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A Small Outbreak of Third Generation Cephem-Resistant *Citrobacter freundii* Infection on a Surgical Ward

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Citrobacter freundii is a member of family *Enterobacteriaceae* and has been associated with nosocomial infections in the urinary, respiratory, and biliary tracts of debilitated hospital patients. *C. freundii* has an inducible chromosomally encoded cephalosporinase that can inactivate cephamycins and cephalosporins. However, most clinical isolates are sensitive to new third generation cephems and carbapenems.

We report here a small outbreak of infection caused by third generation cephem-resistant *C. freundii* on a surgical ward of a university hospital in 2002. We identified four patients with biliary infection and two carrier patients during July and October. All of the infection cases (patients A, B, E, and F) and one colonization case (patient G) underwent surgical procedures prior to the isolation of resistant *C. freundii* (Table 1). *C. freundii* were isolated from bile, wound gauze, ascites, pus, and/or feces of these patients. Epidemiological study using pulsed-field gel electrophoresis (PFGE) revealed that two different PFGE types of strains were involved in the outbreak; PFGE patterns of lanes 1, 2, 3, and 4 were indistinguishable from each other, and the patterns of lanes 5 and 6 were likewise identical (Fig. 1). These types were tentatively designated as a and b, respectively, and strains of both types were found to be highly resistant to third generation cephems including ceftazidime and ceftriaxone, but sensitive to imipenem (Table 2). Type a strain and type b strain showed similar antibiotic susceptibility profiles, suggesting that these strains were evolutionarily related, or that these strains carry similar antibiotic resistant genes on a putative plasmid. The susceptibility profile of type c strain was different from those of type a and b strains.

As previous studies have indicated, third generation cephem-resistance of Gram-negative bacteria are due to the hydrolysis of β-lactams by β-lactamases. These β-lactamases include extended spectrum β-lactamase (ESBL), metallo-β-lactamase, and plasmid-encoded AmpC cephalosporinase (1-3). ESBL confers variable levels of resistance to cefotaxime, ceftazidime, and other broad-spectrum cephalosporins and to monobactams, but has no detectable activity against cephamycins and carbapenems, and is relatively sensitive to sulbactam (1). Plasmid-encoded metallo-β-lactamase hydrolyzes imipenem as well as almost all penicillins and cephems (2). Plasmid-encoded AmpC cephalosporinases of Gram-negative bacteria are uncommon and have broad substrate specificity including cephamycins but not carbapenems, as compared with chromosomally encoded AmpC cephalosporinases (3-5). Types a and b *C. freundii* strains were resistant to cephamycins and β-lactamase inhibitors, and were sensitive to imipenem. It is therefore likely that these third generation cephem-resistant *C. freundii* strains carry a plasmid-encoded AmpC cephalosporinase that hydrolyzes new cephalosporins and cephamycins. However, conjugational studies to transfer the resistances of type a and b *C. freundii* strains to *Escherichia coli* raised no resistant transconjugants, indicating that the resistance to new third generation cephems is rather chromosomal. Attention to nosocomial spread of Gram-negative bacteria that carry this type of resistance is recommended.

Table 1. Profiles of cases involved in the outbreak and of a control case

Patient	Age/Sex	Underlying disease	Infection or colonization	Sources of *C. freundii*	PFGE type
A[1)]	71/F	chologicarcinoma	Infection	bile, wound gauze, pus, others	a
B	59/M	chologicarcinoma	Infection	bile	a
C	70/M	gallbladder cancer	colonization	bile	a
D	58/F	gallbladder cancer	colonization	feces	a
E	50/M	chologicarcinoma	Infection	bile, wound gauze, ascites	b
F	63/M	cholelithiasis	Infection	bile, wound gauze	b
G[2)]	55/F	gallbladder cancer	colonization	feces	c

[1)]: Patients A, B, C, D, E, and F were involved in the outbreak of infection by third generation cephem-resistant *C. freundii*.
[2)]: A control who was a carrier of *C. freundii*.

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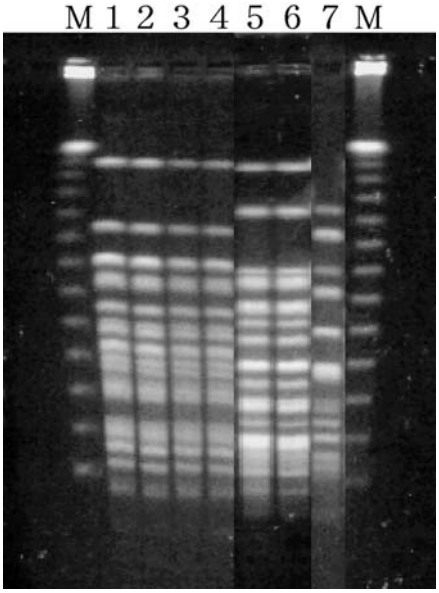

Fig. 1. PFGE analysis of *C. freundii* strains.
Bacterial DNA was extracted, digested with *Xba*I and subjected to PFGE, as previously described (7). Lanes 1, 2, 3, 4, 5, and 6 were isolates from patients A, B, C, D, E, and F, respectively. Lane 7 represents an occasional *C. freundii* isolate from another patient (patient G) in the same ward. M: molecular marker.

Table 2. Antibiotic susceptibility of outbreak strains of *C. freundii*

antibiotics	PFGE type a	PFGE type b	PFGE type c
ampicillin	>128[1)]	>128	64
ampicillin/clavulanic acid	>128	>128	128
ampicillin/sulbactam	128	128	8
piperacillin	64	128	2
piperacillin/clavulanic acid	64	128	2
cefazolin	>128	>128	64
cefotaxime	32	128	0.5
cefotaxime/clavulanic acid	64	128	0.5
ceftazidime	128	>128	0.5
ceftazidime/clavulanic acid	128	>128	0.5
cefmetazole	128	>128	32
ceftriaxone	64	>128	0.5
latamoxef	64	64	0.5
imipenem	0.5	0.5	0.5
aztreonam	32	64	0.5
cefoperazone/sulbactam	8	32	0.5
gentamicin	0.5	1	0.5

[1)]: MICs were determined by an agar dilution method with Muller-Hinton agar (Difco, Detroit, Mich., USA) according to the National Committee for Clinical Laboratory Standards procedure (6).

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