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# Risk Factors for Emergence of Resistance to Broad-Spectrum Cephalosporins among *Enterobacter* spp.

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**Among 477 patients with susceptible *Enterobacter* spp., 49 subsequently harbored third-generation cephalosporin-resistant *Enterobacter* spp. Broad-spectrum cephalosporins were independent risk factors for resistance (relative risk [OR] = 2.3, $P = 0.01$); quinolone therapy was protective (OR = 0.4, $P = 0.03$). There were trends toward decreased risk for resistance among patients receiving broad-spectrum cephalosporins and either aminoglycosides or imipenem. Of the patients receiving broad-spectrum cephalosporins, 19% developed resistance.**

*Enterobacter* spp. are among the most common gram-negative pathogens associated with hospital infections, representing 6% of all nosocomial isolates recovered and 11% of pneumonia isolates (8).

Resistance to β-lactam antibiotics often complicates the treatment of *Enterobacter* infections (2, 6). In a recent report, 36% of *Enterobacter* spp. infections in intensive care units were resistant to broad-spectrum cephalosporins (9). Most commonly, resistance to third-generation cephalosporins in this organism is mediated by chromosomal AmpC cephalosporinase. Although isolates often appear susceptible in vitro, antibiotic pressure can facilitate the emergence of derepressed mutant *Enterobacter* cells which produce AmpC β-lactamases at high levels constitutively (12; A. A. Medeiros, Editorial, Clin. Infect. Dis. **25:**341–342, 1996). In addition, exposure to certain β-lactam antibiotics results in increased synthesis of AmpC and induction of resistance to broad-spectrum cephalosporins.

A landmark study by Chow et al. showed a strong correlation between previous broad-spectrum cephalosporin exposure and the isolation of *Enterobacter* spp. resistant to these agents (2). Although the study demonstrated emergence of resistance during therapy of *Enterobacter* bacteremia with broad-spectrum cephalosporins in 19% of patients, the prospective nature of this study precluded a large enough sample size on which conclusive analysis of risk factors for emergence of resistance could be performed (only six patients showed the emergence of a resistant strain). No study to date has had enough statistical power to study this question comprehensively. We applied here effective analytical methods to a large study cohort to measure the effects of antimicrobial agent exposures on the emergence of broad-spectrum cephalosporin resistance among *Enterobacter* spp. We analyzed antimicrobial risk factors as time-dependent variables (7) so that risk estimates would account for the duration of time an individual was exposed to an antimicrobial agent only after therapy with the agent had commenced, thus decreasing the potential for bias.

The study design was a retrospective cohort. All patients admitted to Beth Israel Deaconess Medical Center, West Campus, Boston, Mass., between October 1993 and September 1997 with cultures positive for *Enterobacter* spp. susceptible to broad-spectrum cephalosporins were included in the study. Patients remained in the cohort until *Enterobacter* spp. resistant to broad-spectrum cephalosporins were isolated (this was the outcome of interest) or until hospital discharge or death. Data were collected from administrative, laboratory, and pharmacy databases. Antibiotics analyzed included narrow-, expanded-, and broad-spectrum cephalosporins (ceftriaxone and ceftazidime were the only broad-spectrum cephalosporins used during the study period); ampicillin; penicillin; aminoglycosides; quinolones; imipenem; piperacillin; ampicillin-sulbactam; and piperacillin-tazobactam. During the course of this study, other agents such as cefepime and meropenem were rarely used at our institution.

Statistical analyses were performed using the SAS software (SAS Institute, Cary, N.C.) system for Windows. Cox proportional-hazard models were used to analyze time-dependent variables and to account for variable durations of time spent in the cohort by study patients.

A total of 477 patients with broad-spectrum cephalosporin-susceptible *Enterobacter* spp. satisfied the criteria for entry into the cohort. Forty-nine patients (10% of the cohort) had broad-spectrum cephalosporin-resistant *Enterobacter* spp. isolated subsequently. Among the initial strains susceptible to broad-spectrum cephalosporins, 343 *E. cloacae* isolates, 108 *E. aerogenes* isolates, and 26 other *Enterobacter* spp. isolates were identified. Resistance emerged subsequently in only two species: in 31 of 343 of the patients with initial *E. cloace* isolates (9%) and in 18 of 108 of the patients with initial *E. aerogenes* isolates (17%) ($P = 0.03$). Among patients in whom resistance emerged, species of resistant and susceptible isolates were identical in all but three patients.

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TABLE 1. Sites of initial isolation of *Enterobacter* spp.[a]

| Site | No. (%) |
|---|---|
| Wounds | 174 (36.5) |
| Respiratory secretions | 119 (24.9) |
| Urine | 102 (21.4) |
| Effusions | 41 (8.6) |
| Blood | 26 (5.5) |
| Vascular line tips | 8 (1.7) |
| Tissue | 7 (1.5) |

[a] Among patients with the emergence of resistant *Enterobacter* spp., susceptible and resistant isolates were recovered from the same anatomic site in all but nine patients. In six of these patients, blood was one site of isolation and the other anatomic sites likely represented the source of bacteremia (four pulmonary, one effusion, and one urine). In the other three patients, the two *Enterobacter* isolates were recovered from anatomically distinct sites and might have been spread via self-inoculation.

The sites of initial *Enterobacter* isolation are listed in Table 1.

Descriptive patient characteristics and crude results are shown in Table 2. Exposure to broad-spectrum cephalosporins was a risk factor for the emergence of resistant *Enterobacter* spp. (relative risk [RR] = 3.3, $P < 0.001$). Nineteen percent of the patients initially treated with these agents subsequently showed the emergence of a resistant *Enterobacter* isolate. Among patients treated with broad-spectrum cephalosporins, resistance emerged significantly more frequently when the initial site of isolation was the blood (4 of 14, 29%) than if the initial site was urine, tissue or wounds (5 of 67, 7%) ($P = 0.04$). Exposure to quinolones was associated with a decreased risk for emergence of broad-spectrum cephalosporin-resistant *Enterobacter* spp. (RR = 0.4, $P = 0.03$).

In multivariate analysis (Table 3), exposure to broad-spectrum cephalosporins remained a strong, independent predictor for the emergence of resistant *Enterobacter* spp. (RR = 2.3, $P = 0.01$), and the association between quinolone exposure and a decreased risk for emergence of resistance was unchanged (RR = 0.4, $P = 0.03$).

When patients treated with broad-spectrum cephalosporins received either imipenem or aminoglycosides, the risk for

TABLE 2. Descriptive characteristics of cohort and univariate analysis

| Variable (U)[a] | Value for patients with ($n = 49$) or without ($n = 428$) emergence of *Enterobacter* spp. resistant to broad-spectrum cephalosporins | | RR (95% CI)[b] | *P* |
|---|---|---|---|---|
| | With | Without | | |
| Demographics | | | | |
| Mean age in yr (SD) | 63.8 (11.5) | 63.5 (14.9) | NA | 0.85 |
| Male gender (%) | 31 (63.3) | 240 (56.1) | 1.3 (0.7–2.3) | 0.37 |
| Comorbidities | | | | |
| Liver disease (%) | 13 (26.5) | 56 (15.1) | 2.7 (1.4–5.1) | 0.003 |
| Lung disease (%) | 9 (18.4) | 47 (11.0) | 1.3 (0.6–2.7) | 0.48 |
| Renal disease (%) | 10 (20.4) | 82 (19.2) | 0.8 (0.4–1.5) | 0.44 |
| Cardiovascular disease (%) | 38 (77.6) | 334 (78.0) | 0.8 (0.4–1.5) | 0.44 |
| Cancer (%) | 13 (26.5) | 73 (17.1) | 1.7 (0.9–3.2) | 0.10 |
| Diabetes (%) | 17 (34.7) | 231 (54.0) | 0.5 (0.3–0.9) | 0.02 |
| AIDS (%) | 0 (0.0) | 7 (1.6) | ND | 1.00 |
| Transplant (%) | 4 (8.2) | 21 (4.9) | 1.1 (0.4–1.4) | 0.95 |
| Median no. of patient comorbidities (IQR) | 2 (1–3) | 2 (1–2) | | |
| Hospital exposures | | | | |
| Median length of hospital stay in days before entry into cohort (IQR) | 7.0 (5–12) | 4.0 (2–11) | NA | 0.005 |
| Median no. days in cohort (IQR) | 8 (4–14) | 7 (4–13) | NA | 0.79 |
| Transfer from other institution (%) | 15 (30.6) | 113 (26.4) | 1.1 (0.6–2.0) | 0.87 |
| ICU stay (%) | 40 (58.0) | 185 (43.2) | 2.8 (1.4–5.5) | 0.003 |
| Surgical procedure (%) | 38 (77.6) | 208 (48.6) | 2.6 (1.3–5.0) | 0.006 |
| Median culture score (1) (IQR)[c] | 1.4 (0.9–1.9) | 0.6 (0.3–1.0) | 3.6 (1.8–7.1)[c] | <0.001[d] |
| Exposures to antimicrobial agents | | | | |
| Ampicillin and penicillin (%) | 22 (44.9) | 184 (43.0) | 1.0 (0.5–1.7) | 0.90 |
| Narrow-spectrum cephalosporins (%) | 12 (24.5) | 115 (26.9) | 0.9 (0.5–1.8) | 0.79 |
| Expanded-spectrum cephalosporins (%) | 3 (6.1) | 31 (7.2) | 1.0 (0.3–3.2) | 0.93 |
| Broad-spectrum cephalosporins (%) | 31 (63.3) | 130 (30.4) | 3.3 (1.8–6.0) | <0.001 |
| Aminoglycosides (%) | 10 (20.4) | 68 (15.9) | 0.9 (0.4–1.9) | 0.73 |
| Quinolones (%) | 6 (12.2) | 116 (27.1) | 0.4 (0.1–0.9) | 0.03 |
| Imipenem (%) | 8 (16.3) | 35 (8.2) | 1.0 (0.5–2.3) | 0.97 |
| Piperacillin (%) | 8 (16.3) | 55 (12.9) | 1.0 (0.5–2.2) | 0.98 |
| Ampicillin-sulbactam (%) | 13 (26.5) | 122 (28.5) | 1.0 (0.5–1.8) | 0.87 |
| Piperacillin-tazobactam (%) | 5 (10.2) | 35 (8.2) | 1.1 (0.4–2.7) | 0.90 |

[a] Units apply to data in columns two and three only. IQR, interquartile range; ICU, intensive care unit.
[b] CI, confidence interval; ND, not determinable; NA, not applicable.
[c] The culture score is the average number of cultures obtained per day.
[d] Analyzed as a dichotomous variable.

TABLE 3. Multivariate analysis

| Variable | RR (95% CI)[a] | *P* |
|---|---|---|
| Broad-spectrum cephalosporins | 2.3 (1.2–4.3) | 0.01 |
| Quinolones | 0.4 (0.1–0.9) | 0.03 |
| Culture score[b] | 3.0 (1.5–6.1) | 0.002 |
| Liver disease | 1.7 (0.9–3.3) | 0.10 |

[a] CI, confidence interval.
[b] See Table 2, footnote *c*. The score was analyzed as a dichotomous variable.

emergence of resistance tended to decrease. These effects were not statistically significant (RR = 0.5, $P = 0.38$ and RR = 0.5, $P = 0.32$, respectively).

This was the first analytical study to comprehensively investigate risk factors for the emergence of resistance to broad-spectrum cephalosporins among *Enterobacter* spp. Isolates were not available for molecular typing, and it is possible that some resistant strains might have been different clones than the initial susceptible isolates. However, species of susceptible and resistant isolates were identical in >90% of cases, and the site of isolation was the same in >80% of cases. Thus, extrapolating from the molecular analyses of Chow et al., it is likely that susceptible and resistant isolates were of the same clone in most instances (2). Therapy with broad-spectrum cephalosporins was a strong risk factor for the emergence of *Enterobacter* spp. resistant to these agents. Similar relationships have been demonstrated in vitro and in the clinical setting (2, 10, 11). Interestingly, exposures to narrow- and expanded-spectrum cephalosporins were not associated with the emergence of resistance, nor was exposure to ureidopenicillins or to β-lactam–β-lactamase inhibitor combination agents. This finding is in accord with some studies (2, 6), but not with others (4, 5). Quinolone therapy was associated with decreased risk for emergence of broad-spectrum cephalosporin-resistant *Enterobacter* spp. This important association has not been previously demonstrated. We did not study the frequency of quinolone resistance. We detected a trend toward a protective effect when patients received both broad-spectrum cephalosporins and either aminoglycosides or imipenem, but this was not statistically significant. These findings deserve further investigation.

Our study suggests that if broad-spectrum cephalosporins are used to treat patients with *Enterobacter*-positive isolates, approximately 19% will develop resistance. This number was identical to the frequency of emergence of resistance among patients with *Enterobacter* spp. bacteremia reported by Chow et al. (2). However, our study found rates of emergence of resistance to be significantly higher in patients with *Enterobacter* bacteremia than in patients whose isolates were initially recovered from tissue, wounds, or urine. Also, resistance occurred more frequently among *E. aerogenes* than *E. cloacae*. Because the emergence of resistance to broad-spectrum cephalosporins in *Enterobacter* spp. is associated with adverse clinical outcomes (3), knowledge of the specific risk factors for resistance should aid in the selection of appropriate antibiotic therapy.

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