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. Similar to


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Transparency declarations
None to declare.

References


6 Shibata N, Doi Y, Yamane K. P. alcaligenes PAM-1-positive MRY13-0052 strain was not categorized as carbapenem resistant. However, 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.


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Colistin susceptibility testing: time for a review

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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. Broth microdilution (BMD) is widely used as a method of MIC estimation in Europe and the USA. Colistin exhibits a varying degree of adherence to organic and inorganic materials due to its polycationic nature, resulting in loss during experimental conditions. Also, polysorbate 80 (P-80), a surfactant widely used as a dispersing agent in BMD panels, may influence the free drug concentration of colistin and hence MIC results. We evaluated the impact of the use of different BMD panels and the presence of P-80 on colistin MIC estimation. A total of 146 clinical isolates collected from a variety of sources and stored at –70°C were evaluated in this study. The isolates included 56 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 × 10⁶ 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.

Acknowledgements
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Transparency declarations
None to declare.

References

Table 1. Colistin MICs for isolates

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Trays</th>
<th>MIC (mg/L)</th>
<th>Geometric mean</th>
<th>Average</th>
<th>SD</th>
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<td></td>
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<td>0.015625</td>
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<td>0.0625</td>
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<tr>
<td>P. aeruginosa, n=168 (56×3)</td>
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<td>22</td>
<td>91</td>
<td>42</td>
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<tr>
<td></td>
<td>NMTT+P-80</td>
<td>29</td>
<td>4</td>
<td>12</td>
<td>125</td>
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<tr>
<td></td>
<td>TCMTT</td>
<td>3</td>
<td>2</td>
<td>26</td>
<td>110</td>
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<tr>
<td></td>
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<td>7</td>
<td>12</td>
<td>33</td>
<td>17</td>
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<td>Acinetobacter spp., n=87 (29×3)</td>
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<td>10</td>
<td>41</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>NMTT+P-80</td>
<td>14</td>
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<td>29</td>
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</tr>
<tr>
<td></td>
<td>TCMTT</td>
<td>4</td>
<td>32</td>
<td>34</td>
<td>14</td>
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<td></td>
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<td>TCMTT+P-80</td>
<td>32</td>
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</tbody>
</table>
Polymyxin B and haemofiltration in an adolescent with leukaemia

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Keywords: clearance, haemofiltration, polymyxin B, sieving coefficient

Sir,

Sandri et al. 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 required renal 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 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 × blood flow × (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.

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References

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

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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. 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. 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 = −36%, AUC = −35% and Cmin = −32%); telaprevir: Cmax = −36%, AUC = −40% and Cmin = −42%)

Based on these data, co-administration of darunavir/ritonavir and telaprevir is not recommended. However, a darunavir/