Letter to the Editor
Clinical Microbiology
Ann Lab Med 2013;33:84-85
http://dx.doi.org/10.3343/alm.2013.33.1.84
ISSN 2234-3806 • eISSN 2234-3814
ANNALS OF LABORATORY MEDICINE

First Detection of VIM-4 Metallo-β-Lactamase-Producing *Citrobacter freundii* in China

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Dear Sir,

Carbapenems are one of the most critically important antimicrobials and are considered as last-choice drugs in clinical settings. However, the emergence and dissemination of Enterobacteriaceae carrying acquired metallo-β-lactamases (MBLs) is being increasingly reported throughout the world, presenting a serious threat to the effectiveness of carbapenem treatment [1]. Acquired MBLs, such as VIM, IMP, and NDM, are associated with different mobile elements, and the genes for VIM-type enzymes are carried as mobile gene cassettes inserted in class 1 integrons. In *Citrobacter freundii*, MBL-mediated resistance is rare and only scattered studies have reported the isolation of a few MBL-producing strains, particularly in the Far East [2]. To our knowledge, there is no report of VIM-producing *C. freundii* in China. In this study, we describe the first identification of the bla_{VIM-4} gene in a clinical *C. freundii* strain isolated from a patient in a hospital in Wuhu, China.

The multidrug-resistant *C. freundii* CF0638 isolate was recovered from the sputum of a 66-year-old man presenting with diabetic foot to a hospital in Wuhu, China, in September 2011. During the period of hospitalization, symptoms of lower respiratory tract infection emerged and this patient received treatment with cefotaxime and ciprofloxacin for 7 days. The effect of the treatment was not satisfactory. The patient continued to be febrile until he was treated with imipenem-cilastatin (3 g/day). Imipenem-cilastatin treatment was continued for a 2-week course, and the symptoms gradually disappeared during the patient's hospital stay.

Susceptibility testing was performed by an agar dilution method according to the CLSI guidelines (2012) [3]. *C. freundii* CF0638 exhibited high-level resistance to most β-lactam antibiotics tested. The minimum inhibitory concentrations (MICs) of imipenem and meropenem were 4 mg/L and 1 mg/L, respectively (Table 1). Positive results of the modified Hodge test with meropenem disc and double-disc synergy test between carbapenems and EDTA indicated the production of an MBL. CF0638 was subjected to PCR analysis using the respective primer pairs and amplifying conditions for bla_{IMP} and bla_{VIM}, as described previously [4], and followed by DNA sequencing. Nucleotide sequence analysis and homology searches were carried out using the BLAST database (http://www.ncbi.nlm.nih.gov). To determine whether the imipenem resistance 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 (4 mg/L).

PCR amplification and nucleotide sequence analysis showed that CF0638 harbored bla_{VIM-4}. Conjugation experiments showed that imipenem resistance was successfully transferred to the recipient *E. coli* J53. PCR analysis of the transconjugant strain was positive for the bla_{VIM-4} gene. Susceptibility test results of *C. freundii* CF0638 and its transconjugant are presented in Table 1.

C. freundii has been recognized as an opportunistic pathogen that is rarely involved in nosocomial infections. In neonates and

Received: July 25, 2012
Revision received: July 26, 2012
Accepted: November 6, 2012

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Table 1. MIC for VIM-4 metallo-β-lactamase-producing *Citrobacter freundii* CF0638 and its transconjugant

Strain	MIC (mg/L)												
	PIP	CTX	CAZ	FEP	FOX	ATM	IMP	MEM	CIP	LVX	GAT	GM	AMK
CF0638	512	128	64	8	>256	>256	4	1	>32	32	16	>256	>1,024
Transconjugant of CF0638	256	128	32	4	64	128	2	0.5	2	1	1	128	512
E. coli J53	<0.5	<0.5	<0.5	<0.25	<0.5	<0.5	<0.25	<0.25	<0.06	<0.125	<0.125	<0.25	<0.25

Abbreviations: MIC, minimum inhibitory concentration; PIP, piperacillin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; ATM, aztreonam; IMP, imipenem; MEM, meropenem; CIP, ciprofloxacin; LVX, levofloxacin; GAT, gatifloxacin; GM, gentamicin; AMK, amikacin.

immunocompromised patients, however, invasive infections such as bacteremia, meningitis, brain abscesses, pneumonia, endocarditis, and intra-abdominal sepsis have also been reported [5]. VIM-4 is a single amino-acid variant of VIM-1 β-lactamase. VIM-4 was first described in *Pseudomonas aeruginosa* in Greece [6], but was subsequently identified as the most common integron-encoded MBL in Enterobacteriaceae species in several European countries. *C. freundii* isolates are usually susceptible to carbapenems and only a limited number of carbapenem-resistant strains have been identified. Prompt and accurate detection of MBL-producing *C. freundii* strains is necessary to prevent the dissemination of resistance vectors to more virulent pathogens.

Authors' Disclosures of Potential Conflicts of Interest

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

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