The Role of Systemic Antibiotics in Acquiring Respiratory Tract Colonization With Gram-Negative Bacteria in Intensive Care Patients: A Nested Cohort Study

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Objective: Colonization of the respiratory tract with Gram-negative bacteria in intensive care patients increases the risk of subsequent infections. Application of systemic antibiotics may prevent colonization with Gram-negative bacteria, but this effect has never been quantified. The objective of this study was to determine associations between systemic antibiotic use and acquisition of respiratory tract colonization with Gram-negative bacteria in ICUs.
Design: A nested cohort study.
Setting: A university hospital and a teaching hospital.
Patients: Patients with ICU stay of more than 48 hours and absence of respiratory tract colonization with Gram-negative bacteria on ICU admission.
Interventions: None.
Measurements and Main Results: Acquisition was determined through protocolized surveillance. Associations were investigated with Cox regression models with antibiotics as a time-dependent covariate. In all, 250 of 481 patients (52%) acquired respiratory tract colonization with Gram-negative bacteria after a median of 5 days (interquartile range, 3–8 d) (acquisition rate, 77.1/1,000 patient-days at risk). Antibiotic exposure during ICU admission was present in 78% and 72% of the patients with and without acquired Gram-negative bacteria colonization, respectively. In Kaplan-Meier curve analysis, the median times to acquisition of Gram-negative bacteria were 9 days (95% CI, 7.9–10.1) and 6 days (95% CI, 4.8–7.2) in patients receiving and not receiving antibiotics, respectively. In time varying Cox regression analysis, however, the association between acquired colonization and systemic antibiotics was not statistically significant (hazard ratio, 0.90; 95% CI, 0.70–1.16).
Conclusions: Among patients not colonized with Gram-negative bacteria in the respiratory tract at admission to ICU, systemic antibiotics during ICU stay were not associated with a reduction in acquisition of Gram-negative bacteria carriage in the respiratory tract during the ICU stay. (*Crit Care Med* 2014; XX:00–00)
Key Words: antibiotic resistance; antibiotics; cross infection; Gram-negative bacteria; intensive care; respiratory tract infections

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Trial registration: ISRCTN75875670.

This work was performed at University Medical Center Utrecht, The Netherlands, and St Elisabeth Hospital Tilburg, The Netherlands.

Dr. Jongerden's institution received grant support from The Netherlands Organisation for Health Research and Development (ZonMw, project number 62300037). Dr. Bonten consulted for Crucell (consultant for Antibtiotic Resistance Program). His institution received grant support from the Netherlands Organisation for Health Research and Development. The remaining authors have disclosed that they do not have any potential conflicts of interest.

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DOI: 10.1097/CCM.0000000000000768

Infections caused by Gram-negative bacteria (GNB) are associated with increased morbidity and mortality and higher healthcare costs, especially in ICUs (1–4). ICU-acquired infections are almost always preceded by colonization, which is defined as the presence of microorganisms without generating adverse clinical effects (5, 6). Especially mechanically ventilated (MV) patients are frequently colonized with GNB in the respiratory tract, with rates varying from 50% to 91% (5, 7–14).

Modulation of the respiratory tract flora is a widely used infection prevention measure, either through topical application of chlorhexidine (15, 16) or antibiotics (17, 18). It has

been suggested that exposure to systemic antibiotics is a risk factor for acquiring colonization with GNB, mostly in studies that addressed single pathogens (i.e., *Pseudomonas aeruginosa*, *Acinetobacter baumannii,* or *Stenotrophomonas maltophilia*), in the circumstance of a nosocomial outbreak, or in single-center studies (19–23). Still, the independent contribution of systemic antibiotics to the acquisition of respiratory tract colonization has not been determined. We, therefore, aimed to assess the effects of systemic antibiotics (in the absence of using topical preventive measures) on acquisition with any GNB in ICU patients.

MATERIALS AND METHODS

Design

Acquisition of colonization with GNB was evaluated in two hospitals between January 2007 and February 2008. This cohort study was nested in a prospective crossover trial on cross-transmission, in which closed and open suction systems were implemented unit wide for all eligible patients during periods of 6 months (24). The study was approved by the institutional review board of both hospitals, and a waiver for informed consent was granted. Reporting was done according to the Strengthening the Reporting of Observational Studies in Epidemiology statement (25).

Study Population

Four mixed ICUs participated in the study: two units from a university hospital with 10 beds (four single rooms and six on the ward) and eight beds (one single room and seven on the ward) and two 8-bed units from a teaching hospital (all single rooms). Patients with a length of ICU stay of more than 48 hours were eligible for study inclusion. For analysis, we included only patients with at least two microbiological cultures from respiratory tract samples and without GNB colonization at admission. Patients with unknown colonization status at admission were excluded. Use of infection control measures (such as contact precautions) in case of carriage with highly resistant bacteria was based on the guidelines of the Dutch Infection Prevention Working Party (26, 27). Selective Digestive Decontamination (SDD), Selective Oropharyngeal Decontamination (SOD), and chlorhexidine oropharyngeal decontamination were not used during the period of this study.

Outcomes

Primary outcome of our study was the first event of acquired colonization with any GNB in the respiratory tract. GNB included were *P. aeruginosa*, *Acinetobacter* species, *S. maltophilia*, and Enterobacteriaceae (*Escherichia coli*, *Enterobacter* species, *Klebsiella* species, *Citrobacter* species, *Proteus* species, and *Serratia* species).

Acquired colonization was defined as documentation of GNB in a respiratory tract sample obtained at least 48 hours after ICU admission and preceded by documented absence of GNB previously (13). Colonization at admission was defined as growth of GNB from an endotracheal aspirate sample (or throat swab in the absence of endotracheal aspirate) in a sample obtained within 48 hours of ICU admission. When the first culture grew, GNB was taken more than 48 hours after ICU admission, and colonization status at admission was considered unknown. Patient-days at risk for acquiring colonization was defined as all days in ICU in which the patient did not have documented colonization with any of the selected GNB. To determine acquired colonization with individual marker pathogens, all noncolonized patient-days for that specific pathogen were considered at risk.

Data Collection

Colonization was determined by surveillance cultures of endotracheal aspirates (in MV patients) or oropharyngeal swabs (nonventilated patients) that were obtained at admission and twice weekly thereafter (every Monday and Thursday) until discharge from ICU. The samples were analyzed according to local protocol, and isolated marker pathogens were stored at –80°C. Results were communicated to the medical staff according to standard microbiological reporting practices.

The antimicrobials used in the ICUs were registered (name, dose, and frequency) and classified according to the Anatomical Therapeutic Chemical/Defined Daily Dose (DDD) system by the World Health Organization (index 2011) (28). Based on this system, the antibiotic density was calculated, expressing the DDD (the assumed average maintenance dose per adult per day) (28) per 1,000 patient-days at risk.

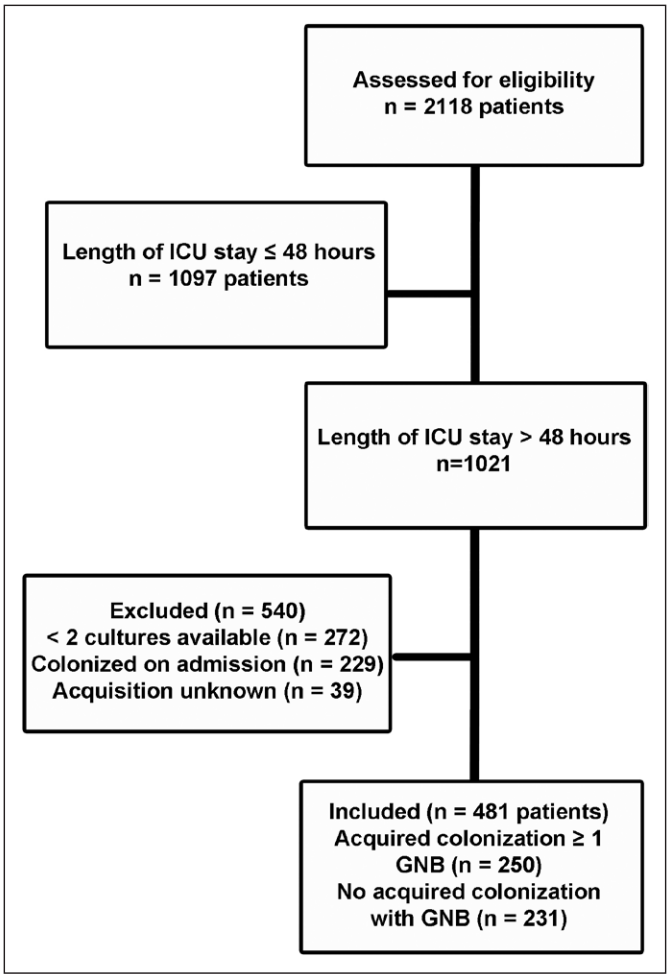

Figure 1. Flowchart of study inclusion. GNB = Gram-negative bacteria.

TABLE 1. Characteristics and Outcomes of Patients Who Did and Did Not Acquire Colonization

Variable	Without GNB	Acquired GNB[a]	*p*[b]
Patients (*n* = 481)	231	250	
Gender, male, *n* (%)	133 (58)	153 (61)	0.42
Age, median (IQR)	59 (46–70)	61 (48–72)	0.16
Hospital, % patients			0.00
H1	55	45	
H2	39	61	
Diagnosis, surgical, *n* (%)	80 (35)	76 (30)	0.32
Cardiovascular	30 (13)	31 (12)	0.15
Neurology/neurosurgery	53 (23)	41 (16)	
Respiratory	65 (28)	68 (27)	
Gastrointestinal	17 (7)	24 (10)	
Trauma	26 (11)	55 (22)	
Sepsis	17 (7)	19 (8)	
Other	23 (10)	12 (5)	
Acute Physiology and Chronic Health Evaluation II score, mean (SD)	19 (7.2)	20 (7.2)	
Antibiotics at admission, *n* (%)	68 (29)	52 (21)	0.03
Antibiotics during ICU stay, *n* (%)[c]	167 (72)	195 (78)	0.15
Duration antibiotics use, median days (IQR)[c]	3 (0–6)	4 (2–7)	0.18
Isolation, *n* patients (%)[c]	15 (7)	20 (8)	0.52
Isolation days, median (IQR)	4 (3–6)	6 (3–9)	0.37
Mechanical ventilation, *n* (%)	201 (87)	243 (97)	0.00
Duration of mechanical ventilation, median days (IQR)	4 (2–7)	12 (7–22)	0.00
Suction system (closed), *n* (%)[d]	123 (53)	137 (55)	0.73
Tracheostomy, *n* (%)[c]	15 (7)	24 (10)	0.21
Length of ICU stay, median days (IQR)	6 (4–10)	14 (8–25)	0.00
Length of hospital stay, median days (IQR)	19 (11–37)	32 (19–57)	0.00
Hospital stay before ICU admission, median days (IQR)	0 (0–3)	0 (0–3)	0.38
Hospital stay after ICU admission, median days (IQR) of patients who survived ICU	11 (5–25)	19 (9–38)	0.00
ICU mortality, %	26 (11)	58 (23)	0.001
Hospital mortality, %	40 (17)	79 (32)	0.00

GNB = Gram-negative bacteria, IQR = interquartile range.

[a]Acquired colonization with ≥ 1 GNB.

[b]*p* value based on *t* test (age, duration of antibiotic use, isolation days, duration of mechanical ventilation, length of stay), Mann-Whitney test (Acute Physiology and Chronic Health Evaluation II score), and chi-square test (gender, hospital, diagnosis, antibiotics at admission or during ICU stay, isolation, mechanical ventilation, suction system, tracheostomy, and ICU and hospital mortality).

[c]Before acquisition or otherwise discharge from ICU.

[d]Suction system: according to study protocol in the crossover trial (24).

Data Analysis

Descriptive analyses were used to summarize demographic and clinical information (age, gender, Acute Physiology and Chronic Health Evaluation [APACHE] II score, admission diagnosis, antibiotic use before acquisition, isolation before acquisition, and mechanical ventilation) and outcomes (length of ICU stay, length of hospital stay, and survival status). For univariate analysis, continuous variables were tested with Kolmogorov-Smirnov test for normal distribution. Parametric data were expressed as mean (SD) and *t* test was used to test

for significant differences, whereas nonparametric data were expressed as median (interquartile range [IQR]) and tested by using Mann-Whitney test. Dichotomous variables were analyzed by using chi-square test.

Kaplan-Meier survival curves were used to determine differences in time to acquisition of GNB between patients who received and did not receive antibiotics. Since systemic antibiotic use may vary between patients and during the course of admission, Cox regression models with time-dependent covariates were used to estimate hazard ratios (HR) and their corresponding 95% CI, with days in ICU until first event of acquisition (days at risk) as time variable and use of systemic antibiotics prior to acquisition or discharge as time-dependent variable. Patients without acquired colonization were censored at discharge from the unit or at the time of death. Once colonized, patients were considered no longer at risk for acquisition with that pathogen. It has been suggested that increased age (29), higher severity of illness (29–31), mechanical ventilation (22), and longer length of stay (20, 30) are associated with higher colonization rates. Therefore, we included age, APACHE II score, and mechanical ventilation (dichotomous) as potential risk factors for acquisition. In addition, we added hospital (due to differences in performance and materials), admission diagnosis (surgical or medical), endotracheal suction system (open or closed system), and gender as potential risk factors. All covariates were tested univariately, and variables with p value of less than 0.05 were included in a multivariate model. Systemic antibiotics were included in the model regardless of significance level, since it was the determinant. Multicollinearity was tested in advance, and all covariates were included ($r < 0.8$).

Imputation was used for missing data using an expectation-maximization (EM) analysis with the Impute function in SPSS software (version 20; Somers, New York, NY), with inclusion of study period, age, gender, diagnosis, and mechanical ventilation as key variables in the imputation model. A total of 21 APACHE II scores were missing (4.4% of all values). EM analysis revealed that data were missing at random, meaning that differences in missing data are related to the observed data, and missing values were replaced by imputed values. Apart from increasing the sample size, imputation corrects for possible bias due to selective missing values (32, 33). Statistical analyses were performed with IBM SPSS version 20 (IBM; Somers) and SAS version 9.2 (SAS Institute, Cary, NC). A p value of less than 0.05 was considered statistically significant.

RESULTS

From January 2007 to February 2008, 1,021 patients were admitted more than 48 hours to one of the participating

TABLE 2. Acquired Gram-Negative Bacteria (% of Patients Who Acquired Colonization)

Pathogen	*n* (%)	Acquisition/1,000 Patient-Days at Risk
Pseudomonas aeruginosa	74 (30)	22.7
Stenotrophomonas maltophilia	42 (17)	12.9
Acinetobacter species	34 (14)	10.4
Enterobacter species	79 (32)	24.3
Klebsiella species	71 (28)	21.8
Escherichia coli	57 (23)	17.5
Serratia species	26 (10)	8.0
Proteus species	22 (9)	6.8
Citrobacter species	13 (5)	4.0
Other Gram-negative bacteria[a]	30 (12)	9.2

[a]Other Gram-negative bacteria: Moraxella, Morganella, Achromobacter, Hafnia, Pantoea, Alcaligenes, Burkholderia, Eikenella, Leclercia, Ochrobactrum, and Providencia.

TABLE 3. Antimicrobial Density in Patients With and Without Acquired Gram-Negative Bacteria[a]

Variable	Antimicrobial Density (Defined Daily Dose/1,000 Patient-Days at Risk)		
	Total	No GNB Acquisition	With GNB Acquisition
Antimicrobial group[b]			
Penicillin-like antibiotics[c]	859	687	1,034
Cephalosporins[d]			
First generation	31	29	33
Second generation	86	63	108
Third generation	159	180	139
Aminoglycosides[e]	63	39	89
Quinolones[f]	94	94	95
Carbapenems[g]	89	45	134
Glycopeptides[h]	28	15	41
Sulfamethoxazole/trimethoprim	49	78	19
Other[i]	158	136	180
Total	1,616	1,365	1,871

GNB = Gram-negative bacteria.

[a]Antibiotic density is defined daily doses per 1,000 patient-days at risk.

[b]The antimicrobials used in the ICUs were divided by class and group according to Anatomical Therapeutic Chemical classification defined by the World Health Organization, index 2011.

[c]Penicillin-like antibiotics: amoxicillin-clavulanic acid, piperacillin-tazobactam, flucloxacillin, amoxicillin, benzylpenicillin, and piperacillin.

[d]Cephalosporins: cefazolin (first generation), cefuroxim (second generation), and ceftriaxone and ceftazidime (third generation).

[e]Aminoglycosides: gentamycin and tobramycin.

[f]Quinolones: ciprofloxacin, levofloxacin, moxifloxacin, and ofloxacin.

[g]Carbapenems: meropenem and imipenem/cilastatin.

[h]Glycopeptides: vancomycin and teicoplanin.

[i]Other: metronidazol, clindamycin, rifampicin, erythromycin, colistin, and azithromycin.

ICUs, of whom 540 were excluded mainly because an insufficient number of cultures was obtained ($n = 272$ with either one [$n = 193$] or no [$n = 79$] cultures available) (**Fig. 1**).These were mainly patients with a short length of ICU stay (median, 3 d; IQR, 3–4 d), frequently admitted in-between surveillance culture days. In total, 481 patients (with 6,252 patient-days) were included, mostly male patients (60%) with a median age of 60 years (IQR, 46–71 yr) (**Table 1**). Both hospitals had heterogeneous ICU populations with comparable patient characteristics (gender, age, and admission diagnosis); only APACHE II scores were significantly different (mean, 20.3 and 18.6 for hospital 1 and 2, respectively; SD, 7.3 and 6.9). From included patients, 2,059 respiratory tract cultures were available for analysis (965 marker pathogens isolated).

Acquired Colonization

In total, 250 patients (52%) acquired colonization with at least 1 GNB, yielding an overall acquisition rate of 77.1/1,000 patient-days at risk. Most patients acquired colonization with one (50%) or with two (29%) pathogens, mainly with *Enterobacter* species (32%) or *P. aeruginosa* (30%) (**Table 2**). Median time until colonization with the first GNB was 5 days (IQR, 3–8 d), and the median time before half of the study population acquired colonization was 8 days (IQR, 7–9 d).

Effect of Antibiotic Use on Acquired Colonization

Of all patients who acquired colonization with GNB, 78% received systemic antibiotics prior to acquisition, as compared with 72% of patients who did not acquire GNB colonization ($p = 0.15$). Expressed in DDD per 1,000 patient-days at risk, antimicrobial usage densities were 1,871 and 1,365 in patients who acquired colonization with GNB and patients who did not, respectively, with marked differences for different classes of antibiotics (**Table 3**).

In Kaplan-Meier curve analysis, the median times to acquisition of GNB were 9 days (95% CI, 7.9–10.1) and 6 days (95% CI, 4.8–7.2) in patients receiving and not receiving antibiotics, respectively (**Fig. 2**). In time-dependent Cox regression analysis, however, the association between acquired colonization and systemic antibiotics was not statistically significant (HR, 0.90; 95% CI, 0.70–1.16) (**Table 4**). Furthermore, acquired GNB colonization was associated with hospital (HR, 0.58; 95% CI, 0.45–0.75). In exploratory analyses for *P. aeruginosa* (74 patients who acquired colonization) and Enterobacteriaceae (194 patients), comparable results were identified, with no statistically significant association between systemic antibiotics and acquired colonization (HR, 0.96; 95% CI, 0.59–1.58 for *P. aeruginosa* and HR, 0.84; 95% CI, 0.63–1.12 for Enterobacteriaceae, respectively).

DISCUSSION

In two Dutch ICUs, the median duration until acquisition of colonization with GNB in the respiratory tract, among patients not colonized with GNB at the time of ICU admission, was 5 days. Acquisition occurred in 52% of the patients before ICU discharge. Antibiotic use was associated with a longer duration until acquired GNB colonization; however, risk of acquisition was not associated with systemic antibiotic use during ICU stay.

Our findings suggest that systemic antibiotics neither increase nor prevent respiratory tract colonization with GNB, which seems in contrast to results from other studies, in which previous use of antibiotics or selected classes of antibiotics (i.e., Carbapenems or third-generation Cephalosporins) were identified as risk factors for acquiring multidrug-resistant *P. aeruginosa* (34), *Acinetobacter* species (19, 35, 36), *S. maltophilia* (23), or GNB (20). In these studies, defined daily doses or use of antibiotics in the patient population was not quantified, and antibiotic exposure was not treated as a time-dependent covariate in statistical analysis, which hampers a comparison with our findings. The percentage of patients receiving antibiotics in our study (75%) is comparable to results from the Extended Prevalence of Infection in Intensive Care (EPIC) II study, in which 71% of the patients received antimicrobial agents on the day of study (37). In addition, overall antibiotic density in our study

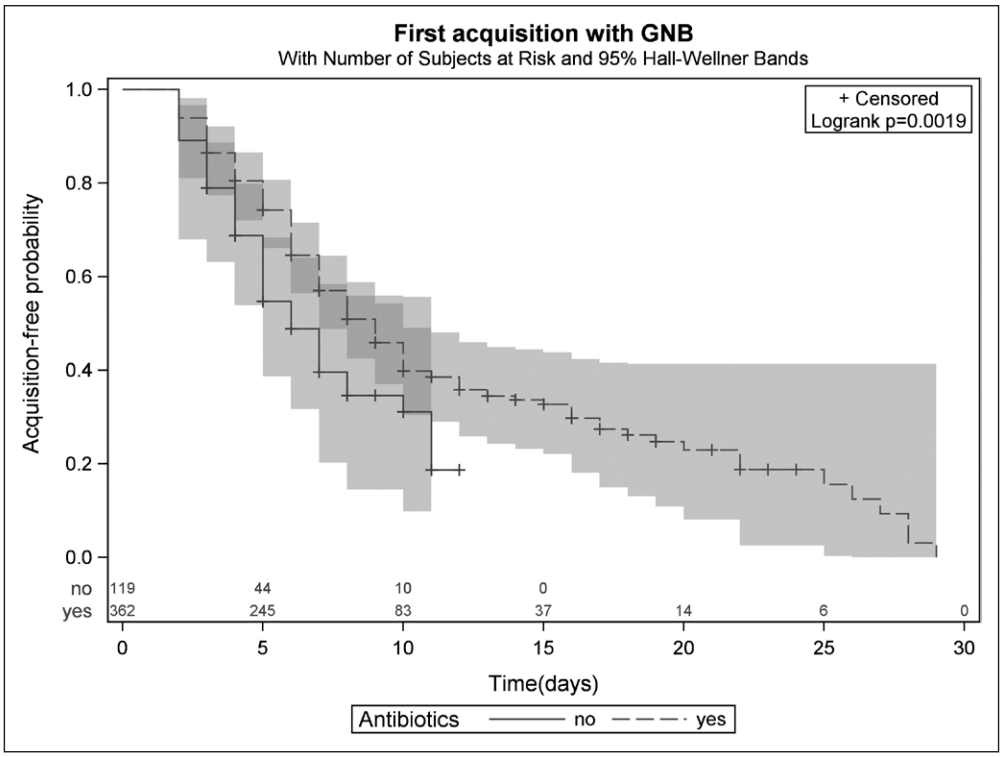

Figure 2. Kaplan-Meyer curve of days at risk until first acquisition with Gram-negative bacteria (GNB). No = no antibiotics prior to acquisition with GNB, yes = antibiotics prior to acquisition with GNB.

TABLE 4. Risk Factors for Acquiring Colonization With ≥ 1 Gram-Negative Bacteria

Potential Risk Factors	Univariate Analysis, HR (95% CI)	Multivariate Analysis, HR (95% CI)
Systemic antibiotics[a]	0.86 (0.67–1.11)	0.90 (0.70–1.16)
Hospital 1	0.59 (0.46–0.76)	0.58 (0.45–0.75)
Gender (male)	1.09 (0.84–1.41)	
Age (yr)	1.00 (0.997–1.01)	
Diagnosis (surgical)	0.94 (0.71–1.23)	
Acute Physiology and Chronic Health Evaluation II score	1.01 (0.99–1.02)	
Mechanical ventilation (yes)	2.25 (1.06–4.77)	1.82 (0.86–3.87)
Suction system[b] (closed)	1.04 (0.80–1.34)	

HR = hazard ratio.
[a]Prior to acquisition or otherwise discharge from ICU.
[b]Suction system: according to study protocol in the crossover trial (24).

(1,616 DDD per 1,000 patient-days at risk) seems higher as compared with figures reported from other European ICUs, with reported medians of 1,254–1,380 DDD per 1,000 patient-days (38–40). However, we only monitored antibiotic administration until acquisition; overall, DDDs did not deviate from other ICUs (DDD 1,165 per 1,000 patient-days) (24).

In this study, there were considerable differences in GNB colonization dynamics between participating ICUs, despite the presence of heterogeneous ICU populations with comparable patient characteristics in which only APACHE II scores were statistically significant different. Yet, acquisition of GNB occurred in 39% and 61% of the patients in hospital 1 and 2, respectively (HR, 0.58; 95% CI, 0.45–0.75). The difference in microbiology and in acquisition between hospitals might be due to local ecology.

Comparison of the overall acquisition rate with GNB (52% in our study) to rates reported in previous studies is difficult, since other studies focused on selected, usually multiresistant, GNB-like metallo-β-lactamase producing GNB (20) or cephalosporin-resistant GNB (31). The reported rate of acquired respiratory tract colonization with *P. aeruginosa* (28%) in one study (41) was in range with our results (30%), whereas for *S. maltophilia*, we found a higher colonization rate as compared with the 2% reported in French ICU (23). For the other pathogens, comparison was hampered due to incomparability of studied GNB.

Although systemic antibiotics do not seem to be associated with GNB colonization in the respiratory tract, application should be restricted. Due to antibiotic selective pressure and antibiotic susceptibility, differences in acquisition of individual pathogens might occur (42, 43). In the same patient population, we have previously demonstrated the effects of different antibiotics to select for antibiotic resistance in *P. aeruginosa* (44). And in Dutch ICUs, where SDD and SOD are frequently used, the routine use of systemic prophylaxis during the first 4 days in ICU as part of SDD does not offer additional benefits in patient outcome as compared with patients receiving SOD (17, 45). As a matter of fact, most patients receiving SOD also received systemic antibiotics prescribed for clinical reasons (17).

Strengths of our study include the detailed microbiological monitoring and the large sample size. Furthermore, our results were not confounded by other interventions that might influence acquisition of GNB, such as the use of topical antibiotics (as in SDD or SOD) or oropharyngeal application of chlorhexidine. A study limitation is that we only focused on colonization with GNB in patients with a length of ICU stay of more than 48 hours, rather than on actual infections. However, respiratory tract infections are almost always preceded by respiratory tract colonization. Therefore, risk factors for colonization may well be considered risk factors for subsequent ventilator-associated pneumonia (6).

CONCLUSIONS

In conclusion, of patients not colonized with GNB at the time of ICU admission, 52% acquired respiratory tract colonization before ICU discharge. Systemic antibiotics during ICU stay were not associated with a reduction in acquisition of GNB carriage in the respiratory tract during ICU stay.

ACKNOWLEDGMENTS

We thank David Ong for analytical advice and Fieke Kloosterman, Piet Vos, and Twan Verhoeven for assistance in collecting data.

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