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Enterobacteriaceae Bloodstream Infections: Presence of Integrons, Risk Factors, and Outcome▽

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A prospective observational study was conducted to identify factors associated with bloodstream infections (BSIs) caused by integron-carrying *Enterobacteriaceae* and to evaluate the clinical significance of integron carriage. Consecutive patients with *Enterobacteriaceae* BSIs were identified and followed up until discharge or death. Identification of blood isolates and susceptibility testing were performed by the Wider I automated system. *int-1*-specific PCR, conserved-segment PCR, and DNA sequencing were used to determine the presence, length, and content of integrons. The relatedness among the isolates was examined by pulsed-field gel electrophoresis. Two hundred fifty episodes of *Enterobacteriaceae* BSI occurred in 233 patients; 109 (43.6%) were nosocomial, 82 (32.8%) were community acquired, and 59 (23.6%) were health care associated. Integrons were detected in 11 (13.4%) community-acquired, 24 (40.7%) health care-associated, and 46 (42.2%) nosocomial isolates. Integron-carrying organisms were more likely to exhibit resistance to three or more classes of antimicrobials (odds ratio [OR], 9.84; 95% confidence interval [95% CI], 5.31 to 18.23; $P < 0.001$) or to produce extended-spectrum β-lactamases (OR, 5.75; 95% CI, 2.38 to 13.89; $P < 0.001$) or a VIM-type metallo-β-lactamase (P, 0.003). Inter- or intraspecies integron transfer and cross-transmission of integron-carrying clones were observed. Use of cotrimoxazole (OR, 4.77; 95% CI, 1.81 to 12.54; $P < 0.001$) and a nosocomial or other health care setting (OR, 3.07; 95% CI, 1.30 to 7.22; P, 0.01) were independently associated with BSIs caused by integron-carrying *Enterobacteriaceae*. Patients with a nonurinary source of bacteremia (OR, 9.46; 95% CI, 2.77 to 32.32; $P < 0.001$) and a Pitt bacteremia score of ≥4 (OR, 23.36; 95% CI, 7.97 to 68.44; $P < 0.001$) had a significantly higher 14-day mortality rate, whereas integron carriage did not affect clinical outcomes. These findings may have implications affecting antibiotic policies and infection control measures.

The pattern of life-threatening infections is changing over time, along with our clinical practices and antibiotic usage. In recent years, infections caused by multidrug-resistant gram-negative bacilli have increasingly been recognized as important causes of morbidity and mortality among hospitalized patients (19, 27). Antimicrobial resistance may develop through mutations in chromosomal DNA or through acquisition of resistance genes carried by plasmids or transposons. A substantial proportion of these resistance genes in gram-negative bacilli are packaged as discrete small mobile units into DNA structures called integrons (3, 4, 14, 25, 28). These genetic structures operate as a general gene-capture system and provide a powerful mechanism for the acquisition and dissemination of antimicrobial resistance genes. Integrons possess three essential components in the 5′-conserved segment (5′ CS), including an *int* gene, encoding an integrase; a specific recombination site (*attI* site); and a promoter that directs transcription of the genes carried in the cassette. Most class 1 integrons contain an additional resistance gene, *sulI*, in the 3′ CS, which confers resistance to sulfonamides. More than 60 different gene cassettes conferring resistance to commonly used antimicrobial agents, antiseptics, and disinfectants have been described to be carried by class 1 integrons.

Previous reports have focused on the genetic structure of integrons and have emphasized their role in the acquisition and dissemination of antimicrobial resistance among gram-negative bacilli (11, 13, 17, 18). The aim of the present study was to identify the risk factors associated with bloodstream infections (BSIs) caused by integron-carrying *Enterobacteriaceae* and to evaluate the consequences of these genetic elements on the patient outcome.

MATERIALS AND METHODS

Study design. A prospective observational study was conducted between November 2003 and June 2005 in a 500-bed tertiary care hospital located in the Athens metropolitan area. Consecutive patients with *Enterobacteriaceae* BSIs were identified by daily communication with the clinical microbiology laboratory. The medical records of all patients who had one or more blood cultures positive for *Enterobacteriaceae* and a clinical course consistent with bacteremia were reviewed upon notification and twice a week until discharge or death. Patients with polymicrobial bacteremia were excluded. Pertinent information regarding demographic characteristics, underlying disease, severity of illness, use of immunosuppressive drugs, invasive procedures, length of hospitalization, prior hospitalizations, and prior antibiotic use for at least 5 days over the last 4 months was abstracted in a predesigned form. Antibiotic use for the bacteremia episode was also recorded. The end point was mortality 14 days after the onset of bacteremia or the end of the hospital stay. Patients discharged from the hospital in good condition before 14 days were considered survivors. The study was approved by the institutional review board of the hospital.

Microbiology. Identification of blood isolates and susceptibility testing with commonly used antimicrobials were performed with a Wider I automated system

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(Dade Behring MicroScan, West Sacramento, CA). All isolates were tested for extended-spectrum β-lactamase production by the double-disk diffusion method (Bio-Rad, Marnes-la-Coquette, France), using CLSI (formerly NCCLS) performance standards (16). Production of metallo-β-lactamases was detected by the double-disk diffusion method (imipenem/imipenem plus EDTA) as described previously (22).

Detection and analysis of integrons. Genomic DNAs for PCR amplification were prepared with a QIAamp DNA Mini kit (QIAGEN GmbH, Hilden, Germany). Integrons were detected by PCR amplification of the class 1 integrase-specific *int-1* gene (11). The sizes of the variable regions of integrons were determined by CS-PCR, using primers for the 5′ CS and 3′ CS regions, as described previously (12). Analysis of the CS-PCR products was performed by restriction fragment length polymorphism (RFLP) analysis and sequencing. Briefly, the CS-PCR products were cut from the agarose gel, purified by a QIAquick gel extraction kit (QIAGEN GmbH, Hilden, Germany), and digested with the restriction endonucleases HpaII and MseI. The integrons were classified into groups according to their RFLP patterns. One representative CS-PCR product from each distinct RFLP group was sequenced using an automated sequencer (Avant 3100; Applied Biosystems) to determine the gene cassette content. The results were interpreted by the WU-BLAST 2 program of the European Bioinformatics Institute (www.ebi.ac.uk). Integrons exhibiting the same RFLP patterns were considered to contain identical gene cassettes.

PFGE. The relatedness among integron-positive isolates was examined by pulsed-field gel electrophoresis (PFGE) after XbaI digestion of genomic DNAs (Fermentas Gmb, St. Leon-Rot, Germany), using a CHEF DRIII apparatus (Bio-Rad Laboratories, Athens, Greece) as previously described (9; http://0-www.cdc.gov.mill1.sjlibrary.org/pulsenet/protocols/ecoli_salmonella_shigella_protocols.pdf). Dendrograms of similarity based on the Dice coefficient and clustering by the unweighted-pair group method using average linkages, allowing for 1% tolerance in band positions (1.5% for *Klebsiella pneumoniae*), were constructed using GelCompar I software (Applied Maths, Sint-Martens-Latem, Belgium). Isolates with PFGE pattern similarities of 80% or higher were assigned to the same type (26). Indices of diversity (DI) were calculated by dividing the number of types observed by the number of isolates typed.

Definitions. An episode of BSI was defined as the period of 14 days from the time of collection of the first positive blood culture. BSIs were classified into the following three categories according to the system of Friedman et al. (5): nosocomial (positive cultures obtained from patients already hospitalized for 48 h or longer), health care associated (positive cultures obtained at admission or within 48 h if the patient had received intravenous therapy at home, attended a hospital or hemodialysis clinic, received intravenous chemotherapy, been hospitalized in the preceding 3 months, or resided in a nursing home), and community acquired (positive culture obtained at admission or within 48 h of hospitalization for patients not fulfilling the criteria for health care-associated infection). The infection was determined to be urinary, intra-abdominal, vascular catheter related, pulmonary, soft tissue, primary BSI, or unknown according to Centers for Disease Control and Prevention definitions (6). The severity of bacteremia was assessed at the time of the first positive blood culture by using the Pitt bacteremia score, as described previously (21). To obtain comparisons regarding the severity of underlying illnesses, all patients were classified into one of the following three categories, according to the system of McCabe and Jackson (15): rapidly fatal, ultimately fatal, and nonfatal. Patients on immunosuppressive medication and patients with AIDS or neutropenia were classified as immunocompromised. Appropriate antibiotic therapy for an episode of bacteremia was defined as treatment with at least one antibiotic that had in vitro activity against the infecting organism, initiated within 48 h of the initial positive blood culture.

Statistical analysis. The data were processed and analyzed by using SPSS statistics software, version 12, for Microsoft Windows. For univariate analysis, the χ^2 or Fisher's exact test was used to compare categorical variables, and Student's *t* test or the Mann-Whitney U test was used to compare continuous variables. Variables that were statistically significant at a 10% level were included in a multiple logistic regression model to test for independent associations with integron carriage, 14-day mortality, and in-hospital mortality. Only the first episode of bacteremia for each patient was included in the analysis for mortality. Odds ratios (OR) and 95% confidence intervals (95% CI) are presented when appropriate. A *P* value of <0.05 was considered statistically significant.

RESULTS

During the study period, a total of 250 instances of *Enterobacteriaceae* BSI occurred in 233 patients, including 117 males and 116 females (Table 1). The mean patient age was 64.7 years (range, 17 to 97 years). One hundred nine cases (43.6%) were nosocomial, 82 (32.8%) were community acquired, and 59 (23.6%) were health care associated. The median duration of hospitalization before the onset of bacteremia was 10.34 days. The probable source of bacteremia was the genitourinary tract in 118 instances, the gastrointestinal system in 46 cases, an intravascular catheter in 10 cases, the skin or soft tissue in 8 cases, and the lungs in 7 cases. In 61 instances, no definite portal of entry could be detected. Of all BSIs, 60.8% (152 of 250 cases) were caused by *Escherichia coli*, 18.4% (46 of 250 cases) were caused by *K. pneumoniae*, 6% (15 of 250 cases) were caused by *Proteus mirabilis*, 5.2% (13 of 250 cases) were caused by *Enterobacter cloacae*, 4.4% (11 of 250 cases) were caused by *Serratia marcescens*, and 5.2% (13 of 250 cases) were caused by other *Enterobacteriaceae* species.

TABLE 1. Characteristics of 233 patients with *Enterobacteriaceae* BSIs

Characteristic	No. of patients (%)[a]
Age (yr) (mean ± SD)	64.73 ± 17.02
Gender	
Male	117 (50.2)
Female	116 (49.8)
Underlying disease	
Hematological malignancy	47 (20.2)
Solid tumor	28 (12)
Heart disease	47 (20.2)
Lung disease	23 (9.9)
Diabetes	55 (23.6)
Renal failure	68 (29.2)
Cirrhosis	13 (5.6)
Immunosuppression	85 (36.5)
None	19 (8.2)
Severity of underlying disease	
Rapidly fatal	36 (15.5)
Ultimately fatal	133 (57.1)
Nonfatal	64 (27.5)
Pitt bacteremia score	
0–2	158 (67.8)
3–5	53 (22.8)
>5	22 (9.4)

[a] Unless indicated otherwise.

Presence and analysis of integrons. Overall, 32.4% (81 of 250 cases) of *Enterobacteriaceae* BSIs were caused by integron-carrying organisms. This proportion was higher for nosocomial (42.2%) and health care-associated (40.7%) infections than for community-acquired infections (13.4%). Integrons were carried by 27.6% of *E. coli* isolates, 42.9% of *Klebsiella* isolates, 44.4% of *Enterobacter* isolates, 33.3% of *P. mirabilis* isolates, 16.7% of *Serratia* isolates, and 75% of isolates of other species. Susceptibilities of integron- and non-integron-carrying organisms to commonly used antimicrobials are shown in Table 2. Notably, integron-carrying organisms were more likely to exhibit resistance to three or more classes of antibiotics (OR, 9.84; 95% CI, 5.31 to 18.23; $P < 0.001$) or to produce extended-spectrum β-lactamases (OR, 5.75; 95% CI, 2.38 to 13.89; $P < 0.001$) or VIM-type metallo-β-lactamases (*P*, 0.003). The mean size of integrons derived from isolates causing community-

TABLE 2. Antimicrobial resistance profiles of *Enterobacteriaceae* according to integron carriage

Antibiotic	% of isolates with resistance		*P* value
	Integron positive	Integron negative	
Ampicillin	96.3	60	<0.001
Piperacillin	90.2	32.1	<0.001
Amoxicillin-clavulanate	59.2	27.8	<0.001
Piperacillin-tazobactam	31.2	8.1	<0.001
Cefuroxime	46.9	21.9	<0.001
Cefoxitin	40	16.6	<0.001
Cefotaxime	41.3	6.9	<0.001
Ceftazidime	36.3	4.9	<0.001
Cefepime	29.5	6.2	<0.001
Imipenem	7.6	1.2	0.033
Gentamicin	19.8	4.8	<0.001
Tobramycin	41.8	8.5	<0.001
Aztreonam	26.9	9.4	<0.001
Amikacin	29.7	4.2	<0.001
Ciprofloxacin	49.4	6.9	<0.001
Meropenem	6	2	0.207
Colimycin	12.5	12.5	1.000
Cotrimoxazole	87.7	14.5	<0.001

acquired BSIs was smaller than that of integrons derived from isolates causing hospital-acquired or health care-associated BSIs (1,090 ± 475 bp versus 1,535 ± 735 bp; *P*, 0.043). Analysis of the CS-PCR products revealed 19 distinct groups of integrons. The sizes of the variable regions and the gene cassettes of each group are presented in Table 3. The most commonly detected gene cassettes were those encoding resistance to aminoglycosides and trimethoprim. Nine integrons contained the bla_{VIM} gene, encoding resistance to carbapenems. Eleven isolates contained more than one different integron. For 20 isolates, the variable region of the integrons was not amplified.

PFGE. The high association of integron carriage with nosocomial and health care-associated infections, known to be due frequently to dissemination of specific clones, prompted us to examine the genotypic diversity of our isolates. The first isolate from each patient was typed. In particular, 37 *E. coli* isolates (88% of all *int-1*-positive *E. coli* isolates), 17 *K. pneumoniae* isolates (81%), 6 *E. cloacae* isolates (100%), 3 *P. mirabilis* isolates (60%), and 2 isolates each of *Enterobacter aerogenes* (100%), *Morganella morganii* (100%), and *S. marcescens* (100%) were typed. For *E. coli*, with an overall DI of 90%, PFGE showed that the vast majority of isolates (31 in total [84% of all typed *E. coli* isolates]) belonged to unique types (Table 3). There were only three types represented by two *E. coli* isolates each (six isolates [16%]). In contrast, 71% (12 isolates) of all *K. pneumoniae* isolates (DI = 50%) typed clustered in one of three types containing two to eight isolates each; the remaining five isolates belonged to unique types. Finally, four *E. cloacae* isolates (67% of all typed; DI = 67%) belonged to one of two types; the remaining two isolates displayed unique patterns. All isolates belonging to the four remaining species (*E. aerogenes*, *M. morganii*, *P. mirabilis*, and *S. marcescens*) were of unique types.

Risk factors. Table 4 presents the factors associated with infections caused by integron-carrying organisms. Patients with BSIs due to integron-carrying organisms were more likely to have had nosocomial (OR, 4.71; 95% CI, 2.25 to 9.88; $P < 0.001$) or health care-associated (OR, 4.43; 95% CI, 1.95 to 10.05) infection or longer hospitalization ($P < 0.001$) or to have been hospitalized in the intensive care unit (ICU) ($P < 0.001$). In addition, patients with BSIs caused by integron-carrying organisms were more likely to have had an invasive procedure (OR, 2.69; 95% CI, 1.55 to 4.67; *P*, 0.001) or to have received antibiotics in the preceding 4 months (OR, 5.51; 95% CI, 2.91 to 10.43; $P < 0.001$). Age, gender, the Pitt bacteremia score, and the primary site of infection were not associated with the presence of integrons, while an association of marginal significance was found with the severity of underlying disease. Prior administration of aztreonam (OR, 8.75; 95% CI, 0.96 to 79.38; *P*, 0.039), cotrimoxazole (OR, 6.79; 95% CI, 3.15 to 14.65; $P < 0.001$), β-lactam/β-lactamase inhibitors (OR, 3.22; 95% CI, 1.81 to 5.72; $P < 0.001$), quinolones (OR, 2.73; 95% CI, 1.28 to 5.79; *P*, 0.014) or aminoglycosides (OR, 2.28; 95% CI, 1.14 to 4.57; *P*, 0.025) significantly increased the risk for integron carriage, whereas prior administration of cephalosporins (OR, 1.73; 95% CI, 0.92 to 3.24; *P*, 0.097), carbapenems (OR, 2.12; 95% CI, 0.94 to 4.74; *P*, 0.081), glycopeptides (OR, 2.12; 95% CI, 0.94 to 4.74; *P*, 0.081), macrolides (OR, 1.61; 95% CI, 0.54 to 4.80; *P*, 0.391), metronidazole (OR, 1.56; 95% CI, 0.74 to 3.27; *P*, 0.243), or clindamycin (OR, 2.06; 95% CI, 0.86 to 4.88; *P*, 0.106) was not significantly associated with the presence of integrons. By multivariate analysis, prior administration of cotrimoxazole (OR, 5.14; 95% CI, 2.09 to 12.62; $P < 0.001$) and a nosocomial or other health care setting (OR, 3.07; 95% CI, 1.30 to 7.22; *P*, 0.01) were independently associated with integron carriage.

Outcome. One hundred eighty-five patients were discharged home, and 48 died (all-cause in-hospital mortality, 20.6%). The all-cause 14-day mortality rate among the patients infected with integron-carrying organisms was 15.1% (11 of 73 patients died), and that among patients infected with non-integron-carrying organisms was 10.6% (17 of 160 patients died). By univariate analysis, as shown in Table 5, patients with a nonurinary source of bacteremia, a Pitt bacteremia score of ≥4, and infection with VIM-producing organisms or multidrug-resistant organisms had a higher risk of dying. By multivariate analysis, only the severity of bacteremia, as assessed by a Pitt bacteremia score of ≥4 (OR, 23.36; 95% CI, 7.97 to 68.44; $P < 0.001$) and a nonurinary source of bacteremia (OR, 9.46; 95% CI, 2.77 to 32.32; $P < 0.001$), was independently associated with 14-day mortality. When we used mortality at the end of the hospital stay as an end point, the factors that had independent associations were a Pitt bacteremia score of ≥4 (OR, 11.61; 95% CI, 4.75 to 28.38; $P < 0.001$), a nonurinary source of bacteremia (OR, 7.24; 95% CI, 2.72 to 19.30; $P < 0.001$), and the severity of underlying disease (OR, 4.20; 95% CI, 1.16 to 15.11; *P*, 0.028).

DISCUSSION

Class 1 integrons are found extensively among gram-negative isolates obtained from human, animal, and environmental sources (4, 7, 8, 13, 14, 28). Several ecologic niches, however, facilitate the dissemination of integron-carrying organisms and promote the spread of integrons among different bacterial species. In the present study, the prevalence of integrons was

TABLE 3. Gene cassettes of integron-positive isolates according to bacterial species and PFGE type

Bacterial species	PFGE type (no. of isolates)	No. of integrons	CS-PCR length (bp)	Gene cassette(s)
E. coli	eA (2)	1	1,700	*dfrA17-aadA5*
	eB (1)	1	800	*dfrA7*
	eB (1)	1	700	*dfrA5*
	eC (2)	1	1,700	*dfrA17-aadA5*
	Nongroupable (6)	1	1,700	*dfrA17-aadA5*
	Nongroupable (3)	1	1,900	*dfrA12-orfF-aadA2*
	Nongroupable (2)	1	1,600	*aadA1-dfrA1*
	Nongroupable (2)	1	800	*dfrA7*
	Nongroupable (2)	1	700	*dfrA5*
	Nongroupable (1)	1	2,200	*aadB-cmlA*
	Nongroupable (1)	1	2,000	bla_{OXA1}-*aadA1*
	Nongroupable (1)	1	1,000	*aadA1*
	Nongroupable (1)	1	2,100	*aadA2-sat*
	Nongroupable (12)			Not determined
	Not done (1)	1	1,700	*dfrA17-aadA5*
K. pneumoniae	kA (3)	3	1,900, 3,200, 1,500	*dfrA12-orfF-aadA2*, bla_{VIM-1}-*aacA7-dhfrI-aadA*, *dfrA1-aadA1*
	kA (2)	2	3,200, 800	bla_{VIM-1}-*aacA7-dhfrI-aadA*, *aacA4*
	kA (1)	2	1,900, 1,600	*dfrA12-orfF-aadA2*, *aadA1-dfrA1*
	kA (1)	3	1,900, 3,200, 800	*dfrA12-orfF-aadA2*, bla_{VIM-1}-*aacA7-dhfrI-aadA*, *aacA4*
	kA (1)	2	1,900, 3,200	*dfrA12-orfF-aadA2*, bla_{VIM-1}-*aacA7-dhfrI-aadA*
	kB (1)	1	1,900	*dfrA12-orfF-aadA2*
	kB (1)	1	700	*sat*
	kC (1)	1	3,200	*aacA4-aacC1-aadA1a*
	kC (1)			Not determined
	Nongroupable (2)	1	1,000	*aadA1*
	Nongroupable (1)	1	1,000	*aadA2*
	Nongroupable (1)	1	800	*aacA4*
	Nongroupable (1)	1	1,900	*dfrA12-orfF-aadA2*
	Not done (1)	1	800	*aacA4*
	Not done (1)	1	1,000	*aadA1*
E. cloacae	cA (2)			Not determined
	cB (2)	2	1,600, 1,700	*aadA1-dfrA1*, *aacA4*-bla_{VIM-1}
	Nongroupable (1)	1	3,200	*aacA4-dfrII-aadA1a*
	Nongroupable (1)	1	1,000	*aadA2*
P. mirabilis	Nongroupable (2)	1	3,200	*aacA4-aacC1-aadA1a*
	Nongroupable (1)			Not determined
E. aerogenes	Nongroupable (1)	1	3,200	*aacA4-aacC1-aadA1a*
	Nongroupable (1)	2	1,900, 1,500	*dfrA12-orfF-aadA2*, *dfrA1-aadA1*
S. marcescens	Nongroupable (2)			Not determined
M. morganii	Nongroupable (1)	1	1,700	*dfrA17-aadA5*
	Nongroupable (1)	1	1,500	*aadB-catB3*
Proteus stuartii	Not done (1)	1	1,700	*dfrA14-aadA1*

significantly higher among *Enterobacteriaceae* causing hospital-acquired and health care-associated BSIs than among *Enterobacteriaceae* causing community-acquired BSIs. Nosocomial and other health care settings, prolonged hospital stay, admission to ICU, and invasive procedures were all factors positively associated with integron carriage. Importantly, nosocomial and other health care settings remained independent factors in multivariate analysis. Our results correspond with those reported by others and indicate that the nosocomial environment, particularly the ICU setting, promotes integron dissemination and amplifies the existing reservoir of integrons in the community (10, 17, 24).

In the present study, we observed two routes of integron dissemination, namely, inter- or intraspecies integron transfer and cross-transmission of integron-carrying clones from patient to patient. In particular, some integrons, such as *aacA4-aacC1-aadA1a*, have invaded more than one species (*K. pneumoniae*, *P. mirabilis*, and *E. aerogenes* in this case), while others, e.g., *dfrA17-aadA5*, have spread in different clones within the same species (*E. coli* in this case). On the other hand, cross-transmission of integron-carrying clones also occurred, mainly in the nosocomial environment, where the spread of bacteria from patient to patient is more efficient than that in the community. These results are in agreement with

TABLE 4. Univariate analysis of factors associated with BSIs by integron-carrying *Enterobacteriaceae*

Variable	No. of patients with variable/total no. of patients (%)[a]		OR (95% CI)	*P* value
	Integron-positive BSIs (n = 81)	Integron-negative BSIs (n = 169)		
Age (yr) (mean ± SD)	61.27 ± 19.17	65.55 ± 16.09		0.152
Gender				
Male	38/125 (30.4)	87/125 (69.6)	0.83 (0.49–1.41)	0.589
Female	43/125 (34.4)	82/125 (65.6)		
Type of BSI				<0.001
Community acquired	11/82 (13.4)	71/82 (86.6)	1	
Health care associated	24/59 (40.7)	35/59 (59.3)	4.43 (1.95–10.05)	<0.001
Nosocomial	46/109 (42.2)	63/109 (57.8)	4.71 (2.25–9.88)	<0.001
Antibiotic use in preceding 4 mo				
Yes	66/141 (46.8)	75/141 (53.2)	5.51 (2.91–10.43)	<0.001
No	15/109 (13.8)	94/109 (86.2)		
Cotrimoxazole use in preceding 4 mo				
Yes	26/37 (70.3)	11/37 (29.7)	6.79 (3.15–14.65)	<0.001
No	55/213 (25.8)	158/213 (74.2)		
Hospital location				<0.001
Medical ward	66/213 (31)	147/213 (69)	1	
Surgical ward	8/30 (26.7)	22/30 (73.3)	0.81 (0.34–1.91)	0.631
ICU	7/7 (100)	0/7 (0)		<0.001
Time from admission to bacteremia (days) (mean ± SD)	15.79 ± 23.32	7.73 ± 16.92		<0.001
Time from bacteremia to discharge (days) (mean ± SD)	18.93 ± 28.17	14.96 ± 14.95		0.048
Source of BSI				0.395
Urinary tract	33/118 (28)	85/118 (72)	1	
Unknown	23/61 (37.7)	38/61 (62.3)	1.56 (0.81–3.00)	0.184
Abdomen	16/46 (34.8)	30/46 (65.2)	1.37 (0.66–2.84)	0.393
Intravenous catheter	5/10 (50)	5/10 (50)	2.58 (0.70–9.48)	0.155
Soft tissue	1/8 (12.5)	7/8 (87.5)	0.37 (0.04–3.11)	0.358
Lung	3/7 (42.9)	4/7 (57.1)	1.93 (0.41–9.10)	0.405
Bacterial species				0.108
E. coli	42/152 (27.6)	110/152 (72.4)	1	
K. pneumoniae	21/49 (42.9)	28/49 (57.1)	1.96 (1.01–3.83)	0.053
Other	18/49 (36.7)	31/49 (63.3)	1.52 (0.77–3.00)	0.228
Severity of underlying disease				0.091
Nonfatal disease or no disease	14/65 (21.5)	51/65 (78.5)	1	
Ultimately fatal disease	51/144 (35.4)	93/144 (64.6)	1.99 (1.01–3.96)	0.047
Rapidly fatal disease	16/41 (39)	25/41 (61)	2.33 (0.98–5.52)	0.054
Invasive procedure				
Central venous catheter	28/49 (57.1)	21/49 (42.9)	3.72 (1.95–7.11)	<0.001
Urinary catheter	32/66 (48.5)	34/66 (51.5)	2.59 (1.45–4.64)	0.001
Mechanical ventilation	9/11 (81.8)	2/11 (18.2)	10.44 (2.20–49.52)	0.001
Pitt bacteremia score (mean ± SD)	2.36 ± 2.67	2.01 ± 2.07		0.942

[a] Unless stated otherwise.

previous observations. Recently, Nijssen et al. demonstrated that the dominant route of integron transfer was cross-transmission of integron-carrying organisms from patient to patient (17, 18). Alternatively, other investigators have shown that inter- or intraspecies integron transfer occurs very efficiently among *Enterobacteriaceae* within hospital settings (10).

Several findings from our study support the hypothesis that exchange of gene cassettes between different integrons occurs in the hospital environment. Firstly, the integrons circulating in the community were shorter and contained smaller numbers of gene cassettes than the integrons carried by nosocomial isolates. Secondly, and more importantly, some integrons derived

TABLE 5. Univariate analysis of factors associated with crude 14-day mortality for 233 patients with *Enterobacteriaceae* BSIs

Variable	No. of patients with variable/total no. of patients (%)		OR (95% CI)	*P* value
	Survivors (n = 205)	Nonsurvivors (n = 28)		
Integron				
Yes	62/73 (84.9)	11/73 (15.1)	1.49 (0.66–3.37)	0.386
No	143/160 (89.4)	17/160 (10.6)		
Size of integron (bp)				
≥1,800	20/23 (87)	3/23 (13)	0.58 (0.14–2.46)	0.516
<1,800	31/39 (79.5)	8/39 (20.5)		
Extended-spectrum β-lactamase production				
Yes	19/23 (82.6)	4/23 (17.4)	1.63 (0.51–5.20)	0.494
No	186/210 (88.6)	24/210 (11.4)		
VIM production				
Yes	6/9 (66.7)	3/9 (33.3)	3.98 (0.94–16.92)	0.045
No	202/228 (88.6)	26/228 (11.4)		
Multidrug resistance[a]				
Yes	75/91 (82.4)	16/91 (17.6)	2.31 (1.04–5.15)	0.041
No	130/142 (91.5)	12/142 (8.5)		
Bacterial species				0.161
E. coli	129/142 (90.8)	13/142 (9.2)	1	
K. pneumoniae	39/45 (86.7)	6/45 (13.3)	1.53 (0.54–4.28)	0.421
Other	37/46 (80.4)	9/46 (19.6)	2.41 (0.96–6.09)	0.062
Severity of underlying disease[b]				0.477
Nonfatal	59/64 (92.2)	5/64 (7.8)	1	
Ultimately fatal	115/133 (86.5)	18/133 (13.5)	1.85 (0.65–5.22)	0.247
Rapidly fatal	31/36 (86.1)	5/36 (13.9)	1.90 (0.51–7.08)	0.337
Pitt bacteremia score				
≥4	32/52 (61.5)	20/52 (38.5)	13.51 (5.48–33.32)	<0.001
<4	173/181 (95.6)	8/181 (4.4)		
Source of bacteremia				0.004
Urinary tract	109/112 (97.3)	3/112 (2.7)	1	
Unknown	43/57 (75.4)	14/57 (24.6)	11.83 (3.23–43.23)	<0.001
Abdomen	37/42 (88.1)	5/42 (11.9)	4.91 (1.12–21.55)	0.035
Intravenous catheter	6/9 (66.7)	3/9 (33.3)	18.17 (3.01–109.79)	0.002
Soft tissue	5/7 (71.4)	2/7 (28.6)	14.53 (1.96–107.51)	0.009
Lung	5/6 (83.3)	1/6 (16.7)	7.27 (0.64–82-89)	0.110
Immunosuppression				
Yes	76/85 (89.4)	9/85 (10.6)	0.80 (0.35–1.87)	0.680
No	129/148 (87.2)	19/148 (12.8)		
Nosocomial infection				
Yes	83/98 (84.7)	15/98 (15.3)	1.70 (0.77–3.75)	0.223
No	122/135 (90.4)	13/135 (9.6)		
Appropriate antibiotic therapy				
Yes	182/207 (87.9)	25/207 (12.1)	0.95 (0.27–3.39)	1.000
No	23/26 (88.5)	3/26 (11.5)		

[a] Resistance to three or more classes of antimicrobial agents.
[b] As classified by McCabe and Jackson (15). When in-hospital mortality was used as an end point, the severity of underlying disease was an independent factor associated with mortality.

from organisms causing hospital-acquired infections contained combinations of gene cassettes that were found as single cassettes in other resident integrons. These data indicate that the hospital environment may not only promote integron dissemination but also could favor the exchange of gene cassettes between different integrons, as suggested by other investigators (10).

Another important finding of the present study was the link between antibiotic use and integron carriage. Although the use of several groups of antimicrobial agents was associated with integron carriage, prior administration of cotrimoxazole exhibited the strongest association and remained an independent factor in multivariate analysis. This novel observation may be

explained by the fact that the majority of integrons examined in the present study contained the *dfr* gene cassettes, in addition to the *sul* gene cassettes that are almost always present in class 1 integrons (11). These two gene families inactivate trimethoprim and sulfamethoxazole, respectively, resulting in resistance to cotrimoxazole. Consequently, the use of cotrimoxazole exerts a strong selection pressure upon integron-carrying organisms and may coincidentally select and maintain other resistance determinants linked to *sul* and *dfr* gene cassettes within the same integron, transposon, or other genetic element. This hypothesis is supported by our finding that integron-carrying organisms had reduced susceptibility not only to antimicrobial agents for which the respective gene cassettes were contained within the integron(s) but also to other classes of agents. For example, none of the integrons examined in the present study contained an extended-spectrum β-lactamase gene, but a strong correlation between integrons and extended-spectrum β-lactamase production was observed. Similar results have been reported in previous studies (10, 11, 13, 29).

Divergent opinions have been expressed concerning the hypothesis that infections caused by multidrug-resistant organisms may have worse prognoses (1, 2, 20, 23). The importance of integron carriage with regard to this hypothesis has not been addressed previously. In our study, no significant difference was detected between integron-carrying and non-integron-carrying organisms with respect to 14-day mortality. Nevertheless, when we looked at the subgroup of patients infected with integron-carrying organisms that harbored the bla_{VIM} gene, which confers resistance to almost all available β-lactam agents, a significant effect on the mortality rate was observed in univariate analysis; this effect was not maintained in multiple logistic regression analysis. This may be due to the limited power of our study, since only nine patients were infected with organisms carrying such integrons.

The data presented here underline the importance of antibiotic use, especially of cotrimoxazole, in selecting integron-carrying *Enterobacteriaceae* and emphasize the role of the hospital and other health care environments in the dissemination of such organisms. Whether certain integrons, particularly those carrying multidrug resistance determinants, have an impact on survival is a question that needs further evaluation.

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