Isolation of colistin-resistant *Hafnia alvei*

Colistin belongs to the polymyxins, a group of polypeptide antibiotics which includes polymyxins A, B, C, D and colistin (polymyxin E). Of these, only polymyxins B and E have been employed for therapy of human infections (Gales et al., 2001; Kasiakou et al., 2005; Tan & Ng, 2006). Colistin is mainly administered as colistin sulphomethate sodium; the active drug is then released after hydrolysis and removed by glomerular filtration (Muyembe et al., 1973). Bactericidal activity is due to binding of cell membrane phospholipids and subsequent rapid permeability changes, leading to leakage of cell contents. Interestingly, this process is not dependent on bacterial metabolic activity (Gales et al., 2001; Kasiakou et al., 2005; Tan & Ng, 2006). Colistin emerged in the early 1960s (Catchpole et al., 1997; Kasiakou et al., 2005) as an alternative for treatment of multidrug-resistant *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* infections, but due to its neurotoxicity (neuromuscular blockade, dizziness, nausea, convulsions, coma) and nephrotoxicity (Gales et al., 2001; Jones et al., 2005; Kasiakou et al., 2005; Tan & Ng, 2006) it was displaced in the 1970s by the less toxic aminoglycosides, carboxypenicillins and cephalosporins.

Clinical use of colistin has therefore been limited to use in oral non-absorbable compounds, nebulized formulations, and topical preparations for therapy of otitis, conjunctivitis and skin infections (Jones et al., 2005; Kasiakou et al., 2005; Tan & Ng, 2006) it was displaced in the 1970s by the less toxic aminoglycosides, carboxypenicillins and cephalosporins.

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susceptible *A. baumannii* strains have been successfully treated (Bassetti *et al.*, 2008) with intravenous colistin sulphomethate sodium plus rifampicin. Also, neither renal failure (among patients with normal baseline renal function) nor neurotoxicity were documented, so the role of colistin as a safe therapeutic option against difficult-to-treat Gram-negative pathogens was emphasized (Bassetti *et al.*, 2008).

Interestingly, none of the three patients studied had received colistin prior to the isolation of the polymyxin-resistant *H. alvei* strains. This was surprising, as cross-resistance between colistin and antimicrobial compounds other than polymyxins has never been described, so previous exposure to carbapenems, ceftazidime, amikacin and ciprofloxacin (which all of the patients had received during hospitalization) could not explain the development of colistin resistance. One hypothesis is that previous administration of antibiotic compounds other than polymyxins may have altered membrane phospholipids, which led to lack of colistin activity due to irreversible modification of the bacterial target site. In fact, *in vitro* colistin resistance appeared to be a stable character, as it was documented even after thawing out and subculturing each strain many times. Anyway, this hypothesis is unlikely, given that colistin activity is the same as disinfectant activity (where no bacterial metabolism is required), so that alteration of cell membrane lipids (which is also a mechanism for so-called disinfectant resistance) is known to be reversible once the disinfectant is removed. Another possibility is that there was plasmid-mediated transfer of resistance genes, involving polymyxin resistance. The presence of mixed Gram-negative flora in the enteric environment may contribute to spread of resistance by DNA exchange. This has been described for diffusion of ESBL genes, as well as for co-transferred aminoglycoside, fluoroquinolone, tetracycline and cotrimoxazole resistance, but never for reduced susceptibility to colistin (Savini *et al.*, 2008). Both of these hypotheses then remain just speculative for the moment. Finally, it is likely that the two polymyxin-resistant *H. alvei* strains may have acquired resistance due to exposure of previously colonized patients to colistin. The two isolates could then have spread within the nosocomial environment and colonized two of the patients studied. The authors consider this hypothesis as the most plausible of the three mentioned. If this is what has really occurred, besides focusing on the first isolation of polymyxin-resistant *H. alvei* strains our findings further emphasize the need for the implementation of infection control measures to limit the nosocomial spread of uncommon organisms and the emergence of drug resistance among them.

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**Table 1.** MIC values (µg ml⁻¹) for the *H. alvei* strains and their interpretation

<table>
<thead>
<tr>
<th>FOX</th>
<th>AMP</th>
<th>AUG</th>
<th>CTX</th>
<th>CAZ</th>
<th>CPO</th>
<th>IPM</th>
<th>MRP</th>
<th>AK</th>
<th>CIP</th>
<th>TM/SMX</th>
<th>TE</th>
<th>CS</th>
</tr>
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<tbody>
<tr>
<td>HA4</td>
<td>≤4 (S)</td>
<td>8 (S)</td>
<td>≤1 (S)</td>
<td>≤1 (S)</td>
<td>0.25 (S)</td>
<td>≤2 (S)</td>
<td>≤0.25 (S)</td>
<td>20 (S)</td>
<td>&gt;16 (R)</td>
<td>&gt;16 (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA112</td>
<td>≤4 (S)</td>
<td>8 (S)</td>
<td>≤1 (S)</td>
<td>≤1 (S)</td>
<td>0.25 (S)</td>
<td>≤2 (S)</td>
<td>≤0.25 (S)</td>
<td>20 (S)</td>
<td>4 (S)</td>
<td>&gt;16 (R)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HA14</td>
<td>≤4 (S)</td>
<td>8 (S)</td>
<td>≤1 (S)</td>
<td>≤1 (S)</td>
<td>0.25 (S)</td>
<td>≤2 (S)</td>
<td>≤0.25 (S)</td>
<td>20 (S)</td>
<td>4 (S)</td>
<td>≤0.5 (S)</td>
<td></td>
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