



Original article

Epidemiology of *Aeromonas* spp. isolated from stool in a tertiary hospital in Cantabria, Northern Spain, in the last five years



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ABSTRACT

Introduction: The role of *Aeromonas* species in gastrointestinal disease is controversial. The aim of this study was to know the epidemiological distribution of *Aeromonas* spp. isolated from stool in our health area, determine the existence of diarrhea as a significant symptom, identification of existing species in our environment and association as co-pathogen.

Methods: It was a retrospective descriptive study of isolates of *Aeromonas* spp. in feces (2016–2020). The protocol for these isolates included coproculture, identification by MALDI-TOF (Vitek-MS®, BioMérieux) and confirmation by multiplex PCR.

Results: A total of 366 *Aeromonas* spp. isolates were analyzed being *Aeromonas caviae* the most prevalent species (289, 78.7%). A total of 58 (15.8%) co-infections were identified, being more frequent in pediatric age (49;84.5%) ($p=0.01$) and mostly associated with *Campylobacter* spp.

Discussion: *Aeromonas* spp. prove to be a gastrointestinal pathogen more frequently associated with co-infections in pediatric age, evidencing its appearance especially with *Campylobacter* spp.

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Epidemiología de *Aeromonas* spp. aisladas de heces en un hospital terciario en Cantabria, norte de España, en los últimos 5 años

RESUMEN

Introducción: El papel de las especies de *Aeromonas* en la enfermedad gastrointestinal es muy controvertido. El objetivo de este estudio fue conocer la distribución epidemiológica de *Aeromonas* spp. aislada de heces en nuestra área de salud, determinar la existencia de diarrea como síntoma significativo, la identificación de especies existentes en nuestro entorno y la asociación como copatógeno.

Métodos: Se trata de un estudio descriptivo retrospectivo de aislados de *Aeromonas* spp. en heces (2016–2020). El protocolo para estos aislamientos incluía coprocultivo, la identificación por MALDI-TOF (VITEK®MS, bioMérieux) y la confirmación por PCR multiplex.

Resultados: Se analizaron un total de 366 aislados de *Aeromonas* spp., siendo *Aeromonas caviae* la especie más prevalente (289; 78,7%). Se identificaron un total de 58 (15,8%) coinfecciones, siendo significativamente más frecuentes en edades pediátricas (49; 84,5%) ($p=0,01$) y asociadas principalmente con *Campylobacter* spp.

Discusión: *Aeromonas* spp. resulta ser un patógeno gastrointestinal que se asocia con mayor frecuencia a coinfecciones en la edad pediátrica, evidenciando su aparición especialmente con *Campylobacter* spp.

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Palabras clave:

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Introduction

The genus *Aeromonas* belongs to the *Aeromonadaceae* family and comprises a group of Gram-negative bacteria widely distributed

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in aquatic environments, but its great capacity for adaptation also makes *Aeromonas* species able to colonize terrestrial environments, so these microorganisms can be identified from different sources, such as soils, plants, fruits, vegetables, birds, reptiles, amphibians, among others.¹ The genus *Aeromonas* comprises of two different groups of bacteria. One is non-motile psychrophilic *Aeromonas salmonicida* and the other group comprising of three mesophilic motile spp. *Aeromonas hydrophila*, *Aeromonas caviae*, and *Aeromonas sobria*.² Some species of *Aeromonas* are known to be pathogenic in both mammals and reptiles or fish, particularly in humans, Aeromonads, are known to cause gastroenteritis and extraintestinal diseases such as septicemia, skin, eye, wound and respiratory tract infections.³ It has not always been easy to identify *Aeromonas* spp. by traditional biochemical tests in clinical microbiology laboratories. Today, thanks to the new technologies used such as mass spectrometry, which is increasingly used because it is faster, simpler and with high precision with respect to traditional methods, make it easier identifying them. Some *Aeromonas* species are found forming part of the human gastrointestinal microbiota, varying the rates of frequency from 0% to 4% in persons with no disease to 0.8–7.4% in persons with diarrheal illness.⁴ The prevalence in case of diarrhea in pediatric age is around 4.6%⁵ and also it has been described as a co-pathogen at 1.9–57.7% in pediatric age.^{6,7}

The objective of this study was to know the epidemiological distribution of *Aeromonas* spp. in our health area, to determine the most significant symptom associated with *Aeromonas*, frequency of association as co-pathogen, identification of existing species as well as age of distribution or existence of seasonal association in our environment.

Material and methods

Retrospective descriptive study, through the laboratory information system (SIL), of isolates of *Aeromonas* spp. in stool between 2016 and 2020 in the Microbiology Department of the University Hospital Marqués de Valdecilla, Santander, that serves a total of 313.040 census population. The protocol for these isolates included inoculated on Yersinia Selective Agar (CIN Agar; BD, Heidelberg, Germany) and incubated at 37 °C for 24 h. Identification was performed by MALDI-TOF system (Vitek-MS[®], BioMerieux) and most of them were confirmed by multiplex PCR developed by Perssons S et al.⁸ The statistical analysis was performed by Fisher statistical test with SPSS program.

Results

A total of 366 isolates of *Aeromonas* spp. were analyzed. It was 1.1% of the total of coprocultures analyzed and 15.8% of all enteropathogens identified in the years under study, behind *Campylobacter* spp. (57.8%) and *Salmonella enterica* (25.2%), but at the same time far ahead of other genera such as *Yersinia enterocolitica* (1.3%) or *Shigella* spp. (0.5%). *A. caviae* was the most prevalent species (78.5%), at a large distance from other species such as *A. sobria/veronii* (9.0%) or *A. hydrophila* (2.4%). Without taking into account the enteropathogenic *Escherichia coli* serotypes that are identified at the express request of the clinician.

No significant differences were found in sex distribution (190; 52.0% women) nor in the frequency of infection in adults (>16 years) with respect to pediatric age (185; 50.5%), with a mean age of infection of 34 years (range 0–98 years). Diarrhea was found as the most common referred symptom (162; 44.0%), followed by acute gastroenteritis (78; 21.3%). A total of 58 co-infections were identified (15.93%), being significantly more frequent in pediatric ages (49; 84.5%) ($p=0.05$) and mostly associated with *Campylobacter* spp. (75.9% of the total co-infections and 75.5% in pediatric age).

A. caviae (51, 87.9%) was the most frequently isolated species as a copathogen regardless of age.

Antimicrobial sensitivity to the most commonly used antibiotics showed good activity to cotrimoxazole (92.9%), ciprofloxacin (86.1%) and cefepime (98.9%).

The 52.0% of the studied samples were requested from health centers, followed by emergency service for both pediatric 12.6% and adult age 10.9%. The most frequently observed symptom was diarrhea 44.2%, being bloody in 4.6% of cases, followed by acute gastroenteritis in 21.3%.

In the cases accumulated by months, there is a clear increase in the incidence in summer, especially in the month of August (15.8%), being significant this increase for adults ($p=0.02$) and children ($p=0.04$) (see Fig. 1).

Discussion

Aeromonas spp. ranks third in terms of incidence of enteropathogenic bacteria in our area of influence, as opposed to other series in the 1990s, possibly due to the fact that in those years it was not yet considered a primary pathogen. But in more recent series this enteropathogen does not appear possibly because it is not included in the molecular syndromic panels of recent massive introduction in clinical diagnostic laboratories.⁹ It is true that its finding in association with another enteropathogen,⁷ as well as its known role as part of the usual intestinal microbiota¹⁰ or doubts about its possible pathogenicity mechanism,¹¹ relegate it to the identification of classical enteropathogens. Even more in case of isolation of *A. caviae* in pediatric age where it is not given much attention, although published series show that *A. caviae* is the most frequently isolated species¹² a fact that coincides with our population.

In the present study, *Campylobacter* spp. showed a significant association with *Aeromonas* spp. particularly in the younger age group, as other studies in our country.¹³ Is reported *Aeromonas* spp. as a part of a polymicrobial infection, which raises the question of whether it is behaving as a nonpathogenic “co-traveler”, perhaps acquired from the same source as the true pathogen, or is interacting with other agents to cause disease. The almost exclusive appearance of the species *A. caviae* and its lower virulence can indicate its appearance in feces as a colonizer. Pathogens are commonly reported in literature to coexist with *Aeromonas* are rotavirus, *Shigella*, *Campylobacter*, and *Escherichia coli* O157:H7.^{12,14} This feature in identifying *Aeromonas* can help the microbiologist and pediatrician be alert to a possible primary enteropathogen.

But the pathogenicity is clear both by its association with diarrhea, as in this and other works and by the identification of virulence genes especially in species such as *Aeromonas hydrophila* or *Aeromonas sobria*.¹⁵

We can conclude that *Aeromonas* spp. is an enteropathogen that appears very frequently in the feces of patients with digestive symptoms both in our health area and in other published case series. Circumstance that should be taken into account when deciding to diagnose patients with gastrointestinal pathology with syndromic panels in which *Aeromonas* spp is not included.

The appearance of *Aeromonas* spp. in feces of patients at an early age, in many cases, is associated with the appearance of a classic primary copathogen, in our case *Campylobacter* spp. This finding could alert in the active search by molecular techniques for primary pathogens if they are not isolated by stool culture, especially in those cases that are associated with diarrhea, bloody diarrhea or acute gastroenteritis.

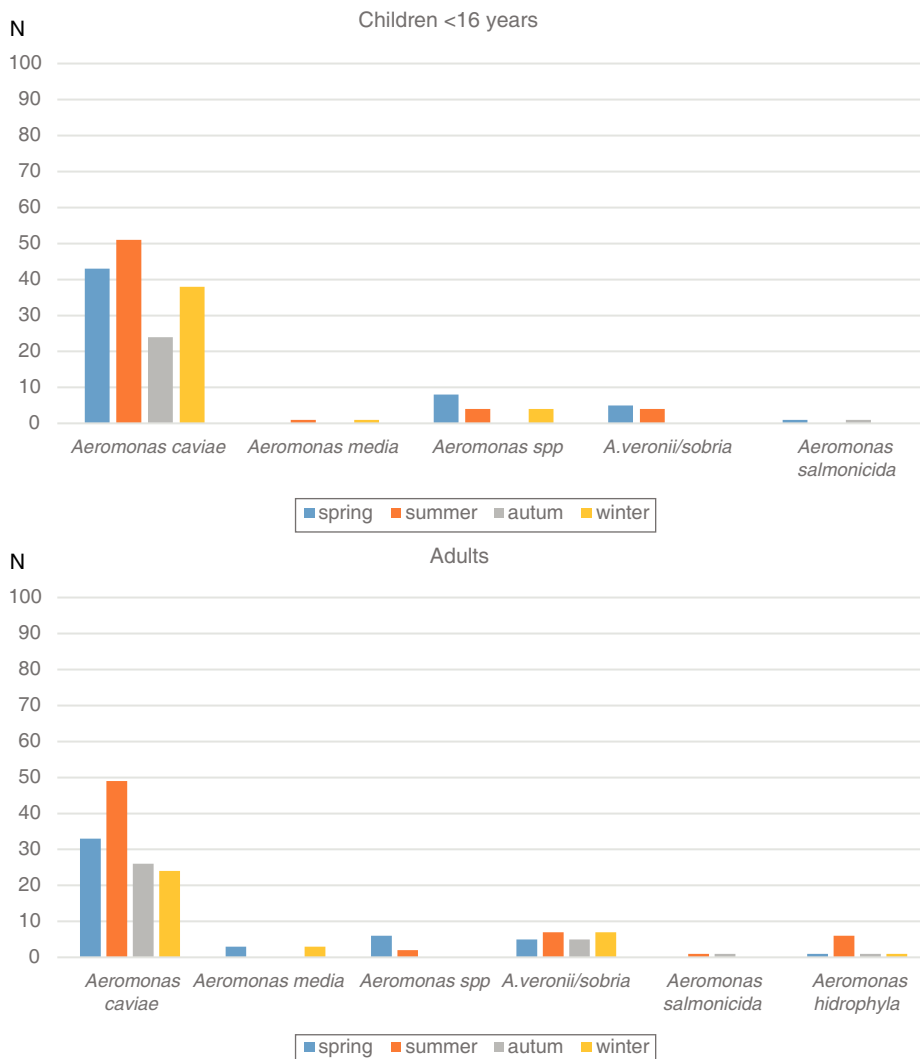


Fig. 1. Seasonal distribution of *Aeromonas* spp. in children and adults.

Transparency declarations

All authors have nothing to declare. This study has not been financially supported by any Diagnostic/Pharmaceutical company.

Ethical approval

Not applicable.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Gonçalves Pessoa RB, de Oliveira WF, Marques DSC, Dos Santos Correia MT, de Carvalho EMMM, Coelho LCBB. The genus *Aeromonas*: a general approach. *Microb Pathog.* 2019;130:81–94, <http://dx.doi.org/10.1016/j.micpath.2019.02.036>.
2. Praveen PK, Debnath C, Pramanik AK, Shekhar S, Dalai N, Rai R. Antibiotic sensitivity and virulence potential study of *Aeromonas* species isolated from retail fish and chicken in and around Kolkata. *J Cell Tissue Res.* 2014;14:4613–6, <http://dx.doi.org/10.14202/vetworld.2016.6-11>.
3. Bhowmick UD, Bhattacharjee S. Bacteriological, clinical and virulence aspects of *Aeromonas*-associated diseases in humans. *Pol J Microbiol.* 2018;67:137–49, <http://dx.doi.org/10.21307/pjm-2018-020>.
4. Igbinosa IH, Igumbor EU, Aghdasi F, Tom M, Okoh AI. Emerging *Aeromonas* species infections and their significance in public health. *Sci World J.* 2012;2012:625023, <http://dx.doi.org/10.1100/2012/625023>.
5. Zhang H, Pan F, Zhao X, Wang G, Tu Y, Fu S, et al. Distribution and antimicrobial resistance of enteric pathogens in Chinese paediatric diarrhoea: a multi-centre retrospective study, 2008–2013. *Epidemiol Infect.* 2015;143:2512–9, <http://dx.doi.org/10.1017/S0950268814003756>.
6. García Vera C, García Ventura M, Del Castillo Aguas G, Domínguez Aurrecoechea B, Esparza Olcina MJ, Martínez Rubio A, et al. Acute bacterial gastroenteritis: 729 cases recruited by a Primary Care national network. *An Pediatr (Barc).* 2017;87:128–34, <http://dx.doi.org/10.1016/j.anpedi.2016.04.020>.
7. Pazzaglia G, Sack RB, Salazar E, Yi A, Chea E, Leon-Barua R, et al. High frequency of coinfecting enteropathogens in *Aeromonas*-associated diarrhoea of hospitalized Peruvian infants. *J Clin Microbiol.* 1991;29:1151–6, <http://dx.doi.org/10.1128/JCM.29.6.1151-1156.1991>.
8. Persson S, Al-Shuweli S, Yapici S, Jensen JN, Olsen KE. Identification of clinical *aeromonas* species by *rpoB* and *gyrB* sequencing and development of a multiplex PCR method for detection of *Aeromonas hydrophyla*,

- A. caviae*, *A. veronii*, and *A. media*. J Clin Microbiol. 2015;53:653–6, <http://dx.doi.org/10.1128/JCM.01963-14>.
9. Valenzuela C, Legarraga P, Peña A, Arenas A, Berkowitz L, Ramírez G, et al. Etiologic and clinical characterization of community acquired gastroenteritis in adult patients in a Chilean emergency room by the FilmArray GI panel. PLOS ONE. 2018;13:e0207850, <http://dx.doi.org/10.1371/journal.pone.0207850>.
 10. Pazzaglia G, Escalante JR, Sack RB, Rocca C, Benavides V. Transient intestinal colonization by multiple phenotypes of *Aeromonas* species during the first week of life. J Clin Microbiol. 1990;28:1842–6, <http://dx.doi.org/10.1128/JCM.28.8.1842-1846.1990>.
 11. Tomás JM. The main *Aeromonas* pathogenic factors. ISRN Microbiol. 2012;2012:256261, <http://dx.doi.org/10.5402/2012/256261>.
 12. Figura N, Marri L, Verdiani S, Ceccherini C, Barberi A. Prevalence, species differentiation, and toxigenicity of *Aeromonas* strains in cases of childhood gastroenteritis and in controls. J Clin Microbiol. 1986;23:595–9, <http://dx.doi.org/10.1128/JCM.23.3.595-599.1986>.
 13. Del Valle de Toro A, Santos-Pérez JL, Navarro-Marí JM, Gutiérrez-Ferández J. Epidemiological data description of pediatric patients with diarrhea by *Aeromonas* spp. and the antibiotic susceptibility of this agent. Rev Argent Microbiol. 2020;52:22–6, <http://dx.doi.org/10.1016/j.ram.2019.03.003>.
 14. Sinha S, Shimada T, Ramamurthy T, Bhattacharya SK, Yamasaki S, Takeda Y, et al. Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata, India. J Med Microbiol. 2004;53:527–34, <http://dx.doi.org/10.1099/jmm.0.05269-0>.
 15. Silva LCAD, Leal-Balbino TC, Melo BST, Mendes-Marques CL, Rezende AM, Almeida AMP, et al. Genetic diversity and virulence potential of clinical and environmental *Aeromonas* spp. isolates from a diarrhea outbreak. BMC Microbiol. 2017;17:179, <http://dx.doi.org/10.1186/s12866-017-1089-0>.