

## Faecal Carriage of Extended-Spectrum $\beta$ -Lactamase (ESBL)- Producing *Aeromonas* species

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### Abstract

The mesophilic aeromonads are emerging as important pathogens in humans, causing a variety of extra intestinal and systemic infections. ESBL-producing organism can cause severe infections, but they are also isolated from the stool of asymptomatic subjects. A total of 10 strains of *Aeromonas* spp., isolated from stools were tested for ESBL production by the double-disk synergy test. The faecal carriage rate of ESBL-producing *Aeromonas* spp., was 30% (3/10). This is the first paper where ESBL-producing *Aeromonas* spp., isolated from an asymptomatic patient is reported and with a high level of faecal carriage. These results suggest that the faecal carriage of ESBL-producing *Aeromonas* spp., occurs at high rates in this geographic area. The finding of ESBL-producers indicates a potential risk of dissemination of resistant bacteria outside hospitals.

**Keywords:** *Aeromonas* species; Extended-Spectrum- $\beta$ -Lactamase; Faecal Carriage

**Abbreviations:**  $\beta$ : beta; ESBL: extended-spectrum beta-lactamase; AMC: amoxicillin-clavulanate; CTX : cefotaxime; FEP: cefepime; ATM: aztreonam.

### Introduction

*Aeromonas* species, aquatic Gram-negative bacilli, distributed globally and ubiquitously in the natural environment, may be implicated in a variety of human diseases in community or hospital settings, such as gastroenteritis, septicemia, abdominal/peritoneal sepsis, hepatobiliary tract infections, and catheter-related infections [1].

An increase in resistance levels of the genus, particularly to  $\beta$ -lactam antimicrobial agents, not only has been observed in clinical isolates, but also in environmental strains [2,3]. The most common mechanism of antibacterial resistance is the production of three chromosomally encoded  $\beta$ -lactamases, which have been described and identified in different *Aeromonas* [4]. Another important class of  $\beta$ -lactamases addressed is a Class A extended-spectrum  $\beta$ -lactamases (ESBLs), which has been increasingly reported in both clinical and environmental aeromonads [1,5,6].

ESBL enzymes are encoded by transferable conjugative plasmids, which often code resistance determinants to other classes of antimicrobial agents and are also responsible for the dissemination of resistance to other Gram-negative bacteria in the community and in the hospital. Infection caused by ESBL-producing bacteria is an emerging problem in the community setting in many parts of the world. Several reports have addressed faecal carriage of these organisms during nosocomial out breaks [7].

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Recent studies have shown a significant increase of faecal carriage of ESBL-producing isolates during no outbreak situation in hospitalized patients and the establishment of these isolates in the community with corresponsiveness to non-antibiotics, including quinolones, represent an opportunity for these isolates to become endemic and, these faecal strains can serve as reservoir to pathogens in the spread of resistance. The risk of infection with multi-resistant bacteria, and thus the need for usage of last resort antibiotics, such as carbapenems and colistin, in the treatment of common infections [8,9,10].

The knowledge about commensal microbiota and resistance is limited, asymptomatic faecal carriage of ESBL-producing bacteria in the community has been reported from several countries and continents with widely differences in carriage rates between geographic areas and study population characteristics [7,9,11,12,13,14].

Because faecal carriage is a key factor in the epidemiology of ESBL-producing bacterial infection [14], we investigated the prevalence of faecal carriage of ESBL-producing *Aeromonas* spp., isolated from asymptomatic patients and from patients with diarrhoea.

### Materials and Methods

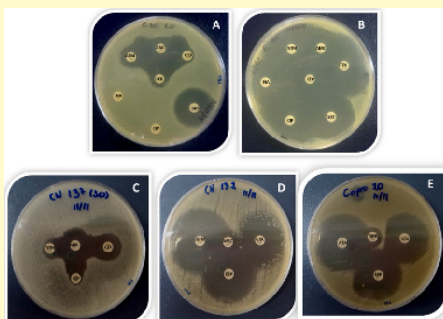
A total of 10 strains of *Aeromonas* spp., pertaining to the collection Laboratory of Gastrointestinal and Urinary Syndromes "Lcda. Luisa Vizcaya", University of Los Andes, Venezuela, was used. The strains were isolated from children under five years old; 6 strains were isolated from children with diarrhoea and identified as *A. hydrophila* (6/10), and 4 from asymptomatic children identified as *A. caviae* 4/10. *A. hydrophila* strains isolated from children with diarrhoea were not enteropathogenic. These were present but were not the cause of infection.

*Aeromonas* isolates were tested for ESBL production by the double-disk synergy test in which an amoxicillin-clavulanate (AMC) (20  $\mu$ g/10  $\mu$ g) disk was placed in the centre with cefotaxime (CTX) (30  $\mu$ g), cefepime (FEP) (30  $\mu$ g), aztreonam (ATM) (30  $\mu$ g) disk at 20 mm distance from AMC: Strains producing ESBL, were defined as those showing synergism between AMC and any one of CTX and FEP [15].

Quality control was carried out by using *Escherichia coli* ATCC 621 (positive control) and *Escherichia coli* ATCC 25922 (negative control).

### Results

The results of ESBL-producing *Aeromonas* species are shown in (Figure 1C, 1D, 1E). In total, only 3 isolates (30%) produced ESBL of which, 2 (66.66%) were strains of *A. hydrophila* non enteropathogenic isolated from patients with diarrhoea and 1 *A. caviae* (33.33%) from the asymptomatic group isolates.



**Figure 1:** Detection of ESBL-production by the double disk synergy test. A *Escherichia coli* ATCC 621 (positive control), B *Escherichia coli* ATCC 25922 (negative control), C and D *Aeromonas hydrophila* isolated from children with diarrhoea. E *A. caviae* isolated from asymptomatic children.

AMC: amoxicillin-clavulanate (20  $\mu$ g/10  $\mu$ g), CTX: cefotaxime (30  $\mu$ g), FEP: cefepime (30  $\mu$ g), ATM: aztreonam (30  $\mu$ g), SXT: trimethoprim-sulfamethoxazol (20  $\mu$ g/10  $\mu$ g), CIP: Ciprofloxacin (5  $\mu$ g), NA: Nalidixic acid (30  $\mu$ g).

### Discussion

The increase in antimicrobial resistance of pathogenic bacteria is a major problem worldwide. The rapidly increasing resistance in Gram-negative bacteria is a particularly serious problem, leading to increased mortality, morbidity and health systems costs [9]. ESBLs are the most important factors contributing to Gram-negative bacilli, resistance to broad-spectrum  $\beta$ -lactam antibiotics [11].

The occurrence of ESBL-producing isolates has increased. Faecal carriage of ESBL-producing isolates has mainly been detected in nosocomial outbreak, but recent studies have shown a significant increase in ESBL producers among community bacterial isolates and, asymptomatic faecal carriage of ESBL-producing bacteria in the community has been reported from several countries [9,11,12,13,14].

This study demonstrates the presence of ESBL in faecal strains of *Aeromonas* spp., from both patients with diarrhoea and asymptomatic patients; although the *Aeromonas* strains were isolated from patients with diarrhoea these were not the producers of the infectious condition. The acquisition of ESBL genes in aeromonads may result from horizontal gene transfer by mobile genetic elements between aeromonads and coexistent bacteria in aquatic microenvironments and ESBL-producing aeromonads have been increasingly reported in recent years [1].

The presence of ESBL-producing *Aeromonas* spp., in the gut, as well as, has been described for ESBL-producing *Enterobacteriaceae*, not only contributes to the difficulty of treating extra intestinal infections, but can also result in the transfer of antibiotic-resistance determinants to other strains of *Aeromonas* and other organisms within the gastrointestinal tract. Their presence increases the risk of transmission to other individuals because of human-to-human transmission or through the environment. The emergence of ESBL-producing organism in the community could also be caused by over used of antibiotics in community patients. Community acquired strains possessing ESBLs might be selected from the existing gastrointestinal microbiota, when they are exposed to broad-spectrum antimicrobial agents [7].

High levels of antibiotics resistance, as found in this study, have a multifactorial explanation. Antibiotic use in humans as well as in animals is probably one of the most important factors, but also anthropogenic activities and lifestyles factors such as hygiene, crowding, and transportation, should be considered [9].

ESBL determinants have been detected not only in clinical isolates but also in commensal bacteria from human and animals and in isolates from products of the food chain and suggesting the presence of environmental reservoir for these resistance determinants. Low-level gut colonization occurs in the community, via the food chain. These determinants of resistance may be located in transferable plasmids. The transferable nature of this resistance is particularly worrisome, and treatment options for infections caused by these organisms are very limited and this may account in part for the association between fluoroquinolones resistance and expanded-spectrum cephalosporins [16].

Normal intestinal microbiota is the major source from which common hospital-and community-acquired infections originate, and these faecal strains can serve as reservoir to pathogens in the spread of resistance [16]. The existence of ESBL-producing organism in the gut of healthy individuals has clinical implications, as intestinal tract colonization is a prerequisite for infection by ESBL-producers [7].

This is the first paper where ESBL-producing *Aeromonas* spp., isolated from asymptomatic patient and a high level of faecal carriage are reported (30%).

The scope of this study was limited to identify ESBL-producers by standard phenotypic methods. Subsequently examined to verify these ESBL-producing isolates and to determine the nature of these ESBL-producing *Aeromonas* species.

## Conclusion

Our results suggest that the faecal carriage of ESBL-producing *Aeromonas* spp., occurs at high rates in this geographic area. These findings are a good reason to conduct larger studies looking into dynamics and implications of faecal carriage.

The increase in occurrence of ESBL-producing *Aeromonas* species emphasizes the importance of constant surveillance of *Aeromonas* isolates to determine the prevalence of antibiotic resistance and continuous monitoring and evaluation of emerging antibiotic resistance in bacteria such as *Aeromonas* species is of great importance.

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## Conflict of interest

The author(s) declare that they have no competing interests.

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