Aeromonas spp.: an emerging pathogen?

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Summary

The aim of this study is to identify and monitor the presence of *Aeromonas* spp. strains in stool cultures. We analyzed 5564 stool cultures from September 2012 to August 2013. Sixty-three patients were positive for *Aeromonas* spp. The most frequent symptoms were: diarrhea (46.0%) and abdominal pain (12.7%). Pediatric subjects were 28. Samples' microscopic examination showed leukocytes in 38.1% of cases. It is still controversial whether *Aeromonas* are responsible for human gastroenteritis, but their presence in faecies of symptomatic patients supports their etiologic role. We propose search for toxins by polymerase chain reaction to identify strains that require an antibiotic therapy.

Introduction

Aeromonas spp. are Gram-negative rods, facultative anaerobic, non-spore-forming, ubiquitous bacteria (2,3,4). *Aeromonas* spp. strains have been isolated in different aquatic environments including treated drinking water, in the soil and in many foods, including meat and milk (2,6). The genus *Aeromonas* shares many biochemical features with *Enterobacteriaceae*, from which it can be differentiated by the oxidase test, positive for Aeromonas (2,4). The genus includes several species, including the mesophilic *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas sobria*, *Aeromonas veronii*, *Aeromonas schubertii* and psychrophilic *Aeromonas salmonicida*, forced fishes' pathogen, not correlated with human disease (2,3,4,6). The mesophilic species have shown to possess several virulence factors (adhesins, hemolysins, cytotonic and cytotoxic enterotoxins), all of them probably involved in human disease (2,4). Primary toxins produced are hemolysins, among which the most significant is the aerolysin expressed by many strains of *A. hydrophila* and *A. sobria*. Furthermore it has been demonstrated the presence of at least one citotonic toxin with activity similar to cholera toxin (5). *Aeromonas* spp. strains can cause gastroenteritis, skin and soft tissue infections and bacteremia in immunocompromised patients (2,4). In particular *A. hydrophila* was associated with two distinct types of gastroenteritis: a cholera-like form with watery diarrhea and a dysenteric form (2,4). Several factors, including patient's age, state of immunocompetence, presence of concomitant diseases, infectious load and virulence factors expression affect the pathogenicity of *Aeromonas* spp. (5). Many studies have found an increase of *Aeromonas* spp. isolation from fecal samples during the hottest months of the year, an event that can reasonably be attributed to the fact that the mesophilic species grow optimally at high temperatures, thereby leading to an increase in the concentration of bacteria in freshwater ecosystems and in home drinking water (3). Gastrointestinal infections caused by *Aeromonas* spp. are generally self-limiting and antibiotic therapy is usually used in cases of severe and not responsive infections (2,4).

Aeromonas spp. strains are universally considered resistant to penicillins, due to inducible betalactamase production, while usually show themselves sensible to aminoglycosides, tetracyclines, chloramphenicol, trimethoprim-sulfamethoxazole and quinolones. They are also sensible to second and third cephalosporins generation (2,4). *Aeromonas* spp. strains are commonly isolated from fecal samples of children aged less than 5 years (2). It was shown that the number of cases of infection by *Aeromonas* may be underestimated because of sample's inadequate management, both in preanalytic phase than in the culture method (4). The purpose of this study is to identify and monitor the presence of such strains in stool cultures of patients attending the operative unit of Microbiology and Virology of Padua.

Materials and Methods

The analysis was carried out in the period between September 2012 and August 2013 on the 5564 stool culture requests from Hospital of Padua departments and clinics and by external users related to our structure. The samples were collected in tubes with screw cap containing Cary-Blair transport medium modified (FecalSwab™, Brescia, Italy) and then they were inoculated with the automated WASP® BD™

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(Becton, Dickinson, Franklin Lakes, NJ, USA) on *Aeromonas/Yersinia* Agar, selective differential medium. This medium is a variation of CIN agar (agar cefsulodin-irgasan-novobiocin) that not only promotes the growth of *Yersinia enterocolitica* but also of *Aeromonas* spp., because of a lower content of cefsulodin (1). The plates were incubated under aerobic conditions at a temperature of 25±2°C for 48 hours. *Aeromonas* spp. strains produce light colonies with pink to red center, similar to *Yersinia*. On such media other *Enterobacteriaceae* are able to grow, such as *Citrobacter* and *Serratia*, not always distinguishable on the basis of colony morphology. For strain's identification we used BioMerieux Vitek® 2 andVitek® MS, while for the antibiogram we used BioMerieux Vitek® 2 (BioMerieux, Marcy-l'Étoile, France).

Results

In our records 63 patients were positive for *Aeromonas* spp., and more precisely 55 for *A. hydrophila* and 8 for *A. sobria*. Among these patients, 28 were male (44.4%) and 35 were females (55.6%). It is important to note that the paediatric subjects (aged less than 14 years) were 28 (44.4%) and in particular those aged less than 3 years accounted for 31.3% of the total. The foreigners were 9 patients (14.3%). From the data that we have collected from the medical history card, when available, and from interviews with the patients or the doctors, the symptoms that we found most frequently was distributed as follows: 29 patients reported diarrhea (46.0%); 8 patients complained of abdominal pain (12.7%).

As for the 26 remaining patients, representing the 41.3%, unfortunately no anamnestic information was available whatsoever, whilst the coproculture screening was carried out for other unspecified purposes.

In 9 cases we found a co-infection with other intestinal pathogens, in detail: 4 *Campylobacter* spp. (culture method); 2 *Yersinia enterocolitica* (culture method); 1 *Salmonella* spp. (culture method); 1 *Clostridium difficile* (rapid enzyme immunoassay); 1 *Rotavirus* (rapid enzyme immunoassay).

As a further study we carried out a microscopic examination of the samples and we detected the significant presence of leukocytes in 38.1% of cases.

As for the antibiogram we tested the following molecules: ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefotaxime, cefepime, ciprofloxacin, and gentamicin. All strains were resistant to ampicillin and amoxicillin/clavulanic acid; 12 strains were also resistant to piperacillin/tazobactam, 1 strain was resistant to cefotaxime and 1 strain showed resistance to gentamicin.

Discussion and Conclusions

It is still controversial whether *Aeromonas* spp. strains are responsible for human gastroenteritis, but their presence in faeces of symptomatic patients, in the absence of other known pathogens, supports their etiologic role. *Aeromonas* ubiquitous nature in aquatic environments makes it possible the interaction with humans continuous and inevitable. It is therefore conceivable that some *Aeromonas* spp. strains, presenting the right set of virulence factors, are likely to induce gastrointestinal diseases.

In our situation, and in selected cases guided by a proper medical history, we propose to combine the culture method with PCR research of specific toxins of *Aeromonas* spp. to identify pathogenic strains with the aim of a precise etiological diagnosis as well as for a contingent antibiotic treatment.

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