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Emergence of Antibiotic Resistance during Therapy for Infections Caused by *Enterobacteriaceae* Producing AmpC β-Lactamase: Implications for Antibiotic Use▽

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***Enterobacter* spp., *Serratia marcescens*, *Citrobacter freundii*, and *Morganella morganii* are characterized by chromosomally encoded AmpC β-lactamases and possess the ability to develop resistance upon exposure to broad-spectrum cephalosporins. To determine the incidences of the emergence of resistance during antimicrobial therapy for infections caused by these organisms and the effect of the emergence of resistance on patient outcomes, all patients who were admitted to the Asan Medical Center (Seoul, Republic of Korea) from January 2005 to June 2006 and whose clinical specimens yielded *Enterobacter* spp., *S. marcescens*, *C. freundii*, or *M. morganii* were monitored prospectively. The main end point was the emergence of resistance during antimicrobial therapy. A total of 732 patients with infections were included for analysis. The overall incidence of the emergence of antimicrobial resistance during antimicrobial therapy was 1.9% (14/732). Resistance to broad-spectrum cephalosporins, cefepime, extended-spectrum penicillin, carbapenem, fluoroquinolones, and aminoglycosides emerged during treatment in 5.0% (11/218), 0% (0/20), 2.0% (2/100), 0% (0/226), 0% (0/153), and 1.1% (1/89) of patients, respectively. The emergence of resistance to broad-spectrum cephalosporins occurred more often in *Enterobacter* spp. (8.3%, 10/121) than in *C. freundii* (2.6%, 1/39), *S. marcescens* (0%, 0/37), or *M. morganii* (0%, 0/21). Biliary tract infection associated with malignant bile duct invasion was significantly associated with the emergence of resistance to broad-spectrum cephalosporins ($P = 0.024$ at a significance level of 0.042, by use of the Bonferroni correction). Only 1 of the 14 patients whose isolates developed resistance during antimicrobial therapy died. The emergence of resistance was more frequently associated with broad-spectrum cephalosporins than with the other antimicrobial agents tested, especially in *Enterobacter* spp. However, the emergence of resistance was associated with a low risk of mortality.**

Enterobacter spp., *Serratia marcescens*, *Citrobacter freundii*, and *Morganella morganii* have emerged as major causes of nosocomial infections caused by gram-negative bacteria. According to recent data from the SENTRY antimicrobial resistance surveillance program, *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., and *M. morganii* ranked 4th, 6th, 11th, and 12th, respectively, among the gram-negative organisms that cause bloodstream infections (3). These organisms are characterized by inducible resistance mediated by the chromosomal AmpC β-lactamase (12). This type of resistance can emerge rapidly during antimicrobial therapy (2, 9, 10), thus limiting the choice of antimicrobial agents that can be used to treat infections caused by these organisms.

A landmark study by Chow et al. showed the emergence of resistance to broad-spectrum cephalosporins in 19% of *Enterobacter* blood isolates from patients receiving antimicrobial agents of this class (2). Chow et al. suggested that it may be prudent to avoid treatment with broad-spectrum cephalosporins, regardless of the in vitro susceptibility of isolates (2). That study, however, had several limitations, including a small number of patients ($n = 129$), of whom only 31 were treated with broad-spectrum cephalosporins. In addition, it did not include other organisms that produce the AmpC β-lactamase or patients who had been treated with fluoroquinolones, and it included only patients with bacteremia. It should also be noted that the use of carbapenems has sharply increased in recent years. Although several retrospective studies partly investigated these issues, there have been no prospective studies with large numbers of patients. We therefore prospectively assessed the incidences of resistance and the factors related to the emergence of resistance to antimicrobial therapy for infections caused by *Enterobacter* spp. *S. marcescens*, *C. freundii*, and *M. morganii*.

MATERIALS AND METHODS

Design, study population, and setting. All patients who were admitted to the Asan Medical Center, a 2,200-bed tertiary-care affiliated teaching hospital in Seoul, Republic of Korea, from January 2005 to June 2006 and whose clinical specimens yielded *Enterobacter* spp., *S. marcescens*, *C. freundii*, or *M. morganii* were prospectively identified and monitored until the time of discharge. This study was approved by the institutional review board of the Asan Medical Center.

Data collection. The data collected prospectively included patient demographic characteristics, the underlying disease or condition, the significance of the isolates recovered (pathogen versus colonizer), the type of infection, antibiogram findings, the antimicrobial therapy received, and the outcome. All patients classified as having infections and who received adequate therapy (see "Definitions" below) were included for analysis.

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Definitions. Infection was considered community acquired if a positive culture was obtained within 48 h of admission for patients who had not been hospitalized during the previous 6 months. The type of infection was defined in accordance with the criteria of the Centers for Disease Control and Prevention (4). Patients who did not meet the criteria for infection were considered colonized and were excluded from the data analysis. When the isolate was resistant to any of the broad-spectrum cephalosporins tested (cefotaxime, ceftriaxone, and ceftazidime), it was regarded as resistant to broad-spectrum cephalosporins. Intermediately resistant strains were considered resistant. Adequate therapy was defined as the use of an antimicrobial agent to which the organism was susceptible in vitro for more than 24 h within 5 days after the sample positive by culture was obtained (2). The outcome was evaluated at the time of discharge. Death was considered infection related if a patient died during the phase of an active infection and no other cause of death was identified.

Microbiological and molecular methods. All initial and subsequent clinical isolates of *Enterobacter* spp., *S. marcescens*, *C. freundii*, or *M. morganii* were collected. Stock cultures were stored at −70°C in 30% glycerol. Species were identified by using the NEG Combo type 21 panel (Dade Behring), and antimicrobial susceptibilities were determined by use of the MicroScan system (Dade Behring) and the standard criteria of the Clinical Laboratory Standards Institute (CLSI). All isolates were tested for extended-spectrum β-lactamase (ESBL) production by the combined disk test by using 30 μg of cefotaxime, ceftazidime, or cefepime alone or in combination with 10 μg clavulanate (5, 7, 11). *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive controls for ESBL production, respectively. For the second or subsequent isolates of the same species obtained from the same patient, the MICs of paired isolates to cefotaxime, ceftriaxone, ceftazidime, cefepime, ciprofloxacin, aztreonam, piperacillin-tazobactam, and imipenem were confirmed by the agar dilution method, in accordance with the CLSI guideline. All paired isolates were compared by pulsed-field gel electrophoresis (PFGE). PFGE of genomic DNA digested with XbaI was performed with a CHEF DRIII system (Bio-Rad, Hercules, CA). As determined by restriction fragment analysis, the strains were defined as clonal, related, or unrelated strains according to the description of Tenover et al. (13).

Statistical analysis. Bivariable and multivariable analyses for determination of the independent risk factors for mortality were performed by using logistic regression models. Forward selection was used. Odds ratios were estimated by exponentiation of the regression coefficients and calculation of the 95% confidence intervals (CIs). Variables with P values of <0.1 in the bivariable analysis were included in the multivariable analysis. For adjustments for multiplicity, the significance levels of the P values were determined according to the Bonferroni correction. All tests were performed by using SPSS software (version 12.0; SPSS, Inc.).

RESULTS

Study population. During the study period, a total of 1,867 cases were identified, of whom 832 were classified as colonizations and 303 were inadequately treated, leaving 732 cases for analysis.

Patients characteristics. The epidemiological characteristics and the underlying disease/condition of the 732 patients are shown in Table 1. Sixty-three percent were male, and the median age was 57.0 years (range, neonate to 89 years). The most common underlying illness was solid cancer (35.7%), followed by biliary disease (22.7%), neurologic disease (18.9%), and diabetes mellitus (18.3%). Among the predisposing factors, 535 patients (73.1%) had a history of hospital admission, 209 (28.6%) had a biliary drainage catheter, 197 (26.9%) had an indwelling urinary catheter, 192 (26.2%) had a central venous catheter, and 182 (24.9%) had undergone surgery within the previous 1 month. Of these 732 infection episodes, 535 (73.1%) originated in the hospital.

Microbiological and clinical features. Table 2 shows the microbiological and clinical features of the 732 patients. Of the 732 initial isolates, 443 (60.5%) were *Enterobacter* spp. (287 *Enterobacter cloacae*, 143 *E. aerogenes*, 11 *E. agglomerans*, and 2 *E. asburiae* isolates), 130 were *C. freundii*, 113 were *S. marcescens*, and 46 were *M. morganii.* Pneumonia was the most common type of infection (26.4%), followed by biliary tract infection (23.4%), urinary tract infection (15.3%), primary bacteremia (10.7%), skin and soft tissue infection (10.5%), and intra-abdominal infection (8.6%). Bacteremia accompanied the primary infection in 202 cases (27.6%). Twenty-eight patients (3.8%) presented with septic shock at the time of the initial positive culture. The MicroScan susceptibility testing system indicated that the organisms that caused 299 (40.8%) infections were resistant to one or more of the broad-spectrum cephalosporins, with 87 (11.9%) being resistant to cefepime, 149 (20.4%) to piperacillin-tazobactam, 98 (13.4%) to ciprofloxacin, and 46 (6.3%) to imipenem.

TABLE 1. Epidemiological characteristics of 732 patients with infections caused by *Enterobacter* spp., *S. marcescens*, *C. freundii*, or *Morganella morganii*

Characteristic[a]	No. (%) of patients
Male gender	461 (63.0)
Underlying disease[b]	
Solid cancer	261 (35.7)
Biliary disease	166 (22.7)
Hypertension	164 (22.4)
Neurologic disease	138 (18.9)
Diabetes mellitus	134 (18.3)
Hematologic malignancy	45 (6.1)
Liver cirrhosis	44 (6.0)
End-stage renal disease	27 (3.7)
Solid organ transplantation	27 (3.7)
Multiple trauma	25 (3.4)
Alcoholism	17 (2.3)
Chronic obstructive pulmonary disease	13 (1.8)
Bone marrow transplantation	9 (1.2)
McCabe and Jackson criteria[c]	
Nonfatal disease	452 (61.7)
Ultimately fatal disease	238 (32.5)
Rapidly fatal disease	42 (5.7)
Underlying condition[b]	
Prior hospital admission (within 6 mo)	535 (73.1)
Presence of biliary drainage catheter	209 (28.6)
Presence of indwelling urinary catheter	197 (26.9)
Presence of central venous catheter	192 (26.2)
Recent surgery (within 1 mo)	182 (24.9)
Prior ICU care (within 1 mo)	155 (21.2)
Presence of mechanical ventilation	108 (14.8)
Cancer chemotherapy (within 1 mo)	104 (14.2)
Leukopenia (<4,000 leukocytes/mm^3)	79 (10.8)
Immunosuppressive therapy[d]	62 (8.5)
Location of acquisition	
Hospital	535 (73.1)
Community	197 (26.9)
Ward at the time of bacteremia	
Medical ward	334 (45.6)
Surgical ward	148 (20.2)
Emergency room	116 (15.8)
Medical ICU	76 (10.4)
Surgical ICU	58 (7.9)

[a] The median age of the patients was 57 years (range, neonate to 89 years).
[b] Some patients had more than one underlying disease.
[c] The median Charlson cormorbidity index score was 2.0 (range, 0 to 14).
[d] Receipt of steroid therapy for more than 10 days or the use of other immunosuppressant (tacrolimus, mycophenolate mofetil, azathioprine, cyclosporine, or OKT-3) for more than 1 week during the previous 1 month.

TABLE 2. Microbiological and clinical features of 732 patients with infections caused by *Enterobacter* spp., *S. marcescens*, *C. freundii*, or *Morganella morganii*

Characteristic	No. (%) of patients
Organism	
Enterobacter spp.	443 (60.5)
E. cloacae	287 (39.2)
E. aerogenes	143 (19.5)
E. agglomerans	11 (1.5)
E. asburiae	2 (0.3)
C. freundii	130 (17.8)
S. marcescens	113 (15.4)
M. morganii	46 (6.3)
Type of infection	
Pneumonia	193 (26.4)
Biliary tract infection	171 (23.4)
Urinary tract infection	112 (15.3)
Primary bacteremia	78 (10.7)
Skin and soft tissue infection	77 (10.5)
Intra-abdominal infection	63 (8.6)
Central nervous system infection	3 (0.4)
Catheter-associated infection	3 (0.4)
Other	32 (4.4)
Concomitant bacteremia	202 (27.6)
Septic shock	28 (3.8)
Resistance to:	
Cefuroxime	493 (67.3)
Cefotaxime	276 (37.7)
Ceftriaxone	264 (36.1)
Ceftazidime	264 (36.1)
Broad-spectrum cephalosporin[a]	299 (40.8)
Cefepime	87 (11.9)
Aztreonam	262 (35.8)
Piperacillin	311 (42.5)
Piperacillin-tazobactam	149 (20.4)
Ciprofloxacin	98 (13.4)
Gentamicin	114 (15.6)
Tobramycin	142 (19.4)
Amikacin	64 (8.7)
Imipenem	6 (0.8)
ESBL production	65 (8.9)

[a] Resistance to cetotaxime, ceftriaxone, or ceftazidime.

TABLE 3. Treatment of patients with infections caused by *Enterobacter* spp., *S. marcescens*, *C. freundii*, or *Morganella morganii*

Treatment[a]	No. (%) of patients
Monotherapy	621 (84.8)
Carbapenem	198 (27.0)
Broad-spectrum cephalosporin	182 (24.9)
Fluoroquinolone	105 (14.3)
Extended-spectrum penicillin	69 (9.4)
Aminoglycoside	33 (4.5)
Cefepime	14 (1.9)
Extended-spectrum cephalosporin	10 (1.4)
Aztreonam	5 (0.7)
Trimethoprim-sulfamethoxazole	3 (0.4)
Tigecycline	2 (0.3)
Combination therapy	111 (15.2)
Broad-spectrum cephalosporin + aminoglycoside	27 (3.7)
Extended-spectrum penicillin + fluoroquinolone	23 (3.1)
Extended-spectrum penicillin + aminoglycoside	10 (1.4)
Carbapenem + aminoglycoside	10 (1.4)
Carbapenem + fluoroquinolone	10 (1.4)
Broad-spectrum cephalosporin + fluoroquinolone	6 (0.8)
Cefepime + aminoglycoside	4 (0.5)
Carbapenem + extended-spectrum penicillin	4 (0.5)
Others	20 (2.7)

[a] The median duration of therapy was 10.0 days (range, 1 to 125 days).

floxacin, 64 (8.7%) to amikacin, and 6 (0.8%) to imipenem. Sixty-five strains (8.9%) were positive by the ESBL production test.

Treatment. The treatment regimens are shown in Table 3. Of the 732 patients, 621 (84.8%) received monotherapy and 111 (15.2%) received combination therapy. The most common monotherapy was a carbapenem (27.0%, 198/621), followed by a broad-spectrum cephalosporin (24.9%, 182/621), a fluoroquinolone (14.3%, 105/621), an extended-spectrum penicillin (9.4%, 69/621), an aminoglycoside (4.5%, 33/621), and cefepime (1.9%, 14/621). The most commonly used combination therapy was a broad-spectrum cephalosporin plus an aminoglycoside (3.7%, 27/621), followed by an extended-spectrum penicillin plus a fluoroquinolone (3.1%, 23/621), an extended-spectrum penicillin plus an aminoglycoside (1.4%, 10/621), carbapenem plus an aminoglycoside (1.4%, 10/621), and carbapenem plus a fluoroquinolone (1.4%, 10/621). The median length of therapy was 10.0 days (range, 1 to 125 days).

Emergence of resistance during therapy. In 17 patients (2.3%), we identified a second or subsequent isolate of the same species with resistance to an antimicrobial agent to which it was initially susceptible. PFGE showed that three pairs were unrelated clones, indicating superinfection with second strains. Thus, the overall incidence of the emergence of antimicrobial resistance during antimicrobial therapy was 1.9% (14/732). Resistance to broad-spectrum cephalosporins, cefepime, extended-spectrum penicillin, carbapenem, fluoroquinolones, and aminoglycosides emerged during treatment in 5.0% (11/218), 0% (0/20), 2.0% (2/100), 0% (0/226), 0% (0/153), and 1.1% (1/89) of the patients, respectively (Table 4). Resistance emerged mostly in *Enterobacter* spp. (13/14, 92.9%), but there was one case of the emergence of resistance in *C. freundii*. In no case did resistance emerge in *S. marcescens* or *M. morganii*. Six cases of emergence of resistance were associated with biliary tract infection, four with skin and soft tissue infection, three with pneumonia, and one with intra-abdominal infection. Combination therapy, ESBL production, and concomitant bacteremia were not associated with the emergence of resistance. The second resistant isolate was obtained a median of 7 days (range, 3 to 28 days) after the first isolate was obtained.

Since resistance emerged primarily during treatment with a broad-spectrum cephalosporin (11/14, 78.6%), we analyzed the factors associated with the emergence of resistance during broad-spectrum cephalosporin therapy (Table 5). The overall incidence of the emergence of resistance during broad-spectrum cephalosporin therapy was 5.0% (11/218), with 8.3% (10/121), 2.6% (1/39), 0% (0/37), and 0% (0/21) for *Enterobacter* spp., *C. freundii*, *S. marcescens*, and *M. morganii*, respectively. Biliary tract infection (11.5%, 6/52) was significantly associated

TABLE 4. Emergence of resistance during therapy

Characteristic	No. of patients with emergence of resistance to the therapy/total no. of patients in the group (%)	
	All patients	Patients with bacteremia
Overall	14/732 (1.9)	5/202 (2.5)
Antimicrobial agent		
Broad-spectrum cephalosporin	11/218 (5.0)	4/54 (7.4)
Cefepime	0/20 (0)	0/6 (0)
Extended-spectrum penicillin	2/100 (2.0)	1/18 (5.6)
Carbapenem	0/226 (0)	0/98 (0)
Ciprofloxacin	0/153 (0)	0/27 (0)
Aminoglycoside	1/89 (1.1)	0/22 (0)
Organism		
Enterobacter spp.	13/443 (2.9)	5/125 (4.0)
E. cloacae	10/287 (3.5)	2/88 (2.3)
E. aerogenes	3/143 (2.1)	3/32 (9.4)
E. agglomerans	0/11 (0)	0/4 (0)
E. asburiae	0/2 (0)	0/1 (0)
C. freundii	1/130 (0.8)	0/34 (0)
S. marcescens	0/113 (0)	0/33 (0)
M. morganii	0/46 (0)	0/10 (0)
Type of infection		
Pneumonia	3/193 (1.6)	1/23 (4.3)
Biliary tract infection	6/171 (3.5)	2/74 (2.7)
Urinary tract infection	0/112 (0)	0/12 (0)
Primary bacteremia	0/78 (0)	0/74 (0)
Skin and soft tissue infection	4/77 (5.2)	1/3 (33.3)
Intra-abdominal infection	1/63 (1.6)	1/14 (7.1)
Central nervous system infection	0/3 (0)	0
Catheter-associated infection	0/3 (0)	0/2 (0)
Other	0/32 (0)	
Monotherapy vs combination therapy		
Monotherapy	11/621 (1.8)	3/171 (1.8)
Combination therapy	3/111 (2.7)	2/31 (6.5)
Concomitant bacteremia		
Bacteremia positive	5/202 (2.5)	6/202 (3.0)
Bacteremia negative	9/530 (1.7)	
ESBL production		
ESBL positive	2/65 (3.1)	0/21 (0)
ESBL negative	12/667 (1.8)	5/181 (2.8)

TABLE 5. Emergence of resistance during broad-spectrum cephalosporin therapy

Characteristic	No. of patients with emergence of resistance to the therapy/total no. of patients in the group (%)	
	All patients	Patients with bacteremia
Overall	11/218 (5.0)	4/54 (7.4)
Organism		
Enterobacter spp.	10/121 (8.3)	4/30 (13.3)
E. cloacae	8/65 (12.3)	2/18 (11.1)
E. aerogenes	2/51 (3.9)	2/10 (20.0)
E. agglomerans	0/4 (0)	0/2 (0)
E. asburiae	0/1 (0)	0
C. freundii	1/39 (2.6)	0/8 (0)
S. marcescens	0/37 (0)	0/10 (0)
M. morganii	0/21 (0)	0/6 (0)
Type of infection		
Biliary tract infection	6/52 (11.5)	2/22 (9.1)
Urinary tract infection	0/46 (0)	0/6 (0)
Pneumonia	0/43 (0)	0/2 (0)
Skin and soft tissue infection	4/29 (13.8)	1/1 (100)
Intra-abdominal infection	1/18 (5.6)	1/5 (20.0)
Primary bacteremia	0/17 (0)	0/17 (0)
Catheter-associated infection	0/1 (0)	0/1 (0)
Other	0/12 (0)	
Monotherapy vs combination therapy		
Monotherapy	10/181 (5.5)	3/41 (7.3)
Combination therapy	1/37 (2.7)	1/13 (7.7)
Concomitant bacteremia		
Bacteremia positive	4/54 (7.4)	4/54 (7.4)
Bacteremia negative	7/164 (4.3)	
ESBL production		
ESBL positive	1/5 (20.0)	0
ESBL negative	10/213 (4.7)	4/54 (7.4)

with the emergence of resistance ($P = 0.024$ at the significance level of 0.042 by use of the Bonferroni correction), and skin and soft tissue infection (13.8%, 4/29) was associated with a strong tendency toward significance ($P = 0.043$). However, combination therapy, ESBL production, and concomitant bacteremia were not associated with the emergence of resistance. Resistance emerged in 4 of 30 patients (13.3%) with bacteremia caused by *Enterobacter* spp.

Analysis of risk factors for infection-related mortality. The overall and infection-related mortality rates were 10.8% (79/732) and 5.7% (42/732), respectively. In patients with bacteremia, the overall and infection-related mortality rates were 12.9% (26/202) and 8.4% (17/202), respectively. Univariate analysis showed that the factors that were significantly correlated with infection-related mortality were acquisition of the infection in an intensive care unit (ICU), solid cancer, liver cirrhosis, rapidly fatal or ultimately fatal disease, leukopenia, immunosuppressive therapy, septic shock, non-urinary tract infection, pneumonia, resistance to a broad-spectrum cephalosporin, and combination therapy. Multivariate analysis showed that the independent risk factors for infection-related mortality were ICU acquisition (adjusted odds ratio [AOR], 4.0; 95% CI, 1.58 to 10.06), septic shock at the time of an initial positive culture (AOR, 5.71; 95% CI, 1.98 to 16.49), pneumonia (AOR, 2.93; 95% CI, 1.09 to 7.92), and resistance to a broad-spectrum cephalosporin (AOR, 3.02; 95% CI, 1.33 to 6.89). In patients with bacteremia, septic shock was the only independent risk factor for infection-related mortality (AOR, 5.71; 95% CI, 1.21 to 26.95). Of the 14 patients in whom resistance emerged during antimicrobial therapy, only 1 died. Of the 218 patients who received broad-spectrum cephalospo-

rin therapy, 9.1% (1/11) of the patients in whom the therapy was associated with the emergence of resistance died, whereas 1.0% (2/207) of the patients in whom the therapy was not associated with the emergence of resistance died ($P = 0.144$).

DISCUSSION

We found that the emergence of resistance during antimicrobial therapy was mainly confined to *Enterobacter* spp., although the amount of the AmpC β-lactamase produced by *S. marcescens*, *C. freundii*, and *M. morganii* can also be increased upon exposure to various antimicrobial agents (1). The lack of emergence of resistance in *S. marcescens*, *C. freundii*, or *M. morgani* suggests that broad-spectrum cephalosporins or other antimicrobial agents might be safely used for the treatment of infections caused by these bacteria. Our finding, however, showing that the incidence of the emergence of resistance varied according to the organism involved is very difficult to explain and indicates a need for additional in vitro and large-scale clinical investigations.

Consistent with the findings described in previous reports (2, 9, 10), the emergence of resistance during antimicrobial therapy was frequently associated with broad-spectrum cephalosporin therapy. For *Enterobacter* spp., the incidence of the emergence of resistance during broad-spectrum cephalosporin therapy was substantial (8.3% for all cases and 13.3% for bacteremic cases), although it was lower than the 19% reported previously (2). Interestingly, the incidence of resistance varied according to the type of infection, with most cases of resistance associated with biliary tract infection and, possibly, skin and soft tissue infection. It was surprising that resistance emerged in patients with biliary tract infection, which is frequently relieved by drainage. After a careful review of the individual cases, we found that most of these patients, except for one with calculous cholangitis, had unresectable malignant bile duct invasion. Although all these patients underwent percutaneous bile duct drainage, adequate drainage was not possible. Most patients with calculous biliary tract infection did not show the emergence of antibiotic resistance. These results indicate that it may be prudent to avoid broad-spectrum cephalosporin treatment in patients with biliary tract infections associated with malignant bile duct invasion, as well as those with skin and soft tissue infections. However, this finding, that different rates of the emergence of resistance according to the anatomical site of infection, is difficult to explain. It could be a bias associated with the insufficient number of cases or a limitation of the observational type of study. Several other factors, such as the bacterial inoculum size and the rate of penetration of antimicrobial agents, should also be considered. This issue requires further investigations.

It is still unclear if combination therapy can prevent the emergence of resistance in gram-negative organisms. In a prospective, case-controlled observational study of organisms producing the AmpC β-lactamase, episodes of resistance were less frequent in patients treated with a broad-spectrum cephalosporin plus an aminoglycoside than in those treated with a broad-spectrum cephalosporin alone (6). In our study, however, the incidence of the emergence of resistance did not differ significantly between patients receiving monotherapy and those receiving combination therapy, which is consistent with the findings of previous cohort studies (2, 8). Even if combination therapy cannot prevent the emergence of resistance, it may be considered, since it is likely that a particular strain would become resistant to only one of the two agents but remain susceptible to the other (2).

We found a low rate of mortality among patients in whom resistance emerged during antimicrobial therapy (7.1%, 1/14). However, the number of patients in whom resistance emerged was too small to be able to reach a reliable conclusion.

This study had several limitations. First, it had an observational design, in which the antimicrobial type, dose, schedule, and changes were decided primarily by the treating physicians. Second, we did not measure the absolute amounts of AmpC β-lactamase in paired isolates. Plasmid-mediated β-lactamases other than the AmpC β-lactamase or other resistance mechanisms may contribute to the emergence of resistance. ESBL production is one possible mechanism. However, only 2 of the 65 patients with ESBL production showed the emergence of resistance. Finally, our study was conducted in a single center, indicating that additional multicenter studies are required to validate our findings.

In summary, our results suggest that (i) the emergence of resistance during antimicrobial therapy for infections caused by organisms producing the AmpC β-lactamase was mainly confined to *Enterobacter* spp.; (ii) for infections caused by *Enterobacter* spp., it might be prudent to avoid the use of broad-spectrum cephalosporins in patients with biliary tract infections associated with malignant bile duct invasion and, possibly, skin and soft tissue infections; and (iii) combination therapy might not prevent the development of resistance. Further larger-scale investigations are warranted.

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