**Incidence and clinicoepidemiological characteristics of *Aeromonas*-associated gastroenteritis in Northern Israel**

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**Abstract**

**Background:** The role of *Aeromonas* spp. in gastroenteritis is controversial due to its wide range of clinical presentations and variable prevalence in asymptomatic patients. The aims of the present study were to assess the incidence rate of *Aeromonas*-associated gastroenteritis (AAG) in Northern Israel as a novel pathogen diagnosed in microbiology laboratories, compare the rate with that in an asymptomatic population, and examine the role of *Aeromonas* spp. in AAG by comparing clinical and epidemiological characteristics between AAG and *Campylobacter*-associated gastroenteritis (CAG).

**Methods:** This study was conducted at Emek Medical Center, which serves a population of 0.5 million people in Northern Israel, from January 2020 to April 2023. The study comprised two case-control studies: 1) a comparison of the prevalence and demographic characteristics of patients with AAG and age-matched asymptomatic controls, and 2) a comparison of the demographic and clinical characteristics of patients with CAG and AAG.

**Results:** In the AAG and asymptomatic case-control study, 282 (4.81%), 411 (4.27%), and 425 (4.24%) of AAG patients had *Aeromonas* isolated in their stools as the sole pathogen in 2020, 2021, and 2022, respectively, versus 5 (4.9%) of the 102 asymptomatic controls (2022). In the AAG and CAG case-control study, comparison of the clinical gastrointestinal and demographic characteristics of 243 patients with *Campylobacter* spp. infections with those of 70 patients with *Aeromonas* spp. infection revealed that AAG patients had lower percentages of diarrhea (95.5% vs. 85.7%, p=0.004), fever (61.3% vs. 31.4%, p<0.001), shivering (33.3% vs. 7.1%, p<0.001), abdominal pain (81.9% vs. 57.1%, p<0.001), muscle pain (28.4% vs. 4.3%, p<0.001), headache (32.1% vs. 5.7%, p<0.001), and nausea (38.6% vs. 20%, p=0.004). In addition, patients with *Aeromonas* were characterized by more underlying diseases (44.3% vs. 25.5%, p=0.002), a higher PCR Ct value (34.94±3.73 vs. 29.28±5.2, p<0.001), and a longer duration of illness (26±42.02 vs. 9.81±9.77 days, p=0.003).

**Conclusions:** We did not find substantial evidenceindicating that *Aeromonas* spp. is a true enteropathogen in positive cases of AAG, suggesting that it is an occasional finding in stool samples. Unlike CAG, chronic gastrointestinal symptoms with a high Ct value and no fever were more common in AAG cases, with clinical symptoms of acute gastroenteritis seen in a small number of those cases.

**Introduction**

The genus Aeromonas comprises Gram-negative rod-shaped, facultative anaerobic, and oxidase-positive bacteria. Aeromonas species are widely distributed in freshwater, estuarine, and marine environments and grow at a wide range of temperatures (0 to 42ºC), although they are isolated with increasing frequency during warmer months.

Aeromonas species cause a wide spectrum of symptoms in warm- and cold-blooded animals, including fish, reptiles, amphibians, and mammals. In humans, gastroenteritis is the main presentation. Wound infections, bacteremia, and septicemia have also been described (1,2). The most common species associated with human infections include *A. hydrophila*, *A. caviae*, and *A. veronii* complexes (3–6). The most common presentation of *Aeromonas*-associated gastroenteritis (AAG) is abdominal pain, fever, vomiting, and nausea (5,7), which can lead to acute self-limiting but significant diarrhea (63.1%) or chronic gastrointestinal disease (36.9%) (2,3).

The role of Aeromonas as a gastrointestinal pathogen is controversial (8). While *Aeromonas* spp. can be isolated from 0% to 4% of the asymptomatic population, it has been found in 0.8%–7% (5,6,9) through 1%–60% (1,10) of symptomatic persons. Moreover, in recent years, the enteropathogen role of *Aeromonas* was re-evaluated and confirmed in adults in a human challenge study (10). An epidemiologic investigation of an AAG outbreak in Brazil documented growth of Aeromonas in stool culture as the sole pathogen in 25% of cases. Finally, a Spanish review reported growth of Aeromonas in stool culture as the sole pathogen causing 2% of cases of traveler's diarrhea (2). On the other hand, a pediatric study found differences in the prevalence of *Aeromonas*-positive stool cultures in children by age and region, with *Aeromonas* isolated as the sole pathogen in less than 5% of cases (9).

In 2019, Clalit Health Services, the largest health insurance service in Israel, serving about 60% of the insured population, changed the routine workflow for gastroenteritis diagnosis in all clinical microbiology laboratories from the conventional culture-based method to a molecular-based method. All stool samples were analyzed using multiplex PCR for six enteropathogens: *Campylobacter* spp., *Salmonella* spp., *Shigella* spp./enteroinvasive *Escherichia coli* (EIEC), *Vibrio* spp., *Yersinia enterocolitica*, and *Aeromonas* spp. To the best of our knowledge, no studies of the epidemiology and clinical significance of *Aeromonas* spp. in Israel have been published. However, the new diagnostic workflow for bacterial stool pathogens gave us the opportunity to evaluate the clinical and epidemiological significance of *Aeromonas* spp. in Northern Israel.

Accordingly, in the present study, we assessed the incidence rate of AAG in Northern Israel as a novel pathogen diagnosed in microbiology laboratories and compared the rate to that in the asymptomatic population. In addition, we examined the role of *Aeromonas* spp. in AAG by comparing the clinical and epidemiological characteristics of AAG to those of *Campylobacte*r-associated gastroenteritis (CAG). We hypothesized that *Aeromonas* gastroenteritis would have specific clinical characteristic and that the incidence rate would be similar to that reported in the latest literature.

**Materials and Methods**

**Setup and population**

The present study was conducted at Emek Medical Center (EMC), Afula, Israel, by the microbiology laboratory and Infectious Diseases Unit teams. As part of Clalit Health Services, the EMC laboratory serves as a regional laboratory in Northern and Eastern Israel for a population of about 0.5 million, primarily located in rural settlements and with a similar proportion of Arabs and Jews. From the last week of December 2019, the EMC microbiology laboratory replaced stool culture as the routine workup of bacterial gastroenteritis with PCR-based diagnosis, expanding the diagnosis of bacterial enteropathogens.

Due to the transition to the molecular diagnostic routine and the expansion of bacterial enteropathogen diagnosis, a corresponding study was conducted to examine the impact of this change (11), in addition to the current study, which focuses on *Aeromonas* spp. as a causative agent of gastroenteritis.

**Study design**

This study combined the following: (1) a prospective cohort study of the yearly incidence rates of laboratory-diagnosed AAG between January 1, 2020, and October 30, 2022: and (2) two prospective case-control studies conducted from November 2020 to April 2023 comparing the prevalence and demographic characteristics of AAG patients vs. age-matched asymptomatic controls and comparing the demographic and clinical characteristics of AAG and CAG. CAG case data were recently published in an article comparing the clinical characteristics of bacterial gastroenteritis cases diagnosed solely by PCR to those diagnosed by both PCR and culture (11). In both case-control studies, eligible cases in AAG and CAG groups were contacted by an infectious diseases physician once the PCR results were received to request their consent to participate in the study. The study and telephone-based consent procedure were approved by the local ethics committee. Following their consent, a telephone questionnaire was completed by study personnel (see Supplemental Material). This questionnaire included sociodemographic data, general medical history, diarrheal characteristics, and other symptoms, prior antimicrobial treatment, and food exposure. Patients in the asymptomatic control group were asked by the study personnel for their consent to participate in the study and completed the same questionnaire as the case group; their stool samples were sent to the same laboratory.

Inclusion criteria:

Symptomatic Case Group: Positive stool samples for *Aeromonas* spp. or *Campylobacter* spp. as the sole pathogen. Samples with more than one enteropathogen were excluded.

Asymptomatic Control Group: 1) Asymptomatic volunteers of all ages; 2) no history of antimicrobial treatment in the month preceding their participation; and 3) EMC workers or their relatives. The control group participants were matched by age with the case group (<1, 1–5, 5–18, 18–60, >60 years).

**Laboratory methods**

AAG and CAG group samples

Stool samples were transported from the community clinics and tested every day. On weekends, samples were refrigerated at 4°C until testing. Stool samples were suspended in ASL buffer (Qiagen, Hilden, Germany) and then DNA extraction was conducted using the STARMag Universal Cartridge Kit (Seegene, Dusseldorf, Germany) on the STARLET automated extraction platform (Seegene). Bacterial enteropathogens were tested using the Allplex™ GI-Bacteria(I) PCR assay (Seegene, Seoul, South Korea) (12) until October 30, 2022, and then using an Allplex™ GI-EB Screening Assay. The Allplex™ GI-Bacteria(I) PCR Assay detects seven enteropathogens: *Shigella* spp./EIEC, *Salmonella* spp., *Campylobacter* spp., *Aeromonas* spp., *Yersinia* *enterocolitica,* *Vibrio* spp., and *Clostridium difficile* toxin B. The Allplex™ GI-EB Screening Assay detects seven enteropathogens, including *Shigella* spp./EIEC, *Salmonella* spp., *Campylobacter* spp., *E. coli* O157, STEC (*stx1/2*), and *Clostridium difficile* toxin A/B.

Control group samples

All stool samples of the control group were tested using both culture and PCR. As for the stool samples of the case group (see above), they were tested using PCR and cultured on SS agar plates (Hylabs, Rehovot, Israel) after enrichment in alkaline peptone water with 0.5 M NaCl and cephalothin (10 mg/L) overnight (13,14). Both the enrichment broth and SS agar plates were incubated at 36ºC for 24 h. Suspicious colonies for *Aeromonas* were identified using the MALDI Biotyper Sirius system (Bruker Daltonics, Bremen, Germany).

**Statistical analysis**

Categorical variables of the two study groups were compared using the chi-squared test. Continuous variables were compared using one-way ANOVA and t-test and are reported as means and standard deviations (SD). Multivariate logistic regression was performed to establish independent predisposing factors and predict variables of enteric morbidity due to *Aeromonas* and included all variables identified in the univariate analysis with a p-value < 0.05. Incidence rates were calculated for 2020 and 2021. The incidence rate for 2022 was standardized because of a lack of data for November and December 2022. Data analysis was performed using SPSS® version 28.0.1.1 (14) (SPSS Inc., Chicago, IL, USA).

**Results**

All stool samples sent to the microbiology laboratory for bacterial enteropathogens from January 2020 until October 2022 were included in the calculation of the positivity and incidence rates of *Aeromonas* spp. These rates and those of *Campylobacter* spp. are summarized in Table 1. In 2020, 2021, and 2022, the positivity rates of *Aeromonas* spp. as the sole pathogen were 4.81%, 4.27%, and 4.24%, respectively (not significant). In addition, 1.49%, 1.02%, and 1.01% of stool samples were positive for additional pathogens, such as *Campylobacter* spp., *Salmonella* spp., and *Shigella* spp., respectively. The incidence rate of positive stool samples for *Aeromonas* spp. as the sole pathogen increased from 0.49 in 2020 to 0.7 in 2021 and to 0.86 in 2022 per 1,000 population. The incidence rate in 2022 was standardized based on these results (p<0.001). During the study period, we enrolled 70 patients with *Aeromonas* spp. infection, 102 age-matched *Aeromonas* spp. asymptomatic controls, and 243 patients with *Campylobacter* spp. infection.

***Aeromonas* gastroenteritis versus asymptomatic carriers: age-matched case-control study**

*Aeromonas* spp. was detected as the sole pathogen in 1,118 of all 25,498 stool samples (4.38%) sent to the microbiology laboratory from symptomatic patients and in 5 of the 102 asymptomatic controls (4.9%). Comparison of the demographic characteristics of the AAG group (N=70) and the asymptomatic control group (N=102) revealed that the mean (SD) ages of the AAG and control groups were 18.54 (1.0) and 18.94 (4.38) months, respectively (p=0.916).

The AAG group had a nonsignificantly lower percentage of females compared with the control group (52.9% vs. 63.7%, p=0.154) and fewer members of an ethnic/religious group other than Jewish (12.9% vs. 58.8%, p<0.001). In addition, the mean PCR Ct value of the AAG group was lower than that of the positive samples (N=3) of the control group (34.94 vs. 37.95, p=0.0171).

***Aeromonas* and *Campylobacter*** **gastroenteritis: case-control study**

The clinical and demographic characteristics of the patients with AAG versus CAG are summarized in Table 2. Patients in both AAG and CAG groups had similar demographic characteristics, except for a younger age in the *Aeromonas* group (younger than 2 years old: 58.6% vs. 27.2%, respectively) (data not shown).

The predisposing factors and clinical characteristics differed between patients with *Aeromonas* spp. and *Campylobacter* spp. gastroenteritis. Patients with *Aeromonas* spp. had more underlying diseases (p=0.002), a higher Ct value (p<0.001), and more prolonged diarrhea (> 10 days) than patients with CAG (62.5% vs. 23.5%) (p<0.001). Accordingly, the Ct value was lower in individuals with short-term diarrhea (<10 days) for both patient groups (Figure 1). Compared with CAG, AAG was characterized by a lower percentage of patients with diarrhea and nausea (p=0.004 for both) and lower rates of fever, shivering, abdominal pain, muscle pain, skin rash, and headache (p<0.001 for all) (Table 2).

Multivariate analyses of the predisposing factors identified a higher Ct value (odds ratio [OR]=1.23, 95% confidence interval [CI] 1.14–1.32, p<0.001), recent restaurant dining (OR=0.38, 95% CI 0.18–0.79, p=0.01), and prolonged duration of diarrhea (> 10 days) (OR=5.9, 95% CI 2.46–10.9, p<0.001) as predictive variables for AAG vs. CAG.

**Discussion**

In this prospective study, we compared the prevalence of *Aeromonas* spp. in AAG and the incidence rate of AAG and its clinical characteristics with those of the traditional enteropathogen *Campylobacter* spp. Before 2020, AAG was not diagnosed in Israel in the Clalit Health Services microbiology laboratories and its status as a true enteropathogen was unknown. With the transition to molecular diagnosis and use of molecular multiplex panels, the range of pathogens has expanded beyond what was previously detected by culture, such as *Campylobacter* spp., *Salmonella* spp., *Shigella* spp., and *Yersinia* spp., to include *Aeromonas* spp. and *Vibrio* spp.

Our findings indicate that the positivity rates of *Aeromonas* spp. as the sole pathogen did not change from 2020 to 2022 and ranged from 4.24% to 4.81% in symptomatic patients and was 4.9% in asymptomatic individuals. However, the incidence rate increased from 0.49 in 2020 to 0.7 in 2021 and to 0.86 in 2022 per 1,000 population (p<0.001). This increase might be explained by the restrictions on outdoor activities in 2020 and early 2021 due to the COVID-19 pandemic, followed by the resumption of the previous routine. The same phenomenon was reported in a corresponding study (11).

The prevalence of *Aeromonas* spp. varies among studies and is influenced by factors such as geographic location, population characteristics, and laboratory diagnostic methods. Industrialized countries show a lower prevalence, from 2%–10% to 0%–4% among symptomatic and asymptomatic adult populations, respectively (1,15–17). Moreover, the geographic variability is also seen in pediatric populations, with prevalences varying from 0.002% in symptomatic children in Denmark (18) to 30% in India (19), with an overall pooled prevalence of 4.2% (95% CI 3.1%–5.6%) (20).

The Global Enteric Multicenter Study (GEMS) compared stool cultures among 12,110 children with diarrhea and 17,291 matched control children at seven global sites. In this study, *Aeromonas* spp. was associated with diarrhea only in Pakistan and Bangladesh. In contrast, in Africa and India, the isolation rates never exceeded 1% (9). Another finding of the GEMS study is that *Aeromonas* spp. was isolated as the sole pathogen in less than 5% of cases, with *Shigell*a spp. found to be the most common co-isolate. Factors responsible for these regional differences in prevalence remain to be determined, in addition to the potential role of *Aeromonas* spp. as a co-infecting pathogen (9).

The prevalence of *Aeromonas* spp. is also influenced by age. The GEMS study found rates ranging from 19% in the 0- to 11-month-old age group to 29% in the 24- to 59-month-old group (9), higher than in other studies in the adult population (1,16-18). Similar findings were published in an Israeli study, with 94% of stool samples positive for *Aeromonas* spp. in children younger than 3 years old and 78% positive in those younger than 1 (21). In a study conducted in Australia, *Aeromonas* spp. was the most common enteropathogen in children aged 6–18 months, with a high detection rate between ages 0–4 years (7). Our study results are consistent with the literature. Most of the positive *Aeromonas* spp. samples were detected in the pediatric population: 60% and 41.4% of positive *Aeromonas* spp. samples in symptomatic patients were in patients younger than 3 and 1 years old, respectively.

To determine the clinical relevance of *Aeromonas* spp. in gastroenteritis, we evaluated the epidemiological and clinical characteristics of patients diagnosed with AAGcompared to patients diagnosed with CAG. In contrast to recognized enteropathogens such as *Shigella* spp., *Salmonella* spp., and *Campylobacter* (22), evidence on *Aeromonas* spp. being a cause of gastroenteritis is limited in recent literature (1,8,10,15), yet it has been regularly described as a true enteropathogen (10). Our study results do not support this trend because there was no difference in the positivity rate for *Aeromonas* spp. as the sole pathogen between symptomatic and asymptomatic populations. This is supported by our results documenting significant differences in clinical characteristics, with patients with AAG presenting fewer clinical characteristics compared with patients with CAG.

Various clinical studies have defined AAG as ranging from acute self-limited diarrhea lasting up to 1 or 2 weeks to a more prolonged illness or chronic gastroenteritis lasting more than a month (1,8,9). It may also be accompanied by abdominal pain, fever, vomiting, and nausea (1). The clinical presentation of CAG is usually acute diarrhea lasting up to 2 weeks with abdominal pain and diarrhea in most infected patients. Fever, muscle pain, and headache occur in most patients with CAG, whereas vomiting and bloody diarrhea are less frequent (23). We found that AAG displayed milder symptoms compared with CAG, and fewer patients had diarrhea (85.7% in AAG vs. 95.5% in CAG, p=0.004). The combination of the clinical characteristics of AAG, including milder symptoms of gastroenteritis such as abdominal pain, fever, headache, dizziness, weakness, and chronic diarrhea diagnosed later in the illness, as well as the same prevalence in both symptomatic and asymptomatic individuals, suggests an occasional finding of *Aeromonas* in stool samples compared with CAG. Notably, the illness lasted more than 10 days in over 60% of AAG cases but was prolonged in just 23.4% of CAG cases. Although we could not prove that *Aeromonas* is a true enteropathogen, we noted a difference in the average Ct value in positive *Aeromonas* samples: 34.94 (N=70) vs. 37.95 (N=3) (p=0.171) in symptomatic and asymptomatic cases, respectively. A high Ct value might indicate a residual bacterial remnant in the stool or low-level bacterial colonization in the asymptomatic control group compared to the symptomatic group that showed lower Ct values.

In November 2022, the microbiology laboratory standard of procedure for testing stool was changed from a multiplex PCR panel to a different bacterial panel that did not include *Aeromonas* spp. Following this change, we had to stop enrollment of patients to the AAG group.

**Conclusion**s

Our study results do not support our hypothesis that *Aeromonas* spp. is a true enteropathogen in all positive cases and indicate that this bacterium is probably an occasional finding in the stool. Unlike other classic enteropathogens, chronic gastrointestinal symptoms with a high Ct value and no fever seem to be more common in AAG, with acute gastroenteritis in fewer cases. Considering these factors, testing for *Aeromonas* spp. should be conducted in settings where outbreaks of gastroenteritis and food poisoning are more common. Until additional data are available, we suggest against the routine testing for *Aeromonas* spp. A larger study is required in patients diagnosed with *Aeromonas* by PCR as the sole pathogen in a stool sample to determine if there is a cut-off Ct value that could differentiate colonization from true infection, which will be a useful tool for physicians to avoid unnecessary treatment.

**Disclosure statement:**

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| --- | --- | --- | --- | --- |
|  | *Aeromonas* aloneN (%) | *Aeromonas* with other pathogensN (%) | *Campylobacter* aloneN (%) | Incidence rate of *Aeromonas* |
| 2020 | 282 (4.81) | 87 (1.49) | 341 (5.82%) | 0.49 |
| 2021 | 411 (4.27) | 98 (1.02) | 635 (6.60%) | 0.7 |
| Oct. 2022 | 425 (4.24) | 101 (1.01) | 643 (6.41%) | 0.86\* |

**Table 1: Positivity and incidence rates of *Aeromonas*-associated gastroenteritis, January 2020–November 2022**

**\* Standardized**

|  |  |  |  |
| --- | --- | --- | --- |
|  | *Aeromonas* PCR (N=70) | *Campylobacter* PCR (N=243) | p-value |
| Age (year), mean (median) | 18.54 (1.0) | 21.93 (13.00) | 0.3 |
|  |
| Female sex, n (%) | 37 (52.9) | 110 (45.3) | 0.3 |  |
|  |
| Member of a minority group1, n (%) | 9 (12.9) | 52 (21.4) | 0.1 |  |
|  |
| PCR Ct value, mean (SD) | 34.94 (3.73) | 29.28 (5.2) | <0.001 |  |
|  |
|  |
| Underlying morbidities, n (%) | 31 (44.3) | 62 (25.5) | 0.002 |  |
| Antimicrobial treatment, n (%) | 28 (40%) | 117 (48%) | 0.228 |  |
| Regular pharmaceutical therapy, n (%) | 30 (42.9) | 77 (31.8) | 0.087 |  |
| Hospitalization within 1 month, n (%) | 4 (5.7) | 9 (3.7) | 0.458 |  |
| Gastroenteritis in family members, n (%) | 23 (32.9) | 56 (23.0) | 0.096 |  |
| Antimicrobial treatment, n (%) | 28 (40) | 117 (48.1) | 0.228 |  |
| Recent (<1 month) restaurant dining, n (%) | 26 (45.6) | 162 (67.8) | 0.009 |  |
| Mean duration of illness, days | <10 | 24 (37.5%) | 182 (76.5%) | <0.001 |  |
| >10 | 43 (64.2%) | 58 (24.2%) |  |
| Fever, n (%) | 22 (31.4) | 149 (61.3) | <0.001 |  |
| Shivering, n (%) | 5 (7.1) | 81 (33.3) | <0.001 |  |
| Diarrhea, n (%) | 60 (85.7) | 232 (95.5) | 0.004 |  |
| Vomiting, n (%) | 20 (28.6) | 65 (26.7) | 0.763 |  |
| Abdominal pain, n (%) | 40 (57.1) | 199 (81.9) | <0.001 |  |
| Rash, n (%) | 26 (37.1) | 31 (12.8) | <0.001 |  |
| Nausea, n (%) | 14 (20) | 94 (38.6) | 0.004 |  |
| Muscle pain, n (%) | 3 (4.3) | 69 (28.4) | <0.001 |  |
| Headache, n (%) | 4 (5.7) | 78 (32.1) | <0.001 |  |
| Weakness, n (%) | 34 (48.6) | 177 (72.8) | <0.001 |  |

**Table 2: Demographic and clinical characteristics of *Aeromonas*-associated gastroenteritis and *Campylobacter*-associated gastroenteritis (univariate analysis)**

1-member of an ethnic/religious group other than Jewish

Figure 1: Duration of illness and mean Ct value of AAG (N=64) and CAG (N=238)