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Identification of ACT-1 Plasmid-Mediated AmpC β-Lactamase Producing *Citrobacter freundii* from a Chinese Patient

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Dear Sir,

Enterobacteriaceae, other than *Escherichia* spp. and *Klebsiella* spp., can cause serious nosocomial infections and appear to have become increasingly drug resistant. *Citrobacter freundii*, belonging to the Enterobacteriaceae family, is a prominent opportunistic pathogen responsible for serious infections in immunocompromised individuals. *C. freundii* is characterized by the presence of chromosomally-encoded AmpC β-lactamases and possesses the ability to develop resistance on exposure to broad-spectrum cephalosporins [1]. Resistance to third-generation cephalosporins in *C. freundii* has been attributed to the production of extended-spectrum β-lactamases (ESBLs), but resistance mediated by plasmid-mediated AmpC β-lactamase (pAmpCs) has not been reported to the best of our knowledge.

A specific *C. freundii*, designated CF0513, was isolated from the wound drainage fluid of a 75-yr-old man who suffered from chest trauma and was admitted to a hospital in Wuhu, China, in December 2008. During the period of hospitalization, symptoms of lower respiratory tract infection emerged. This patient was treated with cefotaxime for 7 days, but the treatment was not found to be effective The patient was then administered imipenem-cilastatin (500 mg intravenous, q8 hr) for a period of 7 days, and the symptoms of lower respiratory tract infection gradually disappeared during his hospitalization. The bacterial species identification was performed using the Vitek 2 system (BioMérieux, Marcy l'Etoile, France) and confirmed using API 20E identification kit (BioMérieux, Marcy l'Etoile, France). CF0513 was found to be resistant to all the β-lactams (except carbapenems), and to ciprofloxacin. Further, the minimum inhibitory concentrations (MICs) of various antimicrobial agents were determined by the agar dilution method, and the susceptibility data were interpreted in accordance with the recommendations of the Clinical Laboratory Standards Institute, 2012 [2]. CF0513 showed elevated MICs for all β-lactams, except for imipenem and meropenem (<0.25 mg/L), and it was also resistant to ciprofloxacin, levofloxacin, gatifloxacin, gentamicin, and amikacin (Table 1).

The presence of AmpC was determined by a modified 3-dimensional test [3]. Plasmid DNA was extracted using a Qiagen Plasmid Purification kit (QIAGEN, Hilden, Germany), and *ampC* gene amplification was carried out by multiplex PCR using plasmid DNA as a template [4]. The association with mobile elements (*IntI1,* IS*CR1*, and IS*Ecp1*) and other resistance genes (*qnr, aac(6')-Ib-cr, qepA,* bla_{TEM}, bla_{SHV}, bla_{CTX-M}; and 16S rRNA methylase genes such as *armA, rmtA, rmtB, rmtC, rmtD*, and *rmtE*) was investigated by PCR amplification [5-8]. The PCR assay showed that CF0513 carried bla_{ACT-1}. In addition, CF0513 co-harbored $bla_{CTX-M-14}$, *armA*, IS*Ecp1*, and *IntI1* (Table 1). DNA sequence analysis showed that bla_{ACT-1} was associated with an IS*Ecp1* mobile element downstream.

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Table 1. Characteristics of clinical isolate *Citrobacter freundii* CF0513 and the transconjugate

Strain	Plasmid		Mobile elements	Resistance genes	MIC (mg/L)							
	rep typing	size (kb)			PIP	CTX	CAZ	FEP	FOX	ATM	GM	AMK
Citrobacter freundii CF0513	IncL/M	50	IS*Ecp1*, *IntI1*	bla_{ACT-1}, $bla_{CTX-M-14}$, *armA*	512	64	64	8	>256	128	>128	32
Transconjugate of CF0513	IncL/M	50	IS*Ecp1*, *IntI1*	bla_{ACT-1}, $bla_{CTX-M-14}$, *armA*	256	32	32	4	256	64	128	32
E. coli J53	–	–	–	–	<0.5	<0.5	<0.5	<0.25	<0.5	<0.5	<0.25	<0.25

Abbreviations: MIC, minimum inhibitory concentration; PIP, piperacillin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; ATM, aztreonam; GM, gentamicin; AMK, amikacin.

Plasmid analysis revealed that CF0513 only harbored a 50 kb plasmid. *Escherichia coli* V517 was used as a reference marker. To determine whether the AmpC phenotype was transferable, conjugation experiments were carried out in Luria-Bertani (LB) broth with sodium azide-resistant *Escherichia coli* J53 as the recipient. Transconjugants were selected on LB agar plates supplemented with sodium azide (100 mg/L) and cefotaxime (2 mg/L). The AmpC phenotype was successfully transferred to *E. coli* J53. The transconjugant plasmids showing an AmpC phenotype were classified according to their incompatibility group, using the PCR replicon-typing scheme described by Carattoli et al. [9]. The plasmid profile revealed that the CF0513 transconjugate carried a bla_{ACT-1}-positive plasmid that belonged to the IncL/M incompatibility type. The plasmid was also the same size as that of the original strain CF0513. To assess if plasmid p0513, encoding bla_{ACT-1}, was stable, both the donors and transconjugants were serially split and regrown up to 10 times on separate LB agar plates with cefotaxime (8 mg/L). The plasmid persisted even after the last generation, which indicates that p0513 is highly stable.

A wide variety of plasmid-mediated β-lactamases, especially ESBLs and pAmpCs, have been identified worldwide. However, other than *Escherichia* spp. and *Klebsiella* spp., they are rarely investigated in China among Enterobacteriaceae where the resistance problem has increased dramatically. *C. freundii*, which belongs to the Enterobacteriaceae family, is a prominent opportunistic pathogen and characterized by chromosomally-encoded AmpC β-lactamases. To our knowledge, there are no reports of resistance caused by plasmid-mediated AmpC β-lactamases in *C. freundii*. In our study, we describe an isolated *C. freundii* carrying the ACT-1 type pAmpCs. Furthermore, the bla_{ACT-1} gene was carried by an IncL/M plasmid and was associated with $bla_{CTX-M-14}$, *armA*, IS*Ecp1*, and *IntI1*. Due to these characteristics, *C. freundii* CF0513 was also resistant to aminoglycosides, such as gentamicin and amikacin. To our knowledge, this is the first report of an isolated *C. freundii* producing ACT-1 AmpC β-lactamase.

In conclusion, the bla_{ACT-1} gene associated with IS*Ecp1* found on a transmissible plasmid in *C. freundii* is cause for serious concern, as this insertion element facilitates the mobilization and expression of ACT-1-mediated resistance. This is alarming, since horizontal transfer played a role in the dissemination of these multidrug resistant isolates. Such strains, with acquired resistance, further limit the therapeutic options in clinical work. Further investigation of the association between mobile elements and resistance genes is warranted.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

REFERENCES

1. Choi SH, Lee JE, Park SJ, Choi SH, Lee SO, Jeong JY, et al. Emergence of antibiotic resistance during therapy for infections caused by *Enterobacteriaceae* producing AmpC beta-lactamase: implications for antibiotic use. Antimicrob Agents Chemother 2008;52:995-1000.
2. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-Second informational supplement, M100-S22. Wayne, PA: Clinical and Laboratory Standards Institute, 2012.
3. Yong D, Park R, Yum JH, Lee K, Choi EC, Chong Y. Further modification of the Hodge test to screen AmpC β-lactamase (CMY-1)-producing strains of *Escherichia coli* and *Klebsiella pneumoniae*. J Microbiol Methods 2002;51:407-10.
4. Pérez-Pérez FJ and Hanson ND. Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 2002;40:2153-62.
5. Park YJ, Kim SY, Yu JK, Kim SI, Uh Y, Hong SG, et al. Spread of *Serratia marcescens* coharboring *aac(6′)-Ib-cr*, bla_{CTX-M}, *armA*, and bla_{OXA-1} carried by conjugative IncL/M type plasmid in Korean hospitals. Microb Drug Resist 2009;15:97-102.
6. Kim HB, Park CH, Kim CJ, Kim EC, Jacoby GA, Hooper DC. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. Antimicrob Agents Chemother 2009;53:639-45.
7. Voets GM, Fluit AC, Scharringa J, Cohen Stuart J, Leverstein-van Hall MA. A set of multiplex PCRs for genotypic detection of extended-spectrum β-lactamases, carbapenemases, plasmid-mediated AmpC β-lactamases and OXA β-lactamases. Int J Antimicrob Agents 2011;37:356-9.

8. Yang J, Ye L, Wang W, Luo Y, Zhang Y, Han L. Diverse prevalence of 16S rRNA methylase genes *armA* and *rmtB* amongst clinical multidrug-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates. Int J Antimicrob Agents 2011;38:348-51.
9. Carattoli A. Resistance plasmid families in *Enterobacteriaceae*. Antimicrob Agents Chemother 2009;53:2227-38.

http://dx.doi.org/10.3343/alm.2013.33.1.86