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, carbapenem antibiotics 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; Tan & Ng, 2006). For this reason, our knowledge of colistin is limited. It exerts activity against Gram-negative bacteria (including extended-spectrum β-lactamase (ESBL)-producing bacteria) (Catchpole et al., 1997), except for *Proteus* spp., *Serratia* spp., *Providencia* spp., *Burkholderia pseudomallei* and *Neisseria* spp., which are known to be intrinsically resistant to colistin. Also, Gram-positive and anaerobic organisms show intrinsic polymyxin resistance (Kasiakou et al., 2005; Li et al., 2005; Markou et al., 2003). In addition, resistant *P. aeruginosa*, *Acinetobacter, Edwardsiella tarda*, *Enterobacter, Klebsiella* and *Salmonella* strains have been reported (Catchpole et al., 1997; Kasiakou et al., 2005; Muyembe et al., 1973; Shimizu et al., 1977). Interestingly, cross-resistance with antimicrobials other than polypeptide compounds has not been described so far. Recently, the paucity of novel antibiotics against multidrug-resistant organisms has renewed interest in the possible use of polymyxin E.

Three *Hafnia alvei* strains were isolated from faeces of hospitalized leukaemia patients from January 2004 to June 2007. Identifications were obtained by VITEK 2 (card ID-GNB) and confirmed by the mini API system (both by bioMérieux). Susceptibilities were provided by VITEK 2 (AST-N021, AST-N022 and AST-N041 cards; bioMérieux), and confirmed by performing a disc diffusion test on Mueller–Hinton agar (bioMérieux) (NCCLS, 2003) (see Table 1). Cefoxitin, ampicillin, amoxicillin–clavulanate, cefotaxime, ceftizidime, cefpirome, imipenem, meropenem, amikacin, ciprofloxacin, cotrimoxazole, tetracycline and colistin discs were used (discs from Liofilchem). Plates were incubated for 24 h at 36 °C in ambient air. Of particular note, two of the three *H. alvei* isolates were found to show colistin resistance. Also, E-test with cefotaxime plus cefoxitine/clavulanate and ceftizidime plus ceftazidime/clavulanate (AB BIODISK) was carried out on Mueller–Hinton agar, showing the absence of ESBL production. *H. alvei* causes uncommon nosocomial infections including cholecystitis, airway, urinary tract, liver and pancreatic infections, sporadic cases of enteritis, conjunctivitis, joint and wound infections, pleuritis, peritonitis and bacteraemia (Catchpole et al., 1997; Janda & Abbott, 2006; Jones et al., 2005; Kasiakou et al., 2005; Li et al., 2005). This organism commonly shows susceptibility to quinolones, aminoglycosides, chloramphenicol, cotrimoxazole, cefepime, aztreonam and carbapenem, whereas susceptibility to tetracycline is variable. Penicillin and narrow-spectrum cephalosporin resistance has been reported, and has been found to be due to a natural high-level constitutive chromosomal *Bush* group 1 β-lactamase (resistant to oxyiminocephalosporins) or to a low-level inducible *Bush* group 1 cephalosporinase (susceptible to oxyimino-cephalosporins). An uncommon AmpC β-lactamase may be responsible for ceftazidime and cefotaxime resistance, and for reduced susceptibility to cefpirome (Girlich et al., 2000; Janda & Abbott, 2006; Thomson et al., 1993). To our knowledge, *H. alvei* colistin resistance has not been described before.

The three patients studied suffered from diarrhoea, while abdominal pain and fever were absent. In all of the three cases *H. alvei* was collected with high bacterial counts (>100 c.f.u. per plate) but as part of the mixed flora. Also, no other organism (such as *Shigella* spp., *Yersinia* spp., *Salmonella* spp., *Clostridium difficile*) of known enteric pathogenicity was isolated, and *C. difficile* antigen and A/B toxins were not detected by the immunoenzymic *Clostridium difficile* Panel test (BioMite). Therefore, the role of *H. alvei* as the agent of the episodes of diarrhoea remained unclear. Also, *H. alvei* is a member of the family *Enterobacteriaceae*, but its enteropathogenicity remains controversial at present (Janda & Abbott, 2006).

Though reports concerning *H. alvei* isolation are still uncommon, emergence of colistin resistance represents a serious clinical and microbiological concern, as an increased incidence of hospital infections caused by multidrug-resistant Gram-negative organisms has been observed over the past years in Italy as well as worldwide, and has justified the reintroduction of colistin as a promising alternative in the treatment of severe life-threatening infections. Particularly, critically ill patients admitted to medical and surgical intensive care units and suffering from pneumonia and bloodstream infections caused by multidrug-resistant but colistin-
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>
</thead>
<tbody>
<tr>
<td>HA4</td>
<td>≤4</td>
<td>8</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1</td>
<td>≤0.25</td>
<td>≤2</td>
<td>≤0.25</td>
<td>≤20</td>
<td>≥16(R)</td>
<td>≥16(R)</td>
</tr>
<tr>
<td>HA112</td>
<td>≤4</td>
<td>8</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1</td>
<td>≤0.25</td>
<td>≤2</td>
<td>≤0.25</td>
<td>≤20</td>
<td>4</td>
<td>≥16(R)</td>
</tr>
<tr>
<td>HA14</td>
<td>≤4</td>
<td>8</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1</td>
<td>≤1</td>
<td>≤0.25</td>
<td>≤2</td>
<td>≤0.25</td>
<td>≤20</td>
<td>4</td>
<td>≤0.5</td>
</tr>
</tbody>
</table>

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 ciprofloxazole 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.

Acknowledgements

We express our gratitude to Dr Claudio Cappetti (Liolifchem srl, Roseto degli Abruzzi, Italy) and to Mrs Annarita Perfetti from soup to nuts.

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