out; first, these strains were not detected in Bariloche until 2010 and, second, they harboured blaKPC-2 in the Variant 1a.\textsuperscript{6} ST162 was isolated from a patient with no recent history of travel. Interestingly, this patient shared the same ward simultaneously with two other patients undergoing infections due to KPC-producing \textit{Enterobacter cloacae} and \textit{K. pneumoniae}. In these isolates, the bla\textsubscript{KPC-2} genetic environment matched that of ST162. However, bla\textsubscript{KPC-2} in \textit{E. cloacae} and \textit{K. pneumoniae} was associated with plasmids of different sizes (40 and >150 kb, respectively)\textsuperscript{6} from that detected in ST162. Thus, we speculated that the surge of ST162 could be due to bla\textsubscript{KPC-2} mobilization among different plasmids (i.e. transposition).

Clonal complexes CC111 and CC235 have been reported as the major clones involved in the global dissemination of extended-spectrum and metallo-\beta-lactamases in \textit{P. aeruginosa}.\textsuperscript{8} However, we observed that the main dissemination of KPC was mediated by a new clone (ST654) not related to both CC111 and CC235. ST654 is endemic in Singapore,\textsuperscript{9} where it has been associated with IMP-1 and IMP-26; it has also been reported in Sweden as a VIM-2 producer, although the isolate was imported from Tunisia.\textsuperscript{10} Unlike other Latin American regions, such as Puerto Rico and Colombia, where different PFGE types and STs have been identified,\textsuperscript{1,4} the dissemination of KPC-producing \textit{P. aeruginosa} in Argentina is mainly associated with a single clone. These findings confirm that ST654 plays an important role in the global spread of carbapenemases, either metallo-\beta-lactamases or KPC. Thus, the worldwide dissemination of KPC-producing \textit{P. aeruginosa} of ST654 might be expected and should be monitored.

Acknowledgements
Part of this work was presented at the Fifty-first Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, 2011 (Abstract no. C2-1700).

\textbf{Pseudomonas aeruginosa KPC Group}

\textbf{Funding}
This work was supported by the regular federal budget of the Ministry of Health of Argentina.

\textbf{Transparency declarations}
None to declare.

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\textit{J Antimicrob Chemother} 2012
doi:10.1093/jac/dkr593
Advance Access publication 18 January 2012

\textbf{In vitro activity of tigecycline against multidrug-resistant Gram-negative blood culture isolates from critically ill patients}

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\textbf{Keywords:} MIC, VITEK 2C, intensive care unit

Sir,

With increasing resistance to currently available antibiotics and decreasing numbers of newer antimicrobial agents, tigecycline

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represents a new therapeutic alternative. Tigecycline (a 9-t-butyIglycylam ide derivative of minocycline) is a glycylcycline antimicrobial agent having potent in vitro activity against most of the multidrug-resistant (MDR) Gram-positive and Gram-negative bacteria, except for *Pseudomonas aeruginosa* and Proteae.1,2 Moreover, tigecycline is not affected by the mechanisms of resistance to tetracycline (efflux and ribosomal protection).3

As in other tertiary care hospitals in India, drug resistance in bacteria has been an obstacle to treatment in intensive care units. Carbapenems have been the last resort in treating MDR Gram-negative infections. An increase in carbapenem resistance in Gram-negative organisms has also been noted recently.3 This study was conducted to evaluate the in vitro activity of tigecycline against extended-spectrum β-lactamase (ESBL)-producing and/or MDR Gram-negative bacteria isolated from the blood of critically ill patients.

From May 2010 to February 2011, blood culture was performed using the automated BacT/Alert blood culture system (bioMérieux, Marcy l’Étoile, France) from 827 critically ill patients admitted to the intensive care unit of St John’s Medical College and Hospital, Bangalore, India. Only one isolate per patient was identified. Non-repetitive Gram-negative bacilli (n = 69; 8.34%) were isolated from 827 patients. The isolates were identified as *Escherichia coli* (n = 20), *Klebsiella pneumoniae* (n = 9), *Enterobacter cloacae* (n = 2), *Proteus mirabilis* (n = 2), *Serratia marcescens* (n = 1), *Citrobacter koseri* (n = 1), *Acinetobacter baumannii* (n = 18), *Acinetobacter lwoffii* (n = 1), *Pseudomonas aeruginosa* (n = 11), *Pseudomonas putida* (n = 1), *Achromobacter xylosoxidans* (n = 2) and *Chromobacterium indologenes* (n = 1), using routine phenotypic microbiological methods and an automated identification system (VITEK 2, bioMérieux).

MICs of antibiotics were determined using an automated system (VITEK 2), following the manufacturer’s guidelines. For analysis of in vitro activity of tigecycline, ESBL-producing strains of Enterobacteriaceae [85% of the *E. coli* (n = 17) and 77.8% of the *K. pneumoniae* (n = 7)] and MDR (resistant to at least three classes of commonly used antimicrobials) strains of non-fermenting Gram-negative bacteria [84.2% of the *Achromobacter* spp. (n = 16) and 100% of the *Pseudomonas* spp. (n = 12)] were included. ESBL production for Enterobacteriaceae was determined by the VITEK 2 ESBL confirmation test (AST-GN25 card; bioMérieux) and/or double disc synergy test using cefotaxime and cefotaxime/clavulanic acid discs. For tigecycline a susceptibility breakpoint of ≤2 mg/L was applied for all the Gram-negative isolates, according to CLSI guidelines. The study was approved by the institutional ethics review board of St John’s Medical College and Hospital, Bangalore, India.

Tigecycline showed good potency, inhibiting 100% of the ESBL-producing *E. coli*, 85.7% of the ESBL-producing *K. pneumoniae* and 93.7% of the MDR *A. baumannii* at a concentration of ≤2 mg/L (Table 1). A tigecycline MIC₉₀ of 2 mg/L was noted for *A. baumannii*, a result similar to those of other studies.4,5 As expected, 100% of the *P. aeruginosa* (MIC₉₀ and MIC₉₀ of 16 mg/L) and *Proteus mirabilis* showed resistance to tigecycline, irrespective of being MDR or not. However, a single strain of MDR P. *putida* isolated was susceptible to tigecycline (MIC 2 mg/L). It should be noted that the MIC₉₀ for *K. pneumoniae* was higher compared with the other Enterobacteriaceae tested, as also observed by Souli et al.6 Carbapenem resistance was observed in 28.6% of ESBL-producing *K. pneumoniae*, 100% of MDR *Acinetobacter* spp. and 66.7% of MDR *Pseudomonas* spp. The results of our study support the previous studies showing good in vitro activity of tigecycline against MDR organisms,1,5,6 with a few exceptions.3

Good in vitro potency of tigecycline was observed against ESBL-producing *E. coli*, *K. pneumoniae* and MDR *Acinetobacter* spp. Tigecycline can play a key role in tackling MDR organisms in the critical care units of tertiary care hospitals.

### Acknowledgements

We thank the staff of the intensive care unit for their help and support.

### Funding

This work was supported by ‘Council of Scientific and Industrial Research’ (CSIR), India.

### Transparency declarations

None to declare.

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The combination of chloroquine and minocycline, a therapeutic option in cerebrospinal infection of Whipple’s disease refractory to treatment with ceftriaxone, meropenem and co-trimoxazole

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Keywords: Tropheryma whipplei, T. whipplei, cerebral Whipple’s disease

Sir,

Whipple’s disease is a chronic infection caused by Tropheryma whipplei. In a prospective study, the CNS was found to be involved in 38.5% of the cases. Treatment is not always successful.1–6 Co-trimoxazole (trimethoprim/sulfamethoxazole) has been reported to be significantly more effective than tetracycline;2 however, resistance to co-trimoxazole has also been observed.2–6 More recently, there have even been reports describing resistance of CNS infections to treatment with ceftriaxone, a bactericidal antibiotic penetrating the blood–brain barrier.1,7

In 2002, a patient presented with diarrhoea, weight loss to 64 kg, anaemia (8.4 g/dL haemoglobin) and erythrocyte sedimentation rate of 33 mm/h. The medical history revealed relapsing arthritis since 1991 and pericarditis necessitating pericardial resection in 1993. Gastrointestinal biopsies disclosed periodic acid-Schiff (PAS)-positive macrophages typical of untreated Whipple’s disease in the mucosa of the duodenum and the ileum and in the submucosa of the colon. The patient had no cerebral symptoms. However, microscopic examination of centrifuged CSF obtained by spinal puncture showed a PAS-positive macrophage typical of Whipple’s disease (histopathology carried out by Dr Reinhard Golz, Wuppertal), and the PCR to T. whipplei in the CSF was positive. The patient was admitted to a prospective treatment trial, as reported in Feurle et al.7 He was randomized to 2 g of ceftriaxone infused intravenously once daily for 2 weeks, followed by oral co-trimoxazole at a dosage of 160/300 mg twice daily for 12 months. While the patient recovered from all signs and symptoms of Whipple’s disease, the PCR for T. whipplei remained positive in the CSF for 5.5 years despite additional treatment with 1 g of meropenem infused intravenously thrice daily for 2 weeks followed by co-trimoxazole for another year.1 After a further year of co-trimoxazole, the CSF was still positive in the T. whipplei PCR, while the patient was receiving this treatment. PAS-positive macrophages in duodenal mucosal biopsies and in the CSF had disappeared. CT and magnetic resonance imaging of the brain did not reveal any structural abnormality. At this time, the patient had no symptoms of systemic or cerebral Whipple’s disease.

The presence of T. whipplei in the CSF was confirmed by sequence analysis of the amplification product, and the viability was established by culture in MRCS fibroblasts and in axenic medium. In vitro susceptibility of the cultured strain to ceftriaxone, meropenem, tetracyclines and co-trimoxazole is shown in Table 1. The risk of this patient developing symptomatic cerebral disease seemed unpredictable. After obtaining written informed consent for an individual treatment attempt, this patient was treated with chloroquine and tetracycline, a combination suggested previously.8 Chloroquine (or hydroxychloroquine) enhances antimicrobial activity of tetracyclines by raising the pH of the phagolysosomes within macrophages by inhibiting the pH of the phagolysosomes within macrophages.8 Minocycline was selected as the tetracycline in this case as this compound has been reported to cross the blood–brain barrier well.9,10 Pollock et al.,11 taking advantage of this pharmacokinetic property, were probably the first to treat a patient with cerebral Whipple’s disease with minocycline. They did not combine it with chloroquine. Others prefer the combination of doxycycline and chloroquine.6

In the present study, the dosage of chloroquine, based on a body weight of 83 kg, was 1725 mg of chloroquine phosphate in divided doses orally on day 1, and 575 mg on the second to the fourth day, followed by one 250 mg chloroquine phosphate tablet once daily for 45 days. A serum concentration of 165 μg/L chloroquine (therapeutic range 20 to 200 μg/L) was obtained. Minocycline was given at a dosage of one 100 mg minocycline tablet twice daily for 45 days. This dosage had to be reduced to 100 mg daily from day 5 to day 8 because of vertigo. Vestibular toxicity manifesting as dizziness, ataxia and nausea is a

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Table 1. In vitro antimicrobial susceptibility of the cultured T. whipplei strain, assessed by methods reported previously7

<table>
<thead>
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<th>Antibiotic</th>
<th>axenic medium</th>
<th>MRCS fibroblasts</th>
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<td>Co-trimoxazole</td>
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