A severe case of Aeromonas veronii biovar sobria travellers’ diarrhoea characterized by Vibrio parahaemolyticus co-isolation

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We report a severe case of travellers’ diarrhoea in a patient returning from Ecuador to Italy with the concomitant presence of Aeromonas veronii biovar sobria and Vibrio parahaemolyticus in their faeces. Based on diagnostic results, epidemiological information and the clinical outcome, we conclude that the real aetiological agent was A. veronii biovar sobria, while V. parahaemolyticus was only transient in the intestine of the patient.

Case report
A previously healthy 45-year-old woman resident in Central Italy, 6 days before her return flight from Ecuador, consumed raw vegetables and about 18 h later she presented with severe watery diarrhoea (more than 10 stools in 24 h), vomiting, abdominal cramps, hypotension and a fever ranging between 37.5 and 38.5 °C. On 15 July 2009, as soon as she arrived in Italy, she was hospitalized due to the persistence of the symptoms and dehydration. The patient reported that since the onset of symptoms she had followed a mostly liquid diet, sometimes having consumed white meat and grilled marine fish, and she had not taken any medication. Hydration therapy and empiric treatment with ciprofloxacin (400 mg twice daily), both intravenous, were immediately started.

Prior to antibiotic treatment, stool and blood cultures were performed. The blood cultures were negative. From the

Introduction
Vibrio parahaemolyticus and Aeromonas species are aquatic micro-organisms associated with gastroenteritis worldwide (Faruque & Nair, 2006; Janda & Abbott, 2010). For V. parahaemolyticus, seafood, in particular shellfish, represents a common source of human infection (Ottaviani et al., 2008, 2010), while for aeromonads, the more common source is drinking water or vegetables contaminated by irrigation processes (Khajanchi et al., 2010; Janda & Abbott, 2010). In V. parahaemolyticus the pathogenicity is closely linked to the action of thermostable direct haemolysin (TDH) and/or TDH-related haemolysin (Okuda et al., 1997). However, Aeromonas species may produce different toxins such as aerolysin (AerA), cytolytic enterotoxin (ACT), cytotoxic enterotoxin (AST), heat-labile cytotoxic enterotoxin (ALT), as well as many enzymes that are considered virulence factors, and may even possess a type III secretion system (Janda & Abbott, 2010). Due to the lack of a suitable animal model for Aeromonas gastrointestinal infection, the specific role that each of these putative virulence factors, alone or in combination, may play in the development of diarrhoea is unknown (Janda & Abbott, 2010). However, the mouse model is the most commonly used to experimentally test the animal enteropathogenicity of Aeromonas species and the suicide phenomenon is its effective phenotypic marker (Namdari & Bottone, 1988; Ottaviani et al., 2006). Both V. parahaemolyticus and A. veronii biovar sobria have been recognized as possible causes of travellers’ diarrhoea (Ahn et al., 2011; Vila et al., 2003; Yates, 2005) but, to the best of our knowledge, co-infection by these two species, in travellers or other individuals, has never been reported worldwide.

Here, we report a severe case of travellers’ diarrhoea characterized by the co-isolation of A. veronii biovar sobria and V. parahaemolyticus isolates from the patient’s faeces.
patient's faeces, in the absence of other enteric pathogens, including viruses and parasites, two different Gram-negative, oxidase-positive bacterial species were isolated. Few colonies of the first micro-organism grew on thiosulfate citrate bile salt sucrose agar, while a large number of colonies of the second organism grew on 5% sheep blood agar supplemented with ampicillin (30 μg ml⁻¹) (ASBA 30). These strains were presumptively identified as *V. parahaemolyticus* and *A. veronii*, respectively, by using the API ID 32 GN commercial system (bioMérieux) and standardized biochemical protocols (Abbott et al., 2003; Ottaviani et al., 2010, 2011). The species identification was confirmed by a PCR approach (Ottaviani et al., 2010, 2011; Sen, 2005). The biovar sobria of the *A. veronii* strain was identified on the basis of acid production from salicin, aesculin hydrolysis, arginine dihydrolase and ornithine decarboxylase tests (Ottaviani et al., 2011). LPS (O) and capsular (K) antigens of *V. parahaemolyticus* were determined by the slide agglutination test using specific commercial antisera (Denka Seiken), following the manufacturer's instructions. Molecular tests using PCR were performed to detect *tdh* and *trh* genes of the *V. parahaemolyticus* isolate and *aerA, ast, alt* and *act* genes of the *A. veronii* biovar sobria isolate (Ottaviani et al., 2010, 2011). For both isolates, as markers of virulence, tests for the cytotoxicity against Caco-2 cells, adhesiveness on HEP-2 cells and the invasivity on HT29 cells were performed (Couto et al., 2007; Ottaviani et al., 2011). For the *A. veronii* biovar sobria isolate, as additional markers of animal enteropathogenicity and virulence, the suicide phenomenon at 30 and 37°C, and the β-haemolytic activity on ASBA 30 medium, respectively, were evaluated (Albert et al., 2000; Chopra et al. 1993; Namdari & Bottone, 1988). For both isolates, susceptibility to 13 antimicrobial agents (amoxicillin–clavulanic acid, cefoperazone, cephalaxin, cephalothin, chloramphenicol, ciprofloxacin, colistin, doxycycline, erythromycin, nalidixic acid, oxolinic acid, tetracycline, trimethoprim–sulphamethoxazole) was determined by using the disc diffusion method, according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2006).

The *V. parahaemolyticus* isolate belonged to the serotype OUT (untypable): K29 and was *tdh* and *trh* negative, adhesive, not cytotoxic and not invasive. It was resistant to erythromycin, amoxicillin–clavulanic acid, nalidixic acid, oxolinic acid, chloramphenicol and ciprofloxacin. The *A. veronii* biovar sobria isolate was *act* positive, β-haemolytic, non-suicidal at 30 and 37°C, adhesive, cytotoxic (Fig. 1) and not invasive. It was resistant to erythromycin and amoxicillin–clavulanic acid.

During the first 48 h of hospitalization, the general condition of the patient improved and the fever ceased, so, on the basis of the antibiograms and characterization of the isolates, on day 3 treatment with ciprofloxacin was confirmed and switched to oral (500 mg twice daily for 3 days). The symptoms ceased on day 4. On day 5, repeated stool cultures were performed, with negative results. On day 6 the patient was discharged. Two sets of repeated stool cultures were performed after the patient had been discharged; both yielded negative results.

**Discussion**

*V. parahaemolyticus* is considered a primary enteropathogen (Faruque & Nair, 2006), while the clinical relevance of aeromonads as diarrhoeagenic agents is often questioned (Lamy et al., 2009), particularly when they are isolated concurrently with other enteropathogens (Chu et al., 2006).

However, *V. parahaemolyticus* gastroenteritis is usually self-limited and lasts 3 days (Faruque & Nair, 2006), while gastroenteritis caused by *Aeromonas* species frequently results in subacute or chronic forms, in particular, in travellers returning from Africa, Asia and Latin America (Ahn et al., 2011; Vila et al., 2003; Willoughby et al., 1989; Yates, 2005). In this regard, many reports indicate that the use of antibiotics can improve the course of acute gastroenteritis induced by *Aeromonas* species, avoiding subacute or chronic forms, or invasive complications.

**Fig. 1.** Adhesiveness (a) and cytotoxicity (b) of the *A. veronii* biovar sobria isolate on HEP-2 and Caco-2 cells, respectively, after 3 h incubation.
(Figuera, 2005; Figueras et al., 2007; Vila et al., 2003; Willoughby et al., 1989). In contrast, for V. parahaemolyticus, there is no evidence that antibiotic treatment decreases the severity of the length of the illness (Faruque & Nair, 2006).

In this case study, the epidemiological investigation suggests that the most probable source of infection of the A. veronii biovar sobria strain was raw vegetables or drinking water consumed by the patient a few hours before the onset of symptoms, and of V. parahaemolyticus, marine fish, probably undercooked, consumed in the days following the development of disease. However, the possibility that V. parahaemolyticus could have been ingested in other food consumed by the patient before the onset of symptoms, of which we had no specific information, cannot be excluded. The V. parahaemolyticus isolate lacked the tdh and trh genes, while the A. veronii biovar sobria isolate had the act gene and biological activities associated to its expression, such as cytotoxicity on Caco-2 cells and β-haemolysis (Albert et al., 2000; Chopra et al. 1993). Moreover, this Aeromonas strain, being non-suicidal, also showed potential animal enteropathogenicity (Namadi & Bottone, 1988). Diagnostic results, in line with epidemiological data, suggest that, although adhesive properties of both micro-organisms allowed their adhesion to the intestinal tract of the patient, the V. parahaemolyticus strain was only transient, while the A. veronii biovar sobria isolate exerted its pathogenic action, probably by the expression of the act gene, not excluding the possible involvement of other functional genes not specifically investigated here. Although, in this case the classic Koch’s postulates have not been fulfilled, due to the lack of a suitable animal model for human infection by aeromonads, a lot of clinical evidence also supports our hypothesis of pathogenesis, such as the persistence of the disease over 10 days, the large amount of A. veronii biovar sobria, but not of V. parahaemolyticus, in the patient’s faeces during the acute phase of the infection, the rapid positive response of the patient to ciprofloxacin, to which A. veronii biovar sobria, but not V. parahaemolyticus, is susceptible and the resolution of the symptoms concomitant with the clearance of A. veronii biovar sobria.

In this case study, only by integrating diagnostic results with epidemiological investigation data, could we determine the most probable aetiology of this illness and direct appropriate therapeutic and diagnostic strategies in order to avoid any possible complications in the patient and extra healthcare costs related to further investigations.

In conclusion, the co-isolation of gastrointestinal pathogens from stools of patients with severe diarrhoea requires not only the identification of the micro-organisms, but also the investigation of their pathogenic potential. Only in this way is it possible to link the pathogens to the illness, choose effective treatments and disseminate accurate information regarding the pathogenesis. Finally, we believe that this article will increase awareness in the scientific community that Aeromonas species can induce severe diarrhoea in the right circumstances and host, while the isolation of V. parahaemolyticus from a diarrhoeal patient might not necessarily be related to the disease.

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References


