

Positioning *Aeromonas* Infection in Inflammatory Bowel Disease: A Retrospective Analysis

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Keywords

Aeromonas · Inflammatory bowel disease · Crohn's disease · Ulcerative colitis · GI infections · Immunology · Microbiology and inflammatory bowel disease

Abstract

Background and Aim: *Aeromonas* are Gram-negative rods known to cause a spectrum of diseases. Inflammatory bowel disease (IBD) is an idiopathic complex condition resulting from interaction of multiple factors. *Aeromonas* infection in association with IBD is still largely unknown. We aim to look for the significance of *Aeromonas* infection and for significant differences between IBD and non-IBD patients. **Methods:** A retrospective observational analysis was performed of all patients positive for *Aeromonas* in stool cultures, during a 10-year period, from a tertiary and university hospital. **Results:** Fifty patients were included, 56% male with a mean age of 42.1 years. Thirty-eight (76%) were non-IBD and 12 (24%) IBD patients. IBD patients were more frequently under immunosuppressors. Two patients were asymptomatic and 44% developed mild, 44% moderate, and 16.7% severe infection. The main strains isolated were *Aeromonas hydroph-*

ila/caviae. Bacterial co-isolation was found in 4 non-IBD and histological findings of cytomegalovirus in 2 IBD patients. Non-IBD patients presented more frequently with fever and IBD patients with bloody diarrhea and abdominal pain. There was higher tendency for severe infection rate in IBD patients with higher antimicrobial therapy use. Steroids were exclusively used in the IBD group. From IBD, 4 patients had the diagnosis of ulcerative colitis and 9 of Crohn's disease with colonic involvement. Of these patients, 5 received IBD diagnosis after the acute episode of *Aeromonas* infection. **Conclusions:** Clinical presentation of *Aeromonas* infection differs between IBD and non-IBD patients. Non-IBD patients had milder severity of infection with less use of antibiotics. *Aeromonas* infection seems to greatly contribute to IBD manifestation.

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Infeção por *Aeromonas* e doença inflamatória intestinal: uma análise retrospectiva

Palavras Chave

Aeromonas · Doença inflamatória intestinal · Doença de Crohn · Colite ulcerosa · Infeção gastrointestinal · Imunologia · Microbiologia e doença inflamatória intestinal

Resumo

Introdução: A etiologia da Doença Inflamatória Intestinal (DII) é complexa e resultante da interação de diversos fatores, nomeadamente microbiológicos. A infeção por *Aeromonas* caracteriza-se por um espectro alargado de manifestações clínicas. O papel da infeção por *Aeromonas* na DII não está caracterizado. **Objetivos:** Avaliar o significado da infeção por *Aeromonas* na DII e as diferenças com a infeção em doentes não-DII. **Métodos:** avaliação retrospectiva e observacional de todos os doentes com isolamento microbiológico de *Aeromonas* em amostras fecais num período de 10 anos, num hospital terciário. **Resultados:** foram avaliados 50 doentes, 56% do sexo masculino, com idade média de 42.1 anos. Doze (24%) com diagnóstico de DII e trinta e oito (76%) não-DII. Os doentes com DII encontravam-se mais frequentemente sob imunossupressão. Dois doentes foram assintomáticos, 44% desenvolveram doença ligeira, 44% moderada e 16.7% severa, havendo maior tendência para infeção severa nos DII. Os doentes não-DII apresentaram mais frequentemente febre e os DII diarreia sanguinolenta e dor abdominal. O uso de antimicrobianos foi superior no grupo DII e a utilização de corticoesteroides foi exclusiva nestes doentes. Isolamento concomitante de outros agentes microbiológicos ocorreu em 4 doentes não-DII e 2 com DII tinham histologia compatível com infeção por Citomegalovírus. Da população DII, 4 eram Colite Ulcerosa e 9 Doença de Crohn com envolvimento cólico. Destes, 5 receberam o diagnóstico após a infeção por *Aeromonas*. **Conclusão:** A apresentação clínica da infeção por *Aeromonas* foi distinta entre as populações DII e não-DII, sendo que os doentes DII apresentaram doença mais severa e maior utilização de antimicrobianos. A infeção na DII ocorreu essencialmente em doentes com envolvimento cólico.

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Introduction

The *Aeromonas* genus, belonging to the Aeromonadaeae family, comprises facultatively anaerobic Gram-negative bacteria widely present in aquatic environments, soil, and food goods (such as meat, shellfish, and dairy products), making the gastrointestinal tract an understandable reaching point for *Aeromonas* [1, 2]. However, there has been discordant information concerning its role as a gastrointestinal pathogen, since its isolation in stool from asymptomatic individuals is not uncommon [3]. Of the 17 phenospecies of the genus *Aeromonas*, the most frequently isolated from human feces are *A. hydrophila*, *A. caviae*, *A. veronii*, and *A. trota* [4–6]. The most common manifestation of *Aeromonas* infection is diarrhea, usually acute and self-limited, but other presentations such as bloody diarrhea and abdominal pain, or chronic more indolent diarrhea, have been described [7]. Although most of the infections occur in immunocompromised patients, it can also cause disease in healthy individuals [8].

Although the ultimate cause of inflammatory bowel disease (IBD) is still unknown, there are many components interacting in its network of pathogenic mechanisms like environmental factors, genetic susceptibility, dysregulated immune response, and microbiological factors [9, 10]. Regarding the latter, both alterations in intestinal microbiota and infections by external agents might play a role in IBD onset and its flares [11, 12]. Dysbiosis can also result from commensal flora that, although normal in speciation, possess more subtle virulence factors such as enteroadherence, or the lack of diversity of the fecal microbiome [13, 14].

Considering the specific bacteria related to IBD onset, *Campylobacter* is probably the one with more data, while the heterogeneity of the studies does not allow to draw consistent conclusions [15]. Although data are mainly based on case report studies, some authors have suggested that *Aeromonas* can be a trigger of flares in IBD [16–18] and as well as a trigger to develop de novo chronic colitis in patients with no previous history of IBD [7, 19]. To our knowledge, only 2 recent studies involving IBD patients have tried to correlate the diagnosis and severity of the disease with *Aeromonas* infection [7, 8].

Our aim was to further look for the significance of *Aeromonas* infection as well as for significant differences between IBD and non-IBD patients.

Materials and Methods

Patients and Clinical Information

A retrospective analysis of data was performed of inpatients and outpatients with a positive stool culture for *Aeromonas*, between January 2009 to January 2019, in a tertiary and university hospital. Patients of all ages were included. Clinical data were obtained retrospectively from the electronic medical records.

Baseline data included age at *Aeromonas* fecal detection, sex, comorbidities (namely the presence of IBD and transplantation status), ongoing drugs (namely immunosuppressants), recent travels, and previous abdominal surgery. Clinical characteristics at the time of *Aeromonas* detection were also analyzed: the presence of symptoms attributed to gastrointestinal infection such as nausea, vomiting, diarrhea, number of bowel movements, abdominal pain, and fever; endoscopy findings, if performed; presence of histopathological features of cytomegalovirus (CMV) infection in colon biopsies; other bacterial co-identification in stool cultures; treatment applied (namely antibiotics and steroids); disease severity and/or death related to the infection.

The indication for treatment was done according to the physician's criteria. Disease severity was defined as: (a) mild self-limited gastroenteritis, as a gastrointestinal infection that resolved spontaneously without the need for antibiotics; (b) moderate gastroenteritis, as a gastrointestinal infection that required antibiotic treatment; and (c) severe gastroenteritis, defined by a gastrointestinal infection complicated with septicemia and/or renal impairment. Death related to the infection was defined as the occurrence of death over the duration of infection and/or antibiotic therapy.

Stool samples were processed for bacterial culture using GN broth and selective media: Macconkey agar, Macconkey agar with sorbitol, Salmonella-Shigella agar, CIN (Cefsulodin-Irgasan-Novobiocin) agar, and Campyloset agar. In certain patients, a blood agar with an ampicillin disc was also used. The culture media were incubated at 35 °C and observed daily for 48 h, except for the Campyloset media. Suspected colonies of *Aeromonas* spp. in CIN media and those that grew close to the ampicillin disc and, simultaneously, showed a positive oxidase reaction, were identified. The identification was made in automated systems: phenotypical methods (Vitek2® – bioMérieux) or by mass spectrometry technique (MALDI-TOF MS® – bioMérieux).

IBD and Non-IBD Patients

Data from all patients was analyzed, and two groups (IBD and non-IBD patients) were created for comparison. The IBD group included all patients with the diagnosis of IBD previously and after *Aeromonas* isolation. Individuals for whom both ulcerative colitis (UC) and Crohn's disease (CD) were recorded on successive dates were categorized according to the latest.

Statistical Analysis

Descriptive analysis was performed considering absolute and relative frequencies for categorical variables or mean and standard deviation for quantitative variables. Association between qualitative variables was performed based on the χ^2 test (with Yates' correction) or Fisher's exact test (when assumptions could not be verified in 2 × 2 tables). Group comparison according to quantitative variables was performed using the independent-sample *t* test or Mann-Whitney test (variable with relevant skewness). In all analyses, a significance level of 0.05 was considered and SPSS version 26 was used.

Results

Baseline Characteristics

Stool isolation of *Aeromonas* was identified in 53 patients during the 10-year period of the study. Fifty patients were included and analyzed. Three were excluded due to the absence of minimal clinical information.

Our population consisted mainly of individuals of male sex (54%) with a mean age of 42.1 years (1–89 years old). Fourteen (28%) patients were under 18 years old. Baseline characteristics are presented in Table 1.

Twelve patients (24%) had the diagnosis of IBD, with 7 (14%) having it diagnosed previously and 5 (10%) after *Aeromonas* fecal isolation. Five patients had ongoing chemotherapy treatment during *Aeromonas* isolation and 2 had advanced liver disease. Thirteen patients (26%) were immunosuppressed considering therapeutics as calcineurin inhibitors, AZA, systemic steroids, and biological therapy as infliximab. Six patients (12%) were previously submitted to organ transplantation. At the time of *Aeromonas* isolation, 4 patients were under azathioprine (AZA), 6 under topical or systemic steroids, and 6 under calcineurin inhibitors. Only 1 IBD patient was under combined therapy with infliximab and AZA. Only 1 non-IBD patient was under AZA for autoimmune hepatitis. Also, the only IBD patient under calcineurin inhibitors was a liver transplant recipient.

No patient had recent travel history. Twelve patients (24%) presented a history of previous abdominal surgery, with 3 having a previous hemicolectomy, 1 a cholecystectomy, 3 liver transplants, 1 reno-pancreatic transplant, and 2 gastric bypasses.

Clinical Presentation in IBD and Non-IBD Patients

The clinical and microbiological characteristics at the time of *Aeromonas* isolation are shown in Table 2. From the overall sample, 48/50 (96%) patients were found to have symptoms that could be attributed to the presence of *Aeromonas*. Only 1 IBD and 1 non-IBD patient were considered asymptomatic: concerning the IBD patient, the stool culture was required in the course of the diagnosis of IBD, as part of a protocol required at the first consultation. This patient was sent to IBD consultation due to chronic diarrhea and abdominal pain in the past year but was asymptomatic at the time of consultation. Concerning the non-IBD patient, the stool culture was required in the course of the postrenal transplantation protocol.

The most frequent strains isolated in both groups were *A. hydrophila/caviae* (distinction not possible with the

Table 1. Baseline characteristics

	Total n (%) or mean (SD)	IBD n (%) or mean (SD)	Non-IBD n (%) or mean (SD)	p value
Total	50 (100.0)	12 (24.0)	38 (76.0)	–
Age, years	42.1 (28.6)	40.2 (23.5)	42.7 (30.3)	0.794 ^c
Female sex	23 (46.0)	4 (33.3)	19 (50.0)	0.498 ^a
IBD diagnosis				
Previous to <i>Aeromonas</i> isolation	7 (14.0)	7 (58.3)	–	–
Posterior to <i>Aeromonas</i> isolation	5 (10.0)	5 (41.7)	–	–
Comorbidities at the moment of <i>Aeromonas</i> fecal isolation				
Arterial hypertension	14 (28.0)	3 (25.0)	11 (28.9)	1.000 ^b
Dyslipidemia	7 (14.0)	1 (8.3)	6 (15.8)	1.000 ^b
Diabetes	4 (8.0)	1 (8.3)	3 (7.9)	1.000 ^b
Chronic renal failure	3 (6.0)	0 (0.0)	3 (7.9)	1.000 ^b
Cirrhosis	2 (4.0)	0 (0.0)	2 (5.3)	1.000 ^b
Cancer	5 (10.0)	0 (0.0)	5 (13.2)	0.319 ^b
Transplant	6 (12.0)	1 (8.3)	5 (13.2)	1.000 ^b
Ongoing drugs at the moment of <i>Aeromonas</i> fecal isolation				
PPI	18 (36.0)	5 (41.7)	13 (34.2)	0.735 ^b
Chemotherapy	5 (10.0)	0 (0.0)	5 (13.2)	0.319 ^b
Immunosuppressed patients	13 (26.0)	7 (58.3)	6 (18.8)	0.073 ^b
Immunosuppressant drugs				
AZA	4 (8.0)	3 (25.0)	1 (2.6)	0.038 ^b
Topical or systemic steroids	6 (12.0)	4 (33.3)	2 (5.3)	0.024 ^b
Calcineurin inhibitors	6 (12.0)	1 (8.3)	5 (13.2)	0.294 ^b
Biologic therapy (anti-TNF)	1 (2.0)	1 (8.3)	0 (0.0)	0.240 ^b
Previous abdominal surgery	12 (24.0)	1 (8.3)	11 (28.9)	0.248 ^b

AZA, azathioprine; IBD, Inflammatory bowel disease; PPI, proton pump inhibitors; SD, standard deviation; ^a χ^2 test; ^b Fisher Test; ^c Independent sample Student's *t* test.

routinely used methods). Symptoms presented at the time of *Aeromonas* isolation differ among both groups, with IBD patients presenting more frequently with bloody diarrhea (83.3 vs. 10.5%, $p < 0.001$) and abdominal pain (75.0 vs. 34.2%, $p = 0.032$). The presence of fever was more common among patients without IBD (0.0 vs. 34.2%, $p = 0.022$). IBD patients were more frequently under AZA (25.0 vs. 2.6%, $p = 0.038$) and systemic or topical steroids (33.3 vs. 5.3%, $p = 0.024$) when compared with non-IBD patients. Immunosuppressive therapy with calcineurin inhibitors was more often used in non-IBD patients (8.3 vs. 13.2%, $p = 0.294$).

The majority of patients presented a mild-moderate *Aeromonas* infection with a proportionally higher tendency for severe episodes in IBD patients (27.3 vs. 13.5%, $p > 0.05$). There were 8 (16.7%) severe cases, 7 of which concerned immunocompromised patients. Nineteen patients (41.7% were IBD and 36.8% non-

IBD) needed to be admitted to the hospital for management of severe infection and symptomatic control in moderate cases.

Concerning the treatment of the acute episode, IBD patients were more frequently submitted to antibiotics (83.3 vs. 36.8%, $p = 0.013$), and quinolones were the most frequently used class in both (36% overall). Only IBD patients were treated with systemic or topical steroids (33.3 vs. 0.0%, $p = 0.002$), and all of these patients were also under antibiotics. Four (8%) *Aeromonas*-resistant strains were identified (3 [7.9%] in non-IBD vs. 1 [8.3%] in IBD). Other fecal bacteria were identified only in 4 non-IBD patients (10.5%): 3 children, two 1-year-olds and one 8-year-old, presented *Campylobacter jejuni*, and a 73-year-old patient presented also with *Salmonella*. All patients progressed favorably with no deaths observed.

Table 2. Clinical presentation features in IBD and non-IBD patients

	Total n (%) or median (SD) or median (IQR)	IBD n (%) or median (SD) or median (IQR)	Non-IBD n (%) or median (SD) or median (IQR)	p value
<i>Aeromonas</i> strain				–
<i>Hydrophila/caviae</i>	32 (64.0)	9 (75.0)	23 (60.5)	–
<i>Hydrophila</i>	12 (24.0)	1 (8.3)	11 (28.9)	
<i>Aeromonas spp.</i>	3 (6.0)	1 (8.3)	2 (5.3)	
<i>Aeromonas veronii</i>	3 (6.0)	1 (8.3)	2 (5.3)	
Symptoms	48 (96.0)	11 (91.7)	37 (97.4)	0.426 ^b
Diarrhea	46 (92.0)	10 (83.3)	36 (94.7)	0.240 ^b
Bloody diarrhea	14 (8.0)	10 (83.3)	4 (10.5)	<0.001 ^b
Abdominal pain	22 (44.0)	9 (75.0)	13 (34.2)	0.032 ^a
Nausea and vomiting	21 (42.0)	4 (33.3)	17 (44.7)	0.717 ^a
Fever	13 (26.0)	0 (0.0)	13 (34.2)	0.022 ^b
Bowel movements/day				–
<5	18 (36.0)	2 (16.7)	16 (42.1)	
5–10	24 (48.0)	9 (75.0)	15 (39.5)	
>10	4 (8.0)	0 (0.0)	4 (10.5)	
Severity				0.582 ^a
Mild	21 (44.0)	4 (36.4)	17 (45.9)	
Moderate	21 (44.0)	5 (45.5)	16 (43.2)	
Severe	8 (16.7)	3 (27.3)	5 (13.5)	
Hospital admission	19 (38.0)	5 (41.7)	14 (36.8)	1.000 ^a
Treatment				
Antibiotics	24 (48.0)	9 (75.0)	14 (36.8)	0.013 ^a
Quinolone	18 (36.0)	9 (75.0)	9 (23.7)	–
Carbapenem plus quinolone	3 (6.0)	1 (8.3)	2 (5.3)	
Carbapenem plus vancomycin	1 (2.0)	0 (0.0)	1 (2.6)	
Amoxicillin and clavulanic acid	1 (2.0)	0 (0.0)	1 (2.6)	
Systemic or topical steroids	4 (8.0)	4 (33.3)	0 (0.0)	0.002 ^b
Other treatments				–
No treatment	18 (36.0)	1 (8.3)	17 (44.7)	
Mesalazine	6 (12.0)	5 (41.7)	1 (2.6)	
Probiotic	2 (4.0)	0 (0.0)	2 (5.3)	
Resistant strains identified	4 (8.0)	1 (8.3)	3 (7.9)	–
Dead	0 (0.0)	–	–	–

IBD, inflammatory bowel disease; IQR, interquartile range; SD, standard deviation; ^a χ^2 test; ^b Fisher test; ^c Independent sample Student's *t* test.

IBD Diagnosis and *Aeromonas* Infection

Clinical characteristics of IBD patients and *Aeromonas* infection are presented in Table 3. From the IBD patient subgroup, 7 had the diagnosis of IBD previously and 5 after the *Aeromonas* isolation. From the latter, only 1 had the infection and subsequent diagnosis of IBD at pediatric age (patient 8, Table 3).

From those with a previously established diagnosis of IBD, 3 were UC patients and 4 CD patients (Table 3). Only 1 patient with CD was under combined therapy with

infliximab and AZA (patient 4, Table 3), and the remaining were under AZA monotherapy at the time of *Aeromonas* isolation. UC patients were under mesalamine. One patient (patient 9, Table 3) was under tacrolimus due to a previous liver transplant. Five patients were treated with antibiotics, and 2 of them simultaneously with systemic steroids. One patient was treated only with topical steroids. Three patients needed hospital admission, with one requiring ganciclovir treatment due to concomitant histological features of CMV on colonic biopsy (patient 5,

Table 3. *Aeromonas* isolation in IBD patients

Patient	Age, sex	CD, (MC)/UC	Ongoing IBD treatment	De novo IBD	Main symptoms	Endoscopic/imaging features	Treatment	Hospitalization	<i>Aeromonas</i> strain
1	24, F	CD (A2L1B2)	AZA	No	Bloody diarrhea	–	Topical steroids	No	<i>Aeromonas hydrophila/caviae</i>
2	28, F	CD (A2L1B1)	AZA Topical steroids	No	Diarrhea, abdominal pain	–	Ciprofloxacin	No	<i>Aeromonas hydrophila/caviae</i>
3	36, F	CD (A2L2B1)	None	Yes	Diarrhea, abdominal pain	Colonic mucosa with congestion and aphthoid erosions	Mesalamine	No	<i>Aeromonas hydrophila</i>
4	76, M	CD, (A3L1+L4 B2)	Infliximab AZA	No	Abdominal pain, nausea, vomiting	Ileal and jejunal segmental stenosis with transmural hyperenhancement and small loop dilation	Impipenem, systemic steroids	Yes	<i>Aeromonas hydrophila/caviae</i>
5	16, M	CD (A1L2B1)	AZA	No	Bloody diarrhea, abdominal pain	Friable and congestive mucosa with erosions	Ciprofloxacin, systemic steroids, ganciclovir	Yes	<i>Aeromonas hydrophila/caviae</i>
6	64, F	CD (A3L2B)	None	Yes	Bloody diarrhea, weight loss	Colonic mucosa with deep ulcers on colon	Ciprofloxacin, systemic steroids, ganciclovir	Yes	<i>Aeromonas spp</i>
7	31, M	CD (A2L2B1)	None	Yes	Bloody diarrhea	Decreased vascular pattern, erosions	Mesalamine	No	<i>Aeromonas hydrophila/caviae</i>
8	15, M	CD A1L2B1	Mesalamine, systemic steroids	Yes	Bloody diarrhea, abdominal pain	Decreased vascular pattern, aphthoid erosions	Ciprofloxacin, mesalamine	Yes	<i>Aeromonas hydrophila/caviae</i>
9	27, M	UC	Mesalamine	No	Diarrhea, abdominal pain, weight loss	Mayo endoscopic subscore 2	Ciprofloxacin	Yes	<i>Aeromonas sobria</i>
10	62, M	UC	Mesalamine one month previous	Yes	Bloody diarrhea	Mayo endoscopic subscore 2	Ciprofloxacin	No	<i>Aeromonas hydrophila/caviae</i>
11	12, F	UC	Mesalamine	No	Bloody diarrhea	–	Ciprofloxacin, mesalamine	No	<i>Aeromonas hydrophila</i>
12	62, M	UC	Mesalamine	No	–	–	No additional	No	<i>Aeromonas spp</i>

CD, Crohn disease; F, female; IBD, inflammatory bowel disease; UC, ulcerative colitis; M, male; MC, Montreal classification.

Table 3). The only patient under infliximab presented with intestinal sub-occlusion with a quinolone-resistant *A. hydrophila/caviae* isolation. All patients progressed favorably, and none needed to escalate IBD therapy after the *Aeromonas* acute episode. Seven patients were submitted to endoscopic evaluation. However, histological data was compatible with IBD, with no other specific features. Immediately after the acute episode, only clinical and analytical data were used to assess the remission, namely the symptoms reported by the patients, CRP, and calprotectin.

Concerning the 5 patients with a diagnosis of IBD after the episode of *Aeromonas* isolation, 4 of them were diagnosed with colonic CD and 1 with UC. Two of them were admitted to the hospital with 1 showing simultaneous histological features of CMV infection on colonic biopsy (patient 6, Table 3), being treated with antibiotics, systemic steroids, and ganciclovir. The other 3 received the IBD diagnosis in an ambulatory setting due to the persistence of chronic diarrhea in which *Aeromonas* was isolated in the first stool culture requested for the workup. From this subgroup of patients with de novo IBD diagnosis, 2 required posterior escalation therapy to infliximab (patients 6 and 10, Table 3).

Discussion/Conclusion

The pathogenic role of *Aeromonas* in human enterocolitis is still controversial as is the association of *Aeromonas* infection with IBD development or as a flare trigger. To our knowledge, a few case reports have been published, with only 2 similar case series available [7, 8].

The global prevalence of *Aeromonas* gastrointestinal infection ranges from 2 to 88% and carriage status in healthy individuals from 1 to 45% [7]. Stool isolation rates differ depending, among others, on geography, food habits, and isolation methods [20]. The prevalence of infection in developed countries ranges from 0.8 to 7.4% and carriage rate from 0 to 4% [20], indicating a possible higher prevalence in developing countries. Although no specific data exists for Portugal, we had a relatively low number of *Aeromonas* stool isolation considering the period covered. The evolution of *Aeromonas* detection methods through the years may have a role in it, since the majority (56%) of our isolates were identified between 2014 and 2019, coinciding with the introduction of mass spectrometry methods. In this study ($n = 50$), 2 patients were asymptomatic carriers (4%), concordant with the reported carriage rate in healthy individuals in developed countries [20].

The reports of the most predominant clinical species of *Aeromonas* have changed over the years. Improved molecular methods led to the conclusion that 95.4% of the strains associated with the clinical disease were *A. caviae* (37.3%), *A. veronii* (23.5%), *A. dhakensis* (21.5%), and *A. hydrophila* (13.1%) [21]. In our study, the 3 identified species are concordant with the most associated with clinical disease in other studies [7, 8]. Nevertheless, since *A. dhakensis* cannot be identified by the biochemical methods and mass spectrometry used in our laboratory, we cannot exclude that there might be a misidentification as *A. caviae*, *A. hydrophila*, or *A. veronii*. Although our data reinforce the predilection of this finding in non-IBD patients (96.2%), we have interestingly shown higher isolation of *A. hydrophila*. This could be explained by the higher number of patients under 18 years old and different geographical factors. It should, however, be kept in mind that the frequency of each strain differs according to the country. In our study, no relation was found between the strains and severity of the clinical manifestations.

When compared with a recent similar study performed in a tertiary hospital in Spain ($n = 98$) [8], the mean age of our population was lower (42 vs. 62 years-old) as well as the proportion of female sex (46 vs. 51%). This difference can be explained mainly by the fact that only adult patients were involved in the Spanish study. On the other hand, we presented a higher percentage of isolates in patients with previous IBD diagnosis (14 vs. 11%) with very similar cases of CD (8 vs. 7 patients) and an equal number of UC patients ($n = 4$).

The main comorbidities found in our group of patients were malignancies (10%), pharmacological immunosuppression (34%), and liver diseases (4%). *Aeromonas* is known to cause gastrointestinal symptoms in both immunocompetent and immunocompromised individuals with predisposing factors reported, such as diabetes, hematological malignancies, or hepatobiliary diseases [20]. Overall, the severity of the infection in our population was mainly mild-moderate with 17% severe. The percentage of severe infection was greater in IBD patients (27.3 vs. 13.5%), also reported by others [7]. Of the 5 non-IBD patients that presented with severe infection, 2 were undergoing chemotherapy, 2 had previous abdominal surgery, and the last was an HIV patient with no antiretroviral therapy adherence. The reported mortality rate can reach 30% in the set of *Aeromonas*' bacteremia [21]. Fortunately, there were no reported deaths attributed to the infection in our study.

The antibiotic susceptibility profile for *Aeromonas* does not appear to have changed substantially [22]. In the

present study, only 4 isolates were resistant to antibiotics usually active against aeromonads, according to EUCAST breakpoints [23]. Two patients had a strain resistant to ciprofloxacin and cotrimoxazole, another patient to cotrimoxazole, and the last one to ciprofloxacin. There was no identified resistance to carbapenems, a fortunate sign regardless of the reports describing an increased *Aeromonas* resistance to the latter [24]. Resistant strains were more common in non-IBD patients (75 vs. 25%). This could be explained by the fact that the mean age of non-IBD patients was higher, which usually implies a significantly higher exposure to antibiotics and previous hospital admissions.

In our analysis, IBD patients represented 24% of our population with a predominance of male sex and a tendency for younger age when compared to non-IBD patients. In the previous series, IBD patients represented 11–14% of all *Aeromonas*-positive patients with the same age trend [7, 8]. Symptomatic *Aeromonas* infection was seen in almost all patients (96%). Distinctive symptoms between the two groups were the presence of bloody diarrhea and abdominal pain which was significantly more common in IBD patients. This was in line with previous findings suggesting that a colonic involvement in the vast majority of IBD might explain the high rates of bloody diarrhea in these patients [7].

A higher proportion of IBD patients presented a severe clinical infection which followed the trend of previous studies [7]. Three IBD patients presented a more severe disease: one was under tacrolimus after liver transplant due to primary sclerosing cholangitis; the other patient was under combined therapy with infliximab and AZA; finally, a 64-year-old female with de novo CD and severe endoscopic features with concomitant CMV colitis. Almost half of non-IBD patients present a mild disease, which is a very similar result compared to the 44% previously reported [7, 16]. IBD population significantly needed more antibiotic therapy and steroids, following the trend of higher severity of infection. This might be explained by the lower threshold to antibiotic therapy in patients with IBD, namely in immunocompromised, regardless of clinical, analytical, and endoscopic features.

Regarding the place of *Aeromonas* infection in the natural history of the disease, the scarcely reported literature on the subject poses it as a potential trigger to flare and for the de novo IBD diagnosis. In our study, from those with IBD, 7/12 were diagnosed previously to *Aeromonas* isolation. A role for *Aeromonas* as a trigger to IBD flare was previously reported in a patient diagnosed with mild

ulcerative proctitis, who presented a severe colitis refractory to steroid therapy with favorable evolution under antibiotic therapy directed to the isolated *Aeromonas* [16]. On the other hand, other reports had postulated a putative role of *Aeromonas* infection for the development of de novo IBD diagnosis [7, 12]. It remains under discussion if the infection prompts IBD development or naturally unmasks an underlying disease with previous sub-clinical activity. The mechanisms inherent to these inter-relationships are unknown, but *Aeromonas*-associated intestinal dysbiosis could possibly lead to reduced bacterial diversity and, in those genetically susceptible, result in IBD onset. In our population, we also found concomitant CMV infection (Table 3, patient 6). Giving the epidemiology of CMV as a flare-causing pathogen in IBD, the endoscopic features (deep ulcers on colon) and the significant prevalence of *Aeromonas* carriage status, it is plausible that the *Aeromonas* isolated was not responsible for causing the disease.

In our population, we observed the subsequent diagnosis of IBD in 5/12 patients after *Aeromonas* infection. Lobatón et al. [7] also described 2 cases of diarrhea and abdominal pain with *Aeromonas* detection simultaneously to CD diagnosis. Similarly, more than 30 years ago, an association was proposed between *Aeromonas* infection and the new onset of 3 cases of UC [19].

Although it represents one of the few studies relating *Aeromonas* infection and IBD patients, there are some limitations to address. The retrospective character of the study, data retrieval from a unicentric microbiology database, and the loss of 3 patients due to insufficient clinical information might bias the interpretation of our results. It is important to mention that only 7 IBD patients with flares were submitted to endoscopic evaluation, which, to some extent, could compromise the conclusion. Also, the analysis of the time between *Aeromonas* isolation and the first signs of IBD would be important in the evaluation of the possible causality between the two diseases in future larger studies. It is important to mention the heterogeneity of the control group, mainly the inclusion of pediatric patients that could compromise the comparisons that were made. The reduced sample and the bias to request stool culture mainly in symptomatic patients do not allow us to infer about the overall and ecological scenario of *Aeromonas* identification in our population.

In conclusion, *Aeromonas* infection appears to play an important role in IBD activity. This infection might contribute as one more piece in the interactome unsolved puzzle for IBD. Our results reinforce that *Aeromonas* in-

fection can be a trigger for IBD flare or de novo IBD diagnosis, supporting the importance of fecal culture analysis. Our results alluding with the lack of data on *Aeromonas* infection and IBD might indicate an overlook of this infection.

Statement of Ethics

The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

The authors declare that they have followed the protocols of their work center on the publication of patient data.

The authors declare that no patient data appear in this article.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

T.P.G. and J.A.S. equally contributed to the manuscript. T.P.G. and M.S. conceived and designed the study. T.P.G., J.A.S., S.N., D.F. collected, analyzed, and interpreted data. P.C., P.L., I.P., and M.S. performed critical review of the manuscript.