of *Stenotrophomonas maltophilia* (63.3% and 62.1% identity, respectively, relative to the *S. maltophilia* strain IAM 1566 protein) (Figure 1). *S. maltophilia* is a Gram-negative bacterium found in a variety of environments, including soil, water and plants, and is therefore a potential reservoir of the MBL gene. Similar to variety of environments, including soil, water and plants, and is therefore a potential reservoir of the MBL gene. 

The combination of PAM-1-mediated β-lactam hydrolysis with genetic mutations that decrease outer-membrane permeability could confer high-level carbapenem resistance, leading to major concern for the treatment of *P. alcaligenes* infection.

**Acknowledgements**

We thank Kumiko Kai and Yoshie Taki for technical assistance. We are grateful to the participating medical institution for providing the strain and clinical information.

**Funding**

This work was supported by a Grant-in-Aid for Young Scientists (A) (25713017) from the Ministry of Education, Culture, Sports, Science and Technology, Japan and H24-Shinkou-Ippan-010 from the Ministry of Health, Labour and Welfare, Japan.

**Transparency declarations**

None to declare.

**References**


**J Antimicrob Chemother** 2014
doi:10.1093/jac/dkt503
Advance Access publication 29 December 2013

Colistin susceptibility testing: time for a review

Mahableswar Albur*, Alan Noel, Karen Bowker and Alasdair MacGowan

Bristol Centre for Antimicrobial Research and Evaluation, Department of Microbiology, Limewalk Building, Southmead Hospital, North Bristol NHS Trust, Westbury-on-Trym, Bristol BS10 5ND, UK

*Corresponding author. Tel: +44-117-323-8671; Fax: +44-117-323-8513; E-mail: msalbur@hotmail.com

**Keywords**: polysorbate 80, microtitre plates, MICs

Sir,

Colistin has re-emerged as an important antimicrobial in recent times owing to limited therapeutic options against carbapenem-resistant Gram-negative bacteria. Current guidelines (BSAC, CLSI and EUCAST) recommend routine colistin susceptibility testing by estimation of MIC because the disc diffusion test does not reliably detect low-level resistance. 

Bacteremia caused by *Pseudomonas aeruginosa*, 29 Acinetobacter spp. and 61 Enterobacteriaceae. The MIC testing was carried out on two different types of polystyrene microtitre trays (MTTs), namely non-coated V-bottom MTTs (NMTTs; costar 3896; Corning, NY, USA) and tissue-culture-coated round-bottom MTTs (TCMTTs; costar 3799; Corning). The MICs of colistin for the isolates were determined using the CLSI broth dilution method using colistin sulphate. MIC determination was carried out by using an initial bacterial inoculum of 5 × 10⁵ cfu/mL in Mueller–Hinton broth with or without P-80 (final P-80 concentration of 0.002%) on both types of MTT. The experiments were done in triplicate, and quality control was assured by concurrent testing of *P. aeruginosa* ATCC 27853 as a control, with all results within the range published by the CLSI.  

MICs for the isolates in both types of MTT with or without P-80 are shown in Table 1. The NMTT MICs (mean 0.54 ± 0.58) were
significantly lower than the TCMTT MICs (mean 2.84 ± 1.93) (P<0.0001; 95% CI –2.5 to –2.1). The tissue coating on MTTs, achieved by means of excess negative electric charge, resulted in an overall 5.3-fold increase in MIC value, probably due to decreased free colistin concentration within the microwells. The differences in MIC results were seen among all types/groups of isolates (3.2, 5.5 and 9.4, respectively, for P. aeruginosa, Enterobacteriaceae and Acinetobacter spp.). The addition of P-80 to NMTTs significantly decreased the colistin MIC (mean 0.09 ± 0.09) by 6-fold (P<0.0001; 95% CI 0.4–0.5). Although there was a relatively smaller decrease (1.24-fold) in the mean MIC determined using TCMTTs with added P-80 (mean 2.3 ± 1.5), this was also statistically significant (P<0.001; 95% CI –0.31 to –0.75). Comparing the MICs determined using NMTTs and TCMTTs containing P-80, there was an even bigger difference in the MIC result than without P-80. There were 25.6-fold differences in the mean MIC results between NMTTs and TCMTTs containing P-80, compared with just 5-fold differences without P-80 (P<0.0001; 95% CI –2.35 to –2.1).

In conclusion, colistin MIC results were greatly influenced by the characteristics of the MTTs. Also, the addition of a commonly used surfactant agent such as P-80 not only significantly altered the result in a single type of MTT but also exponentially exacerbated the difference when tested on different types of MTT panel. The effect of the make-up of MTTs and the presence of P-80 on MIC results were similar among all types/groups of isolates (i.e. P. aeruginosa, Acinetobacter spp, and Enterobacteriaceae). A recent study comparing BMD (with or without P-80), the Etest and the agar dilution method against 50 clinical isolates of multidrug-resistant Gram-negative bacilli showed significant variability among colistin MIC results. At this present time when the therapeutic use of colistin is on the increase with an anticipated rise in colistin resistance, a review of the methodology for colistin MIC testing is urgently needed.9

Acknowledgements
This research work was presented as an abstract at the Fifty-second Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, USA, 2012 (Abstract D-793).

Funding
This research work received support from ‘The Showering Fund’, Department of Pathology, North Bristol NHS Trust, Bristol, UK.

Transparency declarations
None to declare.

References
Polymyxin B and haemofiltration in an adolescent with leukaemia

J. Scott Baird*

Department of Pediatrics, Division of Critical Care Medicine, Columbia University, New York, NY, USA

*Corresponding author. Present address: Morgan Stanley Children’s Hospital of New York-Presbyterian, 3959 Broadway, CHN 10-24, New York, NY 10032-3784, USA. Tel: +1-212-305-8548; Fax: +1-212-342-2293; E-mail: jsb106@columbia.edu

Keywords: clearance, haemofiltration, polymyxin B, sieving coefficient

Sir,

Sandri et al. 1 described the clearance of polymyxin B recently in two patients during continuous venovenous haemodialysis, but data during haemofiltration are not available. An adolescent with relapsed (high-risk) acute lymphocytic leukaemia received a stem cell transplant and developed persistent shock within 24 h. The patient was anuric and received replacement therapy (RRT), as well as vasopressors and polymyxin B for presumptive multidrug-resistant, Gram-negative bacterial sepsis. The polymyxin B dose was variable during the first week of RRT due to changing renal function and support. A dose of 100 mg (1 mg/kg/day) polymyxin B by intravenous infusion was given on days 11 and 12 of RRT. On day 13 of RRT (continuous venovenous haemofiltration via a Prismaflex system with an M100 haemofilter and Prismasol BGK 4/2.5 replacement fluid), post-filter (before replacement fluid) and in the ultrafiltrate (replacement fluid), the concentrations of polymyxin B were 123.3, 117.2 and 0 ng/mL, respectively; these levels were measured when the haemofilter had been in use for ~42 h. The extraction ratio was 0.05, the sieving coefficient was 0 and the haemofilter clearance [extraction ratio x blood flow x (1 – haematocrit)] was 8 mL/min; RRT clearance was thus exclusively via haemofilter adsorption. Further data regarding haemofilter sieving and adsorption of polymyxin B are needed.

Columbia University Medical Center’s IRB exempted this report (AAAM4509) from review.

Darunavir and telaprevir drug interaction: total and unbound plasma concentrations in HIV/HCV-coinfected patients with cirrhosis

Adrian Curran1*, Josep Maria Guiu2, Esteban Ribera1 and Manuel Crespo1

1Hospital Universitari Vall d’Hebron, Barcelona, Spain; 2Institut de Recerca Vall d’Hebron, Barcelona, Spain

*Corresponding author. Infectious Diseases Department, Hospital Universitari Vall d’Hebron, Universitat Autònoma de Barcelona, Passeig Vall d’Hebron 119-129, 08035, Barcelona, Spain. Tel: +34932746090; Fax: +34932746204; E-mail: acurran@vhebron.net

Keywords: pharmacokinetics, hepatic cirrhosis, antiretroviral treatment

Sir,

Telaprevir is an NS3/4A protease inhibitor approved for the treatment of chronic hepatitis C virus (HCV) genotype 1. 1 Telaprevir is primarily metabolized by cytochrome 450 3A4 (CYP3A4). Also, telaprevir is a potent inhibitor of CYP3A4 and intestinal P-glycoprotein, resulting in increased concentrations of CYP3A substrates. 1 Darunavir is mainly metabolized by CYP450 and ritonavir is a potent CYP450 inhibitor. Significant drug–drug interactions have been described in healthy volunteers between telaprevir (750 mg/8 h) and darunavir/ritonavir (600/100 mg/12 h), resulting in decreases in plasma concentrations of both drugs (darunavir: Cmax = −40%, AUC = −40% and Cmin = −42%; telaprevir: Cmax = −36%, AUC = −35% and Cmin = −32%). 2,3 Based on these data, co-administration of darunavir/ritonavir and telaprevir is not recommended. 1 However, a darunavir/