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REVIEW ARTICLE

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The pathogenesis of ventilator-associated pneumonia: I. Mechanisms of bacterial transcolonization and airway inoculation

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Abstract Ventilator-associated pneumonia (VAP) is an infection of the lung parenchyma developing in patients on mechanical ventilation for more than 48 h. VAP is associated with a remarkably constant spectrum of pathogenic bacteria, most of which are aerobic Gram-negative bacilli (AGNB) and, to a lesser extent *Staphyloccus aureus.* Most authorities agree that VAP develops as a result of aspiration of secretions contaminated with pathogenic organisms, which appear to be endogenously acquired. These pathogens gain access to the distal airways by mechanical reflux and aspiration of contaminated gastric contents and also by repetitive inoculation of contaminated upper airway secretions into the distal tracheobronchial tree. Persistence of these organisms in the upper airways involves their successful colonization of available surfaces. Although exogenous acquisition can occur from the environment, the rapidity at which critically ill patients acquire AGNB in the upper airways in conjunction with the low rate of AGNB colonization of health-care workers exposed to the same environment favors the presence of endogenous proximate sources of AGNB and altered upper airway surfaces that are rendered receptive. Proximate sources of AGNB remain unclear, but potential sites harboring AGNB prior to illness include the upper gastrointestinal tract, subgingival dental plaque, and the periodontal spaces. Following illness or antibiotic therapy, competitive pressures within the oropharynx favor AGNB adherence to epithelial cells, which lead to oropharyngeal colonization. Similar dynamic changes in contiguous structures (oropharynx, trachea, sinuses, and the upper gastrointestinal tract) lead to the transcolonization of these structures with pathogenic bacteria. Following local colonization or infection, these structures serve as reservoirs of AGNB capable of inoculating the lower airways. As the oropharynx becomes colonized with AGNB, contaminated oropharyngeal secretions reach the trachea, endotracheal tube, and ventilator circuit. Contaminated secretions pooled above the endotracheal tube cuff gain access to the trachea and inner lumen of the endotracheal tube by traversing endotracheal tube cuff folds. Amorphic particulate deposits containing AGNB form along the endotracheal tube and are capable of being propelled into the distal airways by ventilator-generated airflow or by tubing manipulation. Bacteria embedded within this type of amorphous matrix are particularly difficult for the host to clear. If host defenses fail to clear the inoculum, then bacterial proliferation occurs, and the host inflammatory response

progresses to bronchopneumonia. By understanding the mechanisms involved in the pathogenesis of VAP, new strategies may be developed to prevent this significant complication of mechanical ventilation.

Key words Ventilator-associated pneumonia · Colonization · Aspiration defense mechanisms · Inflammatory response

Introduction

An understanding of the pathogenesis of ventilator-associated pneumonia (VAP) has evolved during recent years. As demonstrated by clinical epidemiologic studies, VAP is associated with a remarkably constant bacterial spectrum of aerobic Gram-negative bacilli (AGNB), and, to a lesser extent *Staphylococcus* species, which implicates unique host-bacterial interactions (Table 1). These organisms apparently transcolonize the oropharynx, sinuses, upper gastrointestinal tract and trachea in large numbers prior to the development of nosocomial pneumonia. Although the origin of these AGNB is unclear, potential sites harboring AGNB prior to illness include gingival plaque, the periodontal pits, and the upper gastrointestinal tract. Upon illness, successful colonization of the oropharynx and trachea by AGNB occurs as alterations in host defenses, changes in bacterial adherence profiles to mucosal surfaces, and fluctuating competitive pressures favor AGNB colonization. Inoculation of these colonizing AGNB into the distal lung parenchyma occurs as con-

Table 1 Concordance of bacterial isolates associated with pneumonia

	Medical legal autopsies, normal lungs Mays, 1969 [3]	All hospitalized patients Mays, 1969 [3]	Nosocomial pneumonia, all wards[a] Bartlett, 1986 [43]	Necrotizing pneumonia Mays, 1969 [3]	Ventilator-associated pneumonia[b,c] Pooled data[d]
	154 isolates from 108 lung tissues in 54 autopsies	608 isolates from 672 lung tissues in 226 autopsies	287 isolates from 159 patients with nosocomial pneumonia	46 isolates from 36 lung tissues having necrosis	154 isolates from 115 episodes of VAP
	% Lung tissue	% Lung tissue	% Patients	% Lung tissue	% Events VAP
Total AGNB[e]	15%	54%	73 (46%)	89%	96 (83%)
Pseudomonas spp.	2%	16%	9%	50%	30%
Acinetobacter spp.					19%
Escherichia coli	9%	18%	14%	17%	8%
Klebsiella spp.	2%	8%	14%	17%	6%
Enterobacter spp.		6%	4%	13%	5%
Proteus spp.		4%	11%	7%	11%
Serratia marcescens					1%
Other GNB	2%	2%	3%	3%	3%
Miscellaneous Gram-neg	N/A	N/A	27 (17%)	N/A	16 (14%)
Hemophilus influenzae			17%		9%
Legionella pneumophilia					1%
Branhamella catarrhalis					4%
Total staphylococci	11%	12%	42 (26%)	14%	33 (29%)
Staphylococcus aureus	5%	10%	26%	9%	27%
Coagulase-negative	6%	2%		5%	2%
Total streptococci	100%	22%	60 (38%)	19%	16 (14%)
Streptococcus pneumoniae	7%	1%	31%		2%
Streptococcus faecalis	7%	7%	7%	8%	1%
β-hemolytic streptococci	10%	3%		3%	9%
α-hemolytic streptococci	76%	11%		8%	1%
Total anaerobic	N/A	N/A	56 (35%)	N/A	3 (2%)
Yeasts/fungus	2%	2%	N/A	6%	5 (4%)

[a] Specimens obtained by either transtracheal aspirates (before antibiotics), empyema fluid culture (before antibiotics), or positive blood cultures

[b] Pneumonia developed >48 h after mechanical ventilation

[c] Specimens obtained by either protected brush catheter, transthoracic needle lung specimen, pleural fluid cultures or blood cultures

[d] Pooled data from Dreyfuss [8], Jimenez [91] and Fagon [92]

[e] Aerobic gram-negative bacilli

taminated oropharyngeal secretions reach the trachea and endotracheal tube. These contaminated secretions are then propelled into the distal airways by inspiratory airflow from mechanical ventilation or endotracheal tube manipulation. In the distal airways, the bacterial inoculum and host inflammatory response interact. If the host inflammatory response can contain the inoculum, then bacterial proliferation is insufficient to overcome bacterial clearance, and the inoculum is eliminated. If the host inflammatory response is overwhelmed, then bacterial proliferation exceeds bacterial clearance, leading to the development of bacterial pneumonia. This article reviews current concepts involving the transcolonization of the upper airways and subsequent inoculation of AGNB into the distal lung parenchyma, which initiates VAP.

Clinical epidemiology

Pathways to lung infection

Bacteria may gain access into the lung from (1) the airways which can occur with aspiration of oropharyngeal secretions or by the inhalation of bacterial aerosols; (2) the bloodstream which can occur with pneumonia following bacteremia (e.g., with tricuspid valve endocarditis); or

Table 2 Bacterial isolates from proximate sites: concordance supports transcolonization

	Oropharynx	Periodontal disease	Dental plaque	Sinusitis	Gastric aspirates	Tracheal aspirates	Condensate
	AGNB from critically ill patients Johanson, 1969 [49]	AGNB from patients with periodontitis Slots, 1990 [53]	Dental plaque from critically ill patients Scannapieco, 1992 [52]	Nosocomial sinusitis Caplan, 1982 [64]	Patients on mechanical ventilation Atherton, 1978 [12]	Patients on mechanical ventilation Atherton, 1978 [12]	Patients on mechanical ventilation Craven, 1984 [89]
	122 isolates from 104 patients	468 isolates from 427 patients	18 isolates from 22 patients	41 isolates from 32 patients	15 isolates from 10 patients	13 isolates from 10 patients	63 isolates from 20 patients
Total AGNB[a]	122	468	16 (89%)	34 (83%)	11 (74%)	9 (70%)	48 (76%)
Pseudomonas spp.	16%	20%	44%	29%	6%	8%	√
Acinetobacter spp.		6%					√
Escherichia coli	17%	6%		8%	13%	8%	
Klebsiella spp.	23%	18%	11%	17%	20%	23%	√
Enterobacter spp.	21%	27%	6%	17%			
Proteus spp.	7%	2%	6%	12%	33%	23%	
Serratia marcescens	8%	2%	17%				√
Other GNB	8%	20%	5%				
Misc Gram-neg	N/A	N/A	0%	N/A	N/A	1 (8%)	
Hemophilus influenzae						8%	
Total staphylococci	N/A	11/21 (52%)	2/18 (11%)	1 (2%)	1 (7%)	3 (23%)	13 (21%)
Staphylococcus aureus		52%[†]	11%	2%	7%	23%	√
Coagulase-negative							√
Total streptococci	N/A	N/A	N/A	4 (10%)	1 (7%)	1 (8%)	√
Streptococcus pneumoniae						8%	
Streptococcus faecalis				3%	7%		
β-hemolytic streptococci				7%			
α-hemolytic streptococci							
Total anaerobic	N/A	N/A	N/A	2 (5%)	N/A	N/A	
Yeasts/fungus	N/A	5/21 (23%)[b]	N/A	N/A	2 (13%)	N/A	2 (3%)

[a] Aerobic gram-negative bacilli – number of isolates (percentage of isolates in parenthesis)
[b] From Rams et al. 11/21 patients

(3) direct extension of a contiguous infection which can occur with a pleural space infection [1]. Most episodes of nosocomial pneumonia are suspected to originate from aspiration of pathogenic oropharyngeal bacteria into the distal bronchi followed by bacterial proliferation [2, 3]. The host then responds with an inflammatory reaction. If the rate of bacterial proliferation in the inoculi exceeds the rate of host inflammatory clearance, the inflammatory response spreads into the contiguous bronchiole wall leading to bronchiolitis. Further centripetal inflammation from the bronchiole wall involves the alveolar septi and airspaces leading to bronchopneumonia.

In the normal host, the lung is repeatedly exposed to oropharyngeal flora during life, and yet few develop pneumonia. Normal adults frequently aspirate oropharyngeal secretions during sleep [4], and yet the bronchi and pulmonary parenchyma of persons without lung disease are remarkably free of bacteria [3]. However, autopsy cultures of the pulmonary parenchyma of persons dying from nonmedical causes demonstrate the ubiquitous presence of oropharyngeal flora. Of 82 such autopsies, 100% of lung cultures (of which all specimens were free of acute or chronic inflammation) yielded normal pharyngeal flora (alpha-hemolytic *Streptococcus spp.*, γ-hemolytic *Streptococcus spp.*, *Streptococcus pneumoniae*, and *Neisseria spp.*), while 15% also yielded AGNB [3]. The studies suggest that the normal host frequently aspirates oropharyngeal contents and that their defenses prevent significant tissue inflammation from occurring during life by sustaining net clearance of these bacterial inoculi from the lower airways.

Hospitalized patients appear to be somewhat different. Out of 287 consecutive autopsies from hospitalized patients (of which half had not received antibiotics) the incidence of lung tissue cultures yielding normal pharyngeal flora was reduced (21%) and the incidence of AGNB was increased (62%), even though most tissue samples were free of acute inflammation (81%), chronic inflammation (97%), or necrosis (94%) [3] (Fig. 1 and Table 1). Furthermore, the recovery of AGNB from lung tissue, even in large numbers, could not reliably predict the presence of histologic pneumonia [3].

These findings demonstrate that the majority of hospitalized patients retain the capacity to manage aspirated bacteria as effectively as a normal host. Major differences with hospitalized patients appear to be the high recovery rate of AGNB bacteria, which are not regarded as oropharyngeal bacteria in the normal host and the high rate (19%) of parenchymal inflammation. This increased rate of AGNB recovery results from a combination of increased oropharyngeal colonization with AGNB and high rates of aspiration. Despite their high incidence of recovery from noninflammed lung, AGNB remain important pathogens in nosocomial pneumonia. When nosocomial pneumonia with acute inflammation and necrosis were found at autopsy, the likelihood of recovering AGNB especially *Pseudomonas aeruginosa* [3] from lung tissue was significant. The increased rate of pneumonia found

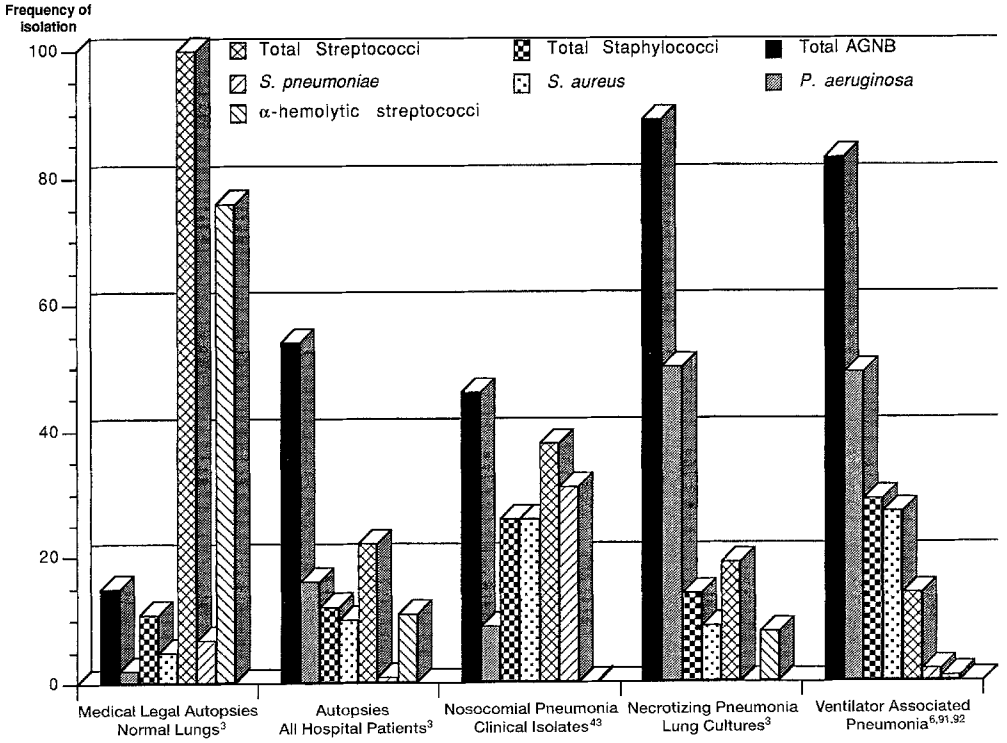

Fig. 1 Frequency of bacterial isolation from lung samples. The distribution of bacterial isolates shifts from predominately alpha hemolytic streptococcal species in normal lung tissues to predominately AGNB and *Pseudomonas* species in ventilator associated pneumonia

in hospitalized patients is most likely related to a patient's limited capacity to sustain containment of repetitive pathogenic bacterial inoculi. The distribution of bacterial recovery ranging from patients with normal lungs to patients with VAP is summarized in both Fig. 1 and Table 1.

Early pneumonia: aspiration of gastric contents

Mechanical bolus aspiration of gastric contents appears to be a prominent mechanism in patients who develop pneumonia within the first 48 h of admission to the hospital. Similar organisms are found both within tracheal secretions and gastric contents soon after admission to intensive care units and these organisms are different than initial oropharyngeal organisms [5]. Further, patients with early pneumonia admitted to intensive care units from out-patient settings have tracheal secretions containing 65% mixed flora and gram-positive rods while those admitted from in-patient settings have tracheal secretions containing 64% AGNB [6]. These suggest that patients admitted from out-patient settings have bolus aspiration of gastric contents containing refluxed duodenal secretions with intestinal bacteria and that these organisms have not yet 'transcolonized' the upper airways.

Colonization preceding nosocomial pneumonia

The concept that nosocomial pneumonia develops from the aspiration of colonized oropharyngeal or tracheal secretions is supported by studies demonstrating upper airway colonization with AGBN preceding the development of nosocomial pneumonia. In a study where healthy baboons were intubated and placed on mechanical ventilation, the oropharynx in all animals became colonized with AGNB within 24 h, and all animals subsequently developed fatal pneumonia. On autopsy, pathogens recovered from lung samples were the same organisms previously colonizing the oropharynx [7]. In a study of 73 patients requiring mechanical ventilation for greater than 48 h, VAP (as defined bronchoscopically with protected brush specimens) developed in 26%. Of these, 14 of 18 responsible bacteria were found in the patient's oropharynx or trachea during the 72 h preceding pneumonia [8]. Further, in a prospective clinical study of 213 hospitalized patients, nosocomial pneumonia developed in 23% of patients colonized with AGNB but in only 3.3% of patients not colonized with AGNB [2].

Sinusitis can occur in patients with nasopharyngeal intubation (nasogastric tubes or nasotracheal intubation) and serve as a reservior for AGNB capable of causing pneumonia [9]. Maxillary sinusitis was prospectively identified by cultures of sinus aspirates in 28 of 507 MICU patients. All the sinus aspirate cultures yielded AGNB growth. Pneumonia, as defined bronchoscopically with protected brush specimens, developed in 10 of the 28 patients. The organisms causing pneumonia had been previously isolated from the maxillary sinus aspirates in 6 of the 10 patients with pneumonia [9].

The stomach can also serve as a reservoir for AGNB, seeding the oropharynx and trachea, leading to nosocomial pneumonia [10–14]. In the first report suggesting the stomach as a source of tracheal AGNB, Atherton and White demonstrated stomach colonization with AGNB 2 to 9 days post ileus, followed by tracheal colonization 1 to 3 days later [12]. In another study with simultaneous cultures of the trachea, oropharynx and stomach, the first isolation of AGNB occurred equally between the three sites [13].

Collectively, these studies demonstrate the dynamic transcolonization of the oropharynx, sinuses, trachea, and upper intestinal tract occurs. This transcolonization

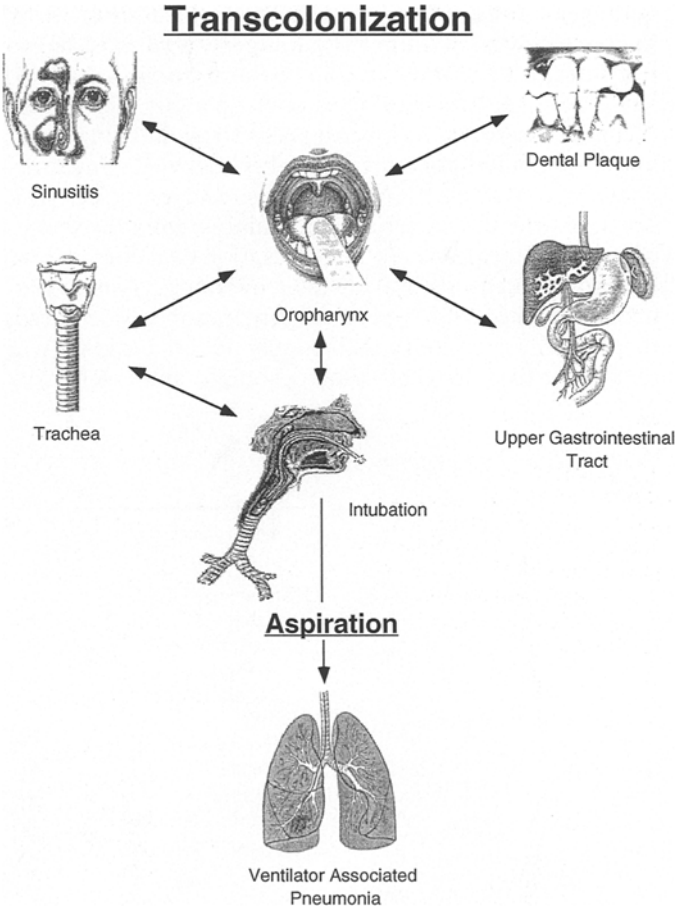

Fig. 2 Pathogenesis of ventilator associated pneumonia. Transcolonization of the oropharynx occurs with contiguous structures such as the sinuses, trachea, gastric contents, and periodontal areas. Contamination of the inner and outer surfaces of the endotracheal tube with oropharyngeal and tracheal secretions occurs as does contamination of the ventilator circuits. During mechanical ventilation, inoculi of these contaminated secretions enter the distal airway and may induce an inflammatory response leading to pneumonia

frequently involves large numbers of bacteria and often precedes the development of nosocomial pneumonia (Fig. 2). Clearly, some combination of factors contributes to the successful colonization and proliferation of AGNB bacteria in these patients. The initial step of this interaction is the adherence of bacteria to the mucosal surfaces.

Mechanisms of colonization

Adherence

"Bacteria stick to and grow on almost any surface. Within minutes after submerging a solid object in sea water or fresh water, the surfaces become colonized by adherent microorganisms, and the earliest organisms to adhere are bacteria" [15]. Most bacteria are capable of exploiting an available niche by first adhering to a presented surface and then proliferating. Attachment to host surfaces remains the initial event in the pathogenesis of most bacterial infectious diseases in animals and humans [15]. To survive on mucosal surfaces, bacteria must first attach themselves securely to mucosal cells to avoid being swept away by luminal contents through swallowing, sneezing, coughing, ciliary action, peristalsis, and excretion [15]. Furthermore, the bacteria must maintain an adhesive capacity to allow persistent recolonization of a continuously desquamated mucosal surface. In contrast, once colonization is successful and tissue penetration has occurred, the bacteria must loose their highly adhesive capacity to reduce the likelihood of being opsonized and ingested by phagocytic leukocytes, a process termed phase variation (as occurs with the uropathogen *Proteus mirabilis*) [16]. Bacterial colonization with subsequent tissue penetration and infection are thus the results of complex interactions between the bacterium and host tissue.

Adhesins and receptors

Most pathogenic bacteria possess cell surface structures that interact with host tissue, leading to virtually irreversible binding. The specific bacterial structures are termed adhesins, and the target host structures are termed receptors. Streptococcal species bind to host receptors via bridging macromolecules (e.g., lipoteichoic acid), while AGNB bind to host receptors via filamentous extensions termed fimbriae. Fimbriae are complex proteinacious projections that have adhesive properties. Domains on these fimbriae specifically recognize carbohydrate moieties of host glycoproteins or glycolipids and bind with ligand specificity. Most binding domains are localized at the tips of fimbriae, where adhesin-receptor interactions are maximized [17]. Multiple fimbrial types can be found on the same bacterium and influence tissue binding. Fimbriae have been demonstrated to be involved in the adherence of AGNB to the gastrointestinal tract, urinary tract, airway epithelium, and even to other bacteria [17].

The inherent specificity of the adhesin-receptor interaction may be involved in tissue trophism, or the apparent preference of a particular bacterium for a particular host tissue. *Streptococcus mutans,* for example, is

Table 3 Upper airway tissue trophism

	Organisms
Nasopharynx	
Epithelium	*Streptococcus pyogenes* [16]
Oropharynx	
Epithelium	
Buccal	*Streptococcus pyogenes*
Tongue	*Streptococcus salivarius* [44]
Nonkeritinized	*Streptococcus mitis*
Other	
Tooth surface	*Streptococcus mutans* [44]
	Actinomyces viscosus [51]
Plaque	*Bacteroides melaninogenicus*
Other bacteria	
Actinomyces viscosis	*Bacteroides melaninogenicus*
	Pseudomonas aeruginosa
	Streptococcus pyogenes, agalactiae [51]
Normal gingival crevice	*Streptococcus* and *Actinomyces*
Gingivitis	Anaerobic GNB:
	Fusobacterium, Bacteroides, Campylobacter, Veilloniella, Haemophilus
	Spirocetes
Periodontitis	As with Gingivitis + aerobic GNB (see Table 2)
Trachea	
Epithelium	*Pseudomonas aeruginosa* [26]
Mucus	Mucoid *Pseudomonas* alginate positive

found in abundance in dental plaque but only sparsely on epithelial cells of the tongue [16] (Table 3). It may also explain both the differences in pathogenicity of different bacterial strains (different adhesins) on the same host species and the differences in susceptibility of different host species (different receptors) to the same bacterial strain.

In addition to fimbrial adhesins, *Pseudomonas spp.* may also possess additional nonfimbrial cell surface adhesins [18, 19]. Interestingly, all *Pseudomonas* adhesins might be under the regulatory control of a single gene, rpoN. By controlling both fimbrial and nonfimbrial adhesin expression as well as flagella expression, the rpoN gene may be a regulatory gene for phase variation [18]. After motile bacteria reach and adhere to mucosal surfaces, invasion of deeper tissues could be followed by down regulation of the rpoN gene with the coregulated loss of adhesins and flagellins, thereby allowing the bacterium to avoid suicidal attachment to phagocytic cells.

Altered host receptors

Profiles of bacterial adherence to upper airway epithelium can be altered by a variety of insults, such as airway intubation, previous influenza infection, or treatment of the epithelium with various proteases or elastases [16]. The changing profile of bacterial adherence for a particular tissue most likely results from alterations in host tissue receptors. These changes appear induced by either the expression of different receptors or by the unmasking of previously unavailable receptors [16, 21].

Adherence studies have demonstrated that normal resident oropharyngeal bacteria have greater affinity for buccal epithelium than for ciliated tracheal epithelium, suggesting differences in adhesin receptors between these two host tissues [17, 22]. Clinical studies of hospitalized patients have also demonstrated a different colonization pattern between the trachea and oropharynx [2, 23, 24]. In particular, *Pseudomonas aeruginosa* has increased binding affinity to ciliated tracheal epithelial cells compared to buccal epithelium [25], colonizes the trachea with higher frequencies than the oropharynx [2, 23] and can possibly colonize the trachea without prior oropharyngeal colonization [23, 24]. Given a concomitant exposure of *Pseudomonas aeruginosa* to these various surfaces, preferential binding and subsequent colonization of the trachea may occur without colonization of the oropharynx due to the increased binding affinity of *Pseudomonas aeruginosa* for this surface.

Variations in strain also affect *Pseudomonas* adherence. Nonmucoid strains bind preferentially to nonciliated tracheal epithelium while mucoid strains have increased binding affinity to ciliated epithelium. Mucoid *Pseudomonas* secretes a glycocalyx (alginate) that binds to both the epithelial cilia and the bacterial surface, forming an adhesive bridge between the cilia and bacterium [26]. Most clinical isolates of *Pseudomonas aeruginosa* are of the nonmucoid strain, and all subsequent referrals to *Pseudomonas aeruginosa* will be of the nonmucoid variety unless otherwise noted.

Since adhesin-receptor interactions involve ionic charges [16, 17, 25], changes in pH would be expected to influence this interaction [25]. Grant et al. [26] demonstrated that *Pseudomonas* binding to nonciliated tracheal epithelial cells was maximal at an acidotic pH of 6.4. This maximal binding at an acidotic pH may have clinical significance. Karnad et al. [27] evaluated the tracheal pH of patients having tracheostomies and found that with the average tracheal pH decreased from 7.2 to 6.8 with the development of pneumonia and fell as low as 6.2. These data suggest that *Pseudomonas* binding to tracheal epithelium increases with the development of pneumonia and the resultant drop in tracheal pH, leading to persistence of colonization or superinfection.

Fibronectin, proteases, and the oropharynx

Fibronectin may be involved in modulating oropharyngeal bacterial flora by providing binding sites for the adhesion of oral streptococci while inhibiting adhesion of AGNB [28, 29]. Fibronectin normally coats the luminal surface of buccal epithelium but not deeper layers of the stratified epithelium. This indicates that buccal epithelial cells acquire the fibronectin coating from saliva rather than from the production of fibronectin by epithelial cells [29]. Whole-saliva fibronectin originates from salivary glands (primarily submandibular and sublingual) and crevicular fluid [30–33]. Crevicular fluid is a transudate of serum that issues from the crevices between the teeth and gums and contains proteins in proportion with that of serum. Concentrations of crevicular fluid fibronectin are similar to plasma fibronectin levels, reflecting transudation of serum proteins in this fluid.

Once secreted into the oropharynx, fibronectin bathes and coats the luminal surface of buccal epithelium, while the remaining free salivary fibronectin undergoes proteolytic degradation [30]. The buccal surface fibronectin coating provides an adhesive surface for the binding of oral streptococci through classic adhesin-receptor interactions that utilize a bridging macromolecule, lipoteichoic acid (LTA). LTA is a specialized surface macromolecule secreted by streptococci. It has a polyanionic backbone that forms ionic complexes with streptococcal surface fibrillar proteins and a lipid end that binds to buccal cell fibronectin [15]. Through these interactions, the LTA molecule forms an effective bridge between the streptococci and buccal epithelium, resulting in streptococcal adherence [15].

Decreases in salivary fibronectin content or alterations in salivary fibronectin structure adversely influence streptococcal adherence while promoting AGNB adherence

[34]. During illness such as sepsis, decreases in serum fibronectin may result in decreases in whole-saliva fibronectin and promote oropharyngeal colonization with AGNB. Increased proteolytic cleavage of buccal fibronectin, which can occur with oropharyngeal inflammation, serves to reduce available binding sites for streptococci while promoting AGNB adherence, leading to a shift in oropharyngeal colonization patterns. Buccal epithelial cells obtained from patients colonized with AGNB have increased in-vitro binding of AGNB with decreased binding of streptococci compared to buccal epithelial cells obtained from patients not colonized with AGNB, possibly as a result of fibronectinolysis [35]. The increase in oropharyngeal AGNB found in hospitalized patients may occur as the result of increased salivary fibronectinolytic activity originating from proteases of multiple sources.

There are many bacteria in the oropharynx that secrete proteases capable of degrading fibronectin, including *Pseudomonas aeruginosa* [36]. Gram-positive pathogenic bacteria capable of degrading fibronectin include *Staphylococcus aureus, Staphylococcus epidermidis, Clostridium sporogenes, Propionibacterium acnes,* and *Peptococcus prevotii* [37]. Of 116 normal oral bacterial species tested, all gram-negative bacteria possessing fibronectinolytic activity belonged to the *Bacteroides spp.* [37]. These oral bacterial species proliferate and predominate the bacterial spectrum of patients with periodontal disease and, as will be discussed, may play a role in supporting AGNB colonization in the oropharynx.

Altered oropharyngeal colonization may be partially attributed to alterations in gingival flora with resultant increases in fibronectinolytic bacteria. Subsequent fibronectinolysis leads to loss of receptors for inhibitory alpha-streptococcus while removing barriers for AGNB adherence to buccal epithelium. In a study evaluating the role of cell-associated fibronectin, salivary protease activity, and AGNB buccal ahderence, Woods et al. [21] evaluated patients with acute respiratory failure (ARF) and patients undergoing coronary artery bypass surgery. In patients with ARF, increases in in-vitro *Pseudomonas* adherence were associated with concomitant increases in salivary protease activity and decreased cell surface fibronectin. In patients undergoing cardiac surgery, a parallel transient increase in *Pseudomonas* adherence occurred on postoperative days 1 and 2, with associated fibronectin on the same postoperative days. When sputum from the ARF patients was incubated with buccal cells from normal volunteers, increases in *Pseudomonas* adherence and decreases in cell surface fibronectin occurred, indicating fibronectinolysis activity in the sputum [21].

The role of cell-associated fibronectin and oropharyngeal AGNB colonization was also studied by Dal Nogare et al. [38]. Of 16 patients undergoing cardiac surgery, 6 became colonized with AGNB within 72 h postsurgery. These colonized patients demonstrated concomitant increases in salivary elastase activity and increased fibronectin digestive activity over noncolonized patients. Enzyme inhibition studies demonstrated that most of the fibronectin digestive activity was due to polymorphonuclear neutrophil (PMN) elastase. The increased fibronectinolytic activity was not due to reduced levels of antileukoprotease, the major elastase inhibitor found in saliva. These authors suggested that PMN migrate via crevicular fluid into the saliva where they release elastase. The released elastase then digests buccal epithelial cell fibronectin, and the loss of fibronectin allows gram-negative bacilli to adhere to and colonize the upper airways [38].

Staphyloccocus aureus

Studies of *Staphylococci aureus* adherence mechanisms have focused on the particular talent of *S. aureus* for adhering to wounds, intravascular devices, and native heart valves. These structures have in common tissue injury and the formation of fibrin clots associated with those inflammatory reactions. These clots are derived from platelets and platelet-derived factors, host coagulation proteins, and fibrin polymers. It has been demonstrated that *S. aureus* has specific receptor sites for fibronectin, fibrinogen, and fibrin and binds to a platelet-derived glycoprotein, thrombospondin [39–41]. In addition, the cell coat contains protein A, which binds Fc fragments of immunoglobulin and immune complexes. *S. aureus* can also bind to washed epithelial cells [39, 42]. Together, *S. aureus* can adhere to and colonize surfaces that have mucosal injury with associated platelet aggregation and fibrin clot formation. Interestingly, heparin can significantly reduce *S. aureus* binding to these fibrin clots [40].

Intravascular dissemination of *S. aureus* infections is common, with resultant endocarditis, osteomyelitis, and pulmonary infections being well known complications of distant focus of *S. aureus* infection such as cellulitis. For this reason, VAP with *S. aureus* can occur both from classic transcolonization of the upper airways with subsequent lower airway inoculation and from blood-born sources. This may account for the fact that *S. aureus* is found in only 80% of sputum in patients with *S. aureus* pneumonia [43].

Summary

Bacteria interact with available mucosal surfaces through adhesins that bind to host surface receptors. These adhesin-receptor interactions play an important role in defining the bacterial population on a given available surface. Competitive pressures are constantly in force that favor those organisms with the best adhesive capacity for a particular surface. Changes in either the bacterial adhesins or host surface receptors can open new niches for

the adherence and proliferation of bacteria normally found only sparingly at a given anatomic site. Once a competitive advantage has been established, the favored bacterial species can dominate the colonization pattern. Further competitive pressure can then be exerted on neighboring anatomic structures by the large numbers of organisms competing for binding sites. In addition, fluctuating local host defenses, inflammation, and adherence capabilities in adjacent anatomic sites promote the transcolonization of these bacterial species to contiguous surfaces.

Transcolonization: anatomic considerations

Oropharynx

As the site of origin of most respiratory secretions entering the distal airways, the oropharynx plays a pivotal role in the pathogenesis of nosocomial pneumonia. Normal host defenses operate in the oropharynx to promote a stable bacterial population. This stable flora can undergo rapid fluctuations with changes in the balances of the oropharyngeal ecology. These changes can occur with gingival disease, periodontal disease, antibiotic therapy, and critical illness.

Host defenses

Oropharyngeal host defenses start with salivary flow and content. Whole saliva is composed of crevicular fluid and salivary gland secretions (Table 4). As previously discussed, crevicular fluid is a transudate of serum that issues from the crevices between the teeth and gums and contains proteins in proportion to that of serum. Crevicular fluid is the major source of whole saliva IgG and a significant source of salivary fibronectin. Other components of crevicular fluid include various products (including proteases) from the bacteria and PMN that reside within the crevices. The constituents of crevicular fluid can be altered by changes in serum content (e.g., as with increases in specific serum IgG) by changes in the crevicular bacterial population or by the presence of periodontal inflammation [44].

Salivary gland secretions are responsible for the great majority of whole-saliva IgA and IgM. They contain nonimmune antimicrobial agents such as fibronectin, lysozyme, lactoferrin, and agglutinins (parotid saliva glycoproteins and mucins) and provide salivary peroxidases. Other components of whole saliva are products of PMN origin such as myeloperoxidase, lysozyme, and lactoferrin. Whole-saliva sediment, which contains oral bacteria, exhibits higher enzymatic activity than whole-saliva supernatant since salivary bacteria are the sole origin of numerous salivary proteases. Enzyme activity of whole

Table 4 Components of whole saliva

Crevicular fluid
Transudate of serum originating from the crevises between the gums and teeth
IgG (80% of salivary IgG)
Serum proteases: (lysozyme, lactoferrin)
Fibronectin
Neutrophils
Bacteria
Salivary gland
Parotid gland secretions
Salivary peroxidase (hypothiocyanite)
Histidine-rich polypeptides
IgM and IgA
Agglutinins
saliva glycoproteins
mucins
β2-microglobulin
fibronectin
Lysozyme, lactoferrin
Alpha-mannosidase
Submandibular and sublingual glands
Fibronectin
Neutrophils
Found in crevicular and salivary gland secretions; increases with inflammation
Myeloperoxidase
Lysozyme
Lactoferrin
Bacteria
Trypsin
Butryate esterase
β-Galactosidase
alpha-Galactosidase
Desquamated epithelium

Table 5 Incidence of aerobic gram-negative bacilli

	Recovery (%)
Orophayrnx	
Buccal	
Normal	11
Moderate illness	35
Moribund	73
Subgingival	
Periodontitis	14 ($5\% > 10^5$ cfu/ml)
Upper intestinal	
Duodenal	
Normal (counts $\leqslant 10^2$/ml)	40
Dysbiosis (counts $> 10^5$/ml)	20
Gastric	
pH > 4.0	19
pH < 4.0	58
Tracheal	
Normal	Sterile
Endotracheal intubation	89
Chronic tracheostomy	78

saliva is greater in individuals with inflammatory periodontal disease, possibly as a result both of increased PMN activity and increased bacterial counts [45]. As discussed, increases in salivary protease activity may influence abnormal oropharyngeal colonization through fibronectinolysis.

Despite potential variations in salivary content, reduced salivary flow rate (normal is approximately 0.6 ml/min) [46] always results in reduced defense capacity and increased susceptibility to oral diseases and is the only factor found to bear a direct relationship with the development of dental caries [47]. Other mechanisms of oral defense include swallowing and mastication, mechanical friction provided by oral hygiene, continuous desquamation of surface epithelium, the presence of sIgA, and oral colonization with inhibitory bacteria such as alpha streptococcus [2, 16, 17, 44].

Bacteriology

For bacteria to successfully colonize the oropharynx, they must overcome the cleansing activity of the oral fluid flow [44]. To avoid being swept away by oral secretions, bacteria must be able either to adhere strongly to oropharyngeal structures or find stagnant areas to inhabit. Stagnant environments can be found in dental sulci, fissures and pits, carious lesions, and other sites within the oral cavity, including periodontal pockets. To overcome losses due to oral fluid flow, desquamation, and phagocytosis, the bacteria must proliferate at rates high enough to compensate for losses and repopulate newly exposed surfaces.

Normally, the oropharynx is relatively free of pathogenic organisms. Oropharyngeal cultures from normal volunteers, hospital staff in contact with patients, and patients on psychiatric wards all harbor AGNB infrequently, with an incidence of AGNB recovery of 2% for single cultures and 6% for multiple cultures [4]. Healthy personnel exposed to patients with AGNB infections had no more oropharyngeal colonization (8.5%) than nonexposed control personnel (11%) [48]. Even when healthy volunteers were exposed to massive numbers of AGNB via gargling bacterial solutions, bacterial retention [4] was minimal.

In contrast, when moderately ill orthopedic patients were surveyed with a single oropharyngeal culture, the incidence of recovery of AGNB was 16%; with moribund patients, the incidence of recovery was 57% [49]. Of 90 critically ill patients admitted directly to a medical intensive care unit, the respiratory tract (oropharynx, trachea, or sputum) was colonized in 26% on the first hospital day [2]. When followed prospectively, 37% of 213 patients admitted to a medical intensive care unit developed AGNB oropharyngeal colonization [2]. Following endotracheal intubation for greater than one week, 45% of 75 patients had AGNB recovered from buccal swabs [24]. Possible reservoirs for AGNB colonizing the oropharynx include the upper gastrointestinal tract, sinuses, and subgingival plaque.

Gingivitis

Oral surfaces available for bacterial attachment include the clean tooth, dental plaque, other microorganisms, and epithelial cells [44]. The healthy gingival crevice harbors scant microflora dominated by gram-positive organisms, *Streptococcus mutans* and *Actinomyces viscosus.* These organisms normally adhere to clean tooth surfaces. When allowed to proliferate, they form palisading colonies on the tooth, resulting in dental plaque [50]. These organisms, in turn, provide binding sites for other bacteria, particularly *Bacteroides melaninogenicus* [44], *Streptococcus pyogenes,* and *Pseudomonas aeruginosa* [51]. This binding has been shown to involve classic fimbrial adhesin-receptor binding with *Actinomyces viscosus* fimbriae [51]. Upon cessation of an oral hygiene regimen (which most certainly occurs in the intubated patient), bacterial plaque (primarily deposits of *S. mutans* and *A. viscosus*) builds on teeth within 72 h and is followed by the development of gingivitis. As gingivitis develops, there is a shift at the gingival surface from primarily *Streptococcus* and *Actinomyces spp.* to substantial numbers of AGNB, motile forms and spirochetes [44]. As the number of *A. viscosis* increases within the plaque, there is an increase in the available binding sites for *Bacteroides melaninogenicus,* which can become the predominant cultivable organism. The presence of *B. melaninogenicus* directly correlates with the development of periodontitis [44].

The importance of dental plaque as a potential reservoir for AGNB in critically ill patients has only recently been appreciated. Over 300 named species of bacteria have been isolated from dental plaque and the periodontal spaces. AGNB can be recovered from cultures of dental plaque in 16% of healthy volunteers. Following illness or systemic antibiotics, the incidence of AGNB recovery increases. Scannapieco et al. [52] cultured dental plaque for respiratory pathogens from critically ill patients admitted to a medical intensive care unit. Of the 34 patients, 22 had dental plaque cultures yielding AGNB or *Staphylococcus aureus* (Table 2), 21 of the 22 were colonized on admission to the MICU, with 11 having been admitted to the MICU directly from the emergency room. The rapidity at which these patients developed dental plaque colonization with AGNB suggests that the AGNB were endogenously acquired. It is likely that AGNB are normally present in dental plaque, but their relatively low numbers makes detection difficult. Following changes in local ecologic pressures associated with illness, these bacteria proliferate and become a prominent component of the dental plaque microflora.

Periodontal disease

If dental plaque is allowed to accumulate, subgingival inflammation progresses to periodontitis. This is accompanied by large increases in the number of GNB, with the majority being anaerobic. Slots et al. [53] obtained subgingival bacterial cultures from 3050 patients with periodontitis, most of whom received various forms of periodontal therapy, including antibiotic therapy. They found that 427 patients (14%) contained facultative enteric GNB, which comprised greater than 5% of the total bacterial population in 159 (5%). In these subgingival cultures were found most organisms commonly associated with nosocomial pneumonia: *Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Enterobacter agglomerans, Acinetobacter* spp., and *Escherichia coli* (Table 2). Using a similary sampling technique, Rams et al. [54] demonstrated that 11 of 21 patients with periodontitis harbored *Staphylococcus* species. These studies demonstrate that the organisms commonly isolated in patients with nosocomial pneumonia, such as enteric AGNB and *Staphylococcus,* are frequently found in patients with periodontal disease, which has a high prevalence in the adult population. Although dental caries have been associated with pulmonary abscesses, the relationship between periodontal disease and oropharyngeal colonization in hospitalized patients, especially in those patients requiring intubation and mechanical ventilation, has not been examined. However, edentulous patients also acquire nosocomial pneumonia, which indicates that other mechanisms contributing to oropharyngeal AGNB colonization exist.

Influence of systemic antibiotics

Rapid changes in the normal oropharyngeal flora can occur following antibiotic therapy, possibly resulting from the loss of inhibitory alpha-streptococci [1, 55]. Sprunt and Redman [55] evaluated 15 patients undergoing open-heart surgery who received antibiotic therapy for approximately one week. Prior to any therapy, all patients were colonized with *Streptococcus viridans,* while none had AGNB. During antibiotic therapy postsurgery, streptococci became undetectable, and enteric coliform AGNB became 70–100% of cultivable aerobic bacteria, even though the AGNB were sensitive to the given antibiotics. Following cessation of antibiotic therapy, alpha-streptococci reappeared with 6 days with disappearance of the AGNB. This pattern was also demonstrated in 2 non-operative patients being treated with penicillin for endocarditis and osteomyelitis. However, in patients harboring streptococci resistant to the given antibiotics, neither a decrease in streptococci nor an increase in AGNB occurred following antibiotic therapy. In a similar fashion, Scannapieco et al. [52] performed serial cultures of dental plaque and oropharyngeal scrapings in critically ill patients. They demonstrated a significant correlation between the use of systemic antibiotics and recovery of AGNB.

Increases in AGNB recovery from the respiratory tracts of critically ill patients have also been demonstrated to correlate with antibiotic therapy. In 38 medical intensive care patients, isolation of AGNB from the oropharynx, trachea or sputum increased from 55% on initiation of antibiotic therapy to 79% during or after antibiotic therapy [2]. Further, following antibiotic therapy, sensitivity patterns of colonizing organisms were not significantly altered [57]. Collectively, these studies demonstrate that antibiotic therapy is associated with an increase in oropharyngeal and upper respiratory tract colonization with AGNB in a variety of clinical situations and that this increase in AGNB colonization may be due to loss of normal inhibitory bacterial flora, which allow the AGNB to exploit a newly opened niche.

Tracheobronchial tree

Host defenses

The lower airways are protected from bacterial colonization and remain sterile in the normal host [3]. The major defense mechanism of the tracheobronchial tree is mucociliary clearance and to a minor degree, sIgA. Normal mucus is produced from goblet cells and submucosal glands and is composed of a periciliary (or sol) layer lying in contact with the ciliated epithelium and a viscous mucus (or gel) layer that floats on the sol layer. In addition to neutrophils and macrophages [58], the composition of the mucus includes locally produced antimicrobial lysozyme, bronchotransferrin, sIgA, and antiproteases. In the sol layer, these immunoglobulins and lysozymes bind and degrade bacteria, preventing their interaction with surface epithelium. Mucus is produced in a daily volume of approximately 10 ml in a normal state and can increase to 300 ml/24 h with exacerbations of chronic bronchitis [58]. Mucociliary clearance involves the coupling of ciliary beating and mucus viscosity. Depending on the method of measurement, the velocity of mucus flow ranges from 4.4 mm/min radioaerosol techniques to 21.5 mm/min utilizing cinebronchofiberscopic techniques [58]. In addition to mucociliary clearance, cough is effective in clearing the airway of mucus with its associated debris and may contribute close to 50% of mucus clearance [58] in chronic bronchitis.

Mucociliary transport

Using a cinebronchofiberscopic technique, Sackner et al. [59] evaluated the effects of intubation and supplemental

oxygen therapy on tracheal mucus velocity (TMV) in a dog model. Interestingly, there was no difference in TMV following intubation for up to 30 h. With the addition of oxygen, however, there were significant reductions: 100% oxygen decreased TMV by 84% at 6 h; 75% oxygen decreased TMV by 42% at 9 h; and 50% oxygen decreased TMV by 51% at 30 h. These changes were histologically correlated with acute tracheal and bronchial inflammation, sloughing of ciliated epithelium, and exposure of the underlying basement membranes [59]. This combination of loss of mucociliary clearance with exposure of underlying basement membranes may promote bacterial colonization, particularly *Pseudomonas* spp.

sIgA

The role of sIgA in bacterial defense can be appreciated by the association of decreases in sputum sIgA with both recurrent respiratory tract infections in children and increased tracheal epithelial bacterial adherence [60]. Although sIgA is capable of binding to and agglutinating bacteria for which it was specifically directed, sIgA is not considered to be bactericidal, mediate complement-dependent bacterial lysis, bind to macrophages, or enhance phagocytosis [61]. However, human sIgA does interfere with bacterial adherence to epithelial surfaces in vitro and in vivo [61, 62]. Decreasing adherence may reduce the capacity of a micro-organism to repopulate and compensate for losses. Bacterial proteases, such as those from *S. pneumoniae* and *H. influenzae*, have the capacity to degrade free IgA [62]. Altered airway colonization or airway inflammation following intubation may increase mucosal protease activity capable of degrading sIgA, subsequently promoting AGNB colonization.

Mucosal injury

Different experimental models have suggested that injured tracheal epithelium and loss of ciliated cells may predispose to *Pseudomonas* infection. Utilizing mouse trachea and nonmucoid *Pseudomonas aeruginosa,* Ramphal et al. [63] demonstrated that *Pseudomonas* adhered poorly to normal tracheal epithelium. Following infection with influenza virus, the adherence of *Pseudomonas* to desquamated or desquamating epithelium increased. This increased binding may have been due to a virally-induced alteration in receptor expression [15] or to the availability of previously masked receptors. Notably, there was no change in the adherence to basal or regenerating cells. Following tracheal injury with intubation, there was again increased adherence of *Pseudomonas* to desquamating epithelium and the exposed basement membrane, suggesting that *Pseudomonas* adheres to injured epithelium or denuded epithelial surfaces [63]. Similarly, *Pseudomonas* binding to tracheal epithelium increases when the epithelium is in monolayers, possibly as a result of a decrease in blocking mucins or fibronectin and more accessible receptors [20]. Further, in-vitro binding of *Pseudomonas* to epithelial monolayers occurs preferentially to nonciliated epithelial cells, possibly due to increased receptor availability or expression [26].

The increased *Pseudomonas* binding to injured tracheal epithelium has clinical relevance. In a clinical study by Niederman et al. [23], 15 patients with long term tracheostomies had simultaneous buccal and tracheal cultures biweekly. Of tracheal cultures, 37 (76%) had colonization with AGNB and 28 (57%) with *Pseudomonas* spp. This was in contrast to the buccal site, which harbored AGNB in 18 (37%), with only 9 (18%) being *Pseudomonas* spp. This tissue trophism of *Pseudomonas* for the tracheal site may be due to tracheostomy-induced airway injury and conversion of a primarily ciliated epithelium to a predominately nonciliated epithelium [26].

Sinusitis

Pathogenesis

Nosocomial sinusitis has been shown to be associated with nasopharyngeal intubation involving either nasotracheal tubes or nasoenteric tubes. There is little doubt that nasopharyngeal intubation is the major factor precipitating nosocomial sinusitis by blocking sinus outflow through the nasal ostia [9, 64, 66]. Irritation and mucosal edema induced by nasopharyngeal intubation may further lead to infection by impeding osteal outflow and decreasing sinus ventilation [64]. Following nasal intubation, most patients develop rhinitis characterized by a nonpurulent nasal discharge, congestive engorgement of the inferior turbinates, and nasal blockage [65]. The clinical diagnosis of sinusitis can be made by the combination of mucopurulent nasal discharge, fever, and leukocytosis or by sinus aspirate cultures [64, 66]. Although the presence of mucopurulent nasal discharge is specific for sinusitis, it is commonly absent in critically ill patients with nosocomial sinusitis [66, 67]. Nosocomial sinusitis is frequently an occult infection, can present solely as ongoing hypermetabolism of undetermined etiology (fever, leukocytosis, continued negative nitrogen balance despite seemingly adequate nutritional support, increased CO_2 production or O_2 consumption, or the presence of triglyceride intolerance) [66]. The diagnosis of sinusitis therefore requires clinical suspicion confirmed by sinus imaging and drainage procedures.

Microbiology

The microbial etiology of community-acquired sinusitis is *S. pneumoniae* and unencapsulated strains of *H. influen-*

zae in approximately 50% of all cases [68]. In contrast, the microbial flora from sinus aspirates of patients with nosocomial sinusitis are predominately polymicrobial and predominately AGNB (Table 2). Caplan and Hoyt [64] demonstrated that 46% of pathogens isolated from sinus aspirates had been previously isolated from orotracheal cultures.

Interestingly, most patients shown to develop nosocomial sinusitis had previously been receiving systemic antibiotics [64–66]. These antibiotics often had activity against organisms subsequently isolated from sinus aspirates [64]. Because sinusitis developed while patients were on antibiotics to which the organisms were susceptible and because the sinusitis failed to clear until the nasopharyngeal tubes were removed, mechanical obstruction of the osteo with impaired sinus drainage appears to be the major factor in the pathogenesis of nosocomial sinusitis. Removal of the nasopharyngeal tubes alone without any other specific therapy clears the sinusitis in a majority of patients, although a significant proportion of patients require concomitant drainage procedures [64–66].

Association with nosocomial pneumonia

Nosocomial sinusitis is frequently associated with nosocomial pneumonia [9, 67, 69]. In several studies of patients with nosocomial sinusitis and pneumonia, the same pathogens isolated from sinus drainge fluid were found in 48 to 60% of distal bronchopulmonary aspirates obtained with protected brush specimens in patients with pneumonia [9, 67, 69]. The mechanisms by which sinusitis contributes to the development of nosocomial pneumonia remain to be defined, but possible mechanisms include the bolus aspiration of mucopurulent sinus discharge into the distal airways or chronic seeding of AGNB into the oropharynx (transcolonization), as previously discussed (Fig. 2).

Upper gastroinestinal tract

Bacterial ecology

The upper gastrointestinal tract is normally colonized with bacteria [70, 71]. This duodenal reservoir can serve as a proximate source of bacteria capable of colonizing the stomach. Following a reduction in gastric acidity with either antacid or H_2-blocker treatment, gastric contents become a favorable medium for bacterial proliferation [72]. Numerous studies of hospitalized patients have demonstrated increased gastric colonization with AGNB following treatment with H_2-blockers or antacids [12, 13, 72–74]. A recent review demonstrated a significant correlation with gastric AGNB colonization and the risk for developing nosocomial pneumonia in the intensive-care-unit patient [10].

H_2-blockers in ambulatory patients

The normal flora of the duodenum can vary widely and possibly affect gastric colonization. Although normal duodenal aspirates contain $\leq 10^3$ aerobic bacteria/ml, several patterns of dysbiosis (in which duodenal aspirates contained $\geq 10^6$ obtained bacteria/ml, including coliform AGNB and gram-positive bacteria) have been identified in 20% of normal ambulatory patients [70, 71]. Gastric cultures from ambulatory patients with peptic ulcer disease treated with H_2-blockers have been shown to contain predominately gram-positive bacteria, including enterococci, lactobacilli, *S. salivarius,* micrococci, and staphylococci [75]. AGNB were conspicuously absent or found only in low levels ($< 10^2$ bacteria/ml). The increase in enterococcal recovery with an absence of oropharyngeal flora (with the exception of *S. salivarius*) suggested to the authors that the increased colonization was of fecal origin [75], possibly originating from the duodenum.

H_2-blockers in hospitalized patients

Gastric aspirates from hospitalized patients being treated with H_2-blockers contain a very different flora, with cultures yielding predominantly AGNB [72, 73]. Increased levels of bacterial growth were further demonstrated to correlate with increased gastric pH [11, 14, 72]. This gastric overgrowth following treatment with H_2-blockers or antacids usually preceded the development of nosocomial pneumonia and was shown to occur before, after, or simultaneously with oropharyngeal colonization [12, 13, 72, 73]. The mechanisms that promote gastric overgrowth with AGNB rather than gram-positive bacteria in hospitalized patients treated with H_2-blockers remains to be explored. Perhaps the reduction in gastric acidity following treatment with H_2-blockers provides a favorable environment for bacterial growth. The type of bacterial overgrowth would then be determined by the type of organisms inoculating the stomach, either from the duodenum or from swallowed saliva. In nonhospitalized patients being treated with H_2-blockers, gram-positive bacteria dominate the gastric flora [75], possibly reflecting a predominance of gram-positive bacteria in the duodenum refluxing into the stomach or in the oropharyngeal saliva being swallowed at the time of treatment. However, hospitalized patients have increased gastric colonization with AGNB, possibly reflecting increased AGNB in the duodenum at the time of treatment. An interesting alternative explanation for this AGNB gastric overgrowth could be an increase in gingival and periodontal AGNB, which contaminate the oropharyngeal saliva. This contaminated saliva is swallowed into a favorable gastric environment, allowing bacterial proliferation and successful AGNB gastric colonization.

Gastric reflux

Reflux of gastric contents contaminated with duodenal bacteria and mechanical aspiration of these contents into the tracheobronchial tree may account for VAP that develop within the first several days of intubation without oropharyngeal colonization. Inglis et al. [5] performed plasmid typing and DNA analysis of bacterial isolates obtained from gastric, tracheal, and oropharyngeal secretions from 100 consecutive intubated patients admitted to their intensive care unit (ICU). Nineteen patients had AGNB isolated from tracheal secretions, 11 of which had identical AGNB isolated from gastric secretions, either simultaneously or shortly before tracheal acquisition. Further, 5 patients had identical gastric and tracheal AGNB on admission to the ICU. Oropharyngeal cultures at admission yielded 26 patients with AGNB in which all isolates differed from the AGNB obtained from the tracheal secretions, indicating that tracheal AGNB originated from mechanical aspiration of gastric contents early in the course of the patients' illnesses. Further, these authors demonstrated that gastric bilirubin concentrations correlated with gastric AGNB cultures, indicating duodenal reflux as a probable source of gastric AGNB [5].

Mucosal ischemia

Although most authors suspect that nosocomial pneumonia develops from aspiration of contaminated secretions, translocation of enteric bacteria may be mechanism in the pathogenesis of nosocomial pneumonia. Fiddian-Green and Baker [76] demonstrated an association between gastric mucosal acidosis and the development of nosocomial pneumonia in critically ill patients [76]. This suggests that ischemic mucosal injury and its associated translocation of enteric bacteria and toxins may contribute to the pathogenesis of nosocomial pneumonia in the critically ill.

Preventive strategies

Gastric colonization with AGNB has been implicated in the pathogenesis of nosocomial pneumonia by transcolonization of AGNB from the stomach to the oropharynx and trachea followed by aspiration of contaminated secretions. In an attempt to reduce the incidence of nosocomial pneumonia, multiple clinical trials are currently underway to assess methods of decreasing gastric colonization with AGNB. Reductions in the rate of gastric colonization with concomitant reductions in clinically defined nosocomial pneumonia have been attained through the use of selective decontamination of the upper digestive tract and oropharynx with topically applied antibiotics [74]. It remains interesting that this therapy reduces colonization and reduces the incidence of nosocomial pneumonia based on clinical definitions but has not affected mortality.

Potential risk factors for the aspiration of gastric contents include the supine position and the length of time patients are in this position. Aspiration of gastric contents can be reduced by placing patients in a semirecumbent position. Torres et al. [77] instilled technetium sulfur colloid intragastrically via indwelling nasogastric tubes. Increased radioactivity from tracheal secretions was demonstrated while patients were supine, with tracheal counts increasing with time spent in this position. By placing patients in a semirecumbent position, the radioactivity of tracheal secretions was significantly reduced. It would be interesting to evaluate whether elevating the head of the bed to provide a semirecumbent position can reduce the incidence of VAP, since this may be a simple, no-cost prophylactic measure [77].

Airway inoculation

Endotracheal intubation

As previously discussed, airway intubation, mechanical ventilation, and the use of ventilator circuits are the most frequent risk factors associated with the development of nosocomial pneumonia in hospitalized patients. When patients are intubated with endotracheal tubes having large volume cuffs, virtually all will aspirate oropharyngeal contents [78–80]. These low-pressure, large-volume cuffs were developed to replace high-pressure, low-volume cuffs. Requiring greater than 68 cm water pressure to achieve clinical seal, the high-pressure, low-volume cuffs induce high rates of tracheal injury after prolonged usage (above 50 cm water pressure, capillary mucosal blood flow over cartilaginous ridge ceases, leading to ischemic mucosal injury) [79, 81]. The low-pressure, large-volume cuffs, which can achieve clinical seals at pressures below 30 cm water pressure and have much lower rates of tracheal injury, are now exclusively used in the ICU setting [79, 81]. When fully inflated, these cuffs have diameters 1.5–2.0 times the diameter of the average adult male trachea [80]. When inflated in a trachea to achieve a clinical seal, the excess material folds on itself in randomly oriented folds, often spanning the length of the cuff. Stagnant oropharyngeal secretions pooled above the cuff can then track along these folds and gain access to the lower airway, providing a direct route for tracheal colonization and bolus aspiration from the oropharynx. Photographs of endotracheal tube cuffs in 30 patients, all of whom had documented aspiration of oropharyngeal secretions past the cuffed endotracheal tubes, demonstrated that these cuff folds contained oropharyngeal secretions

[80]. Furthermore, increases in cuff pressures to 50 cm H_2O pressure did not obliterate these folds or prevent aspiration. In attempts to decrease the size of the folds, thinner, more pliable polyurethane cuffs were tested and shown to decrease the incidence of aspiration [79].

Another factor that predisposes to aspiration of oropharyngeal secretions around endotracheal tube cuffs is temporary cuff deflation. This intentionally occurs during extubation but effectively can also occur with changes in the diameter of a compliant trachea during ventilation. As transmural tracheal pressures change during both mechanical and spontaneous ventilation, the tracheal diameter changes. When a high-volume cuff is inflated at a constant pressure, changes in tracheal diameter may cause cuff water pressure to decrease from 30 to 5 cm H_2O [79]. Transient changes in cuff pressure to levels below that of the hydrostatic pressure of secretions pooled above the cuff may then allow bolus entry of these secretions into the lower airways [79]. Further, changes in tracheal diameter during ventilation may induce the cuff channels to expand and contract, thereby milking cuff-fold contents into the trachea.

Mehta [81] demonstrated that during extubation 33% of patients aspirated secretions that had been pooled above the endotracheal tube cuff following cuff deflation. Multiple extubation techniques were unsuccessful in reducing this high incidence of aspiration. These techniques included the use of oropharyngeal suctioning alone, in conjunction with endotracheal tube suctioning, and even with the addition of suctioning above the cuff with a specially designed endotracheal tube. However, the incidence of aspiration during these postoperative extubations could be decreased if the cuff was initially placed snugly against the vocal cords to prevent the accumulation of pooled secretions in the space between the cuff and vocal cords or by extubating patients in a 6–10 degree, head-down position with nasopharyngeal and endotracheal suctioning [81].

Utilizing an endotracheal tube designed to allow frequent suctioning of pooled subglottic secretions through a separate evacuating channel, Mahul et al. [82] demonstrated that regular mechanical drainage of these subglottic secretions lowered the incidence of VAP. In 145 patients intubated for more than 3 days, 70 received hourly subglottic secretion drainage. Nosocomial pneumonia, as defined with quantitative cultures of bronchoalveolar lavage specimens obtained from infiltrates developing greater than 48 h after intubation, occurred in 21 of the 75 control patients (28.0%) and in only 9 of the 70 study patients (12.8%) [82].

The surfaces of endotracheal tubes are frequently contaminated. Infected oropharyngeal secretions bath the outer surface of the endotracheal tube, reach the trachea via the cuff folds, and subsequently ascend the inner lumen of the endotracheal tube. When endotracheal tubes remain in place for more than 24 h, all develop an organic coating or biofilm formed by the deposition of secretions from both patient and bacteria. When viewed under electron microscopy, this biofilm is thick and imbedded with bacteria [83]. Positive bacterial cultures were obtained from the inner surfaces in 73% of 45 endotracheal tubes, with 30% yielding bacteria frequently found in nosocomial pneumonia (i.e., *Pseudomonas aeruginosa, Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas mirabilis, Pseudomonas stuartii* and *Staphylococcus* spp.) [84]. Of the 33 endotracheal tubes having bacteria cultured from the inner surfaces, 94% had bacteria isolated from both the inner and outer surfaces.

Nodular projections of these bacterial colonies can be identified on the inner and outer surfaces of endotracheal tubes and are capable of being detached and launched into the distal airways by airflow generated with normal inspiratory flow rates used in clinical practice [84]. Following a single ventilatory cycle with a maximal flow rate of 1 l/s through endotracheal tubes recently removed from patients, Inglis et al. [84] demonstrated dispersion of cultivable bacteria up to 45 cm from the tip of the endotracheal tube. During routine mechanical ventilation, recurrent showers of bacterial inoculi apparently are capable of being generated with each inspiratory cycle, a situation that could lead to VAP.

Bacteria imbedded in an amorphous nidus as a form of bolus inoculation was successfully used by Cash et al. [85] to produce chronic *Pseudomonas* lung infections in a rodent model. *Pseudomonas* bacteria were first enmeshed in agar beads and then instilled intratracheally. The animals subsequently developed chronic lung infections. On histology, the infected agar beads were found embedded within terminal bronchioles with a surrounding inflammatory reaction characteristic of bronchopneumonia, a pattern that closely resembled the findings of clinical *Pseudomonas* bronchopneumonia. Further, this form of bolus inoculation with infected amorphous particles was more effective in initiating chronic bronchopneumonia than pure bacterial aerosols [85, 86]. These studies demonstrate that bacterial bolus inoculation into the distal airways are highly effective in producing bronchopneumonia, especially when the bacteria are enmeshed in amorphous material (as with endotracheal tube glycocalyx deposits) [85, 86].

Ventilator circuits

In the 1960s, several outbreaks of nosocomial pneumonia were caused by nebulization equipment that generated small-particle bacterial aerosols. Through improved policies for maintenance of nebulizer equipment, nosocomial pneumonia from nebulizer equipment is now restricted to isolated epidemic outbreaks [86, 87]. Still, the potential for respiratory equipment to contribute to nosocomial

pneumonia exists, and use of this equipment remains a significant risk factor for the development of nosocomial pneumonia [88]. Because manual spirometers, resuscitator bags, and ventilator circuits are in contact with the endotracheal tube, they have the potential to become contaminated with infected respiratory secretions. Craven et al. [87] demonstrated that the parts of the ventilator circuit closest to the patient have both the highest rates of contamination and the highest bacterial colony counts. Quantitative cultures of cotton swabs from the inner surfaces of four different circuit components were obtained in 58 ventilator circuits. Greater than 1000 colony-forming units (cfu)/swab were found in 71% of the swivel adapters and 43% of the proximal tubing swabs but in none of the distal tubing sampled. After 24 h in use, 80% of the ventilator circuits were colonized, with median levels of bacterial growth being 7×10^4 cfu/ml. Furthermore, moisture within the ventilator circuit tubing precipitates out in the form of condensate and accumulates at mean rates of 30 ml/h. This condensate is frequently contaminated. At 24 h, 80% of condensates were shown to be contaminated, with a mean bacterial growth of 2×10^5 cfu/ml [89]. The majority of the bacterial isolates obtained from these ventilator circuit sites and from the condensate had previously been isolated from sputum cultures, with a distribution of 76% AGNB (*Pseudomonas, Acinetobacter, Klebsiella, Serratia*), 21% gram-positive cocci (*S. aureus, S. epidermitis* and *Streptococcus*), and 3% yeasts [89].

The high frequency of colonization of ventilator circuits and condensate with the pathogenic organisms that cause nosocomial pneumonia (see Tables 1 and 2) argues again that these sites can be sources of airway inoculi leading to nosocomial pneumonia. The potential for large-volume airway inoculation exists if the condensate is accidentally poured into the patient's endotracheal tube, a situation that can easily happen with ventilator tubing manipulation [8, 89, 90]. Dreyfuss et al. [8] demonstrated that not changing ventilator circuits for the duration of mechanical ventilation (mean duration of MV, 10 days; range, 4–29 days) did not increase the incidence of VAP compared to circuit changes every 48 hours. Further, Craven et al. [89] demonstrated that changing ventilator circuits every 24 h rather than every 48 h increased the risk of pneumonia. These authors state that the risk of spillage of a heavily contaminated condensate into the tracheobronchial tree by ventilator tubing manipulation is decreased by reducing the frequency of circuit changes, thus potentially decreasing the incidence of pneumonia.

Nebulizers

In-line nebulizers are gravity dependent sumps that can easily collect ventilator tube condensate. Once contaminated, these nebulizers can aerosolize bacteria into the distal small airways. In a study investigating this process, Craven et al. [87] demonstrated high levels of contamination ($>10^3$ organisms/ml) in 68% of nebulizers, and true bacterial aerosols were generated in 71% of nebulizer reservoirs. Small-particle bacterial aerosols (less than 3 μm, a size capable of reaching the terminal alveoli) accounted for 37–85% of particles generated. Although bacterial aerosols are less efficient than bolus inoculation in inducing pneumonia in animal models, the potential exists for aerosols to produce diffuse inoculation of pathogenic bacteria into the distal airways [7]. Aerosol-administered organisms, (having a wider dispersion of organisms within the lungs) may be more susceptible to host defenses such as phagocytosis than are bacteria enmeshed in an amorphous nidus that has been aspirated and lodged in larger airways [86]. This may, in part, explain the poor correlation between the high rate of nebulizer contamination and the relatively low rate of nosocomial pneumonia [88].

Summary

The development of nosocomial pneumonia involves multiple factors ranging from altered host defenses, acquisition of AGNB flora in the upper gastrointestinal and upper respiratory tract, increased lung parenchymal exposure to these AGNB, and impaired host clearance. Adherence mechanisms are involved in normal colonization and appear to be altered in disease. Large amounts of AGNB are found in 5% of subgingival plaque from patients with periodontitis and appear as a result of increased receptor availability. This reservoir could be a source of AGNB leading to oropharyngeal colonization during illness. Large numbers of AGNB are also normally found in duodenal aspirates, providing a proximate source of AGNB capable of colonizing the stomach. Evidence indicates that gastric colonization with AGNB is a precursor to oropharyngeal AGNB colonization and that increases in gastric AGNB occur with antacid therapy or H_2-blocker therapy. Large numbers of bacteria are involved in the transcolonization of the stomach, oropharynx, sinuses, and trachea, and this transcolonization may promote the subsequent development of nosocomial pneumonia.

AGNB colonization of the oropharynx occurs in hospitalized patients and can be viewed as a marker of an impaired host. Mechanisms of colonization include loss of protective, inhibitory, normal resident alpha-*Streptococcus* due to either antibiotic therapy or degradation of oral epithelial fibronectin, a large glycoprotein necessary for alpha-streptococcal adherence. With increases in salivary protease activity (originating from crevicular fluid, parotid secretions, neutrophils, or even oropharyngeal bacteria) leading to fibronectin degradation and the loss of inhibitory bacterial species, the oropharyngeal ecology favors AGNB adherence with subsequent colonization

with these bacteria. *Pseudomonas* demonstrates tissue trophism for the injured tracheal surface and can colonize the trachea without prior successful oropharyngeal colonization.

Altered host defenses involve mechanical instrumentation such as endotracheal and nasogastric intubations, which breach normal barriers to provide a direct conduit for bacterial spread into the distal airways. Environmental contamination, especially with ventilator circuit tubing, nebulizers, and condensate, can provide direct, large-volume airway inoculations with contaminating AGNB. Airway inoculation can also occur through the use of high-volume, low-pressure endotracheal tube cuffs having the longitudinal folds that provide direct channels to the trachea for secretions pooled in the oropharynx. Endotracheal tubes also become coated with an amorphous glycocalyx colonized with oropharyngeal AGNB, which can be directly inoculated into the distal airways in bolus form. Once the oropharynx is colonized with AGNB, transcolonization of adjacent structures with these organisms occurs, including endotracheal tubes, ventilator circuits, the trachea, and sinuses. Eventually, the lung parenchyma is exposed to these pathogenic organisms, and nosocomial pneumonia may then develop.

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