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Epidemiology of Intestinal Colonization by Members of the Family *Enterobacteriaceae* Resistant to Cefotaxime in a Hematology-Oncology Unit

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Intestinal colonization by members of the family *Enterobacteriaceae* resistant to cefotaxime was surveyed for 3 years in a hematology-oncology unit. Of 416 patients, 66 (15.9%) were colonized, each with a different strain. The incidence of intestinal carriage was not correlated with cefotaxime consumption in the ward but was strongly associated with individual exposure to cefotaxime.

Cefotaxime, a broad-spectrum cephalosporin exhibiting high activity against members of the family *Enterobacteriaceae* (10) and good beta-lactamase stability (13), is often used as first-line empiric therapy in leukemic patients (4, 5, 9). It suppresses endogenous intestinal members of the *Enterobacteriaceae* (8, 18). However, strains of *Enterobacteriaceae* resistant to cefotaxime (CTX-R) have been described with increasing frequency (2, 3, 6, 12, 14, 18). We previously showed that intestinal colonization is the major harbinger of gram-negative bacteremia in neutropenic patients with hematological malignancies (17). Consequently, intestinal colonization of these patients by CTX-R strains of *Enterobacteriaceae* might induce bacteremia caused by these resistant bacteria. This is why, in the present work, we analyzed the epidemiology of intestinal colonization by CTX-R strains of *Enterobacteriaceae* in a hematology-oncology unit using methods that we previously described (1). We also compared the CTX-R strains of *Enterobacteriaceae* isolated from fecal samples with those isolated from the blood of patients who developed bacteremia.

The study started on 1 January 1981, when cefotaxime was first introduced in empirical antibiotic combination therapy in the hematology-oncology unit of our institution, and ended on 31 December 1983. The number of patients discharged was 1,030 (mean hospital stay ± standard deviation, 12.0 ± 2.1 days).

Fecal samples were usually obtained twice a week from inpatients with fewer than 10^2 leukocytes per μl. In all, 2,009 fecal samples from 416 such patients (34.7 ± 7.6 patients per quarter) were analyzed. Total members of the *Enterobacteriaceae* (1) and CTX-R strains of *Enterobacteriaceae* (18) were counted, as previously described. Student's *t* test was used for comparison of mean log values of bacterial counts.

At least three blood specimens were drawn for culture at the onset of all febrile episodes (fever of >38.5°C for 6 h or more). A total of 3,959 blood culture samples were analyzed. Bacteremia was defined as the recovery of a bacterial strain from one or more blood cultures during periods of fever. All the fecal CTX-R strains of *Enterobacteriaceae* and blood isolates were biotyped by the API 20E system (API, La Balme les Grottes, France), and the MICs of cefotaxime were determined by the method of Steers et al. on Mueller-Hinton agar (16). Susceptibility to amikacin, ampicillin, carbenicillin, cefazolin, chloramphenicol, colistin, gentamicin, kanamycin, minocycline, nalidixic acid, streptomycin, sulfamethoxazole, tetracycline, tobramycin, and trimethoprim was determined by the disk-diffusion technique (11).

Members of the *Enterobacteriaceae* isolated from cases of bacteremia which occurred in the same unit during the 3 years preceding the study period were also analyzed. During this period, referred to below as the precefotaxime period, the consumption of cefotaxime was negligible.

A case-control study was done to investigate the connection between intestinal carriage of CTX-R strains of *Enterobacteriaceae* and previous exposure to antibiotics. The study group included the 416 patients who had at least one stool culture analyzed during the period studied. The results of all their stool cultures were reviewed. Cases were defined as patients carrying intestinal CTX-R strains of *Enterobacteriaceae* in at least one stool culture and hospitalized for at least 10 days before this carriage was detected. This period is referred to as the surveillance period. Controls were chosen among the patients in the study group in whom no intestinal carriage of CTX-R strains of *Enterobacteriaceae* was detected. For the controls, the surveillance period was defined as the interval between the day of admission and the day on which the last stool culture before discharge was analyzed. Cases and controls were matched first for primary diagnosis and second for equivalence of both the surveillance period and the number of stool cultures analyzed during that period. Two controls were matched with each case (an adequate number of controls was available). Exposure to antibiotics during the surveillance period was determined from individual charts. Analyses were done by standard procedures for matched triplets (15).

In all, 80 fecal strains of CTX-R members of the *Enterobacteriaceae* (36 *Enterobacter* sp., 19 *Citrobacter* sp., 18 *Escherichia coli*, 5 *Proteus* sp., 1 *Klebsiella pneumoniae*, and 1 *Serratia marcescens*) were isolated from 66 of the 416 patients (15.9%) studied. No cluster of colonization was observed, and several biotypes were identified within each species (data not shown). This suggests that colonization did not originate from a common source of contamination. No statistically significant trend in the isolation rate of CTX-R strains (2 to 15 strains per quarter) was observed over the study period ($r' = 0.57$ [Spearman's]; data not shown). This

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TABLE 1. Exposure to antibiotics during surveillance period of 33 intestinal carriers of CTX-R strains of *Enterobacteriaceae* (cases) and 66 noncarriers (controls)

Antibiotic exposure	% Exposed		Frequency[a] of eight possible exposure outcomes[b] among case-control triplets[c]						Chi-square	Odds ratio[d]	95% Confidence limits[e]
	Cases	Controls	+++	+−+ or ++−	+−−	−++	−+− or −−+	−−−			
Cefotaxime	88	59	12	12	5	0	3	1	7.6	7.3	1.8–30.0
Beta-lactams	30	39	3	5	2	2	11	10	0.5	0.6	0.1–2.4
Aminoglycosides	90	74	16	12	2	1	1	1	3.9	5.3	1.0–28.3
Vancomycin	30	29	0	4	6	2	11	10	0.01	1.0	0.6–1.8
Macrolides	27	35	0	6	3	5	6	13	0.2	0.7	ND[f]
Co-trimoxazole	0	11	0	0	0	1	6	26	3.0	0	ND
Colistin	3	5	0	0	1	0	6	26	0.45	0.33	ND

[a] Data are numbers of patients.
[b] +, Exposed; −, not exposed.
[c] Case, control 1, control 2.
[d] Mantel-Hentzel estimate.
[e] Test-based method.
[f] ND, Not done.

rate was not correlated with the consumption of cefotaxime in the unit ($r = 0.16$ [least-squares linear regression analysis]), which ranged from 347 to 2,844 g per quarter ($r' = 0.65$, $P = 0.05$ [Spearman's]). CTX-R strains of *Enterobacteriaceae* predominated in at least one fecal sample in 40 of 66 of the carriers (60%). When these strains did not predominate, their counts were much lower than the total counts of *Enterobacteriaceae* (5.39 ± 1.19 versus 8.14 ± 1.09 $\log_{10}$ CFU/g of feces; $P = 0.01$; individual data not shown). A total of 31 episodes of bacteremia caused by strains of *Enterobacteriaceae* (22 *E. coli*, 4 *Klebsiella* sp., 2 *Enterobacter* sp., 1 *Salmonella* sp., and 2 *Citrobacter* sp.) were documented during the study period in 24 patients. In comparison, 59 episodes of bacteremia were caused in 47 patients by strains of *Enterobacteriaceae* during the precefotaxime period. The proportions of the various species of *Enterobacteriaceae* found in the blood samples were not statistically different for the two periods. The MICs of cefotaxime for 50% of isolates tested (MIC_{50}s) in the respective groups of blood isolates for the study and precefotaxime periods were identical (0.03 μg/ml); the MIC_{90} was slightly higher (0.12 versus 0.5 μg/ml) during the study period because, in two patients treated with cefotaxime, strains resistant to more than 16 μg of cefotaxime per ml (1 *Citrobacter freundii*, 1 *Enterobacter cloacae*) emerged and predominated in the feces. Similar organisms were isolated from the blood of these patients.

The percentages of strains resistant to the other antimicrobial agents tested were not statistically different (Bonferroni's inequality) between the blood isolates of the study period and those of the precefotaxime period (data not shown).

As expected, the MIC_{50} and MIC_{90} of cefotaxime for the fecal CTX-R strains of *Enterobacteriaceae* (8 and 32 μg/ml, respectively; data not shown) were much higher than those for the blood isolates ($P < 0.01$ [Wilcoxon signed-rank test]). Resistance to the other beta-lactam antibiotics (ampicillin, carbenicillin, and cefazolin) also increased markedly in the CTX-R fecal strains of *Enterobacteriaceae* ($P < 0.01$ in all three instances). However, the pattern of resistance of these strains to the other antibiotics tested was not statistically different from that of the blood isolates (Bonferroni's inequality; data not shown).

A total of 33 of the 66 intestinal carriers of CTX-R strains of *Enterobacteriaceae* (50%) (12 acute lymphocytic leukemia patients, 15 acute nonlymphocytic leukemia patients, 4 lymphosarcoma patients, 1 idiopathic aplasia patient, 1 lung carcinoma patient) met the criteria for inclusion in the case-control study. Mean surveillance periods were 25.6 ± 11.3 and 25.8 ± 11.0 days for cases and controls, respectively. Mean ages (23.6 ± 15.3 and 25.4 ± 17.5 years), percentages of males (72.7 and 58.0), and numbers of stool cultures per patient during the surveillance period (6.3 ± 3.2 and 6.3 ± 3.3) were not statistically different for cases and controls. Neutropenia (<200 neutrophils per μl) was present in 91% of the cases and 74% of the controls ($P = 0.01$). During the surveillance period, only exposure to cefotaxime or to an aminoglycoside was associated with intestinal carriage of CTX-R strains of *Enterobacteriaceae* (Table 1). In fact, one aminoglycoside was associated with cefotaxime in 66 of 68 (97%) of the patients treated with cefotaxime.

The patients in the case-control study (i.e., 33 cases and 66 controls) were divided into two groups: those exposed to cefotaxime ($n = 68$) and those not exposed ($n = 31$). The Kaplan-Meier method (7) was used separately for these two groups to estimate the period during which patients were free of colonization by CTX-R strains of *Enterobacteriaceae*. The results (Fig. 1) showed that as few as 26 ± 11% of those exposed to cefotaxime might be free of colonization by CTX-R strains of *Enterobacteriaceae* after 1 month of treatment and, conversely, that 77 ± 13% of those not so exposed might remain free of such colonization after 1 month of surveillance.

The incidence of intestinal carriage of CTX-R strains of *Enterobacteriaceae* was not correlated with cefotaxime consumption in the ward. However, we showed that patients exposed to cefotaxime were at significantly increased risk of being colonized by CTX-R strains of *Enterobacteriaceae*. As for the association observed between colonization and exposure to aminoglycosides, this might have been a result of the frequent use of antibiotic combinations. The predominance of CTX-R strains among fecal *Enterobacteriaceae* in 60% of the carriers indicated actual colonization (19) and an increased risk of translocation of these strains to the bloodstream (17). Indeed, CTX-R strains of *Enterobacteriaceae* predominated in the feces of the two patients in whom translocation of these strains was documented. No such case occurred during the precefotaxime period.

In conclusion, our results indicated that neutropenic patients treated with cefotaxime are at risk of intestinal colo-

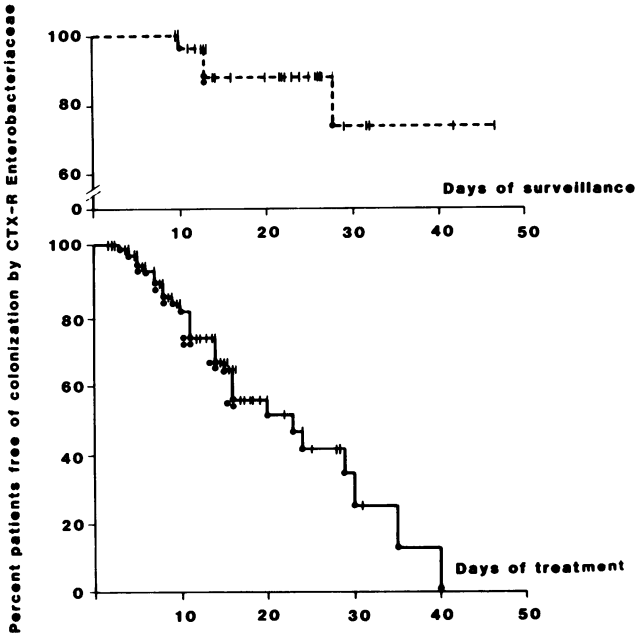

FIG. 1. Kaplan-Meïer curves for intestinal colonization by CTX-R strains of *Enterobacteriaceae* in 68 patients exposed to cefotaxime (——) and in 31 patients not exposed (– – –) in a hematology-oncology unit. Status at the end of the follow-up period: ●, colonized by CTX-R strains of *Enterobacteriaceae*; |, not colonized.

nization and bacteremia by CTX-R strains of *Enterobacteriaceae*. Because these strains are also resistant to other beta-lactam antibiotics, this might lead to therapeutic problems. Therefore, the feces of such patients should be analyzed to detect intestinal colonization by CTX-R strains of *Enterobacteriaceae*, and subsequent therapy during febrile episodes should take into account the possibility of resistance.

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