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Antibiotic Usage and Risk of Colonization and Infection with Antibiotic-Resistant Bacteria: a Hospital Population-Based Study▽

Evelina Tacconelli,[1]* Giulia De Angelis,[1] Maria Adriana Cataldo,[1] Elisabetta Mantengoli,[2] Teresa Spanu,[1] Angelo Pan,[3] Giampaolo Corti,[4] Anna Radice,[5] Lucia Stolzuoli,[2] Spinello Antinori,[5] Franco Paradisi,[4] Giampiero Carosi,[3] Roberto Bernabei,[1] Massimo Antonelli,[1] Giovanni Fadda,[1] Gian Maria Rossolini,[2] and Roberto Cauda[1]

Departments of Infectious Diseases, Microbiology, Gerontology and Intensive Care and Anesthesiology, Università Cattolica Sacro Cuore, Rome, Italy[1]; Departments of Molecular Biology, Section of Microbiology, and Infectious Diseases, University of Siena, Siena, Italy[2]; Institute of Tropical and Infectious Diseases, Spedali Civili, University of Brescia, Brescia, Italy[3]; Infectious Disease Unit, University of Florence School of Medicine, Florence, Italy[4]; and Department of Clinical Sciences L. Sacco, Section of Infectious Diseases and Immunopathology, University of Milan, Milan, Italy[5]

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Accurate assessment of risk factors for nosocomial acquisition of colonization by antibiotic-resistant bacteria (ARB) is often confounded by scarce data on antibiotic use. A 12-month, nested, multicenter cohort study was conducted. Target ARB were methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and ciprofloxacin-resistant *Pseudomonas aeruginosa* (CR-PA). Nares and rectal swabs were obtained before and after starting antibiotics. Pulsed-field gel electrophoresis was done to define genetic relatedness of the strains. Primary outcomes were (i) the mean time, in days, for acquisition of target ARB colonization in patients previously not colonized; (ii) the rate of acquisition per 1,000 antibiotic-days according to different classes of antibiotics; (iii) the rate of infection caused by the same bacteria as those previously isolated in screening samples; and (iv) the risk factors for ARB acquisition. In total, 6,245 swabs from 864 inpatients were processed. The rate of acquisition was 3%, 2%, and 1% for MRSA, VRE, and CR-PA, respectively. The rate of acquisition of ARB per 1,000 antibiotic-days was 14 for carbapenems, 9 for glycopeptides, and 6 for broad-spectrum cephalosporins and quinolones. The highest rates of acquisition were observed for carbapenems in dialyzed and diabetic patients. Four risk factors were independently associated with acquisition of target ARB: use of carbapenems, age of >70 years, hospitalization for >16 days, and human immunodeficiency virus infection. During the 30-day follow-up, 4 among 42 patients newly colonized by ARB (9%) suffered from an infection due to the same bacteria as those isolated in a previous screening sample. Colonizing and infecting strains from single patients were genotypically identical. Identifying ARB colonization early during antibiotic therapy could target a high-risk hospitalized population that may benefit from intervention to decrease the risk of subsequent nosocomial infections.

The control of nosocomial antibiotic-resistant infections is a public health priority worldwide. Meta-analyses have documented that bloodstream infection caused by methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended-spectrum β-lactamase-producing *Enterobacteriaceae*, and multidrug-resistant *Acinetobacter baumannii* are associated significantly with mortality (7, 9, 11, 24). Numerous papers have demonstrated that prior antimicrobial drug exposure is a strong risk factor for colonization and infection due to a drug-resistant pathogen (2, 25, 27). However, the association between antibiotic therapy and the acquisition of antibiotic-resistant bacteria (ARB) is still unclear and is often confounded by scarce data on antibiotic usage. In our opinion, two major questions are still unsolved. When does an antibiotic select colonizing ARB in a hospitalized patient? Is there a direct correlation between hospital antibiotic usage, acquisition of ARB colonization, and subsequent bacterial infection in a single patient? Results from retrospective studies were not able to answer these complex questions. Previous studies were strongly heterogeneous in defining colonization and infection. In particular, the extension of time at risk in single studies ranged from a few days up to 1 year. A few small prospective studies are available (10, 17). In a double-blind clinical trial, the acquisition of stool colonization with resistant *Enterobacteriaceae* occurred significantly after treatment with piperacillin-tazobactam (10).

To the best of our knowledge, no previous hospital-based studies have assessed temporal changes in nasal and intestinal colonization in the predominance of resistant and susceptible bacterial strains in patients after starting antibiotic therapy. Accordingly, the main goals of our study were to compare rates of ARB acquisition in different treatment groups and to define the temporal relationship between the start of antibiotic therapy, the acquisition of new colonization in patients previously not colonized, and the development of a bacterial infection

* Corresponding author. Mailing address: Departments of Infectious Diseases, Università Cattolica del Sacro Cuore, Largo A. Gemelli 8, 00168, Rome, Italy. Phone: 39-06-30154945. Fax: 39-06-3054519. E-mail: etacconelli@rm.unicatt.it.

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caused by the same strain as that isolated in a screening sample. Target ARB were MRSA, VRE, and ciprofloxacin-resistant *Pseudomonas aeruginosa* (CR-PA). Primary outcomes were (i) the mean time, in days, for acquisition of target ARB colonization in patients previously not colonized; (ii) the rate of acquisition per 1,000 antibiotic-days according to different classes of antibiotics; (iii) the rate of infection caused by the same bacteria as those previously isolated in screening samples; and (iv) risk factors for ARB acquisition.

MATERIALS AND METHODS

Study size, subjects, and design. A 12-month, nested, multicenter cohort study was performed in seven wards in five university hospitals in Italy during a period commencing in January 2006. The following five hospitals were involved: Agostino Gemelli Hospital, Catholic University of Medicine, Rome; University Hospital of Siena, Siena; L. Sacco Hospital, University of Milan, Milan; Spedali Civili Hospital, University of Brescia, Brescia; and Careggi Hospital, University of Florence, Florence. The departments ranged from 400 to 1,500 hospital admissions per year. Infection control policies to prevent the spread of ARB did not differ among centers and followed international guidelines (3, 4, 19). The policies included active surveillance, staff education, contact precautions, and hospital formulary management. Local ethical committee approvals were obtained in all centers. MRSA was endemic in all hospitals, with methicillin resistance being detected in 17% to 37% of invasive isolates. Resistance to vancomycin in enterococci ranged from 5% to 10% of invasive isolates, while resistance to ciprofloxacin in *P. aeruginosa* ranged from 50% to 75% of invasive isolates.

Inpatients were eligible for participation in the study if they started antibiotics during their hospital stay. Antibiotic treatment was determined using clinical evidence of bacterial infection and/or microbiological findings. Antibiotics were given per os and/or intravenously and included penicillins (including beta-lactam/beta-lactamase inhibitor combinations), glycopeptides (teicoplanin and vancomycin), narrow-spectrum to broad-spectrum cephalosporins, clindamycin, metronidazole, cotrimoxazole, macrolides, carbapenems (imipenem and meropenem), quinolones (levofloxacin and ciprofloxacin), aminoglycosides, chloramphenicol, rifampin (rifampicin), and linezolid. Exclusion criteria were prescription of an antibiotic for surgical prophylaxis, refusal to participate, antibiotic therapy in the previous 48 h, and pregnancy. All patients were interviewed by an infectious diseases fellow before enrolling in the study. Data were prospectively recorded on a standardized form.

Definitions and variables. The following variables were recorded at the study enrollment (i.e., first day of antibiotic and screening sample): patient demographics; date of hospital admission, hospital service, date and reason of admission, transfer from another hospital or ward, and residence in a long-term care facility or nursing home; history of alcoholism or intravenous drug abuse; ambulatory status; history of cirrhosis, diabetes, neoplastic disease, chronic renal failure, human immunodeficiency virus (HIV) infection, or bone marrow transplantation; presence of prosthetic heart valves or vascular reconstructive grafts; and presence of invasive devices. Hospitalizations, intensive care unit (ICU) admissions, and surgical procedures within the previous 30 days were also recorded. Antibiotic therapy was studied according to reason for antibiotic therapy, type, dosage, route of administration, and duration. A composite score of comorbid illnesses was derived using the Charlson score (5).

Surveillance samples (anterior nose and rectum) were taken on the first day of antibiotic (t_0) and after 2 (t_1), 4 (t_2), 7 (t_3), 15 (t_4), and 30 (t_5) days. Follow-up cultures were performed in outpatient clinics if the patient was discharged from the hospital. Carriage was defined as positive isolation from one or more screening samples. Acquisition was defined as a conversion from a negative baseline sample to a positive screening sample during follow-up. Serial colonizing ARB in single patients were genotypically defined. Nasal and rectal cultures were also obtained from the staff of wards at the beginning and at the end of the study. This group included nurses and all staff and research-dedicated doctors having contact with patients. These cultures were handled in the same manner as those of the patient's cultures.

All patients included in the study were followed to determine whether they developed clinical infection with ARB. Patients were followed during the hospitalization and afterwards for a total of 30 days from the inclusion in the study. ARB infection was defined as recovery of the organism from either normally sterile sites or nonsterile sites concomitant with diagnosis of infection by the primary physician caring for the patient. All patients developing bacterial infections were followed until the resolution of the disease. Nosocomial and health care-associated infections were defined using the Centers for Disease Control and Prevention definitions (12). All target ARB recovered from surveillance cultures and clinical samples were further characterized for genetic relatedness.

To identify risk factors for the acquisition of target ARB, a nested case-control study was performed. Designation as a case patient was based on whether the patient with negative cultures at baseline acquired a new colonization by target ARB during the follow-up. The control group was composed of patients with negative cultures at baseline and from whom ARB were not isolated. Antibiotic therapy was analyzed until the new colonization in cases and for the duration of treatment among controls.

Microbiological studies. All centers followed the same microbiological protocol. Nasal swabs were streaked onto *S. aureus* ID and chromID MRSA media (bioMérieux, Marcy l'Etoile, France), which are selective for *S. aureus* and MRSA, respectively. To detect the presence of VRE and *P. aeruginosa*, the rectal specimens were plated onto Columbia, chromID VRE, and MacConkey agar plates (bioMérieux). Clinical samples were processed by conventional methods. Identification to the species level and antimicrobial susceptibility testing were carried out with the Vitek 2 system (bioMérieux, Inc, Hazelwood, MO). Conventional manual biochemical methods were used when the automated system provided indeterminate results. Results of susceptibility testing were interpreted in accordance with Clinical Laboratory Standards Institute (CLSI) guidelines (6). All target ARB recovered from surveillance cultures or clinical samples were stored at −70°C and shipped to a central laboratory (Siena) for further phenotypic and genotypic characterization. In cases of patients acquiring both susceptible and resistant strains, both isolates were frozen and genotyped. Glycopeptide resistance genotypes were identified by the PCR method as described by Depardieu et al. (8). Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed as described by Kondo et al. (18). Genotyping of MRSA and VRE isolated by macrorestriction analysis through pulsed-field gel electrophoresis (PFGE) was carried out as previously described (28). Genotyping of *P. aeruginosa* by PFGE was performed by the method of Römling et al. (23).

Statistical analysis. Quantitative variables were tested for normal distribution and compared by means of the two-tailed t test. Differences in groups were assessed by use of the χ^2 test and Fisher's exact test. The precision of relative risk was determined by calculating a 95% confidence interval. A P value less than 0.05 was considered statistically significant. The assessable population for each target organism included all treated patients from whom data from baseline screening and at least one follow-up screening were available. The incidence of colonization was defined by the number of new cases of colonization for 1,000 days of antibiotic therapy. Only patients with a negative baseline (t_0) were included in the longitudinal analysis. The length of exposure to antibiotic therapy for patients newly colonized by ARB was defined by the number of days between inclusion in the study (i.e., first screening sample) and the date of the first positive sample. The length of exposure to antibiotic therapy for patients not colonized by ARB was defined by the number of days between inclusion in the study and the date of antibiotic withdrawal. The time-to-event analysis (in days) between inclusion in the study and the acquisition of colonization was done using the Cox proportional hazard regression model, in which the number of days until acquisition of ARB was the dependent variable. Potential risk factors for ARB acquisition were analyzed by univariate analysis. Variables from the univariate analysis with a P value of less than 0.01 were considered for inclusion in multivariate logistic regression analysis. Backward stepwise logistic regression was performed, and the model that was considered biologically plausible and had the lowest −2-log likelihood ratio was chosen as the final model. Results are presented as multivariate (adjusted) risk ratios. The Hosmer and Lemeshow goodness-of-fit test was used to assess model fit. Statistical analysis was performed using the software program Inter-cooled Stata (Stata Statistical Software, version 8.0).

RESULTS

Patient characteristics. Eight hundred ninety-six patients were asked to participate in the study; 32 of them refused enrollment. A total number of 6,245 samples (3,143 nasal and 3,102 rectal) were collected. The most important epidemiological features of patients included in the study are illustrated in Table 1. Mean duration of hospitalization (standard deviation [SD]) before baseline screening was 5 days (11 days). The most commonly prescribed antibiotics were broad-spectrum cephalosporins (31%), followed by quinolones (29%) and macrolides

TABLE 1. Demographic and clinical characteristics of 864 inpatients included in the study

Variable	No. (%) positive or mean ± SD
Male sex	492 (57)
Age (yr)	50 ± 23
Total length of hospitalization (days)	20 ± 20
Previous ambulatory visit(s)[a]	248 (29)
Previous hospitalization(s)[a]	203 (24)
Residence in long-term care facility or nursing home	24 (3)
Previous ICU admission[a]	44 (5)
Previous surgery[a]	82 (9)
HIV infection	178 (21)
Tracheotomy[b]	32 (4)
Dialysis	11 (1)
Central venous catheter[b]	81 (9)
Urinary catheter[b]	148 (17)
Diabetes	103 (12)
Cancer	109 (13)
Chronic renal failure	55 (6)
Cirrhosis	54 (6)
Transplantation	13 (1.5)

[a] Within 30 days.
[b] At study enrollment.

TABLE 2. Diagnosis and type of antibiotics at study enrollment

Variable	No. (%)
Most common diagnosis for antibiotic prescription[a]	
Pneumonia	292 (34)
Skin infection	84 (10)
Urinary infection	80 (9)
Bacteremia	44 (5)
Meningoencephalitis	33 (4)
Abdominal infection	32 (4)
Most common etiological agent(s)[b]	
Staphylococcus aureus	43 (12)
Enterococci	41 (11)
Pseudomonas aeruginosa	42 (11)
Other gram-positive bacteria	113 (31)
Other gram-negative bacteria	124 (34)
Class or type of antibiotic	
Broad-spectrum cephalosporins	270 (31)
Quinolones	251 (29)
Macrolides	165 (19)
Glycopeptides	115 (13)
Carbapenems	105 (12)
Metronidazole/clindamycin	74 (9)
Penicillins	37 (4)
Cotrimoxazole	32 (4)
Rifampin	25 (3)
Linezolid	17 (2)
Narrow-spectrum to expanded-spectrum cephalosporins	11 (1)
Chloramphenicol	6 (1)

[a] Data available for 856 patients.
[b] Of 864 suspected bacterial infections, 363 (42%) were etiologically proven during hospitalization.

(19%). Reasons for prescribing antibiotics and etiological agents are shown in Table 2.

Overall, 9,300 days of antibiotic therapy were analyzed. Mean duration of therapy (SD) was 8.6 (6.2) days for carbapenems, 7.9 (6.6) days for broad-spectrum cephalosporins, 9.1 (6.6) days for quinolones, 8.5 (6.4) days for glycopeptides, 8.1 (5.8) days for macrolides, and 8.9 (5.6) days for piperacillin-tazobactam. Of the 864 patients who had culture screening performed, 47 (5%) had at least one screening sample positive for MRSA, 16 (2%) had at least one screening sample positive for VRE, and 15 (2%) had at least one screening sample positive for CR-PA.

Baseline colonization (t_0). Before starting antimicrobial therapy, 140 patients (16%) harbored *S. aureus* in the nose, 434 patients (50%) harbored *Enterococcus* species, and 24 patients (3%) harbored *P. aeruginosa* in rectal samples. At baseline, the prevalence of target ARB was 4%. In particular, the baseline rate was 3%, 1%, and 0.5% for MRSA, VRE, and CR-PA, respectively. Twenty-four percent of patients colonized by ARB at t_0 had at least one course of therapy within the 15 days before their inclusion, for the most part with quinolones.

New colonization in patients with negative results at t_0. Two hundred eighty-three patients (283/696; 40%) acquired new colonization during the 30-day follow-up. Seventy-four patients acquired colonization with staphylococci (52 with methicillin-susceptible *S. aureus* and 22 with MRSA), 28 acquired colonization with *P. aeruginosa* (17 with ciprofloxacin-susceptible *P. aeruginosa* and 11 with CR-PA), and 10 acquired colonization with VRE. One patient acquired both MRSA and VRE. The rate of acquisition was 3% (22/724), 2% (10/430), and 1% (11/840) for MRSA, VRE, and CR-PA, respectively. A high percentage of new acquisition (40%) was detected within 48 h of the start of antibiotic therapy. MRSA acquisition appeared after a mean (SD) of 8 (4) days (range, 2 to 16), VRE appeared after 6 (6) days (range, 2 to 19), and CR-PA appeard after 9 (8) days (range, 2 to 28) of antibiotic therapy.

The incidence of new colonization by ARB was calculated for 1,000 antibiotic-days. Table 3 shows the incidence of acquisition according to antibiotic classes, length of therapy (stratified by 5, 10, and 15 days), and high-risk classes of patients. The highest risk for ARB acquisition was observed for the use of carbapenems, with 14 new cases for 1,000 antibiotic-days. The incidence increased in the presence of the following risk factors: dialysis and diabetes, ICU admission, and cirrhosis. The incidence of acquisition was 9 for glycopeptides, 6 for broad-spectrum to fourth-generation cephalosporins, and 6 for quinolones. For MRSA acquisition, the highest risks were observed for carbapenem and macrolide usage (eight new cases for 1,000 antibiotic-days for both classes).

Infections with target ARB. Follow-up was completed in 85% of included patients. If a patient did not perform the last screening sample, he or she was contacted by telephone, whenever possible, in order to obtain information. During the 30-day follow-up, antibiotics were prescribed by physicians for 145 patients (17%) with suspected bacterial infections. In 82 of those patients (57%), a microbiologically proven bacterial infection was detected. None of the patients suffered from a relapsing disease. Among the microbiologically proven infections, 39 (48%) were caused by *P. aeruginosa*, 26 (32%) by *S. aureus*, and 10 (12%) by enterococci. The sites of infection included the lung (40 patients), urine (27 patients), bloodstream (20 patients), and skin and soft tissues (four patients). Fourteen episodes (14/82, 17%) were caused by the same bacterium as that isolated in a previous surveillance sample. In particular, 4 out of 42 (9%) patients with new ARB colonization developed an infection due to the same bacterium ac-

TABLE 3. Incidence of acquisition for 1,000 antibiotic-days by antibiotic class, patient risk factor, and duration of therapy for the overall target ARB (i.e., MRSA, VRE, and CR-PA) and specific for MRSA

Antibiotic class and risk factor[a]	ARB incidence/1,000 days of antibiotic				MRSA incidence overall
	Overall	By duration of therapy:			
		5 days	10 days	15 days	
Carbapenems	13.8	18.3	13.2	7.6	7.9
Dialysis	29.4				
Diabetes	28.6				
ICU	22.8				
Cirrhosis	20.4				
Broad-spectrum cephalosporins	5.8	5.1	3.5	13.5	2.4
Chronic renal failure	27.3				
Cancer	15.8				
HIV infection	10.9				
Cirrhosis	10.6				
Age of >70 yrs	8.1				
Quinolones	5.9	6.6	5.2	17.2	3.1
Age of >70 yrs	8.3				
Glycopeptides	9.2	11.3	8.0	21.7	3.2
HIV	19.5				
Cirrhosis	15.1				
Macrolides	5.8	7.2	10.9	6.3	8.2
Chronic renal failure	22.7				
Cancer	16.8				
Piperacillin-tazobactam	6.5	11	3.1		3.5
Age of >70 yrs	16.2				

[a] Only relevant risk factors are reported.

TABLE 5. Variables associated in multivariate analysis with ARB acquisition

Risk factor	Adjusted relative risk (95% CI[a])	*P* value
Length of hospitalization[b]	2.5 (1.2–5.1)	0.01
HIV infection	2.1 (1.1–4.4)	0.04
Use of carbapenems	2 (1–3.8)	0.04
Age of >70 yrs	1.5 (1.1–2)	0.008

[a] CI, confidence interval.
[b] More than 16 days before the study enrollment.

quired after the start of antibiotic therapy. Two infections were due to MRSA (skin and ventilation-associated pneumonia), and two were due to CR-PA (urinary tract infection and pneumonia). The mean interval (SD) between the first positive sample and the appearance of infection was 13 (9) days (range, 4 to 30). The patient with CR-PA pneumonia died.

Staff screening. Infection control surveillance cultures were obtained from 139 ward staff. Cultures from two nurses were positive for MRSA. Both nurses were successfully treated with mupirocin, showing negative cultures posttreatment.

Risk factors for ARB acquisition. A nested case control study was performed to define the inpatients at risk of acquiring ARB colonization among those starting antibiotics. Seven hundred fifteen patients were included: 42 cases and 673 controls. Table 4 shows the main epidemiological and clinical variables in cases and controls. In the multivariate analysis (Table 5), the use of carbapenems, age of >70 years, HIV infection, and length of stay (LOS) of >16 days before enrollment remained significant predictors of subsequent acquisition

TABLE 4. Risk factor analysis including 42 cases and 673 controls

Variable	No. (%) or mean ± SD for group		*P* value
	Cases	Controls	
Age (yr)	55 ± 22	49 ± 23	0.07
Male sex	28 (67)	444 (56)	0.2
Length of hospitalization before enrollment (days)	14 ± 27	4 ± 9	0.02
Previous hospitalization	19 (45)	173 (22)	0.0005
Previous ambulatory visit[a]	12 (29)	220 (28)	0.9
Residence in long-term care facility or nursing home	2 (5)	20 (3)	0.4
Previous ICU admission[a]	6 (14)	34 (4)	0.003
Diabetes	6 (14)	91 (12)	0.6
HIV infection	12 (29)	163 (21)	0.2
Urinary catheter[b]	13 (31)	124 (16)	0.01
Tracheotomy[b]	4 (10)	23 (3)	0.02
Central venous catheter[b]	6 (14)	70 (9)	0.2
Cirrhosis	2 (5)	50 (6)	0.7
Cancer	5 (12)	99 (13)	0.9
Chronic renal failure	5 (12)	42 (5)	0.07
Dialysis	1 (2)	8 (1)	0.4
Treatment with class/type of antibiotic			
Broad-spectrum cephalosporins	12 (29)	249 (37)	0.2
Narrow-spectrum to expanded-spectrum cephalosporins	1 (2)	10 (1)	0.4
Metronidazole/clindamycin	3 (7)	37 (6)	0.7
Carbapenems	12 (29)	81 (12)	0.002
Quinolones	17 (40)	224 (33)	0.3
Glycopeptides	9 (21)	96 (14)	0.2
Macrolides	10 (24)	151 (22)	0.8
Penicillins	10 (24)	164 (24)	0.9

[a] Within 30 days.
[b] At study enrollment.

of target bacteria when adjusting for other variables such as colonization pressure, age, sex, and comorbidities (McFadden R^2, 0.68).

Molecular analysis. SCC*mec* analysis showed that 67% of MRSA strains were type I, 17% were type IV, 4% were type II, and 3% were type III. All VRE tested showed the presence of the *vanA* gene. PFGE analysis of target ARB showed that all strains were genotypically different, except for one strain of CR-PA identified in three patients in Rome and in two patients in Brescia.

Six patients colonized with methicillin-susceptible *S. aureus* at t_0 acquired colonization with MRSA (5%). Three patients colonized with vancomycin-susceptible enterococci (1%) and two patients colonized with ciprofloxacin-susceptible *P. aeruginosa* (10%) at baseline acquired VRE and CR-PA, respectively. Eleven patients initially colonized with resistant flora (11/36, 30%) harbored susceptible organisms in a subsequent sample. The PFGE analysis done to compare susceptible and resistant strains isolated in different screening samples in an individual patient did not show any correlation, suggesting that all new ARB colonizations were new acquisitions regardless of colonization with susceptible bacteria. Colonizing and infecting strains in an individual patient were shown to be genotypically identical. Staff screening and patient samples did not show any correlation.

DISCUSSION

This study was designed to assess the acquisition of antibiotic-resistant microorganisms in hospitalized patients after starting antibiotics. Overall, including 864 patients for a total of 6,245 samples, we found that 5% of patients were newly colonized by ARB after starting antibiotic therapy. A significantly high percentage of isolations appeared within 48 h of the start of therapy. This colonization occurred more rapidly for VRE (on average after 6 days of therapy) and later for MRSA and CR-PA (after 9 days of therapy). A small percentage of patients showed an initial colonization by susceptible strains followed by a colonization by resistant strains. The use of carbapenems, age of more than 70 years, HIV infection, and a LOS of more than 16 days before the start of the antibiotic therapy were independently associated with the acquisition of ARB after adjusting for major confounders.

In vitro and in vivo studies have already documented that exposure to carbapenems increases the risk of isolation of ARB (1, 13, 14, 29). In our study the use of carbapenems was also associated with the highest incidence of colonization for 1,000 antibiotic-days (14 new cases). This value rises in the presence of dialysis, diabetes, ICU admission, and cirrhosis.

Not unexpectedly, an age of more than 70 years and previous LOS of more than 16 days were strongly associated with ARB acquisition. A number of factors contribute to an increased risk of colonization and infection among the elderly population, including alteration in the immune system, malnutrition, and social and economic factors (16, 20, 26). Older age might also be a marker for other risk factors not covered in our study. Prolonged duration of hospitalization almost doubled the risk for ARB acquisition, probably due to patients' risk factors (having more severe underlying conditions) and hospital-related risk factors such as colonization pressure.

Several immunological HIV-related alterations and epidemiological risk factors predispose infected patients to ARB colonization and infection. A recent case control study observed that a low CD4 cell count, a history of multiple hospitalizations, and exposure to beta-lactams were statistically significant risk factors for MRSA bloodstream infection in HIV-infected patients (30).

Our study showed that 9% of patients newly colonized by ARB developed an infection by the 30-day follow-up. A previous study of ICU patients showed that 25% of patients previously colonized with extended-spectrum beta-lactamase-producing bacteria had a subsequent extended-spectrum beta-lactamase-positive clinical culture (15). The results from the genotyping support the concept that colonizing bacteria provide the subsequent infection. Of course, typing concordance cannot be used to definitively exclude the possibility that the infecting strain originated from other sources such as health care workers or patients not undergoing antibiotic therapy. However, it is important to underline that genotypic analysis did not show any correlation between the patients' strains within the same center. Our data further underline that antibiotic therapy has a role not only in the development of new colonization but also in the emergence of nosocomial infections caused by the same strains. Previous studies have already documented that colonization is a risk factor for antibiotic-resistant infections (21, 22), but this is the first study that correlates this event directly to a previous antibiotic therapy cycle.

There were several shortcomings in our study. First, the data set on which we performed our analysis was limited by the small number of patients colonized with ARB. This might have hampered our ability to fully check for possible confounding factors and might have underestimated the role of specific antibiotic or combination therapy. Although the conclusions based on these data are statistically significant, confirmation of our findings in a larger clinical trial would be desirable. In particular, our study was not able to distinguish the effect of monotherapy from that of combination therapy. Data were analyzed for the most commonly used associations such as glycopeptides plus cephalosporins or carbapenems with no significant results. Second, the lack of combined sites of screening and of stool samples might have underestimated the relationship of some antibiotics with gastrointestinal absorption. We cannot exclude the possibility that ARB were already present in the gut or in the nose of the patients and were not detected. Third, intermittent colonization, not detected at study enrollment, might have played a role. Lastly, we did not detect infections after more than 30 days of antibiotic therapy.

Our findings contribute toward a better understanding of the effect of antibiotic therapy on the acquisition of antibiotic-resistant microorganisms and development of infections in hospitalized patients, although more studies with a greater sample size are needed. The risk of acquiring ARB colonization varies between different classes of antibiotics and is highest for dialysis and diabetes patients after carbapenem usage. The risk associated with a selected antibiotic is also strictly related to the preexisting condition of the patient, length of hospitalization, and age. We believe that the information obtained from prospectively monitoring the colonization status of high-risk patients after starting antibiotics could directly ben-

efit patient care. Readily available colonization information could impact empirical therapy for nosocomial infections and might suggest the application of a decolonization protocol for high-risk patients such as dialysis patients. Further studies would be needed to confirm results and to validate the potential benefits of prospective screening of inpatients after antibiotic therapy. Controlled, prospective, clinical trials should measure colonization acquisition rates of ARB and include rates of subsequent infections in their analysis of adverse effects and economical modeling.

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