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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; 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, ceftazidime, 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 cefotaxime/clavulanate and ceftazidime 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 carbapenems, 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 oxyimino-cephalosporins) 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 (Biosite). 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-

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Table 1. MIC values (μg ml^{-1}) for the *H. alvei* strains and their interpretation

S, Susceptible; R, resistant; FOX, cefoxitin; AMP, ampicillin; AUG, amoxicillin–clavulanate; CTX, cefotaxime; CAZ, ceftazidime; CPO, cefpirome; IPM, imipenem; MRP, meropenem; AK, amikacin; CIP, ciprofloxacin; TM/SMX, cotrimoxazole; TE, tetracycline; CS, colistin.

	FOX	AMP	AUG	CTX	CAZ	CPO	IPM	MRP	AK	CIP	TM/SMX	TE	CS
HA4	⩽4 (S)	8 (S)	8 (S)	⩽1 (S)	⩽1 (S)	⩽1 (S)	⩽1 (S)	⩽0.25 (S)	⩽2 (S)	⩽0.25 (S)	⩽20 (S)	**⩾16 (R)**	**⩾16 (R)**
HA112	⩽4 (S)	8 (S)	8 (S)	⩽1 (S)	⩽1 (S)	⩽1 (S)	⩽1 (S)	⩽0.25 (S)	⩽2 (S)	⩽0.25 (S)	⩽20 (S)	4 (S)	**⩾16 (R)**
HA14	⩽4 (S)	8 (S)	8 (S)	⩽1 (S)	⩽1 (S)	⩽1 (S)	⩽1 (S)	⩽0.25 (S)	⩽2 (S)	⩽0.25 (S)	⩽20 (S)	4 (S)	⩽0.5 (S)

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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