The presence and sequence of endotracheal tube colonization in patients undergoing mechanical ventilation

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ABSTRACT: Endotracheal tube colonization in patients undergoing mechanical ventilation was investigated.

In the first part of this prospective study, the airway access tube was examined for the presence of secretions, airway obstruction and bacterial colonization, in cases undergoing extubation or tube change. In the second part of the study, the sequence of oropharyngeal, gastric, respiratory tract and endotracheal tube colonization was investigated by sequential swabbing at each site twice daily for 5 days in consecutive noninfected patients.

In the first part, it was noted that all airway access tubes of cases undergoing extubation had secretions lining the interior of the distal third of the tube which were shown on scanning electron microscopy to be a biofilm. Gram-negative micro-organisms were isolated from these secretions in all but three cases. In the second part, it was noted that the sequence of colonization in patients undergoing mechanical ventilation was the oropharynx (36 h), the stomach (36–60 h), the lower respiratory tract (60–84 h), and thereafter the endotracheal tube (60–96 h). Nosocomial pneumonia occurred in 13 patients and in eight cases identical organisms were noted in lower respiratory tract secretions and in secretions lining the interior of the endotracheal tube.

The endotracheal tube of patients undergoing mechanical ventilation becomes colonized rapidly with micro-organisms commonly associated with nosocomial pneumonia, and which may represent a persistent source of organisms causing such infections.
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In 1972, JOHANSON *et al.* [1] were the first to note an increase in oropharyngeal colonization by micro-organisms in patients with increasing severity of illness, and to document thereafter that there was an increased risk of the development of nosocomial pneumonia in patients who had Gram-negative oropharyngeal colonization. Subsequent to the findings by these investigators and many others, it has long been recognized that there is a direct relationship between oropharyngeal colonization and nosocomial pneumonia [2–9]. However, despite numerous studies there is still considerable ongoing debate as to the primary site of colonization, as well as the exact sources and routes of this colonization process [10–13]. Increasing research interest over the past few years has focused on the possible role of direct tracheal colonization [5, 7], as well as the potential role of the endotracheal tube (ETT) itself in the colonization process [14–17].

Bacterial adherence to objects, such as intravenous catheters, by means of the so-called biofilm, in the causation of various nosocomial infections, has been studied over several years [14, 15]. In 1986, SOTTILE *et al.* [14] used scanning electron microscopy (SEM) and qualitative cultures to assess bacterial adhesion to the interior of polyvinylchloride ETTs. They examined biofilm colonization of ETTs, which they considered may act as a reservoir for contamination of the tracheobronchial tree [14]. In their study of 25 patients in the intensive care unit (ICU) under-

going extubation, the ETT was completely covered in 84% of cases and partially covered in 16% by an amorphous bacterium-containing matrix [14]. They suggested that there may be a relationship between nosocomial pulmonary infections and bacterial adherence to the interior surface of the ETT [14]. Several investigators have subsequently studied this process further [15–17] and have suggested not only that the formation of a biofilm may be an important mechanism of ETT obstruction, but also a potential source of pulmonary infection [16, 17].

The main purpose of the current investigation was to determine more precisely the pattern and sequence of ETT colonization in patients in the ICU undergoing mechanical ventilation. In the first part of the study, the presence of colonization and biofilm formation within the interior of the ETT was examined, and in the second part ETT colonization was investigated simultaneously with oropharyngeal, gastric and lower respiratory tract colonization, to determine by which routes micro-organisms may colonize the ETT.

Materials and methods

Patient data

This was a prospective study conducted in two parts at the Hillbrow Hospital Intensive Care Unit, Johannesburg,

South Africa. Permission to undertake these investigations was obtained from the Committee for Research on Human Subjects of the University of the Witwatersrand. The following data were recorded in each case: age and sex of the patient, reasons for mechanical ventilation, type of humidification used (humidifier in the ventilator circuit or heat and moisture exchanger), duration of ventilation, means of respiratory access (oral or nasal ETT or tracheostomy), method of stress ulcer prophylaxis (histamine type 2-receptor blockers or barrier preparation, *i.e.* sucralfate), the occurrence or not of nosocomial pneumonia, together with data of suspected nosocomial pathogens, using criteria similar to those previously described [10, 11].

The diagnosis of nosocomial pneumonia was based on the presence after ≥ 48 h of ventilation of all the following criteria: 1) radiological evidence of new pulmonary infiltrates; 2) fever of >38°C taken rectally; 3) leukocytosis of $>10 \times 10^9$ cells·L^{-1}; 4) purulent tracheobronchial secretions; and 5) no evidence of infection elsewhere. Organisms cultured in significant amounts (2+ (20–100 colonies) and 3+ (>100 colonies) growth) from good quality specimens of secretions obtained by sterile suctioning of the lower respiratory tract through the ETT or tracheostomy tube were considered to be likely causative pathogens, as previously described [10].

Part 1 of the study

In the first part of the study, the airway access tubes (ETT, nasotracheal or tracheostomy tubes) of 21 consecutive patients undergoing extubation for either tube change or following mechanical ventilation were studied. On extubation, the interior of the tube was immediately sampled using a sterile throat swab, and these specimens submitted for microbiological analysis. Thereafter, the tubes were inspected for evidence of secretions that coated and/or obstructed the interior of the lumen, the latter being assessed by attempting to pass a standard ETT suction catheter through the lumen of the tube. The degree of tube obstruction (mild, moderate or severe) and the site of these secretions (distal, middle or proximal third of the tube) were recorded.

In addition, SEM of the secretions lining the interior of the ETT of a patient following extubation was performed using standard techniques [18]. Briefly, the distal half of the tube was placed in 3% gluteraldehyde and stored at 4°C for 48 h. After fixation, the tube was rinsed in Millonigs buffer. Samples were taken 1.5 cm from the tip, divided longitudinally, and processed for SEM as follows. Primary fixation was in 3% gluteraldehyde in Millonigs buffer (pH 7.4) for 48 h. Post-fixation was in 1% osmium tetroxide in Millonigs buffer (pH 7.4) for 1 h. This was followed by dehydration in graded ethanol 50, 70, 90 and 100%. Two samples were dried in a Hitachi critical point apparatus (Hitachi HCP/2, Koki Company Ltd., Tokyo, Japan) and a further two samples placed in hexamethyldisilazane (HMDS) for 5 min. The samples were then gold coated with an SEM Coating Unit E5100 (Polaron Cool Sputter Coater, Watford, UK) and viewed with a JSM 840 SEM (Jeol JSM 840, Jeol Pty Ltd, Tokyo, Japan). The critical point drying and HMDS methods were performed simultaneously to compare the result of the two methods, and the HMDS technique gave better results.

The swabs taken from the interior of the tube were plated onto blood and McConkey agar which were incubated and read after 24 and 48 h. Each morphological type of colony present was picked off and identified according to standard microbiological protocols. The growth of micro-organisms was quantitated as follows: 1) no growth; 2) 1–5 colonies as scanty growth; 3) 6–20 colonies as 1+ growth; 4) 20–100 colonies as 2+ growth; and 5) >100 colonies as 3+ growth. The isolation of organisms with 1+ growth was considered to represent significant colonization. Susceptibility of all isolated micro-organisms to 14 antibiotic agents was measured using the disc diffusion method. These tests of antibiotic susceptibility were undertaken for comparative purposes, as a measure of the likelihood that the colonizing organisms were the same as those considered responsible for the nosocomial infections.

Part 2 of the study

In the second part of the study, 10 consecutive noninfected patients admitted to the ICU for mechanical ventilation, of a duration of at least 5 days, were recruited. Sampling of the oropharynx (using a sterile throat swab), nasogastric tube (through suction catheter), interior of the airway tube (throat swab) and endotracheal secretions (sterile suction catheter) was undertaken immediately on admission and at 08:00 h and 16:00 h daily for 5 days. All specimens were immediately plated onto McConkey agar only (to suppress and exclude commensal upper respiratory tract flora) and incubated and read after 24 and 48 h. Each morphological type of colony present was picked off and identified according to standard microbiological protocols. The growth of micro-organisms was quantitated and the susceptibility to 14 antibiotic agents was measured in a similar manner to that described in part 1 of the study.

Results

Part 1 of the study

In the first part of the study, 21 patients were recruited who were extubated on 24 occasions. The duration of ventilation varied between 12 h and 12 days. The clinical features of these patients, as well as the reasons for mechanical ventilation are shown in table 1. There was no apparent relationship between the type of humidification or method of stress ulcer prophylaxis used and the presence or absence or nature of tube secretions.

Secretions lining the interior of the ETT or tracheostomy tubes were noted in all cases, which was the cause of obstruction in 11 cases, including two tracheostomy tubes. These secretions were noted from as early as 12 h (in one patient in whom significant growth of Gram-negative organisms (3+ growth) were cultured from the secretions), but were more pronounced from 48 h. The site of secretions was invariably in the distal third of the tube beginning just above the distal open end (10 cases) and in one tube additionally in the middle third, but never in the proximal portion. These secretions caused severe tube obstruction in one case (unable to pass a suction catheter beyond the secretions).

Table 1. – Clinical features and reasons for mechanical ventilation of 21 patients enrolled in the first part of the study undergoing mechanical ventilation in the intensive care unit

Characteristics	Number
Mean age (range) yrs	42.2 (25–86)
Male/female	11/10
Duration of ventilation days	0.5–12
Reasons for ventilation	
Respiratory failure	14
Pneumonia	4
Trauma	4
Polyneuropathy	2
Asthma	2
Drug overdose	1
Surgical sepsis	1
Postoperative	7
Surgery	6
Trauma	1
Airway access tube	
Orotracheal	5
Nasotracheal	11
Tracheostomy	4
Humidification	
Humidifier in the circuit	15
Heat and moisture exchanger	6
Stress ulcer prophylaxis	
Sucralfate	11
Histamine-2-blocker	7
Nil	3

Figures 1a and b are SEMs of the ETT biofilm lining the interior lumen of the airway access tube of a patient following extubation. In addition to demonstrating dense amorphous material covering most of the surface of the tube, there are clumps of material projecting into the lumen, as well as micro-organisms appearing to adhere to this matrix. Micro-organisms were isolated from these secretions in all but three tubes (patients mechanically ventilated for 3, 9 and 12 days, respectively). The organisms isolated (sometimes multiple) are shown in table 2.

Nosocomial pneumonia was diagnosed in 10 of these patients between 5–12 days following initiation of mechanical ventilation. The organisms isolated (sometimes multiple) in secretions obtained by suctioning of the lower respiratory tract of these cases and deemed to be the likely cause of the pneumonia were *Pseudomonas aeruginosa* (6 cases), *Acinetobacter anitratus* (5 cases) *Klebsiella pneumoniae* (3 cases), *Proteus mirabilis* (3 cases) and *Enterobacter* spp. (1 case). As can be seen from table 2, in six of these patients at least one of the micro-organisms deemed to have been responsible for the previous nosocomial pneumonia was still found colonizing the ETT on extubation, following apparently successful treatment of the pneumonia.

Second part of the study

In the second part of the study, 10 male patients were recruited. The clinical features are shown in table 3. All patients had previously been well with no evidence on admission of any infection. In only one patient were no organisms isolated from any of the sites sampled over the 5-day period. Nine of the 10 cases subsequently became
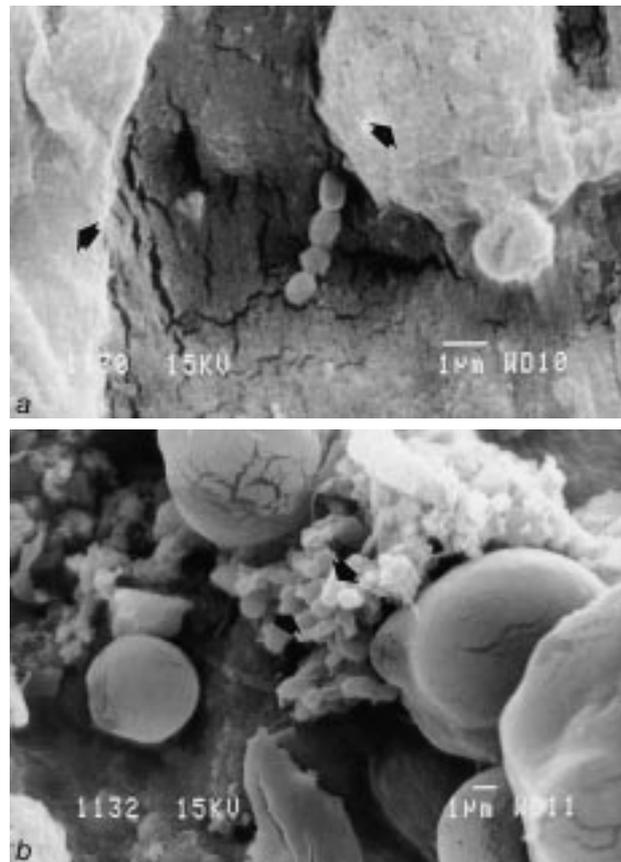

Fig. 1. – The appearance of a biofilm on scanning electron microscopy of the interior of an endotracheal tube of a patient following extubation. a) Shows the dense amorphous material covering much of the interior of the tube, with additional material projecting into the lumen of the tube (arrow). b) Shows micro-organisms apparently adhering to the amorphous matrix (arrow).

colonized with various micro-organisms, Gram-negative bacilli being the most common (*A. anitratus*, *K. pneumoniae*, *P. aeruginosa*, *Escherichia coli* and *Enterobacter* spp.), although Gram-positive micro-organisms were also sometimes noted (*Staphylococcus aureus*, *S. epidermidis* and *Streptococcus bovis*).

The isolation of colonizing organisms from the various sites sampled appeared to follow a set pattern, whether these were Gram-positive or Gram-negative isolates. The oropharynx was the first site to be colonized most commonly at 36 h. Thereafter, the stomach became colonized (36–60 h) and then the lower respiratory tract (beginning at 36 h, but being most abundant between 60–84 h). Isolation of organisms from the ETT itself began at 48 h (scanty isolation), but occurred in significant amounts from 60–96 h.

While the numbers of individual isolates were very small, there was a tendency for Gram-positive organisms to colonize the oropharynx early, between 12–36 h, and to appear rapidly thereafter (12–24 h later, respectively) in the stomach and then in secretions from the lower respiratory tract. No Gram-positive isolates were found to colonize the ETT in significant amounts (only scanty growth). Colonization of the oropharynx with *A. anitratus* occurred later, between 36–48 h, and these organisms progressively colonized the stomach, thereafter appearing in endotracheal

Table 2. – Micro-organisms (sometimes multiple) isolated from the interior of the airway access tube of patients undergoing mechanical ventilation in the intensive care unit

Part 1: following extubation or tube change	
Acinetobacter anitratus	9
Klebsiella pneumoniae	6
Pseudomonas aeruginosa	5
Proteus mirabilis	3
Escherichia coli	2
Enterobacter spp.	2
Morganella morgani	1
Enterococcus faecalis	2
Streptococcus viridans	2
Staphylococcus aureus	1
Part 2: during mechanical ventilation	
A. anitratus	4
K. pneumoniae	3
Enterobacter spp.	1

Table 3. – Clinical features and reasons for mechanical ventilation of 10 patients enrolled in the second part of the study undergoing mechanical ventilation in the intensive care unit

Characteristics	Number
Mean age (range) yrs	34.9 (18–61)
Male/female	10/0
Reasons for ventilation	
Trauma	10
Airway access tube	
Orotracheal	10
Humidification	
Heat and moisture exchanger	10
Stress ulcer prophylaxis	
Sucralfate	3
Histamine-2-receptor blocker	7

secretions and all these isolates were subsequently noted within the ETT by 96 h. The pattern of colonization with *Pseudomonas* spp. and *Klebsiella* spp. was not clear-cut. Other Enterobacteriaceae colonized the oropharynx and stomach from 48 h, only occasionally appearing in the lower respiratory tract and in endotracheal secretions. Figure 2 shows the time sequence of colonization of the oropharynx, stomach, lower respiratory tract and ETT in four patients colonized with *A. anitratus* and four patients colonized with staphylococcal isolates.

Nosocomial pneumonia was diagnosed in three patients, occurring after 48 h. In one case, *P. aeruginosa* was deemed to be the causative pathogen, being cultured from lower respiratory tract secretions. In this patient, no organisms were isolated from within the ETT. In two cases, *A. anitratus* was deemed responsible for the infection. In both patients, the organisms isolated from the lower respiratory tract were identical to those isolated from the interior of the ETT, as documented by same speciation and identical antimicrobial susceptibility patterns. In one of the cases significant colonization of the ETT and lower respiratory tract by *A. anitratus* occurred simultaneously at 60 h, and was followed by clinical evidence of nosocomial pneumonia at 96 h. In the second case, significant colonization of the lower respiratory tract by this organism occurred at 72 h, and of the ETT at 84 h, followed by clinical evidence of nosocomial pneumonia at 96 h.
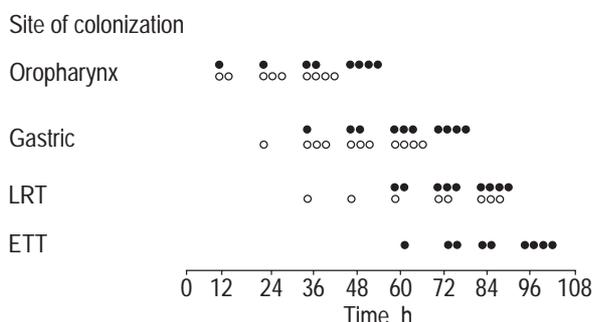

Fig. 2. – The time sequence of colonization of the oropharynx, the stomach (gastric), the lower respiratory tract (LRT) and the endotracheal tube (ETT) by *Acinetobacter anitratus* (Gram-negative isolates; ●) in four patients and by staphylococcal isolates (Gram-positive isolates; ○) in four patients.

Discussion

In this study, it was documented that the interior of the ETT of patients undergoing mechanical ventilation rapidly became colonized with Gram-negative micro-organisms which commonly appeared to survive within a biofilm. While it appears that colonization of the ETT may begin from as early as 12 h, it is most abundant at 96 h. This investigation further suggests that the common sequence of bacterial colonization of patients undergoing mechanical ventilation is firstly the oropharynx/upper gastrointestinal tract, followed by the lower respiratory tract, leading on to ETT colonization. Colonization of the ETT with micro-organisms commonly causing nosocomial pneumonia appears to persist in many cases despite apparently successful treatment of the previous pneumonia.

This study confirms previous findings that the oropharynx is an important early site of colonization by bacterial pathogens in severely ill patients, which occurred in these cases, and has been demonstrated in many other studies by 48 h [7]. The next site at which micro-organisms were noted was in the upper gastrointestinal tract, occurring progressively between 36–60 h. The present study suggests that colonization of the upper gastrointestinal tract is a common and early occurrence. The role of the stomach as a reservoir for nosocomial pneumonia is still a controversial topic. While some investigators suggest that gastric colonization is less important in the pathogenesis of ventilator-associated pneumonia [11, 19], even in cases undergoing chronic mechanical ventilation [13], other meta-analyses [12] and reviews [20, 21] have concluded that there is major evidence in the literature in favour of the gastric origin of at least some pulmonary infections.

Thereafter colonizing micro-organisms were found in lower respiratory tract secretions obtained through sterile suctioning through the ETT. This was noted from as early as 36 h, but was most prominent between 60–84 h. Isolation of micro-organisms from within the ETT itself was noted from as early as 48 h (scanty isolation), while colonization in significant amounts was most apparent at 96 h. It is interesting to note that the appearance of micro-organisms in lower respiratory tract secretions of these patients almost invariably preceded their appearance in the interior of the ETT. This suggests that colonization of the ETT in these patients occurred from the lower respiratory tract, as has been suggested by others [16, 17]. This

conclusion is strengthened by two additional observations in this study. Firstly, ETT colonization occurred despite the use of heat and moisture exchangers (incorporating a bacterial filter) within the ventilator circuit of some of these cases, which would be expected to prevent direct contamination of the ETT from the exterior or from the ventilator tubing. These devices would not, however, be expected to prevent colonization occurring from the lower respiratory tract below. Secondly, colonization was invariably noted to occur in the distal part of the tube of all these patients, which is where such secretions would be expected to accumulate if they appeared in this site as a result of direct spread of secretions from the lower respiratory tract by means of the mucociliary escalator.

A definite pattern to the colonization process emerged with the various micro-organisms, even though there were small patient numbers. There appeared to be a tendency for Gram-positive isolates to colonize the oropharynx early, between 12–36 h, and to appear rapidly thereafter in the stomach and then in lower respiratory tract secretions. No Gram-positive isolates were found to colonize the ETT in significant amounts. Colonization of the oropharynx with *A. anitratus* occurred later, between 36–48 h and these organisms progressively colonized the stomach, the lower respiratory tract and then the ETT (by 96 h). The pattern of colonization with the other micro-organisms was less clear-cut. Figure 2 shows the sequence of colonization of the various sites in four patients colonized with *A. anitratus* and four cases colonized with staphylococcal isolates.

On SEM, it was demonstrated that the micro-organisms colonizing the interior of the ETT adhere within a biofilm (fig. 1a and b). In 1986, SOTTILE *et al.* [14] systematically studied 25 patients in ICU undergoing extubation and found that in 84% of cases the ETT was completely covered and in 16% partly covered by an amorphous bacterium-containing matrix. They were among the first to suggest that there may be a relationship between nosocomial pulmonary infections and bacterial adherence to the interior of the ETT. A study in neonates undergoing prolonged mechanical ventilation failed to demonstrate a clinical correlation between ETT colonization and nosocomial pneumonia [15]. Nevertheless, others have suggested that the biofilm may not only represent a mechanism of tube obstruction, but also be a source of nosocomial pulmonary infection [14–17]. It has been reported that organisms adhering in the biofilm in the interior of the ETT may enter the lungs *via* aerosolization of particles during the ventilatory process or following mechanical dislodging of these secretions at the time of airway suctioning [5, 6, 14–17].

Nosocomial pneumonia occurred in a total of 13 patients in these two study groups. Because of the study design, it was only possible to relate the time of ETT colonization to development of nosocomial pneumonia in patients enrolled in the second part of this study. Three of these patients developed nosocomial pneumonia. In one of these, no organisms were noted in the ETT. In a second case, significant colonization of the lower respiratory tract and ETT had occurred simultaneously with *A. anitratus* at 60 h, followed by clinical evidence of nosocomial pneumonia, deemed to be due to the same organism, at 96 h. In a third patient, colonization of the lower respiratory tract with *A. anitratus* occurred at 72 h, followed by ETT colonization with the same organism at 84 h, followed by nosocomial pneumonia, deemed to be due to the same organism, at 96

h. Thus, in two of these cases, nosocomial pneumonia followed ETT colonization. In the first part of the study, 10 patients developed pneumonia and in six of these, despite apparently successful treatment of the infection, at least one of the micro-organisms deemed to have been responsible for the pneumonia was still found colonizing the ETT on extubation. Thus, persistence of micro-organisms in the ETT occurred in several cases even after apparently successful treatment of the previous nosocomial pneumonia. While this series is far too small to make any definitive comment, it would be interesting to speculate that colonization of the ETT may precede nosocomial pneumonia in a number of patients or, more importantly, be a persistent source of organisms that may cause recurrent pneumonia, much in the same way as it has been suggested in recent studies that recurrent *P. aeruginosa* pneumonia may be due to persistence and relapse of these strains in the lower respiratory tract [22].

With regard to the management of ETT biofilm formation and colonization, a number of options have been considered. While regular ETT changes may seem appropriate, recent studies have suggested that this may be associated with a higher incidence of nosocomial pneumonia [4, 22]. The reasons for this are not entirely clear, but may relate to the passing of the airway access tube through areas (*e.g.* the naso- or oropharynx) that are already colonized in critically ill cases with common nosocomial pathogens. Others have suggested that the interior of the ETT could be "brushed" using a specially developed wire mesh instrument or that specific materials could be developed for airway access tubes that impede biofilm formation, these being steps which may prevent nosocomial pneumonia [5, 16, 17].

In conclusion, it has been noted that endotracheal tube colonization and biofilm formation occurs in many patients undergoing mechanical ventilation, from a very early stage. Biofilm formation may in many cases precede the development of nosocomial pneumonia, and perhaps more importantly, represent a persistent source of organisms causing recurrent infections. Further studies are needed to clarify the exact role of endotracheal tube colonization in the pathogenesis of nosocomial pneumonia, since measures aimed at addressing the problem of endotracheal tube colonization and biofilm formation may help impact further on this ongoing problem.

References

1.   Johanson WG, Pierce AK, Sanford JP, *et al*. Nosocomial respiratory infections with Gram-negative bacilli: the significance of colonization of the respiratory tract. *Ann Intern Med* 1972; 77: 701–706.
2.   Chastre J, Trouillet JL. Nosocomial pneumonia. *Curr Opin Infect Dis* 1995; 1: 194–201.
3.   A'Court C, Garrard CS. 1–Nosocomial pneumonia in the intensive care unit: mechanisms and significance. *Thorax* 1992; 47: 465–473.
4.   Craven DE, Steger KA. Nosocomial pneumonia in the intubated patient: new concepts on pathogenesis and prevention. *Infect Dis Clinic North Am* 1989; 3: 843–866.

5. Campbell DG Jr, Niederman MS, Broughton WA, *et al*. Hospital-acquired pneumonia in adults: diagnosis, assessment of severity, initial antimicrobial therapy, and preventative strategies. *Am J Respir Crit Care Med* 1995; 153: 1711–1725.

6. Craven DE, Steger KA. Epidemiology of nosocomial pneumonia: new perspectives of an old disease. *Chest* 1995; 108 (Suppl.): 1S–16S.

7. Estes RJ, Meduri GU. The pathogenesis of ventilator-associated pneumonia: I. Mechanisms of bacterial transcolonization and airway innoculation. *Intensive Care Med* 1995; 21: 365–383.

8. Niederman MS. Bacterial adherence as a mechanism of airway colonization. *Eur J Clin Microbiol Infect Dis* 1989; 8: 15–20.

9. Niederman MS. The pathogenesis of airway colonization: lessons learnt from the study of bacterial adherence. *Eur Respir J* 1994; 17: 1737–1740.

10. de Latorre FJ, Pont T, Ferrer A, *et al*. Pattern of tracheal colonization during mechanical ventilation. *Am J Respir Crit Care Med* 1995; 152: 1028–1033.

11. Bonten MJM, Gaillard CA, van Tiel FH, Smeets HEW, van der Geest S, Stobberingh EE. The stomach is not a source for colonization of the upper respiratory tract and pneumonia in ICU patients. *Chest* 1994; 105: 878–884.

12. Heyland D, Mandell LA. Gastric colonization by Gram-negative bacilli and nosocomial pneumonia in the intensive care unit: evidence for causation. *Chest* 1992; 101: 187–193.

13. Palmer LB, Donelan SV, Fox G, Bellemore E, Greene WH. Gastric flora in the chronically mechanically ventilated patients: relationship to upper and lower airway colonization. *Am J Respir Crit Care Med* 1995; 151: 1063–1067.

14. Sottile FD, Marrie TJ, Prough DS, *et al*. Nosocomial pulmonary infection: possible aetiologic significance of bacterial adhesion to the endotracheal tube. *Crit Care Med* 1986; 14: 265–270.

15. Diaz-Blanco J, Clawson RC, Roberson SM, *et al*. Electron microscopic evaluation of bacterial adherence to polyvinyl chloride endotracheal tubes used in neonates. *Crit Care Med* 1989; 17: 1335–1340.

16. Inglis TJJ, Millar MR, Jones JG, *et al*. Tracheal tube biofilm as a source of bacterial colonization of the lung. *J Clin Microbiol* 1989; 27: 2014–2018.

17. Inglis TJJ, Tit-Meng L, Mah-Lee N, *et al*. Structural features of tracheal tube biofilm, formed during prolonged mechanical ventilation. *Chest* 1995; 108: 1049–1052.

18. Goldstein JI, Yakowitz H, eds. Practical Scanning Electron Microscopy. Electron and Ion Microprobe Analysis. New York, Plenum Press, 1997.

19. Bonten MJM, Gaillard CA, de Leeuw PW, Stobberingh EE. Role of colonization of the upper intestinal tract in the pathogenesis of ventilator-associated pneumonia. *Clin Infect Dis* 1997; 24: 309–319.

20. Torres A, El-Ebiary M, Soler N, Monton C, Fabegras N, Hernandez C. Stomach as a source of colonization of the respiratory tract during mechanical ventilation: association with ventilator-associated pneumonia. *Eur Respir J* 1996; 9: 1729–1735.

21. Torres A, Aznor R, Gatell JM, *et al*. Incidence, risk, and prognosis factors of nosocomial pneumonia in mechanically ventilated patients. *Am Rev Respir Dis* 1990; 142: 523–528.

22. Rello J, Mariscal D, March F, *et al*. Recurrent *Pseudomonas aeruginosa* pneumonia in ventilated patients. *Am J Respir Crit Care Med* 1998; 157: 912–916.