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

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Abstract

Background: The role of Aeromonas spp. in gastroenteritis is controversial, owing to its wide range of clinical presentations and variable prevalence in asymptomatic patients.

The aim of this study was to assess the incidence rate of *Aeromonas* -Associated Gastroenteritis (AAG) in northern Israel as a novel pathogen diagnosed in microbiology laboratories and compared it to its prevalence in asymptomatic population, and to examine the role of *Aeromonas* spp. in AAG by comparing the clinical and epidemiological characteristics of AAG to those of *Campylobacter*-Associated Gastroenteritis (CAG).

Methods: This study was conducted at the Emek Medical Center, serving a population of 0.5 million people in northern Israel from January 2020 to April 2023. The study included two case-control studies: 1) comparing the prevalence and demographic characteristics of AAG and age-matched asymptomatic controls, and 2) comparing demographic and clinical characteristics of CAG and AAG.

**Results:** AAG and asymptomatic case-control **s**tudy**:**282(4.81%),411 (4.27%), and 425 (4.24%) of AAG patients had Aeromonas isolated in their stools as a sole pathogen in 2020, 2021, and 2022 respectively compared to 5 (4.9 %) of asymptomatic controls (2022). AAG and CAG case-control **s**tudy: Clinical gastrointestinal and demographic characteristics of 243 patients with *Campylobacter* spp. infections compared to 70 patients with *Aeromonas* spp infectionwere characterized by a lower percentage of patients with 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) respectively. In addition, Patients with *Aeromonas* were characterized by more underlying diseases (44.3% vs. 25.5%, p=0.002), a higher 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: According to our study, we did not find a substantial evidenceto indicate *Aeromonas* spp is a true enteropathogen in positive cases of AAG, suggesting being an occasional finding in stool samples. Unlike to *Campylobacter*-associated gastroenteritis, chronic gastrointestinal symptoms with high CT value and no fever were more common in AAG cases, with clinical symptoms of acute gastroenteritis were seen in a small number of those cases.

**Introduction**

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

Aeromonas species cause a wide spectrum of symptoms among warms and cold-blooded animals, including fish, reptiles, amphibians, and mammals. In humans, gastroenteritis is the main presentation. Wound infections, bacteremia, and septicemia were also described(1,2). The most common species associated with human infections include *A. hydrophila*, *A. caviae*, and *A. veronii* complexes (3–6). Most common presentation of *Aeromonas*-Associated Gastroenteritis (AAG) is abdominal pain, fever, vomiting, and nausea (5,7), which might be acute self-limited disease, 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 among 0%-4% of asymptomatic population, it was found in 0.8%-7% (5,6,9) through 1%-60% (1,10) of symptomatic persons. Moreover, during last years, the enteropathogen role of *Aeromonas* has been re-evaluated and confirmed in adults in a human challenge study(10). Epidemiologic investigation of an AAG outbreak in Brazil documented growth of Aeromonas in stool culture as a sole pathogen in 25% of cases. A Spanish review reported growth of Aeromonas in stool culture as a sole pathogen causing 2% of cases of traveler's diarrhea (2).

On the other hand, a pediatrician study found regional and age different prevalence of *Aeromonas*-positive stool culture in children, while *Aeromonas* was isolated as the sole pathogen in less than 5% of cases (9).

In 2019, Clalit Health Services, (CHS), the major health insurance service in Israel, serving about 60% of insured population, changed the routine workflow for diagnosing gastroenteritis in all clinical microbiology laboratories, from conventional culture-based method to a molecular-based method. All stool samples were diagnosed using multiplex PCR for six enteropathogens*: Campylobacter spp.*, *Salmonella spp.*, *Shigella spp/*EIEC, *Vibrio spp.*, *Yersinia enterocolitica,* *and Aeromonas spp*.

Until now, to the best of our knowledge, no studies about epidemiology and clinical significance of *Aeromonas* spp. in Israel were published.

The introduction of the new diagnostic workflow for bacterial stool pathogens, gives us the opportunity to evaluate the clinical and epidemiological significance of *Aeromonas* spp. in Northern Israel.

In this study, we assessed the incidence rate of AAG in Northern Israel as a novel pathogen diagnosed in microbiology laboratories and compared it to the prevalence in 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 assume that *Aeromonas* gastroenteritis would have specific clinical characteristic and the incidence rate would be similar to the data reported in the updated literature.

**Materials and Methods**

**Setup and population**

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

Due to the transition to molecular diagnostic routine and the expansion of bacterial enteropathogens diagnosis, a corresponding study was conducted to examine the impact of this change (11), alongside with this current study that focused on *Aeromonas* spp. as a causative agent of gastroenteritis.

**Study design**

This study was combined of the following: (1) A prospective cohort study evaluating the yearly incidence rates of laboratory-diagnosed AAG between January 1st 2020 to October 30th 2022. (2) Two prospective case-control studies conducted from November 2020 to April 2023: a study comparing the prevalence and demographic characteristics of AAG patients vs. age-matched asymptomatic control group and a study comparing demographic and clinical characteristics of AAG and CAG. The data of CAG cases was recently published in a paper that compared 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, asking for their consent to participate in the study. Study and phone-consent procedure were approved by the local ethics committee. Following their consent, a phone questionnaire was completed by study personnel (see Supplemental Material) including sociodemographic data, general medical history, diarrheal characteristics and other symptoms, prior antimicrobial treatment and food exposure. Patients in the asymptomatic control group who were asked by the study's personnel for their consent to participate in the study, completed a questionnaire as the case group, and stool samples were sent to the same laboratory.

Inclusion criteria :

Symptomatic Case Group: Positive stool samples for *Aeromonas* spp. or *Campylobacter* spp. as a 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 during the month preceding their participation, and 3) EMC workers or family 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 tested. Stool samples were suspended in ASL buffer (Qiagen, Hilden, Germany) followed by DNA extraction that was conducted using the STARMag Universal Cartridge Kit (Seegene, Duesseldorf, Germany) on the STARLET automated extraction platform (Seegene). Bacterial enteropathogens were tested by the Allplex™ GI -Bacteria(I) PCR assay (Seegene, Seoul, South Korea) (12). Until October 30th , 2022, and afterward replaced to Allplex™ GI-EB Screening Assay. Allplex™ GI -Bacteria(I) PCR assay detects seven enteropathogens, including *Shigella* spp./*Enteroinvasive Escherichia coli* (EIEC), *Salmonella* spp., *Campylobacter* spp., *Aeromonas* spp., *Yersinia* *enterocolitica,* *Vibrio* spp. and *Clostridium difficile* toxin B. Allplex™ GI-EB screening Assay detects seven enteropathogens, including *Shigella* spp./*Enteroinvasive Escherichia coli* (EIEC), *Salmonella* spp., *Campylobacter* spp., *Escherichia coli* O157, STEC (*stx1/2*) and *Clostridium difficile* toxin A/B.

Control group samples

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

**Statistical analysis**

Categorical variables of the two study groups were compared using the Chi2 test and One-Way ANOVA for categorical variables and continuous variables reported as means and standard deviations (SD) and t-test. 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. The incidence rates were calculated for 2020 and 2021. The incidence rate for 2022 was standardized because of 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 positivity and incidence rate of *Aeromonas* spp*.*. Incidence and positivity rates of *Aeromonas* spp. and *Campylobacter* spp. are summarized in Table 1. In 2020, 2021, and 2022, the positivity rates of *Aeromonas* spp. as a sole pathogen were 4.81%, 4.27% and 4.24% respectively (N.S.) 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 a sole pathogen had increased from 0.49 in 2020 to 0.7 in 2021 and to 0.86 per 1000 population. The incidence rate in 2022 was standardized based on those results (p<0.001). During the study period, a total of 70 patients with *Aeromonas* spp. infection, 102 age-matched *Aeromonas* spp. asymptomatic control group and 243 patients with *Campylobacter* spp. infection, were enrolled.

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

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

The AAG group had a lower percentage of females compared to the control group (52.9% vs. 63.7%, p=0.154, N.S), and less members of an ethnic-religious group other than Jews (12.9% vs. 58.8%, p<0.001). In addition, the mean PCR Ct value of the AAG group was lower compared to 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 from both AAG and CAG groups had similar demographic characteristics, except for younger age in *Aeromonas* vs. *Campylobacter* group (58.6% under 2 years old vs. 27.2%, respectively) (data not shown).

The predisposing factors and the clinical characteristics differed between patients with *Aeromonas* spp. Vs. *Campylobacter* spp. gastroenteritis. Patients with *Aeromonas* spp. had more underlying diseases (p=0.002), higher CT value (p<0.001) and more prolonged diarrhea (> 10 days) than the CAG (62.5% vs. 23.5%) (p<0.001). Accordingly, the CT value was lower in short-term diarrhea (<10 days) for both patients groups (Figure 1).

Compared to CAG, AAG was characterized by a lower percentage of patients with diarrhea and nausea (p=0.004 for both), and lower rate of fever shivering, abdominal pain, muscle pain, skin rash and headache (p<0.001 for all), respectively (Table 2).

Multivariate analyses for the predisposing factors identify higher CT value (O.R. =1.23, 95% C.I. 1.14-1.32, p<0.001), recent restaurant dining (O.R. =0.38, 95% C.I. 0.18-0.79, p=0.01) and prolonged duration of diarrhea (> 10 days) (O.R. =5.9, 95% C.I. 2.46-10.9, p<0.001) as predictive variables for AAG vs. CAG.

**Discussion**

In this prospective study, we evaluated the prevalence of *Aeromonas* spp. in AAG, the incidence rate of AAG and its clinical characteristics, compared to traditional enteropathogens, such as *Campyobacter* spp. Before 2020, AAG was not diagnosed in Israel in the Clalit Health Services microbiology laboratories, thus was unknown as a true enteropathogen. With the transition to molecular diagnosis and using molecular multiplex panels, the panel of pathogens previously reported by culture, such *as Campylobacter* spp., *Salmonella spp.*, *Shigella* spp., and *Yersinia* spp., was expanded to include *Aeromonas* spp. and *Vibrio* spp.

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

The prevalence of *Aeromonas* spp. varies in different studies and is influenced by factors as geographic location, population characteristics, and laboratory diagnostic methods.

Industrialized countries showed lower prevalence, from 2%-10% to 0%-4% among symptomatic and asymptomatic adult populations, respectively (1,15–17). Moreover, the geographic variability was also seen in pediatric population showing a 0.002% prevalence 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 these study, *Aeromonas* spp. was associated with diarrhea only in Pakistan and Bangladesh. In contrast, in Africa and India, rates of isolation never exceeded 1% (9). Another finding in the GEMS study was 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, as also 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 months age group to 29% in the 24 to 59 months age group (9), higher than reported in other studies in adult population (1,16-18).

Similar findings were published in an Israeli study, documenting 94% of stool samples positive for *Aeromonas* spp. in children under three years old and 78% under one(21). In another 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 pediatric population: 60% and 41.4% of positive *Areomonas* spp. samples in symptomatic patients were in patients younger than three and one year old respectively.

In order 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.

Differ from recognized enteropathogens as *Shigella* spp. *Salmonella* spp. and *Campylobacter* (22), evidence on *Aeromonas* spp. being a cause for gastroenteritis is limited in recent published literature (1,8,10,15), yet it has been more described as a real enteropathogen(10). Our study results do not support this trend, since 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, while AAG presented less clinical characteristics, compared to CAG.

Various clinical studies defined AAG as ranging from acute self-limited diarrhea lasting up to one or two weeks, to a more prolonged illness or chronic gastroenteritis lasting more than one 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 up to two weeks with abdominal pain and diarrhea in the majority of infected patients. In CAG, fever, muscle pain, and headache occur in most patients, whereas vomiting and bloody diarrhea are less frequent (23). We found that AAG displayed milder symptoms compared to CAG, and less cases had diarrhea (85.7% in AAG vs. 95.5% in CAG, p=0.004). The combination of AAG's clinical characteristics, including milder symptoms of gastroenteritis like 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, can suggest an occasional finding of *Aeromonas* in stool samples, compared to CAG. Notably, the illness lasted more than 10 days in over 60% of AAG cases, while in CAG it was prolonged only in 23.4% of cases. Although we could not prove that *Aeromonas* is a true enteropathogen as described above, we note 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 symptomatic group who showed lower CT values.

In November 2022, the microbiology laboratory standard of procedure for testing stool was changed from 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. have been found to be a true enteropathogen in all positive cases, probably representing an ocasional finding in stool. Unlike other classic enteropathogens, chronic gastrointestinal symptoms with high CT value and no fever seem to be more common in AAG with acute gastroenteritis in a less cases. Considering these factors, *Aeromonas spp*. should be tested in settings where outbreaks of gastroenteritis and food poisoning are more common. Until additional data is available, we suggest not to test routinely *Aeromonas* spp. A larger study is required in patients diagnosed with *Aeromonas* by PCR as a single pathogen in 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:**

No potential conflict of interest was reported by the authors.

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| --- | --- | --- | --- | --- |
|  | *Aeromonas*  N(%) alone | *Aeromonas* with other pathogens N(%) | *Campylobacter*,  alone N(%) | 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 rate and *Aeromonas*-associated gastroenteritis incidence, 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 gender, 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 values, 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 one 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 (one 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 of *Campylobacter*-associated gastroenteritis (univariate analysis)**

1-member of an ethnic-religious group other than Jews

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