J Antimicrob Chemother 2017; **72**: 317–319
doi:10.1093/jac/dkw378
Advance Access publication 12 September 2016

Isolation of seven distinct carbapenemase-producing Gram-negative organisms from a single patient

Julie Creighton[1]*, Helen Heffernan[2] and Julia Howard[1]

[1]*Canterbury Health Laboratories, Christchurch, New Zealand;* [2]*Antibiotic Reference Laboratory, Institute for Environmental Science and Research (ESR), Wellington, New Zealand*

*Corresponding author. Tel: +64-33640117; Fax: +64-33640238; E-mail: julie.creighton@cdhb.health.nz

Sir,

Carbapenemase-producing organisms (CPO) continue to spread around the globe at an alarming rate.[1,2] People travelling to, and especially hospitalized in, areas that have high prevalence rates of MDR organisms (MDRO), such as ESBL- and carbapenemase-producing Gram-negative organisms, are at risk of acquiring these bacteria and subsequent colonization or infection.[3] In low-prevalence countries, such as New Zealand (NZ), this risk factor has recently been identified for the first cases of *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae* reported in NZ.[4] Current reports of CPO in NZ are rare, with a low number of patients found to have cocarriage of more than two CPO and coproducing strains infrequently encountered.[5] Here, we report the isolation of seven distinct CPO, together with an ESBL-producing *Pseudomonas aeruginosa* and *Proteus mirabilis* with an ACC-type AmpC β-lactamase, from a patient recently returned to NZ from a Romanian hospital.

In November 2015, a NZ tourist was transferred to Christchurch Hospital after spending 2 months hospitalized in Bucharest, Romania. Due to travel history, screening samples were collected on admission. For detection of ESBL/CPO, faeces, multiple burn wound swabs and urine and tracheal aspirate samples were cultured onto CHROM Agar ESBL/VRE (Fort Richard Laboratories, NZ) as well as MacConkey agar with a 10 μg meropenem disc placed in the first quadrant. For MRSA, nose, groin and perineum swabs were placed in an in-house enrichment broth before subculturing onto MRSA Select (Bio-Rad, France). For VRE screening, faeces were also pre-enriched before subculturing onto VRE Select (Bio-Rad). Isolates were identified using MALDI-TOF (Bruker Daltonics, USA). Antimicrobial susceptibility testing was performed with Phoenix™ (BD Diagnostics, USA) or MIC gradient strips (bioMérieux, France or Liofilchem, Italy) using EUCAST breakpoints. Initial laboratory investigation included phenotypic ESBL detection, CarbaNP and GeneXpert Carba-R (Cepheid, USA), as previously described.[6] All unique carbapenem-resistant isolates as well as a ceftriaxone-resistant ESBL-negative *P. mirabilis* were sent to the ESR national reference laboratory for identification of β-lactamase genes by PCR and sequencing and strain typing by PFGE.

In total, seven distinct CPO were isolated from samples taken on admission and day 4 catheter urine: two distinct OXA-23-like-producing *Acinetobacter baumannii* strains; OXA-48-producing *K. pneumoniae*; *K. pneumoniae* coproducing OXA-48 and NDM-1; KPC-2-producing *K. pneumoniae*; NDM-1-producing *Providencia stuartii*; and VIM-2-producing *P. aeruginosa* (Table 1). In addition, the VEB-1 ESBL gene was identified in a further carbapenem-resistant *P. aeruginosa*, which is the first VEB-1-producing *P. aeruginosa* reported in NZ. The *P. mirabilis* was found to be carrying bla_{ACC} cephalosporinase, which is the first description of bla_{ACC} from NZ. PFGE demonstrated that any isolates of the same species were distinct (data not shown). No VRE or MRSA were found in any sample.

All CPO, as well as the ESBL-producing *P. aeruginosa*, expressed broad-range multidrug resistance, leaving alarmingly few treatment options (Table 1). Although no isolates were pan-resistant, the NDM-1-producing *P. stuartii* was susceptible to only fosfomycin and colistin was the only option for both *P. aeruginosa* strains and one of the OXA-23-like-producing *A. baumannii* strains. Interestingly, both OXA-23-like-producing *A. baumannii* strains displayed high-level carbapenem resistance, despite the IS*Aba*1 being detected in only one of the strains.

Cocarriage of multiple CPO from a single patient has been reported by other authors. Ding *et al.*[7] reported the identification of four distinct CPO in a patient from eastern China, with *Escherichia coli*, *K. pneumoniae*, *Enterobacter aerogenes* and *A. baumannii* carrying bla_{NDM-1}, bla_{KPC-2}, bla_{IMP-1} and bla_{OXA-23}, respectively. Similarly, Hammerum *et al.*[8] detected four carbapenemases (NDM-7, OXA-181, NDM-5 and OXA-23) in three different Gram-negative species, in a patient who was transferred to Denmark from a hospital in India. A total of six CPO were reported by Drieux *et al.*[9] in a patient who was transferred from Greece to France; however, only two different carbapenemases (KPC-2 and VIM-1) were found amongst five bacterial species. The prevalence and dissemination of CPO in some countries can be difficult to ascertain due to several factors including a lack of national standards for patient screening, inadequate laboratory detection protocols and non-mandatory reporting. The 2013 European surveillance data suggested only sporadic cases of CPO in Romania; however, by 2015 the rating for Romania had escalated to inter-regional spread and the true burden may be underestimated.[10]

We would suggest that the collective finding of seven distinct CPO from a single patient is highly unusual and would imply a heavy burden of a diverse variety of CPO in Romania. This study highlights the risk that patients who have been hospitalized in endemic countries pose for the importation of CPO into low-prevalence countries. In order to reduce the spread of MDRO, it is imperative that all patients with recent hospitalization overseas, possibly extending to recent travel to CPO-endemic areas, be screened for MDRO on admission to NZ hospitals and that laboratories have sensitive and robust procedures to rapidly identify CPO.

Table 1. MDR organisms isolated and susceptibility testing results [MIC (mg/L)]

Antimicrobial[a,b]	*A. baumannii* OXA-23-like	*A. baumannii* OXA-23-like/ ISAba1	*K. pneumoniae* OXA-48	*K. pneumoniae* OXA-48/NDM-1	*K. pneumoniae* KPC-2	*P. stuartii* NDM-1	*P. aeruginosa* VIM-2	*P. aeruginosa* VEB-1	*P. mirabilis* ACC
Ertapenem	NA	NA	**>4**[c]	**>4**	**>4**	**>4**	NA	NA	≤0.25
Imipenem Etest	**>32**	**>32**	**8**	**16**	**4**	**>32**	**>32**	**>32**	NT
Meropenem Etest	**>32**	**>32**	**>32**	**16**	**4**	**>32**	**>32**	**>32**	NT
Piperacillin/tazobactam	**>64/4**	**>64/4**	**>64/4**	**>64/4**	**>64/4**	**>64/4**	**>64/4**	**32/4**	≤4/4
Ceftolozane/tazobactam MIC Test Strip	**>256/4**	**>256/4**	**>256/4**	**>256/4**	1/4	**>256/4**	**>256/4**	**>256/4**	NT
Cefepime	NA	NA	**>16**	**>16**	**>16**	**>16**	**>16**	**>16**	1
Cefoxitin	NA	NA	**>32**	**>32**	**16**	**>32**	**>32**	**>32**	≤4
Aztreonam	NA	NA	**>16**	≤0.5	**>16**	**4**	**>16**	**>16**	≤0.5
Gentamicin	**>8**	**>8**	2	**>8**	≤1	**>8**	**>8**	**>8**	**>8**
Tobramycin	**>8**	≤2	**>8**	**>8**	≤2	**>8**	**>8**	**>8**	**>8**
Amikacin	**>32**	**>32**	**16**	**>32**	≤4	**>32**	**>32**	**16**	≤4
Chloramphenicol	NA	NA	8	**>32**	8	**16**	**>32**	**>32**	**>32**
Colistin	≤1	≤1	**>4**	**>4**	≤1	NA	≤1	≤1	NA
Fosfomycin	NA	NA	≤16	≤16	≤16	≤16	64	64	≤16
Tigecycline	**4**	**4**	**2**	**2**	**2**	NA	**2**	**>4**	NA
Sample site	head wound thigh wound foot wound	sacral wound	tracheal aspirate chest wound	sacral wound	faecal screen	catheter urine	faecal screen groin wound thigh wound	tracheal wound sacral wound thigh wound	groin wound

NA, not applicable as species intrinsically resistant; NT, not tested.
[a]All isolates were resistant to ceftazidime, ceftriaxone, ciprofloxacin and trimethoprim/sulfamethoxazole.
[b]MIC determined by Phoenix with the NMIC-404 panel unless otherwise specified as Etest (bioMérieux) or MIC Test Strip (Liofilchem).
[c]MIC values in bold indicate resistant or non-susceptible.

Acknowledgements

Grateful thanks to the staff at the Antibiotic Reference Laboratory, ESR, for performing the molecular characterization and PFGE tests on these study isolates.

Funding

This study was completed as part of our routine work at Canterbury Health Laboratories. The work at ESR was funded by the NZ Ministry of Health.

Transparency declarations

None to declare.

References

1 Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin Microbiol Infect* 2014; **20**: 821–30.

2 Tängdén T, Giske C. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. *J Intern Med* 2015; **277**: 501–12.

3 Hassing RJ, Alsma J, Arcilla MS *et al*. International travel and acquisition of multidrug-resistant Enterobacteriaceae: a systematic review. *Euro Surveill* 2015; **20**: pii=30074.

4 Heffernan H, Dyet K, Munroe S *et al*. First cases of KPC-type carbapenemase-producing bacteria in patients in New Zealand hospitals. *J Glob Antimicrob Resist* 2014; **2.4**: 330–3.

5 Institute of Environmental Science and Research (ESR). Enterobacteriaceae with Acquired Carbapenemases, 2015. Porirua: ESR, 2016. https://surv.esr.cri.nz/PDF_surveillance/Antimicrobial/ACE/2015Carbap.pdf.

6 Creighton J, Jayawardena S. Comparison of four phenotypic tests, three biochemical tests and Cepheid Xpert Carba-R for detection of carbapenemase enzymes in Gram-negative bacteria. *NZ J Med Lab Sci* 2015; **69**: 81–5.

7 Ding B, Hu F, Yang Y *et al*. Four carbapenem-resistant Gram-negative species carrying distinct carbapenemases in a single patient. *J Clin Microbiol* 2015; **53**: 1031–3.

8 Hammerum AM, Littauer P, Hansen F. Detection of *Klebsiella pneumoniae* co-producing NDM-7 and OXA-181, *Escherichia coli* producing NDM-5 and *Acinetobacter baumannii* producing OXA-23 in a single patient. *Int J Antimicrob Agents* 2015; **46**: 597–8.

9 Drieux L, Bourgeois-Nicolaos N, Cremniter J *et al*. Accumulation of carbapenemase-producing Gram-negative bacteria in a single patient linked to the acquisition of multiple carbapenemase producers and to the in vivo transfer of a plasmid encoding VIM-1. *Int J Antimicrob Agents* 2011; **38**: 179–80.

10 Albiger B, Glasner C, Struelens MJ *et al*. The European Survey of Carbapenemase-producing Enterobacteriaceae (EuSCAPE) working group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries, May 2015. *Euro Surveill* 2015; **20**: pii=30062.

J Antimicrob Chemother 2017; **72**: 319–321
doi:10.1093/jac/dkw361
Advance Access publication 2 September 2016

Pharmacokinetics of colistin methanesulfonate (CMS) in burn patients

Silvia Corcione[1]*†, Lorena Baietto[1]†, Valeria Malvasio[2], Maurizio Stella[2], Giovanni Di Perri[1], Antonio D'Avolio[1]‡ and Francesco Giuseppe De Rosa[1]‡

[1]Department of Medical Sciences, University of Turin, Infectious Diseases at Amedeo di Savoia Hospital, Turin, Italy; [2]Department of Plastic Surgery, Burns Unit, CTO Hospital, Turin, Italy

*Corresponding author. Tel: +39-011-4393828; Fax: +39-011-4393996; E-mail: corcione.silvia@gmail.com
†Both authors equally contributed to the work.
‡Both authors equally contributed to the work.

Sir,

Colistin is now widely used to treat infections caused by MDR Gram-negative bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*.[1] The pharmacokinetics (PK) of colistin, available for parenteral use as colistin methanesulfonate (CMS), which requires metabolization to the active form of colistin, are highly variable especially amongst critically ill patients with varying degrees of renal impairment.[2] Colistin has a concentration-dependent activity and narrow therapeutic window; therefore, therapeutic drug monitoring may represent a useful clinical tool to optimize individual dosage and plasma concentrations, possibly optimizing the antibacterial effect, minimizing the emergence of resistance and reducing colistin-induced side effects.[1] An average steady-state plasma colistin concentration of 2 mg/L has been considered a reasonable target value for infective pathogens with an MIC <1 mg/L.[1] Few PK data are available in burn patients: a population PK analysis by Lee *et al*.[3] and three case reports were published.[4–6] As the clinical interest in optimizing colistin plasma concentrations is increasing, we aimed to describe the PK of colistin treatment in eight patients with *Acinetobacter* infections admitted to a burn unit.

Patients were treated with CMS twice daily by intravenous infusion after appropriate loading dose adjustment for kidney impairment, which occurred in the majority of patients.[7] Blood samples were collected at 0, 1, 3, 5 and 12 h after administration, at steady-state. Colistin and CMS plasma concentrations were determined by two previously reported chromatographic methods with some modifications.[8,9] Colistin was extracted using protein precipitation with acetonitrile containing 0.1% trifluoroacetic acid; the supernatant was diluted 1:1 with water. The CMS concentration was calculated by subtraction of colistin from total colistin after hydrolysis with sulphuric acid (0.5 M). The analysis was performed using an UPLC/MS-MS system. Chromatographic separation was done on an ACQUITY UPLC HSS T3 1.8 μm (2.1×150 mm) column (Waters, Milford, MA, USA), protected by an ACQUITY UPLC Column