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Oropharyngeal or Gastric Colonization and Nosocomial Pneumonia in Adult Intensive Care Unit Patients

A Prospective Study Based on Genomic DNA Analysis

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Colonization of the digestive tract has been supposed to be the source of many hospital-acquired infections, especially nosocomial pneumonia. To assess the relationship between oropharyngeal and gastric colonization and subsequent occurrence of nosocomial pneumonia, we prospectively studied 86 ventilated, intensive care unit (ICU) patients. Oropharyngeal or gastric colonizations were detected and quantified on admission and twice weekly during ICU stay. When nosocomial pneumonia was suspected on clinical grounds (new chest X-ray infiltrate and purulent tracheal secretions), diagnosis was assessed on fiberoptic bronchoscopy with quantitative cultures of a protected specimen brush sampling and/or a plugged telescoping catheter sampling yielding $\geqslant 10^3$ cfu/ml of at least one microorganism. Bacterial strains responsible for colonization and infection (*Acinetobacter baumannii*, *Pseudomonas aeruginosa*, Enterobacteriaceae, and *Staphylococcus aureus*) were compared using pulsed-field electrophoresis. A total of 31 cases (36%) of pneumonia were diagnosed. Oropharyngeal colonization, detected either on admission or from subsequent samples, was a predominant factor of nosocomial pneumonia as compared with gastric colonization. For instance, oropharyngeal colonization with *A. baumannii* yielded a 7.45-fold estimated increased risk of pneumonia as compared with patients not yet or not identically colonized ($p = 0.0004$). DNA genomic analysis demonstrated that an identical strain was isolated from oropharyngeal or gastric samples and bronchial samples in all but three cases of pneumonia, due to *S. aureus*. These findings provide better knowledge of the pathophysiology of nosocomial pneumonia in mechanically ventilated patients. Garrouste-Orgeas M, Chevret S, Arlet G, Marie O, Rouveau M, Popoff N, Schlemmer B. Oropharyngeal or gastric colonization and nosocomial pneumonia in adult intensive care unit patients: a prospective study based on genomic DNA analysis. AM J RESPIR CRIT CARE MED 1997;156:1647–1655.

Bacterial colonization of the airway is crucial for the development of nosocomial pneumonia (1) that usually follows microinhalation of oropharyngeal or gastric secretions colonized with potentially pathogenic microorganisms (2). There is a general consensus that nosocomial pneumonia is a secondary endogenous infection. However, the importance of these various sources, i.e., oropharynx or stomach, is still controversial. Previous studies demonstrated an association between gastric colonization and the subsequent occurrence of nosocomial pneumonia (3, 4). However, this association has been recently debated (5, 6). Colonization of the oropharynx rather than stomach has been more frequently detected at the same time pneumonia was diagnosed, and appeared to be due to the same bacterial species (6).

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The demonstration requires that bacterial identity must be established. Antibiotyping is often inadequate for the purpose of differentiating strains. Recently, molecular techniques have been developed for discriminating strains in infectious diseases (7), such as genomic fingerprinting, that provide an overall view of bacterial organization of the chromosome (8). DNA fingerprinting patterns by pulsed-field gel electrophoresis (PFGE) are therefore proposed as a useful tool to investigate the source, transmission, and spread of nosocomial infections. Within the past few years, these techniques have been applied to the genetic and epidemiologic analysis of many clinically relevant bacterial pathogens and have shown DNA polymorphism within the species *Acinetobacter baumannii* (9), *Staphylococcus aureus* (10, 11), *Escherichia coli* (12), *Enterobacter cloacae* (13), and *Pseudomonas aeruginosa* (14). To our knowledge, no prospective study using this technique has been carried out to study digestive colonization and nosocomial pneumonia in intensive care unit (ICU) patients.

The main objectives of this study were to describe the oropharyngeal and gastric colonizations in an adult ICU population receiving mechanical ventilation for at least 48 h and to assess the relationship between colonization and occurrence

of nosocomial pneumonia. Serial quantitative cultures of the oropharynx and the stomach and molecular analysis by genomic fingerprinting of bacteria responsible for colonization and nosocomial pneumonia served to provide us with better knowledge of the pathophysiology of ventilator-associated pneumonia.

METHODS

Patients

This prospective study was conducted at Hôpital Saint-Louis (Paris, France), a referral care university teaching hospital with a 20-bed medical-surgical ICU. From December 1991 to July 1992, 86 consecutive patients who were mechanically ventilated over at least 2 d starting from the day of admission were enrolled in the study. Patients were excluded if they were neutropenic, had pneumonia on admission, had received previous selective digestive decontamination, or had undergone gastric or esophageal surgery.

Patients had nasogastric tubes and started on enteral feeding as necessary. Treatment with sucralfate or H2-receptor antagonist was not routinely used and depended on the preference of the patient's physician. Antacids were never used. The oropharyngeal cavity was disinfected four times a day with a povidone-iodine solution. Ventilators were equipped with heat- and moisture-exchanging bacterial filters, and only one ventilator circuit was used for each patient throughout the study.

For each patient, the chart was reviewed for the following parameters: age, sex, severity of illness on admission using the Simplified Acute Physiology Score (SAPS) (15), severity of underlying disease using the McCabe's score (16), duration of ICU stay, duration of mechanical ventilation, duration of stress ulcer therapy, use of systemic antibiotics, admission diagnosis, immunosuppression, and ICU outcome. Patients were clinically examined daily for the occurrence of nosocomial pneumonia.

As this study was only epidemiologic and as no invasive measure was required to study patients, the Institutional Review Board of Hôpital Saint-Louis waived the need for informed consent.

Study Design

The detection of colonizing microorganisms was assessed on oropharyngeal and gastric samples both collected on admission day or within the first 48 h of ICU stay and then twice weekly until removal of the nasogastric tube. Colonization on admission was defined as the isolation of at least one microorganism in oropharyngeal and/or gastric site $\geqslant 10^2$ cfu/ml in the first 48 h after admission. Colonization was considered as ICU-acquired only when there was no colonization on admission and when the same potentially pathogenic microorganism at a concentration $\geqslant 10^2$ cfu/ml was detected from at least two consecutive samples, oropharyngeal or gastric, from the same site. Gastric juice pH was measured on every sample with pH paper.

Distal bronchial sampling was performed upon request by the attending physician in case of clinical suspicion of nosocomial pneumonia because the patient fulfilled the following criteria: recent and persistent clinical infiltrates on the chest X-ray and purulent tracheal secretions associated with fever ($\geqslant$ 38.5° C) or hypothermia ($\leqslant$ 36.5° C), leukocytosis ($\geqslant$ 10,000/mm^3), or leukopenia ($\leqslant$ 4,000/mm^3). All patients were sedated with midazolam and fentanyl. Curarization was used as necessary when optimal muscle relaxation was required. After an endotracheal suctioning, the fiberoptic bronchoscope was introduced through an adapter (Bodai suction-safe Y; Sontek Medical, Lexington, MA) and advanced under direct vision to the bronchus according to the radiographic location of the infiltrate on the X-ray. Neither suction nor topical anesthetics were used. A telescoping brush catheter or a plugged catheter was introduced through the inner channel to collect samples following a standardized technique described elsewhere (17, 18).

The diagnosis of nosocomial pneumonia was established using the association of clinical and radiologic criteria (19) and a positive quantitative culture ($\geqslant 10^3$ cfu/ml) of the protected specimen from either a plugged catheter (17) or a telescopic brush catheter (18). When the protected samples were positive but below the 10^3 cfu/ml threshold, these episodes were considered as distal bronchitis. The sampling method depended on the preference of the patient's physician. The decision of introducing or modifying antibiotic therapy was left to the patient's physician, according to the severity of the patient's condition and the evidence of infection.

Microbiologic Processing

Oropharyngeal samples were treated for 15 min with an equal volume of mucolytic agent and then vortexed; 20 μl of each sample were dispensed to a series of culture media including bile-esculin agar, mannitol salt agar, Sabouraud plus gentamicin agar, and Drigalski agar (BioMerieux S.A., Marcy l'Etoile and Sanofi Diagnostics Pasteur, S.A., Marnes-la-Coquette, France), selected respectively for *Enterococcus* sp., *S. aureus*, fungi, and gram-negative bacilli. For gastric aspirates, 1 ml of sample was buffered with 9 ml of phosphate-buffered saline (pH 7.4) and then vortexed, and 0.1 ml was dispensed to the same selective culture media described above. Incubation was at 37° C except for Sabouraud media (30° C). After the primary inoculation on solid media, the buffered gastric samples and homogenized salivary samples were stored at 4° C for 24 h for subsequent dilutions. After overnight incubation, all colonies were counted and, if confluent culture was observed, serial 10-fold dilutions of sample were plated in appropriate media. All microorganisms were identified by standard microbiologic methods, and susceptibility testing was performed on Mueller-Hinton agar plates by the disk diffusion technique (Sanofi Diagnostics Pasteur S.A.). Extended-spectrum β-lactamases (ESBL) were detected using the double-disk synergy test (20). One colony of each strain was selected for susceptibility testing and stored for further investigation (e.g., PFGE analysis) either at room temperature in peptone, meat extract agar (Sanofi Diagnostics Pasteur S.A.) for *S. aureus*, *Enterococcus*, and Enterobacteriaceae isolates or at −70° C in brain-heart infusion broth for *Acinetobacter* and *Pseudomonas* strains.

Distal bronchial samplings were cultured quantitatively as described elsewhere (17, 18).

Chromosomal DNA Analysis by PFGE

Chromosomal DNA was prepared as previously described (21). Overnight cultures were harvested and resuspended in Pett IV solution (1 M NaCl, 10 mM Tris [pH 7.6]). The suspensions were mixed with an equal volume of 1.6% InCert agarose (FMC Bioproducts, Rockland, ME) and allowed to solidify in 100-μl molds. Plugs were incubated in 1 ml of lysis buffer (6 mM Tris [pH 7.6], 1 M NaCl, 0.1 M EDTA [pH 7.4], 0.5% Brij 58, 0.2% chromosomal desoxycholate, 0.5% sarcosyl) (Sigma Chimie, Saint-Quentin Fallavier, France) with 1 mg/ml of lysozyme or 40 U/ml of lysostaphin (Sigma Chimie) and 20 μg/ml of DNase-free RNase (Boerhinger Mannheim France S.A., Meylan, France). After 18 h at 37° C, the lysis buffer was removed and plugs were incubated in 1 ml of ESP buffer (0.5 M EDTA [pH 9], 1% sarcosyl) supplemented with 50 μg/ml of proteinase K (Boerhinger Mannheim) for 18 h at 50° C. Plugs were then washed four times with 1 ml of TE buffer (10 mM Tris [pH 8], 1 mM EDTA [pH 8]) and digested with 40 U of *Sma* I or *Xba* I for 6 h according to the recommendations of the supplier (Boerhinger Mannheim). Fragments of DNA were separated using PFGE in a 1% agarose gel (Agarose Gold; FMC Bioproducts) in TBE buffer (0.0445 M Tris [pH 8], 0.0445 M boric acid, 0.001 M EDTA [pH 8]) at 12° C using the Pulsaphor system (Pharmacia France S.A., Saint-Quentin en Yvelines, France). Running conditions were 200 V for 16 h with pulse times ranging from 10 to 25 s. DNA size marker was lambda ladder (Bio-Rad S.A., Ivry-sur-Seine, France). After electrophoresis, gels were stained with ethidium bromide, washed, and photographed under ultraviolet light.

We systematically compared by PFGE clinical strains isolated from distal bronchial samples and oropharyngeal and/or gastric samples, located closer to infection.

Statistical Analysis

Time to occurrence of nosocomial pneumonia from the date of ICU admission, either overall or specifically for each main responsible bacteria (*A. baumannii*, *P. aeruginosa*, *S. aureus*) and for Enterobacteriaceae was estimated by the Kaplan-Meier method (22). Patients free of pneumonia were censored at the time of their death or discharge from the ICU. Patients who developed pneumonia with other microorganisms or species than those specifically studied were censored at the time of pneumonia.

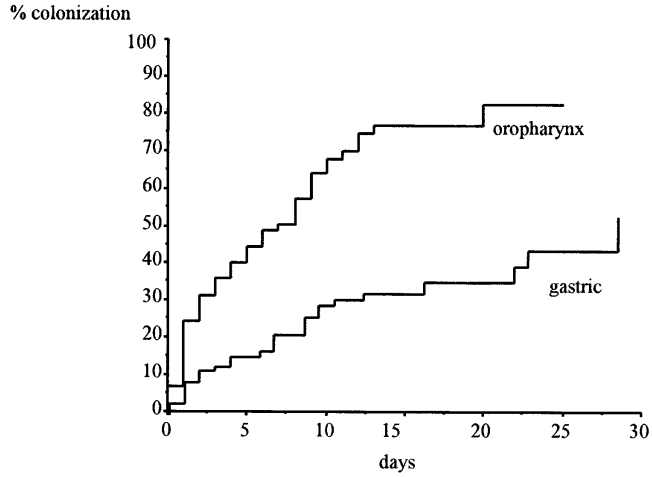

Figure 1. Kaplan-Meier estimation of time to gastric and oropharyngeal colonizations after study inclusion.

We estimated the prevalence of oropharyngeal and/or gastric colonization in the sample globally and then specifically for each bacteria responsible for colonization. To determine risk factors for pneumonia, we first studied the prognostic value of several baseline characteristics, i.e., assessed at the time of ICU admission or within the first 48 h of admission, using the log-rank test (23). Secondly, variables found to be associated with the occurrence of pneumonia at the 5% level were entered into a multivariate Cox model to summarize prognostic information (24). Finally, we studied the predictive value of either specific oropharyngeal or gastric colonization for the subsequent (or simultaneous) occurrence of similar microorganism pneumonia, using the Cox model in which each colonization was introduced alone as a so-called time-dependent covariate, owing to the possible delayed occurrence of colonization after ICU admission (24). This allowed us to compute the relative risk of developing specific pneumonia after colonization in identically colonized patients, as compared with remainders (not yet or not identically colonized patients).

RESULTS

Patients

The study included 86 patients (62 men, 24 women) 19 to 89 yr of age (mean ± SD: 57.4 ± 17); 49 were referred from other wards, with a previous mean hospitalization period of 4.5 ± 8 d (range: 1 to 48 d). All the patients were mechanically ventilated on the day of admission, 66 by the oropharyngeal route, 19 by the nasopharyngeal route, and one by a previous tracheostomy. The median length of mechanical ventilation was 11 d (range: 2 to 65 d). The severity of illness on admission was high, as displayed by a 14.2 ± 4 mean SAPS value (range: 6 to 27). Using the McCabe's score, 47 of 86 (55%) patients had no fatal disease, 25 of 86 (29%) had an ultimately fatal disease, and 14 of 86 (16%) had a rapidly fatal disease. Thirty-seven (43%) patients were immunocompromised, due to AIDS (11%), malignant hematologic disease or cancer (21%), and other conditions with impairment in host defenses related to prolonged steroid (5%) or immunosuppressive (2%) therapy or diabetes mellitus (5%). Among the 86 patients, 24 were surgical patients, 13 of whom were unscheduled. The most frequent diagnoses on admission in the 62 medical patients were acute respiratory failure (56.5%), neurologic conditions (19%), shock (8%), metabolic disorders (5%), and acute renal failure (3%). Thirty patients (35%) had been receiving antibiotics before their ICU admission, half of them with a combination regimen. The most common antibiotic therapy prior to the study consisted of amoxicillin-clavulanate plus aminoglycoside. Of the 86 studied patients, 45 (52%) died in the ICU.

Colonization

On admission, oropharyngeal samples (OS) were missing from five patients and gastric samples (GS) from three patients. Bacterial colonization on admission was demonstrated in 49 of 86 (57%) patients. Bacteria were cultivated from oropharyngeal and gastric samples from 44 of 81 (54.3%) and 27 of 83 (32.5%) patients, respectively. *Klebsiella pneumoniae* with ESBL and *A. baumannii* resistant to third-generation cephalosporins were identified on admission in eight and five patients, respectively. Colonization with Enterobacteriaceae, *Pseudomonas* sp., *S. aureus*, or *Enterococcus* sp. was demonstrated in oropharyngeal samples in 30 (35%), three (3.5%), 13 (15%), and eight (9%) patients, respectively, and in gastric samples in 15 (18%), two (2%), two (2%), and nine (11%) patients, respectively. Yeast colonization was detected in 51 patients on admission without a predominant site of colonization (OS: 44 samples; GS: 40 samples).

Bacterial colonization acquired during ICU stay, irrespective of the involved type of bacterial strains, was demonstrated in 44 of 86 (51%) patients. Time to occurrence of bacterial colonization since ICU admission is displayed in Figure 1. The median time for oropharyngeal colonization was 7 d, as compared with 30 d for gastric colonization. Bacteria were mainly

TABLE 1

ACQUIRED BACTERIAL COLONIZATION: LOCATION OF THE MICROORGANISMS IN THE 44 CARRIER PATIENTS

Colonizing Microorganisms	Patients with OC (*n*)	Patients with GC (*n*)	Patients with BC (*n*)	Colonized Patients (*n*)
*A. baumannii**	7	0	1	8
K. pneumoniae ESBL	12	0	3	15
Enterobacteriaceae	9	5	8	22
Pseudomonadaceae	8	2	1	11
S. aureus	17	0	3	20
Enterococcus sp.	2	1	1	4
Total number of patients with colonization in the site	22	5	17	

Definition of abbreviations: OC = oropharyngeal colonization; GC = gastric colonization; BC = both oropharyngeal and gastric colonization; ESBL = extended-spectrum β-lactamase.

* Resistant to third-generation cephalosporins and aztreonam.

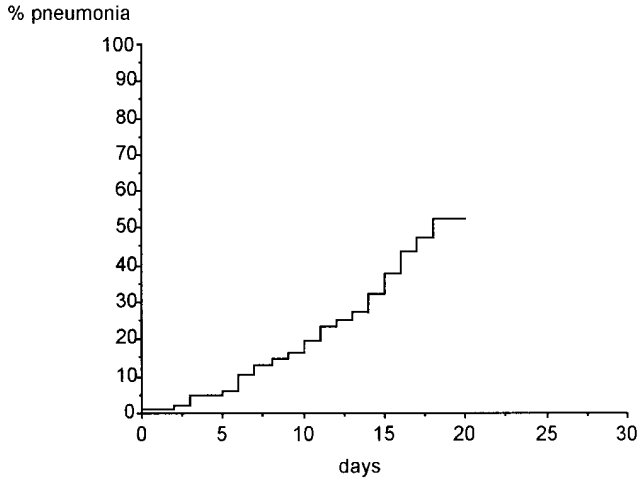

Figure 2. Estimated time to occurrence of pneumonia after enrollment in the study.

cultivated in the oropharyngeal site, alone in 22 patients, and in conjunction with the gastric site in 17 other patients. On the other hand, gastric colonization alone was detected in only five patients. The total number of patients with bacterial acquired colonization, with the different microorganisms located in either site or both sites, is listed in Table 1. Acquired yeast colonization was demonstrated in 15 patients.

Gastric pH on admission day in the 86 patients was 2.82 ± 3 (range: 1 to 9). The mean gastric pH obtained twice weekly during ICU stay was not significantly different whether or not patients had gastric colonization: 5.71 ± 2 (range: 1 to 9) versus 5.0 ± 2 (range: 1 to 10), respectively.

Nosocomial Pneumonia

Incidence and bacteriologic results. In 30 of the 86 patients, 37 episodes of respiratory infection occurred. According to our criteria, 31 were nosocomial pneumonias (30 bacterial, one fungal), giving a crude total 36% incidence of nosocomial pneumonia, and six were distal bronchitis. When we examined the bacterial episodes only and excluded the fungal one, we diagnosed 36 respiratory infections in 29 patients. Of these 29 patients, 23 had 25 nosocomial pneumonias, three patients had three distal bronchitis, and three had both infections (five nosocomial pneumonias and three distal bronchitis). Of the 26 patients who developed bacterial pneumonia, 15 of 26 (58%) died.

The bacteria responsible for the 30 nosocomial pneumonias were mainly gram-negative bacilli: *A. baumannii*, n = 11; Enterobacteriaceae, n = 8 (*E. coli*, n = 3; *E. cloacae*, n = 2; *E. aerogenes*, n = 1; *K. pneumoniae*, n = 2, with one strain producing ESBL); *P. aeruginosa*, n = 9; and *S. aureus*, n = 13. Nosocomial pneumonia was polymicrobial in 11 of 30 (37%) bacterial episodes. Bacteria responsible for the six episodes of distal bronchitis were: *S. aureus*, n = 3; *P. aeruginosa*, n = 3; and *A. baumannii*, n = 1. All *S. aureus* strains were resistant to methicillin, aminoglycosides, and fluoroquinolones.

Three (9%) of the 31 episodes of nosocomial pneumonia were early-onset cases, i.e., occurring within 4 d after admission, while the remainders, called late-onset pneumonias, occurred from the fourth day until the thirtieth day. The median time for pneumonia was 18 d. The Kaplan-Meier rate of patients free of pneumonia was 80% at day 10 and 56% at day 16, as reported in Figure 2.

Baseline risk factors for pneumonia. Two of the 11 baseline parameters were selected by the log-rank test as being individually associated with the risk of developing pneumonia. The time to occurrence of pneumonia was indeed shortened in the case of rapidly progressing underlying disease ($p = 0.05$) or need of parenteral feeding ($p = 0.05$). In contrast, neither age ($p = 0.47$), duration of hospitalization prior to ICU admission ($p = 0.75$), severity of illness ($p = 0.31$), immunosuppression ($p = 0.20$), enteral feeding ($p = 0.44$), antibiotic therapy prior to the admission ($p = 0.12$), nor the use of sucralfate ($p = 0.28$) or H2-blockers ($p = 0.99$) were retained as predictive for the

TABLE 2

COLONIZATION ON ADMISSION AND ACQUIRED COLONIZATION* IN 86 PATIENTS AND BACTERIAL RESPIRATORY INFECTION† WITH IDENTICAL MICROORGANISMS‡

	Colonization on Admission	Acquired Colonization	Infection after Colonization on Admission/Total Infections§	Infection after Acquired Colonization/Total Infections§
Oropharynx				
A. baumannii‖	3 (3.5%)	8 (9.3%)	1/12	7/12
K. pneumoniae ESBL¶	4 (4.6%)	15 (18%)	0/1	1/1
Enterobacteriaceae	30 (35%)	17 (20%)	3/7	2/7
Pseudomonadaceae	3 (3.5%)	9 (10.5%)	1/12	4/12
S. aureus	13 (15%)	20 (23.2%)	0/16	11/16
Enterococcus sp.	8 (9.3%)	3 (3.5%)	0	0
Gastric				
A. baumannii‖	2 (2.4%)	1 (1.1%)	0/12	1**/12
K. pneumoniae ESBL¶	6 (6.9%)	3 (3.5%)	0/1	1**/1
Enterobacteriaceae	15 (18%)	13 (15%)	1/7	1**/7
Pseudomonadaceae	2 (2.4%)	3 (3.5%)	0/12	1/12
S. aureus	2 (2.4%)	3 (3.5%)	0/16	3**/16
Enterococcus sp.	9 (10.5%)	2 (2.4%)	0	0

* Number of patients (percent).
† Nosocomial pneumonia and distal bronchitis.
‡ Bacterial identity was assessed by PFGE.
§ Number of infections with demonstrated relationship to colonization/number of infections.
‖ Resistant to third-generation cephalosporins and aztreonam.
¶ ESBL = extended-spectrum β-lactamase.
** Also detected in the oropharynx.

TABLE 3

BACTERIAL CHARACTERISTICS IN 29 PATIENTS WITH 36 INFECTIONS: COMPARISON OF COLONIZING AND INFECTING STRAINS WITH PFGE

Patient No.	Admission Colonization	Acquired Colonization	Infection	Time from Admission to Infection	Colonization Close to Infection[¶]	Time from Acquired Colonization to Infection	Pulsotype
1	0	*A. baumannii**	Pneumonia	9	10^6 *A. baumannii**	Simultaneous	Identical
		S. aureus[†]	*A. baumannii*		10^7 *S. aureus*[†]	4 d before	Identical
			S. aureus				
2	10^5 *C. freundii*[‡]	0	Pneumonia	6	0		
			E cloacae				
3	0	*K. pneumoniae**	Distal bronchitis	7	10^3 *S. aureus**	3 d before	Identical
		*S. aureus**	*S. aureus*				
4	10^5 *K. pneumoniae**	*P. aeruginosa**	Pneumonia	6	10^9 *K. pneumoniae**		
	10^4 *E faecalis**	*S. aureus**	*A. baumannii*		10^2 *K. pneumoniae*[‡]		
4	10^5 *K. pneumoniae**	*P. aeruginosa**	Distal bronchitis	30	10^3 *P. aeruginosa**	7 d before	Identical
	10^4 *E faecalis**	*S. aureus**	*P. aeruginosa* 06		10^8 *S. aureus**		
5	0	*K. pneumoniae*[†]	Pneumonia	3	10^9 *K. pneumoniae*[†]	Simultaneous	Identical
		S. aureus[†]	*K. pneumoniae*		10^9 *S. aureus**	Simultaneous	Identical
		Enterococcus[†]	*S. aureus*		10^7 *S. aureus*[‡]	Simultaneous	Identical
6	0	*A. baumannii**	Pneumonia	6	10^9 *A. baumannii**	Simultaneous	Identical
			A. baumannii				
			P. aeruginosa				
7	10^6 *E coli**	*A. baumannii**	Pneumonia	4	10^6 *E coli**	4 d before	Identical
	10^5 *M. morganii*[†]	*K. pneumoniae*[†]	*E coli*		10^3 *M. morganii*[‡]		
7[§]	10^6 *E coli**	*A. baumannii**	Pneumonia	7	0		
	10^5 *M. morganii*[†]	*K. pneumoniae*[†]	*P. aeruginosa* 01				
8	0	*K. pneumoniae**	Pneumonia	16	10^8 *K. pneumoniae**		
			A. baumannii				
			P. aeruginosa 05				
9	0	*A. baumannii**	Pneumonia	10	10^8 *A. baumannii**	4 d before	Identical
			A. baumannii				
10	10^8 *E aerogenes**	0	Pneumonia	15	0		Identical
			E aerogenes				
11	10^4 *E coli*[†]	*S. aureus*[†]	Pneumonia	5	10^8 *S. aureus**	4 d later	Identical
			S. aureus		10^8 *S. aureus*[‡]		
12	10^3 *K. oxytoca**	*A. baumannii**	Pneumonia	7	10^8 *A. baumannii**	3 d before	Identical
		*S. aureus**	*A. baumannii*		10^8 *S. aureus**	3 d before	Identical
			S. aureus				
12[§]	10^3 *K. oxytoca**	*A. baumannii**	Pneumonia	14	10^9 *S. aureus**	1 d later	Identical
		*S. aureus**	*S. aureus*				
12	10^3 *K. oxytoca**	*A. baumannii**	Distal bronchitis	25	10^9 *S. aureus**	Simultaneous	Different
		*S. aureus**	*S. aureus*				
13	0	*K. pneumoniae*[†]	Pneumonia	19	0		
			P. aeruginosa 02				
14	10^7 *A. baumannii**	*K. pneumoniae**	Pneumonia	12	10^3 *A. baumannii**	12 d before	Identical
		*S. aureus**	*A. baumannii*		10^2 *K. pneumoniae**		
					10^5 *S. aureus**		
15	0	*K. pneumoniae*	Pneumonia	17	10^4 *K. pneumoniae**	5 d before	Identical
		with ESBL[†‖]	*K. pneumoniae*		10^7 *K. pneumoniae*[‡]		
		*S. aureus**	with ESBL[‖]		both with ESBL[‖]		
16	0	*S. aureus**	Pneumonia	3			
		E cloacae[†]	*S. aureus*		10^8 *S. aureus**	4 d later	Identical
		E faecalis[‡]	*E cloacae*		10^4 *E cloacae**	4 d later	Identical
17	0	*A. baumannii**	Pneumonia	9	10^6 *A. baumannii**	4 d before	Identical
		S. aureus[†]	*A. baumannii*				
		*P. aeruginosa**					
17	0	*A. baumannii**	Distal bronchitis	16	10^8 *S. aureus**	5 d before	Identical
		S. aureus[†]	*S. aureus*				
		*P. aeruginosa**					
17[§]	0	*A. baumannii**	Pneumonia	29	10^5 *P. aeruginosa**	18 d before	
		S. aureus[†]	*S. aureus*				
		*P. aeruginosa**					

(*continued*)

occurrence of nosocomial pneumonia. Similarly, the estimated time to occurrence of pneumonia did not rely on the baseline gastric pH.

Relationship between Colonization and Infection

The relationship between colonization and infection is reported in Tables 2 and 3.

Table 2 reports, for each bacterial species, the number of infected patients in whom bacterial identity, as assessed by PFGE, was established between the infecting strain and the colonizing strain, according to time and site of detection of colonization. This was plotted against the total number of infections due to the same bacterial species. This permitted us to segregate infections due to organisms colonizing on admission

TABLE 3

CONTINUED

Patient No.	Admission Colonization	Acquired Colonization	Infection	Time from Admission to Infection	Colonization Close to Infection¶	Time from Acquired Colonization to Infection	Pulsotype
18	10^4 *M. morganii*‡	*P. aeruginosa**	Pneumonia	8		2 d before	
		*S. aureus**	*S. aureus*		10^8 *S. aureus**		Identical
			P. aeruginosa 06		10^8 *P. aeruginosa**		Identical
19	10^3 *P. aeruginosa* O_2*	*K. pneumoniae*†	Pneumonia	13			
	10^6 *E. faecalis**	*S. aureus**	*P. aeruginosa* 02				
			S. aureus		10^6 *S. aureus**	Simultaneous	Different
20	0	0	Distal bronchitis	20	0		
			P. aeruginosa 01				
21	0	*S. aureus**	Pneumonia	16	10^5 *S. aureus**	2 d later	Different
			S. aureus				
22	10^8 *E. coli**	0	Pneumonia	8	10^8 *E. coli**	8 d before	Identical
	10^5 *S. aureus**		*E. coli*		10^8 *E. coli*‡		
23	10^8 *K. pneumoniae**	*P. aeruginosa**	Pneumonia	14	10^8 *P. aeruginosa**	4 d before	Identical
			P. aeruginosa				
23§	10^8 *K. pneumoniae**	*P. aeruginosa*†	Pneumonia	24	10^8 *P. aeruginosa*‡	Simultaneous	Identical
			P. aeruginosa		10^8 *K. pneumoniae*‡		
			A. baumannii				
24	10^4 *Hafnia alvei**	0	Distal bronchitis	16	0		
			P. aeruginosa				
			A. baumannii				
25	0	*K. pneumoniae**	Pneumonia	14	10^4 *K. pneumoniae**		
			S. aureus				
26	0	*S. aureus**	Pneumonia	12	10^5 *A. baumannii**	5 d before	Identical
		*A. baumannii**	*A. baumannii*		10^7 *S. aureus**		
		E. coli‡			10^5 *K. oxytoca**		
		*K. oxytoca**			10^8 *E. coli*‡		
27	10^2 *K. oxytoca**	*S. aureus**	Pneumonia	12			
	10^4 *E. coli*‡		*E. coli*		10^2 *E. coli**		Identical
			S. aureus		10^4 *S. aureus**	1 d before	Identical
28	0	*S. aureus**	Pneumonia	15			
		*P. aeruginosa**	*P. aeruginosa* 06		10^5 *P. aeruginosa**	Simultaneous	Identical
			S. aureus		10^6 *S. aureus**	7 d before	Identical
29	0	*A. baumannii*†	Pneumonia	20	10^5 *A. baumannii**	Simultaneous	Identical
			A. baumannii		10^6 *A. baumannii*‡		

* Oropharyngeal colonization.
† Both oropharyngeal and gastric colonization.
‡ Gastric colonization.
§ Patient with a second episode of nosocomial pneumonia.
‖ ESBL = extended-spectrum β-lactamase.
¶ Colonization close to infection: results of samples (oropharyngeal and gastric) close to infection which were analyzed by PFGE.

or acquired later during ICU stay. For instance, the majority of *A. baumannii* and *S. aureus* pneumonia occurred after previous colonization in the unit. Microorganisms responsible for infections were recovered more often from the oropharynx site than from the gastric site. This relationship was reported especially in infections due to organisms acquired during ICU stay.

Table 3 details, for each patient, the relationship between colonization and infection, according to time of colonization, either detected on admission or acquired in ICU, time of detection of infection, time between colonization and infection with identical bacteria, and results of molecular analysis. In 36 bacterial episodes in 29 patients (the fungal episode was not included), the bacteria responsible for infection was also recovered from oropharyngeal samples in 17 (47%) of these 36 episodes, from both oropharyngeal and gastric samples in six (16%), and only once from gastric samples. Colonization was detected, previously or simultaneously, in all patients except four. In three of these patients (Patients 11, 12, and 16), the last sample before pneumonia was missing, while in Patient 21, all the digestive samples were sterile before pneumonia occurred. The identical bacteria was recovered from oropharyngeal samples in all these patients and from gastric sample (Patient 11) after pneumonia with a delay of 4, 1, 4, and 2 d in Patients 11, 12, 16, and 21, respectively.

We thereafter separately assessed the influence of both oropharyngeal and gastric colonization on the subsequent development of pneumonia, according to the bacteria responsible for each carriage, through the use of Cox modeling. Only one of these colonization patterns was associated with a subsequent increased risk of developing nosocomial pneumonia with similar bacteria, as assessed by PFGE. This consisted in oropharyngeal carriage with *A. baumannii* that yielded a 7.45-fold estimated increased risk of pneumonia as compared with that of remainders ($p = 0.0004$).

Chromosomal DNA Analysis

The results of comparison by PFGE of one colony of the bronchial samples and one colony of the oropharyngeal and/or gastric samples (when oropharyngeal was negative) closer to infection are shown in Table 3 for all episodes of nosocomial pneumonia and distal bronchitis.

In patients infected with Enterobacteriaceae sp. or *P. aeruginosa*, the same strain, as assessed by PFGE, was isolated for

each patient in bronchial samples and oropharyngeal or gastric samples (data not shown). The strains of Enterobacteriaceae sp. or *P. aeruginosa* were different according to PFGE from one patient to another.

As shown in Figure 3, all strains of *A. baumannii* isolated from oropharyngeal or bronchial samples corresponded to the same clone. In lanes B and H, the two DNA bands larger than 200 kb were in fact present in the gel but unfortunately damaged by DNase and not detectable on the photograph.

Concerning methicillin-resistant *S. aureus*, the situation was more difficult to analyze. In 15 episodes of pneumonia in 12 patients, strains of methicillin-resistant *S. aureus* were isolated in the same period (less than 4 d) from bronchial samples and oropharyngeal or gastric samples. In all except three cases, infecting and colonizing strains were identical. In three episodes (Patients 12, 19, and 21), infecting strains differed from colonizing strains by only one DNA fragment (Figure 4). In Patient 12, three episodes were observed. Two episodes of pneumonia with the same strain were diagnosed with PFGE with a 7-d interval between the end of the first treatment and the new clinical episode. This strain was recovered from oropharyngeal samples as well only during the first episode. Subsequently, 11 d later, distal bronchitis was observed and a different strain (more than six different DNA fragments) was isolated from both bronchial and oropharyngeal samples (Figure 4).

DISCUSSION

This study has shown a positive bacterial relationship between colonization of the digestive tract and nosocomial pneumonia and has highlighted the possible role of the oropharyngeal site as one of the main bacterial digestive reservoirs. This relation was microbiologically established by using genomic DNA analysis demonstrating bacterial identity between digestive colonizing strains and strains responsible for nosocomial pneumonia.

The importance of each digestive reservoir, either oropharyngeal or gastric, was a subject of controversy for many years. For a long time, oropharyngeal colonization with gram-negative bacilli was considered to increase with the severity of illness in patients (25) and reported as a risk factor for acquiring nosocomial pneumonia (1, 26). During the 1980s, the evidence of a gastric reservoir emerged in many reports (2–4) which focused on its role as a source of oropharyngeal colonization by gram-negative bacilli. However, recent studies (5, 6, 27, 28) have demonstrated that the gastric reservoir was not a main reservoir in the process of acquiring nosocomial pneumonia.

Our study demonstrating predominant oropharyngeal colonization is in line with the most recent reports. Colonization on admission was detected in 58% and 37% of our patients in the oropharyngeal or gastric site, respectively. The majority of the isolated strains were Enterobacteriaceae, probably in accordance with the high proportion (56%) of previously hospitalized in other wards. Using sequential samplings of oropharyngeal and gastric secretions, the importance of the oropharyngeal reservoir was also demonstrated when colonization was acquired during ICU stay. Oropharyngeal colonization alone was the most frequent event in 50% of the patients, whereas colonization of the gastric site alone was observed in 11% of the patients. Oropharyngeal colonization was clearly predominant for *A. baumannii, K. pneumoniae, Pseudomonas* sp., and *S. aureus* (Table 1). The lack of gastric colonization did not appear to be related to the acidity of the stomach. However, this analysis was hampered by the fact that gastric pH was only measured twice weekly.

The incidence of nosocomial pneumonia was 36%, higher than reported in previous studies using the same diagnostic

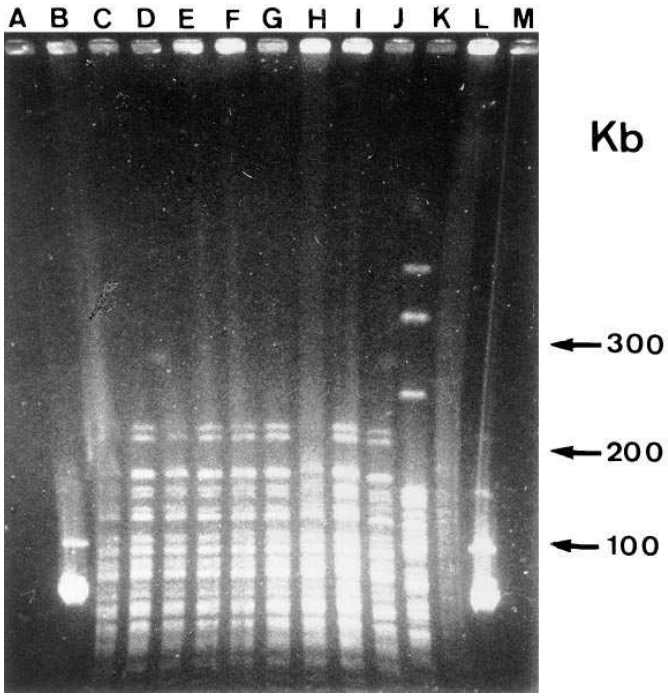

Figure 3. Pulsed-field gel electrophoresis of strains of *A. baumannii* isolated from bronchial samples (BS) and oropharyngeal samples (OS). Lanes A and M: phage lambda DNA ladder; lane B: BS of Patient 1; lane C: OS of Patient 1; lane D: BS of Patient 6; lane E: OS of Patient 6; lane F: BS of Patient 8; lane G: OS of Patient 8; lane H: BS of Patient 17; lane I: OS of Patient 17; lane J: BS of Patient 23; lanes K and L: strains of *A. baumannii* isolated from urinary tract infection in patients staying in the urology unit.

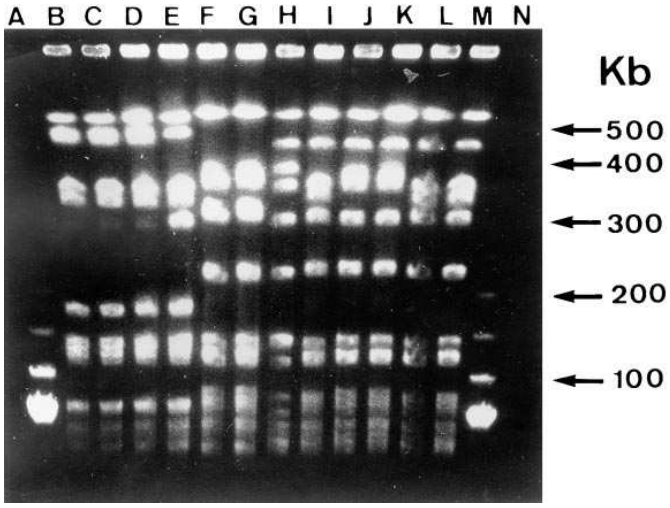

Figure 4. Pulsed-field gel electrophoresis of strains *S. aureus* isolated from bronchial samples (BS) and oropharyngeal samples (OS). Lanes A and N: phage lambda DNA ladder; lane B: BS1 of Patient 12; lane C: OS1 of Patient 12; lane D: BS2 of Patient 12; lane E: OS2 of Patient 12; lane F: BS3 of Patient 12; lane G: OS3 of Patient 12; lane H: BS of Patient 19; lane I: OS of Patient 19; lane J: BS of Patient 5; lane K: OS of Patient 5; lane L: BS of Patient 27; lane M: OS of Patient 27.

criteria (9%, 21%) (17, 29). This can be explained by our population of seriously ill patients with sustained mechanical ventilation and therefore at increased risk of nosocomial pneumonia (29). The high incidence of *A. baumannii* and *P. aeruginosa* pneumonia, which can be responsible for an attributable mortality independent of the underlying disease (30), probably accounts for a 58% death rate among patients with nosocomial pneumonia.

This study, to our knowledge, represents the first prospective study of nosocomial pneumonia in ventilated patients, assessing digestive colonization by DNA analysis to compare colonizing and infecting strains. We demonstrated that in the majority of cases, nosocomial pneumonia was a secondary endogenous infection with identical colonizing strains. In each case of gram-negative pneumonia, PFGE clearly demonstrated that identical strains were recovered from digestive colonized sites and bronchial samples. Strains of *E. coli*, *K. pneumoniae*, and *P. aeruginosa* were different according to PFGE from one patient to another. On the contrary, the same *A. baumannii* strain was detected in several patients, indicating the diffusion of an epidemic strain. In most cases of *S. aureus* pneumonia, identical strains were identified by PFGE from bronchial and oropharyngeal samples. In the other cases, the strains isolated from oropharyngeal samples differed from bronchial samples by only one fragment. Similar results were observed by Prevost and associates (31), indicating that some patients can harbor multiple methicillin-resistant *S. aureus* strains in different body sites (e.g., nares and tracheal secretions). Another explanation was that only one colony of each colonizing strain in the different samples was selected for PFGE, so some colonizing strains might have been undetected.

However, we did not design the study to analyze the specific role of each reservoir, oropharyngeal, gastric, or tracheal, in the acquisition of nosocomial pneumonia. For this reason, the tracheal site has not been studied. Moreover, daily digestive samplings, rather than weekly samplings, should be preferable to elucidate the different sources of bacteria that enter the lung during the colonization process. Three recent studies (27, 28, 32) focused on this particular point in a similar population of ventilated patients in trying to detect the initial site of colonization using sequential oropharyngeal, gastric, and tracheal samples. They demonstrated that the gastro-pulmonary route was not important for the development of nosocomial pneumonia (27). The oropharynx, alone or in association with the trachea, was the initial site of colonization for, respectively, 13 and 14 microorganisms out of the 58 microorganisms causing nosocomial pneumonia in which the sequence of colonization was reported (27). The upper respiratory tract was supposed to be an important reservoir for *Pseudomonas* sp., as reported in past studies (33) or recently (28, 32). However, genetic bacterial identity was not assessed in these studies to demonstrate definitively the role of each reservoir. Our study cannot demonstrate, in the absence of tracheal cultures, the real importance of the oropharyngeal colonization in the respiratory colonization process. However, we demonstrated a relationship for oropharyngeal *A. baumannii* colonization: when *A. baumannii* was previously isolated as a colonizing strain, the estimated increased risk of nosocomial pneumonia was 7.45-fold. This is in accordance with the well-known primary location of *A. baumannii* in the posterior pharynx in the colonization process.

The specific epidemiologic context existing at the time of this study must be kept in mind to explain the high rate of multiresistant colonizing and infecting strains. The epidemic situation for *A. baumannii* and *K. pneumoniae* with ESBL was well known during the same period in other French (20, 34, 35) and European (36) ICUs. For methicillin-resistant *S. aureus*, the situation has been endemic for many years in France, especially in the ICU setting, as reported recently (37). This epidemiologic context probably affected the pattern of our results, in particular the strong relationship between colonization and infection with *A. baumannii*, as reported recently (36).

In summary, the results of this study demonstrate, under the circumstances tested, the bacterial identity between colonizing digestive strains, especially in the oropharyngeal site, and strains responsible for nosocomial pneumonia. Similar techniques of molecular typing have to be more widely used in the future to allow better assessment of the role of each reservoir as a source of subsequent nosocomial pneumonia in mechanically ventilated patients.

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