Case Report

Graft versus host disease-related *Hafnia alvei* colonization and probable infection

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We describe the case of a graft versus host disease (GvHD) patient, in whom *Hafnia alvei* was cultured as a single organism, and at high bacterial counts from stool samples, from the onset of the disease until its resolution. This case is a further example of the contentious role of this species in causing human intestinal disease. Furthermore, it focuses on enteric damage by GvHD as a risk factor for acquiring *H. alvei* colonization, and probably infection.

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A 9-year-old female patient with Fanconi's anaemia was admitted to Spirito Santo Hospital, Pescara, Italy, on 4 October 2007, in order to receive a haematopoietic stem cell transplantation (using unrelated cord blood cells). Prophylaxis with cotrimoxazole was administered from 4 to 15 October 2007. Also, standard immunosuppressive pre-transplantation treatment with cyclosporine and low-dose corticosteroids was started on 13 October 2007, and the patient was still receiving this treatment on 1 February 2008. Stool samples were cultured twice a week, and showed the presence of commensal enteric flora (mostly represented by *Escherichia coli*). On 30 October 2007, *Hafnia alvei* appeared in the stool sample for the first time, at elevated bacterial counts (>200 c.f.u. per plate) and as heavy pure growth. Identification at 99% certainty was made using the Vitek2 system and confirmed by the mini API system (both instruments by bioMérieux). The isolate showed susceptibility to cefoxitin ($\text{MIC} \leq 4 \mu\text{g ml}^{-1}$), cefepime ($\text{MIC} \leq 1 \mu\text{g ml}^{-1}$), imipenem ($\text{MIC} \leq 1 \mu\text{g ml}^{-1}$), meropenem ($\text{MIC} \leq 0.25 \mu\text{g ml}^{-1}$), amikacin ($\text{MIC} \leq 2 \mu\text{g ml}^{-1}$), ciprofloxacin ($\text{MIC} \leq 0.25 \mu\text{g ml}^{-1}$), levofloxacin ($\text{MIC} \leq 0.25 \mu\text{g ml}^{-1}$) and tetracycline ($\text{MIC} 4 \mu\text{g ml}^{-1}$), but resistance to ampicillin ($\text{MIC} \geq 32 \mu\text{g ml}^{-1}$), piperacillin ($\text{MIC} \geq 256 \mu\text{g ml}^{-1}$), amoxicillin/clavulanate ($\text{MIC} \geq 32 \mu\text{g ml}^{-1}$), ampicillin/sulbactam ($\text{MIC} \geq 32 \mu\text{g ml}^{-1}$), piperacillin/tazobactam ($\text{MIC} \geq 128 \mu\text{g ml}^{-1}$), ceftazidime ($\text{MIC} \geq 64 \mu\text{g ml}^{-1}$) and

cotrimoxazole ($\text{MIC} \geq 320 \mu\text{g ml}^{-1}$). MICs were determined by the Vitek2 system. On 18 November 2007, the patient experienced acute diarrhoea and strong cramp-type abdominal pain. Plate cultures from faeces grew *H. alvei* (>200 c.f.u. per plate) as a single organism again. No other organisms (bacteria, fungi, viruses or parasites) of known intestinal pathogenicity were detected. Also, *Clostridium difficile* was not found in the culture, and *C. difficile* A/B toxins were not revealed by the immunoenzymic test we performed (*C. difficile* panel; Biosite). *H. alvei* as the probable causative agent for the enteritis was considered uncertain, since expression of enteropathogenicity by this organism is still controversial at present (Janda & Abbott, 2006). Also, typical acute graft versus host disease (GvHD) skin lesions appeared, so that enteric GvHD was considered as the most likely cause. As the patient also suffered from *Staphylococcus aureus* bacteraemia (data not shown) during the hospitalization period, ceftazidime, amikacin and teicoplanin were started on 24 November 2007 as parenteral combined therapy (10 day therapy). Antimicrobials led to the resolution of the bacteraemia, whereas the abdominal pain and diarrhoea persisted. Also, despite the documented susceptibility of *H. alvei* to amikacin, the species was cultured as a single organism from a new stool sample (>200 c.f.u. per plate). Finally, enteric GvHD was documented by intestinal endoscopy and biopsy on 3 December 2007. Low-dose corticosteroids were changed to high-dose corticosteroids, and anti-lymphocyte mAbs were started, leading to a gradual relief of symptoms within 1 week. On 17 December 2007, corticosteroids were changed

Abbreviation: GvHD, graft versus host disease.

back to a low-dose treatment, whilst mAbs were stopped. Interestingly, antimicrobials had been stopped already, on 4 December 2007, whereas *H. alvei* disappeared from stool samples within 10 days of the clinical resolution (the last positive culture was obtained on 20 December 2007).

H. alvei is a rare human pathogen. Some cases of gastroenteritis reported in the literature have been suspected to be due to this organism, but its role as an agent of enteric infection is still uncertain (Gunthard & Pennekamp, 1996; Janda & Abbott, 2006). Isolation from respiratory secretions is more common; although most of the isolates from airways do not seem to be clinically significant, sporadic reports of pneumonia, bronchopneumonia and pulmonary abscesses that were most likely due to *H. alvei* have been described (Gunthard & Pennekamp, 1996; Janda & Abbott, 2006). The organism is occasionally found in the urinary tract, usually as a commensal, although in a few cases it was considered to be clinically significant (Gunthard & Pennekamp, 1996; Janda & Abbott, 2006). Rare bloodstream infections in which *H. alvei* was isolated from blood cultures have been reported. Bacteraemias were mostly community acquired, and the times of the first positive blood cultures usually ranged from 1 to 41 days after hospitalization. In some cases, the organism has been isolated from blood and hepatic abscesses, pancreatic pseudocyst fluid, pleural fluid, and central venous catheters, at the same time; although the source of bacteraemias mostly remained unknown, the main origin of the bloodstream infections was thought to be the respiratory or intestinal tract (Gunthard & Pennekamp, 1996; Janda & Abbott, 2006; Liu *et al.*, 2007; Rodriguez-Guardado *et al.*, 2006). Neonatal infections related to silent *H. alvei* vaginal carriage by mothers and a meningitis case in a 1-year-old patient have been reported too. Very few reports exist in the literature regarding wound colonization by this organism, and we found just two reports concerning *H. alvei* endophthalmitis (Gunthard & Pennekamp, 1996; Janda & Abbott, 2006). Some cases of isolation of this organism from abscesses, and one from a patient with septic arthritis, are known, but its role as a pathogen was uncertain since it was recovered as a part of a mixed bacterial flora (Gunthard & Pennekamp, 1996; Janda & Abbott, 2006). Finally, two cases of cholecystitis, a report of spontaneous bacterial peritonitis and a case of endocarditis have been described (Hazouard *et al.*, 2006; Janda & Abbott, 2006; Loulergue *et al.*, 2007). Interestingly, an outbreak of probable haemolytic uraemic syndrome has been cited by Janda and colleagues, in which a *H. alvei* strain producing a Vero cell active cytolytic toxin was isolated from faeces (Crandall *et al.*, 2006).

H. alvei is known to be an uncommon nosocomial pathogen, but little has been written regarding its role as an opportunistic agent of infections in immunocompromised patients. Particularly, no data exist at present about GvHD as a possible risk factor for acquiring *H. alvei* colonization and/or infection. Acute GvHD usually occurs after allogeneic stem cell transplant, and represents a reaction of donor-derived T cells against host skin, liver

or gut. Enteric disease is suspected when the patient develops signs or symptoms such as cramp-type abdominal pain, diarrhoea, nausea and vomiting. However, as these are non-specific symptoms, endoscopy, biopsy and histological confirmation, such as by cultures, are needed, in order to exclude competing diagnoses. Damages by GvHD are represented by oedema, mucosal sloughing and possible bleeding, and histopathology usually documents crypt-cell necrosis and dropout with crypt abscess. Therapy is mostly based on immunosuppression and steroids, so that the risk of opportunistic infections has to be carefully weighed, as a number of deaths due to infectious complications have been reported in the literature (Ferrara *et al.*, 2003; Jacobsohn & Vogelsang, 2007).

Limited clinical, epidemiological and laboratory data are available at present regarding the possible pathogenicity of *H. alvei*. In the case reported here, the pathogenicity of *H. alvei* remained uncertain, despite the finding of elevated bacterial counts and the isolation of the organism as heavy pure growth, and notwithstanding antimicrobial and steroid treatment, clinical improvement and disappearance of the organism from cultures proceeded at the same pace. Anyway, it seemed to be interesting that the organism appeared in faeces just a few weeks before the onset of symptoms, and it disappeared from faeces within 10 days of the clinical resolution. The role of amikacin in eradicating colonization was unclear, since antimicrobial therapy did not seem to be successful prior to the beginning of anti-GvHD treatment. Whether the use of steroids and immunosuppressive compounds alone, without an antimicrobial, would have been followed by *H. alvei* eradication from the gut remains unknown. Perhaps, cotrimoxazole prophylaxis played a remarkable role in replacing normal flora with *H. alvei* (it is notable that the organism showed resistance to the cited drug), whereas immunosuppressive treatment might have altered mucosal immune defence, thus inducing overgrowth of the organism. Finally, enteric damage by GvHD may have increased adherence of the pathogen to the epithelium, and biofilm formation, probably resulting in the failure of amikacin to eradicate colonization before GvHD resolution. In turn, a role for *H. alvei* in increasing mucosal damage cannot be excluded. In fact, onset of infectious inflammation-related mucosal changes as a risk factor for development of GvHD can be suspected, such as eventual cross-reaction between host defence mechanisms (leukocytes, immunoglobulins) and gut-wall antigens on the one hand, and host immune response and *H. alvei* antigens on the other. This case further confirms that the behaviour of this organism as an enteric commensal or pathogen is contentious, and many questions remain unanswered.

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