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Emergence of Imipenem-Resistant Gram-Negative Bacilli in Intestinal Flora of Intensive Care Patients

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Intestinal flora contains a reservoir of Gram-negative bacilli (GNB) resistant to cephalosporins, which are potentially pathogenic for intensive care unit (ICU) patients; this has led to increasing use of carbapenems. The emergence of carbapenem resistance is a major concern for ICUs. Therefore, in this study, we aimed to assess the intestinal carriage of imipenem-resistant GNB (IR-GNB) in intensive care patients. For 6 months, 523 consecutive ICU patients were screened for rectal IR-GNB colonization upon admission and weekly thereafter. The phenotypes and genotypes of all isolates were determined, and a case control study was performed to identify risk factors for colonization. The IR-GNB colonization rate increased regularly from 5.6% after 1 week to 58.6% after 6 weeks in the ICU. In all, 56 IR-GNB strains were collected from 50 patients: 36 *Pseudomonas aeruginosa* strains, 12 *Stenotrophomonas maltophilia* strains, 6 *Enterobacteriaceae* strains, and 2 *Acinetobacter baumannii* strains. In *P. aeruginosa*, imipenem resistance was due to chromosomally encoded resistance (32 strains) or carbapenemase production (4 strains). In the *Enterobacteriaceae* strains, resistance was due to AmpC cephalosporinase and/or extended-spectrum β-lactamase production with porin loss. Genomic comparison showed that the strains were highly diverse, with 8 exceptions (4 VIM-2 carbapenemase-producing *P. aeruginosa* strains, 2 *Klebsiella pneumoniae* strains, and 2 *S. maltophilia* strains). The main risk factor for IR-GNB colonization was prior imipenem exposure. The odds ratio for colonization was already as high as 5.9 (95% confidence interval [95% CI], 1.5 to 25.7) after 1 to 3 days of exposure and increased to 7.8 (95% CI, 2.4 to 29.8) thereafter. In conclusion, even brief exposure to imipenem is a major risk factor for IR-GNB carriage.

Intestinal flora form a major reservoir of Gram-negative bacilli (GNB), including members of the family *Enterobacteriaceae* and nonfermenters such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, all of which are potentially pathogenic for patients hospitalized in intensive care units (ICUs). These GNB are increasingly resistant to antibiotics and particularly to broad-spectrum cephalosporins, because of the global spread of extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae* and because of the emergence of *Enterobacteriaceae* and *P. aeruginosa* producing high levels of AmpC cephalosporinase (1–3). Carbapenems are currently the only active beta-lactams effective against these bacteria; this has led to an increase in their use not only for documented infections but also for empirical treatment of acquired hospital infections such as those occurring in ICU patients (3). Thus, selective pressure for carbapenem resistance has spread progressively (4); in response, resistance to carbapenems emerged rapidly during the 1990s and continues to increase worldwide not only in nonfermenter GNB but also in *Enterobacteriaceae* (5). Carbapenem resistance in GNB can result from various mechanisms, including (i) selective loss of external membrane permeability (such as OprD porin loss in *P. aeruginosa*) (6); (ii) the combination of impermeability with various broad-spectrum β-lactamases (extended-spectrum β-lactamase and/or cephalosporinase) (7–9); and (iii) carbapenem-hydrolyzing enzymes, i.e., carbapenemases. This last mechanism is of particular concern, because the genes encoding carbapenemases (e.g., bla_{KPC}, bla_{VIM}, bla_{OXA-23}, bla_{OXA-48}, bla_{IMP}, and bla_{NDM}) are carried by transmissible genetic elements with high dissemination potential (10, 11). In addition, carbapenem-resistant GNB are often resistant to other classes of antibiotics such as aminoglycosides, fluoroquinolones, and cotrimoxazole (12), leaving very few therapeutic options (13). ICU-acquired infections due to imipenem-resistant GNB (IR-GNB) are associated with more-severe clinical outcomes and higher morbidity and mortality (14, 15). Control of IR-GNB in the ICU is an important method of preserving carbapenem efficacy (16) but is difficult to achieve because their paths of emergence and dissemination are not yet fully described. Intestinal flora play a central role in the epidemiology of antibiotic-resistant GNB (17, 18), and many ICU patient infections originate in the intestinal tract (19). However, the dynamics, characteristics, and risk factors of intestinal colonization by carbapenem-resistant GNB in ICU patients are still poorly described, which precludes the design of tailored control measures. In order to clarify this aspect, we pro-

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spectively analyzed the epidemiology of the intestinal carriage of IR-GNB in the ICU.

MATERIALS AND METHODS

Study design. This study was performed from May to October 2008 in the 26-bed medical ICU (M-ICU) and the 18-bed surgical ICU (S-ICU) of Bichat-Claude Bernard University hospital (Paris, France), a large secondary care teaching hospital with 950 beds. We benefited from the fact that rectal swabs are collected on a routine basis upon admission of each patient to these units and weekly thereafter, for screening of carriage of ESBL-producing *Enterobacteriaceae*. This process is part of a package that aims to control the spread of multiresistant bacteria and also applies isolation procedures and contact precautions to all colonized patients (20).

Policy of antibiotic use in participating ICUs. No selective decontamination protocol was used. Semiinvasive and invasive diagnostic strategies were used to determine the cause of infections. Ventilator-associated pneumonia (VAP) and postoperative peritonitis accounted for over 80% of all nosocomial infections observed. Risk factors for multidrug-resistant (MDR) pathogens were assessed when choosing empirical therapy (21). When present, broad-spectrum antibiotics were used according to the current guidelines (21, 22), combining imipenem (1 g every 8 h [q8h], in the absence of renal failure) and aminoglycoside or fluoroquinolone, plus glycopeptide or linezolid if β-lactam-resistant Gram-positive cocci were suspected. Deescalation was considered when microbiological results became available. The duration of treatment followed current guidelines (21–23). The levels of procalcitonin in plasma were monitored, and the results were used as previously described (23).

Microbiology. During the study period, all rectal swabs from patients admitted for the first time to the ICU were additionally screened for IR-GNB by plating on Drigalski agar (Bio-Rad, Marne-la-Coquette, France) with imipenem and ertapenem Etest strips (bioMérieux, Marcy l'Etoile, France) laid top-to-tail; the plates were incubated at 37°C for 24 h. This method is similar to that described previously except for the use of broth enrichment (24). All GNB (*Enterobacteriaceae* and nonfermenters) growing within the inhibition ellipse of the imipenem strip up to 2 mg/liter and all *Enterobacteriaceae* growing within the inhibition ellipse of the ertapenem strip up to 0.5 mg/liter were collected. These cutoffs were chosen according to the imipenem and ertapenem susceptibility breakpoints defined by the Antibiogram Committee of the French Society of Microbiology (CA-SFM) (http://www.sfm-microbiologie.org). *Escherichia coli* ATCC 25922 (MIC for ertapenem, 0.004 to 0.016 mg/liter and for imipenem, 0.064 to 0.25 mg/liter) and *Pseudomonas aeruginosa* ATCC 27853 (MIC for imipenem, 0.064 to 0.25 mg/liter) were used as controls. Only the first resistant isolate of each species was analyzed. Identification was performed using API 20E for *Enterobacteriaceae* and API 20NE for nonfermenter GNB identification (ID) systems (bioMérieux, Marcy l'Etoile, France). The MICs of imipenem and ertapenem were determined using Etests (bioMérieux, Marcy l'Etoile, France), in accordance with the manufacturer's recommendations. MICs were interpreted according to the recommendations of the CA-SFM. All strains with confirmed resistance or intermediate susceptibility to imipenem or ertapenem were studied further. For these strains, susceptibility to amoxicillin, amoxicillin-clavulanic acid, ticarcillin, ticarcillin-clavulanic acid, piperacillin, piperacillin-tazobactam, cefalotin, cefoxitin, cefotaxime, ceftazidime, cefepime, aztreonam, ertapenem, imipenem, gentamicin, amikacin, tobramycin, nalidixic acid, ofloxacin, ciprofloxacin, co-trimoxazole, and tigecycline was tested using the disk diffusion method on Mueller-Hinton medium (Bio-Rad, Marne-la-Coquette, France); results were interpreted according to the recommendations of the CA-SFM.

Carbapenem resistance mechanisms were analyzed as follows. *P. aeruginosa* strains were first screened for the presence of carbapenemase by positive double-disk synergy tests (DDSTs) between imipenem and EDTA (10 μl, 100 mM), and/or ceftazidime and EDTA. Positive strains were further screened using PCR for carbapenemase genes, including $bla_{\text{VIMgroup1}}$, $bla_{\text{VIMgroup2}}$, $bla_{\text{IMPgroup1}}$, $bla_{\text{IMPgroup2}}$, and bla_{GES} as described previously (25). Amplification products were sequenced and submitted to the National Center for Biotechnology Information library for identification (http://blast.ncbi.nlm.nih.gov). Overproduction of intrinsic β-lactamase AmpC was assessed phenotypically by restoration of susceptibility to ceftazidime on Mueller-Hinton agar plates containing 1,000 mg/liter cloxacillin. Porin OprD-deficient mutants and overproduction of MexAB efflux system were detected as described previously (26).

Enterobacteriaceae strains were first screened for carbapenemase genes, including $bla_{\text{VIMgroup1}}$, $bla_{\text{VIMgroup2}}$, $bla_{\text{IMPgroup1}}$, $bla_{\text{IMPgroup2}}$, bla_{KPC}, $bla_{\text{OXA-48}}$, and bla_{GES} as described previously (25, 27–29). In the absence of carbapenemase, intermediate strains and strains resistant to ertapenem but susceptible to imipenem were not taken into account in further analysis. In contrast, the presence of ESBL was detected in imipenem-resistant strains using the double-disk synergy test as described previously (30), and overproduction of intrinsic or plasmid cephalosporinase AmpC was detected by studying susceptibility on cloxacillin agar also as described previously (31). ESBL and AmpC-positive strains were then screened for the presence of bla_{TEM}, bla_{SHV},$bla_{\text{CTX-Mgroup1}}$, $bla_{\text{CTX-Mgroup2}}$, $bla_{\text{CTX-Mgroup8/25}}$, $bla_{ampC\text{-CITgroup}}$ $bla_{ampC\text{-ENTgroup}}$, and bla_{DHA} genes as described previously (32). Amplification products were sequenced and analyzed as described previously (32). We also used the laboratory database to check whether any of the patients with imipenem-resistant (IR) *Enterobacteriaceae* previously had strains from the same species with the same resistance pattern except for carbapenems (imipenem-susceptible [IS] *Enterobacteriaceae*) isolated from a clinical specimen. If they did, outer membrane protein (OMP) patterns of the IS and IR strains were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and compared as described previously (33).

In *Acinetobacter baumannii*, $bla_{\text{VIMgroup1}}$, $bla_{\text{VIMgroup2}}$, $bla_{\text{IMPgroup1}}$, $bla_{\text{IMPgroup2}}$, bla_{KPC}, $bla_{\text{OXA-23}}$, $bla_{\text{OXA-40}}$, and $bla_{\text{OXA-58}}$ carbapenemase genes were detected by PCR (25, 27–29).

Carbapenem resistance in *Stenotrophomonas maltophilia* is natural and was not further investigated.

Genetic relatedness between *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, and *S. maltophilia* strains and between pairs of IR and IS *Enterobacteriaceae* was determined and analyzed using the semiautomated repetitive-sequence-based PCR (rep-PCR) DiversiLab system (bioMérieux, Marcy l'Etoile, France). Strains were subcultured on Trypticase soy agar (Oxoid, Dardilly, France) at 37°C for 24 h. DNA was extracted using the UltraClean microbial DNA isolation kit (Mo Bio Laboratories, Saint Quentin en Yvelines, France) as described by the manufacturer. The concentration of extracted DNA was determined using a Nanodrop ND1000 spectrophotometer (Labtech). DNA amplification was performed using the designated DL fingerprinting kit for each species in accordance with the manufacturer's instructions. After amplification, PCR products were separated by electrophoresis using microfluidic lab-on-a-chip technology (Agilent bioanalyzer 2100) and analyzed by the DiversiLab software (v3.4) using Pearson correlation coefficient pairwise pattern matching and the unweighted-pair group method using average linkage (UPGMA) clustering. Strains with 97% similarity or more were considered indistinguishable (34).

Risk factor analysis. Carrier patients were defined as those with at least one rectal swab test-positive result for IR-GNB. Carriage detected more than 48 h after ICU admission was considered ICU-acquired GNB. The period between admission and acquisition of IR-GNB carriage was defined as the surveillance period. Each ICU-acquired IR-GNB carrier was matched with a control patient chosen from IR-GNB-negative patients, who were present in the same ICU for at least as long as the surveillance period of the case patient. Demographic data, characteristics at admission (including ICU, patient's location prior to admission, comorbidities, and McCabe and SAPS [Simplified Acute Physiology Score] II scores calculated as described previously (35, 36), and prior colonization with ESBL-producing *Enterobacteriaceae*), antibiotic exposure during ICU stay, and outcome (ICU stay duration and death) were collected for IR-GNB carriers and noncarrier controls. Case and control characteristics were compared using R software (version 2.13.0

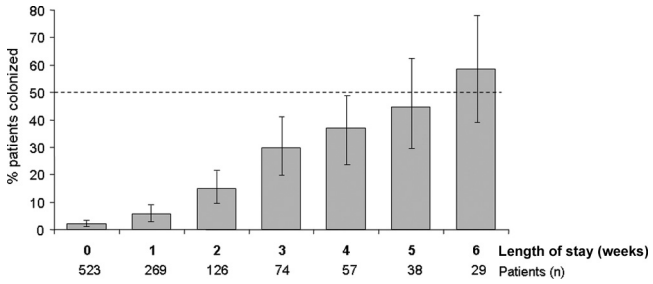

FIG 1 Rates of intestinal colonization by imipenem-resistant gram-negative bacilli in intensive care patients. Bars indicate observed rates ± standard deviation (SD) (error bars).

[http://www.cran.r-project.org]). Bivariate analysis of discrete variables was performed using the two-sided Pearson's chi-squared test and Fisher's exact test ($\alpha = 0.05$). Student's *t* test, and Wilcoxon test were used for continuous variables ($\alpha = 0.05$). Variables with univariate *P* values of <0.15 were included in the multivariate analysis, which was performed using descending stepwise logistic regression.

RESULTS

Study population and prevalence of IR-GNB carriage. There were 523 first admissions to the 2 ICUs during the study period (363 to the medical and 160 to the surgical ICU).

The male-to-female ratio was 1.80, the median age was 58 years (range, 17 to 95 years), and the median SAPS II score was 37 (range, 6 to 120). The median length of ICU stay was 6 days (2 to 136 days). Two hundred fifty-four patients (48.6%) were screened only once (upon admission), while 269 (51.4%) patients had at least 2 swabs (range, 2 to 19 swabs). IR-GNB prevalence at admission was 2.1% (11/523), 1.9% in M-ICU versus 2.5% in S-ICU (not statistically significant [NS]). The acquisition rate was 14.5% (39/269), 14.4% (24/167) in M-ICU versus 14.7% (15/102) in S-ICU (NS). Thirty-four patients acquired a single IR-GNB, 5 acquired 2 IR-GNB, and a single patient who was already a carrier upon admission acquired a second IR-GNB during the hospital stay. The median time between admission in ICU and acquisition of carriage was 15 days (range, 3 to 135 days). At the first weekly screening after admission, the prevalence of IR-GNB carriers was 5.6% (15/269). This value increased to 15.1% (19/126), 29.7% (22/74), 36.8% (21/57), 44.7% (17/38), and 58.6% (17/29) after 2, 3, 4, 5, and 6 weeks of hospitalization, respectively (Fig. 1).

Strain characteristics. In all, 56 IR-GNB strains were isolated from 50 patients. Nine *P. aeruginosa* strains and 2 *Stenotrophomonas maltophilia* strains were isolated upon admission, while 27 *P. aeruginosa* strains, 10 *S. maltophilia* strains, 3 *K. pneumoniae* strains, 2 *A. baumannii* strains, 1 *Enterobacter aerogenes* strain, 1 *Enterobacter cloacae* strain, and 1 *Hafnia alvei* strain were acquired later. The acquisition rates of IR *P. aeruginosa*, *S. maltophilia*, *Enterobacteriaceae*, and *A. baumannii* carriage were 10.0% (27/269), 3.7% (10/269), 2.2% (6/269), and 0.7% (2/269), respectively ($P < 0.001$). The median times between admission and acquisition of colonization were 13 (12 to 19) days, 15 (4 to 135) days, 21 (3 to 101) days, and 58.5 (52 to 65) days for *Enterobacteriaceae*, *P. aeruginosa*, *S. maltophilia*, and *A. baumannii* strains, respectively (NS).

For *P. aeruginosa* strains, imipenem resistance was due to the association of chromosomally encoded resistance mechanisms (inactivation of the OprD gene alone [$n = 19$] with hyperexpression of AmpC [$n = 6$], overproduction of the MexAB efflux system [$n = 4$], or both [$n = 2$]) except for 4 strains (11%) producing a VIM-2 carbapenemase and 1 strain producing a GES-9 ESBL (Table 1).

No carbapenemase was found in any *Enterobacteriaceae* strains. Among IR *Enterobacteriaceae* strains, 2 *K. pneumoniae* strains produced a plasmid-encoded cephalosporinase DHA-1 and 1 *K. pneumoniae* strain produced an ESBL CTX-M-15, 2 *Enterobacter* spp. overproduced natural AmpC and carried ESBL SHV-12 (in *E. cloacae*) or TEM-24 (*E. aerogenes*), and 1 *Hafnia alvei* strain overproduced AmpC only (Table 1). The MICs for ertapenem and imipenem are presented in Table 1, according to

TABLE 1 Mechanisms of resistance and MICs for imipenem and ertapenem of 56 isolated imipenem-resistant Gram-negative bacilli

Species	No. of strains	Resistance mechanisms[a]		MIC (mg/liter)[b]	
		Enzymes	Other	Imipenem	Ertapenem
P. aeruginosa	19		OprD−	6–>32	ND
	6	AmpC++	OprD−	16–>32	ND
	4		OprD− MexAB efflux ++	24–>32	ND
	2	AmpC++	OprD− MexAB efflux ++	24–32	ND
	1	GES-9	OprD−	>32	ND
	4	VIM-2		>32	ND
Enterobacteriaceae					
K. pneumoniae	2	DHA-1	OMP−	24–32	>32
	1	TEM-1 CTX-M15	NP	3	>32
E. aerogenes	1	TEM-24 AmpC++	OMP−	16	>32
E. cloacae	1	SHV-12 AmpC++	OMP−	32	>32
H. alvei	1	AmpC++	NP	4	32
A. baumannii	2			6–12	ND
S. maltophilia	12	Wild type		ND	ND

[a] OprD−, loss of OprD porin; AmpC++, hyperexpression of AmpC chromosomal cephalosporinase; MexAB efflux ++, hyperexpression of MexAB-OprM system efflux; OMP−, loss or reduced expression of outer membrane protein; NP, OMP analysis not performed.
[b] ND, not determined.

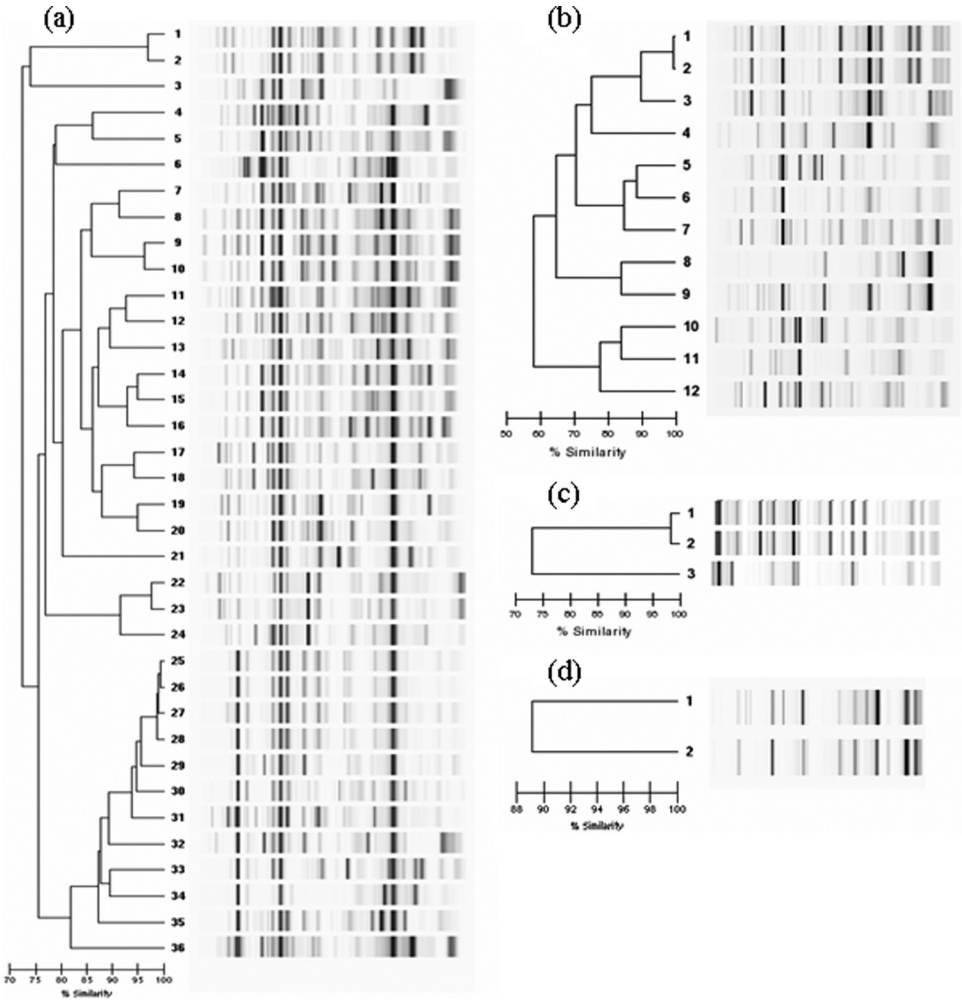

FIG 2 Dendrogram and rep-PCR fingerprints of *Pseudomonas aeruginosa* (a), *Stenotrophomonas maltophilia* (b), *Klebsiella pneumoniae* (c), and *Acinetobacter baumannii* (d).

resistance mechanisms. No carbapenemase was identified in *A. baumannii* strains.

Three pairs of IR and IS *Enterobacteriaceae* of the same species and isolated from the same patients were available for OMP analysis. Strains from each pair had indistinguishable rep-PCR pattern (data not shown). Compared to the susceptible strain, one DHA-1-producing IR *K. pneumoniae* strain lacked a major 35-kDa OMP, the *E. aerogenes* strain lacked a major 37-kDa OMP, and the *E. cloacae* strain had reduced expression of a major 37-kDa OMP.

High genetic diversity was observed among the 36 *P. aeruginosa* strains, except for 1 cluster of 4 strains carrying VIM-2 carbapenemase. Two of the 3 *K. pneumoniae* strains, which were producing DHA-1, were indistinguishable. In contrast, the 2 IR *A. baumannii* strains were different. High genetic diversity was also observed among the 12 *S. maltophilia* strains except for 2 with indistinguishable patterns (Fig. 2).

Risk factors for IR-GNB colonization. This case control study included 36/39 cases; 3 IR-GNB acquirers (2 in M-ICU and 1 in S-ICU) with a surveillance period of >60 days were excluded due to a lack of proper controls. Univariate analysis showed that the only risk factor associated with the acquisition of IR-GNB carriage was prior exposure to imipenem ($P < 0.001$), and this was confirmed by multivariate analysis. In addition, a dose-dependent relationship was identified between length of exposure and likelihood of colonization. The increase in risk, which was already at 5.9 (95% CI, 1.5 to 25.7) over controls in cases that received 1 to 3 days of imipenem treatment, increased further to 7.8 (95% CI, 2.4 to 29.8) in those who received more than 3 days of imipenem treatment. In contrast, prior exposure to penicillin conferred protection (odds ratio [OR] = 0.3; 95% CI, 0.1 to 0.8) (Table 2). This analysis was repeated in the subgroup of patients colonized with IR *P. aeruginosa*; the ORs for IR *P. aeruginosa* carriage acquisition were 5.3 (95% CI, 1.0 to 38.4] and 6.8 (95% CI, 1.2 to 50.2) when patients received fewer than or more than 3 days of imipenem treatment, respectively (Table 3). No risk factor analysis was performed for other species because the numbers of cases were too low.

DISCUSSION

Our main finding is that even a short exposure to imipenem was followed by a significant increase in carriage of IR-GNB. The risk of acquisition was 5.9 times higher in carriers than in controls who received only 1 to 3 days of imipenem treatment and increased to

TABLE 2 Univariate and multivariate analysis of risk factors associated with intestinal colonization of imipenem-resistant Gram-negative bacilli[a]

Characteristic or outcome	No. of individuals or parameter value (%, unless range is specified)		Univariate OR[b]	Univariate P[c]	Multivariate OR[d]
	Carrier patients ($n = 36$)	Controls ($n = 36$)			
Sociodemographic characteristics					
Age, yr [avg (range)]	58.3 (30–86)	59.9 (37–86)		0.57	
Sex ratio (F/M)[e]	0.33	0.64		0.31	
Characteristics at admission					
Type of ICU				1.00	
Surgical	14 (38.9)	14 (38.9)	1.0		
Medical	22 (61.1)	22 (61.1)	1.0 (0.3–2.9)		
Origin				0.69	
Home	8 (22.2)	10 (27.8)	1.0		
Hospital	27 (75.0)	24 (66.7)	1.4 (0.4–4.8)		
Other	1 (2.8)	2 (5.6)	0.6 (0.1–14.4)		
Cancer	2 (5.6)	2 (5.6)	1.0 (0.1–14.5)	1.00	
HIV	3 (8.3)	4 (11.1)	0.7 (0.1–4.7)	1.00	
Respiratory failure	7 (19.4)	3 (8.3)	2.6 (0.5–17.2)	0.31	
Renal failure	6 (16.7)	1 (2.8)	6.8 (0.8–330.4)	0.11	
Cardiac failure	3 (8.3)	4 (11.1)	0.7 (0.1–4.7)	1.00	
Obesity	6 (16.7)	6 (16.7)	1.0 (0.2–4.2)	1.00	
Pulmonary transplantation	3 (8.3)	2 (5.6)	1.5 (0.2–19.5)	1.00	
Diabetes mellitus	4 (11.1)	5 (13.9)	0.8 (0.1–4.0)	1.00	
Cirrhosis	2 (5.6)	2 (5.6)	1.0 (0.1–14.5)	1.00	
McCabe scores				0.07	
0	7 (19.4)	15 (41.7)	1.0		
≥1	29 (80.6)	21 (58.3)	2.9 (0.9–10.0)		
SAPS II [median (range)]	48.5 (13–120)	41 (13–104)		0.36	
ESBL carriage	8 (22.2)	6 (16.7)	1.4 (0.4–5.7)	0.77	
Surveillance period,[f] days [median (range)]	13.5 (3–52)	12.5 (3–52)		0.97	
Antibiotic treatments					
Exposure time to antibiotics, days [median (range)]	11.5 (0–51)	9.0 (0–37)		0.84	
Penicillin exposure	8 (22.2)	16 (44.4)	0.4 (0.1–1.1)	0.08	0.3 (0.1–0.8)
Penicillin and β-lactamase inhibitor exposure	17 (47.2)	20 (55.6)	0.7 (0.3–2.0)	0.64	
Cephalosporin exposure	20 (55.6)	17 (47.2)	1.4 (0.5–3.9)	0.64	
Imipenem exposure	28 (77.8)	14 (38.9)	5.4 (1.8–17.8)	<0.01	
Days of imipenem exposure				<0.01	
0	8 (22.2)	22 (61.1)	1.0		1.0
1 to 3	10 (27.8)	6 (16.7)	4.4 (1.1–20.5)		5.9 (1.5–25.7)
4 to 21	18 (50.0)	8 (22.2)	6.0 (1.7–23.3)		7.8 (2.4–29.8)
Fluoroquinolone exposure	9 (25.0)	8 (22.2)	1.2 (0.3–4.0)	1.00	
Aminoglycoside exposure	25 (69.4)	21 (58.3)	1.6 (0.6–4.8)	0.46	
Glycopeptide exposure	20 (55.6)	11 (30.6)	2.8 (1.0–8.4)	0.06	
Metronidazole exposure	5 (13.9)	6 (16.7)	0.8 (0.2–3.6)	1.00	
Macrolide exposure	5 (13.9)	3 (8.3)	1.8 (0.3–12.3)	0.71	
Colistin exposure	5 (13.9)	2 (5.6)	2.7 (0.4–30.4)	0.43	
Outcome					
ICU stay, days [median (range)]	33.5 (4–173)	15.5 (5–137)		0.14	
Death	13 (36.1)	11 (30.6)	1.3 (0.4–3.9)	0.80	

[a] Imipenem-resistant Gram-negative bacilli including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Enterobacteriaceae*, and *Acinetobacter baumannii*.
[b] Odds ratios (ORs) are given with the 95% confidence intervals in parentheses.
[c] Bivariate analysis was performed using Pearson's chi-squared, Fisher's exact, Wilcoxon, or Welch test ($\alpha = 0.05$).
[d] Variables with univariate P value of <0.15 were included for multivariate analysis, which was performed using descending stepwise logistic regression.
[e] F, female; M, male.
[f] Period of surveillance, period between admission and acquisition of imipenem-resistant Gram-negative bacilli.

7.8 in those who received treatment for longer. It was known that imipenem exposure was associated with the emergence of resistance to imipenem (37, 38), but the risk associated with short-treatment duration (1 to 3 days) was unknown. Our results suggest that prescribing carbapenems empirically before the availability of bacteriological results in patients suspected of GNB infections susceptible only to this class of beta-lactams, i.e., ESBL producers, may increase the likelihood of developing IR-GNB in-

TABLE 3 Univariate and multivariate analysis of risk factors associated with intestinal colonization of imipenem-resistant *Pseudomonas aeruginosa*

Characteristic or outcome	No. of individuals or parameter value (%, unless range is specified)		Univariate OR[a]	Univariate P^b	Multivariate OR[c]
	Carrier patients ($n = 22$ (%))	Controls ($n = 22$)			
Sociodemographic characteristics					
Age, yr [avg (range)]	57.3 (30–74)	60.9 (37–80)		0.28	
Sex ratio (F/M)[d]	0.57	0.29		0.31	
Characteristics at admission					
Type of ICU				1.00	
Surgical	8 (36.4)	8 (36.4)	1.0		
Medical	14 (63.6)	14 (63.6)	1.0 (0.2–4.1)		
Origin				1.00	
Home	6 (27.3)	5 (22.7)	1.0		
Hospital	16 (72.7)	17 (77.3)	0.8 (0.2–3.8)		
Other					
Cancer	1 (4.5)	2 (9.1)	0.5 (0.1–10.0)	1.00	
HIV	3 (13.6)	1 (4.5)	3.2 (0.2–182.0)	0.61	
Respiratory failure	5 (22.7)	2 (9.1)	2.9 (0.4–33.8)	0.41	
Renal failure	3 (13.6)	1 (4.5)	3.2 (0.2–182.0)	0.61	
Cardiac failure	2 (9.1)	3 (13.6)	0.6 (0.1–6.2)	1.00	
Obesity	4 (18.2)	5 (22.7)	0.8 (0.1–4.2)	1.00	
Pulmonary transplantation					
Diabetes mellitus	2 (9.1)	4 (18.2)	0.5 (0.1–3.6)	0.66	
Cirrhosis	1 (4.5)	1 (4.5)	1.0 (0.1–82.1)	1.00	
McCabe scores				0.12	
0	5 (22.7)	11 (50.0)	1.0		1.0
≥1	17 (77.3)	11 (50.0)	3.3 (0.8–15.8)		5.0 (1.1–29.0)
SAPS II (median [range])	55 (15–120)	41 (13–86)		0.05	
ESBL carriage	3 (13.6)	5 (22.7)	0.5 (0.1–3.3)	0.70	
Surveillance period,[e] days [median (range)]	13 (4–37)	13 (3–37)		0.91	
Antibiotic treatments					
Exposure time to antibiotics, days [median (range)]	10.5 (2–23)	8.5 (0–37)		0.52	
Penicillin exposure	6 (27.3)	8 (36.4)	0.7 (0.1–2.8)	0.75	
Penicillin and β-lactamase inhibitor exposure	8 (36.4)	12 (54.5)	0.5 (0.1–1.9)	0.36	
Cephalosporin exposure	13 (59.1)	12 (54.5)	1.2 (0.3–4.7)	1.00	
Imipenem exposure	17 (77.3)	10 (45.5)	3.9 (0.9–18.9)	0.06	
Days of imipenem exposure				0.09	
0	5 (22.7)	12 (54.5)	1.0		1.0
1 to 3	8 (36.4)	6 (27.3)	3.1 (0.6–18.3)		5.3 (1.0–38.4)
4 to 21	9 (40.9)	4 (18.2)	5.1 (0.9–35.1)		6.8 (1.2–50.2)
Fluoroquinolone exposure	6 (27.3)	4 (18.2)	1.7 (0.3–9.6)	0.72	
Aminoglycoside exposure	14 (63.6)	12 (54.5)	1.4 (0.4–5.8)	0.76	
Glycopeptide exposure	12 (54.5)	5 (22.7)	3.9 (0.9–18.9)	0.06	
Metronidazole exposure	4 (18.2)	3 (13.6)	1.4 (0.2–10.9)	1.00	
Macrolide exposure	2 (9.1)	1 (4.5)	2.1 (0.1–129.6)	1.00	
Colistin exposure	4 (18.2)	2 (9.1)	2.2 (0.3–26.9)	0.66	
Outcome					
ICU stay, days [median (range)]	27.5 (9–173)	15.0 (5–137)		0.25	
Death	11 (50.0)	7 (31.8)	2.1 (0.5–8.8)	0.36	

[a] ORs are given with the 95% confidence intervals in parentheses.
[b] Bivariate analysis was performed using Pearson's chi-squared, Fisher's exact, Wilcoxon, or Welch test ($\alpha = 0.05$).
[c] Variables with univariate *P* value of <0.15 were included for multivariate analysis, which was performed using descending stepwise logistic regression.
[d] F, female; M, male.
[e] Period of surveillance, period between admission and acquisition of imipenem-resistant *P. aeruginosa*.

fections that will be even more difficult to treat. Given that the number of such patients is increasing dramatically, this issue should be investigated further.

Our results also show that the acquisition rate of IR-GNB carriage was high (14.5%) and that carriage prevalence increased steadily during the ICU stay. Approximately 60% of patients hospitalized for 6 weeks or more had IR-GNB in their digestive flora.

Previous studies on digestive colonization in ICU patients have focused on *P. aeruginosa*. Among our ICU patients, the acquisition rate of IR *P. aeruginosa* carriage was 10%, similar to the 12% reported in a previous study (38) but higher than the 4.3% reported in another study (37). These studies also showed that prior carbapenem use was associated with acquisition of IR *P. aeruginosa* carriage, with ORs ranging from 3.4 to 7.8 (37, 38). These

results were very similar to our results indicating that the ORs for IR *P. aeruginosa* carriage acquisition were 3.4 and 6.8 in patients who received less than or more than 3 days of imipenem treatment, respectively. However, in the present study, we did not limit our analysis to *P. aeruginosa* but included all IR-GNB, as all species can cause serious infections in ICU patients (39). A previous study comparing the impact of antibiotic therapy on digestive flora did not observe the emergence of carbapenem resistance in *Enterobacteriaceae* of 132 patients receiving ertapenem treatment for 4 to 14 days (40). However, ertapenem and imipenem have different spectra of activity, and their impact on commensal flora appears to be different (41). The fact *P. aeruginosa* was the most prevalent of the isolated species may be explained by the ease with which this species is known to become resistant.

Although not all carbapenemase genes possibly associated with the various species were searched for, we observed great diversity among the species and mechanisms of resistance that emerged after imipenem exposure. Acquired IR-GNB were not restricted to *P. aeruginosa* but also included *S. maltophilia*, *A. baumannii*, and *Enterobacteriaceae*. In terms of mechanisms of resistance, few carbapenemase-producing GNB were isolated. Most IR *P. aeruginosa* bacteria were resistant because of unrelated chromosomal mechanisms, i.e., inactivation of the OprD gene alone, together with hyperexpression of AmpC or with overproduction of the MexAB efflux system, as previously described in France (26, 42). In contrast, imipenem resistance in *Enterobacteriaceae* was due to hyperproduction of AmpC cephalosporinase or the production of ESBL associated with the loss of porins, also as described previously (7–9). However, this may change in future because of the worldwide diffusion of carbapenemase-producing GNB (43, 44). In hospitals where carbapenemase-producing bacteria are becoming endemic (45, 46), they might also be selected under imipenem exposure. Strikingly, we observed very few cases of cross-transmission. Except for 4 strains of VIM-2-producing *P. aeruginosa*, 2 strains of *S. maltophilia*, and 2 DHA-1-producing *K. pneumoniae* strains, all strains were genetically different, suggesting unique emergence of resistance in each individual patient. This may be a result of our active infection control program, but it also emphasizes that selection of mutants from the susceptible bacterial population is a major mechanism of resistance emergence during treatment. This type of emergence and dissemination of resistance can be reduced not only by isolation procedure but also by reduction of exposure of intestinal flora to antibiotics. Some reports suggest that this can be achieved by administering oral beta-lactamases (47, 48) or other compounds (49), as companions for antibiotic treatments, a concept recently termed "eco-evo drugs" (50).

In summary, although the study was restricted to patients from a single hospital, which can be a limit, our results indicate that imipenem use in ICUs is associated with the emergence of IR-GNB in commensal flora even after short-duration treatment. Reducing intestinal flora exposure to carbapenems is a major issue in the control of emergence and spread of resistance. Empirical carbapenem treatments should be initiated only when necessary and deescalation, considered soon as possible.

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We declare that we have no conflicts of interest

This study was part of "usual care" and did not raise any ethical issues.

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