Do not routinely change the breathing circuit attached to a hygroscopic condenser-humidifier or heat-moisture exchanger while it is in use on a patient.

CATEGORY IB

3. Wall Humidifiers

a. Follow manufacturers' instructions for use and maintenance of wall oxygen humidifiers unless data show that the modification in their use or maintenance poses no threat to the patient and is cost-effective.

CATEGORY IB

b. Between patients, change the tubing, including any nasal prongs or mask, used to deliver oxygen from a wall outlet.

CATEGORY IB

4. Small-Volume Medication Nebulizers: 'In-line' and Hand-held Nebulizers

a. 1) Between treatments on the same patient, disinfect, rinse with sterile water, or air-dry small-volume medication nebulizers.

CATEGORY IB

2) No recommendation for using tap water as an alternative to sterile water to rinse reusable small-volume medication nebulizers between treatments on the same patient.

UNRESOLVED ISSUE

b. Between patients, replace nebulizers with those that have undergone sterilization or high-level disinfection.

CATEGORY IB

c. Use only sterile fluids for nebulization, and dispense these fluids aseptically.

CATEGORY IA

d. If multidose medication vials are used, handle, dispense, and store them according to manufacturers' instructions.

CATEGORY IB

5. Large-Volume Nebulizers and Mist Tents

a. Do not use large-volume room-air humidifiers that create aerosols (eg, by venturi principle, ultrasound, or spinning disk) and thus are really nebulizers, unless they can be sterilized or subjected to high-level disinfection at least daily and filled only with sterile water.

CATEGORY IA

b. Sterilize large-volume nebulizers that are used for inhalation therapy, eg, for tracheotomized patients, or subject them to high-level disinfection between patients and after every 24 h of use on the same patient.

CATEGORY IB

c. 1) Use mist-tent nebulizers and reservoirs that have undergone high-level disinfection and replace them between patients.

CATEGORY IB

2) No recommendation regarding the frequency of changing mist-tent nebulizers and reservoirs while in use on one patient

UNRESOLVED ISSUE

6. Other Devices Used in Association with Respiratory Therapy

a. Between patients, sterilize or subject to high-level disinfection portable respirometers, oxygen sensors, and other respiratory devices used on multiple patients.

CATEGORY IB

b. 1) Between patients, sterilize or subject to high-level disinfection reusable hand-powered resuscitation bags (for example, Ambu bags).

CATEGORY IA

2) No recommendation regarding the frequency of changing hydrophobic filters placed on the connection

port of resuscitation bags.

UNRESOLVED ISSUE

7. Anaesthesia Machines, and Breathing Systems or Patient Circuits

- a. Do not routinely sterilize or disinfect the internal machinery of anesthesia equipment.

CATEGORY IA

b. Clean and then sterilize or subject to high-level liquid chemical disinfection or pasteurization reusable components of the breathing system or patient circuit (eg, tracheal tube or face mask; inspiratory and expiratory breathing tubing; y-piece; reservoir bag; humidifier and tubing) between uses on different patients, by following the device manufacturers' instructions for their reprocessing.

CATEGORY IB

c. No recommendation for the frequency of routinely cleaning and disinfecting unidirectional valves and carbon dioxide absorber chambers.

UNRESOLVED ISSUE

d. Follow published guidelines and/or manufacturers' instructions regarding in-use maintenance, cleaning, and disinfection or sterilization of other components or attachments of the breathing system or patient circuit of anesthesia equipments.

CATEGORY IB

e. Periodically drain and discard any condensate that collects in the tubing of a breathing circuit, taking precautions not to allow condensate to drain towards the patient. After performing the procedure or handling fluid, wash hands with soap and water or with a waterless handwashing preparation.

CATEGORY IB

f. No recommendation for placing a bacterial filter in the breathing system or patient circuit of anesthesia equipment.

UNRESOLVED ISSUE

8. Pulmonary-Function Testing Equipment

a. Do not routinely sterilize or disinfect the internal machinery of pulmonary-function testing machines between uses on different patients.

CATEGORY II

b. Sterilize or subject to high-level liquid chemical disinfection or pasteurization reusable mouthpieces and tubings or connectors between uses on different patients, or follow manufacturers' instructions for their reprocessing.

CATEGORY IB

B. Interruption of Person-to-Person Transmission of Bacteria

1. Handwashing. Wash hands after contact with mucous membranes, respiratory secretions, or subjects contaminated with respiratory secretions, whether or not gloves are worn. Wash hands before and after contact with a patient who has an endotracheal or tracheostomy tube in place, and before and after contact with any respiratory device that is used on the patient, whether or not gloves are worn.

CATEGORY IA

2. Barrier Precautions

a. Wear gloves for handling respiratory secretions or objects contaminated with respiratory secretions of any patient.

CATEGORY IA

b. Change gloves and wash hands between patients; after handling respiratory secretions or objects contaminated with secretions from one patient and before contact with another patient, object, or environmental surface; and between contacts with a contaminated body site and respiratory tract of, or respiratory device on, the same patient.

CATEGORY IA

c. Wear a gown when soiling with respiratory secretions from a patient is anticipated, and change the gown after such contact and before providing care to another patient.

CATEGORY IB

3. Care of Patients with Tracheostomy

a. Perform tracheostomy under sterile conditions.

CATEGORY IB

b. When changing a tracheostomy tube, use aseptic technique and replace the tube with one that has undergone sterilization or high-level disinfection.

CATEGORY IB

4. Suctioning of Respiratory Tract Secretions

a. No recommendation for wearing sterile rather than clean gloves when suctioning a patient's respiratory secretions.

UNRESOLVED ISSUE

b. If the open suction system is employed, use a sterile single-use catheter.

CATEGORY II

c. Use only sterile fluid to remove secretions from the suction catheter if the catheter is to be used for re-entry into the patient's lower respiratory tract.

CATEGORY IB

d. No recommendation for preferential use of the multi-use closed-system suction catheter or the single-use open-system catheter for prevention of pneumonia.

UNRESOLVED ISSUE

e. Change suction collection tubing (up to the canister) between patients.

CATEGORY IB

f. Change suction collection canisters between uses on different patients except when used in short-term care units.

CATEGORY IB

III. Modifying Host Risk for Infection

A. Precautions for Prevention of Endogenous Pneumonia

Discontinue enteral-tube feeding and remove devices such as endotracheal, tracheostomy, and/or enteral (i.e., oro- or naso-gastric, or jejunal) tubes from patients as soon as the clinical indications for these are resolved.

CATEGORY IB

1. Prevention of Aspiration Associated with Enteral Feeding

a. If there is no contraindication to the manoeuvre, elevate at an angle of 30-45° the head of the bed of a patient at high risk of aspiration pneumonia, eg. a person receiving mechanically assisted ventilation and/or has an enteral tube in place.

CATEGORY IB

b. Routinely verify appropriate placement of the feeding tube.

CATEGORY IB

c. Routinely assess the patient's intestinal motility (eg. by auscultating for bowel sounds and measuring residual gastric volume or abdominal girth) and adjust the rate and volume of enteral feeding to avoid regurgitation.

CATEGORY IB

d. No recommendation for the preferential use of small-bore tubes for enteral feeding.

UNRESOLVED ISSUE

e. No recommendation for administering enteral feeding continuously or intermittently.

UNRESOLVED ISSUE

f. No recommendation for preferentially placing the feeding tubes, eg. jejunal tubes, distal to the pylorus.

UNRESOLVED ISSUE

2. Prevention of Aspiration Associated with Endotracheal Intubation

a. No recommendation for using oro-tracheal rather than naso-tracheal tube to prevent nosocomial pneumonia.

UNRESOLVED ISSUE

b. No recommendation for routinely using an endotracheal tube with a dorsal lumen above the endotracheal cuff to allow drainage (by suctioning) of tracheal secretions that accumulate in the patient's subglottic area.

UNRESOLVED ISSUE

c. Before deflating the cuff of an endotracheal tube in preparation for tube removal, or before moving the tube, ensure that secretions are cleared from above the tube cuff.

CATEGORY IB

3. Prevention of Gastric Colonization

a. If stress-bleeding prophylaxis is needed for a patient with mechanical assisted ventilation, use an agent that does not raise the patient's gastric pH.

CATEGORY II

b. No recommendation for selective decontamination of a critically ill, mechanically ventilated, or ICU

patient's digestive tract with oral and/or intravenous antimicrobials to prevent Gram-negative bacillary (or *Candida* spp.) pneumonia.

UNRESOLVED ISSUE

- c. No recommendation for routine acidification of gastric feeding to prevent nosocomial pneumonia.

UNRESOLVED ISSUE

B. Prevention of Postoperative Pneumonia

1. Instruct preoperative patients, especially those at high risk of developing pneumonia, regarding frequent coughing, taking deep breaths, and ambulating as soon as medically indicated in the postoperative period. High-risk patients include those who will receive anaesthesia, especially those who will have abdominal, thoracic, head, or neck operation, or who have substantial pulmonary dysfunction, such as patients with chronic obstructive lung disease, a musculoskeletal abnormality of the chest, or abnormal pulmonary function tests.

CATEGORY IB

2. Encourage postoperative patients to cough frequently, take deep breaths, move about the bed, and ambulate unless it is medically contraindicated.

CATEGORY IB

3. Control pain that interferes with coughing and deep breathing during the immediate postoperative period by using systemic analgesia, including patient-controlled analgesia, with as little cough-suppressant effect as possible; appropriate support for abdominal wounds, such as tightly placing a pillow across the abdomen; or regional (eg, epidural) analgesia.

CATEGORY IB

4. Use an incentive spirometer or intermittent positive pressure breathing on patients at high risk of developing postoperative pneumonia.

CATEGORY II

C. Other Prophylactic Procedures for Pneumonia

1. **Vaccination of Patients.** Vaccinate patients at high risk for complications of pneumococcal infections with pneumococcal polysaccharide vaccine. High-risk patients include ≥ 65 yr old; adults with chronic cardiovascular or pulmonary disease, diabetes mellitus, alcoholism, cirrhosis, or cerebrospinal fluid leaks; and children and adults with immunosuppression, functional or anatomic asplenia, or HIV infection.

CATEGORY IA

2. **Antimicrobial Prophylaxis.** Do not routinely administer systemic antimicrobial agents to prevent nosocomial pneumonia.

CATEGORY IA

3. Use of rotating 'kinetic' beds or continuous lateral rotational therapy

No recommendation for the routine use of 'kinetic' beds or continuous lateral rotational therapy (ie, placing patients on beds that turn on their longitudinal axes intermittently or continuously) for prevention of nosocomial pneumonia in patients in the ICU, critically ill patients, or patients immobilized by illness and/or trauma.

UNRESOLVED ISSUE

Indications for antibiotic use in ICU patients: a one-year prospective surveillance

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Abstract

Background

The high prevalence of nosocomial infections in critically ill ICU patients is associated with high antibiotic consumption. Besides its economic impact, there is the constant threat of selection and induction of antibiotic resistance. Surveillance studies recording the incidence of infections, antibiotic use, and antimicrobial susceptibilities of pathogens supply vital information regarding infection control and prevention of antibiotic resistance.

Methods

In order to analyze antibiotic consumption we recorded antibiotic use in a general ICU during one year by categorizing the indications for antibiotic use into three groups: (I) prophylaxis; (II) therapy for a bacteriologically proven infection (BPI); (III) therapy for a non-bacteriologically proven infection (non-BPI). Bronchoscopic techniques were used to diagnose pneumonia. In practice, BPI must be treated, but a proportion of antibiotics prescribed for non-BPI may be unnecessary. The subdivision in BPI and non-BPI may help to identify these cases.

Results

In all, 515 patients were admitted to ICU and 36% of these had at least one infection. Of all infections, 53% were ICU-acquired and 99% of these occurred in intubated patients. Antibiotics were prescribed in 61% of admissions. Of all antibiotics prescribed for therapy, 49% were for respiratory tract infections, 19% for abdominal infections and 13% for sepsis *e.c.i.*. Categorized by indication, 59% of all antibiotic prescriptions were for BPI, 28% for non-BPI, and 13% for prophylaxis. A theoretical reduction of 25% in the number of non-BPI prescriptions would result only in a 7% decrease of total antibiotic use.

Conclusions

We conclude that almost all antibiotics prescribed were for intubated patients and for BPI. Respiratory infections were the single most common infection and accounted for 49% of all antibiotics used. Therefore, in our setting, prevention of respiratory tract infections is probably the most effective mode to reduce antibiotic use.

Introduction

The last decade has witnessed the increasing problem of antibiotic resistance as a consequence of excessive or inappropriate antibiotic use. Indeed, contemporary literature has addressed the doom scenario of 'the post-antimicrobial era'¹.

Collecting data on motives for antibiotic use is the first step in managing this problem. Therefore, surveillance studies regarding the incidence of infections, antibiotic prescription policies and knowledge about antimicrobial susceptibilities of pathogens are crucial¹⁻⁵.

Critically ill patients treated in ICUs frequently have an infection or are prone to develop new infections. As a corollary, the total antibiotic consumption is approximately ten times greater in ICU wards than in general hospital wards⁶. A considerable part of total hospital antibiotic consumption and cost are for antibiotics use, in ICU^{3,4}. Data from the National Nosocomial Infection Surveillance System (NNISS) describe 'rough' changes in epidemiology and antibiotic susceptibility of pathogens in US hospitals over a 20 year period, while the European Prevalence of Infection in Intensive Care study (EPIC) determined prevalence of infections, antibiotic use, and susceptibilities of isolated pathogens in a continent-wide point-prevalence study⁵. Only few detailed longitudinal surveillance studies exist on infections and antibiotic use in ICUs^{6,7}. As a result, little is known about the indications for and costs of antibiotic therapy for different patient populations within a general ICU. We, therefore, prospectively analyzed antibiotic use and related costs in a general ICU ward over a one-year period. Special emphasis was given to the indications for antibiotic use, which were divided into bacteriologically proven infections, non-bacteriologically proven infections, and prophylactic use.

Patients and methods

Setting

The University Hospital Maastricht is a 700-bed facility. Critically ill medical, surgical, neurological/neurosurgical and trauma patients requiring haemodynamic monitoring and/or mechanical ventilation are admitted to one of two similar general ICUs. The total capacity is 15 beds of which two are reserved for paediatric patients. Coronary-care patients and patients recovering from cardiac surgery are cared for in other separate units as are neonates. The ICU is managed by a multidisciplinary staff (internal medicine, surgery, anaesthesiology), with daily assistance from the departments of medical microbiology, pulmonology and radiology. During the study period no changes in the composition of the medical staff occurred.

Data collection

Study population

From January 1, 1994 through December 31, 1994 all adult patients (age > 15 years), ventilated or nonventilated, who had been admitted to ICU were studied prospectively. On admission the following data were recorded: age, sex, medical specialty, pre-existent diseases, APACHE II-score⁸, and length of hospital stay before admission to the ICU. Subsequently, the number of days in ICU, number of days on mechanical ventilation, surgical procedures, type of nutrition and mortality were monitored. During ICU stay, parameters of infection (temperature, leucocyte count and differentiation, X-ray results, culture results) and types and doses of prescribed antibiotics, with the exception of topical applications, were registered daily. Selective decontamination of the digestive tract (SDD) was not used during the study period.

Microbiology

Semi-quantitative and quantitative microbiological analysis of samples collected for culture were performed according to standard microbiological methods^{9,10}. Besides the cultures performed of samples that were collected on indication, all patients had routine urine and sputum cultures taken twice weekly (monday and thursday). Antibiotic susceptibility was determined by means of a microbroth dilution method according to the NCCLS guidelines. *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and ATCC 35218, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as reference strains. The criteria for susceptibility and resistance, according to the NCCLS guidelines, were used to determine the percentages of strains susceptible to the most frequently used agents.

Data analysis

All collected data were analyzed by one investigator according to the definitions described below. The multidisciplinary staff of the ICU wards were not informed of the study to prevent bias.

Definitions

Infections

The Centers for Disease Control definitions for surveillance of nosocomial infections, were used¹¹. However, an additional classification was made based upon the culture results as described below in order to categorize infections and associated antibiotic use in groups in which a clinical suspicion of infection was or was not microbiologically confirmed. More detailed criteria were used for pneumonia (see below). Infections occurring within 48 h of admission to ICU were considered non-ICU-acquired (i.e. community- or hospital-acquired). Infections that were clinically suspected and diagnosed more than 48 h after admission to ICU were considered to be ICU-acquired.

A clinical suspicion of infection was based on the following infection parameters: (I) temperature $>38^{\circ}\text{C}$ or $<35.5^{\circ}\text{C}$; (II) leukocytosis $>10 \times 10^3/\text{mm}^3$ with or without left-shift in differential count or leukopenia $<3 \times 10^3/\text{mm}^3$. In addition, clinical findings such as a new or progressive infiltrate on chest X-ray, cloudy urine, purulent sputum or phlebitis in the absence of above mentioned criteria were considered indicative of possible infection. If a clinical suspicion of infection was present, samples of the suspected site of infection (urinary tract, respiratory tract, blood, wound drainage, ascites, cerebrospinal fluid, catheters etc.), were taken for culture. Depending on the culture results, the infections were divided into two groups: (I) non-bacteriologically proven infection (non-BPI): all cases of clinical suspicion of infection, with negative or nonsignificant bacterial culture results, but necessitating antibiotic therapy in view of the clinical condition of the patient; (II) bacteriologically proven infection (BPI): all cases of clinical suspicion of infection with significant culture results of the samples collected from the suspected infection site that were treated with antibiotics.

Decisions regarding the presence of a clinical suspicion of infection, the orders to take samples for culture, the interpretation of culture results, and the institution of antibiotic therapy were made by the primary care team assisted by the department of medical microbiology.

Pneumonia

When pneumonia was clinically suspected, bronchoscopy with protected specimen brush and bronchoalveolar lavage were performed as described elsewhere¹². The diagnosis of pneumonia was established according to the criteria listed in Table 3.1. When criteria A and B were met it was considered a non-bacteriologically proven pneumonia and the infection regarded as non-BPI. When criteria A, B and C were met, pneumonia was bacteriologically proven and the infection considered to be a BPI.

Antibiotic use

Antibiotic use was analyzed either in prescriptions or in courses. The term 'prescription' was used to describe the uninterrupted use of a single antibiotic. A 'course' was defined as an episode of clinical or suspected infection in which antibiotics, either consecutively or in combination, were prescribed.

Antibiotic consumption was analyzed in prescribed daily doses (PDD) or as PDD per 100 bed days⁶. The costs of antibiotic therapy were calculated using the prices per PDD paid by the hospital pharmacy, including the costs of monitoring serum antibiotic concentrations for aminoglycosides and vancomycin (average three times weekly).

The indications for antibiotic use were categorized into three groups: (I) prophylaxis, either for a surgical procedure or endocarditis; (II) therapy for a BPI; (III) therapy for a non-BPI.

Table 3.1 Criteria used in the diagnosis of pneumonia

Criteria	
A	≥ 3 positive of the following 4: <ol style="list-style-type: none">1. rectal temperature above 38.0°C or below 35.5°C2. blood leukocytosis ($>10 \times 10^3/\text{mm}^3$) and/or left shift or blood leukopenia ($<3 \times 10^3/\text{mm}^3$)3. >10 leukocytes per high-power field in Gram stain of tracheal aspirate4. positive culture from tracheal aspirate
	and
B	new, persistent or progressive infiltrate on chest radiograph
	and
C	≥ 1 positive of the following 3: <ol style="list-style-type: none">1. positive quantitative culture of a sample obtained by BAL (cut-off point $\geq 10^4$ cfu/ml) or PSB (cut-off point $\geq 10^3$ cfu/ml)2. positive blood culture unrelated to another source and obtained within 48 h before and after respiratory sampling3. positive pleural fluid culture in the absence of previous pleural instrumentation

BAL, Bronchoalveolar Lavage; PSB, Protected Specimen Brush.

A + B = non-bacteriologically proven pneumonia. A + B + C = bacteriologically proven pneumonia.

Statistical analysis

Data are expressed as absolute numbers with or without percentages, as means with standard deviation or as medians with ranges. Antibiotic costs are expressed in Dutch florins (Dfl) (100 Dfl approximately equals 60 US\$ and £40 sterling). Frequency comparisons were performed by chi-square test or Fisher's Exact test. A probability value less than 0.05 was considered to denote statistical significance.

Results

Patient characteristics

During 1994, 469 patients were admitted to ICU. Since 30 patients were admitted twice and eight patients three times, the total number of admissions was 515 (Table 3.2).

Infections

Of all patients admitted to ICU, 36% had at least one infection during ICU stay: 19% had ≥ 1 BPI, 13% had ≥ 1 non-BPI and 4% had both BPI and non-BPI.

In all, 179 BPI were observed in 121 patients: 77 (43%) of these originated in the respiratory tract, 29 (16%) were of abdominal origin and 73 (41%) originated from other sites. Of all BPI, 164 (92%) occurred in intubated patients and the majority of these infections ($n=116$, 71%) were ICU-acquired. Among nonintubated patients, 15 BPI were diagnosed of which one was ICU-acquired (Table 3.3).

Table 3.2 Characteristics of the patients

	All (n=515)	Intubated (n=276)	Nonintubated (n=239)
Median age (range)*	61 (16-93)	63 (16-90)	56 (19-93)
Male/Female*	279/190	166/88	113/102
Median APACHE II (range)†	15 (0-50)	18 (2-50)	9 (0-40)
Days on ICU, median (range)	3 (1-75)	7 (1-75)	2 (1-12)
Number of patient bed days (%)	4091	3434 (84%)	657 (16%)
Medical speciality:			
- medical‡	209	107	102
- surgery§	259	137	122
- trauma	47	32	15
Surgical procedures:			
- abdominal surgery	110	90	20
- thoracic surgery	26	23	3
- neurosurgery	97	21	76
Mortality in ICU (%)*	102 (22%)	92 (33%)	10 (4%)
Mortality after ICU discharge (%¶)*	32 (9%)	20 (11%)	12 (5%)

* Second and third admissions not analyzed (first admissions, n=469).

† APACHE II score was determined for 386 admissions.

‡ Including pulmonology, cardiology, neurology and anesthesiology.

§ Including neurosurgery, ENT, cardiopulmonary surgery, gynecology and urology.

¶ Percentage of patients that died after discharge from ICU to hospital ward.

A non-BPI emerged 95 times in 86 patients: in 64 (67%) cases this related to the respiratory tract, in 16 (17%) cases to the abdomen. There were nine (10%) cases of clinical sepsis and six (6%) cases originated from other sites. As with BPI, the majority of non-BPI were seen in intubated patients (n=81, 85%), and 29 (36%) of these were ICU-acquired (Table 3.3). All non-BPI (n=14) seen in nonintubated patients were non-ICU-acquired.

All diagnoses of BPI of the respiratory tract were established on positive quantitative culture results of samples obtained by protected specimen brush and/or bronchoalveolar lavage. Twenty-two patients were treated for pneumonia despite nonsignificant culture results from bronchoscopy. These cases, therefore, did not fulfil the criteria for BPI. Although bronchoscopy is not recommended within 48 h after institution or change of antibiotic therapy, this procedure was, in fact, performed within this time period in nine patients. Despite negative culture results, new antibiotics were prescribed for these patients. Although the remaining 13 patients had not received antibiotics before bronchoscopy and had negative or nonsignificant culture results, their clinical condition necessitated antibiotic therapy in the opinion of the physician in charge. In 42 (66%) cases of a non-BPI of the respiratory tract no bronchoscopy was performed, either because of logistical problems (n=12), or because the infection was considered to be community- or

hospital-acquired (non-ventilator-associated) in which cases we do not routinely perform bronchoscopy ($n=30$). Moreover, a number of these patients are not intubated. During the study period, another 48 bronchoscopies were performed when pneumonia was suspected, but culture results were negative and no antibiotic treatment was instituted. These episodes were not labelled as non-BPI.

Microorganisms and antibiotic susceptibility

Among the isolated microorganisms considered as the causative agent of infection (Table 3.4), Gram-negative bacteria were isolated most frequently (mainly *Pseudomonadaceae* and *E. coli*).

All isolates cultured during 1994 from samples from both ICUs were analyzed to determine the percentage of isolates of *P. aeruginosa*, *E. coli*, *S. aureus*, and *E. faecalis* that were susceptible to the tested antibiotics. All strains of *S. aureus* were susceptible to methicillin and flucloxacillin, and all isolates of *E. faecalis* were susceptible to amoxicillin and vancomycin. Of the Gram-negative microorganisms, all isolates of *E. coli* were susceptible to ceftazidime, ciprofloxacin and gentamicin and >90% were susceptible to cefuroxime and co-amoxiclav. More than 95% of *P. aeruginosa* isolates were susceptible to gentamicin, piperacillin, ceftazidime and ciprofloxacin; all isolates were susceptible to imipenem.

Indications for Antibiotics

Antimicrobial therapy was prescribed in 312 (61%) of 515 admissions. Among intubated patients, 74% received antibiotics as compared with 45% of non-intubated patients (chi-square test, $p<0.00001$).

In all, 710 prescriptions of antibiotics were recorded; 574 (81%) prescriptions for intubated and 136 (19%) for nonintubated patients. In the intubated patients, 51% of antibiotic prescriptions were used for BPI, as compared with 31% for non-BPI and 18% for prophylaxis. For nonintubated patients these percentages were 19%, 15% and 66% respectively. Moreover, intubated patients, when compared with nonintubated patients, were treated with more prescriptions (means of 3 and 1 per patient, respectively), more courses of antibiotics (means of 2 and 1 per patient, respectively), and for a longer period (means of 11 and 3 d, respectively).

Patients were treated with antibiotics for 2430 of 4091 patient days (59%). Intubated patients received antibiotics for 2145 of 3434 patient days (63%), and nonintubated patients for 285 of 657 patient days (43%) (chi-square test, $p<0.00001$). Of all prescriptions of antibiotics indicated for therapy, 49% were administered for respiratory tract infections, 19% for abdominal infections and 13% for sepsis *e.c.i.*

Respiratory tract infections, the most important site of infections in ICU patients, accounted for 41% of total antibiotic use for BPI and for 63% of antibiotic use for non-BPI.

Table 3.3 Bacteriologically proven and non-bacteriologically proven infections

Sites	Intubated n=276		NonIntubated n=239		All admissions n=515
	Non-ICU-acquired	ICU-acquired	Non-ICU-acquired	ICU-acquired	
Respiratory tract	- BPI - non-BPI	17 32	58 23	2 9	77 64
Abdominal	- BPI - non-BPI	12 12	15 2	2 2	29 16
Sepsis e.c.i.	- BPI - non-BPI	5 6	10 2	2 1	17 9
Tissue (wound, bone etc.)	- BPI - non-BPI	9 -	7 1	3 1	19 2
IV-line-related sepsis	- BPI - non-BPI	- -	11 -	- -	11 -
Central nervous system	- BPI - non-BPI	3 -	1 1	3 1	7 2
Urosepsis	- BPI - non-BPI	1 2	5 -	- -	6 2
Urinary tract	- BPI - non-BPI	1 -	4 -	1 -	6 -
Bacteraemia	- BPI - non-BPI	- -	5 -	- 1	6 -
Endocarditis	- BPI - non-BPI	- -	- -	1 -	1 -
All	- BPI - non-BPI	48 52	116 29	14 14	179 95

BPI, bacteriologically proven infection; non-BPI, non-bacteriologically proven infection.

Table 3.4 Microorganisms isolated according to type and site of infection

Microorganisms	Type of infection		Site of infection		
	BPI	non-BPI	Respiratory	Abdominal	Other
<i>E. faecalis</i>	27	2	6/29	10/29	13/29
<i>S. aureus</i>	33	11	26/44	1/44	17/44
Other <i>Staphylococcus</i> spp.	19	-	-	4/19	15/19
<i>Streptococcus pneumoniae</i>	8	11	18/19	-	1/19
Other <i>Streptococcus</i> spp.	18	2	7/20	4/20	9/20
<i>E. coli</i>	41	10	27/51	15/51	9/51
Other facultative aerobic Gram-negative species	44	7	25/51	11/51	15/51
Pseudomonadaceae	45	14	45/59	4/59	10/59
Others: - Anaerobes	8	-	-	6/8	2/8
- <i>Bacillus cereus</i>	1	-	1/1	-	-
- <i>Corynebacterium</i> spp.	1	-	-	-	1/1
- Cytomegalovirus	1	-	1/1	-	-
- Fungi	6	2	4/8	1/8	3/8
- <i>Haemophilus influenzae</i>	9	10	19/19	-	-
- <i>Legionella</i> spp.	2	-	2/2	-	-
- <i>Neisseria meningitidis</i>	5	-	2/5	-	3/5
No microorganisms isolated	-	45	16/45	15/45	14/45
Number of isolates/total number of isolates	268/337	69/337	183/337	56/337	98/337

BPI, bacteriologically proven infection; non-BPI, non-bacteriologically proven infection.

Types of antibiotics

The five most frequently used antibiotics in BPI (expressed as percentage of total number of prescriptions used) were: co-amoxiclav (26%), aminoglycosides (24%), piperacillin (8%), ciprofloxacin (8%) and glycopeptide antibiotics (6%). For the group of non-BPI the most frequently used antibiotics were quite similar: co-amoxiclav (38%), aminoglycosides (23%), piperacillin (10%), ciprofloxacin (5%) and metronidazole (4%). The five most frequently used antibiotics for prophylaxis were: flucloxacillin (43%), cefuroxime (17%), aminoglycoside antibiotics (14%), co-amoxiclav (13%) and metronidazole (9%). The frequent use of flucloxacillin in this group is due to the routine prophylaxis with this agent in neurosurgical patients.

Total consumption in PDD and costs of antibiotic therapy

Altogether, 3767 PDD were prescribed for a total amount of Dfl 212,823. In intubated patients, 3387 (90%) PDD were prescribed corresponding to Dfl 200,195 (94% of total cost). In nonintubated patients 380 (10%) PDD were prescribed thus accounting for Dfl 12,629 (6% of total cost).

The largest proportion of PDD (58%) were used for BPI, accounting for 68% of all costs for antibiotic use. Approximately 29% of total PDD were prescribed for non-BPI which represented 25% of the total costs. Finally, only 13% of all PDD (7% of all costs) were administered for prophylactic use.

Overall, β -lactam antibiotics accounted for 56% of the usage in PDD, corresponding to 56% of the total cost. The aminoglycoside antibiotics accounted for 21% of the usage in PDD, but corresponded to only 12% of the total cost (Table 3.5).

The antibiotic consumption was 92.1 PDD/100 bed days accounting for 52 Dfl/bed day. Comparison of these ratios for the three groups of patients yielded the following results: 84.9 PDD/100 bed days and 47 Dfl/bed day for medical patients, 100.5 PDD/100 bed days and 56 Dfl/bed day for surgical patients and 80.5 PDD/100 bed days and 52 Dfl/bed day for trauma patients.

Discussion

The results of this one-year longitudinal surveillance show that 36% of patients admitted to a general ICU had at least one infection during their ICU stay. Of all ICU-acquired infections, 99% occurred in intubated patients. As a result, 90% of total antibiotic use and 95% of the costs for antibiotics were spent in this group. Furthermore, the respiratory tract was by far the most important site of infection, accounting for 43% of BPI and 67% of non-BPI. Under the circumstances tested, reduction of antibiotic use may be achieved most successfully by shortening duration of therapy for respiratory tract infections or reducing prescriptions for BPI by means of infection prevention.

Table 3.5 Antibiotic use in prescribed daily doses and cost according to indication

Antibiotics	Prescribed daily doses				Costs in Dutch florins*			
	All	PRO	BPI	non-BPI	All	PRO	BPI	non-BPI
Aminoglycoside antibiotics†	774	67	460	238	26208	2339	16544	7325
β-lactam antibiotics								
- penicillin	50	-	47	3	1601	-	1505	96
- flucloxacillin	283	167	97	19	4687	2766	1606	315
- amoxicillin	61	6	33	22	554	54	300	200
- co-amoxiclav	1094‡	79	585	426	37021‡	2673	19796	14416
- piperacillin	314	6	184	124	35457	678	20777	14002
- co-ticarcav	105	-	72	33	19963	-	13689	6274
- cephalosporins	211	61	115	35	17680	2381	12428	2871
- aztreonam/imipenem	6	-	6	-	1253	-	1253	-
Ciprofloxacin	273	-	215	58	19262	-	15170	4092
Chloramphenicol	35	-	28	7	275	-	220	55
Clindamycin	23	-	18	5	1536	-	1202	334
Cotrimoxazole	31	-	10	21	264	-	85	179
Erythromycin	76	-	30	46	332	-	131	201
Glycopeptide antibiotic†	196	19	173	4	35065	2403	32156	506
Metronidazole	104	51	34	19	749	367	245	137
Rifampin	31	-	29	2	763	-	714	49
Other antimicrobial agents	100	15	60	25	10153	1530	5814	2809
Total PDD and cost	3767	480	2196	1087	212823	15191	143635	53861

PRO, prophylaxis; BPI, bacteriologically proven infection; non-BPI, non-bacteriologically proven infection.

* 100 Dutch florins approximately equals 60 US\$ and £40 sterling.

† The costs for routine serum-antibiotic titer (3/wk) were taken into account.

‡ Co-amoxiclavulanate was prescribed once for unknown reason for 4 d.

The methods used in the present study provide a simple way to analyze incidence and prevalence of infection and the amount of antibiotic use. We feel that this method of registration would be suitable for others to use, both in other intensive care wards, and in general hospital wards. The most important aspect in this regard is, in our opinion, the distinction between BPI and non-BPI. In addition, by using predefined criteria for infection, the impact of different types of infection on antibiotic use and costs can be easily calculated and flaws such as the variations in interpretation of diagnostic criteria for infection, inevitable in large multi-centre studies, are avoided. The EPIC study was performed by questionnaire and the investigators had to rely on the correct use of the CDC definitions for nosocomial infections by physicians of the 1417 participating ICUs^{5,13}. Difficulties in diagnosis of infection in critically ill patients pertain, in particular to respiratory tract infections¹⁴. Moreover, differences between centres regarding antibiotic susceptibilities weaken the relevance of resistance data obtained from a continent-wide study. This is clearly demonstrated by the antibiotic susceptibilities of the pathogens in our ICU: all *S. aureus* strains were methicillin-sensitive, 97% of *P. aeruginosa* strains were susceptible to gentamicin and 100% to imipenem, and all enterococci were susceptible to amoxicillin and vancomycin. The relation between antibiotic use and the emergence of antibiotic resistance has been demonstrated frequently^{15,16}. Our data, therefore, suggest that the relative lack of significant resistance in nosocomial pathogens in our ICU is a result of restrictive antibiotic policy in our hospital for many years.

Despite major differences in study epidemiology we compared several outcome data from our study with those from the EPIC study⁵. In the EPIC study, which was a one-day point-prevalence study, the prevalence of infection was 45%. This probably means that 45% of the patients were treated for infection (at the day of the study). In our study, 36% of the patients had at least one infection during their ICU stay, but were treated for infection on 48% of all patient days, similar to the percentage found in the EPIC study. Infections of the respiratory tract were diagnosed most frequently in both studies, 65% of all infections in the EPIC study and 51% in the present one. Urinary tract infections also are frequently implicated in nosocomial infections^{2,7}. This type of infection was seen in 18% of patients in the EPIC study but only 5% of patients in our study. This difference may be explained by our policy to perform routine bladder irrigation with chlorhexidin 0.02% if a bladder catheter is *in situ* and twice weekly routine replacement of urinary catheters in case of asymptomatic bacteriuria (i.e. positive urinary cultures without clinical evidence of infection). Antimicrobial therapy is instituted only when urine cultures are persistently positive in spite of the above mentioned regimen and clinical signs of infection develop. Another interesting difference between the EPIC study and ours, is the use of cephalosporins. Forty-four per cent of patients on antibiotics received cephalosporins in the EPIC study as compared with only 8% in the present study. The excellent susceptibility patterns of the isolates cultured in our study partly explains this difference.

Many conditions are known to mimic the signs of infection, especially in critically ill patients¹⁷. Consequently, antibiotics will often be prescribed unnecessarily. Although it is clear that BPI must be treated, the distinction between BPI and non-BPI, according to the definitions applied in the present study, may help to identify unnecessary antibiotic use. However, due to delay in obtaining culture results and the possibility of false negative results, we accept that in a proportion of the non-BPI treatment should be given anyway. Reduction in antibiotic use in this group of infections may only be achieved by decreasing the prescriptions. In our setting even an estimated reduction of 25% in antibiotic prescriptions for non-BPI would only result in a 7% decrease in the total number of PDD in ICU and a 6% reduction in total antibiotic costs. Therefore, in our ICU, a significant reduction in antibiotic use could be achieved either by preventing infection or by shortening the duration of antibiotic therapy.

Few data are available on the optimal duration or route of antibiotic therapy for ICU-acquired infections. For example, although the guidelines of the American Thoracic Society advise that community-acquired pneumonia is treated for 7-10 d, no comparative trials on this issue are available¹⁸. Therefore, future studies evaluating the minimal duration of therapy for nosocomial infections may eventually lead to a decrease in antibiotic use.

Prevention of infections has the highest potential to reduce the enormous antibiotic use in ICUs. Surveillance of infection may help to determine the most prevalent infection and the most important pathogens involved. In our ICU, and many other European ICUs, respiratory infections have the highest incidence. The use of more specific criteria to diagnose nosocomial pneumonia, for instance with bronchoscopic techniques, may help to rationalise antibiotic use, although a prospective study addressing this question is not yet available.

Implementation of control of infection measures are critical in order to limit the possibility of cross-infections¹⁹. Many other specific measures have been shown to reduce the incidence of nosocomial pneumonia²⁰. However, their true impact and cost-effectiveness are as yet unclear.

In conclusion, surveillance studies on antibiotic use, indications for prescription, incidence of infection and distinguishing between bacteriologically proven and non-bacteriologically proven infections may help to develop local strategies to reduce antibiotic use. The findings of this study suggest that in our setting prevention of respiratory tract infections has the greatest potential to reduce overall antibiotic use and costs. In addition, studies evaluating the optimal duration of antibiotic therapy may help to reduce unnecessary antibiotic use.

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4

Implementation of bronchoscopic techniques in the diagnosis of ventilator-associated pneumonia to reduce antibiotic use

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Abstract

Background

In intensive care units a large proportion of antibiotics are prescribed for presumed episodes of ventilator-associated pneumonia (VAP). VAP is usually diagnosed on a combination of clinical, radiographic and microbiologic criteria with a high sensitivity but low specificity for VAP. As a result, patients may receive antibiotics unnecessarily. Specificity can be increased by the addition of quantitative cultures of samples of protected specimen brush (PSB) and bronchoalveolar lavage (BAL) to the diagnostic criteria.

Methods

We prospectively analyzed the effects of implementation of PSB and BAL in the diagnosis of VAP on antibiotic prescription. PSB and/or BAL were performed in patients who fulfilled the clinical, radiographic and microbiologic criteria for VAP. Based on quantitative cultures of PSB and/or BAL, patients were categorized in three groups: VAP microbiologically proven (group 1; $n=72$); clinical suspicion of VAP not confirmed microbiologically (group 2; $n=66$); and patients in whom bronchoscopy could not be performed (group 3; $n=17$).

Results

In group 1, antibiotic therapy was instituted empirically in 40 patients (56%) (group 1a) and after obtaining culture results in the other 32 patients (group 1b). Adjustment of therapy, based on culture results, occurred in 14 (35%) patients in group 1a. In group 2, empiric therapy was instituted in 34 (52%) patients (group 2a) and discontinued within 48 h in 17 of them (50%). In group 3, 17 (100%) patients were treated with antibiotics. Among the 66 patients in whom a clinical suspicion of VAP was not confirmed, only 18 (27%) were treated with antibiotics, and antibiotic therapy was withheld in 48 (35%) of 138 patients who underwent bronchoscopy. Withholding of antibiotic therapy had no negative effect on the recurrence of a clinical suspicion of VAP or on mortality rates.

Conclusions

We conclude that addition of bronchoscopic techniques to the criteria for VAP may help to reduce antibiotic use. However, the definite benefits and cost-effectiveness of these techniques should be analyzed in a randomized study.

Introduction

More than 50% of antibiotic use in intensive care units (ICUs) is instituted to treat presumed respiratory tract infections, especially in mechanically ventilated patients¹. Establishing the diagnosis of ventilator-associated pneumonia (VAP), however, is difficult². Generally, a combination of clinical parameters such as fever, leukocytosis, a positive culture of tracheal aspirate and an infiltrate on chest X-ray are used for this purpose. Although the combination of these criteria may have a high sensitivity, specificity is low³. As a result, many patients may unnecessarily receive antibiotics. The addition of quantitative cultures of samples obtained with bronchoscopic techniques, such as protected specimen brush (PSB) and bronchoalveolar lavage (BAL), to these clinical parameters increases specificity and may decrease unnecessary antibiotic use.

Although many studies have determined predictive values of positive or negative results from samples obtained by PSB and BAL for the diagnosis of pneumonia in selected groups of ventilated patients, little data is available on the clinical consequences of the implementation of these techniques in daily practice. We prospectively studied whether adding the results of PSB and BAL to the generally used criteria for diagnosing VAP influenced antibiotic prescription by intensive care physicians. In addition, we analyzed some costs associated with diagnosis and antimicrobial therapy of VAP.

Methods

The study was performed in the 16-bed general ICU of the University Hospital Maastricht. All patients admitted between January 1 and December 31, 1994 were included. Demographic characteristics and APACHE II score were recorded on admission, and clinical parameters were followed daily. All episodes of infection and antibiotic use were registered prospectively.

In mechanically ventilated patients, a clinical suspicion of pneumonia was defined by the criteria listed in table 4.1. These clinical, microbiologic and radiographic criteria were used as an indication to perform bronchoscopy with PSB and BAL as described elsewhere⁴. Depending on the results of bronchoscopy, the cases of a clinical suspicion of pneumonia were divided into three groups. Group 1 contained those patients for whom the clinical suspicion of pneumonia was microbiologically proven (i.e., when quantitative cultures of PSB yielded $\geq 10^3$ cfu/ml and/or of BAL yielded $\geq 10^4$ cfu/ml). Group 2 contained those patients in whom the clinical suspicion of pneumonia was not confirmed (i.e., when the quantitative cultures of PSB and BAL were sterile or below the above mentioned cut-off points). Finally, group 3 contained those patients that had not undergone bronchoscopy.

Table 4.1 Criteria of a clinical suspicion of pneumonia

Criteria	
A	<p>≥ 3 positive of the following 4:</p> <ol style="list-style-type: none"> 1. rectal temperature above 38.0°C or below 35.5°C 2. blood leukocytosis ($>10 \cdot 10^9/\text{mm}^3$) and/or left shift or blood leukopenia ($<3 \cdot 10^9/\text{mm}^3$) 3. >10 leukocytes per high-power field in Gram stain of tracheal aspirate 4. positive culture from tracheal aspirate <p>and</p>
B	new, persistent or progressive infiltrate on chest radiograph

The diagnosis of a clinical suspicion of pneumonia was made when the criteria A and B were were positive.

Antibiotic therapy was prescribed by the responsible attending critical care physician, and was in no way dictated by protocol. However, the choice of compounds was guided by the hospital formulary. In general, the results of Gram staining of samples obtained by BAL were available within 2 h after bronchoscopy, preliminary culture results were available after 24 h and define culture results with *in vitro* susceptibility testing results were available after 48 h. Empirical therapy was directed towards pathogens isolated from clinical and surveillance cultures of tracheal aspirates, and patients with VAP were treated with antibiotics for 2 wk. Surveillance cultures of tracheal aspirates were obtained on admission and twice weekly from all patients. In case of a suspicion of VAP, clinical cultures from tracheal aspirates were obtained unless surveillance cultures had been taken within 24 h. In the follow-up of each patient, the duration of antibiotic therapy and the recurrence of a new clinical suspicion of pneumonia was registered.

In case of a clinical suspicion of pneumonia, antibiotic therapy was only instituted after bronchoscopy had been performed. When a patient was receiving antibiotics >48 h for another unrelated infection or for prophylaxis on the day that the criteria for a clinical suspicion of pneumonia were met, antibiotics were not changed until bronchoscopy.

Antibiotic consumption was analyzed in prescribed daily doses (PDD). The costs of antibiotic therapy were calculated using the prices per PDD paid by the hospital pharmacy, including the costs of monitoring serum antibiotic concentrations for aminoglycosides and vancomycin (average three times weekly). The costs of bronchoscopy as used in this study (\$453.00) (1 United States dollar = 1,70 Dutch florin), were the actual costs calculated by the hospital and included physician's charges and costs for material and microbiologic analysis.

Data are presented as mean values $\pm$ SD and analyzed by the chi-square test for categorical variables and Student's *t* test for parametric continuous variables.

Results

Study population

A total of 469 patients (515 admissions) were admitted to the ICU during the study period, and 274 (58%) patients were intubated and mechanically ventilated for a mean duration of 13 d. The majority of the patients were admitted because of medical ($n=182$, ventilated $n=89$) or surgical ($n=197$, ventilated $n=140$) reasons. The criteria for a clinical suspicion of pneumonia were met in 155 patients. Bronchoscopy was performed in 138 cases: BAL was done in 126 episodes, PSB in 104 cases; both procedures were done in 92 cases. No bronchoscopy was performed in 17 episodes, because of medical contraindications such as hypoxemia despite ventilation with high positive end expiratory pressure (PEEP) and high FiO_2 ($n=6$), critical clinical situation ($n=5$), neurological objections ($n=2$), coagulopathy ($n=1$), or technical problems ($n=3$). The mean APACHE II scores on admission were comparable for patients in each of the three groups: 19 ± 6 for group 1, 19 ± 7 for group 2 and 18 ± 6 for group 3 ($p=\text{NS}$).

Microbiologic results

Group 1 (patients in whom the clinical suspicion of pneumonia was microbiologically confirmed) contained 72 patients. These episodes of VAP were polymicrobial in 30 cases and were caused by *Pseudomonas aeruginosa* ($n=30$), *Staphylococcus aureus* ($n=18$), Enterobacteriaceae ($n=44$), Streptococci ($n=7$), *Haemophilus influenzae* ($n=7$), and *Xanthomonas maltophilia* ($n=1$). In 42 patients (58%), culture results of tracheal aspirates and bronchoscopy were concordant (e.g., the same species of microorganisms were isolated from tracheal aspirates and bronchoscopic samples); in 19 patients (26%), more species of bacteria were isolated from tracheal aspirates than from bronchoscopic samples and in 11 patients (15%), species of bacteria were isolated at concentrations above the threshold for VAP from bronchoscopic samples that had not been cultured from tracheal aspirates. Twenty patients (28%) were on antibiotics when bronchoscopy was performed.

Group 2 (patients in whom the clinical suspicion was not confirmed microbiologically) contained 66 patients. In 35 patients (53%), culture results from tracheal aspirates and bronchoscopic samples were concordant; in 24 patients (36%), more species of bacteria were recovered from tracheal aspirates than from bronchoscopy; and in seven patients (11%), bacteria were isolated from bronchoscopic samples that had not been cultured in tracheal aspirates. The diagnosis of VAP was not confirmed microbiologically because no bacteria grew from bronchoscopic samples ($n=37$, 56%) or because quantitative culture results did not reach the predefined cut-off points ($n=29$, 44%). Six patients without bacterial growth in previously obtained cultures of tracheal aspirates had nonsignificant bacterial growth in bronchoscopic samples. Twenty-eight patients (42%) were on antibiotics when bronchoscopy was performed, but in all cases for >48 h.

Antibiotic use

In group 1, empiric antibiotic therapy was instituted directly after bronchoscopy in 40 (56%) patients (group 1a) and, in the remaining 32 patients antibiotics were started after culture results were available (group 1b) (Figure 4.1). Antibiotic therapy was adjusted after culture results of bronchoscopy were available in 21 (53%) of 40 patients in group 1a. In 11 patients, the spectrum of empiric antibiotic therapy, which was based on the bacteria isolated from previously obtained tracheal aspirates, could be narrowed. The reasons to adjust empiric antibiotic therapy in the remaining ten patients were as follows: the occurrence of line sepsis at day 2 ($n=2$); abdominal sepsis at day 4 ($n=1$); a drug-induced rash ($n=1$); the choice of an agent with better penetration because the patient also had intra-abdominal abscesses ($n=1$); colonization of tracheal aspirates with a new pathogen at day 5 ($n=1$); clinical deterioration of the patient despite *in vitro* susceptibility for the empirical treatment of the pathogen causing VAP ($n=1$); and *in vitro* resistance to the empiric antibiotic therapy ($n=1$). Finally, in two patients significant numbers of a pathogen that had not been isolated from tracheal aspirates were isolated from bronchoscopic samples and these pathogens were not covered by empirical therapy. Therefore, the changes in antibiotics did reflect a correction of inappropriate empirical antibiotic therapy in three patients. As a result, in patients in whom VAP was microbiologically confirmed by quantitative cultures from bronchoscopic samples, antibiotic therapy was adjusted because of these culture results in 14 patients (19%); by narrowing of the antibiotic spectrum in 11 patients; by adjusting therapy because of a pathogen not isolated from tracheal aspirates in two patients and because of resistance to empirical therapy in another patient.

In patients in group 2, empiric antibiotic therapy was instituted directly after bronchoscopy in 34 (52%) of 66 patients (group 2a), and antibiotic therapy was discontinued within 48 h (when culture results were available) in 17 patients (Figure 4.1). The decision not to discontinue antibiotic therapy in the other 17 patients was made by the attending critical care physicians. In all cases, there was a high clinical suspicion of VAP, and in two patients, this decision was possibly influenced by a concomitant abdominal source of infection. No antibiotic therapy was instituted in 32 patients (group 2b). However, in one of these patients, antibiotic therapy was started 2 d after bronchoscopy because of a deteriorating clinical condition of the patient, despite nonsignificant culture results. This patient was treated with antibiotics for 7 d with a good clinical response and was discharged from ICU 15 d after initiating of therapy. Antibiotic therapy was instituted in 17 (100%) patients in group 3.

Figure 4.1 Number of patients who underwent bronchoscopy, in whom the clinical suspicion of VAP was confirmed or not, and in whom antibiotic therapy was instituted or adjusted.

When patients were analyzed with regard to institution of empirical antibiotic therapy, antibiotics were started immediately after bronchoscopy in 74 patients (groups 1a and 2a). The clinical suspicion of VAP was subsequently confirmed in 40 of these patients (group 1a); antibiotics were continued in all of them, and therapy was adjusted in 14 patients (35%). In group 2a (VAP not confirmed) antibiotics were discontinued within 48 h in 17 patients (50%). In 64 patients, the decision to start antibiotics was postponed until culture results of bronchoscopic samples were available (groups 1b and 2b). Among these patients, the diagnosis of VAP was confirmed in 32 patients (group 1b), and all were treated with antibiotics. In group 2b, one patient received antibiotics.

Consequently, among 66 patients in whom a clinical suspicion of pneumonia was not confirmed by bronchoscopic culture results (group 2), 18 (27%) patients received a full course of antibiotic therapy, while 48 patients (73%) did not; 17 were treated for <48 h, and 31 were not treated. Overall, antibiotic therapy was withheld in 48 (35%) of 138 patients that had undergone bronchoscopy (groups 1 and 2) and in none of 17 patients who did not undergo bronchoscopy (group 3) ($p=0.0002$). In addition, in 14 of 72 patients (19%) in whom VAP was confirmed by bronchoscopy, empiric therapy based on previously obtained tracheal aspirates was adjusted. The mean duration of antibiotic therapy in ICU was 10.6 d in group 1, 8.4 d in group 3, and 9.6 d for those patients in group 2 ($n=18$) who were treated despite nonsignificant culture results. The significant difference in duration of antibiotic therapy between groups 1 and 3 ($p<0.05$) was a result of the fact that more patients in group 3 (10 of 17, 58%) were discharged from ICU while still

receiving antibiotics as compared with patients in group 1 (22 of 72, 31%). In group 2, nine (50%) of 18 patients were still treated with antibiotics for the clinical suspicion of pneumonia when discharged from the ICU.

Recurrence of a clinical suspicion of pneumonia and mortality

A second clinical suspicion of pneumonia occurred in seven (15%) of 48 patients in whom antibiotic therapy was withheld because of bronchoscopy results and in 18 (17%) of 107 patients who had been treated with antibiotics ($p=NS$). The duration until the second clinical suspicion of pneumonia was similar for patients who had or had not received antibiotic therapy; 15.8 and 13.1 d respectively ($p=NS$).

Hospital mortality was 35% (17 of 48) in patients in whom antibiotic therapy had been withheld and 39% (42 of 107) in patients who had received antibiotics ($p=NS$). The APACHE II scores on admission were 19 ± 6 for patients who had been treated initially and 18 ± 6 for those in whom antibiotics had been withheld ($p=NS$). There were no significant differences in recurrence rates of clinical episodes of pneumonia or mortality rates between the three study groups (data not shown). For patients in group 1, mortality rates were 50% ($n=7$) for patients in whom initial antibiotic therapy was changed based on bronchoscopic culture results ($n=14$), 35% ($n=9$) for those with no change of therapy ($n=26$), and 47% ($n=15$) for those who received therapy when culture results were available. Statistical comparison of these groups did not approach significance ($p\geq 0.34$). The delay in starting antibiotics in group 1b did not influence outcome: The mortality rate in this group was 47%, as compared with 40% in group 1a ($p=NS$), and the APACHE II scores in both groups were 20 ± 6 and 19 ± 6 , respectively ($p=NS$). For patients in group 2, mortality rates were comparable for patients with no growth in bronchoscopic samples (28%) and those with growth below the cut-off point for VAP (37%) ($p=NS$).

Cost analysis

The mean costs of antibiotic therapy per episode of VAP, calculated from all episodes of pneumonia that were treated were \$549.00, corresponding to \$39.00 per day of treatment. The actual costs of an episode of bronchoscopy (including physicians' charges and microbiologic analysis) were \$453.00. As a result, 48 episodes of suspected VAP were not treated (projected costs: \$26,352.00) at the expense of 138 episodes of bronchoscopy (actual costs: \$62,514.00). The costs of nursing and pharmacy administration as well as the costs of antibiotics that were given for <48 h (total: \$1,481.00) were not included in this analysis.

Under the circumstances tested, the introduction of bronchoscopic techniques would only be cost-effective if the costs of bronchoscopy would be \$191.00, when the proportion of patients in whom therapy would be withheld would increase to 114 (83%) of 138, or when the costs of antibiotic therapy per episode of VAP would be \$1,302.00, corresponding to \$93.00 per day.

Discussion

The results of this study suggest that implementation of bronchoscopic techniques to the criteria for diagnosing VAP may help to reduce antibiotic use in ICUs. In addition, empiric antimicrobial therapy was adjusted in 19% of the patients with proven VAP, in most cases by narrowing the antibiotic coverage. In patients with a clinical suspicion of pneumonia but with nonsignificant quantitative cultures from respiratory samples obtained by bronchoscopic techniques, withholding of antibiotic therapy was not associated with an increased mortality rate or a higher recurrence rate for VAP.

Treatment of respiratory infections accounts for the largest part of antibiotic consumption in ICUs. In a recent one-day point-prevalence study of nosocomial infections in 1,047 ICUs in 17 European countries including 10,038 patients, 44.8% of all patients had one or more infection, mostly nosocomial, and 64.7% of these infections were respiratory tract related¹. On the day of study, 62.3% of all patients received antibiotics; 51% more than one agent¹. However, in that study, bronchoscopic techniques were not used to diagnose respiratory tract infections, which probably resembles the current situation in many ICUs. As a result, the prevalence of respiratory tract infections may have been overestimated, which may have led to unnecessary antibiotic use.

We implemented bronchoscopic techniques in the routine work up of a clinical suspicion of nosocomial pneumonia in ventilated patients. A clinical suspicion of pneumonia was based on a combination of clinical, radiographic and microbiologic criteria, which are generally used in clinical practice. These criteria have a high sensitivity but a low specificity for VAP^{5,6}. Many studies have demonstrated that addition of quantitative culture results of PSB or BAL increase the specificity for VAP in selected patient groups^{2,6,7}. However, few studies prospectively analyzed the effects of implementation of these new diagnostic techniques on clinical practice. The results of the present study show that addition of bronchoscopic techniques to the diagnostic criteria for pneumonia resulted in adjustment of empirical antimicrobial therapy in 19% of patients with bronchoscopically proven episodes of VAP and in withholding of antibiotics in 48 (35%) of 138 patients. However, the study design employed does not allow us to conclude that bronchoscopy alone was responsible for this reduction. For example, in some patients, fulfilling the criteria of pneumonia, the physicians' clinical judgement allowed them to withhold antibiotics, and bronchoscopy confirmed this clinical judgement. Importantly, withholding of antibiotics in these cases did not result in a higher recurrence rate of a clinical suspicion of pneumonia during the same period of admission and was not associated with a higher mortality rate. The absence of differences in mortality was compatible with similar APACHE II scores on admission in these patient groups. On the other hand, there were 17 patients who were continued on antibiotics because of the physicians' clinical judgement, despite negative results from bronchoscopy. In these cases, the responsible

physicians did feel reluctant to discontinue antibiotics. The decision to institute and continue antibiotic therapy was in the hands of the responsible attending physicians and was in no way prescribed by protocol. Indeed, false-negative results of bronchoscopic techniques have been reported and may occur because of prior antibiotic therapy or because of 'borderline' results of quantitative analysis, possibly indicating that the infection is in an early stage⁸.

Our data confirm data reported by Croce and coworkers⁹. They calculated that addition of bronchoscopic techniques for diagnosing VAP could significantly reduce associated charges. In a pilot study of 10 trauma patients, the diagnosis of VAP was rejected in seven patients. Discontinuation of empiric therapy had no negative influence on the clinical condition of these patients and, in that medical center, withholding of complete therapy resulted in net savings of \$17,060.12⁹. In contrast, other investigators reported that addition of BAL for diagnosing VAP did not improve patient outcome¹⁰. In that study, the adequacy of antibiotic therapy, administered before VAP was diagnosed, seemed to be the most important variable influencing outcome: 50 (77%) of 65 patients in which the clinical suspicion of VAP was confirmed by BAL received antibiotics before bronchoscopy was performed. If these antibiotics were adequate for the microorganisms recovered from BAL, mortality was 38%, whereas mortality rates were 91% and 60% when these antibiotics were inadequate or when no antibiotics had been administered, respectively¹⁰. However, since in that study, 77% of patients with VAP received antibiotics before bronchoscopy, and overall mortality was 67% (similar for patients with and without confirmed pneumonia), with 50% of the nonsurvivors dying within 48 h after diagnosis of VAP, it seems difficult to compare the findings in that population to our situation.

Cost-effectiveness is an important criterion for introduction of a new diagnostic test. The present study shows that, in our ICU, addition of bronchoscopic techniques to the generally used criteria for VAP may have helped to reduce antibiotic therapy in 35% of cases. In 17 patients, antibiotics were discontinued because of bronchoscopy results, but, as discussed above, in those cases that empirical antibiotic therapy was withheld (in 31 patients); bronchoscopy just helped to confirm the clinical judgement of the physician. The superficial analysis of costs as performed should not be regarded as an appropriate cost-benefit analysis. However, the results show that a formal cost-benefit analysis will be influenced by variables that will not be the same among different settings. For instance, antibiotic therapy depends on local susceptibilities of microorganisms, with a general rule that more resistant bacteria must be treated with more expensive antibiotics. In our ICU, all strains of *S. aureus* were susceptible to methicillin, extended-spectrum beta-lactamases had not been isolated, and all strains of *P. aeruginosa* were susceptible to piperacillin and gentamicin. Therefore, the calculated costs for therapy in the present study (\$39.00 per day) probably are low when compared with other centers with more resistant pathogens. For example, in

the study of Croce and coworkers performed in the United States, standard empiric therapy (ceftazidime and vancomycin) costs \$221.56 per day⁹. Another important variable is the proportion of 'negative' culture results of bronchoscopic techniques. The incidence of suspected pneumonia, based on the generally used combination of radiographic, clinical and microbiologic criteria, may seem high in our study. However, our study population consisted of patients with a mean duration of ventilation of 13 d and the proportion in which the clinical suspicion was not confirmed (48%) is similar to or lower than those reported by others. Timsit and coworkers studied 112 patients with a clinical suspicion of VAP and this suspicion was confirmed by quantitative cultures from samples obtained by bronchoscopic techniques in 50%¹¹, and Luna and colleagues reported that BAL did not confirm a clinical suspicion of pneumonia in 49%¹⁰. Others, however, excluded VAP after performance of PSB in 71% of the cases⁷, thereby increasing the 'benefit' of bronchoscopy. In addition, the costs for bronchoscopy, although regarded as expensive, may differ between centers. Interestingly, several recent reports suggested good correlations between quantitative culture results of PSB or BAL with quantitative culture results of samples obtained by deep endotracheal aspiration^{12,13} or blind PSB¹⁴. If these cheaper, and less invasive, techniques prove to be equivalent to quantitative cultures of bronchoscopic techniques, they are much more likely to be cost-effective for diagnosing VAP. Besides the financial aspects of diagnosing VAP, aspects like toxic effects of unnecessary prescribed antibiotics and their long-term influence on antibiotic susceptibilities should be considered. On the other hand, complications of bronchoscopy, although occurring infrequently, include a drop in PaO_2 , arrhythmias, pneumothorax, transient progression of pulmonary infiltrates, and fever^{15,16}. No serious adverse effects of bronchoscopy were encountered during the present study.

In conclusion, bronchoscopic techniques may be helpful and safe tools in the diagnosis of VAP. Under the circumstances tested, implementation of these techniques in the routine workup of a clinical suspicion of VAP influenced clinical practice, decreasing the number of patients receiving antibiotic therapy. Withholding antibiotics to these patients did not affect the recurrence rate of a clinical suspicion of pneumonia and was not associated with a higher mortality rate. The clinical impacts and a formal cost-benefit analysis of these new diagnostic tools should be determined in a prospective randomized fashion.

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Risk factors for pneumonia, and colonization of respiratory tract and stomach in mechanically ventilated ICU patients

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Abstract

Background

Ventilator-associated pneumonia (VAP) is a frequent infection among ICU patients. Colonization of the upper respiratory and intestinal tract is assumed to be important in the pathogenesis of VAP. Although a number of risk factor analyses for the development of VAP have been performed, preceding colonization at different body sites has not been included as a risk factor in any of these analyses.

Methods

To further elucidate the pathogenesis of VAP we determined the risk factors for the development of VAP and colonization of the respiratory tract and stomach with enteric Gram-negative bacteria (EGB) and Pseudomonadaceae in 141 patients receiving mechanical ventilation for at least 3 d, using univariate analysis and the Cox proportional hazards model.

Results

VAP was caused by EGB in 14 patients (10%), and by Pseudomonadaceae in 19 patients (13%). The duration of ventilation was a significant risk factor for VAP caused by EGB and Pseudomonadaceae, and for acquired colonization in oropharynx, stomach and trachea with these species. Of 20 other variables, oropharyngeal colonization with EGB on admission (hazard ratio (HR)=4.5) and an infection on admission (HR=2.7) were selected as risk factors for VAP caused by EGB. Acquired colonization with Pseudomonadaceae in oropharynx (HR=5.0) was the most important risk factor for VAP caused by these species. Gastric colonization with EGB or Pseudomonadaceae were no risk factors for VAP. For acquired oropharyngeal colonization with EGB only the duration of ventilation was a risk factor, whereas preceding colonization of the trachea with Pseudomonadaceae and duration of ventilation were risk factors for acquired oropharyngeal colonization with these species. In the Cox model, only the duration of ventilation was significantly related to acquired gastric colonization with EGB. Preceding colonization of the oropharynx and of the trachea with Pseudomonadaceae were risk factors for acquired colonization with these species in the stomach. Twelve patients with VAP (46%) and 38 without VAP (33%) died ($p=0.21$).

Conclusions

Duration of ventilation and colonization of the upper respiratory tract are the most important risk factors for VAP caused by EGB or Pseudomonadaceae.

Introduction

Ventilator-associated pneumonia (VAP) is a frequent infection among intensive care unit (ICU) patients and has been associated with increased morbidity, mortality and a longer hospital stay for survivors of this condition^{1,2}. In the pathogenesis of this infection, gastric colonization with potentially pathogenic microorganisms is assumed to be important³⁻⁵. In this hypothesis, microorganisms initially colonize the stomach, subsequently colonize the upper respiratory tract, and finally are aspirated into the lungs. Gastric colonization may be stimulated by decreased gastric acidity, as a result of stress ulcer prophylaxis or gastric nutrition^{6,7}.

The use of sucralfate, a stress ulcer prophylactic agent which does not influence intragastric acidity, has been associated with lower gastric colonization rates and a lower incidence of VAP, compared with patients receiving antacids and/or H₂-antagonists^{7,8}. However, in a recent randomized, placebo-controlled, double-blind study, we failed to demonstrate differences in incidence of infection, colonization of stomach and respiratory tract, and levels of intragastric acidity between patients receiving either sucralfate or antacids⁹. Moreover, sequential analysis of colonization strongly suggested that the stomach was not an important source of bacteria causing VAP⁹. These results confirmed previously reported data, indicating that the gastropulmonary route of colonization may not be as important in the pathogenesis of VAP as generally suggested¹⁰⁻¹².

A risk factor analysis of the data, obtained from patients who have been entered in the previously mentioned double-blind study, may provide additional information about the pathogenesis of VAP. Risk factors analyses for the development of VAP and mortality in ICU patients have been performed before¹³⁻¹⁵. Unfortunately, however, preceding colonization at different sites has not been included as a risk factor in any of these analyses. Moreover, the outcomes of these studies may have been flawed by inaccuracies in diagnosis. In all studies the diagnosis was based on a combination of clinical parameters and chest radiographs¹³⁻¹⁵. Therefore, erroneous diagnoses might have occurred in approximately 30% of cases¹⁶.

In order to further elucidate the pathogenesis of colonization and infection of the respiratory tract in mechanically ventilated patients, we studied risk factors for the development of colonization of stomach, oropharynx and trachea, the development of VAP with enteric Gram-negative bacteria (EGB) and Pseudomonadaceae, and death in univariate and multivariate analysis in patients receiving mechanical ventilation for at least 3 d. The results of the comparative study between sucralfate and antacids have been published recently⁹.

Methods

Adult mechanically ventilated patients ($n=141$) admitted to the ICU of the University Hospital Maastricht between August 1992 and August 1993 were included in this analysis. All patients were ventilated for at least 48 h and were prospectively followed. Because of predefined criteria⁹, patients were not eligible for this study in case of massive gastric haemorrhage or bleeding oesophageal varices, after oesophageal or gastric surgery, or when receiving H_2 -antagonists or $H^+K^+ATPase$ inhibitors. Patients were randomly allocated to receive either sucralfate (1 g every 4 h) or antacids (30 ml every 6 h)⁹. Samples of gastric and tracheal aspirates and oropharyngeal swabs were collected on admission and subsequently twice weekly. Additional tracheal aspirates were collected and analyzed as required by the attending physician. The attending physician was unaware of the culture results of oropharyngeal and gastric samples, and the results of pH monitoring. Patients were studied until extubation, death, or the institution of therapy influencing gastric acidity. In general, all patients were in supine position during controlled mechanical ventilation and if possible in semirecumbent position during weaning. There were no changes in body position during the course of the day, and promotility agents were not used.

Informed consent was obtained from the patient or, if this was not possible because of the clinical condition, from a representative of the family. The study protocol was approved by the hospital ethical committee.

Definitions

Ventilator-associated pneumonia

In case of clinical suspicion of pneumonia, bronchoscopy with protected specimen brush (PSB) and bronchoalveolar lavage (BAL) was performed as described elsewhere¹². The diagnosis of VAP was established on the criteria listed in table 5.1. Episodes of pneumonia diagnosed within the first 24 h of admission were considered present on admission.

Colonization

Colonization was defined as the isolation of EGB or Pseudomonadaceae from gastric aspirate, oropharyngeal swab, or tracheal aspirate, in the absence of infection. Colonization on admission was established by a positive culture obtained within 24 h. Colonization was designated as 'acquired' if at least two positive cultures from consecutive samples yielded the same species, without colonization with that species on admission. Patients colonized on admission with one species of EGB might acquire colonization with another species of EGB during the study. Since the group of Pseudomonadaceae consisted almost entirely of *Pseudomonas aeruginosa*, patients colonized with this species on admission were excluded for acquired colonization with Pseudomonadaceae.

Table 5.1 Criteria used for the diagnosis of ventilator-associated pneumonia

Criteria	
A	≥ 3 positive of the following 4: <ol style="list-style-type: none"> 1. rectal temperature above 38.0°C or below 35.5°C 2. blood leukocytosis ($>10 \cdot 10^3/\text{mm}^3$) and/or left shift or blood leukopenia ($<3 \cdot 10^3/\text{mm}^3$) 3. >10 leukocytes per high-power field in Gram stain of tracheal aspirate 4. positive culture from tracheal aspirate
	and
B	new, persistent or progressive infiltrate on chest radiograph
	and
C	≥ 1 positive of the following 3: <ol style="list-style-type: none"> 1. positive quantitative culture of a sample obtained by BAL (cut-off point $\geq 10^4$ cfu/ml) or PSB (cut-off point $\geq 10^3$ cfu/ml) 2. positive blood culture unrelated to another source and obtained within 48 h before and after respiratory sampling 3. positive pleural fluid culture in the absence of previous pleural instrumentation

Pneumonia was diagnosed if A and B and C were positive.

BAL, bronchoalveolar lavage; PSB, protected specimen brush.

Bacteriologic examination

Colonization was analyzed semiquantitatively as described previously⁹. EGB included *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp., *Proteus* spp., *Morganella morganii*, *Citrobacter freundii*; Pseudomonadaceae included *P. aeruginosa* and *Xanthomonas maltophilia*.

Intragastric pH monitoring

Continuous computerized intragastric pH monitoring was performed as described previously⁹. From each measurement the median pH value was calculated.

Administration of enteral feeding

Enteral feeding (Nutrison, Nutricia, The Netherlands) was administered continuously and intragastrically, and delivered in sterile containers, containing volumes for 24 h, by the nutritional service department.

Risk factor analysis

Separate analyses were performed in order to determine risk factors for VAP caused by EGB and Pseudomonadaceae; risk factors for acquired colonization with EGB and Pseudomonadaceae in the stomach, the oropharynx, and trachea; and risk factors for mortality. The analysis of mortality was restricted to mortality in ICU. The risk factors that were analyzed are listed in table 5.2. The APACHE II score was determined as described by Knaus and coworkers¹⁷. Persistence of a

Glasgow Coma Score (GCS) <8 was scored positive when a patient had a GCS <8 (due to coma or sedatives) for at least five of the first 10 d in ICU, or at least half of the days in ICU when the duration of ICU stay was less than 10 d. Sepsis syndrome and septic shock were defined as described by Bone¹⁸. The amounts of gastric residue per 24 h were monitored daily, and a gastric residue >400 ml was tested as risk factor.

Table 5.2 Characteristics of the patients studied

	No. of patients (n=141)
Mean age $\pm$ SD (range)	58 $\pm$ 19 (18-91)
Male/Female	88/53
Admitting service	
Surgery	54
Trauma	20
Medical	33
Pulmonology	20
Neurology	14
mean APACHE II score $\pm$ SD (range)	21 $\pm$ 9 (1-46)
Stress ulcer prophylaxis	
Antacids	74
Sucralfate	67
Intragastric pH on first day of admission	
<3.0	69
$\geq$ 3.0	72
Computerized intragastric pH monitoring	112
mean pH $\pm$ SD (range)	4.2 $\pm$ 2.1 (0.8-7.9)
Infection on admission	39
Sepsis syndrome or septic shock $\leq$ 10 days of admission	25
Persisting Glasgow Coma Score <8	69
Abdominal or thoracic surgery	40
Gastric residue	56
Gastric nutrition	82
Ventilation with PEEP >5 cm	6
Mean number of days in ICU $\pm$ SD (range)	17 $\pm$ 33 (3-350)
Mortality in ICU	50

For the development of VAP caused by EGB or Pseudomonadaceae, colonization with species from the same bacterial group in oropharynx, stomach, or trachea on admission, or acquired before VAP was diagnosed were tested as risk factors. For acquired colonization, colonization on admission and acquired colonization at the other sites with species from the same bacterial group were tested as risk factors as well. In addition, the number of patients with preceding colonization with the same species (rather than species from the same bacterial group) at the two other sites were determined. In order to be a potential risk factor

for the endpoint of a certain analysis (such as development of VAP or acquired colonization), acquired colonization (at the other sites) had to be established before the endpoint was met. As a result, the number of patients with acquired colonization could be different in the separate analyses.

As for acquired colonization, the institution of enteral feeding was analyzed up to the day that the endpoint of analysis was met. Therefore, the number of patients receiving enteral feeding could be different in separate analyses.

In the initial study design, patients were stratified on the first day of admission to ICU based on their initial intragastric pH ($\text{pH} < 3.0$ or $\text{pH} \geq 3.0$)⁹. When possible, continuous intragastric monitoring was performed as well, and the median pH value of this long-term measurement in each individual patient was used in the analysis.

Statistical analysis

Risk factors for VAP, colonization and mortality were identified using *t* test, Mann-Whitney U test or chi-square statistics when appropriate. For binomial variables, crude odds ratios (OR) were calculated by the formula $(P_a/(1-P_a))/(P_b/(1-P_b))$, where *P* is the outcome for patients with one of both variables tested. Variables found to be associated ($p < 0.05$) with the endpoint tested (VAP, colonization, or death) were entered into a Cox proportional hazards model. Each variable was separately analyzed in a Cox regression model, and the best-fitting model was constructed by the simultaneous inclusion of the significantly related variables. Hazards ratios (HR) with corresponding 95% confidence intervals (95% CI) were computed. A hazard ratio of a variable with two categories is the ratio of the two category-dependent probabilities for occurrence the event. For a continuous variable such as age, the hazard ratio is the ratio of two age-dependent probabilities for occurrence the event. Here the two ages differ one unit (i.e., 1 yr).

Results

Patient characteristics

A total of 141 patients were studied (Table 5.2). Seven patients, included in the study, needed H_2 -antagonists or $\text{H}^+\text{K}^+\text{ATPase}$ inhibitors after 4-14 d. However, none of these patients reached an endpoint in any of the risk factor analyses after institution of acid-influencing medication; two patients developed VAP and five acquired colonization, all before institution of medication. Therefore, these patients are included in the analyses. The number of patients with acquired colonization and colonization on admission with EGB or Pseudomonadaceae is listed in table 5.3.

Table 5.3 Colonization with enteric gram-negative bacteria (EGB) and Pseudomonadaceae (Pseu) in patients with and without ventilator-associated pneumonia*

	Colonization with EGB					Colonization with Pseu				
	Patients developing VAP					Patients developing VAP				
	EGB n=7	Pseu n=12	Both n=7	No VAP n=115	Total†	EGB n=7	Pseu n=12	Both n=7	No VAP n=115	Total‡
Oropharynx										
On admission	4	5	5	38‡	52	0	3	1	14‡	17
Acquired	4	6	5	39	58 (4)	2	8	6	21	39 (2)
Stomach										
On admission	2	2	3	25§	32	0	1	1	4§	6
Acquired	4‡	8	4	38	59 (5)	0‡	4	4	16‡	27 (3)
Trachea										
On admission	1	2	3	15‡	21	0	2	1	13‡	16
Acquired	3	3	4	17	33 (6)	1	8	5	25	25 (3)

* In patients with VAP, acquired colonization is analyzed until the day of diagnosis.
† Number of patients acquiring colonization after development of VAP.
‡ Results of 2 patients not available.
§ Results of 8 patients not available.
‡ Results of 1 patient not available.

Ventilator-associated pneumonia

Twenty-six patients (18%) developed an episode of VAP caused by EGB and/or Pseudomonadaceae; in 12 patients VAP was caused by Pseudomonadaceae, in seven patients by EGB, and in seven patients VAP was polymicrobial and species from both groups were isolated. Five other patients developed an episode of VAP caused by *Staphylococcus aureus*, *Streptococcus pneumoniae* or *Haemophilus influenzae*, and were, therefore, not included in the risk factor analyses. None of these five patients developed a second episode of VAP caused by EGB or Pseudomonadaceae. In the 26 episodes of VAP caused by EGB and/or Pseudomonadaceae, 33 pathogens were isolated: 19 Pseudomonadaceae and 14 EGB. Preceding colonization with the same species as isolated from bronchoscopic samples was demonstrated in the oropharynx for 32 (97%) of 33 species (19 Pseudomonadaceae and 13 EGB), in the trachea for 28 (85%) of 33 species (19 Pseudomonadaceae and 9 EGB) and in the stomach for 14 (42%) of 33 species (5 Pseudomonadaceae and 9 EGB).

Enteric Gram-negative bacteria

VAP caused by EGB (n=14) was demonstrated after 8.9±5.3 d (median=7 d). The total duration of ventilation was 22.4±17.8 d (median=16 d) for patients developing VAP with EGB compared with 10.9±14.2 d (median=8 d) for those without VAP caused by EGB (p=0.006). The median duration of hospitalization before admission to ICU was 5 d (range 2-21) and 3 d (range 1-203) for patients with and without VAP caused by EGB respectively (p=NS). VAP was demonstrated in eight

(11%) of 70 patients receiving and six (10%) of 62 patients not receiving enteral feeding ($p=NS$). In univariate analysis, four factors were associated with a higher risk for VAP caused by EGB: acquired colonization with EGB in the trachea (7 (26%) of 27 vs. 7 (6%) of 114, $OR=5.86$, $p=0.006$), an infection on admission to ICU (8 (21%) of 39 vs. 6 (6%) of 102, $OR=4.43$, $p=0.01$), oropharyngeal colonization with EGB on admission (9 (17%) of 52 vs. 5 (6%) of 87, $OR=3.41$, $p=0.03$), and acquired colonization with EGB in the oropharynx (9 (17%) of 54 vs. 5 (6%) of 87, $OR=3.41$, $p=0.04$). In seven (78%) of these nine patients, bacterial species colonizing the oropharynx on admission eventually caused VAP.

The event-free survival was analyzed by the Cox regression model with oropharyngeal colonization with EGB on admission, acquired tracheal colonization with EGB, acquired oropharyngeal colonization with EGB, and an infection on admission as covariates. The event-free survival was negatively influenced by oropharyngeal colonization with EGB on admission ($p=0.006$), an infection on admission ($p=0.06$), and acquired colonization in the trachea ($p=0.08$). The best-fitting model selected oropharyngeal colonization on admission ($HR=4.5$, $95\%CI=1.5-13.7$, $p=0.009$) and an infection on admission ($HR=2.7$, $95\%CI=0.9-7.7$, $p=0.07$) as most important risk factors for VAP caused by EGB (Figure 5.1).

Figure 5.1 Event-free survival analysis of VAP caused by EGB. Group 1=patients without oropharyngeal colonization with EGB on admission and without an infection on admission. Group 2=patients without oropharyngeal colonization with EGB on admission but with an infection on admission. Group 3=patients with oropharyngeal colonization with EGB on admission and without an infection on admission. Group 4=patients with oropharyngeal colonization with EGB on admission and with an infection on admission.

Pseudomonadaceae

VAP caused by *Pseudomonadaceae* ($n=19$) was demonstrated after 11.7 ± 8.6 d (median=8 d). However, the total duration of ventilation for these patients was 30.5 ± 30.1 d (median=19 d) and 9.2 ± 7.9 d (median=7.5 d) for unaffected patients ($p<0.00001$). The median duration of hospitalization before ICU admission tended to be longer for patients developing VAP caused by *Pseudomonadaceae*: 7 d (range 1-203) and 3 d (range 1-125) respectively ($p=0.07$). VAP developed in 14 (18%) of 79 patients with enteral feeding and in five (8%) of 62 patients who had not received enteral feeding ($p=0.09$). VAP caused by *Pseudomonadaceae* was associated with acquired colonization with these species in the oropharynx (14 (38%) of 37 vs. 5 (5%) of 104, $OR=11.59$, $p<0.00001$), the trachea (13 (33%) of 39 vs. 6 (6%) of 102, $OR=7.68$, $p=0.00006$), and the stomach (8 (33%) of 24 vs. 11 (12%) of 114, $OR=3.59$, $p=0.006$), and the presence of sepsis syndrome or septic shock (7 (28%) of 25 vs. 12 (10%) of 116, $OR=3.51$, $p=0.03$). The event-free survival was analyzed in the Cox regression model with acquired colonization with *Pseudomonadaceae* of the oropharynx, the trachea, and the stomach, and the presence of sepsis syndrome or septic shock as covariates. Both acquired colonization of the oropharynx ($p=0.002$) and of the trachea ($p=0.009$) negatively influenced the event-free survival. In the best-fitting model acquired oropharyngeal colonization had an HR of 5.0 (95%CI=1.6-15.3, $p=0.005$) (Figure 5.2).

Figure 5.2 Event-free survival analysis of VAP caused by *Pseudomonadaceae*. Group 1=patients without acquired oropharyngeal colonization with *Pseudomonadaceae*. Group 2=patients with acquired oropharyngeal colonization with *Pseudomonadaceae*.

Acquired oropharyngeal colonization

Enteric Gram-negative bacteria

Acquired colonization in oropharynx with EGB was demonstrated in 58 (41%) patients after 7.8 ± 7.0 d (median=7 d). Patients acquired colonization with EGB were ventilated for 18.7 ± 19.4 d (median=13.5 d) compared with 7.4 ± 8.1 d (median=5 d) ($p < 0.0001$) in the others. Surgical and trauma patients tended to acquire colonization more frequently than patients admitted for medical or neurologic reasons (36 (49%) of 74 compared with 22 (33%) of 67 patients, OR=1.92, $p=0.06$), as did patients receiving enteral feeding (35 (49%) of 72 compared with 23 (34%) of 67 patients, OR=1.86, $p=0.06$). Preceding colonization with the same species was demonstrated in the stomach in 11 (19%), and in the trachea in five (9%) of 58 patients acquiring oropharyngeal colonization ($p=NS$). Moreover, the median durations of hospitalization before ICU admission did not differ statistically between patients acquiring and not acquiring colonization with EGB: 3 d (range 1-59) and 5 d (range 1-203) respectively ($p=NS$).

Pseudomonadaceae

On admission, 18 (13%) of 141 patients were colonized with Pseudomonadaceae in the oropharynx. Because colonization persisted in all of them, these patients were excluded from the analysis of acquired oropharyngeal colonization. Acquired colonization with Pseudomonadaceae was demonstrated in 39 (32%) of 123 patients after 7.7 ± 4.0 d (median=7 d). Patients acquiring oropharyngeal colonization with Pseudomonadaceae were ventilated for 19.8 ± 14.6 d (median=14 d) compared with 7.3 ± 5.8 d (median=6 d) for those without acquired colonization ($p < 0.00001$). Acquired colonization was demonstrated in 24 (36%) of 67 patients receiving enteral feeding and in 15 (27%) of 56 patients who did not ($p=NS$). The median durations of hospitalization before admission to ICU were 3 d (range 1-33) and 4 d (range 1-203) for patients with and without acquired colonization respectively ($p=NS$). Two risk factors were significantly related to acquired colonization with Pseudomonadaceae in univariate analysis. Tracheal colonization preceded oropharyngeal colonization in 12 (71%) of 17 occasions, whereas acquired oropharyngeal colonization was found without tracheal colonization in 27 (25%) of 106 patients (OR=7.35, $p=0.0004$). Furthermore, 27 (42%) of 65 patients with a persistent GCS<8 acquired colonization compared with 12 (21%) of 58 patients (OR=5.28, $p=0.01$).

In the Cox regression model, the duration until acquired colonization was negatively influenced only by preceding colonization of the trachea ($p=0.08$).

Gastric colonization

Enteric Gram-negative bacteria

Fifty-nine (42%) patients acquired gastric colonization with EGB. The duration of ventilation before acquired colonization was 7.9 ± 4.5 d (median=7 d). The total durations of ventilation were 18.7 ± 20.6 d (median=12 d) and 7.9 ± 8.4 d (median=

5 d) for patients who did and did not acquire colonization ($p=0.0001$). The periods of hospitalization before ICU admission were comparable between patients acquiring and not acquiring gastric colonization: 5 d (range 1-203) and 3 d (range 1-125) respectively ($p=NS$). Thirty-six (48%) of 75 patients receiving enteral feeding acquired colonization compared with 15 (23%) of 65 patients who did not ($OR=3.08$, $p=0.002$). Continuous intragastric pH monitoring was performed in 112 out of 141 patients. The pH values of individual measurements were 4.7 ± 2.1 (median=5.4) and 3.9 ± 2.1 (median=3.8) for patients acquiring and not acquiring gastric colonization ($p=0.04$). None of these risk factors were significantly related to acquired gastric colonization in Cox regression analysis.

Pseudomonadaceae

Six (5%) patients were colonized with *Pseudomonadaceae* in the stomach on admission and were, therefore, excluded from this analysis. Acquired gastric colonization with *Pseudomonadaceae* was demonstrated in 29 (21%) of 135 patients after 10.5 ± 5.5 d (median=10 d). The total duration of ventilation was 18.7 ± 20.6 d (median=12 d) for patients with acquired colonization and 7.9 ± 8.4 d (median=5 d) for other patients ($p<0.0001$). Patients with and without acquired gastric colonization with *Pseudomonadaceae* had similar median durations of hospitalization before ICU admission: 4 d (range 1-203) and 4 d (range 1-125) respectively ($p=NS$). Acquired gastric colonization was frequently preceded by colonization at other sites. Colonization of the oropharynx without gastric colonization was found in 37 patients and was followed by gastric colonization in 17 (46%) patients. Twelve other patients (out of 98 patients) acquired gastric colonization without preceding oropharyngeal colonization ($OR=6.23$, $p=0.00006$). Tracheal colonization preceded gastric colonization in 18 (46%) of 39 patients and 11 (11%) of 96 patients acquired gastric colonization without preceding tracheal colonization ($OR=6.88$, $p=0.00003$). Patients admitted because of surgery or trauma had a higher acquisition rate compared with patients admitted for medical or neurologic reasons (20 (29%) of 70 vs. 9 (14%) of 65 ($OR=2.52$, $p=0.04$)).

The event-free survival was analyzed in the Cox regression model with preceding colonization of the oropharynx and the trachea as covariates. Both variables almost equally influenced the duration until colonization; $p=0.09$ for preceding colonization of the oropharynx and $p=0.05$ for the trachea. The best-fitting model included preceding colonization of the trachea ($HR=2.1$, $95\%CI=0.97-4.6$, $p=0.06$) as most important risk factor for acquired gastric colonization with *Pseudomonadaceae* (Figure 5.3).

Figure 5.3 Event-free survival analysis of acquired gastric colonization with *Pseudomonadaceae*. Group 1=patients without preceding colonization of the trachea with *Pseudomonadaceae*. Group 2=patients with preceding colonization of the trachea with *Pseudomonadaceae*.

Tracheal colonization

Enteric Gram-negative bacteria

Acquired tracheal colonization with EGB was observed in 33 patients (23%), after 11.9 ± 14.3 d (median=8 d). The total durations of ventilation were 21.9 ± 24.3 d (median=15 d) for patients who acquired tracheal colonization and 8.7 ± 8.6 d (median=6 d) for those who did not ($p < 0.00001$). The median durations of hospitalization before admission to ICU were 4.5 d (range 1–48) for patients with and 3 d (range 1–203) for patients without acquired tracheal colonization with EGB respectively ($p = \text{NS}$). Moreover, none of the other risk factors were significantly associated with acquired colonization.

Pseudomonadaceae

Sixteen (12%) patients were colonized with *Pseudomonadaceae* on admission and were excluded from this analysis. Acquired tracheal colonization was found in 42 (34%) of 125 patients after 7.7 ± 4.0 d (median=7 d). The total durations of ventilation were 18.7 ± 14.4 d (median=13 d) for patients with acquired colonization and 6.9 ± 5.5 d (median=6 d) for patients who did not acquire colonization ($p < 0.0001$). Again, no statistical significant differences were found in the median number of days of hospitalization before ICU admission: 3 d (range 1–58) for

patients with acquisition and 4 d (range 1-203) for patients without acquisition ($p=NS$). Oropharyngeal colonization without tracheal colonization was found in 19 patients and preceded tracheal colonization in 14 (74%), whereas tracheal colonization occurred without preceding oropharyngeal colonization in 28 (26%) of the remaining 107 patients ($OR=8.11$, $p=0.00008$).

Table 5.4 Risk factors associated with mortality in univariate analysis

Risk factor		Total n/ Nonsurvivors (%)	Odds Ratio of chi-square	Mean $\pm$ SD	p Value of chi-square
Glasgow Coma Score <8	yes	69/38 (55)	5.97		<0.00001
	no	72/12 (17)			
Sepsis syndrome or septic shock	yes	25/18 (72)	6.61		0.00004
	no	116/32 (28)			
Infection on admission	yes	39/21 (54)	3.02		0.005
	no	102/29 (28)			
Oropharyngeal colonization with EGB on admission	yes	52/25 (48)	2.25		0.02
	no	87/25 (29)			
Gastric colonization with EGB on admission	yes	32/16 (50)	2.33		0.04
	no	101/30 (30)			
Administration of enteral feeding	yes	72/12 (17)	5.96		<0.00001
	no	69/38 (55)			
Specialty of admission	surgery/trauma	74/21 (28)	1.93		0.06
	medical/neurology	67/29 (43)			
APACHE II score	survivors			25.3 $\pm$ 9.4	<0.00001
	nonsurvivors			18.0 $\pm$ 7.2	
Age	survivors			62.4 $\pm$ 16.9	0.04
	nonsurvivors			55.6 $\pm$ 19.5	

Mortality

In all, 50 patients (35%) succumbed during their stay in ICU. In the univariate analysis eight factors were significantly related to mortality (Table 5.4): a persistent GCS <8 ($p<0.00001$), a clinical condition of sepsis syndrome or septic shock ($p=0.00004$), an infection on admission ($p=0.005$), oropharyngeal colonization with EGB on admission ($p=0.02$), gastric colonization with EGB on admission ($p=0.04$), and inability to administer gastric nutrition ($p=0.01$). In addition, the mean APACHE II score of patients who died was 25.3 ± 9.4 compared with 18.0 ± 7.2 for surviving patients ($p<0.00001$), and the mean ages were 62.4 ± 16.9 yr and 55.6 ± 19.5 yr ($p=0.04$) for both patient groups respectively. Furthermore, patients admitted for medical or neurologic reasons tended to have a higher mortality rate compared with patients admitted because of trauma or surgery ($p=0.06$). The development of VAP, caused by EGB or Pseudomonadaceae or both, was not associated with mortality: 12 (46%) of 26 patients who developed VAP died, compared with 38 (33%) of 115 patients who did not develop this infection ($p=0.21$). Moreover, neither VAP caused by EGB nor VAP caused by Pseudomonadaceae were associated with mortality ($p=0.57$ and $p=0.24$, respectively).

In univariate analysis, nine variables were associated with mortality (Table

5.4). In Cox regression analysis, the best-fitting model pointed to admission for a medical or neurologic reason (HR=2.2, 95%CI=1.2-4.1, $p=0.01$), age (HR=1.02, 95%CI=1.00-1.04, $p=0.03$), and oropharyngeal colonization with EGB on admission (HR=2.0, 95%CI=1.2-4.1, $p=0.03$) as most important risk factors. The HR of 1.02 for age means that the risk increases with 2% for each year. Furthermore, the ability to administer enteral feeding was a negative risk factor for mortality (HR=0.18, 95%CI=0.09-0.35, $p<0.0001$).

Discussion

The results of these risk factor analyses demonstrate that duration of mechanical ventilation and colonization of the oropharynx and trachea are the most important risk factors for the development of VAP. Colonization of the stomach did not appear to be a risk factor for VAP. In addition, there appeared to be considerable differences in risk factors for colonization with *Pseudomonadaceae* or EGB.

The data of the present study were collected during a randomized placebo-controlled study comparing sucralfate and antacids, which have already been published⁹. Nevertheless, we feel that the results of these risk factor analyses add important new information regarding the pathogenesis of colonization and infection of the respiratory tract in mechanically ventilated patients. A detailed risk factor analysis of infection and colonization with a discrimination between *Pseudomonadaceae* and EGB was beyond the scope of the prior report.

Of 21 variables, two entities were selected as risk factors for the development of VAP caused by EGB and *Pseudomonadaceae*: the duration of mechanical ventilation and colonization of oropharynx and trachea. The relationship between the duration of ventilation and an increased risk for VAP has been reported previously^{19,20}. Moreover, the association between tracheal colonization and VAP seems obvious, since tracheal colonization is one of the diagnostic criteria for VAP.

The results of the present analyses corroborate previous data about the importance of oropharyngeal colonization in the pathogenesis of VAP. Johanson and coworkers studied the same relation in critically ill patients and found an incidence of pneumonia of 23% in colonized and of 3% in noncolonized patients²¹. However, in that study the diagnosis of VAP was not based on quantitative cultures obtained by bronchoscopic techniques.

The oropharyngeal microflora changes at times of illness, with the occurrence of EGB and *Pseudomonadaceae* as the most common shift²². The incidence of oropharyngeal colonization with these species in healthy subjects has been reported to be as low as 2%²³. Moreover, inoculation of the oropharynx with EGB did not result in colonization in healthy volunteers²⁴. However, the incidence of

colonization gradually increased with the severity of illness from 16% in moderately ill patients to 57% in critically ill patients²³. Although the exact mechanisms of oropharyngeal colonization are not well understood, adherence probably depends on changes of the structure of epithelial cells, making the cells 'stickier' for EGB²². Therefore, oropharyngeal colonization with EGB may be regarded as a marker of the severity of underlying illness. Moreover, the fact that an infection on admission also was a risk factor for VAP caused by EGB may represent a clinical condition in which the host is susceptible for other infections as well.

Risk factors for oropharyngeal colonization may also increase the risk for VAP. In the present study acquisition of oropharyngeal colonization with EGB merely was a matter of time, generally occurring during the first week in ICU. For EGB, surgical and trauma patients tended to acquire colonization more frequently compared with medical and neurologic patients, as did patients receiving enteral feeding. The relation between gastric nutrition and subsequent oropharyngeal colonization with EGB could have been a result of retrograde aspiration. Two studies demonstrated microaspiration of radioactive labelled enteral nutrition, especially in patients treated in supine position with a nasogastric tube in place^{25,26}. However, previously reported chronological analyses failed to demonstrate that the pattern of colonization from the stomach to the respiratory tract occurred frequently in mechanically ventilated patients^{9,12,27}. Moreover, although acquired gastric colonization with EGB was associated with enteral feeding and the intragastric pH in the present study, gastric colonization did not appear to be a risk factor for the development of VAP or oropharyngeal and tracheal colonization with EGB. The relation between gastric nutrition and gastric colonization underscores data reported by Pingleton and coworkers⁶ and us²⁸. Therefore, the importance of the gastropulmonary route of colonization for EGB has not yet been elucidated.

In contrast, a clear pattern of colonization was found for *Pseudomonadaceae*. The upper respiratory tract was the initial site for acquired colonization with these species: acquired colonization of the oropharynx was associated with preceding tracheal colonization, and reversely, acquired tracheal colonization was frequently preceded by oropharyngeal colonization. As for EGB, duration of mechanical ventilation also was an important risk factor for colonization with *Pseudomonadaceae*. The importance of the upper respiratory tract as the initial site of colonization was also obvious in the analysis of acquired colonization of the stomach with *Pseudomonadaceae*. Acquired gastric colonization was strongly related to prior colonization of the oropharynx and trachea. These data strongly suggest that colonization with *Pseudomonadaceae* starts in the upper respiratory tract, thereby confirming data reported by Niederman²⁹ and by us¹².

There appeared to be considerable differences in risk factors for colonization with *Pseudomonadaceae* and with EGB. However, the power of these analyses to detect differences in risk factors for VAP caused by either of these species may be small, notably because 50% of the patients developing VAP caused by EGB had a polymicrobial infection in which *Pseudomonadaceae* were also involved. In our

experience a large proportion of VAP is polymicrobial, and larger study populations will be needed to determine whether VAP caused by either of these pathogens have different risk factors.

The method of multivariate analysis to identify risk factors for pneumonia in mechanically ventilated patients has been used before¹³⁻¹⁵. Unfortunately, a comparison of our data with those of other studies is hampered for several reasons. For instance, the duration of mechanical ventilation for patients who did not develop VAP, was remarkably shorter in two other studies. A duration of mechanical ventilation <3 d was reported for 57% of patients in one study¹⁴, and a duration of mechanical ventilation <5 d for 75% of patients in another¹⁵. In the present study, on the contrary, the minimal and median number of days of ventilation for patients not developing VAP were 3 and 6 d respectively. Furthermore, one should bear in mind that in all these studies only clinical criteria in combination with radiography were used to establish the diagnosis of VAP¹³⁻¹⁵, a method with a lower specificity than the additional use of bronchoscopic techniques like PSB and BAL^{16,30}. Finally, in none of these studies was a discrimination made between VAP caused by EGB or caused by *Pseudomonadaceae*.

In these previous three studies, 13 variables were detected by multivariate analysis as risk factors for VAP¹³⁻¹⁵. In the study of Craven and coworkers intracranial pressure monitoring, changing of ventilator filters every 24 h, the fall-winter season, and the use of cimetidine for stress ulcer prophylaxis were significantly associated with the development of VAP¹³. The first two variables could not be included in the present study, and seasonal differences were not found (data not shown). However, the influence of different stress ulcer prophylactic agents could be analyzed. As reported previously, we found no differences in intragastric acidity, colonization of stomach and respiratory tract and incidences of VAP in patients randomized to receive sucralfate or antacids⁹.

None of the five risk factors for VAP as reported by Torres and colleagues¹⁴ (i.e., a duration of mechanical ventilation >3 d, reintubation, ventilation with positive end-expiratory pressure (PEEP) >5 cm H₂O, gastric aspiration, and presence of chronic obstructive pulmonary disease (COPD)), turned out to be significant in the present study. The relation between VAP and duration of mechanical ventilation >3 d is obvious, but patients receiving mechanical ventilation for less than 3 d were excluded in the present study. In addition, reintubation and massive gastric aspiration occurred only seldom and could not be incorporated in the analysis. Ventilation with PEEP >5 cm H₂O was used in six patients only and was not related to the development of VAP. Because the presence of COPD was not monitored, the influence of this risk factor was not analyzed in the present study.

Kollef reported four risk factors for VAP¹⁵. Three of these, an organ system failure index >3, supine head position during the first 24 h of ventilation, and previous antibiotic use, were not included in the present analysis. The other risk

factor, an age >60 yr, did not come up as risk factor.

Several factors, related to severity of illness on admission or to a lack of clinical improvement during ICU stay, were related to mortality. The APACHE II score, an infection on admission, coma, sepsis syndrome or septic shock, and gastric and oropharyngeal colonization with EGB on admission have been related to a poor clinical condition and a grim prognosis^{13,17,23,31-33}. The inability to administer gastric nutrition should, in our opinion, be regarded as an epiphenomenon of the severity of underlying illness. It is our policy to start gastric nutrition as soon as possible in each patient receiving mechanical ventilation. In general, gastric nutrition was withheld only when continuous massive gastric residue was present. This probably reflects a subgroup of severely critically ill patients. Of course, institution of gastric nutrition is delayed in patients with gastric and oesophageal surgery, but these patients were excluded from the present analysis.

In conclusion, the duration of mechanical ventilation and colonization of oropharynx and trachea were the most important risk factors for VAP caused by EGB or Pseudomonadaceae; gastric colonization was not a risk factor for VAP; and the upper respiratory tract was the most important initial site of colonization for Pseudomonadaceae. These data do not support an important role of the gastropulmonary route in the pathogenesis of VAP caused by Pseudomonadaceae or EGB.

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Clinical spectrum of ventilator-associated pneumonia caused by methicillin-sensitive *Staphylococcus aureus*

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Abstract

Background

Pneumonia is the most frequently occurring nosocomial infection among mechanically ventilated intensive care unit (ICU) patients. Due to its association with mechanical ventilation, this infection has also been called ventilator-associated pneumonia (VAP). In most cases, VAP is preceded by colonization of the upper respiratory tract with the causative pathogen. Although *Staphylococcus aureus* frequently causes VAP, the clinical presentation of staphylococcal pneumonia in mechanically ventilated patients has not been described in great detail.

Methods

The incidence of tracheal colonization and its association with VAP caused by methicillin-sensitive *Staphylococcus aureus* (MSSA) was studied prospectively in 530 consecutively admitted mechanically ventilated patients in a general ICU. Furthermore, the clinical spectrum, outcome, and microbiological results of 27 cases of staphylococcal VAP (SVAP) were examined. Ventilator-associated pneumonia was diagnosed with protected specimen brush and/or bronchoalveolar lavage.

Results

On admission, 7% of the patients were colonized with MSSA in the trachea. Acquired tracheal colonization was demonstrated in 10% of the patients and occurred less frequently in patients with a hospital stay >48 h before ICU admission compared with patients admitted directly to the ICU (6% vs. 15%, $p<0.001$). Moreover, colonization was acquired more frequently among trauma and neurologic/neurosurgical patients (22%) compared with surgical and medical patients (7%) ($p<0.0001$). Twenty-one patients (4%) developed SVAP, the incidence being higher in patients colonized in the trachea with MSSA than in those not colonized (21% vs. 1%, $p<0.00001$). Staphylococcal VAP developed more often in trauma and neurologic/neurosurgical patients compared with surgical and medical patients (8% vs. 3%, $p<0.05$). Moreover, patients with a hospital stay <48 h before admission to the ICU had a higher incidence of SVAP compared with those with a longer hospital stay before ICU admission (7% vs. 2%, $p<0.01$). Crude infection-related mortality was 26%.

Conclusions

Preceding colonization with MSSA in the trachea appears to be an important risk factor for the development of SVAP, and patients with a short duration of hospitalization before ICU admission have the highest incidence of VAP caused by MSSA.

Introduction

Pneumonia is the most frequently occurring nosocomial infection among mechanically ventilated intensive care unit (ICU) patients¹, causing increased morbidity, mortality, and health care costs^{2,3}. Due to its association with mechanical ventilation, this infection has also been called ventilator-associated pneumonia (VAP). Quite often, *Staphylococcus aureus* is the cause of VAP^{1,4}. Although trauma and/or neurosurgical patients have been reported to be at greater risk of acquiring staphylococcal VAP (SVAP)⁵⁻⁸, the clinical presentation of staphylococcal pneumonia in mechanically ventilated patients has not been described in great detail. In contrast, several studies have investigated the clinical spectrum of community-acquired pneumonia caused by *S. aureus*. The latter infection has been characterized as a rapidly developing, fulminating disease associated with high mortality, especially when complicating epidemic influenza^{9,10}. In the UK *S. aureus* was among the three pathogens identified as the most common causes of severe community-acquired pneumonia, with 100% mortality reported for cases caused by *S. aureus*¹¹. In line with these observations, Fine et al.¹² found five risk factors associated with a complicated course for patients with community-acquired pneumonia, one of these being a pneumonia caused by a high-risk pathogen such as *S. aureus*.

In the present study the incidence of colonization of the upper respiratory tract by MSSA and its association with the development of VAP caused by this species was determined in 530 mechanically ventilated ICU patients. In addition, the clinical spectrum and outcome of 27 patients with SVAP and the microbiological results are described. The diagnosis of VAP was established on the basis of positive quantitative cultures of samples obtained by protected specimen brush (PSB) and/or bronchoalveolar lavage (BAL). During the entire study period no methicillin-resistant *S. aureus* (MRSA) was isolated.

Patients and Methods

Patients

From January 1992 through June 1994, tracheal colonization and development of VAP were studied prospectively in all mechanically ventilated patients in the general ICU of the University Hospital Maastricht, The Netherlands, in order to assess the incidence of colonization of the upper respiratory tract by MSSA and its association with the development of SVAP. Age, sex, APACHE II score, medical discipline of admission, and period of hospitalization before admission to the ICU were recorded for each patient on admission, and clinical parameters (vital signs, leukocytosis, temperature, etc.) were recorded daily. Colonization of the respiratory tract was monitored by culturing samples of tracheal aspirate on admission and, subsequently, at least twice weekly. Only patients needing mechanical

ventilation with an ICU stay of at least three days were included in the final analysis.

To describe the clinical spectrum and outcome of SVAP, data on all cases of SVAP from January 1992 through June 1995 were collected prospectively as described above ($n=21$). Because a study using topical antimicrobial decontamination of the oropharynx, which influences oropharyngeal colonization, was started in July 1994, data regarding tracheal colonization collected after this date were not used. However, data on the clinical spectrum and outcome of patients developing SVAP during this period were included ($n=6$).

Definition of colonization

Tracheal colonization was defined as the isolation of MSSA from tracheal aspirates without infection. Colonization on admission was established on the basis of a positive culture for MSSA obtained within 24 h after admission to the ICU. Colonization was considered acquired if there were cultures positive for MSSA from at least two consecutive samples but no colonization with MSSA on admission to the ICU.

Definition of pneumonia

In case of clinical suspicion of pneumonia, bronchoscopy with PSB and BAL was performed. The diagnosis was established when (A) at least three of the following criteria were met: (1) rectal temperature $>38.0^{\circ}\text{C}$ or $<35.5^{\circ}\text{C}$, (2) blood leukocytosis ($>10.10^3/\text{mm}^3$) and/or left shift or blood leukopenia ($<3.10^3/\text{mm}^3$), (3) more than 10 leukocytes per high-power field in Gram stain of tracheal aspirate, and (4) a positive culture from tracheal aspirate, in combination with (B) a new or progressive infiltrate on chest radiograph and (C) the presence of (1) a positive quantitative culture of a sample of secretions obtained by BAL (cut-off point $\geq 10^4$ cfu/ml) or PSB (cut-off point $\geq 10^3$ cfu/ml), or (2) positive cultures from blood, or (3) pleural fluid culture unrelated to another source and obtained within 48 h before and after respiratory sampling. The infection was considered ICU-acquired if symptoms began at least 48 h after admission to the ICU¹³. Pneumonia was considered staphylococcal when caused by MSSA solely or, in case of a polymicrobial pneumonia, when quantitative cultures of MSSA were above the cut-off points for PSB and/or BAL or when this species was cultured from blood or pleural fluid.

Bronchoscopy

A fiberoptic bronchoscope (Pentax FB-15H/FB-15X; Pentax Medicals, Japan) was introduced through a special adaptor (Swivel connector, Gibeck Respiration AB, Sweden) and advanced to the bronchial orifice of a lung segment containing a new or persistent infiltrate. A PSB (Microbiology brush; Mill-Rose Laboratories, USA) was then advanced to a wedged, peripheral position after dislodging the distal catheter plug to obtain lower airway secretions for microbiological analysis.

After brushing, the bronchoscope was positioned in the adjacent subsegment and BAL was performed by infusing one aliquot of 20 ml of sterile saline (0.9% NaCl) followed by three aliquots of 50 ml. The liquid recovered after the first aliquot was discarded and the remaining lavage fluid was pooled. In all cases PSB was performed first. Bronchoscopy specimens were transported to the laboratory within 15 min of collection and analyzed within 1 h.

Microbiology

Cultures of tracheal aspirates were taken on the first day of admission and subsequently twice a week. Semiquantitative and quantitative microbiological analyses of the specimens were performed according to routine methods. Susceptibility to flucloxacillin, gentamicin, ciprofloxacin, and amoxicillin-clavulanate was established by determination of minimal inhibitory concentration (MIC) by a microplate broth dilution method according to NCCLS criteria. *S. aureus* ATCC 29213 was used as reference strain.

Mortality

Crude ICU mortality was defined as the percentage of patients dying during their ICU stay and crude hospital mortality as the percentage of patients dying during hospital stay. The crude infection-related mortality was defined as the percentage of patients who died and in whom any contribution of SVAP to the fatal outcome could not be excluded on clinical or histological grounds.

Statistical analysis

Frequency comparisons were performed by chi-square test or Fisher's exact test. A probability value of <0.05 was considered to denote statistical significance. Data are expressed as absolute numbers with percentages or as medians with ranges.

Results

Colonization and infection by methicillin-sensitive *Staphylococcus aureus*

In the 30-month study period between January 1992 through June 1994, 530 mechanically ventilated patients were admitted to the ICU for at least three days. Twelve of these received topical antimicrobial prophylaxis of the oropharynx and stomach with antibiotics acting against MSSA. Topical antimicrobial prophylaxis was started after the first culture samples had been obtained. The median age of the patients studied was 66 yr (range 14-92) and the median duration of ICU stay was eight days (range 3-349). Patients were surgical ($n=231$, 43%), medical ($n=197$, 37%), neurologic or neurosurgical ($n=56$, 11%), or had been admitted because of multiple trauma ($n=46$, 9%).

On admission, colonization of the upper respiratory tract with MSSA was observed in three of 12 patients receiving topical antimicrobial prophylaxis and in 33 of the remaining 518 patients (Table 6.1). Thus, 36 of 530 patients (7%) were colonized

with MSSA on admission.

Because the 12 patients receiving topical antimicrobial prophylaxis and the 33 colonized with MSSA on admission were excluded from analysis, data on acquired colonization of the upper respiratory tract with MSSA were evaluated in the remaining 485 patients (Table 6.1). Acquired colonization was demonstrated in 48 of these 485 patients (10%). Patients who had been hospitalized for longer than 48 h before admission to the ICU acquired colonization with MSSA less often than patients admitted directly to the ICU (15/266 (6%) vs. 33/219 (15%); $p<0.001$). Moreover, acquired colonization occurred more frequently among trauma and neurologic/neurosurgical patients (20/89 (22%)) than surgical and medical patients (28/396 (7%); $p<0.0001$). The median ages of patients who did or did not acquire colonization were comparable: 67 yr (range 17-88) and 66 yr (range 14-92), respectively.

In the 30-month study period, 21 of 518 (4%) patients analyzed developed an episode of SVAP (Table 6.1). The incidence was higher among patients colonized in the trachea with MSSA (either present on admission ($n=33$) or acquired during ICU stay ($n=48$)): 17 of 81 patients (21%) compared with 4 of 437 not colonized patients (1%) developed SVAP ($p<0.00001$). Staphylococcal VAP occurred more frequently in trauma and neurologic/ neurosurgical patients (8/100 (8%)) than surgical and medical patients (13/418 (3%); $p<0.05$), and patients hospitalized <48 h before ICU admission had a higher incidence (16/241 (7%)) compared with those with a longer duration of hospitalization before ICU admission (5/277 (2%); $p<0.01$).

Table 6.1 Number of patients analyzed and incidences of colonization and infection

	Colonized on admission		Acquired colonization		Incidence of SVAP	
	Analyzed	Colonized	Analyzed	Colonized	Analyzed	Infected
Total	530	36 (7%)	485	48 (10%)	518	21 (4%)
Excluded for analysis	0		12 T + 33 C		12 T	
Surgery/medical patients	428		396	28 (7%)	418	13 (3%)
Trauma/neurologic/ neurosurgical patients	102		89	20 (22%)	100	8 (8%)
Hospital stay before ICU admission >48 h			266	15 (6%)	277	5 (2%)
Hospital stay before ICU admission <48 h			219	33 (15%)	241	16 (7%)
Colonized (on admission or acquired)					81	17 (21%)
Not colonized					437	4 (1%)

SVAP, staphylococcal ventilator-associated pneumonia; T, topical antimicrobial prophylaxis; C, colonized on admission.

Clinical presentation and outcome of staphylococcal ventilator-associated pneumonia

Reason for admission, sex, age, presence of pre-existing diseases, and outcome of the 21 patients who developed SVAP (described above) and of six patients who developed SVAP in the period from July 1994 through June 1995 are summarized in table 6.2. The median age of the 27 patients was 54 yr (range 17-91), the male/female ratio was 18/9, and the median APACHE II score was 18 (range 8-31). Fourteen patients had at least one pre-existing disease. Cardiovascular diseases were present in nine patients, respiratory diseases in four patients, and malignancies in five. Severe autoimmune disease and a combination of amyloidosis resulting in chronic renal failure and chronic use of immunosuppressive drugs were present in two additional patients.

The remaining 13 patients had no underlying disease before admission to the ICU, although two were known intravenous drug and/or alcohol abusers. These 13 patients were admitted because of multiple trauma ($n=5$), acute pancreatitis ($n=1$), neurologic disease ($n=1$), elective craniotomy ($n=1$), resuscitation ($n=1$), status epilepticus ($n=1$), ruptured aneurysm of the abdominal aorta ($n=1$) and hemolysis, elevated liver function tests, low platelets (HELLP) syndrome ($n=1$). One patient was admitted because of a suicide attempt in which 10 ml of lampoil was injected intravenously.

In five patients (No. 2, 5, 9, 11, 14) MSSA was not cultured from tracheal aspirates on the day pneumonia was diagnosed. However, in patients 2 and 5, MSSA had previously been isolated from tracheal aspirates. Tracheal aspirate could not be obtained in patient 8 (Table 6.3).

The diagnosis of SVAP was based on positive quantitative cultures from both PSB and BAL in nine patients, once in combination with a positive blood culture. In 13 other patients the diagnosis was based on a positive quantitative culture from BAL only, and in three patients on a positive quantitative culture of PSB only. In the remaining two patients the diagnosis was made after positive blood cultures, in one patient in combination with positive cultures from pleural fluid. Three patients (No. 5, 6, 18) received systemic antimicrobial therapy at the time of bronchoscopy. Despite in vitro susceptibility of MSSA to these agents in two patients (No. 5, 18), quantitative cultures of PSB and BAL were above the cut-off points in both patients.

Ventilator-associated pneumonia seemed to be caused solely by MSSA in six patients (22%). In all other cases VAP was polymicrobial; among the species most frequently isolated in combination with MSSA were *Escherichia coli* ($n=7$), *Haemophilus influenzae* ($n=6$), *Pseudomonas aeruginosa* ($n=5$), and *Klebsiella pneumoniae* ($n=4$).

Table 6.2 Characteristics of patients who developed staphylococcal ventilator-associated pneumonia

Patient	Age	Sex	Pre-existing disease	Reason for admission	Outcome
1	66	M	hypertension / COPD	ruptured AAA	S
2	17	M	none	trauma capitis	S
3	79	M	none	trauma thoracalis	D*
4	28	M	drug and alcohol abuse	resuscitation	S
5	38	F	none	Myasthenia gravis	S
6	62	F	CVA / NIDDM / adenocarcinoma sigmoid	resection sigmoid	D*
7	43	M	alcohol abuse	acute pancreatitis	D†
8	54	M	amyloidosis / renal failure / immunosuppressive drugs	amyloidosis / PCP	D*
9	51	M	none	craniotomy	S
10	28	M	none	trauma thoracalis	S
11	91	F	none	trauma	D†
12	66	F	none	status epilepticus	S
13	40	F	SLE / immunosuppressive drugs	acute renal failure	D†
14	62	M	CAD / COPD	ruptured AAA	D*
15	76	M	aortic aneurysm	trauma capitis/thoracalis	S
16	68	M	CAD / COPD	exacerbation COPD	D*
17	75	M	none	ruptured AAA	D†
18	45	F	breastcarcinoma / BMT	Cytomegalovirus pneumonia	D†
19	71	M	oesophageal carcinoma / COPD	resection oesophagus	D†
20	40	F	epilepsy / Down's syndrome	resuscitation	S
21	77	M	CAD / adenocarcinoma colon	gastrointestinal bleeding	S
22	53	M	CAD	trauma capitis/thoracalis	S
23	72	M	claudicatio intermittens / adenocarcinoma rectum	resection recto-sigmoid	S
24	25	F	none	HELLP syndrome	S
25	24	M	none	trauma capitis	D†
26	31	M	none	suicide attempt	S
27	67	F	hypertension	ruptured AAA	D†

AAA, aneurysm abdominal aorta; BMT, bone marrow transplantation; CAD, coronary artery disease; COPD, chronic obstructive pulmonary disease; CVA, cerebrovascular accident; D, died; F, female; HELLP syndrome, haemolysis, elevated liver function tests, low platelets syndrome; M, male; NIDDM, noninsulin dependent diabetes mellitus; PCP, *Pneumocystis carinii* pneumonia; S, survived; SLE, systemic lupus erythematosus.

* Death directly related to SVAP.

† Death possibly related to SVAP.

‡ Death not related to SVAP.

The duration of stay in ICU until SVAP was diagnosed ranged from 3 to 19 d (median, 9 d). Peak body temperatures ranged from 37.6°C to 40.9°C (median, 39.4°C) on the day of diagnosis. Two patients had normal body temperature, but

one of these (No. 8) was treated with high doses of immunosuppressive drugs. The leukocyte count ranged from $3.6 \times 10^9/l$ to $31.5 \times 10^9/l$ (median, $15.7 \times 10^9/l$), and in four patients more than five immature granulocytes were present in differential count.

In all, 13 of 27 patients died during their hospital stay (crude hospital mortality, 48%), 11 of whom died during ICU stay (crude ICU mortality, 41%). The number of days between the diagnosis of SVAP and death ranged from 1 to 65 d (median, 13 d). The episode of SVAP was unlikely to have directly influenced the fatal outcome in six patients. Four of these six patients died 37, 42, 50, and 65 d after the diagnosis of SVAP had been established, of cardiac failure ($n=2$), septicemia due to endocarditis caused by another microorganism ($n=1$), and septicemia from an abdominal source with intestinal microorganisms ($n=1$). In the remaining two patients signs of bacterial pneumonia were no longer present at autopsy (one patient died 11 d after SVAP was diagnosed due to septicemia caused by *Staphylococcus epidermidis*; the other patient, admitted because of head trauma, died seven days after SVAP was diagnosed due to severe cerebral damage resulting from the initial trauma).

Staphylococcal VAP seemed to be directly related to death in five patients (No. 3, 6, 8, 14, 16). These patients died within six days after SVAP had been diagnosed, except patient No. 8, who died after 13 d (signs of pneumonia were still obvious at autopsy). Patient 19 died because of recurrent episodes of VAP 46 d after the first episode, which was caused by MSSA, had been diagnosed. In addition, patient 17 also suffered from secondary peritonitis and eventually died of multiple organ failure. The contribution of SVAP to the fatal outcome in these two cases, therefore, could not be excluded. Thus, SVAP was unrelated to mortality in six patients and seemed to be directly related in five patients; SVAP was possibly related to mortality in two patients. As a result the crude infection-related mortality in the 27 patients with SVAP was 26% (7/27).

A closer look at these patients reveals some interesting findings. Nine patients were ≤ 55 yr and were admitted without pre-existing diseases. The mortality in this subgroup of patients was 22%; two of these patients died (No. 7 and 25). Death of both patients seemed unrelated to SVAP: patient 7, admitted with severe necrotizing pancreatitis, died in cardiac arrest without suspicion of infection 50 d after pneumonia had been diagnosed; patient 25 died seven days after pneumonia was diagnosed because of severe cerebral damage following the head trauma for which he was admitted, and signs of bacterial pneumonia were no longer present at autopsy. In contrast, three of five patients ≤ 55 yr, but with severe pre-existing diseases died during their ICU stay (No. 8, 13, 18), and in two of these patients (No. 13 and 18) pneumonia was unlikely to have influenced the outcome. Of the remaining 13 patients, all >55 yr of age, eight (62%) died, and five of these deaths seemed to be related directly to the development of SVAP. The relationship between age and crude infection-related mortality of SVAP showed a trend towards significance (Fisher's exact test $p=0.09$).

Table 6.3 Microbiological diagnosis in patients who developed staphylococcal ventilator-associated pneumonia

Patient	Tracheal aspirate	Protected specimen brush	(cfu/ml)	Bronchoalveolar lavage	(cfu/ml)	Blood	Pleural fluid
1	<i>Staphylococcus aureus</i> <i>Haemophilus influenzae</i>	not analyzed		<i>Staphylococcus aureus</i>	(1.10 ⁵)	no growth	not analyzed
2	<i>Haemophilus influenzae</i> <i>Moraxella catarrhalis</i>	<i>Haemophilus influenzae</i> <i>Staphylococcus aureus</i>	(1.10 ⁴) (6.10 ³)	<i>Haemophilus influenzae</i> <i>Staphylococcus aureus</i>	(1.10 ⁴) (3.10 ³)	no growth	not analyzed
3	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	<i>Pseudomonas aeruginosa</i>	(1.10 ⁴)	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	(1.10 ³) (1.10 ⁵)	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>
4	<i>Staphylococcus aureus</i> <i>Streptococcus pneumoniae</i>	<i>Staphylococcus aureus</i>	(4.10 ³)	<i>Staphylococcus aureus</i>	(2.10 ⁴)	no growth	not analyzed
5	<i>Pseudomonas aeruginosa</i> <i>Escherichia coli</i>	<i>Pseudomonas aeruginosa</i> <i>Escherichia coli</i> <i>Staphylococcus aureus</i>	(1.10 ⁴) (2.10 ⁴) (1.10 ⁴)	<i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	(2.10 ⁴) (2.10 ⁴)	not analyzed	not analyzed
6	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	(1.10 ⁵) (1.10 ⁴)	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	(1.10 ⁵) (1.10 ⁵)	no growth	not analyzed
7	<i>Staphylococcus aureus</i> <i>Klebsiella oxytoca</i>	<i>Staphylococcus aureus</i> <i>Klebsiella pneumoniae</i>	(4.10 ¹) (1.10 ¹)	<i>Staphylococcus aureus</i> <i>Klebsiella pneumoniae</i> <i>Haemophilus influenzae</i>	(2.10 ⁴) (4.10 ³) (1.10 ⁵)	no growth	not analyzed
8	not analyzed	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	(1.10 ⁴) (4.10 ³)	<i>Staphylococcus aureus</i>	(5.10 ³) (9.10 ³)	not analyzed	not analyzed
9	<i>Moraxella catarrhalis</i> <i>Pseudomonas aeruginosa</i>	not analyzed		<i>Moraxella catarrhalis</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus aureus</i>	(1.10 ³) (2.10 ⁵) (2.10 ⁵)	no growth	not analyzed
10	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	(9.10 ²)	<i>Staphylococcus aureus</i>	(5.10 ⁴)	no growth	not analyzed
11	<i>Klebsiella pneumoniae</i>	<i>Klebsiella pneumoniae</i> <i>Staphylococcus aureus</i>	(1.10 ¹) (1.10 ¹)	<i>Klebsiella pneumoniae</i> <i>Staphylococcus aureus</i>	(1.10 ³) (1.10 ³)	<i>Klebsiella pneumoniae</i>	not analyzed
12	<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Candida albicans</i>	not analyzed		<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	(1.10 ⁵) (1.10 ⁵)	not analyzed	not analyzed
13	<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Klebsiella pneumoniae</i>	not analyzed		<i>Staphylococcus aureus</i> <i>Escherichia coli</i> <i>Klebsiella pneumoniae</i>	(1.10 ⁵) (1.10 ⁵) (1.10 ⁵)	<i>Klebsiella pneumoniae</i>	not analyzed

Patient	Tracheal aspirate	Protected specimen brush	(cfu/ml)	Bronchoalveolar lavage	(cfu/ml)	Blood	Pleural fluid
14	<i>Proteus mirabilis</i> <i>Enterococcus faecalis</i> <i>Candida albicans</i>	<i>Proteus mirabilis</i> <i>Enterococcus faecalis</i> <i>Enterobacter</i> spp.	(1.10 ⁵) (1.10 ⁵) (5.10 ²)	<i>Proteus mirabilis</i> <i>Enterobacter</i> spp. <i>Staphylococcus aureus</i> <i>Klebsiella pneumoniae</i>	(1.10 ⁴) (1.10 ³) (1.10 ³) (1.10 ³)	not analyzed	<i>Enterococcus faecalis</i>
15	<i>Staphylococcus aureus</i>	not analyzed		<i>Staphylococcus aureus</i> <i>Haemophilus influenzae</i>	(1.10 ⁴) (1.10 ⁵)	no growth	<i>Klebsiella pneumoniae</i> no growth
16	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	not analyzed		not analyzed		<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	not analyzed
17	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	(1.10 ⁵) (1.10 ⁵)	<i>Staphylococcus aureus</i> <i>Escherichia coli</i>	(1.10 ⁵) (1.10 ⁵)	no growth	not analyzed
18	<i>Staphylococcus aureus</i>	not analyzed		<i>Staphylococcus aureus</i>	(1.10 ⁴)	no growth	not analyzed
19	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	(8.10 ³)	<i>Staphylococcus aureus</i>	(3.10 ⁴)	no growth	not analyzed
20	<i>Staphylococcus aureus</i> <i>Streptococcus pneumoniae</i> <i>Haemophilus influenzae</i>	<i>Staphylococcus aureus</i> <i>Streptococcus pneumoniae</i> <i>Haemophilus influenzae</i> <i>Neisseria meningitidis</i>	(5.10 ³) (1.10 ⁴) (1.10 ⁴) (1.10 ⁴)	<i>Staphylococcus aureus</i> <i>Streptococcus pneumoniae</i> <i>Haemophilus influenzae</i> <i>Neisseria meningitidis</i>	(1.10 ⁵) (1.10 ⁴) (1.10 ⁵) (1.10 ⁵)	no growth	not analyzed
21	<i>Staphylococcus aureus</i> <i>Haemophilus parainfluenzae</i>	<i>Staphylococcus aureus</i>	(1.10 ⁵)	<i>Staphylococcus aureus</i>	(1.10 ⁵)	no growth	not analyzed
22	<i>Staphylococcus aureus</i> <i>Streptococcus agalactiae</i>	<i>Staphylococcus aureus</i>	(1.10 ³)	<i>Staphylococcus aureus</i>	(4.10 ³)	no growth	not analyzed
23	<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i> <i>Enterococcus faecalis</i> <i>Klebsiella oxytoca</i>	not analyzed		<i>Staphylococcus aureus</i> <i>Pseudomonas aeruginosa</i>	(1.10 ⁵) (1.10 ⁵)	<i>Staphylococcus aureus</i>	not analyzed
24	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	(4.10 ³)	<i>Escherichia coli</i>	(1.10 ⁴)		
25	<i>Staphylococcus aureus</i> <i>Haemophilus influenzae</i>	<i>Staphylococcus aureus</i> <i>Haemophilus influenzae</i>	(1.10 ⁵) (1.10 ⁴)	<i>Staphylococcus aureus</i> <i>Haemophilus influenzae</i>	(2.10 ⁴) (1.10 ³)	<i>Staphylococcus aureus</i> not analyzed	not analyzed
26	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	(2.10 ³)	<i>Staphylococcus aureus</i>	(1.10 ⁵)	no growth	not analyzed
27	<i>Staphylococcus aureus</i>	<i>Staphylococcus aureus</i>	(5.10 ²)	<i>Staphylococcus aureus</i>	(1.10 ⁵)	no growth	not analyzed

Staphylococcal VAP was accompanied by empyema in two (7%) patients (No. 4 and 14), and cavitation was suspected on chest radiograph and confirmed by computer tomographic scanning in one (4%) patient (No. 13). A recurrent episode of VAP occurred in six patients: in five cases caused by *P. aeruginosa* and in one by *Citrobacter freundii*. In the group of six patients in whom VAP was caused by MSSA alone, three (50%) died. Death was directly related to SVAP in two cases and possibly related in one case.

All cultured isolates of *S. aureus* were methicillin sensitive. All patients, except patient 14, were treated with antibiotics active against MSSA. Amoxicillin-clavulanate ($n=7$) and flucloxacillin ($n=6$) were used most frequently as monotherapy, but were used in combination with gentamicin in two and three patients respectively. Because of concomitant infections, polymicrobial VAP, or the presence of a prosthesis, the remaining eight patients were treated with various combinations of antibiotics: ticarcillin-clavulanate ($n=2$), ticarcillin-clavulanate plus gentamicin ($n=1$), amoxicillin ($n=1$; because of penicillin-sensitive MSSA), cefuroxime plus gentamicin ($n=1$), teicoplanin plus gentamicin ($n=1$), vancomycin ($n=1$), or vancomycin plus ceftazidime ($n=1$). Patient 14 died on the day the definitive culture results of PSB and BAL were available and before antibiotic therapy active against penicillin-resistant MSSA was started. This patient died while receiving piperacillin for pleural empyema and fluconazole for tracheitis.

Discussion

The main feature of this study is that the clinical spectrum of VAP in which MSSA has an etiological role differs markedly from the well-known fulminating community-acquired pneumonia caused by this pathogen. Ventilator-associated pneumonia caused by MSSA was polymicrobial in most cases (78%) and had a rather mild clinical presentation. However, despite these findings, the overall mortality of patients developing this infection was almost 50%, with a crude infection-related mortality rate of 26%. Prior colonization with MSSA in the trachea appears to be an important risk factor, and patients with a short duration of hospitalization before ICU admission had the highest incidence of SVAP.

In the present study the median day of admission to the ICU on which SVAP was diagnosed was day 9. Therefore, the cases of VAP described here cannot be considered as early-onset pneumonia, which is defined as pneumonia occurring in the first four days of mechanical ventilation and which is most frequently caused by species already colonizing the upper respiratory tract before admission to the ICU¹⁴. In a previous study performed in the same ICU and using the same diagnostic methods, MSSA was the most important pathogen associated with VAP, after *P. aeruginosa*¹⁵. In that study the overall incidence of VAP was 22%, and MSSA was involved in 35% of the cases¹⁵. This incidence of pneumonia is

comparable to the incidences reported by others using the same diagnostic techniques⁴.

The clinical course of SVAP in the present study was associated with elevated body temperatures and leukocyte counts, a finding not surprising given that both are diagnostic criteria. However, more severe presentations such as septicemia, pleural involvement, and abscess formation developed in only one patient each. In addition, other infection parameters were observed infrequently: only four patients had more than five immature leukocytes in the differential cell count, and no patient developed leukopenia. The rather mild presentation of nosocomial pneumonia is in contrast to the well-known clinical picture of fulminating community-acquired pneumonia due to MSSA, especially in those recovering from influenza⁹. This rapidly progressive infection has been associated with high incidences of cavitation (25%) and empyema (10%)⁹. Moreover, the clinical presentation as described in the present study is also incompatible with the clinical course of MSSA pneumonia described by Kaye et al.¹⁶, who found 'an infection typical of a severe bacterial infection, with high fevers and usually significant leukocytosis, and positive blood cultures in a third of the patients'. However, these data are difficult to compare to ours, because among the patients studied by Kaye and colleagues¹⁶, 20% had community-acquired pneumonia and the number of patients requiring mechanical ventilation was not specified.

Besides the differences in clinical presentation, two other characteristics of SVAP deserve attention. First, there seems to be a difference between the patient population developing SVAP and that developing community-acquired pneumonia due to MSSA. Community-acquired pneumonia due to MSSA develops in particular in elderly patients, notably following a viral infection of the respiratory tract^{9,17}. In contrast, SVAP more frequently affects young adults who have been hospitalized for a short period before admission to the ICU, such as patients admitted because of trauma or neurologic/neurosurgical pathology. We found that these patients were also at risk for staphylococcal colonization, which is an important risk factor for the development of SVAP. These observations confirm the very few data available on the epidemiology of SVAP. For instance, Espersen and Gabrielsen⁵ reported a higher incidence of nosocomial pneumonia due to *S. aureus* among neurosurgical patients (25.9%) compared with other patients (1.2%). In keeping with these results, Rello and colleagues^{6,7} demonstrated that staphylococcal pneumonia was associated with coma, age <25 yr, the nonuse of steroids, and preceding trauma. In another study, VAP caused by MSSA occurred more frequently in patients with cranioccephalic trauma, whereas VAP due to MRSA more frequently affected patients who had received steroids, had been ventilated for more than six days, had preceding chronic obstructive pulmonary disease, or were >25 yr⁸. Moreover, previous antibiotic therapy appeared to be the most important risk factor for developing VAP caused by MRSA⁸. The results of these studies suggest that patients with a short duration of hospitalization prior to ICU admission are

especially prone to develop SVAP, and that this infection is frequently preceded by tracheal colonization with *S. aureus*. In theory, colonization of the nares with *S. aureus* on admission to ICU might have been present in these patients. For reasons as yet unknown, nasal colonization may spread to tracheal colonization and cause pneumonia. This theory needs further exploration.

In contrast to VAP caused by MSSA, infections caused by MRSA have all the characteristics of ICU-acquired infections. Fortunately, MRSA is not endemic in the Netherlands. The prevalence was 0% in the European Prevalence of Infection in Intensive Care (EPIC) study^{18,19}, and no episode of VAP caused by MRSA has occurred in our hospital to date. A second difference between the two forms of staphylococcal infection may be that VAP is often polymicrobial (78% in the present study). *Pseudomonas aeruginosa* and Enterobacteriaceae were the pathogens most frequently isolated, together with MSSA. This is in accordance with the bacterial flora colonizing the upper and lower respiratory tract of long-term, mechanically ventilated ICU patients²⁰. The polymicrobial etiology of VAP has drawn little attention, although high incidences have been reported in the literature²¹⁻²⁴. In an experimental model Johanson et al.²⁰ investigated nosocomial pneumonia in baboons receiving prolonged mechanical ventilation. For the diagnosis they used cultures of lung homogenates and histologic findings as gold standards. In this model pneumonia was found to be polymicrobial in all cases.

Despite the relatively benign clinical presentation of SVAP and the availability of effective antibiotics, the overall mortality was 48% in the present study, and the crude infection-related mortality rate was 26%. Although the data of this study cannot answer the question of whether a patient died of or with pneumonia, the data do suggest that in young patients, pre-existing diseases rather than pneumonia itself determined the outcome. In contrast, in patients over age 55, four of the eight patients who died succumbed within six days after pneumonia had been diagnosed. This finding corroborates the alleged relationship between VAP and mortality as reported by Fagon et al.³ and by Kappstein et al.².

In conclusion, the results of the present study demonstrate that in most patients SVAP is not a fulminating disease. In many cases the infection is polymicrobial, and patients with a short duration of hospitalization before ICU admission seem to be especially prone to this infection.

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7

Value of phenotyping methods as an initial screening of *Pseudomonas aeruginosa* in epidemiologic studies

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Abstract

Background

Ventilator-associated pneumonia (VAP) is frequently caused by *Pseudomonas aeruginosa*, mostly after colonization of the upper respiratory tract. When studying the epidemiology of *P. aeruginosa* in an endemic setting, determination of similarity of isolates is crucial. Genotyping techniques have become the method of choice for this purpose. However, genotyping is laborious and expensive, and therefore not feasible for all laboratories. The aim of the present study was to determine whether phenotyping techniques could be used as an initial screening method of *P. aeruginosa* in order to select isolates for further genotyping.

Methods

The distinctive capacity of four phenotyping methods (antibiotic susceptibility patterns, serotyping, phage-typing and outer membrane protein (OMP) profile analysis) was determined and compared with pulsed-field gel electrophoresis (PFGE) of enzyme restricted chromosomal DNA. In all, 91 isolates of *P. aeruginosa* were cultured from 10 patients developing VAP caused by this pathogen.

Results

Antibiotic susceptibility patterns were concordant for all isolates. Serotyping yielded five, phage-typing eight, OMP profile analysis nine and PFGE seven distinct types of *P. aeruginosa*. Compared with PFGE, the distinctive capacities were 89% (81/91) for serotyping, 87% (79/91) for phage-typing, and 90% (82/91) for OMP profile analysis. Combination of two or more phenotypic techniques did not increase distinctive capacity. When serotyping results were different, PFGE types also were different (exclusiveness 100%). However, isolates with the same serotype may have various PFGE patterns. In contrast, isolates with similar PFGE patterns could have different phage-types or OMP types.

Conclusions

Of the four phenotyping techniques tested in the present study, serotyping showed the best distinctive properties, for the study of isolates of *P. aeruginosa*. Serotyping provides a good initial selection to reduce the number of isolates that need to be genotyped.

Introduction

Ventilator-associated pneumonia (VAP) is an important nosocomial infection, frequently caused by *Pseudomonas aeruginosa*¹. When studying routes of colonization with *P. aeruginosa* that lead to VAP, determination of similarity of isolates is crucial, especially since colonization may occur with different isolates, both at one particular body site and at different body sites². Comparison of phenotypes, such as antibiotic susceptibility patterns, serotypes, phage-types and outer membrane protein (OMP) types, is relatively easy to perform, but lacks specificity³. Pulsed-field gel electrophoresis (PFGE) of enzyme restricted chromosomal DNA, has a higher specificity and discriminatory power while maintaining epidemiological linkage and has, therefore, become the method of choice to compare bacterial isolates when studying nosocomial outbreaks⁴⁻⁶. However, PFGE is laborious and expensive, and therefore not feasible for all laboratories. The aim of the present study was to determine the usefulness of four methods of phenotyping as an initial method of screening of *P. aeruginosa* in order to select isolates for further genotyping. This was done by determination of typeability, distinctive capacity, and exclusiveness of antibiotic susceptibility patterns, serotyping, phage-typing and OMP profile analysis compared with PFGE of enzyme restricted chromosomal DNA.

Materials and Methods

Isolates

Ninety-one isolates of *P. aeruginosa*, derived from 10 patients who consecutively developed VAP caused by this species, were analyzed: 16 from oropharyngeal swabs (nine patients), 18 from tracheal aspirates (eight patients), 17 from bronchoalveolar lavage (eight patients), 14 from protected specimen brush (eight patients), eight from gastric aspirates (four patients), and 18 from rectal swabs (four patients).

Typing methods

Antibiotic susceptibility patterns were determined by measurement of MIC according to the NCCLS guidelines. Serotyping of *P. aeruginosa* isolates was performed using antisera to the International Antigenic Typing System (IATS) serotypes (Difco Laboratories, USA). Phage-typing of *P. aeruginosa* was performed using the following phages: 7, 16, 21, 24, 31, 44, 68, 73, F7, F8, F10, 109, 119x, 352, 1214, M4, M6, Col21, Col11, Col18. Serotyping and phage-typing were performed on all isolates in a single run. Outer membrane proteins of *P. aeruginosa* were isolated as described previously^{7,8} with minor modifications. The OMP types were examined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Genome fingerprinting was carried out according to

the method described by Grothues et al.⁹. Pulsed-field gel electrophoresis was performed using a CHEF-DRII System (Bio-Rad Laboratories, Richmond, CA, USA) after restriction digestion with *SpeI* (Boehringer Mannheim, Germany). *Saccharomyces cerevisiae* was used as marker.

Comparison of strains

Antibiotic susceptibility pattern, serotype, phage-type, OMP type and enzyme restricted chromosomal DNA patterns, produced by PFGE, were determined for all isolates of *P. aeruginosa*. Outer membrane protein profiles and PFGE patterns were compared visually on enlarged gel photographs to identify bands of identical relative mobility as fragments of identical size. The criteria for bacterial strain typing according to Tenover et al.¹⁰ were used to interpret chromosomal DNA restriction patterns produced by PFGE.

Typeability

Typeability of the various typing techniques was determined by dividing the number of typeable isolates by the total number of isolates, expressed as a percentage.

Distinctive capacity

The results of each phenotypic method were compared with the results of the PFGE of enzyme digested chromosomal DNA, which was regarded as the gold standard. Distinctive capacity was defined as the number of correctly typed isolates by the phenotypic method divided by the total number of isolates as typed by the PFGE technique, expressed as a percentage.

Exclusiveness

For the isolates of each strain type, as defined by each phenotyping technique, it was established whether they were linked to a single or to multiple PFGE types. Exclusiveness of each phenotyping technique was determined by dividing the number of isolates of one particular strain type exclusively linked to a single PFGE type by the total number of isolates, expressed as a percentage.

Results

Antimicrobial susceptibility patterns were concordant for all isolates. The MIC values were: piperacillin ≤ 8 mg/l, ticarcillin/clavulanate ≤ 8 mg/l, ceftazidime ≤ 2 mg/l, imipenem/cilastatin ≤ 1 mg/l, gentamicin ≤ 1 mg/l, ciprofloxacin ≤ 0.5 mg/l. Serotyping yielded five different patterns in 87 isolates; four isolates were polyagglutinable. With phage-typing eight different patterns could be distinguished, arbitrarily labelled 1 to 8. Analysis of OMP profiles yielded nine distinct patterns, arbitrarily labelled 1 to 9 (Figure 7.1). Combination of the four phenotyping me-

thods identified 11 distinct strain types, labelled A to K (Table 7.1). Pulsed-field gel electrophoresis of enzyme restricted chromosomal DNA showed seven different patterns, arbitrarily labelled I to VII (Figure 7.2). Type I was isolated most frequently ($n=47$), and was isolated from five out of 10 patients. Three types (II, III, and VII) were demonstrated in one patient only. Overall typeability of 100% was achieved by antibiotic susceptibility testing, phage-typing, OMP profile analysis and PFGE of enzyme restricted chromosomal DNA. Because of the four polyagglutinable isolates, typeability was 96% (87/91) for serotyping. Determination of antibiotic susceptibility patterns of the 91 isolates had no distinctive capacity, since all isolates had the same susceptibility pattern. Distinctive capacity of serotyping was 89% (81/91), and 93% (81/87) when the four polyagglutinable isolates were excluded. Distinctive capacity of phage-typing was 87% (79/91), and of OMP profile analysis 90% (82/91). Combination of two or more phenotypic typing methods did not increase the distinctive capacity. Serotyping/phage-typing 87% (79/91), serotyping/OMP profile analysis 89% (81/91), and phage-typing/OMP profile analysis 81% (74/91). The combination of serotyping, phage-typing and OMP profile analysis had a distinctive capacity of 81% (74/91).

Figure 7.1 Outer membrane protein profiles of *Pseudomonas aeruginosa* strains of the 10 patients developing VAP, revealing 9 distinct types labelled 1 to 9. M=marker

Table 7.1 Strains of *P. aeruginosa* based on molecular biotyping techniques

Serotype	Phage-type*	OMP type†	Types‡	PFGE type§	No. isolates	No. patients
3	6	4	G	VI	9	2
5	1	9	A	I	33	4
5	1	5	F	I	2	1
5	3	9	C	I	12	1
6	5	6	E	V	6	2
6	8	6	I	III	5	1 [¶]
6	8	2	J	III	2	1 [¶]
7	2	8	B	IV	6	2
11	4	7	D	II	11	1
11	4	1	K	II	1	1
PA	7	3	H	VII	4	1

PA, polyagglutinable.

* Arbitrarily labelled 1 to 8.

† Arbitrarily labelled 1 to 9, see Figure 7.1.

‡ Distinct bacterial types based on serotype, phage-type and OMP type, arbitrarily labelled A to K.

§ Arbitrarily labelled I to VII, see Figure 7.2.

¶ The same patient.

Table 7.2 Exclusiveness of the phenotyping techniques

Serotype	No. of isolates with an exclusive PFGE		Phage-type	No. of isolates with an exclusive PFGE		OMP type	No. of isolates with an exclusive PFGE	
	Yes	No		Yes	No		Yes	No
3	9	-	1	-	35	1	-	1
5	47	-	2	6	-	2	-	2
6	13	-*	3	-	12	3	4	-
7	6	-	4	12	-	4	9	-
11	12	-	5	6	-	5	-	2
PA	4	-	6	9	-	6	6	5
			7	4	-	7	-	11
			8	7	-	8	6	-
						9	-	45
Total	91	-		44	47		25	66
Exclusiveness	91/91 (100%)			44/91 (48%)			25/91 (27%)	

PA, polyagglutinable.

* Serotype 6 was associated with 2 PFGE types (III, V), however, both were exclusively seen in this serotype.

Isolates with different serotypes always differed in their PFGE pattern (≥ 7 band differences; exclusiveness 100%; table 7.2). Moreover, within a group of isolates with one and the same serotype, PFGE analysis showed ≤ 3 band differences in 85 of 91 isolates (93%). In six of 91 isolates (7%), ≥ 7 band differences were

present according to PFGE analysis. In summary, isolates with a different serotype also had a different PFGE type. Isolates with the same serotype did not always have the same PFGE type.

Isolates with different phage-types were associated with different PFGE patterns (≥ 7 band differences) in 79 of 91 isolates (87%). However, in 12 of 91 isolates (13%) two different phage-types were associated with one PFGE type, exclusiveness was 48% (44/91, table 7.2). Within a group of isolates with the same phage-type, PFGE analysis showed ≤ 3 band differences in 100% of isolates. In summary, isolates with a different phage-type did not always have a different PFGE type. Isolates with the same phage-type always had the same PFGE type.

Figure 7.2 Pulsed-field gel electrophoresis patterns of 11 *Pseudomonas aeruginosa* strains following digestion with *SpeI*. The lanes were labelled as the strains A to K (according to the phenotyping techniques) and as PFGE type I to VII. M=marker (*Saccharomyces cerevisiae*).

Different OMP types were associated with different PFGE patterns in 86 of 91 isolates (95%). However, in five of 91 isolates (5%) two different OMP types were associated with one PFGE type, exclusiveness was 27% (25/91, table 7.2). Within a group of isolates with the same OMP types, PFGE analysis revealed ≤ 3 band differences in 86 of 91 isolates (95%). In summary, isolates with a different OMP type did not always have a different PFGE type, and isolates with the same OMP type did not always have the same PFGE type.

Discussion

The results of this study show that phenotyping may well be used as an initial method of screening for epidemiologic studies of *P. aeruginosa* in an endemic setting of intensive care patients. Based on the data presented in this study, we recommend serotyping for this purpose. In case of identical serotypes of strains from different patients, identity should be confirmed by a method of genotyping.

The purpose of this study was not to determine reproducibility and discriminatory power of typing techniques, which has already been studied extensively¹¹⁻¹³. Furthermore, because a number of the studied isolates are closely related, the set of isolates analyzed in the present study was unsuitable for determination of discriminatory power¹⁴. For epidemiologic studies, genotyping with PFGE of enzyme restricted chromosomal DNA of bacterial isolates currently is the typing method of choice. However, this method is expensive and time consuming. In general, phenotyping techniques are less expensive and easier to perform. The starting point of our investigation was to determine sensitivity and specificity of four phenotyping techniques. The best phenotyping technique may be used to make a preselection out of a large series of isolates, thereby reducing the number of isolates that need to be genotyped in epidemiologic studies. The results of four phenotyping techniques were compared with PFGE of enzyme restricted chromosomal DNA, which we considered to be the gold standard. Although, the methods of analysis used (e.g. determination of distinctive capacity and exclusiveness) are not common in microbiological studies, we feel that they are appropriate for these purposes.

Various typing methods have been used to study the epidemiology of *P. aeruginosa*. Serotyping has been used most frequently. However, reported drawbacks of this method are the high frequency of polyagglutinable strains¹⁵, the fact that most clinical isolates belong to a limited number of serotypes¹⁶ and that, especially in cystic fibrosis patients, a considerable proportion of strains are non-typable⁹. The results of the present study partially are in line with this: among the 91 isolates five different serotypes could be distinguished, only four isolates were polyagglutinable. Although phage-typing has been reported to allow fairly detailed strain typing in 85% of clinical isolates, its reproducibility is poor^{3,12}. Phage-typing

of a series of isolates should, therefore, always be performed in one session. Moreover, phage-typing usually can only be performed in reference laboratories where the various phages are in store. Analysis of OMPs, although laborious and time consuming, has been used in epidemiologic studies of *P. aeruginosa*, although several OMP types of *P. aeruginosa* strains showed high degrees of similarity¹⁷. In contrast, Maloney et al demonstrated considerable variation in OMP types between strains isolated from different patients¹⁸. Restriction enzyme analysis with PFGE has been reported to provide the highest discriminatory power between *P. aeruginosa* isolates¹² and has, therefore, been advocated as the method of choice to investigate outbreaks of infections due to this species⁴. To reduce the number of isolates for PFGE when studying the epidemiology of *P. aeruginosa* in an endemic setting, a phenotyping technique as an initial method of screening can be advocated.

Antibiotic susceptibility testing was not discriminatory in our setting because none of the *P. aeruginosa* strains were resistant to the antibiotics tested. In the presence of resistant strains the distinctive capacity of antibiotic susceptibility testing may increase. Serotyping, phage-typing and OMP profile analysis all had a good distinctive capacity, ranging from 87% to 90%. Any combination of these methods did not improve distinctive capacity. Isolates with different serotypes were always associated with different PFGE patterns (exclusiveness=100%). This group-specific relationship between DNA fingerprint and serotype has been described^{12,19}. Moreover, within a group of isolates with the same serotype, 93% of isolates were closely related or indistinguishable. In contrast, within a group of isolates with the same PFGE patterns, different phage-types and OMP types were seen. For phage-typing this phenomenon has been reported before by Grothues et al.⁹. In a study by Boukadida et al.²⁰ both phenotyping and genotyping techniques were used in the analysis of an outbreak of gut colonization by *P. aeruginosa* in six immunocompromised children. They also found different phage-types within a group of isolates with similar PFGE types²⁰. However, serotyping results from that study were difficult to interpret, since 8 of 13 isolates were polyagglutinable²⁰. Of the four phenotypic techniques tested in the present study, serotyping showed the best discriminative properties. The phenotypic technique of choice in our laboratory, therefore, is serotyping, which also is a simple, cheap and fast method, with good reproducibility^{11,13}. For example, when two patients are colonized or infected with *P. aeruginosa* with different serotypes, cross-colonization is highly unlikely. When both patients have strains with similar serotypes, PFGE is needed to confirm similarity.

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8

Colonization with *Pseudomonas aeruginosa* in patients developing ventilator-associated pneumonia

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Abstract

Background

Ventilator-associated pneumonia (VAP) is frequently caused by *Pseudomonas aeruginosa*, and is in most cases preceded by colonization of the respiratory tract with this microorganism. The objective of the study was to determine routes of colonization and genotypic variation of *P. aeruginosa* among mechanically ventilated patients developing VAP caused by *P. aeruginosa*.

Methods

Colonization with *P. aeruginosa* was studied prospectively and chronologically in rectum, stomach, oropharynx and trachea in 10 mechanically ventilated patients. Ventilator-associated pneumonia was diagnosed by bronchoscopic techniques and genotypic similarity of *P. aeruginosa* isolates was determined by pulsed-field gel electrophoresis.

Results

From 10 patients who developed VAP 91 isolates, derived from bronchoscopic samples ($n=31$), tracheal aspirates ($n=18$), oropharyngeal swabs ($n=16$), gastric aspirates ($n=8$), and rectal swabs ($n=18$), were genotyped. Seven different genotypes were identified. Preceding colonization with a similar genotype as the strain causing VAP occurred in the trachea in all patients, in the oropharynx in nine patients, in the stomach in five patients, and in the rectum in two patients. In three of 10 patients, distinct genotypes were demonstrated in isolates cultured either from one site or from different sites. Acquired colonization with *P. aeruginosa* occurred in eight patients, two of these were already colonized on admission with another genotype. The initial site of colonization, with the strain causing VAP, was the upper respiratory tract in eight out of 10 patients.

Conclusions

Critically ill patients developing VAP caused by *P. aeruginosa* are colonized at multiple body sites and may be colonized with multiple genotypes. In this patient population the upper respiratory tract is the predominant initial site of colonization with *P. aeruginosa*.

Introduction

Ventilator-associated pneumonia (VAP) is frequently caused by *Pseudomonas aeruginosa*¹, and in most cases is preceded by colonization of the respiratory tract with this microorganism^{2,3}. The pathogenesis of colonization and infection of the respiratory tract with *P. aeruginosa* in critically ill patients is not completely understood. Results of several studies have suggested that *P. aeruginosa* preferentially colonizes the trachea rather than the oropharynx^{4,5}. However, in these studies two important questions remained unanswered. In patients, colonized with *P. aeruginosa*, it was not determined whether colonization was restricted to the respiratory tract, and whether patients were colonized with a single or with multiple genotypes of *P. aeruginosa*.

We, therefore, prospectively studied colonization with *P. aeruginosa* prior to the development of VAP chronologically in rectum, stomach, oropharynx and trachea in 10 mechanically ventilated patients developing VAP caused by this microorganism. Pneumonia was diagnosed by bronchoscopic techniques and similarity of multiple isolates of *P. aeruginosa* was determined by pulsed-field gel electrophoresis (PFGE) of enzyme restricted chromosomal DNA.

Methods

Design

Rectal and oropharyngeal swabs, and tracheal and gastric aspirates were obtained on admission and subsequently twice weekly from all patients above 15 yr of age and admitted to one of two general intensive care units (ICU) of the University Hospital Maastricht. Out of this cohort, the first 10 patients who developed VAP caused by *P. aeruginosa* were selected, and isolates of *P. aeruginosa* were analyzed. Informed consent was obtained from the patient or, if this was not possible because of the clinical condition, from a representative of the family. The study protocol was approved by the hospital ethical committee.

Microbiology

Semiquantitative and qualitative analysis of aerobic bacteria and yeasts present in the collected samples as well as qualitative and quantitative analysis of samples of bronchoalveolar lavage (BAL) or protected specimen brush (PSB) obtained by bronchoscopy were performed as described before¹. From each culture plate with growth of *P. aeruginosa*, three colonies were analyzed and stored separately until further processing.

Definition of Colonization

Colonization was defined as the isolation of microorganisms in the absence of infection, from tracheal aspirate, gastric aspirate, rectal swab, or oropharyngeal

swab. Colonization on admission was established on the basis of a positive culture obtained within 24 h after admission to the ICU. Colonization was designated as 'acquired' if cultures were positive from at least two consecutive samples but no colonization on admission to the ICU. The initial site of colonization was defined as the site at which colonization was identified first. Simultaneous colonization of several sites is possible. The routes of colonization with *P. aeruginosa* leading to VAP were determined by analyzing colonization of the rectum, the stomach, the oropharynx and the trachea chronologically.

Definition of ventilator-associated pneumonia

In case of a clinical suspicion of pneumonia, bronchoscopy with BAL and PSB was performed as described elsewhere⁶. The diagnosis of VAP was established on the criteria listed in table 8.1.

Table 8.1 Criteria used for the diagnosis of ventilator-associated pneumonia

Criteria
A ≥ 3 positive of the following 4:
1. rectal temperature above 38.0°C or below 35.5°C
2. blood leukocytosis ($>10 \cdot 10^3/\text{mm}^3$) and/or left shift or blood leukopenia ($<3 \cdot 10^3/\text{mm}^3$)
3. >10 leukocytes per high-power field in Gram stain of tracheal aspirate
4. positive culture from tracheal aspirate
and
B new, persistent or progressive infiltrate on chest radiograph
and
C ≥ 1 positive of the following 3:
1. positive quantitative culture of a sample obtained by BAL (cut-off point $\geq 10^4$ cfu/ml) or PSB (cut-off point $\geq 10^3$ cfu/ml)
2. positive blood culture unrelated to another source and obtained within 48 h before and after respiratory sampling
3. positive pleural fluid culture in the absence of previous pleural instrumentation

Pneumonia was diagnosed if A and B and C were positive.
BAL, bronchoalveolar lavage; PSB, protected specimen brush.

Genotype analysis

Restriction digestion of total genomic DNA was performed using *SpeI* (Boehringer Mannheim, Germany) followed by PFGE using a CHEF-DRII System (Bio-Rad Laboratories, Richmond, CA, USA) as described previously⁷. Pulsed-field gel electrophoresis patterns were compared visually on enlarged gel photographs to identify bands of identical relative mobility as fragments of identical size. The absolute number of band differences between isolate pairs was recorded. The criteria for bacterial strain typing according to Tenover et al.⁸ were used to interpret chromosomal DNA restriction patterns produced by PFGE.

Results

All analyzed patients were mechanically ventilated, endotracheally intubated, and had a nasogastric tube in place (Table 8.2). Ventilator-associated pneumonia was polymicrobial in six patients, the species isolated in combination with *P. aeruginosa* being *Staphylococcus aureus* ($n=3$), *Haemophilus influenza* ($n=1$), *Proteus mirabilis* ($n=1$) and in one case *Klebsiella pneumoniae* together with *Candida albicans*. The mean number of days in ICU before VAP was diagnosed was 15 d (range 3-39). Seven patients had received co-amoxiclav before the diagnosis. Ventilator-associated pneumonia was treated with piperacillin ($n=5$), ceftazidime ($n=3$) and co-ticarcav ($n=1$), in two patients in combination with aminoglycosides. One patient died on the day of diagnosis before antibiotic therapy against *P. aeruginosa* was started. This patient was receiving flucloxacillin for *S. aureus* bacteraemia.

Table 8.2 Patient characteristics

Patient	Age (yr)	Sex	Diagnosis on admission	Days in ICU	Outcome
1	28	Male	Cardiopulmonary resuscitation	31	Survived
2	79	Male	Trauma, multiple rib fractures	22	Died
3	62	Female	Sigmoid resection	15	Survived
4	38	Female	Myasthenia gravis	50	Survived
5	44	Male	Trauma, spinal cord lesion C4-C5	106	Survived
6	22	Male	Trauma, hepatic rupture, amputation arm	60	Survived
7	69	Male	Pneumococcal pneumonia	7	Survived
8	68	Female	Faecal peritonitis, blowout caecum	39	Survived
9	73	Female	Exacerbation of Chronic Obstructive Pulmonary Disease	47	Died
10	44	Female	Abdominal sepsis after total proctocolectomy	19	Died

From each patient, one to three isolates per body site colonized or infected with *P. aeruginosa* were analyzed. Unfortunately, eight isolates were lost for genotyping; one from the oropharynx (patient 8), two from the trachea (patient 7, 10), and five from the stomach. In all, 91 isolates were genotyped: 16 from oropharyngeal swabs (9 patients), 18 from tracheal aspirates (8 patients), 17 from BAL (8 patients), 14 from PSB (8 patients), eight from gastric aspirates (4 patients), and 18 from rectal swabs (4 patients). Pulsed-field gel electrophoresis of enzyme restricted chromosomal DNA showed seven different patterns, arbitrarily labelled I to VII. Type I was isolated most frequently ($n=47$), and was isolated from five out of 10 patients. Within the different types, the number of band differences in one on one comparisons ranged from 0 to 3. When different strain types were compared one on one, the number of band differences was always larger than 10. More extensive data about biotyping of these 91 isolates have been presented⁹.

All 10 patients were colonized with *P. aeruginosa* in the trachea before the diagnosis of VAP. Colonization at the other sites, prior to the diagnosis of VAP, was demonstrated in the oropharynx in nine patients and in the stomach in five patients. The rectum was colonized with *P. aeruginosa* in four patients prior to the diagnosis of VAP, however, only in two of these patients with the similar genotype that caused VAP (Table 8.3). In seven patients (Pt. 1,3,4,6,7,8,10) all isolates of *P. aeruginosa*, cultured from different sites showed similar PFGE patterns and were considered to be identical. In the other three patients (Pt. 2,5,9), distinct types were demonstrated in isolates cultured either from one site or from different sites.

Four patients were colonized with *P. aeruginosa* on admission; two in the rectum (Pt. 2 and 9), one in the stomach (Pt. 7) and one in rectum, stomach, trachea and oropharynx (Pt. 5). In patient 5, three distinct types of *P. aeruginosa* were demonstrated on admission. This patient had been hospitalized for 12 d before admission to our ICU. The remaining three patients colonized on admission, had either not been hospitalized previously ($n=2$), or only for two days ($n=1$) before admission to the ICU. Acquired colonization with *P. aeruginosa* occurred in eight patients after a mean of six days (range 3-14). Two of these patients were already colonized with *P. aeruginosa* on admission, but acquired colonization with another genotype during their ICU stay (Pt. 2 and 9).

The initial site of colonization for genotypes causing VAP was the upper respiratory tract (oropharynx and/or trachea) in eight out of 10 patients. However, simultaneous colonization was demonstrated in the rectum and the stomach in two of these patients (Pt. 1 and 5 respectively). The rectum was the initial site for a genotype causing VAP in patient 9. Although two patients (Pt. 2 and 5) were initially colonized with *P. aeruginosa* in the rectum, these isolates were markedly distinct from those colonizing the upper respiratory tract and subsequently causing pneumonia. The stomach was the initial site of a genotype causing VAP in one patient (Pt. 7), and in four other patients, gastric colonization with an identical strain type was demonstrated before VAP was diagnosed. Gastric colonization with *P. aeruginosa*, occurring after pneumonia had been diagnosed, was demonstrated in four patients.

The fact that five gastric isolates could not be genotyped did not influence our findings. Three of the five missing isolates from colonized gastric aspirates were cultured for the first time seven to 17 d after VAP had been diagnosed. Moreover, the missing isolates from oropharynx ($n=1$) and trachea ($n=2$), in three patients, and the other missing isolates of the stomach ($n=2$) in two patients were not obtained from an initial site of colonization. In each case, the patient was already colonized with a genotype, identical to the type causing VAP. Identification of different genotypes at these sites, compared with the genotypes already found at other body sites of the individual patient or compared with the genotypes causing VAP, would only strengthen our observation that critically ill patients can be colonized with multiple genotypes of *P. aeruginosa*.

Table 8.3 Sequences and day of first isolation of *Pseudomonas aeruginosa*

Patient	Strain	Sites of colonization prior to and after ventilator-associated pneumonia (day first isolation)					
		BAL/PSB*					
1	I		Trachea (14)	Rectum (14)	15	Oropharynx (17)	Stomach (22)
2	I VI	Rectum (1)	Oropharynx (8)	Trachea (8)	19		
3	I		Trachea (10)	Stomach (10)	11		
4	V		Oropharynx (7)	Trachea (7)	8	Stomach (25)	
5	I III IV	Rectum (1)	Trachea (1)	Stomach (1)	39		
			Trachea (1)	Stomach (1)	39		
6	VI		Oropharynx (5)	Trachea (8)	14	Stomach (16)	
7	IV		Oropharynx (3)	Trachea (3)	4		
8	I		Oropharynx (4)	Stomach (4)	23		
9	II V	Rectum (1) Trachea (3)	Trachea (3)	Stomach (5)	16		
10	VII		Oropharynx (3)	Trachea (3)	4	Stomach (15)	

BAL, bronchoalveolar lavage; PSB, protected specimen brush.

* Day of isolation from BAL/PSB is day of diagnosis VAP.

Discussion

The results of this study demonstrate that critically ill patients developing VAP due to *P. aeruginosa* are colonized with strains of this species at multiple body sites. In addition, in three of 10 patients, two or three different genotypes of *P. aeruginosa* were found at different body sites. Previous findings that the upper respiratory tract is the most important initial site of colonization for strains causing VAP were confirmed, but no preference for tracheal rather than oropharyngeal colonization was found.

In order to study whether colonization with *P. aeruginosa* in mechanically ventilated patients was restricted to the upper respiratory tract, and whether these patients are colonized with a single or with multiple genotypes, we performed a conceptual study with only 10 patients, but with much attention to monitoring of colonization, accurately diagnosing VAP and genotyping of isolates. We think that our findings add new and important information to the understanding of the complex pathogenesis of infection and colonization of the respiratory tract with *P. aeruginosa*.

Among critically ill patients, colonization of the respiratory tract is facilitated by several variables. A decrease of the local immune mechanisms may allow respiratory epithelial cells to become 'stickier' for pathogenic bacteria¹⁰. A loss of fibronectin, possibly induced by increased levels of proteases¹¹, nutritional deprivation⁴, and changes in the expression of cell surface carbohydrates¹² have been associated with increased adherence of bacteria¹³. Moreover, colonization is also influenced by bacterial factors, such as the presence of pili which facilitate bacterial adherence to epithelial surfaces¹⁴. The analyses of the present study demonstrate the close relationship between colonization and infection of the respiratory tract with *P. aeruginosa*. Colonization with *P. aeruginosa* prior to the diagnosis of VAP was found in the trachea in all patients and in the oropharynx in nine of 10 patients. Both sites were equally frequently the initial sites of colonization. In a previous study of colonization with *P. aeruginosa* we also found that tracheal colonization was an important risk factor for oropharyngeal colonization, and conversely, oropharyngeal colonization was an important risk factor for tracheal colonization². Niederman et al. have identified sequences of colonization with *P. aeruginosa* in four patients, and in all patients the tracheobronchial tree was colonized before the oropharynx⁴. The results of the present study also demonstrate that colonization with *P. aeruginosa* in critically ill patients is not limited to the upper and lower respiratory tract. Whether *P. aeruginosa* also colonizes epithelial cells of other sites more easily during critical illness remains to be established.

Five out of 10 patients acquired colonization and infection with a similar genotype of *P. aeruginosa* (Strain I), which strongly suggests the occurrence of cross-acquisition. The design of the present study did not allow epidemiologic analyses. However, based on this finding we initiated a study to determine the

importance of cross-colonization and cross-infection in our ward

Since genotyping has not been performed in other studies evaluating routes of colonization, the possibility of cross-acquisition in these studies, therefore, has not been determined^{2,4,5}. However, if cross-acquisition is important, the initial colonization of the respiratory tract may have been a result of frequent manipulation of respiratory equipment or nasogastric tubes by hospital personnel with contaminated hands, a route of colonization that has been well established^{15,16}. In contrast, rectum and stomach were evident endogenous sources of strains causing VAP in two of our patients. However, data on the importance of the stomach as a source of bacteria are controversial^{1,2,17,18}. The rectum was the most common primary site of colonization with *P. aeruginosa* among 153 patients admitted to a surgical ICU¹⁹. In that study, 10 patients acquired colonization with *P. aeruginosa* (6.5%), of whom one patient developed pneumonia¹⁹. In another study, Murthy et al. demonstrated that among 186 admissions to three hospital wards, the rectum was the most commonly observed colonization site for *P. aeruginosa*. In all, 20 patients acquired colonization with *P. aeruginosa* in that study, of which only two acquired oropharyngeal colonization²⁰. In both studies, the incidence of colonization and infection with *P. aeruginosa* were lower than those in our ICU¹, and study populations and methods were different when compared with the present study. Furthermore, the absence of data on gastric and tracheal colonization in one study²⁰ and gastric and oropharyngeal colonization in the other¹⁹ precludes a formal comparison of those results with our data.

In conclusion, our results demonstrate that critically ill patients developing VAP caused by *P. aeruginosa* are colonized with these species at multiple body sites and with multiple genotypes, suggesting that it is not only the respiratory tract epithelium that becomes suitable for colonization with *P. aeruginosa* during critical illness. These findings emphasize the need of genotyping when studying the pathogenesis of VAP caused by *P. aeruginosa*. Our data also underscore the importance of the upper respiratory tract as the initial site of colonization with *P. aeruginosa* in this patient population. However, the relative importance of endogenous and exogenous sources for colonization remains to be established.

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Cross-colonization with *Pseudomonas aeruginosa* of patients in an intensive care unit

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Abstract

Background

Ventilator-associated pneumonia (VAP) caused by *Pseudomonas aeruginosa* is usually preceded by colonization of the respiratory tract. During outbreaks, colonization with *P. aeruginosa* is mainly derived from exogenous sources. The relative importance of different pathways of colonization of *P. aeruginosa* has rarely been determined in nonepidemic settings.

Methods

In order to determine the importance of exogenous colonization, all isolates of *P. aeruginosa* obtained by surveillance and clinical cultures from two identical intensive care units (ICUs) were genotyped with pulsed-field gel electrophoresis.

Results

A total of 100 patients were studied, 44 in ICU 1 and 56 in ICU 2. Twenty-three patients were colonized with *P. aeruginosa*, seven at the start of study or on admission and 16 of the remaining 93 patients became colonized during the study. Eight patients developed VAP due to *P. aeruginosa*. The incidence of respiratory tract colonization and VAP with *P. aeruginosa* in our ICU was similar to that before and after the study period, and therefore represents an endemic situation. Genotyping of 118 isolates yielded 11 straintypes: eight in one patient each, two in three patients each, and one type in eight patients. Based on chronological evaluation and genotypical identity of isolates, eight cases of cross-colonization were identified. Eight (50%) of 16 episodes of acquired colonization and two (25%) of eight cases of VAP due to *P. aeruginosa* seemed to be the result of cross-colonization.

Conclusions

Even in nonepidemic settings cross-colonization seems to play an important part in the epidemiology of colonization and infection with *P. aeruginosa*.

Introduction

Ventilator-associated pneumonia (VAP) is the most important nosocomial infection among mechanically ventilated patients in intensive care units (ICUs) and is associated with increased morbidity and mortality, especially when caused by *Pseudomonas aeruginosa*¹. Ventilator-associated pneumonia is almost always preceded by colonization of the upper respiratory tract with the causative microorganism². However, the exact routes of colonization leading to VAP and the relative importance of different pathways of colonization is a matter of controversy^{3,4}.

Respiratory tract colonization with *P. aeruginosa* may originate from endogenous sources such as the intestine or the stomach⁵⁻⁷, or from exogenous sources such as contaminated equipment or other patients colonized with *P. aeruginosa*⁸⁻¹⁰. Understanding the relative importance of these routes of colonization is crucial in order to the development of effective preventive measures against infection. If, for example, cross-colonization occurs frequently, improved compliance with infection control measures will be necessary, but such a measure will be less effective if most infections are from endogenous sources. In the latter case, modulation of bacterial colonization of the gastrointestinal tract may be more appropriate. The clinical observation of three episodes of VAP due to *P. aeruginosa* in a short period of time was the starting point for the present study. Infection was assumed to be the result of cross-colonization of microorganisms. In order to determine the importance of exogenous and endogenous colonization of *P. aeruginosa* we initiated an analysis of colonization and infection with this pathogen.

Patients and methods

Setting

The study was performed in two identical eight-bed intensive care units (ICU 1 and ICU 2) of the University Hospital Maastricht, a 700-bed hospital. Both ICUs contained a mixed population of surgical, medical, neurosurgical, and trauma patients who were randomly admitted. The units have different nursing staff and medical equipment, but the medical staff and paramedic care providers (physiotherapists, radiographic personnel, and the nutrition support team) have patient contacts in both ICUs. No outbreaks of multiple resistant microorganisms have occurred during the last five years.

Patients

All patients admitted to ICU 1 and ICU 2 (ventilated or nonventilated) were studied prospectively with regard to respiratory tract colonization and the development of VAP and other ICU-acquired infections in two study periods: from 6 November

1993 to 15 December 1993 in ICU 1 (40 days) and from 17 November 1993 to 15 January 1994 in ICU 2 (60 days). The following data were recorded on admission: age, sex, medical specialty, length of time in hospital before admission to the ICU, diagnosis on admission, reason for intubation, previous medical history, and APACHE II score¹¹. During the stay in ICU clinical parameters (temperature, leukocyte count, chest radiograph etc.) were recorded daily. All 16 mechanically ventilated patients receiving enteral nutrition with an expected duration of ventilation of ≥ 3 d were included in a prospective study on the influence of continuous and intermittent enteral feeding on respiratory and digestive tract colonization¹². Another six patients were included in a study on sequences of colonization within the patient.

Surveillance cultures

Patients were subjected to microbiological examination of endotracheal aspirates and urine on admission and then twice weekly. In addition, more extensive examination was performed on the patients included in the two prospective studies mentioned above. Gastric aspirates and oropharyngeal swabs were cultured daily from the 16 patients in the study on the effects of intermittent enteral feeding, and from the six patients studied to determine sequences of colonization cultures were obtained twice daily (08.00 and 16.00 hours) from six body sites (nose, oropharynx, sputum, gastric aspirate, rectum, and groin) during their stay in ICU. The results of all clinical cultures were also included in the analysis. No cultures were taken from nonintubated patients with an ICU stay of less than 24 h.

Definitions

Colonization

Colonization was defined as the isolation of microorganisms from specimens taken from any body site in the absence of infection. Colonization on admission was defined as that occurring within 48 h after admission to the ICU. When colonization occurred more than 48 h after admission to the ICU, without colonization on admission, it was defined as acquired colonization.

Ventilator-associated pneumonia

Ventilator-associated pneumonia was diagnosed by bronchoscopy with protected specimen brush (PSB) and bronchoalveolar lavage (BAL). The diagnosis was established when (A) at least three of the following criteria were met, (1) rectal temperature above 38.0°C or below 35.5°C, (2) blood leukocytosis ($>10 \cdot 10^3/\text{mm}^3$) and/or left shift or blood leukopenia ($<3 \cdot 10^3/\text{mm}^3$), (3) more than 10 leukocytes per high-power field in Gram stain of tracheal aspirate, and (4) a positive culture from tracheal aspirate, in combination with (B) a new or progressive infiltrate on chest radiograph, and (C) the presence of (1) a positive quantitative culture of a sample of secretions obtained by BAL (cut-off point $\geq 10^4$ cfu/ml) or PSB (cut-off point $\geq 10^3$ cfu/ml), or (2) positive cultures from blood unrelated to another source

obtained within 48 h before and after respiratory sampling and with the same species as cultured from tracheal aspirate, or (3) positive pleural fluid culture in the absence of previous pleural instrumentation. Pneumonia was considered to be ICU-acquired if symptoms began ≥ 48 h after admission to the ICU.

Endogenous colonization

Endogenous colonization was defined as colonization occurring with a strain of *P. aeruginosa* that had not previously been isolated from another patient.

Exogenous colonization or Cross-colonization

Defined as colonization occurring with a strain of *P. aeruginosa* with similar pulsed-field gel electrophoresis (PFGE) typing results to that of isolates from another patient present in one of both ICUs.

Colonization pressure and antibiotic pressure

For each study day the point prevalence of *P. aeruginosa* colonization in the ICU was calculated as follows: number of patients colonized with *P. aeruginosa* on that day divided by the total number of patients present in the ICU on that day. Subsequently, for each patient who was not colonized with *P. aeruginosa* on admission or at the start of the study, the mean point prevalence of *P. aeruginosa* colonization for all days in the ICU until acquisition of *P. aeruginosa* or until discharge (if the patient did not acquire colonization) was calculated. This calculated number reflects the colonization pressure with *P. aeruginosa* for the period in the ICU that the patient was not colonized.

Antibiotic pressure was determined as follows: the percentage of days that noncolonized patients received antibiotic therapy that provided *P. aeruginosa* a selective growth advantage ((amino)penicillins, first and second generation cephalosporins, macrolides, glycopeptides etc.) was calculated until acquisition of *P. aeruginosa* or until discharge from the ICU.

Microbiology and bacterial typing

Semiquantitative microbiological analysis of surveillance and clinical cultures were performed and PSB and BAL were analyzed quantitatively. All isolated species were identified according to methods routinely used in the medical microbiology laboratory. Specimens were inoculated immediately after sampling, seven days a week. From each culture plate with growth of *P. aeruginosa* three colonies were analyzed and stored separately until further processing. All isolates of *P. aeruginosa* were stored at a temperature of -70°C . From each patient and each body site found to be colonized with *P. aeruginosa* isolates were randomly selected for bacterial typing. The antibiotic susceptibility pattern was determined by measurement of minimal inhibitory concentrations using the micro-broth-dilution method according to the NCCLS guidelines. *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 and 35218 were used as reference strains. Susceptibility to the

following antibiotics was determined: piperacillin, co-ticarclavulanate, ceftazidime, imipenem, gentamicin, tobramycin, amikacin, ciprofloxacin, and ofloxacin. Pulsed-field gel electrophoresis was carried out according to the method described by Grothues et al.¹³ using a CHEF-DRII System (Bio-Rad Laboratories, Richmond, CA, USA) after restriction digestion with *SpeI* (Boehringer Mannheim, Germany). *Saccharomyces cerevisiae* was used as marker.

Pulsed-field gel electrophoresis patterns were compared visually on enlarged gel photographs to identify bands of identical relative mobility as fragments of identical size. The absolute number of band differences between pairs of isolates was recorded. The criteria of Tenover et al.¹⁴ were used to interpret chromosomal DNA restriction patterns produced by PFGE for typing of bacterial strains. Indistinguishable isolates (no band differences) and closely related isolates (2-3 band differences) were considered to be the same genotype, while possibly related and different isolates (4-6 and ≥ 7 band differences, respectively) were considered different genotypes.

Statistical analysis

Data are expressed as absolute numbers with or without percentages, as means with standard deviation (SD), or as medians with ranges. Frequency comparisons were performed by the Mann-Whitney test or chi-square test, a probability value of less than 0.05 being considered statistically significant.

Results

Patients

In all, 100 patients were studied of whom 65 were mechanically ventilated. During the study period in ICU 1 (40 days) 44 patients were studied, 40 patients were admitted and four were present at the start of the study. In ICU 2 (60 days) 56 patients were studied, 50 of whom were admitted and six were present at the onset of the study. The characteristics of the patients admitted to both ICUs were comparable (Table 9.1). Furthermore, the occupancy rate of beds was similar in both ICUs, 91% in ICU 1 and 93% in ICU 2.

Surveillance of colonization

In all, 1370 specimens were taken from 73 patients (243 endotracheal aspirates, 159 urine samples, 383 blood/pus/intravascular lines, 47 urine catheters, 28 PSB/BAL fluid samples, 199 oropharyngeal swabs, 40 rectal swabs, 191 gastric aspirates, 41 nose swabs, and 39 groin swabs). No cultures were taken from 27 patients because their stay in the ICU was less than 24 h and they were not intubated. Most of these patients were postoperative neurosurgical patients and patients admitted after autointoxication. *P. aeruginosa* was isolated from 69 specimens (22 endotracheal aspirates, two urine samples, 15 blood/pus/

intravascular lines, three urine catheters, seven PSB/BAL fluid samples, 11 oropharyngeal swabs, two rectal swabs, five gastric aspirates, one nose swab, and one groin swab) obtained from 23 patients. Eight of them developed VAP. Surveillance cultures were positive for *P. aeruginosa* before this species was isolated from clinical cultures in 77% of cases.

Colonized patients had a longer median stay in the ICU than noncolonized patients (25 vs. 3 d, $p < 0.0001$), were more frequently intubated (96% vs. 62%, $p < 0.01$), and had a longer median duration on mechanical ventilation (17 vs. 2 d, $p < 0.0001$), but did not differ significantly with respect to the other characteristics (data not shown).

Four (4.4%) of the 90 patients admitted to the ICUs were colonized with *P. aeruginosa* on admission, four (10%) of 40 in ICU 1 and none of 50 in ICU 2. In addition, three out of 10 patients who were present in the ICU at the start of the study were already colonized, one in ICU 1 and two in ICU 2. Thus, 93 patients were not colonized with *P. aeruginosa* at the onset of the study or on admission to the ICU, and 16 (17%) of them acquired colonization with *P. aeruginosa*, four of 39 in ICU 1 and 12 of 54 in ICU 2.

Table 9.1 Patient characteristics

	ICU 1	ICU 2	Total
Number of patients studied	44	56	100
Number of colonized patients	9	14	23
- prior to study-period	1	2	3
- on admission	4	0	4
- acquired	4	12	16
Number of pneumonias	3	5	8
Median age (range)	61 (0-92)	61 (0-79)	61 (0-92)
Male/female	25/19	30/26	55/45
Median APACHE II score (range)	21 (5-33)	18 (2-41)	19 (2-41)
Median days in ICU (range)	3 (1-535)	4 (1-68)	4 (1-535)
Hospital stay prior to ICU*	2 (0-147)	2 (0-115)	2 (0-147)
Medical specialty			
- surgery/trauma	12	12	24
- medical	10	17	27
- pulmonology	5	6	11
- neurology/neurosurgery	7	10	17
- paediatrics	4	1	5
- cardiology	3	4	7
- other	3	6	9
Mortality	13 (30%)	14 (25%)	27 (27%)

* Median number of days in hospital before admission to ICU.

Ventilator-associated pneumonia

During the study period eight (8%) of 100 patients developed VAP caused by *P. aeruginosa* (Table 9.1), three in ICU 1 and five in ICU 2, with a mean duration from admission to ICU to diagnosis of VAP of 7.3 d (SD 3.1). The incidence of VAP caused by *P. aeruginosa* was 8% in the total study population. However, the incidence among ventilated patients was 12% (eight of 65) and 20% (eight of 41) for patients ventilated for ≥ 3 d. The overall incidence of VAP caused by *P. aeruginosa* during the total study period was 0.9 per 100 patients days. In periods of two months the incidence of VAP caused by *P. aeruginosa* ranged from 0.4 to 0.9 per 100 patient days in the year following the study period, suggesting that the incidence of VAP during the period of study was not exceptional in our ICU.

Microbiological typing

In all, 118 isolates of *P. aeruginosa* were genotyped, cultured from the following sites: 61 endotracheal aspirates, seven blood samples, two intravascular lines, 21 PSB/BAL fluid samples, 18 oropharynx, and nine stomach samples. In two patients (Pt. B and S) *P. aeruginosa* was cultured only once during ICU stay. Unfortunately these isolates were lost in the process of storage and could not be genotyped.

Antimicrobial susceptibility patterns were similar for isolates of all but one patient (i.e., susceptible to piperacillin, co-ticarcavulanate, ceftazidime, imipenem, gentamicin, tobramycin, amikacin, ciprofloxacin, and ofloxacin). One patient (Pt. J) was colonized with a strain of *P. aeruginosa* resistant to gentamicin and tobramycin (type X), and such a strain was not isolated from any other patient. We assumed therefore that cross-colonization with this strain did not occur and isolates from this patient were not genotyped. Using PFGE, 11 different genotypes could be distinguished (type 1 to 11). Eight genotypes were found in single patients only, whereas one genotype (type 2) was isolated from eight patients and two genotypes (type 3 and type 7) were isolated from three patients. Two patients (Pt. U and W) were colonized with two different genotypes each.

Incidence of cross-colonization

Genotype 2 was isolated from eight patients, five in ICU 1 and three in ICU 2 (Figure 9.1). Patient M (ICU 2) was colonized with genotype 2 at the start of the study. Patient T (ICU 1) acquired genotype 2, presumably via cross-colonization from patient M. Subsequently, genotype 2 colonized patient O (ICU 1) and patient K (ICU 2). Since patients M and K were not in ICU 2 simultaneously, patient K most probably acquired genotype 2 via patient T in ICU 1. Acquisition of genotype 2 by patient D could have been the result of cross-colonization with patients U or R from ICU 1 as donor, or with patient K in ICU 2 as donor. Patients K and D were in ICU 2 simultaneously, but cultures of patient D only became positive six days after discharge of patient K. This delay may have been caused by sampling

error or because colonization initially occurred in numbers below the detection threshold of culture techniques. Three other patients in ICU 1 were colonized with genotype 2 when they were admitted (Pt. Q, R and U). Two of them were admitted directly from the community and one patient had been admitted to a general hospital ward one day before ICU admission. Cultures from all three patients were taken within 24 h of admission. In patient Q, admitted directly from the community, genotype 2 was cultured from the endotracheal aspirate on admission. This patient had been treated in the ICU four months earlier and was colonized with *P. aeruginosa* during that time as well. Unfortunately no strains from that period were genotyped. Thus, four patients (Pt. T and O in ICU 1, and K and D in ICU 2) appeared to have acquired genotype 2 by cross-colonization. At the start of the study, patient H (ICU 2) was colonized with genotype 3. Subsequently, colonization with this genotype was demonstrated in patient A (on the day of the study) and patient I (both in ICU 2). Although cultures from patient I only became positive with genotype 3 after discharge of patients A and H, all three patients were treated simultaneously in ICU 2 for a number of days. Finally, patient C (ICU 2) acquired colonization with genotype 7, presumably from an endogenous source. Subsequently, two other patients in ICU 2 (Pt. F and N) acquired colonization with this genotype. All other patients acquired colonization with a unique genotype which was therefore presumed to be derived from endogenous sources. In all, eight episodes of cross-colonization were demonstrated (four patients with genotype 2, two patients with genotype 3, and two patients with genotype 7). Fifty percent of all patients acquiring colonization with *P. aeruginosa* did so via cross-colonization, the other 50% most probably being colonized from endogenous sources. In two (25%) of eight patients with VAP caused by *P. aeruginosa*, colonization probably occurred via cross-colonization. The three episodes of VAP within a short period of time that initiated this study were caused by three different genotypes of *P. aeruginosa*.

Colonization pressure and antibiotic pressure

A tendency towards a higher rate of cross-colonization in ICU 2 was observed; two of 39 (5%) patients in ICU 1 and six of 54 (11%) in ICU 2 ($p=0.2$; chi-square test). The fact that more episodes of cross-colonization occurred in ICU 2 may be related to the higher colonization pressure in this ward. Mean colonization pressure was 40% (SD 11%) in ICU 2 and 35% (SD 9%) in ICU 1 ($p<0.05$; Mann-Whitney test). The mean antibiotic pressure for patients was comparable in both ICUs. However, mean antibiotic pressure was higher for patients who acquired colonization than for those who did not (68% (SD 45%) and 37% (SD 43%) respectively; $p<0.05$; Mann-Whitney test).

Figure 9.1 Colonization of *P. aeruginosa* genotypes. Each horizontal bar represents the length of time a patient was colonized during the study period. Patient characters (in capitals) and genotypes (in numbers) are depicted within or just adjacent to the bars. Genotypes colonizing more than one patient (type 2, 3 and 7) have marked bars. Blank bars represent the genotypes that were found in single patients only (type 1, 4, 5, 6, 8, 9, 10, and 11). Type X represents the strain resistant to gentamicin and tobramycin.

Discussion

Our findings suggest that cross-colonization may be an important route of colonization and infection for *P. aeruginosa* even when overall incidence rates do not suggest the occurrence of an outbreak. The epidemiology of *P. aeruginosa* may therefore be described as a silent epidemic, sharing similarities with the epidemiology of other nosocomial pathogens such as vancomycin-resistant

enterococci, methicillin-resistant *Staphylococcus aureus*, and ceftazidime-resistant Gram-negative bacilli. The results also show that surveillance in combination with molecular biotyping may be used as a method to perform continuous quality improvement. Finally, our findings emphasize the constant need of improving compliance with infection control measures, and warrant further studies to elucidate the relative importance of endogenous and exogenous colonization with *P. aeruginosa* and to determine variables that influence both routes of colonization and infection.

The incidence of VAP due to *P. aeruginosa* (12% of mechanically ventilated patients) during the period of study is representative for our ICU. During 1994, the year following this study, 58 of 276 (21%) mechanically ventilated patients admitted to our ICU developed VAP, in 28 (48%) of the cases due to *P. aeruginosa*, either alone or in combination with other microorganisms (i.e., 10% of all mechanically ventilated patients)¹⁵. In a large study performed in 1992 and 1993 in our ICU the incidence of VAP was 22%, with *P. aeruginosa* involved in 58% of patients (i.e., 13% of all mechanically ventilated patients)¹⁶. The incidence of VAP has therefore remained fairly stable throughout the years. Moreover, the clinical observation of three episodes of VAP within a short period of time appeared to be caused by three different genotypes. The period under study therefore reflects our endemic setting and not an outbreak. Comparison of incidence rates to those of other ICUs is difficult, mainly because of different patient populations. However, the incidence of VAP ranges from 15% to 24% in settings where VAP was also diagnosed by bronchoscopy¹⁷⁻²⁰, and the proportion caused by *P. aeruginosa* varied from 14% to 55%¹⁷⁻²².

Observations of similar genotypes of *P. aeruginosa* colonizing and infecting several patients strongly suggest the occurrence of cross-colonization, not only between patients in the same ward but also between patients from different wards. Although contaminated equipment might have been a source, regular examinations of nebulizers and sinks were always negative for *P. aeruginosa*, and the polyclonal character of the epidemiology make this possibility fairly unlikely.

The possibility of cross-colonization of *P. aeruginosa* in nonepidemic situations has rarely been studied. Olson and coworkers²³ addressed this issue in a study of 270 patients admitted to a medical/surgical ICU ward; 63 (23%) patients were colonized with *P. aeruginosa* on admission, 33 (16%) acquired colonization, twelve (36%) of whom were found to be due to cross-colonization. In a prospective surveillance study carried out in five ICUs during a six month period, Chetchotisakd et al.²⁴ did not find an important role for cross-colonization, only 14 (10%) of 137 isolates of bacteria (*P. aeruginosa*, *E. coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, enterococci) cultured from patients with suspected infection being acquired by cross-colonization. Although molecular biotyping methods were used to determine identity of isolates, they may have underestimated the true incidence of cross-colonization. Only a single isolate from a suspected site of

infection was analyzed, without including isolates from colonized patients. It is evident that infections with *P. aeruginosa* only form the tip of the iceberg of the complete epidemiology^{2,23}, and its epidemiology can only be studied reliably when surveillance of colonization is included. Moreover, patients may be colonized or infected with multiple genotypes of *P. aeruginosa*, and analysis of a single isolate will not accurately represent the whole epidemiology²⁵.

The difference in the number of cases of cross-colonization between both ICUs in the present study (two in ICU 1 and six in ICU 2) may be associated with the higher colonization pressure in ICU 2 compared with ICU 1. However, differences in compliance with infection control measures, which was not monitored, may be another explanation of this difference. In addition, antibiotic pressure was clearly higher for patients who acquired *P. aeruginosa*. These findings suggest that both endogenous colonization (e.g., intestinal colonization), and exogenous colonization play a role in the epidemiology of respiratory tract colonization with *P. aeruginosa*. In this regard, the epidemiology of *P. aeruginosa* may share similarities with endemic situations of other nosocomial pathogens such as vancomycin-resistant enterococci. These bacteria are also frequently acquired via cross-colonization²⁶ and acquisition seems to be influenced by a high colonization pressure and by a high antibiotic pressure, providing these bacteria with a selective growth advantage²⁷. However, the present study does not allow us to determine the precise relative impact of both exogenous and endogenous colonization of *P. aeruginosa*. Since the study was started abruptly after the clinical suspicion of cross-colonization, surveillance was not uniform in all patients. More importantly, no surveillance cultures of the rectum were performed. Since rectal colonization may be a source of respiratory tract colonization^{7,28,29}, cases of endogenous colonization may have been missed. The relative importance of endogenous and exogenous colonization in the pathogenesis of VAP therefore remains to be established.

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10

Prevention of ventilator-associated pneumonia by oral decontamination: A prospective, randomized, double-blind, placebo-controlled study

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Abstract

Background

Prevention of ventilator-associated pneumonia (VAP) remains a challenge for intensive care medicine. Colonization of the intestinal tract has been assumed to be important in its pathogenesis, but relative impacts of oropharyngeal, gastric or intestinal colonization have not been elucidated. Our aim was to prevent VAP by modulation of oropharyngeal colonization, without influencing gastric and intestinal colonization and without systemic prophylaxis.

Methods

In this prospective, randomized, placebo-controlled double-blind study 87 patients received topical antimicrobial prophylaxis (gentamicin/colistin/vancomycin 2% in Orabase, q6h) in the oropharynx and 139 patients received placebo. Colonization was monitored in oropharynx, trachea, stomach and intestine. Ventilator-associated pneumonia was diagnosed on quantitative cultures obtained via bronchoscopic techniques.

Results

Baseline characteristics were comparable in both groups. Topical prophylaxis eradicated oropharyngeal and tracheal colonization present on admission (75% vs. 4%, $p<0.00001$, and 52% vs. 16%, $p=0.002$, respectively) and prevented acquired oropharyngeal colonization (10% vs. 61%, $p<0.00001$). Colonization rates in stomach and intestine were not affected. Incidences of VAP were 10% in study and 27% in control patients ($p=0.002$), resulting in a relative risk reduction for VAP of 0.62 (95% CI: 0.25-0.81). This was associated with fewer antibiotic courses per patient (0.95 ± 0.68 vs. 1.27 ± 0.87 , $p=0.018$), but with similar durations of ventilation and intensive care unit (ICU) stay. Although ICU mortality tended to be lower among study patients (29% vs. 38%, $p=0.15$), this difference did not persist during follow-up.

Conclusions

Modulation of oropharyngeal colonization resulted in a 62% relative risk reduction for VAP. Oropharyngeal colonization is of paramount importance in the pathogenesis of VAP, and a targeted approach to prevent colonization at this site is a very effective method of infection prevention.

Introduction

Ventilator-associated pneumonia (VAP) is the most frequently occurring nosocomial infection among mechanically ventilated patients, with reported incidences as high as 78%^{1,2}. Usually two types of VAP are distinguished: early onset VAP, when diagnosed within the first four days of mechanical ventilation, and late onset VAP, occurring thereafter. Since VAP has been associated with increased morbidity, longer hospital stay, increased health care costs and higher mortality rates^{3,4}, prevention of this infection is a major challenge for intensive care medicine.

Early-onset VAP is caused by pathogens presumably colonizing the respiratory tract at the time of intubation, such as *Streptococcus pneumoniae*, *Staphylococcus aureus* and *Haemophilus influenzae*. Late-onset VAP is frequently caused by enteric Gram-negative bacteria and *Pseudomonas* spp. These bacteria may be transmitted from exogenous (e.g., other colonized patients or contaminated common sources) or from endogenous sources (e.g., the stomach or intestine). Because of concomitant colonization of the upper respiratory and digestive tract with these bacteria, the gastropulmonary route of colonization has been considered to be important in the pathogenesis of late onset VAP⁵. Based on the presumed relevance of gastric colonization, modulation of gastric colonization has been attempted as a measure to prevent VAP. However, neither the use of sucralfate for stress-ulcer prophylaxis⁶⁻¹¹, nor modulation of enteral feeding^{12,13} have proven unequivocally to reduce the incidence of VAP. In addition, administration of nonabsorbable antibiotics into the stomach and the intestine, reduced colonization at these sites, but did not influence the incidence of VAP¹⁴. Finally, sequential analyses of colonization failed to demonstrate an important role of the gastropulmonary route in several recent studies^{10,13,15-17}.

Selective decontamination of the digestive tract (SDD) decreases incidences of VAP¹⁸⁻²², by eradicating microorganisms from the intestine, the stomach and the oropharynx by nonabsorbable antibiotics, in combination with systemic antibiotic prophylaxis during the first days of intensive care unit (ICU) admission. However, the constant threat of selection and overgrowth of antibiotic resistant microorganisms, lack of formal cost-benefit analyses and absence of beneficial effects on mortality rates have limited a wide-spread use of SDD²³. From a conceptual point of view, it has remained unclear which part of SDD prevents VAP. The importance of gastric and intestinal colonization has been questioned, and systemic antibiotics during the first days of intubation may prevent early-onset but not late-onset VAP²⁴.

We hypothesized that decontamination of the oropharynx, without modulating gastric and intestinal colonization, and without systemic antibiotic prophylaxis, would reduce the incidence of VAP in critically ill intensive care patients.

Methods

Setting

The study was conducted in three ICUs from September 1994 to December 1996. ICU 1 and ICU 2 are located in the University Hospital Maastricht; both harbour a mixed population of medical, surgical, trauma and neurologic patients. ICU 3 is located in the University Hospital Groningen and is a surgical and trauma ICU.

Patients

Adult patients (≥ 16 yr) admitted to one of these ICUs who were intubated within 24 h of admission and who needed mechanical ventilation with an expected duration of >2 d could be included. Patients were randomized to receive either topical antimicrobial prophylaxis (TAP), consisting of an Orabase with 2% gentamicin, 2% colistin and 2% vancomycin or to placebo Orabase without antibiotics. Active medication and placebo could not be visibly distinguished. Orabase was applied in the buccal cavities on a gloved finger, q6h. The application of Orabase was started within 24 h of intubation. Patients in whom the application of Orabase was not possible or contraindicated, were not eligible for the study. No prophylactic antibiotics were administered via the nasogastric tube or systemically as part of the study regimen. Patients were studied until extubation or death. Since $\geq 95\%$ of the first episodes of VAP occur within the first three weeks of ventilation^{8,10}, application of Orabase was limited to 21 d. Patients were evaluable if they had been included in the study for >2 d.

Sucralfate (Ulcogant Suspension, E.Merck, Germany) was used for stress-ulcer prophylaxis, unless patients were receiving H_2 -antagonists or $H^+K^+ATPase$ inhibitors on admission. Stress-ulcer prophylaxis was discontinued when enteral feeding was started. All patients had a nasogastric tube. In general, all patients were in supine position during controlled mechanical ventilation and if possible in semirecumbent position during weaning. Moreover, all ventilated patients received daily chest physiotherapy, and endotracheal suctioning was performed by the nursing staff if necessary.

Study design

This study was a prospective, randomized, double-blind, placebo controlled study. Since patients, physicians and investigators were blinded to treatment allocation during data collection and analyses in fact the study was triple-blinded. Randomization was conducted per hospital and was executed by the department of clinical pharmacy of the University Hospital Maastricht. The inclusion scheme aimed to create two separate control groups in ICU 1 and ICU 2. One control group was studied in the presence of patients receiving TAP and a second control group was studied in an ICU where no TAP was used. After nine months of study the inclusion scheme was reversed for another six months (Figure 10.1). To secure the double-blind study design, only the study-supervisor and the hospital

pharmacist were aware of this inclusion scheme. In ICU 3, all patients were randomized to receive either TAP or placebo during the whole study period (Figure 10.1). This inclusion scheme was chosen to assess whether TAP influences infection rates in control patients treated simultaneously in the same ICU²⁵. The study protocol was approved by the ethical committee of both hospitals. Informed consent was obtained from the patient or, if this was not possible, from a representative of the family.

Figure 10.1 Trial profile.

Data collection

Demographic data (e.g., age, sex, medical specialty, pre-existent diseases and length of hospital stay before admission to ICU) and APACHE II scores²⁶ were recorded on admission. Number of days in ICU and on mechanical ventilation, surgical procedures, parameters of infection (temperature, leukocyte counts and differential counts, chest radiograph interpretations, culture results) and antibiotic use were monitored prospectively. Surveillance cultures were taken on admission and subsequently twice weekly (monday and thursday) of oropharynx, trachea, stomach and rectum. The results of surveillance cultures of oropharynx, stomach and rectum were not reported to the ICU attendings.

Microbiological analysis and monitoring of resistance

Semiquantitative and/or quantitative microbiological analysis of culture samples were performed according to standard microbiological methods^{27,28}. Antibiotic susceptibility was determined by means of a microbroth dilution method according to the NCCLS guidelines. *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and ATCC 35218, *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as reference strains. The criteria for susceptibility and resistance, according to the NCCLS guidelines, were used. Colonization was analyzed for Enterobacteriaceae, Pseudomonadaceae and *Staphylococcus aureus* (i.e., potentially pathogenic microorganisms or PPMO), enterococci and *Candida* spp. Vancomycin-susceptibility was tested for all enterococci.

Definitions

Colonization was defined as the isolation of microorganisms (i.e., bacterial or yeast species) in two or more consecutive specimens of one site, in the absence of infection. Colonization on admission was defined as colonization demonstrated within 24 h after admission to ICU. Eradication of colonization was defined as the disappearance of microorganisms in two or more consecutive cultures from a body site that was colonized on admission, and is expressed as the proportion of colonized patients in whom eradication occurred. Acquired colonization was defined as colonization demonstrated >24 h after ICU admission, in patients without colonization on admission.

All patients were examined daily for the presence of VAP. When VAP was suspected on clinical grounds and chest radiograph, bronchoscopy with protected specimen brush (PSB) and bronchoalveolar lavage (BAL) were performed. The diagnosis of VAP was established on the basis of the criteria listed in table 10.1. Pneumonia was considered ICU-acquired when diagnosed ≥ 48 h after admission to ICU. Pneumonia was classified early-onset when diagnosed within the first 4 d of mechanical ventilation, and late-onset when occurring thereafter.

Nosocomial infections, other than VAP, were diagnosed according to Centers for Disease Control (CDC) definitions²⁹. Antibiotic use was analyzed in courses. A course was defined as an episode of clinical or suspected infection in which antibiotics, either consecutively or in combination, were prescribed.

Outcome variables

The incidence of VAP was the primary outcome variable of the study. Colonization of oropharynx, trachea, stomach and rectum, number of days in ICU and on mechanical ventilation, other nosocomial infections, antibiotic use and mortality were secondary outcome variables.

Statistics

Data are expressed as absolute numbers with or without percentages, as means

with SD or as medians with ranges. Chi-square or Fisher's exact test were used to compare proportions, *t* test or Wilcoxon-Mann-Whitney test to compare means. For each patient the time until event (i.e., diagnosis of VAP or end of study) and death was determined to calculate the probability of remaining without VAP and survival using Kaplan-Meier survival analysis. Groups were compared by log-rank test. Incidence rates of pneumonia were compared using risk ratios with 95% CI. A probability value <0.05 was considered to denote statistical significance and all reported *P*-values are two-sided. Statistical analysis was performed using SPSS/PC statistical package.

Table 10.1 Diagnosis of pneumonia

Criteria	
A	≥ 3 positive of the following 4:
	1. rectal temperature above 38.0°C or below 35.5°C
	2. blood leukocytosis ($>10 \cdot 10^3/\text{mm}^3$) and/or left shift or blood leukopenia ($<3 \cdot 10^3/\text{mm}^3$)
	3. >10 leukocytes per high-power field in Gram stain of tracheal aspirate
	4. positive culture from tracheal aspirate
	and
B	new, persistent or progressive infiltrate on chest radiograph
	and
C	≥ 1 positive of the following 3:
	1. positive quantitative culture of a sample obtained by BAL (cut-off point $\geq 10^4$ cfu/ml) or PSB (cut-off point $\geq 10^3$ cfu/ml)
	2. positive blood culture unrelated to another source and obtained within 48 h before and after respiratory sampling
	3. positive pleural fluid culture in the absence of previous pleural instrumentation

Pneumonia was diagnosed when A and B and C were positive.
BAL, bronchoalveolar lavage; PSB, protected specimen brush.

Results

Patients

During the 28 months study period 213 eligible patients were admitted to ICU 1 and 2. Fifteen patients were not included because of mandibular fixation after facial trauma ($n=2$), severe intraoral mucosal haemorrhages due to thrombocytopaenia ($n=2$), and refusal to give informed consent ($n=11$). Seventeen of 198 included patients were ventilated and/or intubated ≤ 2 d or succumbed within 2 d (Figure 10.1). Although 93% of all longterm ventilated patients were included, the number of patients studied simultaneously was lower than expected. Overall, the median daily proportion of all patients being included was 14% (range 0-86%), which means that per day on average one patient was included in each

7-bed ICU. The low inclusion rate was a result of an unexpected high admittance rate of children and shortterm ventilated postoperative neurosurgical patients, which were not eligible for our study. In addition, several patients remained in ICU long after the 21 d of study. As a result, we were unable to assess whether TAP influences infection rates in control patients. Since the baseline characteristics of both control groups were comparable (data not shown), these patients were grouped. Consequently, there were more control than study patients.

In ICU 3, informed consent was refused by 10 eligible patients. In this ICU 47 patients were included, two of whom were not evaluable since they were ventilated <2 d (Figure 10.1). In all, 226 patients were evaluable; 87 study patients and 139 control patients. Baseline characteristics were comparable for both groups (Table 10.2). The baseline characteristics of the patients who were not evaluable ($n=19$) were comparable to those of evaluable patients (data not shown).

Table 10.2 Baseline characteristics of the study patients and controls

Characteristic	Study Patients ($n=87$)	Controls ($n=139$)
Male/female	59/28	100/39
Mean age (yr; $\pm$ SD)	57.6 $\pm$ 19.0	58.4 $\pm$ 16.5
APACHE II score (mean $\pm$ SD)	21.0 $\pm$ 6.8	21.6 $\pm$ 7.4
Days in hospital prior to ICU; median (range)	2 (0-66)	2 (0-48)
Medical specialty; no. of patients (%)		
medical*	34 (39)	44 (32)
surgery†	29 (33)	59 (42)
trauma	17 (20)	26 (19)
neurology‡	6 (7)	8 (6)
other§	1 (1)	2 (1)
Antibiotic use on admission; no. of patients (%)	41 (47)	58 (42)
Underlying diseases; no. of patients (%)¶		
cardiovascular disease	34 (39)	68 (49)
gastrointestinal disease	22 (25)	29 (21)
respiratory disease	25 (29)	37 (27)
alcoholism or drug abuse	10 (12)	18 (13)
neoplastic disease	13 (15)	15 (11)
diabetes mellitus	12 (14)	24 (17)
neurologic disease	20 (23)	22 (16)
renal insufficiency	4 (5)	6 (4)
immunodeficiency	3 (3)	3 (2)
Reason for intubation; no. of patients (%)		
respiratory failure	32 (37)	41 (30)
trauma	10 (12)	19 (14)
shock or hypoxic acidosis	10 (12)	26 (19)
cardiopulmonary failure	4 (5)	9 (7)
pneumonia on admission	10 (12)	14 (10)
neurologic disease	6 (7)	9 (7)
elective	15 (17)	21 (15)

* Including pulmonology and cardiology.

† Including cardiopulmonary surgery and urology.

‡ Including neurosurgery.

§ Gynaecology and ENT.

¶ More than one condition possible per patient.

Colonization

Colonization rates on admission in oropharynx, trachea, stomach and rectum with PPMO, enterococci and *Candida* spp., were comparable for study and control patients (Table 10.3). TAP eradicated oropharyngeal colonization with PPMO present on admission (75% of study patients vs. 4% in controls; $p<0.00001$) and reduced rates of acquisition of colonization with PPMO at this site (10.3% vs. 60.9%; $p<0.00001$). In addition, TAP was associated with eradication (52.0% of study and 15.8% of control patients; $p=0.002$), and with a tendency towards prevention of acquisition with PPMO in the trachea (35.6% vs. 47.5%; $p=0.08$). Importantly, in both groups colonization rates of the stomach and rectum were not influenced (Table 10.3).

Acquisition of enterococcal colonization occurred more frequently in control patients (28.8% vs. 2.5% in oropharynx; $p<0.00001$, and 35.9% vs. 17.6% in the stomach; $p=0.008$). Acquired enterococcal colonization was comparable in trachea and rectum. Although no antifungal agents were used, rates of acquired colonization with *Candida* spp. were comparable in both groups at all body sites (Table 10.3).

Ventilator-associated pneumonia

During the study period VAP was diagnosed in nine (10.3%) study and 38 (27.3%) control patients ($p=0.002$; Table 10.4), after 9 d (range 2-18) and 6 d (range 2-19) in study and control patients respectively ($p=0.27$). Therefore, administration of TAP resulted in a relative risk for VAP of 0.38 (95% CI; 0.19-0.75), a relative risk reduction of 0.62 (95% CI; 0.25-0.81), and an absolute risk reduction of 0.17 (95% CI; 0.07-0.27), which implies that six patients needed to be treated to prevent one episode of VAP. Approximately 20% of the episodes of VAP were early onset (two of nine study patients and eight of 38 control patients). Ventilator-associated pneumonia was accompanied by bacteraemia in three control and in none of the study patients. In both study groups a similar distribution of etiologic pathogens was observed (Table 10.4). The difference in the incidence of VAP persisted after day 21 (Figure 10.2), demonstrating that ceasing TAP was not associated with a rebound effect on the incidence of VAP. Of the patients that were ventilated for more than 21 d, VAP was diagnosed in one study patient and in four control patients after the application of trial medication was stopped. For the entire ICU stay, administration of TAP resulted in a relative risk for VAP of 0.49 (95% CI; 0.28-0.86), a relative risk reduction of 0.51 (95% CI; 0.14-0.72), and an absolute risk reduction of 0.15 (95% CI; 0.05-0.26). A clinical suspicion of VAP with negative or nonsignificant culture results from bronchoscopy occurred with equal frequency in both study groups (24 (27.6%) and 40 (28.8%) in study and control patients, respectively).

Table 10.3 Colonization data of the study patients and controls*

Variable	On admission		Eradication†		Acquired‡	
	Study	Control	Study	Control	Study	Control
Oropharynx						
Enterobacteriaceae	(n=87) 40 (46)	(n=138) 60 (43)	30 (75)	19 (32) [§]	(n=87) 2 (2)	(n=138) 68 (49) [§]
Pseudomonadaceae	10 (11)	15 (11)	8 (80)	1 (7) [§]	5 (6)	38 (31) [§]
<i>Staphylococcus aureus</i>	13 (15)	16 (12)	13 (100)	7 (44) [§]	2 (3)	8 (7)
PPMO§	52 (60)	75 (54)	39 (75)	3 (4) [§]	9 (10)	84 (61) [§]
<i>Enterococcus</i> spp.	7 (8)	20 (15)	3 (43)	4 (20)	2 (3)	34 (29) [§]
<i>Candida</i> spp.	53 (61)	74 (54)	4 (8)	9 (12)	16 (47)	23 (36)
Trachea						
Enterobacteriaceae	(n=87) 16 (18)	(n=139) 20 (14)	11 (69)	4 (20) [§]	(n=87) 13 (15)	(n=139) 42 (30) [§]
Pseudomonadaceae	3 (3)	9 (6)	0 (0)	0 (0)	21 (25)	38 (29)
<i>Staphylococcus aureus</i>	6 (7)	13 (9)	4 (67)	5 (38)	5 (6)	14 (11)
PPMO§	25 (29)	38 (27)	13 (52)	6 (16) [§]	31 (36)	66 (48) [§]
<i>Enterococcus</i> spp.	0 (0)	1 (1)	0 (0)	1 (100)	0 (0)	2 (1)
<i>Candida</i> spp.	15 (17)	31 (22)	7 (47)	9 (29)	19 (26)	21 (19)
Stomach						
Enterobacteriaceae	(n=80) 13 (16)	(n=135) 29 (21)	3 (23)	5 (17)	(n=70) 24 (34)	(n=123) 58 (47) [§]
Pseudomonadaceae	5 (6)	7 (5)	1 (20)	1 (14)	17 (26)	30 (26)
<i>Staphylococcus aureus</i>	3 (4)	5 (4)	3 (100)	3 (60)	2 (3)	3 (3)
PPMO§	18 (23)	34 (25)	5 (31)	6 (19)	32 (46)	66 (54)
<i>Enterococcus</i> spp.	3 (4)	7 (5)	1 (33)	0 (0)	12 (18)	42 (36) [§]
<i>Candida</i> spp.	53 (66)	78 (58)	4 (8)	10 (13)	16 (69)	27 (54)
Rectum						
Enterobacteriaceae	(n=85) 72 (85)	(n=134) 115 (86)	8 (11)	14 (12)	(n=82) 33 (40)	(n=133) 61 (46)
Pseudomonadaceae	22 (26)	33 (25)	4 (18)	2 (6)	16 (27)	36 (36)
<i>Staphylococcus aureus</i>	3 (4)	4 (3)	3 (100)	3 (75)	3 (4)	3 (2)
PPMO§	76 (89)	119 (89)	5 (7)	2 (2)	42 (51)	77 (58)
<i>Enterococcus</i> spp.	44 (52)	71 (53)	4 (9)	4 (6)	16 (40)	33 (53)
<i>Candida</i> spp.	28 (33)	33 (25)	3 (11)	6 (18)	16 (29)	23 (23)

* Numbers of patients (percent).
† Eradication was analyzed only for colonization present on admission.
‡ Acquired colonization was analyzed for all patients, if culture results were available.
§ PPMO denotes potential pathogenic microorganisms (Enterobacteriaceae, Pseudomonadaceae, and *Staphylococcus aureus*).
¶ Statistically significant difference, P-values: [§] < 0.01, [§] < 0.0001, [¶] < 0.00001.
¶ P-value 0.08

Table 10.4 Outcome data of the study patients and controls*

Variable	Study Patients (n=87)	Controls (n=139)	P Value†
Days studied (mean $\pm$ SD)	10.1 $\pm$ 5.9	10.9 $\pm$ 6.6	NS
Days ventilated; median (range)	10 (1-50)	10 (1-95)	NS
Days in ICU; median (range)	13 (4-54)	14 (4-108)	NS
Days in hospital after ICU admission; median (range)	26 (4-185)	24 (4-280)	NS
Ventilator-associated pneumonia	9 (10)	38 (27)	0.002
polymicrobial	4	18	
pathogens			
<i>Pseudomonas aeruginosa</i>	3	13	
<i>Staphylococcus aureus</i>	3	11	
<i>Haemophilus influenzae</i>	2	6	
Enterobacteriaceae	4	18	
<i>Streptococcus</i> spp.	-	6	
<i>Candida</i> spp	1	4	
other‡	-	6	
Recurrent episode of VAP during study	2 (22)	3 (8)	NS
Nosocomial infections			
total	37 (43)	111 (80)	<0.00001
respiratory tract (excluding VAP)§	11 (13)	30 (22)	0.09
abdominal	4 (5)	11 (8)	0.3
other¶	13 (15)	32 (23)	0.1
Courses of antibiotics (mean $\pm$ SD)			
during study period	0.95 $\pm$ 0.68	1.27 $\pm$ 0.87	0.02
during ICU stay	1.33 $\pm$ 0.76	1.76 $\pm$ 1.30	0.04
Tracheostomy	13 (15)	28 (20)	NS
Enteral feeding	71 (82)	96 (69)	0.04
Sucralfate	53 (61)	105 (76)	0.02
H ₂ -antagonists/H ⁺ K ⁺ ATPase inhibitors	27 (31)	38 (27)	NS
Mortality			
ICU	25 (29)	53 (38)	0.15
Hospital	30 (35)	59 (42)	NS
one year after inclusion in the study	43 (49)	69 (50)	NS
follow-up until 01-01-98	46 (53)	78 (56)	NS

* Numbers (percent), unless stated otherwise

† NS denotes not significant.

‡ Including *Enterococcus faecalis*, *Staphylococcus epidermidis*, *Hafnia alvei*, *Pasteurella* spp., and *Bacillus* spp.

§ Including sinusitis, tracheobronchitis, and lung empyema.

¶ Including urinary tract infections, CNS infections, tissue infections, IV-line related infections and sepsis e.c.i.

Secondary outcome variables

The number of days in study, on mechanical ventilation, in ICU and in hospital were comparable for study and control patients (Table 10.4). There were differences in the use of enteral feeding and sucralfate: study patients received enteral feeding more frequently (81.6% vs. 69.1%; $p=0.04$), and control patients were more likely to receive sucralfate for stress ulcer prophylaxis (75.5% vs. 60.9%; $p=0.02$). H₂-antagonists and/or H⁺K⁺ATPase inhibitors were administered to

approximately 30% of patients in both groups. Mean durations of enteral feeding, use of sucralfate, and H₂-antagonists and/or H⁺K⁺ATPase inhibitors were comparable in both patient groups.

Although ICU mortality tended to be lower in study patients (25 (29%) vs. 53 (38%); $p=0.15$), this difference did not persist during follow-up (Table 10.4; Figure 10.3). In subgroup analyses, mortality rates were similar for trauma patients ($n=43$) receiving and not receiving TAP (12% vs. 15% at ICU discharge).

Figure 10.2 Probability of remaining free of ventilator-associated pneumonia during total ICU stay in study patients and controls ($p=0.003$).

Systemic antibiotic use and resistance

On admission, systemic antibiotics were prescribed for 41 (47%) study and 58 (42%) control patients. Mean numbers of courses of antibiotics per patient, were 0.95 ± 0.68 for study patients and 1.27 ± 0.87 for control patients during study period ($p=0.018$) and 1.33 ± 0.76 vs. 1.76 ± 1.30 ($p=0.044$) respectively, during total ICU stay (Table 10.4).

No vancomycin resistant enterococci (VRE) were isolated in both hospitals before, during or after the study. No increase in the number of patients colonized or infected with microorganisms resistant to gentamicin was observed during the study.

Figure 10.3 Probability of survival during prolonged follow-up in study patients and controls ($p=0.5$).

Discussion

The main feature of this study is that modulation of oropharyngeal colonization, without influencing gastric and intestinal colonization and without systemic antibiotic prophylaxis, resulted in a relative risk reduction of 62% in the incidence of VAP. This finding underscores the pivotal role of oropharyngeal colonization in the pathogenesis of VAP, and strongly suggests that modulation of colonization at this site will effectively prevent VAP.

The present study shows that prevention of colonization of the oropharynx, and not the stomach, reduces the incidence of late-onset VAP. The effects of oropharyngeal decontamination have been studied previously in two smaller studies. In a double-blind study, Pugin et al.² randomized 52 patients to receive either a solution of polymyxin B, neomycin and vancomycin or placebo in the retropharynx. Colonization with aerobic Gram-negative bacteria was significantly reduced in oropharynx and stomach resulting in a relative risk reduction of VAP of 0.79. Rodriguez-Roldan et al.³⁰ used an oropharyngeal paste containing tobramycin, amphotericin B and polymyxin E. Decontamination of oropharynx and trachea was established in 10 of 13 patients receiving active medication and none developed pneumonia. Eleven (73%) of 15 patients receiving placebo medication

developed pneumonia. Our findings expand the results of both studies. Incidences of VAP in the control groups of both studies (78% and 73% respectively) were extremely high, and not comparable to incidences found in other ICU studies^{4,6-11,13}. This is probably due to the use of clinical and microbiological criteria, instead of bronchoscopic techniques, in the diagnosis of VAP, or due to patient selection. Furthermore, since gastric colonization was significantly decreased in the study by Pugin et al., their findings do not contribute to the determination of the relative importance of gastric and oropharyngeal colonization².

Our findings demonstrate that VAP can be prevented effectively by modulation of oropharyngeal colonization. Importantly, the relative risk reduction of VAP in the present study is similar to the relative risk reductions reported in the meta-analyses of SDD (ranging from 53% to 78%)¹⁸⁻²². This strongly suggests that oropharyngeal decontamination, indeed, represents the effective part of SDD, and that the majority of antibiotic use in SDD is unlikely to add beneficial effects. A similar preventive effect on the incidence of VAP can be achieved with only a fraction of the antibiotics used in SDD. The data, therefore, question the concept of SDD, a method of infection prevention that is still used in many ICUs. However, antibiotic use bears the constant threat of induction or selection of resistant microorganisms. Absence of resistance problems and very strict control, as in the present study, are mandatory. This is supported by three studies failing to show any benefit from the full concept of SDD on the incidence of VAP. These studies were executed in France, Spain and South Africa, countries where antibiotic resistance levels of ICU pathogens are much higher and not comparable to our situation³¹⁻³³. For instance, infections due to MRSA were reported in each study. Since infection rates represent the tip of the iceberg, colonization rates were likely to be even higher. Endemic colonization with multiple resistant bacteria will abolish the beneficial effects of any prophylaxis based on antibiotics. This is a scenario that is, unfortunately, emerging.

Ideally, modulation of oropharyngeal colonization should be established with 'nonantibiotic' methods. One potential approach might be the use of chlorhexidine for oropharyngeal decontamination. An oral rinse of 0.12% chlorhexidine reduced the incidence of respiratory tract infections among 353 cardio-surgical patients from 9% in control patients to 3% in patients receiving oropharyngeal decontamination with chlorhexidine³⁴. The difference was mainly caused by a reduction of infections with Gram-negative pathogens. However, how prolonged chlorhexidine use will affect oral, oesophageal and gastric mucosa in critically ill ICU patients is unclear, as is the risk of chlorhexidine resistance after longterm application.

Multiple strategies to prevent VAP have been developed and tested during the last 15 years, and guidelines for the prevention of hospital-acquired pneumonia have been formulated recently²³. In addition to standard infection control measures, several other preventive measures were considered to have probable

effectiveness²³: the use of sucralfate for stress-ulcer prophylaxis, modulation of enteral feeding, positioning of patients, and subglottic secretion drainage. However, no significant differences in the incidence of VAP were reported in the only two prospective, randomized, double-blind placebo-controlled studies comparing sucralfate to antacids and H₂-antagonists^{10,11}. Beneficial effects of intermittent enteral feeding on gastric colonization and on the incidence of VAP are unproven^{12,35}, and effects of acidified enteral feeding and postpyloric feeding on the incidence of VAP have never been determined. Placing patients in semirecumbent instead of supine position may reduce gastroesophageal aspiration, but up till now the effects on the incidence of VAP^{36,37} have not been studied. Finally, suctioning of subglottic secretions which accumulate above the inflated endotracheal cuff has been shown to reduce incidences of early-onset VAP, but not of late-onset VAP^{38,39}.

How should our findings be applied together with the results of other studies on prevention of VAP? There is strong evidence that modulation of oropharyngeal colonization will influence the development late-onset VAP. However, in settings where early-onset represents a bigger problem, other strategies, such as subglottic secretions drainage or targeted systemic prophylaxis during the first 24 h²⁴ may be more appropriate.

In a previous study in our hospital, topical antimicrobial prophylaxis of the oropharynx and stomach with colistin, tobramycin and amphotericin B was associated with overgrowth and infections due to Gram-positive species, such as *Enterococcus faecalis* and coagulase-negative staphylococci^{40,41}, findings that have been reported by others as well^{31,33,42,43}. Since our aim was to perform a conceptual study to determine the effects of modulation of oropharyngeal colonization on the incidence of VAP and to elucidate the pathogenesis of VAP, we included vancomycin in our prophylactic regimen. We are well aware of the emergence of vancomycin resistant enterococci in many countries and the Hospital Infection Control Practices Advisory Committee (HICPAC) recommendations to avoid the prophylactic use of vancomycin⁴⁴. However, neither vancomycin-resistant enterococci (VRE) nor methicillin-resistant *Staphylococcus aureus* (MRSA) had been isolated on a regular basis in the Netherlands and in the ICUs of both hospitals, at the start of the study, and systemic vancomycin was used only sporadically⁴⁵. In addition, the duration of topical antibiotic prophylaxis was limited to a maximum of 21 d in order to minimize the risk of induction of resistant strains. Finally, susceptibilities for vancomycin were determined for all enterococci isolated during the study. A plan of enforced infection control had been developed, had vancomycin resistant strains emerged. With all these control measures, we felt assured to use vancomycin as a prophylactic agent in a setting with low vancomycin use, an overall low incidence of antibiotic resistance, and complete absence of VRE and MRSA. Although acquired resistance in Gram-negative bacteria is also a potential threat of topical antimicrobial prophylaxis, the susceptibilities of Gram-negative bacteria to gentamicin was approximately 90% in

our ICU and this did not decrease during the study.

The reduction in the incidence of VAP was associated with a decrease in antibiotic prescription, but other outcome parameters such as duration of mechanical ventilation, duration of ICU stay and mortality rates were not affected. The effect of VAP on mortality rates remains controversial. Fagon et al.⁴ found an attributable mortality of VAP of 27%, and even of 43% when caused by *Pseudomonas* spp. or *Acinetobacter* spp. Moreover, in a cohort study by the same investigators, nosocomial pneumonia was independently associated with death in mechanically ventilated patients (OR 1.51; 95% CI 1.11-2.03)⁴⁶. However, VAP was not an independent risk factor for death in two other multivariate analyses^{47,48}, and was not associated with increased mortality in two recent matched cohort studies^{49,50}. Furthermore, significant reductions in the incidence of VAP as determined in five meta-analyses of SDD studies, hardly decreased mortality rates¹⁸⁻²². Inappropriate antibiotic use is an important variable contributing to attributable mortality of VAP^{51,52}. Therefore, comparisons of attributable mortality rates of VAP in various studies is difficult. For example, cases of inappropriate antibiotic therapy for VAP due to multiple resistant pathogens occur infrequently in our setting, and occur probably more often in settings with endemic levels of resistant pathogens. Under the circumstances tested, VAP did not have an attributable mortality. However, there was a tendency towards a reduction of ICU-related mortality associated with the 62% relative risk reduction of VAP. However, when prolonging patient follow-up, differences between both study groups vanished completely. Reported mortality rates usually reflect ICU mortality. Our data show that this may overestimate the benefits of an intervention.

Several potential insufficiencies of our study must be addressed. Incidences of VAP may have been influenced by several variables which were not equally distributed in both study groups. For instance, more control patients received sucralfate and study patients were more likely to receive enteral feeding. However, since sucralfate has been associated with lower incidences of VAP in some studies and enteral feeding has been assumed to be a risk factor for VAP, both of these discrepancies would have favoured the control rather than the study patients. If so, the true beneficial effects of oropharyngeal decontamination would have been even higher.

Another concern is whether aspiration of antibiotics into the lungs influenced the diagnostic yield of bronchoscopic samples. However, negative bronchoscopic results obtained because of a clinical suspicion of VAP occurred with equal frequency in both control and study patients, suggesting that leakage of antibiotics in the lower respiratory tract did not occur. Moreover, from a number of study patients samples of tracheal aspirate were obtained for determination of gentamicin and vancomycin levels during the study period (day 3, 7, 14, and 21). All gentamicin levels were below threshold of detection (i.e., <0.03 mg/l). Vancomycin levels were below threshold of detection (i.e., <0.05 mg/l) in most

samples and very low concentrations in the remaining (median 0.25 mg/l)(data not shown).

In conclusion, our study demonstrated that modulation of oropharyngeal colonization, without influencing gastric and intestinal colonization, effectively reduces the incidence of late-onset VAP. This finding proves the pivotal role of oropharyngeal colonization in the pathogenesis of this infection. When compared with other preventive strategies for VAP, prevention of oropharyngeal colonization is by far the most effective. Notwithstanding the impact of VAP on morbidity and antibiotic use, our findings do not support the concept that VAP has attributable mortality.

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General discussion

General discussion

The hypotheses for the studies as described in the present thesis were based on the conclusions of the thesis 'The role of colonization of the upper intestinal tract in the pathogenesis of Ventilator-Associated Pneumonia', by Marc JM Bonten¹. He concluded that in the pathogenesis of ventilator-associated pneumonia (VAP): 1. the gastropulmonary route of colonization was not important, 2. that oropharyngeal colonization might be of pivotal importance, and, 3. that cross-acquisition within the intensive care unit (ICU) might be more important than generally expected.

These conclusions and suggestions were fully confirmed in the present thesis, the main finding being that oropharyngeal colonization is of pivotal importance in the pathogenesis of VAP. Prevention of this infection, which is a major aim of intensive care medicine, can be established by targeted modulation of colonization in the oropharynx.

The last decade has witnessed the increasing problem of antibiotic resistance as a consequence of excessive and/or inappropriate antibiotic use². Surveillance studies on the incidence of infections, indications for antibiotic use and knowledge about antimicrobial susceptibilities of pathogens are indispensable to allow critical assessment of antibiotic use^{2,3}. Antibiotic use in ICUs is approximately tenfold greater than in general hospital wards and is associated with a considerable part of the total hospital antibiotic consumption and costs⁴. In the ICU population described in this thesis, one in every three patients has at least one infection during ICU stay. Since almost all ICU-acquired infections occur in intubated patients on mechanical ventilation, this patient group is responsible for the majority of antibiotic use and related costs. The respiratory tract is by far the most important site of infection and infections in this area account for half of the total ICU antibiotic use. Reduction of ICU antibiotic use, therefore, may be achieved either by shortening the duration of therapy for respiratory tract infections, or by preventing respiratory tract infections and identification of episodes of unnecessary antibiotic use. Prevention of infections can also be expected to decrease related morbidity and mortality.

Since respiratory tract infections are the most common of all ICU-acquired infections, prevention of these has the greatest impact on antibiotic use. Such a reduction may also be facilitated by applying more specific criteria to diagnose nosocomial pneumonia⁵. Adding the results of bronchoscopic examinations to the criteria for diagnosing VAP may help to reduce antibiotic use in patients with a clinical suspicion of pneumonia but with nonsignificant quantitative cultures from bronchoscopic samples. As shown in this thesis, withholding of antibiotics in these patients, indeed, did not increase mortality rates or recurrence rates for VAP. Moreover, in our patients with bronchoscopically proven VAP, empiric antimicrobial therapy was frequently adjusted on the basis of the bronchoscopy data, mostly by narrowing the antibiotic coverage. Implementation of bronchoscopy in the routine work-up of patients with a clinical suspicion of VAP may influence clinical practice

and decrease the number of patients receiving antibiotic therapy.

The most important conclusion of this thesis is that, in addition to duration of mechanical ventilation, colonization of the upper respiratory tract is the most important risk factor for the development of VAP. Relationships between duration of ventilation and increased risk for tracheal colonization and VAP are obvious^{6,7}. Moreover, tracheal colonization is one of the diagnostic criteria for VAP. However, oropharyngeal colonization appeared to be an independent risk factor for VAP. Interestingly, gastric colonization with enteric Gram-negative bacteria (EGB) and Pseudomonadaceae was not a risk factor for VAP. In contrast to the hypothesis of the gastropulmonary route of colonization⁸, oropharyngeal and tracheal colonization were risk factors for acquired gastric colonization with Pseudomonadaceae. Taken together, the data of the risk factor analyses did not support an important role for the gastropulmonary route of colonization in the pathogenesis of VAP. Instead, the oropharynx and trachea were the initial sites of colonization prior to the development of VAP and should be the actual target for prevention. The final study of this thesis demonstrated that modulation of oropharyngeal colonization, without influencing gastric and intestinal colonization and without systemic antibiotic prophylaxis, effectively reduced the incidence of VAP by 62%. These data confirm the results of two small studies on oropharyngeal decontamination by Pugin and coworkers⁹ and by Rodriguez-Roldan and coworkers¹⁰. Notwithstanding the impact of VAP on morbidity and antibiotic use, VAP did not have attributable mortality in our setting. In view of the results of selective digestive tract decontamination¹¹, oropharyngeal decontamination appears to represent the effective part of this regimen. A similar preventive effect on the incidence of VAP can be achieved with only a fraction of the antibiotics used in selective digestive decontamination. Moreover, when compared with other preventive strategies for VAP (e.g., stress-ulcer prophylaxis with sucralfate, modulation of enteral feeding etc.), prevention of oropharyngeal colonization is by far the most effective.

An important part of ICU infections is caused by Enterobacteriaceae, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. These different species are usually lumped together and labelled as potentially pathogenic microorganisms. However, individual species have specific properties with regard to preferred site, time and route of colonization and clinical spectrum of infection. Furthermore, there are differences between the clinical spectra of community-acquired infections and hospital-acquired infections caused by the same species of bacteria (e.g., *S. aureus*). The association between development of VAP and preceding upper respiratory tract colonization, especially in the oropharynx, appears to account for all pathogens.

S. aureus is known to colonize the nares of many people. Old age, diabetes mellitus and prior infection with influenza are risk factors for community-acquired infection with this pathogen¹². In contrast to community-acquired pneumonia, we

found that VAP caused by *S. aureus* occurs more frequently in young patients, admitted to ICU for trauma or neurologic diseases, with a short (≤ 48 h) duration of hospitalization prior to ICU admission. Moreover, when compared with community-acquired pneumonia, VAP caused by *S. aureus* has a less fulminating course.

Enteric Gram-negative bacteria belong to the commensal intestinal flora. An infection on admission to ICU and oropharyngeal colonization with EGB on admission were found to be independent risk factors for VAP caused by these species and were also associated with increased mortality. Severity of illness on admission to ICU probably is a risk factor for oropharyngeal colonization with EGB which may also represent a lack of clinical improvement during ICU stay, a finding that has also been reported by Johanson and coworkers¹³.

Respiratory tract colonization with *P. aeruginosa* on admission to ICU occurs less frequently and mainly strikes patients with chronic obstructive pulmonary disease¹⁴. The risk factor analysis as presented in this thesis showed that acquired respiratory tract colonization with *P. aeruginosa* is, even more than is the case for EGB, strongly related to the duration of mechanical ventilation. The upper respiratory tract is clearly the initial site of colonization for *P. aeruginosa*, before VAP develops. Although suggested by some¹⁵, *P. aeruginosa* did not show a preference for the trachea or oropharynx in our studies. Colonization with *P. aeruginosa* is not limited to the respiratory tract, and many critically ill patients are colonized at multiple body sites and with multiple genotypes. Therefore, genotyping is crucial when studying the pathogenesis and epidemiology of colonization or infection with *P. aeruginosa*. Since genotyping methods are expensive and laborious, these techniques are not feasible for all laboratories. In general, phenotyping techniques are less expensive and relatively easy to perform, but they lack specificity¹⁶. Nevertheless, phenotyping techniques may be used to make a preselection out of a large series of isolates for further genotyping. The appropriate phenotyping technique for this purpose appears to be serotyping, which showed the best discriminative power among four phenotyping techniques (antibiotic susceptibility patterns, serotyping, phage-typing and outer membrane protein profile analysis) in comparison to pulsed-field gel electrophoresis of enzyme restricted chromosomal DNA.

Another important property of *P. aeruginosa* is its ability to survive and multiply in an inanimate, aqueous environment. *Pseudomonas aeruginosa*, therefore, is a notorious pathogen involved in outbreaks originating from exogenous sources (contaminated equipment or colonized patients), spreading rapidly via the hands of health care workers or via equipment used on several patients. Cross-colonization may even be important in nonepidemic (i.e. endemic) situations. In general, several factors have a bearing on this complex phenomenon. For instance, compliance with infection control measures is essential in infection control, and effective handwashing has been shown to reduce the rate of nosocomial infections. Achieving 100% handwashing compliance is unattainable: studies

evaluating compliance report percentages of about 30%¹⁷. Antibiotic use providing certain bacteria with a selective growth advantage, which can be expressed as antibiotic pressure, also plays a role. In addition, the number of patients already colonized (i.e. colonization pressure) may be an important factor in the risk of occurrence of cross-colonization. This means that in the epidemiology of respiratory tract colonization, for *P. aeruginosa*, both endogenous and exogenous colonization play a role. The epidemiology of *P. aeruginosa* may, therefore, be described as a silent epidemic, similar to the endemic nature of other nosocomial pathogens, including the multiple resistant strains (i.e., vancomycin-resistant enterococci, methicillin-resistant *S. aureus* and ceftazidime-resistant Gram-negative bacilli). The relative impact of both exogenous and endogenous colonization of *P. aeruginosa* in the pathogenesis of VAP remains to be established. The studies show that surveillance in combination with molecular biotyping may be used to perform continuous quality improvement and stress the importance of infection control measures.

Answers to the questions

1. *What is the incidence of infections in critically ill patients admitted in our ICU?*
Thirty-six percent of patients admitted to ICU had at least one infection during ICU stay. Of all infections, 53% were ICU-acquired and almost all of these occurred in ventilated patients. Respiratory infections were the single most common infection accounting for 43% of all infections.
2. *What are the indications for antibiotic use and the related costs in a general ICU ward?*
In mechanically ventilated patients 51% of antibiotic prescriptions were used for bacteriologically proven infections, 31% for non-bacteriologically proven infections and 18% for prophylaxis. Half of the prescriptions of antibiotics for therapy were administered for respiratory tract infections, 94% of total antibiotic costs were spent in intubated patients and non-bacteriologically proven infections consumed 25% of total antibiotic costs.
3. *Does addition of quantitative culture results of samples obtained by bronchoscopic techniques to the generally used criteria for diagnosing VAP influence antibiotic prescription?*
The number of patients receiving antibiotic therapy decreased by implementation of bronchoscopic techniques in the routine workup of a clinical suspicion of VAP, without affecting recurrence rates or mortality. In addition, empiric antimicrobial therapy was adjusted in 19% of the patients with proven VAP, in most cases by narrowing the antibiotic coverage.
4. *What are the risk factors for the development of colonization of stomach, oropharynx, and trachea with EGB and Pseudomonadaceae?*
The duration of mechanical ventilation was an independent risk factor for acquired colonization in stomach, oropharynx and trachea. As far as EGB is concerned, surgical and trauma patients tended to become colonized more frequently than medical and neurologic patients, and so did patients receiving

enteral feeding. In the case of *Pseudomonadaceae*, acquired colonization of the oropharynx was associated with preceding tracheal colonization, and vice versa. Acquired gastric colonization was strongly related to prior colonization of the oropharynx and trachea.

5. *What are the risk factors for the development of VAP caused by EGB and Pseudomonadaceae?*

The duration of mechanical ventilation was an independent risk factor for VAP, regardless of the causative pathogen. In addition, oropharyngeal colonization with EGB on admission and an infection on admission were risk factors for VAP caused by EGB. Acquired colonization of the oropharynx with *Pseudomonadaceae* was a risk factor for VAP by these species. Gastric colonization was not a risk factor for development of VAP.

6. *Does the clinical spectrum and outcome of staphylococcal VAP differ from that of staphylococcal community-acquired pneumonia?*

Community-acquired pneumonia due to *S. aureus* particularly develops in elderly people after a viral respiratory tract infection and is associated with high mortality. VAP caused by this pathogen is not a fulminating disease and occurs more frequently in younger patients with a short duration of hospitalization before ICU admission.

7. *Can phenotyping techniques be used as an initial method of screening for P. aeruginosa in order to reduce the number of isolates that need to be genotyped?*

Serotyping has the best distinctive properties for an initial screening compared with antimicrobial susceptibility patterns, phage-typing and outer membrane protein profile analysis.

8. *Is the respiratory tract the most important initial site of colonization with P. aeruginosa or is colonization with this pathogen not restricted to the respiratory tract?*

The upper respiratory tract is the most important initial site of colonization prior to the development of VAP caused by *P. aeruginosa*. Multiple body sites may be colonized with this species.

9. *Are patients admitted to ICU colonized with a single or with multiple genotypes of P. aeruginosa?*

Patients admitted to ICU can be colonized at multiple body sites with multiple genotypes.

10. *Does cross-colonization influence the rates of colonization and infection with P. aeruginosa in an ICU where colonization with this species is endemic?*

Both endogenous and exogenous colonization are significantly involved in the epidemiology of colonization and infection of the respiratory tract with *P. aeruginosa*. Moreover, even in nonepidemic settings cross-colonization seems to play an important role.

11. *Can VAP be prevented by modulation of oropharyngeal colonization, without influencing gastric and intestinal colonization and without systemic prophylaxis?*

Topical antimicrobial prophylaxis of the oropharynx resulted in a 62% relative risk reduction for VAP. Oropharyngeal colonization is of paramount importance in the pathogenesis of VAP, and a targeted approach to prevent colonization at this site is a very effective method of infection prevention.

Concluding remarks and future research

An important question emerging from this thesis as well as from other studies, is whether or not prevention of VAP is worthwhile. Notwithstanding the impact of VAP on morbidity and antibiotic use, the mortality attributable to VAP remains a matter of debate. In our studies, VAP did not come out as a risk factor for mortality, and a 62% relative risk reduction of the incidence of VAP did not reduce mortality. Several factors are relevant to this issue. Although sensitivity and specificity of the diagnosis of VAP increases by adding the results of bronchoscopic examinations, the latter is not a gold standard. A certain percentage of the episodes diagnosed as VAP, may still reflect extensive colonization rather than infection. Moreover, VAP may be a compartmentalized, and not a systemic inflammation. Recent studies analyzing cytokine responses to VAP underscore this hypothesis¹⁸. However, the localized lung process can initiate a systemic response evolving in severe sepsis or multiple organ failure with considerable mortality.

Importantly, the pathogens cultured most frequently in our ICU are susceptible to empiric antimicrobial therapy and so far no resistance problems have been encountered (neither vancomycin-resistant enterococci nor methicillin-resistant *S. aureus* have been isolated in our ICU). Thus, the infections that occur, including VAP, can usually be treated effectively and a fulminating clinical spectrum develops only in a minority. In certain settings, VAP did have attributable mortality when caused by high-risk (*Pseudomonas* spp. or *Acinetobacter* spp.) or multiple resistant pathogens¹⁹. In these cases, inappropriate antibiotic use has been identified as an important variable contributing to attributable mortality of VAP²⁰. Therefore, it may depend upon the occurrence of multiple resistant or high-risk pathogens and its associated antibiotic use whether VAP has attributable mortality. Nevertheless, VAP carries a number of risks and consequences for individual patients and still warrants effective prevention.

According to our studies, prevention of VAP should be targeted on modulation of oropharyngeal colonization. Increased adherence of microorganisms to epithelial cells precedes colonization in critically ill patients and the mechanisms responsible for this increase have, in part, been elucidated. Prevention of adherence of microorganisms to oropharyngeal epithelium, by increasing mucosal immunity, is an attractive alternative to oropharyngeal decontamination. The advantage of this approach is that prevention is established very early in the process of colonization, thereby reducing the necessity for antibiotics. Research on the development and application of local immunomodulators for the oropharynx is still in its infancy.

Due to the constant threat of selection and induction of antibiotic resistance

which is associated with antibiotic use, modulation of oropharyngeal colonization should, ideally, be established with 'non-antibiotic' methods. Future research should determine whether the application of disinfectants (chlorhexidine, povidone-iodine etc.) for oropharyngeal decontamination is as effective as antibiotics and whether these agents are well tolerated by critically ill patients during prolonged application. Moreover, other means by which oropharyngeal colonization can be modulated need to be explored.

Subglottic secretions drainage has shown promising results on the incidence of mainly early-onset VAP, in early trials. Several studies on this technique are now in progress.

Interventions attempting to modulate gastric colonization failed to demonstrate beneficial effects in terms of lowering the incidence of VAP, and studies on the sequences of colonization do not support the importance of the gastropulmonary route of colonization²¹. Nevertheless, the stomach is a potential source of colonization, especially in a patient receiving enteral feeding, and bacteria from the stomach can eventually colonize the respiratory tract and cause VAP in, at least, some patients. Preventive strategies that interfere with the gastropulmonary route of colonization, like enteral feeding and body position, have not been studied frequently. Moreover, some of these studies were poorly designed or had surrogate endpoints. Well designed studies with sufficient monitoring of colonization sequences and bronchoscopic diagnosis of VAP to evaluate these strategies are warranted. A study comparing the effects of supine with semirecumbent body position during mechanical ventilation on colonization and VAP is ongoing in our institute.

Strategies to prevent VAP continue to undergo evaluation. Based on new insights in the pathogenesis of VAP, newly reported risk factors and development of drugs and equipment new strategies are developed and studied. For instance, biological response modifiers, which are immunomodulators that have the ability to modify host defense mechanisms, may increase the host pulmonary defense against invading pathogens. A continuing series of studies on the palliative treatment of the sepsis syndrome with antilipopolysaccharide antibodies (E5, HA-1A), antibodies to tumor necrosis factor, interleukin-1 receptor antagonists, cyclooxygenase inhibitors and platelet-activating factor antagonists are reported, but beneficial effects of these immunomodulators on VAP remain to be established²². Other strategies attempt to prevent the development of VAP or accelerate the resolution of pneumonia once it has been acquired. These include the use of monoclonal antibodies to specific bacterial antigens, granulocyte colony-stimulating factor and interferon-gamma. Moreover, new biomaterials are being tested for application in endotracheal tubes on which the formation of biofilms and colonization with pathogens is decreased.

Another approach in the prevention of VAP is the application of composite interventions. Preliminary results of trials evaluating the combination of preventive strategies (e.g., subglottic secretions drainage and semirecumbency compared

with intubation with a standard endotracheal tube and supine position) have been reported in abstract form^{23,24}.

Surprisingly, a scheduled change of antibiotic classes used for the empiric treatment of suspected Gram-negative bacterial infections reduced the incidence of VAP attributed to antibiotic-resistant Gram-negative bacteria in patients undergoing cardiac surgery²⁵. This concept of 'crop rotation' is intriguing and should be explored further²⁶.

A further question is whether shortening the duration of antimicrobial therapy for respiratory tract infections is possible. A prospective study designed to perform a cost-benefit analysis on the implementation of bronchoscopy as a diagnostic tool in the assessment of VAP is still warranted. Moreover, addition of microscopic analysis of bronchoalveolar lavage fluid to the criteria for the diagnosis of VAP and optimization of the culture techniques of bronchoscopic samples may help to further increase the specificity of the diagnosis. The relative impact of exogenous and endogenous colonization of *P. aeruginosa*, and other species, in the pathogenesis of VAP remains to be established. An interesting approach to the problem of colonization in endemic situations is the development of mathematical models, analyzing the contribution of several factors (e.g. compliance with handwashing protocols, antibiotic pressure, cohorting, colonization pressure) to the endemicity. These models can identify, for individual species, which strategy for infection prevention will prove to be most effective in that setting.

In this thesis, the studies on the diagnosis of VAP demonstrate that increasing the specificity of the diagnosis, by implementation of bronchoscopic techniques, antibiotic use may be rationalized. The studies on the pathogenesis of VAP show that colonization of the oropharynx is the single most important feature in the development of this infection. As a corollary, our preventive trial indicates that VAP can be prevented successfully by modulation of colonization in the oropharynx.

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12

Summary

Summary

Critically ill patients admitted to the intensive care unit (ICU) who require mechanical ventilation are prone to develop ventilator-associated pneumonia (VAP). Development of this infection is associated with a prolonged ICU stay, additional health care costs, and increased antibiotic use, morbidity and mortality. This thesis deals with the pathogenesis, diagnosis, and prevention of VAP.

In **chapter 1** the general aims of the studies and the main questions addressed in this thesis are described.

The pathogenesis of VAP is complex and not completely understood. However, VAP is almost always preceded by colonization of the respiratory tract with microorganisms. In **chapter 2** the pathogenesis of VAP is reviewed, with special emphasis for (endogenous and exogenous) colonization, and the related literature on prevention of this infection has been critically assessed. The gastropulmonary route of colonization, which for many years has been considered to be the main pathway for pathogens that cause VAP, appears to be of limited importance. Oropharyngeal colonization as an antecedent to VAP has regained attention and several studies strongly suggest a pathogenetic role for colonization in the oropharynx. Although intestinal colonization frequently occurs in critically ill patients, secondary colonization of the respiratory tract seems infrequent. The relevance of this rectopulmonary route of colonization remains largely undetermined. In addition to these endogenous sources, some data suggest that cross-colonization from exogenous sources may be an important route of colonization, not only in epidemic situations.

Based on the presumed pathogenesis of VAP several preventive strategies have been studied. Conversely, the results of these studies helped to clarify the pathogenesis of VAP. Two guidelines have been formulated for the prevention of VAP, one by the American Thoracic Society and the other by the Centers for Disease Control and Prevention. Interestingly, a critical assessment of studies that evaluated preventive strategies revealed that selective digestive decontamination (i.e., eradication of microorganisms from intestine, stomach and oropharynx by nonabsorbable antibiotics, which are combined with systemic antibiotic prophylaxis) has proven to prevent VAP most effectively, but due to its potential side-effects and a lack of effect on secondary outcome parameters, this method of infection prevention is not recommended in either guidelines. The use of sucralfate for stress-ulcer prophylaxis is recommended in both guidelines although scientific evidence is failing.

In the first study of this thesis (**chapter 3**) the incidence of infections and indications for antibiotic use in our ICU have been studied. In our population one in every three patients has at least one infection during ICU stay. Almost all ICU-acquired infections occur in ventilated patients and the respiratory tract is by far

the most important site of infection, accounting for half of the total ICU antibiotic use. In the analysis of indications for antibiotic use, the distinction between bacteriologically proven and non-bacteriologically proven infections was helpful to identify unnecessary antibiotic use. Together with the data on antibiotic consumption and infection rates it may help to develop strategies to reduce antibiotic use. In our setting, prevention of respiratory tract infections has the greatest potential to reduce antibiotic use. The application of more specific criteria to diagnose these infections would help to rationalize antibiotic use even further. In **chapter 4** we investigated whether adding the results of protected specimen brush and bronchoalveolar lavage to the generally used criteria for diagnosing VAP influences antibiotic prescription by intensive care physicians. The results of this study show that the number of patients receiving antibiotic therapy decreases by implementation of bronchoscopic techniques in the routine work-up of a clinical suspicion of VAP, without affecting recurrence rates or mortality. In addition, this implementation frequently resulted in adjustment of empiric antimicrobial therapy in patients with proven VAP, in most cases by narrowing the antibiotic coverage.

In **chapter 5** a risk factor analysis for the development of colonization of stomach, oropharynx and trachea, and the development of VAP with enteric Gram-negative bacteria and Pseudomonadaceae was performed. The duration of mechanical ventilation is an independent risk factor for acquired colonization in stomach, oropharynx and trachea and for VAP. Surgical and trauma patients and patients receiving enteral feeding tend to become colonized more frequently with enteric Gram-negative bacteria than others. In the case of Pseudomonadaceae, acquired colonization of the oropharynx is associated with preceding tracheal colonization, and vice versa. Importantly, oropharyngeal colonization with enteric Gram-negative bacteria on admission and acquired colonization of the oropharynx with Pseudomonadaceae are risk factors for VAP by these species. Acquired gastric colonization is strongly related to prior colonization of the oropharynx and trachea, but did not prove to be a risk factor for development of VAP.

The clinical presentation and outcome of staphylococcal VAP is described in **chapter 6**. Preceding colonization with *Staphylococcus aureus* in the trachea appeared to be an important risk factor for the development of staphylococcal VAP. In contrast to community-acquired pneumonia due to *S. aureus*, which particularly develops in elderly people after a viral respiratory tract infection and which is associated with high mortality, VAP caused by this pathogen is not a fulminating disease and occurs more frequently in younger patients with a short duration of hospitalization before ICU admission.

In chapters 7, 8 and 9 some microbiological and clinical aspects of *Pseudomonas aeruginosa* infections are described. Determination of similarity of isolates of *P. aeruginosa* is important when studying routes of colonization. Genotyping techniques are the methods of choice for this purpose but are expensive and

laborious. In **chapter 7** the usefulness of four methods of phenotyping (antimicrobial susceptibility patterns, serotyping, phage-typing, and outer membrane protein profile analysis) as an initial method of screening for *P. aeruginosa* is presented. Serotyping has the best discriminative properties to reduce the number of isolates that need to be genotyped.

The results of these phenotyping and genotyping methods are used in **chapter 8** to study routes of colonization with *P. aeruginosa* leading to VAP. This study shows that the upper respiratory tract is the predominant initial site of colonization with *P. aeruginosa* prior to the development of VAP, and that multiple body sites may be colonized with multiple genotypes of this species.

In **chapter 9** the influence of cross-colonization on the rates of colonization and infection with *P. aeruginosa* in an ICU where colonization with this species is endemic was assessed. It appeared that both endogenous and exogenous colonization are involved in the epidemiology of colonization and infection of the respiratory tract with this species. Moreover, cross-colonization seemed to be an important route of colonization and infection with *P. aeruginosa*, even in nonepidemic settings.

The most important conclusion of chapter 3 of this thesis was that the respiratory tract is by far the most important site of infection in mechanically ventilated ICU patients and that prevention of these infections has the greatest potential to reduce antibiotic use. The multivariate analysis in chapter 5 identified colonization of the upper respiratory tract as an independent risk factor for the development of VAP due to enteric Gram-negative bacteria and Pseudomonadaceae. Moreover, gastric colonization did not prove to be a risk factor for this infection. In addition, chapters 6, 8 and 9 provided evidence that VAP due to *S. aureus* and *P. aeruginosa* was preceded by colonization of the upper respiratory tract. According to the data from these studies and the studies reviewed in chapter 2, we hypothesized that decontamination of the oropharynx alone, without modulating gastric and intestinal colonization, and without systemic antibiotic prophylaxis, reduces the incidence of VAP in critically ill intensive care patients. In **chapter 10** this hypothesis was tested in a prospective, randomized, placebo-controlled, double-blind study. Modulation of oropharyngeal colonization, indeed, effectively reduced the incidence of VAP by more than 60%. Oropharyngeal colonization proved to be of paramount importance in the pathogenesis of VAP, and a targeted approach to prevent colonization at this site is a very effective method of infection prevention.

13

Samenvatting

Samenvatting

Ernstig zieke patiënten die zijn opgenomen op een intensive care (IC) en die mechanische beademing behoeven zijn vatbaar voor het krijgen van een beademings-gerelateerde longontsteking (ook wel 'ventilator-associated pneumonia' of VAP). Deze infectie leidt tot een langere opname duur, een toename van antibiotica gebruik, extra kosten en mogelijk een lagere overlevingskans voor de patiënt. Dit proefschrift behandelt de pathogenese, diagnose en preventie van VAP.

Hoofdstuk 1 beschrijft de doelen van de diverse studies in dit proefschrift en de belangrijkste vragen die we hebben getracht te beantwoorden.

De pathogenese van VAP is zeer complex en niet volledig duidelijk. VAP wordt bijna altijd voorafgegaan door kolonisatie van de bovenste luchtwegen met micro-organismen. **Hoofdstuk 2** bestaat uit een overzicht van de pathogenese van VAP met speciale aandacht voor (endogene en exogene) kolonisatie en een bespreking van de literatuur over preventie van VAP. De gastropulmonale route van kolonisatie, die vele jaren als de belangrijkste route voor het ontstaan van VAP werd beschouwd, lijkt veel minder belangrijk dan altijd werd aangenomen. Kolonisatie van de orofarynx als antecedent van VAP is opnieuw in de belangstelling gekomen en er zijn sterke aanwijzingen dat orofaryngeale kolonisatie een belangrijke rol in de pathogenese van VAP speelt. Hoewel kolonisatie van de tractus digestivus (colon en rectum) universeel voorkomt bij mensen (gezond en ziek) lijkt verspreiding van kolonisatie naar de luchtwegen zelden plaats te vinden. De relevantie van deze rectopulmonale route van kolonisatie blijft grotendeels onbekend. Naast deze endogene bronnen suggereren sommige studies dat kruis-kolonisatie vanuit exogene bronnen (bijvoorbeeld andere patiënten) een belangrijke route van kolonisatie kan zijn, zowel in epidemische als in endemische situaties.

Op basis van deze pathogenetische modellen zijn diverse preventieve strategieën ontwikkeld en vervolgens getoetst. De resultaten van deze studies hebben vervolgens weer bijgedragen tot het ophelderen van de pathogenese van VAP. Richtlijnen voor de preventie van VAP zijn opgesteld door de 'American Thoracic Society' en door de 'Centers for Disease Control and Prevention'. Kritische beschouwing van de trials waarin de preventie van VAP werd bestudeerd suggereert dat selectieve darm decontaminatie (eliminieren van micro-organismen in de maag, darm en orofarynx met behulp van niet-resorbeerbare antibiotica, gecombineerd met systemische profylaxe) de meest effectieve manier van preventie is. Echter, door het potentiële gevaar voor selectie en inductie van antibiotica resistentie en het ontbreken van effect op mortaliteit wordt deze methode van infectie preventie niet aanbevolen. Het gebruik van sucralfaaf voor stress ulcus profylaxe wordt wel door beide richtlijnen aangeraden, terwijl het wetenschappelijke bewijs hiervoor ontbreekt.

In de eerste studie van dit proefschrift (**hoofdstuk 3**) is de incidentie van infecties en indicaties voor antibiotica gebruik in IC bepaald. In onze populatie heeft één op de drie patiënten ten minste één infectie gedurende opname op de IC. Bijna alle IC verworven infecties treden op bij beademde patiënten en de luchtwegen zijn verreweg het belangrijkste focus voor infecties. Voor deze luchtweginfecties wordt de helft van alle antibiotica voorgeschreven. Het onderscheid tussen bacteriologisch bewezen en niet-bacteriologisch bewezen infecties in de analyse van de indicaties voor antibiotica gebruik bleek waardevol ter opsporing van onnodig antibiotica gebruik. Samen met de resultaten van antibiotica consumptie en de incidentie van infecties kunnen strategieën ontwikkeld worden om het antibiotica gebruik terug te dringen. Het toepassen van meer specifieke criteria om de diagnose infectie te stellen zou het antibiotica gebruik verder rationaliseren. In **hoofdstuk 4** is bestudeerd of het toevoegen van de resultaten van 'protected specimen brush' en 'bronchoalveolaire lavage' aan de doorgaans gebruikte klinische criteria voor de diagnose van VAP, het voorschrijven van antibiotica kan beïnvloeden. De resultaten tonen aan dat het aantal patiënten met antibiotica afneemt door het implementeren van de resultaten van deze bronchoscopische technieken in het diagnostisch traject van een klinische verdenking op VAP. Het aantal recidieven van VAP en de mortaliteit worden hierdoor evenwel niet beïnvloed. Bovendien leidt deze implementatie frequent tot het aanpassen van de empirische antimicrobiële behandeling bij patiënten met VAP, in de meeste gevallen door het versmallen van het antimicrobiële spectrum.

In **hoofdstuk 5** is de ontwikkeling van kolonisatie in orofarynx, trachea en maag en de ontwikkeling van VAP veroorzaakt door intestinale Gram-negatieve bacteriën en Pseudomonadaceae door middel van een risico factor analyse bestudeerd. De duur van mechanische beademing is de belangrijkste risico factor voor het verwerven van kolonisatie in de orofarynx, trachea en maag en voor VAP. Chirurgische en trauma patiënten en patiënten die enterale voeding krijgen, raken vaker gekoloniseerd met intestinale Gram-negatieve bacteriën dan andere patiënten. Voor Pseudomonadaceae geldt dat verworven kolonisatie van de orofarynx geassocieerd is met voorafgaande kolonisatie van de trachea, maar ook omgekeerd. Kolonisatie van de orofarynx met intestinale Gram-negatieve bacteriën bij opname op de IC en verworven kolonisatie van de orofarynx met Pseudomonadaceae zijn risico factoren voor VAP veroorzaakt door deze bacteriesoorten. Verworven kolonisatie in de maag is sterk gerelateerd aan voorafgaande kolonisatie van de orofarynx en trachea, maar is geen risico factor voor VAP.

De klinische presentatie en uitkomst van VAP veroorzaakt door *Staphylococcus aureus* is beschreven in **hoofdstuk 6**. Voorafgaande kolonisatie in de trachea bleek een belangrijke risico factor voor *S. aureus* VAP. Een beademings-gerelateerde pneumonie ten gevolge van deze bacteriesoort is geen fulminant ziektebeeld en treedt vooral op bij jongere patiënten met een kort verblijf in het

ziekenhuis voorafgaande aan opname op de IC. Dit in tegenstelling tot een buiten het ziekenhuis opgelopen pneumonie (ook wel 'community-acquired pneumonia') veroorzaakt door *S. aureus*, die manifesteert zich met name bij oudere patiënten na een virale infectie van de luchtwegen en heeft een hoge mortaliteit.

In de hoofdstukken 7, 8 en 9 worden enkele microbiologische en klinische aspecten van infecties veroorzaakt door *Pseudomonas aeruginosa* beschreven. Typering van *P. aeruginosa* isolaten is belangrijk bij de bestudering van routes van kolonisatie. Genotypering is voor dit doel de methode van eerste keuze maar is duur en arbeidsintensief. In **hoofdstuk 7** wordt de toepasbaarheid van vier fenotyperings methodes (antibiogram, serotypering, faagtypering en buiten-membraan-eiwit profiel) als initiële typeringsmethode voor *P. aeruginosa* beschreven. Serotypering is gelet op het hoogste onderscheidend vermogen het meest geschikt als initiële typeringsmethode. Alleen voor isolaten met hetzelfde serotype is verdere genotypering noodzakelijk.

De resultaten van deze feno- en genotyperingen worden in **hoofdstuk 8** toegepast om de routes van kolonisatie van *P. aeruginosa* leidend tot VAP te bestuderen. Deze studie toont aan dat de bovenste luchtwegen de belangrijkste initiële plaats van kolonisatie met *P. aeruginosa* is voorafgaande aan VAP, en dat meerdere plaatsen van het lichaam gekoloniseerd kunnen zijn met meerdere genotypes van deze bacteriesoort.

In **hoofdstuk 9** is de invloed van kruis-kolonisatie op de incidentie van kolonisatie en infectie met *P. aeruginosa* bepaald op een IC waar kolonisatie met deze bacterie endemisch is. Het blijkt dat zowel endogene als exogene kolonisatie betrokken zijn bij de epidemiologie van kolonisatie en infectie van de luchtwegen. Bovendien lijkt kruis-kolonisatie, ook in niet-epidemische situaties, een belangrijke route van kolonisatie en infectie met *P. aeruginosa* te zijn.

In hoofdstuk 3 van dit proefschrift beschreven wij dat de luchtwegen verreweg het belangrijkste infectiefocus vormen bij mechanisch beademde IC patiënten en dat preventie van deze infecties de beste mogelijkheden biedt om antibiotica gebruik te verminderen. De multivariant analyse in hoofdstuk 5 identificeerde kolonisatie van de bovenste luchtwegen als onafhankelijke risico factor voor VAP veroorzaakt door intestinale Gram-negatieve bacteriën en Pseudomonadaceae. Bovendien bleek kolonisatie van de maag geen risico factor voor deze infectie te zijn. Daarnaast leverden hoofdstuk 6, 8 en 9 additioneel bewijs dat VAP veroorzaakt door *S. aureus* en *P. aeruginosa* wordt voorafgegaan door kolonisatie van de bovenste luchtwegen. Naar aanleiding van de resultaten van deze studies en de studies uit het overzicht van hoofdstuk 2 werd de hypothese geformuleerd dat decontaminatie van alleen de orofarynx, zonder modulatie van kolonisatie van maag en darm en zonder systemische profylaxe, de incidentie van VAP bij ernstig zieke IC patiënten zou reduceren. In **hoofdstuk 10** is deze hypothese getest in een prospectieve, dubbel-blind gerandomiseerde, placebo-gecontroleerde studie. Modulatie van orofaryngeale kolonisatie is een effectieve manier om de incidentie

van VAP te reduceren met meer dan 60%. Kolonisatie van de orofarynx is derhalve van cruciaal belang in de pathogenese van VAP en een gerichte benadering ter preventie van kolonisatie op deze plaats is een effectieve methode van infectie preventie.

Dankwoord

Dankwoord

'... U bent familie van dhr. J? Het moment is uiterst ongelukkig maar zou ik U toch even iets mogen vragen? Mijn naam is Dennis Bergmans, ik ben onderzoeker op de intensive care waar Uw vader zojuist is opgenomen. Zoals U gezien heeft wordt hij kunstmatig beademd. Het buisje in zijn mond loopt tot in zijn longen, de beademings-apparatuur die aan de slangen vast zit helpen hem met ademen omdat hij daarvoor zelf nu te ziek is. Een van de problemen die bij beademing kunnen optreden is het ontstaan van een longontsteking. Uit eerder onderzoek is gebleken dat dit bij één op de vier of vijf mensen gebeurt en dat de bacteriën die de longontsteking veroorzaken bijna altijd eerst in de mondholte aanwezig zijn. Momenteel doen wij onderzoek naar hoe we deze longontsteking het beste zouden kunnen voorkomen. Via U, zou ik ook Uw vader willen vragen mee te werken aan dit onderzoek. In het onderzoek wordt, door middel van een mond pasta met daarin antibiotica, getracht de mondholte vrij van bacteriën te houden. Immers, wanneer dit lukt zou ook de kans op een longontsteking kleiner moeten zijn. De mond pasta wordt vier maal per dag door de verpleegkundige in de mond gestreken...'

Bij het verrichten van klinisch wetenschappelijk onderzoek moet 'informed consent' worden verkregen van de patiënt alvorens mag worden aangevangen met de studie. Bij kunstmatig beademde patiënten is dit meestal niet mogelijk door de aanwezigheid van een beademingstube waardoor het onmogelijk is te spreken, door de ernst van de ziekte of door sedatie. In die gevallen mag de directe familie 'informed consent' geven. Het bovenstaande stukje tekst is, in het kort, de informatie die ik patiënten of hun familieleden gaf. Gevolgd door een wat moeizamer verhaal over het hoe en waarom van de dubbel-blinde en vooral ook placebo-gecontroleerde opzet van de studie. De overwegingen om wel of niet deel te nemen aan een onderzoek of daarvoor, in naam van een familielid, toestemming te verlenen blijven vaak onduidelijk en zijn heel persoonlijk. Ter illustratie wil ik twee reacties voorleggen die mij zijn bijgebleven. De dochter van een patiënte die zojuist was opgenomen met ernstige neurologische schade van een hersenbloeding eiste zelf de mond pasta te 'proeven' alvorens te beslissen. Ze wilde weten hoe die mond pasta aanvoelde en smaakte om in te schatten hoe dit voor haar moeder zou zijn. De senior-vice-president van een middelgrote bank en tevens broer van een patiënt die werd opgenomen met een massale maagbloeding zei, met een stem en intonatie die zijn maatschappelijke functie waardig was: 'Natuurlijk kerel, doen, we smeren hem vier keer per dag van die rommel in zijn bek en we slepen hem erdoor!'. Mijn dank aan alle patiënten die hebben toegestemd in de studies die we hebben verricht, maar zeker ook aan alle echtgenoten en echtgenotes, dochters en zonen, vaders en moeders, broers en zussen die de kracht en de moed hadden om op zo'n moeilijk moment over mijn vraag na te denken en een weloverwogen beslissing te nemen voor hun familielid.

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Curriculum vitae

Dennis Bergmans werd geboren op 29 april 1970 te Weert. In 1988 behaalde hij het VWO diploma aan de Philips van Horne Scholengemeenschap te Weert. In hetzelfde jaar startte hij met de studie Geneeskunde aan de Universiteit Maastricht. Als 3e en 4e-jaars student was hij werkzaam als student-assistent bij het skillslab van de faculteit geneeskunde (Drs M Franssen), de vakgroep Chirurgie (Dr L Hofstra en Prof dr PJEHM Kitslaar) en de vakgroepen Interne Geneeskunde/Medische Microbiologie (Dr MJM Bonten en Dr CA Gaillard/Dr EE Stobberingh). Voor de bijdrage aan het onderzoek naar 'Neurale buissluiting' bij de vakgroep Anatomie/Embryologie (Dr H van Straaten) in het kader van het wetenschaps-keuzeblok werd de Studentenprijs 1992 toegekend door het Bestuur van de Stichting Wetenschapsbeoefening der Rijks Universiteit Limburg. Het artsexamen werd behaald in 1997.

Het in dit proefschrift beschreven onderzoek werd grotendeels uitgevoerd tijdens een aanstelling als onderzoeker bij de vakgroep Interne Geneeskunde van het Academisch Ziekenhuis Maastricht van september 1994 tot en met december 1996 onder leiding van Dr MJM Bonten, Dr EE Stobberingh en Prof dr PW de Leeuw. Hiervoor is de studie geneeskunde tijdelijk onderbroken.

Voor een deel van het onderzoek werd de Posterprijs 1998 toegekend door de Nederlandse Internisten Vereniging en in hetzelfde jaar een prijs van de Stichting Hippocrates Studiefonds voor onderzoek verricht vóór het artsexamen. Sedert 1 oktober 1998 is hij in opleiding tot internist in het Diaconessenhuis te Eindhoven (opleider: Dr HR Haak).