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A Nosocomial Outbreak Due to Novel CTX-M-2-Producing Strains of *Citrobacter koseri* in a Hematological Ward

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Citrobacter koseri is a member of the family *Enterobacteriaceae*. Urinary tract infections caused by *C. koseri* have been observed in as many as 12% of all isolates in adults (1). In compromised hosts, *Citrobacter* spp. could cause pneumonitis, empyema (2), biliary infection (3), and bacteremia (4). *Citrobacter* spp. were formerly susceptible to oximinocephalosporins including cefotaxime (3), but recently, *C. koseri* has been reported to have developed resistance to some cephalosporins and cephamycins through the production of an inducible chromosomally-encoded cephalosporinase that can inactivate these agents (5). Most clinically isolated *C. koseri* are susceptible to oximinocephalosporins and carbapenems. Recently, oximinocephalosporin resistance among Gram-negative bacteria has been developed due to the hydrolysis of beta-lactams by beta-lactamases including extended-spectrum beta-lactamases (ESBLs). ESBLs show variable levels of resistance to cefotaxime, ceftazidime, and other broad-spectrum cephalosporins and monobactams. Nosocomial outbreaks due to SHV-4-type ESBL-producing strains and TEM-type ESBL-producing strains of *C. koseri* have already been reported (6,7). We have identified a novel CTX-M-2-type of ESBL among nosocomially isolated *C. koseri* strains, causing a probable outbreak in the hematological ward.

Sixty-eight strains of *C. koseri* were isolated from the blood, urine, feces, sputum, ascites, and pharynx of 31 patients with a hematological malignancy that had lasted over 18 months (Figure 1). *C. koseri* not only colonized but also caused bacteremia, urinary tract infection, enteritis, and peritonitis. These strains showed similar antibiotic susceptibility profiles (Table 1). We collected 5 strains of *C. koseri* from 4 patients (Table 2) and used the double-disk synergy test and plasmid profiling to screen for ESBL-producing strains as reported previously (8,9). All of the 5 strains harbored a plasmid mediating the CTX-M-2 type beta-lactamase gene. Epidemiological study using pulsed-field gel electrophoresis (PFGE) of total DNA prepared from the 5 strains revealed patterns that were indistinguishable from each other (Figure 2). The results suggested that the 5 strains characterized belong to a single epidemic strain.

In general, multiple factors may help to decrease the immunity of patients with hematological malignancies, including impairment of phagocytosis, impaired cellular immunity, and defective production of antibodies. Moreover, intensive chemotherapies usually induce severe granulocytopenia. Thus, bacterial infections are a major cause of complications and death in patients with hematological malignancies. Recently, two studies (10,11) revealed the efficacy of the prophylactic use of quinolon by neutropenic patients. As for febrile neutropenia, empirical antibiotic therapy using cefepime or cefotaxime has been emphasized (12,13). All 31 patients in this study had hematological malignancy and underwent intensive chemoradiotherapy. After that, most of the patients in our ward were administered prophylactic and therapeutic systemic antibiotics such as quinolon, cefepime, and cefotaxime, which might well be associated with the selection of antibiotic-resistant microorganisms. Unlike other members of the family *Enterobateriaceae*, CTX-M-2-producing *C. koseri* might survive in a patient's bowel flora, because of its resist-

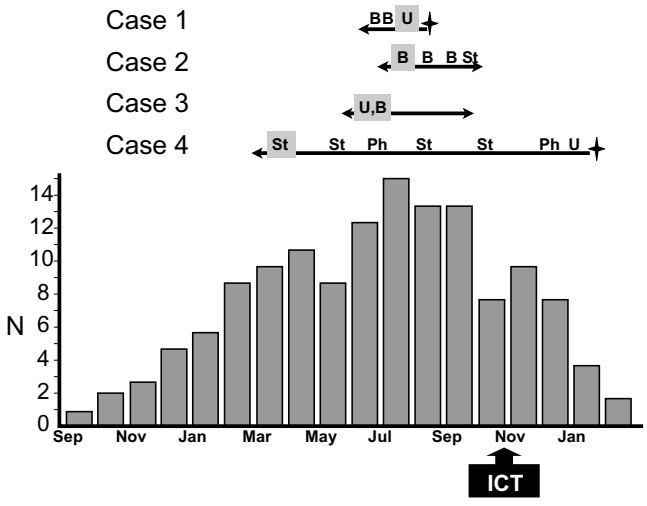

Fig. 1. The number of patients infected and/or colonized with *C. koseri*. Bars indicate the number of patients infected and/or colonized per month with *C. koseri*, the antibiotic susceptibility of which showed the same pattern. Case numbers are identical to those in Table 1. Arrows indicate the duration of each patient's hospitalization in the ward. The bald signs on each arrow indicate the samples, from which *C. koseri* was isolated. The network-breaking characters indicate the samples, from which genetically identical strains were isolated in our study. An infection control team (ICT) intervened in the ward to resolve the outbreak (see article). B, blood; U, urine; St, stool; Ph, pharynx.

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Table 1. Antibiotics susceptibility profile of *C. koseri* isolated in this outbreak

ampicillin	>16
ampicillin/ clavulanate	16
piperacillin	>64
cefazolin	>16
cefotaxime	>16
cefotiam	>32
ceftazidime	>16
cefpirome	>16
cefpodoxime proxetil	>4
cefcapene pivoxil	>1
cefmetazole	>32
flomoxef	32
sulbactam/cefoperazone	>32
aztoreonam	>16
imipenem	<1
gentamicin	<1
amikacin	<1
minomicin	2
levofloxacin	>4

MICs were determined by microdilution method recommended by NCCLS (currently CLSI) guideline with Muller-Hinton broth (Difco, Detroit, Mich., USA) using MicroScan-kit (Dade Behring, West Sacramento, Calif., USA).

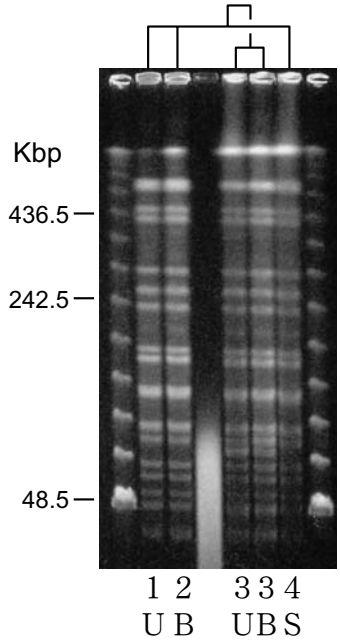

Fig. 2. PFGE analysis of *C. koseri* strains. Bacterial DNA was extracted, digested and subjected to PFGE, as previously described (8,9). Lanes 1, 2, 3, and 4, were sampled from patients Nos. 1, 2, 3, and 4, respectively. U, B, and S indicate urine, blood, and stool, respectively.

Table 2. Profiles of cases involved in the outbreak

No.	Age/Sex	Underlying disease	Therapy/Outcome	Infection	Sources of *C. koseri*
1	71/M	malignant lymphoma	chemotherapy/refractory	sepsis	Urine
2	61/M	adult T-cell leukemia	chemotherapy/partial response	sepsis	Blood
3	25/F	acute lymphoblastic leukemia	bone marrow transplantation	sepsis	Urine, Blood
4	63/F	acute lymphoblastic leukemia	chemotherapy/complete remission	enteritis	Stool

ance to quinolon, cefepime, and cefotaxime. In addition, urinary tract infections tended to be easily associated with urinary catheterization in our cases. We speculated that the situation was as follows. Once *C. koseri* colonizes in the bladder or intestine, it will then disseminate into the blood stream causing severe bacteremia during intensive chemotherapy. The symptoms of sepsis caused by *C. koseri* were often very serious, and could only be cured by appropriate and immediate administration of carbapenem. However, the use of carbapenem in high amounts and at high frequency in our ward could create a grave epidemiological problem.

The number of *C. koseri* infections increased significantly, and standard infection control measures were not effective to stop this outbreak. Therefore, we began to enforce the following precautions. We introduced barrier precautions against not only infected patients but also colonized patients, using disposable gloves and drapes. Mandatory hand washing was done immediately before and after any manipulation involved in the nursing care. Hand hygiene using commercial alcoholic disinfectant (Welpas; Maruishi Pharmaceutical Co., Ltd., Osaka, Japan) was promoted not only for medical workers but also for patients. As for the environment, the water taps were converted to the hands-free types, and all doorknobs and bars for drip injection were sterilized using 70% alcohol twice daily. We also tried to restrict the prophylactic use of quinolon for high-risk patients with neutropenia decreasing under 100/μL which was keeping for more than 1 week. After these procedures, the incidence of *C. koseri* isolation decreased, but this type of infection has not yet been eradicated, as shown in Figure 1. We continue to make an effort to prevent nosocomial transmission of *C. koseri*.

In this report, we emphasize the appearance of *C. koseri* and its new type of drug resistance. We also warn that it is quite difficult to control the outbreak of such antimicrobial-resistant microorganisms in a hematological ward. In the future, we must pay close attention to the nosocomial spread of this type of *C. koseri*, which has demonstrated resistance to a broad spectrum of cephalosporins, cephamycins, and carbapenems.

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