*Journal of Antimicrobial Chemotherapy* (2008) **61**, 219–229
doi:10.1093/jac/dkm453
Advance Access publication 16 November 2007



## Emergence of *Candida tropicalis* resistant to caspofungin

Tim Pasquale[1], Jon Rupert Tomada[1]*, Mahmoud Ghannoun[2], Joseph Dipersio[1] and Hector Bonilla[1]

[1]*Summa Health System, 75 Arch Street, Akron, OH 44304, USA;* [2]*University Case Medical Center, 11100 Euclid Avenue, LKS 5028, Cleveland, OH 44106, USA*

Keywords: echinocandins, invasive candidiasis, candidal oesophagitis

*Corresponding author. Tel: +1-206-3727047; Fax: +1-330-3756608; E-mail: jonrupert@gmail.com

Sir,

*Candida* species is the fourth leading cause of nosocomial bloodstream infection in the USA. The incidence and mortality of invasive candidiasis (IC) remain high despite new antifungal agents. Although *Candida albicans* remains the most common isolated species causing IC, the incidence of IC caused by non-*albicans* species is increasing.[1] Caspofungin, an echinocandin, is approved for treatment of IC.[2] Resistance to caspofungin has been rarely reported.

We admitted a 28-year-old female with acute myelogenous leukaemia (AML) for fever and otitis media. She was started on caspofungin [70 mg intravenous (iv) loading dose followed by 50 mg iv daily] on her fifth hospital day due to persistent fever despite iv broad-spectrum antibiotics. Defervescence was noted and she was started on induction chemotherapy for AML on the sixth hospital day. She again developed fever with symptoms of oesophagitis on her 21st hospital day. Oesophagogastroduodenoscopy with oesophageal biopsy was performed and showed invasive candidal oesophagitis. Caspofungin was switched to liposomal amphotericin B. Her repeat blood cultures grew *Candida tropicalis* susceptible to fluconazole. Amphotericin B was switched to fluconazole and the patient improved slowly. She completed 4 weeks of fluconazole treatment.

We subsequently performed additional *in vitro* MIC testing for echinocandins on the isolated *C. tropicalis* using the CLSI (formerly NCCLS) M27-A2 standard methods.[3] This method has been proven to be reproducible and reliable in the study of MIC trends and patterns of antifungal medications.[4] MICs of caspofungin, micafungin and anidulafungin were 4.0, 8.0 and 1.0 mg/L, respectively.

This is the first documented case of *C. tropicalis* infection clinically resistant to caspofungin. This suggests that all *Candida* species are capable of developing resistance to echinocandins. The CLSI has established an echinocandin MIC of >2 mg/L to identify 'non-susceptible' *Candida* species.[5] Our isolated strain's caspofungin MIC is consistent with this breakpoint.

We also observed cross-resistance of the isolated species to micafungin. This pattern of cross-resistance is similar to the observations made by Mougdal *et al.*[6] for *Candida parapsilosis*. This may suggest a similar mechanism of resistance of *Candida* species to echinocandins, specifically caspofungin and micafungin.

Four mechanisms of reduced susceptibility to caspofungin have been suggested: (i) *fks1* gene mutation; (ii) efflux-based mechanism; (iii) Sbe2p overexpression; and (iv) paradoxical or eagle effect.[1,7] Only the *fks1* gene mutation, however, is proven to cause clinical failure with caspofungin therapy.[1] Further investigation of the *fks1* gene of our isolated strain would help support *Candida* species' similarity of mechanism for echinocandin resistance.

### Funding

None.

### Transparency declarations

None to declare.

### References

**1.** Pfaller MA, Diekema DJ. Epidemiology of invasive candidiasis: a persistent public health problem. *Clin Microbiol Rev* 2007; **20**: 133–63.

**2.** Pappas PG, Rex JH, Sobel JD *et al.* Guidelines for treatment of candidiasis. *Clin Infect Dis* 2004; **38**: 161–89.

**3.** National Committee for Clinical Laboratory Standards. *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeast: Approved Standard M27-A2.* NCCLS, Wayne, PA, USA, 2002.

**4.** Ostrosky-Zeichner L, Rex JH, Pappas PG *et al.* Antifungal susceptibility survey of 2,000 bloodstream *Candida* isolates in the United States. *Antimicrob Agents Chemother* 2003; **47**: 3149–54.

**5.** Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated from Animals: Informational Supplement M31-S1.* CLSI, Wayne, PA, USA, 2007.

**6.** Mougdal V, Little T, Boikov D *et al.* Multiechinocandin- and multiazole-resistant *Candida parapsilosis* isolates serially obtained during therapy for prosthetic valve endocarditis. *Antimicrob Agents Chemother* 2005; **49**: 767–9.

**7.** Stevens DA, Ichinomiya M, Koshi Y *et al.* Escape of *Candida* from caspofungin inhibition at concentrations above the MIC (paradoxical effect) accomplished by increased cell wall chitin; evidence for β-1,6-glucan synthesis inhibition by caspofungin. *Antimicrob Agents Chemother* 2006; **50**: 3160–1.

*Journal of Antimicrobial Chemotherapy*
doi:10.1093/jac/dkm444
Advance Access publication 16 November 2007

## Detection of $bla_{VIM-2}$ carbapenemase in *Pseudomonas aeruginosa* in Ireland

F. Walsh* and T. R. Rogers

*Department of Clinical Microbiology, Trinity College Dublin, St James's Hospital, Dublin 8, Ireland*

Keywords: *P. aeruginosa*, metallo-β-lactamases, MBLs

*Corresponding author. Tel: +353-1-896-3620; E-mail: walshf1@tcd.ie

Sir,

*Pseudomonas aeruginosa* is one of the most frequently isolated Gram-negative pathogens associated with hospital-acquired infections. The carbapenems, imipenem and meropenem, have a broad spectrum of activity and are considered the agents of choice for the treatment of *P. aeruginosa* infections. However, metallo-β-lactamases (MBLs), which readily hydrolyse most β-lactams, including carbapenems, are increasingly being identified among *P. aeruginosa* clinical isolates. The VIM-type MBLs have been widespread in Asia, Southern Europe and North America.[1] Since 2000, the Antibiotic Resistance Monitoring and Reference Laboratory has received approximately 80 isolates, mostly *Pseudomonas* species, from the UK, which tested positive for $bla_{VIM}$ (Dr Neil Woodford, Health Protection Agency, London, personal communication). This study describes the first identification of $bla_{VIM-2}$ in Ireland.

*P. aeruginosa* isolates 32297 and 15488 were isolated from a swab and a sputum sample, respectively, from the same patient at St James's Hospital, Dublin, Ireland. These isolates were 74.3% similar with more than three band differences, as determined by *Xba*I restriction digest and separation using PFGE, and thus considered unrelated. Both isolates were resistant to imipenem and meropenem using the CLSI guidelines.[2] Isolate 32297 had imipenem and meropenem MICs of 64 and 16 mg/L, respectively. Isolate 15488 had imipenem and meropenem MICs of >128 mg/L for both carbapenems. Both isolates had a reduction in imipenem and meropenem MICs of greater than 3 doubling dilutions in the presence of EDTA (320 mg/L), suggesting the presence of an MBL. This was confirmed by the MBL Etest (AB Biodisk, Solna, Sweden). $bla_{VIM}$- and $bla_{IMP}$-positive controls were included in each test. Isoelectric focusing was performed using the methods of Matthew *et al.*[3] $bla_{VIM}$- and $bla_{IMP}$-positive controls were included in the gels. The $bla_{VIM}$ control had pI values of 8.65, 6.85 and 6.75. Isolate 15488 had pI values of 8.65, 7.2 and 6.65 and isolate 33075 had values of 8.15 and 7.85. Both isolates were screened by PCR for the presence of $bla_{VIM}$, $bla_{IMP}$, $bla_{SPM}$ and $bla_{GIM}$ genes. Isolate 15488 was $bla_{VIM}$-positive by PCR. The $bla_{VIM}$ gene was amplified by PCR and sequenced using primers forward 5′-GGC ATC CAA GCA GCA AG-3′ and reverse 5′-AAG CAG ACT TGA CCT GA-3′ for the amplification of the entire gene, which yield an 800 bp PCR product. The PCR product of 15488 had 100% identity with the $bla_{VIM-2}$ gene, GenBank accession number AY943084.1. Sequencing results also revealed that the $bla_{VIM-2}$ gene of 15488 was inserted as a single gene cassette into a class 1 integron. The two isolates were tested phenotypically for the presence of efflux pumps by measuring the meropenem MIC in the presence of efflux pump inhibitors carbonyl cyanide *m*-chlorophenylhydrazone (50 mg/L) and phenyl-arginine-β-naphthylamide (20 mg/L).[4,5] There were no changes in the carbapenem MICs in the presence of the efflux pump inhibitors. The outer membrane proteins of the two clinical isolates, the $bla_{VIM}$ and $bla_{IMP}$ controls and the susceptible strain ATCC 27853 were extracted from exponentially growing cells. Both clinical isolates, in contrast to the susceptible ATCC 27853 strain, had loss of a 46 kDa protein band, which corresponds to the OprD porin. Isolate 33075 was PCR-negative for the tested MBLs, although a reduction in carbapenem MIC in the presence of EDTA has been detected. Such false-positive results could be attributed to the EDTA interference with the cell wall permeability as EDTA affects the regulation of the OprD porin and may facilitate the entry of carbapenems.[6]

To the best of our knowledge, this is the first report of an MBL-producing *P. aeruginosa* isolate from Ireland. The MBL was not associated with an outbreak. The integron did not contain other resistance gene determinants. Although this isolate was not associated with an outbreak, the identification of this carbapenemase on a mobile element poses a threat to infection control. Carbapenem-resistant $bla_{VIM-2}$-producing *P. aeruginosa* clones have emerged in several countries such as Portugal, Poland, Spain, Italy, Greece and Japan and can be attributed to the spread of $bla_{VIM-2}$-producing *P. aeruginosa*. Therefore, continued monitoring and surveillance of *P. aeruginosa* for MBLs is required to minimize the threat of outbreaks. The identification of $bla_{VIM-2}$ in Ireland also indicates that MBLs from their original reports in southern Europe have been further spread within Europe.

## Acknowledgements

We wish to thank the staff of St James's Microbiology Department and Dr Neil Woodford for the donation of the $bla_{VIM}$- and $bla_{IMP}$-positive controls. Part of this study was presented at the Forty-sixth Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, 2006.

## Funding

This project was funded by the University of Dublin, Trinity College.

## Transparency declarations

None to declare.

## References

**1.** Walsh TR, Toleman MA, Poirel L *et al.* Metallo-β-lactamases: the quiet before the storm? *Clin Microbiol Rev* 2005; **18**: 306–25.

**2.** Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Sixteenth Informational Supplement M100-S16*. CLSI, Wayne, PA, USA, 2006.

**3.** Matthew M, Harris AM, Marshall MJ. The use of analytical isoelectric focusing for the detection and identification of β-lactamases. *J Gen Microbiol* 1975; **88**: 169–78.

**4.** Pournaras S, Maniati M, Spanakis N *et al.* Spread of efflux pump-overexpressing, non-metallo-β-lactamase-producing, meropenem-resistant but ceftazidime-susceptible *Pseudomonas aeruginosa* in a region with $bla_{VIM}$ endemicity. *J Antimicrob Chemother* 2005; **56**: 761–4.

**5.** Renau TE, Leger R, Flamme EM *et al.* Inhibitors of efflux pumps in *Pseudomonas aeruginosa* potentiate the activity of the fluoroquinolone antibacterial levofloxacin. *J Med Chem* 1999; **42**: 4928–31.

**6.** Livermore DM, Brown DFJ. *Detection of β-Lactamase-Mediated Resistance*. http://www.bsac.org.uk/susceptibility_testing/powerpoint_presentations.cfm (15 October 2007, date last accessed).