Antibiotic exposure and resistance development in *Pseudomonas aeruginosa* and *Enterobacter* species in intensive care units

David S.Y. Ong, MD, PharmD; Irene P. Jongerden, MSc; Anton G. Buiting, MD, PhD; Maurine A. Leverstein-van Hall, MD, PhD; Ben Speelberg, MD, PhD; Jozef Kesecioglu, MD, PhD; Marc J.M. Bonten, MD, PhD

Objectives: **We quantified the association between antibiotic exposure and acquisition of antibiotic resistance in *Pseudomonas aeruginosa* and *Enterobacter* species in intensive care unit patients.**

Design: **Prospective cohort study.**

Setting and Patients: **In 1201 patients, respiratory tract colonization was determined through regular screening on admission, twice weekly, and on discharge. Primary outcome was the acquisition of antibiotic resistance in previous antibiotic sensitive *P. aeruginosa* and *Enterobacter* species, with acquisition attributable to cross-transmission excluded based on genotyping and epidemiologic linkage. Cox regression analysis, adjusted for covariates, was performed to calculate hazard ratios of patients exposed to antibiotics compared to patients not exposed to antibiotics.**

Methods and Main Results: **In total, 194 and 171 patients were colonized with *P. aeruginosa* and *Enterobacter* species, respectively. Two or more cultures per episode were available for 126 and 108 patients. For *P. aeruginosa*, ceftazidime exposure was associated with 6.3 acquired antibiotic resistance events per 100 days of exposure, whereas incidence rates were lower for ciprofloxacin, meropenem, and piperacillin-tazobactam. In multivariate analysis, meropenem, ciprofloxacin, and ceftazidime were significantly associated with risk of resistance development in *P. aeruginosa* (adjusted hazard ratio, 11.1; 95% confidence interval, 2.4–51.5 for meropenem; adjusted hazard ratio, 4.1; 95% confidence interval, 1.1–16.2 for ciprofloxacin; adjusted hazard ratio, 2.5; 95% confidence interval, 1.1–5.5 for ceftazidime). For *Enterobacter*, ceftriaxone and ciprofloxacin exposure were associated with most antibiotic resistance acquisitions. No significant associations were found in multivariate analysis.**

Conclusions: **Meropenem exposure is associated with the highest risk of resistance development in *P. aeruginosa*. Increasing carbapenem use attributable to emergence of Gram-negative bacteria producing extended-spectrum β-lactamases will enhance antibiotic resistance in *P. aeruginosa*.**

Trial Number: **ISRCTN75875670 (http://www.controlled-trials.com/ISRCTN75875670). (Crit Care Med 2011; 39:000–000)**

Key Words: antibiotics; antibiotic resistance; *Enterobacteriaceae*; intensive care; *Pseudomonas spp*

Nosocomial infections are associated with increased morbidity and mortality in patients treated in intensive care units (ICUs) (1). To treat these infections, many patients need antibiotic treatment, but this is also considered an important cause of emerging antibiotic resistance (2, 3). In ICUs, the problem of antibiotic resistance is even more urgent because of high vulnerability of patients, many invasive procedures, high antibiotic-selective pressure, and high prevalence of resistant bacteria (2). When infections are caused by antibiotic-resistant bacteria, in-hospital mortality rates and length of hospital stay are higher compared to those for infections caused by antibiotic-susceptible bacteria (4).

Infections caused by antibiotic-resistant bacteria in the ICU are almost always preceded by colonization, which may result from either endogenous or exogenous acquisition (5). In case of endogenous acquisition, a patient is already colonized with, initially, undetectable bacterial numbers, which rank increase above detection limits, for instance, because of selective antibiotic pressure. However, it is also possible that antibiotic-susceptible bacteria acquire resistance mechanisms (or start to express resistance traits), changing their phenotype from susceptible to resistant (6). Again, antibiotic exposure is believed to be critical for this process.

Exogenous acquisition is caused by microorganisms from the ICU environment, either inanimate or animate. Resistant bacteria may be transferred from patient to patient, most frequently through temporarily contaminated hands of healthcare workers (7). Although antibiotic-selective pressure may facilitate events of cross-transmission, lapses in adherence to basic hygiene measures must be considered crucial for this mode of transmission of antibiotic resistance.

From the Julius Center for Health Sciences and Primary Care (DSYO, MJMB), University Medical Center Utrecht, Utrecht; Department of Intensive Care Medicine (IPJ, JK), University Medical Center Utrecht, Utrecht; Department of Medical Microbiology (AGB), St. Elisabeth Hospital Tilburg, Tilburg, the Netherlands; Department of Medical Microbiology (MALvH, MJMB), University Medical Center Utrecht, Utrecht; Department of Intensive Care (BS), St. Elisabeth Hospital Tilburg, Tilburg, the Netherlands.

Supported, in part, by the Netherlands Organisation for health research and development (ZonMw project number 62300037).

M.B. has received research funding from the Netherlands Organization of Scientific Research (NWO Vici 918.76.611). The remaining authors have not disclosed any potential conflicts of interest.

For information regarding this article, E-mail: d.s.y.ong@umcutrecht.nl

DOI: 10.1097/CCM.0b013e318225756d

Few studies have quantified the effects of antibiotic exposure on the endogenous selection of antibiotic resistance in *Pseudomonas aeruginosa* (8–10). However, in these studies the role of exogenous acquisition as a cause for resistance acquisition has not been ruled out, thereby obscuring direct effects of antibiotic exposure on endogenous acquisition of antibiotic resistance. Furthermore, other Gram-negative bacteria like *Enterobacter* species have not been rigorously investigated on this specific topic. In this study, we aimed to quantify the occurrence of a phenotype switch from susceptible to resistant in *P. aeruginosa* and *Enterobacter* species in colonized ICU patients.

MATERIALS AND METHODS

Study Design and Patient Population. From January 2007 through February 2008, a prospective cohort study was performed among patients admitted to the ICU for at least 48 hrs and colonized with *P. aeruginosa* and *Enterobacter* species. Four ICUs participated: two units (ten and eight beds, respectively) in the University Medical Center Utrecht and two units (each with eight beds) at St. Elisabeth Hospital in Tilburg, a large teaching hospital. Patients readmitted to the ICU after initially being discharged from the ICU were assigned as new patients in this study. All ICUs had a mixed population of adult patients, including surgical and nonsurgical patients. This cohort study was embedded within a crossover trial evaluating the effects of open and closed endotracheal suctioning on cross-transmission (11). The Institutional Review Board of both hospitals waived the requirement for informed consent because cultures were part of the surveillance program.

Outcome. The primary outcome was the incidence of acquired antibiotic resistance, which was defined as the conversion from carriage with antibiotic susceptible to antibiotic-resistant bacteria in subsequent respiratory tract cultures. The effects of the following antibiotics on antibiotic resistance were assessed: ciprofloxacin, ceftazidime, meropenem, and piperacillin-tazobactam for *P. aeruginosa* and *Enterobacter*, and cotrimoxazol, gentamicin, ceftriaxon, and tobramycin for *Enterobacter*.

To quantify antibiotic use in our study population, the number of defined daily doses (DDDs) per 100 patient-days was calculated according to the ATC/DDD Index 2010 from the World Health Organization Collaborating Centre for Drug Statistics Methodology (12). The number of acquired antibiotic resistance events was expressed per 100 days of antibiotic exposure in which patients were at risk for development of antibiotic resistance. Antibiotic treatment was only considered if it had been prescribed before the date of onset of resistance.

Bacterial Sampling. All patients admitted to the ICU were screened on admission, twice weekly (Monday, Thursday), and on discharge for bacterial colonization of the respiratory tract. All cultures (endotracheal aspirate in mechanically ventilated patients, oropharyngeal swabs in nonventilated patients) were analyzed according to hospital protocol. The following minimum inhibitory concentrations for determining resistant categories for the different antibiotics were used according to the Clinical Laboratory Standards Institute: ciprofloxacin $\geq$4 mg/L; ceftazidime $\geq$32 mg/L; meropenem $\geq$16 mg/L; piperacillin-tazobactam $\geq$128/4 mg/L; cotrimoxazol $\geq$4/76 mg/L; gentamicin $\geq$16 mg/L; ceftriaxone $\geq$64 mg/L; and tobramycin $\geq$16 mg/L (13).

To exclude the occurrence of possible cross-transmission, genotyping was conducted for *P. aeruginosa* and *Enterobacter* species isolates. From patients colonized with one or both species, the first isolate (per pathogen) was genotyped, as were subsequent isolates in case of a change in antibiogram, morphologic differences, or when ten or more cultures with identical antibiograms had been obtained. Cross-transmission was defined as acquired colonization with a genetically identical pathogen and with overlapping time periods to a potential source patient. Genotyping was performed after the trial was finished; therefore, medical staff was not aware of the results during the trial. *P. aeruginosa* isolates were genotyped with multiple-locus variable-number tandem repeats analysis (14), and *Enterobacter* species were genotyped with DiversiLab (15). Multiple-locus variable-number tandem repeats analysis patterns were analyzed with BioNumerics software version 5.10 (Applied Maths), and single locus variants (when the profile varies at one locus) were used as cut-off point for genetic relatedness. For *Enterobacter* species, analysis was performed with DiversiLab software (version 3.4) using 95% similarity as a cut-off point for genetic relatedness.

Data Analysis

To determine acquisition of antibiotic resistance, only patients for whom at least two microbial cultures were available were included in analysis; in patients with only one culture, it was not retrievable whether possible antibiotic resistance was acquired.

To assess the effect of antibiotics administered during ICU admission, Cox proportional hazards models were used. The following covariates were considered for our multivariate models: age, gender, Acute Physiology and Chronic Health Evaluation II score, simultaneous use of other antibiotics, previous use of antibiotics before ICU admission, ICU day of first colonization, and surgical or nonsurgical patient. For every multivariate model, each covariate was tested for confounding by adding it to a univariate model containing the antibiotic exposure variable and examining its effect on the β coefficient of the antibiotic exposure variable. Variables that caused substantial confounding (a change in the β coefficient of $>$10%) were included in the final model. The time interval between first positive culture and the occurrence of resistance acquisition was used as time variable. The date of acquisition was determined as the date on which the first resistant isolate was obtained from the patient. Bivariate analyses with Spearman correlation coefficients (ρ) were performed to rule out multicollinearity among variables entered in multivariate analysis.

Differences in antibiotic resistance acquisitions between patients exposed to antibiotic treatment and patients not exposed to antibiotic treatment were expressed by hazard ratios with corresponding 95% confidence intervals (CIs). Data were analyzed with SPSS version 16.0 for Mac.

RESULTS

In total, 1201 patients were admitted to one of the ICUs for at least 48 hrs, and among them 316 patients were colonized with *P. aeruginosa* or *Enterobacter* species (Fig. 1). In 111 of the colonized patients, only one positive microbial culture with *P. aeruginosa* or *Enterobacter* species was acquired, leaving 205 patients with two or more isolates, corresponding to 126 and 108 patients with *P. aeruginosa* and *Enterobacter* species, respectively. In total, 29 patients had combined colonization.

Patients in the *P. aeruginosa* group were colonized later and had a longer length of stay compared to those colonized with *Enterobacter* species (Table 1). Trauma was more frequently the reason for admission in patients colonized with *Enterobacter* species, whereas a respiratory cause was the most frequent reason in *P. aeruginosa*. The mortality rates in the ICU were 17.5% and 16.7% for patients colonized with *P. aeruginosa* and *Enterobacter* species, respectively (Table 1). Antibiotic exposure was highest to ciprofloxacin in both groups, being 25.9 and 29.7 DDDs per 100 patient-days (Table 2). None of the included patients received aerosolized antibiotics, nor did they receive selective decontamination of the digestive tract or oral antiseptics like chlorhexidine.

Pseudomonas Aeruginosa. Of 126 patients colonized with *P. aeruginosa*, 546

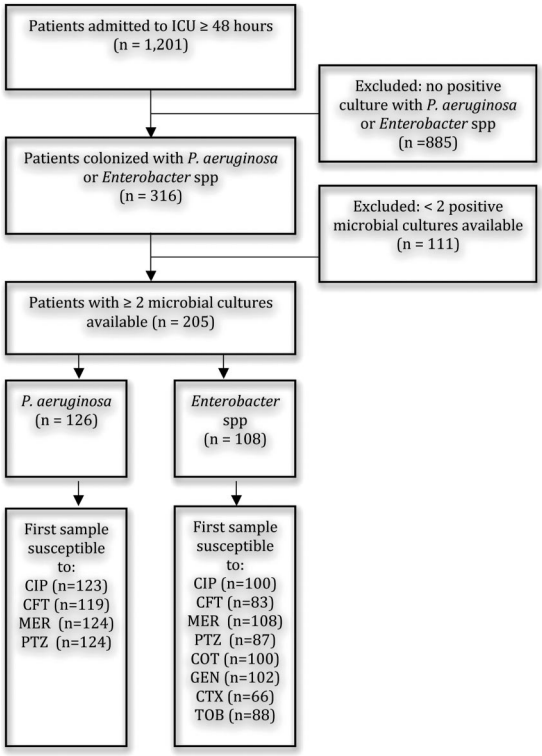

Figure 1. Colonized patients. *CIP*, ciprofloxacin; *CFT*, ceftazidime; *COT*, cotrimoxazol; *CTX*, ceftriaxone; *GEN*, gentamicin; *ICU*, intensive care unit; *MER*, meropenem; *n*, number of patients; *P. aeruginosa*, *Pseudomonas aeruginosa; PTZ*, piperacillin-tazobactam; *spp*, species; *TOB*, tobramycin.

Table 1. Patient characteristics

Characteristic	*Pseudomonas*	*Enterobacter*
No. of patients	126	108
Age in yr, median (IQR)	59 (44−72)	62 (41−75)
Gender % (female)	25	26
Acute Physiology and Chronic Health Evaluation II score, mean (SD)	20.3 (6.5)	19.6 (6.8)
Previous antibiotic use before ICU admission, %	19.5	17.6
Surgical versus not-surgical patient, % surgical	35	37
ICU day of first colonization, median (IQR)	5 (1−15)	4 (1−8)
Ventilation route, number of patients (%)		
Tracheostomy	25 (20)	13 (12)
Endotracheal tube	41 (33)	52 (48)
Endotracheal tube and tracheostomy	59 (47)	43 (40)
No mechanical ventilation	1 (1)	0 (0)
Duration of mechanical ventilation, median days (IQR)	19 (9−29)	16 (9−28)
Suctioning method, number of patients (%)		
Open	66 (52)	47 (44)
Closed	49 (39)	44 (41)
Open and closed	11 (9)	17 (16)
Length of stay, median (IQR)	26 (14−40)	20 (11−36)
Mortality on ICU, %	17.5	16.7
Reason for ICU admission		
Cardiovascular/vascular/circulatory, %	10.3	15.7
Gastrointestinal, %	14.3	11.1
Neurologic, %	4.0	3.7
Neurosurgical, %	4.8	7.4
Pulmonary/respiratory, %	35.7	21.3
Sepsis, %	11.1	8.3
Thorax surgical, %	2.4	0.9
Trauma, %	15.1	28.7
Other, %	2.3	2.9

ICU, intensive care unit; IQR, interquartile range.

cultures were available (median number of follow-up cultures, 3; interquartile range, 1–7). One hundred eighty-nine isolates were selected for genotyping, yielding 81 different *Pseudomonas* multiple-locus variable-number tandem repeats analysis types.

A phenotype switch from susceptible to resistant for one or more antibiotics occurred in 41 patients. Acquisition of resistance to ceftazidime occurred in 29 of 119 episodes (24%) of ceftazidime-susceptible *P. aeruginosa* colonization, corresponding to an acquisition rate of 2.0 (95% CI, 1.3–2.8) per 100 patient-days at risk. Seventeen of 29 patients had been exposed to ceftazidime for a total of 268 days, which yields an acquisition rate of 6.3 (95% CI, 3.4–9.3) per 100 days of antibiotic exposure. Incidence rates were lower for ciprofloxacin, meropenem, and piperacillin-tazobactam, with number of events per 100 days of antibiotic exposure ranging from 2.3 to 2.6 (Table 2).

Five patients (4.0%) had a genotypic match in multiple-locus variable-number tandem repeats analysis type and epidemiologic linkage, suggesting cross-transmission, and therefore were excluded in multivariate analysis. Patients who had meropenem prescribed had the highest risk of development of meropenem resistance, with an adjusted hazard ratio (HR) of 11.1 (95% CI, 2.4–51.5; Table 3). The adjusted HRs for ciprofloxacin and ceftazidime were 4.1 (95% CI, 1.1–16.2) and 2.5 (95% CI, 1.1–5.5), respectively. There appeared no additional risk of piperacillin-tazobactam exposure (adjusted HR, 0.8; 95% CI, 0.2–3.2). Analysis of exposure to any cephalosporin (ceftazidime, cefotaxime, ceftriaxone, cefuroxime, cefazolin) and the development of ceftazidime resistance showed an adjusted HR of 5.9 (95% CI, 1.4−2.5). In this analysis, cross-transmission was defined as genotypical matching and overlapping time periods in the ICU for presumed donor and acceptor. Expanding the time window to 9 days in the definition of cross-transmission resulted in two additional patients with a genotypic match. Excluding these patients in multivariate analyses did not alter the results.

Enterobacter Species. Of 108 patients colonized with *Enterobacter* species, 313 cultures were available (median number of follow-up cultures, 2; interquartile range, 1–4). Of these, 135 isolates were selected for genotyping, yielding 63 different types.

Table 2. Antibiotic use and incidences of acquired antibiotic resistance in *Pseudomonas aeruginosa* and *Enterobacter* species

Antibiotic	Number of Susceptible Episodes[a]	Events[b] (%)	Patient-Days	Events[b] per 100 Patient-Days (95% CI)	Number of Antibiotic-Exposed Episodes (Days of Exposure)	Defined Daily Doses per 100 Patient-Days (95% CI)	Events[c] in Antibiotic-Exposed Episodes	Events[c] per 100 Days of Antibiotic Exposure (95% CI)
Pseudomonas (n = 126)								
Ciprofloxacin	123	11 (8.9)	1803	0.6 (0.3−1.0)	42 (315)	25.9 (23.8−27.9)	8	2.5 (0.8−4.3)
Ceftazidime	119	29 (24.4)	1427	2.0 (1.3−2.8)	45 (268)	19.0 (17.0−21.0)	17	6.3 (3.4−9.3)
Meropenem	124	12 (9.7)	1713	0.7 (0.3−1.1)	24 (221)	14.4 (12.8−16.1)	5	2.3 (0.3−4.2)
Piperacillin-tazobactam	124	18 (14.5)	1594	1.1 (0.6 to −1.7)	23 (229)	13.6 (11.9−15.3)	6	2.6 (0.6−4.7)
Enterbacter (n = 108)								
Ciprofloxacin	100	13 (13.0)	1058	1.2 (0.6 to −1.9)	31 (220)	29.7 (27.0−32.5)	11	5.0 (2.1−7.9)
Ceftazidime	83	14 (16.9)	920	1.5 (0.7−2.3)	18 (121)	7.9 (6.1−9.6)	2	1.7 (−0.6 to 3.9)
Meropenem	108	0 (0.0)	1305	0	22 (262)	24.7 (22.3−27.0)	0	0
Piperacillin-tazobactam	87	11 (12.6)	1080	1.0 (0.4−1.6)	14 (89)	6.1 (4.7−7.6)	2	2.2 (−0.8 to 5.3)
Cotrimoxazol	100	8 (8.0)	1271	0.6 (0.2−1.1)	36 (293)	18.5 (16.4−20.6)	6	2.0 (0.4−3.7)
Gentamicin	102	9 (8.8)	1106	0.8 (0.3 to −1.3)	4 (11)	0.6 (0.2−1.1)	1	9.1 (−7.9 to 26.1)
Ceftriaxone	66	12 (18.2)	643	1.9 (0.8−2.9)	17 (104)	12.8 (10.2−15.3)	5	4.8 (0.7−8.9)
Tobramycin	88	7 (8.0)	979	0.7 (0.2−1.2)	16 (130)	6.7 (5.1−8.2)	1	0.8 (−0.7 to 2.3)

CI, confidence interval.

[a]First isolate susceptible for antibiotic; [b]acquired resistance events in both antibiotic-exposed and nonexposed episodes; [c]acquired resistance events in antibiotic-exposed episodes.

Table 3. Cox regression analysis in patients with *Pseudomonas aeruginosa* and *Enterobacter* species

	Pseudomonas (n = 121)		*Enterobacter* (n = 105)	
	Crude HR (95% CI)	Adjusted HR (95% CI)	Crude HR (95% CI)	Adjusted HR (95% CI)
Ciprofloxacin vs. no ciprofloxacin	2.8 (0.7−10.9)	4.1 (1.1−16.2)[a]	1.7 (0.6−4.7)	1.5 (0.5−4.3)[b]
Ceftazidime vs. no ceftazidime	2.8 (1.3−6.1)	2.5 (1.1−5.5)[c]	1.0 (0.3−3.4)	0.8 (0.2−3.1)[d]
Meropenem vs. no meropenem	8.7 (2.2−33.9)	11.1 (2.4−51.5)[e]	—	—
Piperacillin-tazobactam vs. no piperacillin-tazobactam	2.0 (0.7−5.6)	0.8 (0.2−3.2)[f]	1.1 (0.2−5.3)	1.3 (0.3−6.5)[g]
Cotrimoxazol vs. no cotrimoxazol	n/a	n/a	3.1 (0.6−15.8)	3.1 (0.6−15.8)[h]
Gentamicin vs. no gentamicin	n/a	n/a	2.5 (0.3−20.0)	4.8 (0.5−45.4)[i]
Ceftriaxone vs. no ceftriaxone	n/a	n/a	1.6 (0.5−4.7)	2.4 (0.7−8.9)[j]
Tobramycin vs. no tobramycin	n/a	n/a	0.6 (0.1−5.4)	0.4 (0.04−4.7)[k]

CI, confidence interval; HR, hazard ratio; n/a, not applicable.

[a]Adjusted for gender, previous use of antibiotics, and intensive care unit day of first colonization; [b]adjusted for simultaneous use of other antibiotics, previous use of antibiotics, intensive care unit day of first colonization, and surgical or not surgical patient; [c]adjusted for intensive care unit day of first colonization; [d]adjusted for age, gender, simultaneous use of other antibiotics, previous use of antibiotics, and surgical or not surgical patient; [e]adjusted for Acute Physiology and Chronic Health Evaluation II score; [f]adjusted for age, gender, Acute Physiology and Chronic Health Evaluation II score, simultaneous use of other antibiotics, previous use of antibiotics, intensive care unit day of first colonization, and surgical or not surgical patient; [g]adjusted for age, gender, simultaneous use of other antibiotics, intensive care unit day of first colonization, and surgical or not surgical patient; [h]no adjustment required; [i]adjusted for age, Acute Physiology and Chronic Health Evaluation II score; [j]adjusted for age, Acute Physiology and Chronic Health Evaluation II score, simultaneous use of other antibiotics, and previous use of antibiotics; [k]adjusted for age, gender, Acute Physiology and Chronic Health Evaluation II score, intensive care unit day of first colonization, and surgical or not surgical patient. Number of episodes, excluding episodes with possible cross-transmission.

A phenotype switch from susceptible to resistant for one or more antibiotics occurred in 46 patients. Acquisition of resistance to ciprofloxacin occurred in 13 of 100 episodes (13%) of ciprofloxacin-susceptible *Enterobacter* colonization, corresponding to an acquisition rate of 1.2 (95% CI, 0.6–1.9) per 100 patient-days at risk. Eleven of 13 patients had been exposed to ciprofloxacin for a total of 220 days, which yields an acquisition rate of 5.0 (95% CI, 2.1–7.9) per 100 days of antibiotic exposure. A similar incidence rate was observed for ceftriaxone (4.8 per 100 days of exposure; 95% CI, 0.7–8.9), whereas the incidence rate for cotrimoxazol was lower (2.0 per 100 days of exposure; 95% CI, 0.4−3.7). Incidence rates could not be reliably calculated for other antibiotics because of limited numbers of events (Table 2).

In three patients (2.8%), a genotypic match in Diversilab typing was found. These patients were excluded in multivariate analyses for the reason of possible cross-transmission. Patients with antibiotic exposure were not associated with higher risks for acquiring antibiotic resistance compared to patients without exposure (Table 3). Exposure to any cephalosporin also was not significantly associated with development of ceftazidime resistance (adjusted HR, 1.9; 95% CI, 0.4−2.5).

DISCUSSION

In this study, a phenotypical switch from susceptible to resistant for at least one antibiotic occurred in 41 ICU patients colonized with *P. aeruginosa* and 46 colonized with *Enterobacter* species. For respiratory tract colonization with *P. aeruginosa*, exposure to meropenem was, after adjustment for covariates, associated with the highest risk of resistance development (adjusted HR, 11.1; 95% CI, 2.4−51.5). Among 124 patients colonized with meropenem-susceptible *P. aeruginosa*, meropenem exposure was 14.4 DDD per 100 patient-days, yielding 2.3 resistance acquisition events per 100 days of antibiotic exposure. In contrast, no single event of meropenem resistance acquisition was documented among 108 patients colonized with meropenem-susceptible *Enterobacter* species, despite

meropenem exposure of 24.7 DDD per 100 patient-days.

Few studies have assessed the effects of individual patient antibiotic exposure on the acquisition of antibiotic resistance in *P. aeruginosa* by using time-dependent variables. In a retrospective study in a single tertiary care hospital in the United States, quinolones, third-generation cephalosporins, and imipenem were all associated with acquisition of antibiotic resistance among *Enterobacteriaceae* and *P. aeruginosa* when analyzed at the individual patient level. Among these antibiotics, imipenem was associated with the highest risk (8). In another tertiary care hospital in the United States, emergence of resistance to imipenem and ciprofloxacin among *P. aeruginosa*, after exposure to these antibiotics was considerably higher than the risk of ceftazidime resistance after ceftazidime exposure (9). In a French study of ICU patients, the risk of *P. aeruginosa* resistance to imipenem (and piperacillin-tazobactam, to a lesser extent) was strongly linked to imipenem exposure, and no such risk could be demonstrated for ceftazidime use (10).

Our study differs from these studies in that we investigated a specific patient population (i.e., ICU patients only) instead of a hospital-wide population (8, 9), we used colonization data from protocolized surveillance instead of culture results from samples submitted to the microbiology laboratory for clinical indication (8–10), we meticulously ruled out possible events of cross-transmission through genotyping and epidemiologic linkage, and we included *Enterobacter* species as a separate group in our analysis. Quantifying the occurrence of cross-transmission is important because such events may create nonlinear dynamics, obscuring the direct effects of antibiotic exposure. The standardized surveillance used in our study minimizes the risk of selection bias, because obtaining cultures for clinical reasons is more likely to be performed in the more severely ill patients.

Our findings, together with those from previous studies (8–10), strongly suggest that carbapenems pose a more serious risk to inducing antibiotic resistance in *P. aeruginosa* than other β-lactam antibiotics and fluoroquinolones. Nevertheless, the CIs around the risk estimates were large, which can be attributed to the small number of events. This underscores the difficulties of accurately determining the direct associations between antibiotic use and resistance. Even after inclusion of 1201 consecutive ICU patients and analyzing 1093 microbiological cultures in 205 patients with either *P. aeruginosa* or *Enterobacter*, colonization CIs of HRs were overlapping and we were unable to quantify increased risks for *Enterobacter* species. Naturally, similar studies in settings with higher levels of antibiotic use and higher acquisition rates would have more power to accurately quantify risk associations. Of note, the difficulties to determine these associations on an individual patient level should not be embraced to use aggregated data instead, because this might lead to wrong interpretations (8).

Although the baseline prevalence of antibiotic resistance for *P. aeruginosa* in this study population (1%–6%) was lower compared to that of other ICU populations (5%–37%) (9, 16, 17), it does not affect our findings and or ability to extrapolate to other ICU populations, because we focus on the direct effect of antibiotic exposure on the process of antimicrobial resistance development in previous sensitive bacteria within a single patient.

Our study had a few limitations. First, the date of phenotype switch to antibiotic resistance was determined as the date of the first resistant isolate. Although extensive and regular culturing was conducted in this study, the exact number of days at risk would be slightly lower when the exact day of resistance switch was known, thereby increasing the incidence rates per 100 patient days at risk or per 100 days of antibiotic exposure. However, this would not have altered our HRs significantly, because both exposed and not exposed group of patients would have been equally influenced. Second, inherent to the observational design of our study, results may have been influenced by confounding variables. We attempted to minimize this by adjusting for confounding variables, such as previous and simultaneous antibiotic use, in multivariate analysis. Furthermore, resistance development not only may result from the type of antibiotic or the number of antibiotic exposure days but also may result from the actual dosing of antibiotics. The latter variable, however, was not explicitly included, although dosages are to some extent incorporated in the calculation of DDDs. Finally, we did not investigate the development of multiple antibiotic resistance. Combined resistance acquisition in *P. aeruginosa* was observed in half of meropenem resistance acquisitions and in half of ceftazidime-acquired resistances. In ciprofloxacin and piperacillin-tazobactam, approximately 80% to 90% concerned the development of combined resistance. It is difficult to include multiple resistances in time-dependent analyses, because resistance development for multiple antibiotics did not always occur simultaneously. Furthermore, the numbers of combined resistance development were too low for statistical analysis.

Our findings underscore the potential detrimental consequences of increased usage of carbapenems in ICU patients. With the global emergence of extended-spectrum β-lactamase–producing *Enterobacteriaceae*, it will be challenging to balance the increasing need to treat patients with carbapenems against the risks of creating antibiotic resistance.

CONCLUSION

Meropenem use in ICU patients with *P. aeruginosa* was associated with antibiotic resistance development to meropenem. The association was stronger for meropenem than for other antibiotics. These findings indicate that an increase of carbapenem use as a result of the global emergence of Gram-negative bacteria producing extended-spectrum β-lactamases creates a serious risk for rapid emergence of carbapenem resistance among *P. aeruginosa*. Therefore, antibiotic stewardship to optimize carbapenem use (i.e., to minimize its unnecessary use) is recommended.

ACKNOWLEDGMENTS

We thank all the participating intensive care units, the medical microbiologists at the participating hospitals, and ZonMw.

REFERENCES

1. Vincent JL, Rello J, Marshall J, et al: International study of the prevalence and outcomes of infection in intensive care units. *JAMA* 2009; 302:2323–2329
2. Kollef MH, Fraser VJ: Antibiotic resistance in the intensive care unit. *Ann Intern Med* 2001; 134:298–314
3. Goossens H, Ferech M, Vander Stichele R, et al: Outpatient antibiotic use in Europe and association with resistance: A cross-national database study. *Lancet* 2005; 365:579–587
4. Shorr AF: Review of studies of the impact on Gram-negative bacterial resistance on outcomes in the intensive care unit. *Crit Care Med* 2009; 37:1463–1469
5. Pelupessy I, Bonten MJ, Diekmann O: How to

assess the relative importance of different colonization routes of pathogens within hospital settings. *Proc Natl Acad Sci U S A* 2002; 99:5601–5605
6. Livermore DM: Multiple mechanisms of antimicrobial resistance in Pseudomonas aeruginosa: Our worst nightmare? *Clin Infect Dis* 2002; 34:634–640
7. Nijssen S, Florijn A, Top J, et al: Unnoticed spread of integron-carrying Enterobacteriaceae in intensive care units. *Clin Infect Dis* 2005; 41:1–9
8. Harbarth S, Harris AD, Carmeli Y, et al: Parallel analysis of individual and aggregated data on antibiotic exposure and resistance in gram-negative bacilli. *Clin Infect Dis* 2001; 33:1462–1468
9. Carmeli Y, Troillet N, Eliopoulos GM, et al: Emergence of antibiotic-resistant Pseudomonas aeruginosa: Comparison of risks associated with different antipseudomonal agents. *Antimicrob Agents Chemother* 1999; 43:1379–1382
10. Georges B, Conil JM, Dubouix A, et al: Risk of emergence of Pseudomonas aeruginosa resistance to beta-lactam antibiotics in intensive care units. *Crit Care Med* 2006; 34: 1636–1641
11. Jongerden IP, Buiting AG, Leverstein-van Hall MA, et al: Effect of open and closed endotracheal suctioning on cross-transmission with Gram-negative bacteria: A prospective crossover study. *Crit Care Med* ●●●
12. WHO Collaborating Centre for Drug Statistics. ATC/DDD Index 2011. Available at: http://www.whocc.no/atc_ddd_index/. Accessed January 31, 2011
13. Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing; Twentieth Informational Supplement*. CLSI document M100-S20 (ISBN 1-56238-716-2)
14. Vu-Thien H, Corbineau G, Hormigos K, et al: Multiple-locus variable-number tandem-repeat analysis for longitudinal survey of sources of Pseudomonas aeruginosa infection in cystic fibrosis patients. *J Clin Microbiol* 2007; 45:3175–3183
15. Fontana C, Favaro M, Minelli S, et al: Acinetobacter baumannii in intensive care unit: A novel system to study clonal relationship among the isolates. *BMC Infect Dis* 2008; 8:79
16. Hanberger H, Garcia-Rodriguez JA, Gobernado M, et al: Antibiotic susceptibility among aerobic gram-negative bacilli in intensive care units in 5 European countries. French and Portuguese ICU Study Groups. *JAMA* 1999; 281:67–71
17. Neuhauser MM, Weinstein RA, Rydman R, et al: Antibiotic resistance among gram-negative bacilli in US intensive care units: Implications for fluoroquinolone use. *JAMA* 2003; 289:885–888