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Original Article

Genetic and phenotypic determinants of resistance to antibiotics in *Aeromonas* spp., strains isolated from pediatric patients

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Abstract

Introduction: Intestinal and extraintestinal infections by *Aeromonas* spp., remain controversial, due to the existence of healthy carriers of *Aeromonas* spp. In children under five years old, the diarrhea of infectious origin constitutes the second cause of mortality and remains a major concern for public health. The aim of this work was to detect the pheno/genotype of β-lactamases and class 1 integrons in *Aeromonas* spp., strains isolated from pediatric patients in a tertiary referral hospital in Mexico.

Methodology: Sixty-six strains of *Aeromonas* spp., were isolated from clinical samples of pediatric origin and were identified by RFLP-PCR 16S rRNA. Resistance phenotype according to CLSI, genetic and phenotypic detection of extended-spectrum β-lactamases (ESBL) and metallo−β-lactamases (MBL) was performed. Finally, characterization of class 1 integrons was performed.

Results: *Aeromonas* spp., strains of diarrheic origin were more predominant. A wide heterogeneity was detected, where *A. caviae* was the predominant specie. Second-generation cephalosporins, fluoroquinolones, and nitrofurans had best antimicrobial activity; moreover, antibiotics of the β-lactamic and lincosamides families showed lower inhibitory activity. Phenotypically, prevalences of 4.55% and 3.03% were detected for MBL (intestinal origin) and ESBL (extraintestinal origin), respectively. $bla_{IMIS\text{-}cphA}$ and $bla_{TEM\text{-}1}$ genes, and nineteen class 1 integrons carrying two variants of cassettes corresponding to adenylyl transferases (*aadA*), and dihydrofolate reductases (*dfrA*). Monogenic array with *aadA1* cassette was predominantly.

Conclusions: ESBL and class 1 integrons, in *Aeromonas* collected from pediatric patients, determines a major detection challenge for the clinical microbiology laboratory and represents a remarkable epidemiological risk of nosocomial spread of multidrug-resistant determinants.

Key words: *Aeromonas* spp.*;* antimicrobial resistance; class 1 integrons; β-Lactamases.

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Introduction

Aeromonas is a bacterial group of cosmopolitan distribution and are mainly considered, inhabitants of the aquatic environment [1]. They may be considered opportunistic pathogens, although some authors have described this bacterial genus as a primary human pathogen [2]. Likewise, they can cause intestinal infections with symptoms ranging from watery diarrhea to dysentery or diarrhea with blood [3], and extraintestinal infection like cellulitis, sepsis and wounds, urinary tract, hepatobiliary, among others [4-6]. The main species that cause infection in humans include the species *Aeromonas caviae, Aeromonas hydrophila ,* and *Aeromonas veronii*, while *A. caviae* is frequently isolated in intestinal infections [7]. Gastrointestinal infection by *Aeromonas* spp., remains controversial [4], due to the existence of healthy carriers of *Aeromonas* spp. However, several studies

have shown how these bacteria are the cause of epidemic outbreaks of diarrhea [8], including the traveler's diarrhea form [9]. In children under 5 years old, the diarrhea of infectious origin constitutes the second cause of mortality [10] and remains a major concern for public health worldwide [11]. Currently, the rate of *Aeromonas* diarrheal infections in pediatric patients is constant [12,13], and they may not be identified as a causative agent in routine studies. Furthermore, the extensive and indiscriminate use of antibiotics has resulted in many resistant varieties of *Aeromonas* spp. Several genes involved with virulence and resistance to antibiotics have been identified, which may be associated with non-mobile and mobile genetic elements, such as plasmids, transposons, and integrons [14], generating a serious public health problem. Genes encoding thermolabile (*alt*) and thermostable cytotoxic enterotoxins (*ast*), and cytotoxic and hemolytic enterotoxins (*hylA* and *aerA*) have been characterized as well as the production of inducible chromosomal β-lactamases [15,16], extended spectrum β-lactamases (ESBL), and metallo-β-lactamases (MBL) [17-20]. Class 1 integrons stand out for their great versatility in the capture and diffusion of genetic resistance cassettes, in addition to the participation in resistance in the hospital environment. This versatility contributes significantly to the adaptation of bacteria to different ecological niches, even when the selection pressure is high [21]. Under this context, the objective of this work was to describe the epidemiological characteristics of pediatric patients with *Aeromonas* spp., isolated from different clinical sources, as well as the identification of antibiotic resistance mechanisms to take preventive measures. Implications and consequences of the identification of multiresistant strains *Aeromonas* spp., in patients belonging to vulnerable groups such as pediatric patients are discussed.

Methodology

Aeromonas spp*., strains identification*

Sixty-six non-duplicated strains belonging to the genus *Aeromonas* were isolated from pediatric patients of the Instituto Nacional de Pediatría of the Ministry of Health of Mexico, between April 2000 to April 2008. The strains were isolated from different clinical sources of pediatric patients (feces, blood, urine, peritoneal fluids, and necrotic tissues). Strains were isolated from specimens obtained for routine testing of nosocomial pathogens at the mentioned hospitals, so neither Institutional Review Board (IRB) approval was required, nor was informed consent required from adult patients, or parents, or legal guardians of children. The presumptive bacterial identification of *Aeromonas* strains was carried out by using the automated system MicroScan Walk Away (Dade Behring INC. West Sacramento, CA, USA). Subsequently, genetic confirmation by the RFLP-PCR *16S rRNA* analysis according to Figueras *et al.,* 2000 [22] was performed.

Antimicrobial susceptibility

Antimicrobial resistance to different antibiotics was performed by using the disk diffusion method on Mueller–Hilton agar plates according to the guidelines set by The Clinical and Laboratory Standards Institute (CLSI 100-S21). Antimicrobial susceptibility was performed for eleven different families of antibiotics (β-lactamics and non-β-lactamics): β-Lactams, inhibitors of β-lactamases, aminoglycosides, ansamycins, quinolones, inhibitors of folates, lincosamides, lipopeptides, macrolides, nitrofurans, phenicoles and tetracyclines. *Escherichia coli* ATCC 25922 and *Aeromonas caviae* ATCC 15468^T strains were used as control. Results were inferred as susceptible, intermediate, or resistant by measuring the diameter of the inhibition zone according to the criteria specified by the CLSI (2019) [23]. The frequency of antibiotic resistance, intermediate, and sensibility was calculated and represented in percentage (%).

Phenotypic detection of MBL

Phenotypic detection of MBL was performed by using the double disk diffusion method using imipenem (30 μg/mL) and different concentrations of EDTA (938, 1.406, and 1.874 μg). The interpretation of the test was performed according to Neuwirth *et al.,* 2007 and Arakawa *et al.,* 2000 [24,25]. The same test was performed by using EDTA plus compounds with sulfhydryl groups (thioglycolic acid and β-mercaptoethanol) according to Kim *et al.,* 2007 [26]. *Pseudomonas aeuruginosa* 462/03 UNAM strain was used as a positive control.

Phenotypic detection of ESBL

ESBL detection was carried out by using the double disk diffusion test according to the specifications of Jarlier *et al.,* 1988 [27]. Additionally, disks with aztreonam, ceftazidime, cefotaxime, and amoxaclline-clavulanic acid were used. *Klebsiella pneumoniae* ATCC 700603 was used as a positive control.

Genomic DNA and plasmid isolation

Genomic DNA was extracted by using the InstaGene Matrix (Bio-Rad Laboratories,Mexico City, Mexico), and plasmid DNA was purified by using the

PureLink QuickPlasmid MiniPrep (Invitrogen, Mexico City, Mexico), both according to the manufacturer's protocol. Integrity of genomic and plasmid DNA was visualized on horizontal 0.8 % agarose gels, and were used as templates in PCR assays as follows.

Molecular detection of MBL and ESBL

All the amplification reactions were performed in a Touch-gene Gradient thermal cycler Gene Amp® PCR System 9700 (Applied Biosystems, Mexico City, Mexico). PCR reaction mixtures (50 µL) contained sterile molecular–grade water, 1X Mastermix (Roche, Mexico City, Mexico), 50 pmol of each primer and a total of 100 ng DNA were added to the reaction mixture. All isolates were screened for MBL (bla_{imp} and $bla_{IMIS\text{-}cphA}$) and ESBL (bla_{TEM} and bla_{SHV}) according to previous works (Migliavacca *et al.*, 2002; Henriques *et al.*, 2006, Alinga, 2001; Nüesch-Inderbinen *et al.,* 1996) [28-31] (Table 1). PCR products were visualized and purified by using the PureLink Quick Gel Extraction Kit (Invitrogen,Mexico City, Mexico). *Pseudomonas aeruginosa* 462/03 UNAM, *Aeromonas hydrophila* ATCC 7966^{T}, *Haemophilus influenzae* ATCC 33930, and *Klebsiella pneumoniae* ATCC 700603 were used as positive controls for $bla_{IMP,}$ $bla_{IMIS\text{-}cphA,}$ $bla_{TEM,}$ and bla_{SHV} genes, respectively. DNA Sequencing was performed in the "Instituto de Biología, Laboratorio de Secuenciación Genómica de la Biodiversidad y de la Salud UNAM", by using an ABI PRISM 3100 automated equipment® (PE Applied Biosystems, Foster City, CA). Nucleic acid sequences were compared with the protein sequences on line database (GenBank) by using the BlastX algorithm (http://blast.ncbi.nlm.nih.gov).

Detection of class 1 integrons and their gene cassettes

For screening of class 1 integron elements, all strains were screened as follows: [integrase 5′(*IntI1*)–variable region–(*qacEΔ1–sulI*)3′] by using the primers described in Table 1, and PCR conditions were performed according to Blancarte-Lagunas *et al*., 2020 [32]. The identity of resistance cassettes was also determined by sequencing the PCR amplicons. *Escherichia coli* carrying pAr–32 plasmid ((IncU, class 1 integron (*intI1–aadA2–qacEΔ1/sul1*) In6 (*catA2*)), and *Aeromonas salmonicida* subsp. *salmonicida* 718 carrying pRAS1 plasmid (IncU, class 1 integron (*intI1–dfrA16–qacEΔ1/sul1*), Tn1721 (*TetA*)) were used as positive controls, and *Escherichiae coli* J53–1 (F− met− pro−) as negative control. *A. salmonicida* 718 was kindly provided by Dr. Glenn Rhodes, Centre for Ecology and Hydrology, Lancaster, UK. *E. coli* W3102, was kindly provided by Dr. Henning Sørum, Norwegian School of Veterinary Science, Oslo, Norway.

Results

Description of pediatric population

A population consisting of 66 pediatric patients (internal and external) was included in the study during the period between April 2000 to April 2008. Feacal and extraintestinal samples were obtained from these. The age of pediatric patients was from newborns to 16 years of age. Strains were isolated from specimens obtained for routine testing of nosocomial pathogens at the mentioned hospitals, so neither Institutional Review Board (IRB) approval was required, nor was informed consent required from parents, or legal guardians of children. Diarrheal symptoms (acute watery diarrhea to

Table 1. Primers used in this study.

Primer	Molecular target	Sequence (5′ → 3′)	Size (bp)
IntI1-F	5′-Conserved Segment "Integrase intI1"	GTTCGGTCAAGGTTCTG	923
IntI1-R		GCCAACTTTCAGCACATG	
in-F	Variable region	GGCATCCAAGCAGCAAGC	From 150 to variable
in-B		AAGCAGACTTGACCTGAT	
qacEΔ1-F	3′-Conserved Segment "qacΔE1/sul11"	ATCGCAATAGTTGGCGAAGT	800
sul1-B		GCAAGGCGGAAACCCGCGCC	
16SF	16S rRNA	AGAGTTTGATCATGGCTCAG	1502
16SR		GGTTACCTTGTTACGACTT	
IMP_F	blaIMP	GGAATAGAGTGGCTTAATTCTC	361
IMP_R		GTGATGCGTCYCCAAYTTCACT	
AER_F	blaIMIS-cphA.	GCCTTGATCAGCGCTTCGTAGTG	670
AER_F		GCGGGGATGTCGCTGACGCAG	
TEM_3	blaTEM	AGTGTCGACTTACCAATGCTTAATCAGT	938
TEM_4		AAAGAATTCTAAATACATTCAAATATG	
SHV_A	blaSHV	CGCCGGGTTATTCTTATTTGTCGC	361
SHV_B		TCTTTCCGATGCCGCCGCCAGTCA	

dysenteric illness, abdominal cramps, nausea, vomiting, and fever) were predominant in the population. A description of diagnosis, clinical sources of *Aeromonas* spp., and other characteristics are shown in Table 2. An analysis by diagnosis and age of the patients was performed. The results showed that the youngest groups of patients (in the middle ages of 2.7 and 2.1 years) were those with diarrheal symptoms and urinary tract infections caused by bacteria of the genus *Aeromonas*. A detailed description of the diagnosis and age of the patients is shown in Figure 1.

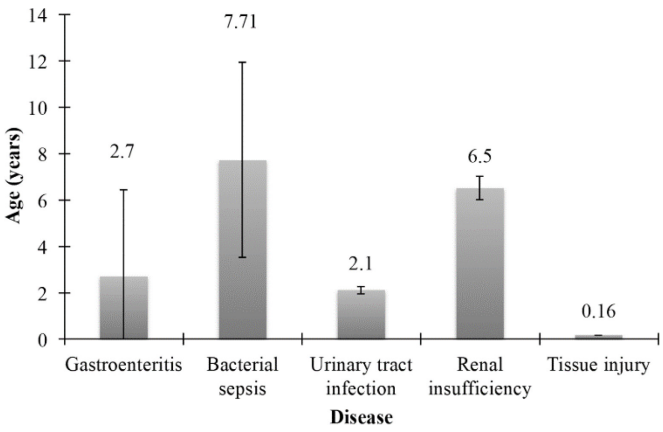
Genetic bacterial identification and origin source

Due to phenotypic methods, they are not able to identify all species of *Aeromonas* genus, and commonly are grouped as *A. hydrophila*, it is necessary to perform standard molecular tests for genetic identification, such as the RFLP-PCR *16S rRNA* analysis. Distribution of *Aeromonas* species and origin source is shown in Table 2. Feces were the most frequent source of isolation, where *A. caviae* was the predominant species, followed by *A. salmonicida, A. hydrophila, A. bestiarum,* and *A. encheleia.* Other clinical sources such as blood, peritoneal fluid, urine, and necrotic tissue were the less frequent sources of the *Aeromonas* species mentioned above.

Antimicrobial resistance

Twenty-four different antimicrobials belonging to eleven families of antibiotics were tested in the 66 *Aeromonas* spp., strains. The resistance percentage analysis against β-lactam antibiotics revealed that 100% of the strains showed resistance to penicillin, ampicillin, and oxacillin. Frequencies limited to β-lactamase inhibitors, 1st generation cephalosporins (amoxicillin-clavulanate and cephalothin) and carbapenemes (imipenem) were observed. The second and third generation cephalosporins and monobactames (cefuroxime, cefotaxime, ceftazidime, and aztreonam) were antibiotics with high antimicrobial activity. Non β-lactam antibiotics that showed lower activity were rifampicin and lincosamines, followed by macrolides and lipopeptides (erythromycin and polymyxin).

Figure 1. Analysis of age versus diagnosis of Mexican pediatric patients who had infection associated with *Aeromonas* spp.



Aminoglycosides showed varied resistance from 9 to 60%, the same was observed with quinolones with frequencies of 3 to 24%. The antibiotics with the highest antimicrobial activity were: cefuroxime, ciprofloxacin, nitrofurantoin, chloramphenicol, and aztreonam. Resistance and sensitive patterns for all strains are shown in Figure 2.

Phenotypic identification of MBL and ESBL

Metallo β-lactamases were detected in 4.55% (3/28) of the imipenem resistant strains. The synergy of the MLB phenotype was observed at the highest concentration of EDTA. The MLB phenotype was detected in *A. caviae* NP-44169, and in *A. hydrophila* INP-F-0050 and INP-408118. Two strains (*A. hydrophila* INP-415677 and *A. caviae* INP-41139) potentially producing ESBL were identified, corresponding to a frequency of 3.03%, these strains presented resistance to cefotaxime, aztreonam, and ceftazidime.

Molecular detection of MBL and ESBL

Chromosomal carbapenemases $bla_{IMIS\text{-}cphA}$ were identified with a frequency of 6.06% (4/66) in *A. salmonicida* INP-11634, *A. hydrophila* INP-408118, *A. hydrophila* INP- 412573, and *A. caviae* INP-402562 with a high homology (100% identity). bla_{TEM}

Table 2. Distribution of Aeromonas spp., strains isolated of Mexican pediatric patients by clinical source and diagnosis.

Diagnosis of pediatric patient	Isolation source	Specie identified by RFLP 16S rRNA gene analysis					TOT
		A. caviae	*A. salmonicida*	*A. hydrophila*	*A. bestiarum*	*A. encheleia*	
Gastroenteritis	Feces	34	10	8	1	1	54
Bacterial sepsis	Blood	2	2	3	0	0	7
Renal insufficiency	Peritoneal fluid	1	1	0	0	0	2
Urinary tract infection	Urine	2	0	0	0	0	2
Tissue injury	Necrotic tissue	0	0	1	0	0	1
	Total	**39**	**13**	**12**	**1**	**1**	**66**

carbapenemase was identified in *A. hydrophila* INP-415677, BLEE producing strain with a homology of 98% identity.

Detection of class 1 integrons and their gene cassettes

Class 1 integrons detection in *Aeromonas* spp., strains was performed under amplification strategy of conserved genes as follows: a first PCR reaction was performed to amplify the integrase ''*intI1*'' (5'CS). Once a positive amplification to first molecular target (923 bp), a second reaction to amplify *''qacEΔ1–Sul1''* (3'CS) was performed (800 bp). Finally, a third reaction was performed to amplify the variable region (flanked by conserved regions (*attI1* and *qacEΔ1*). Nine complete integrons (*intI1*/variable region/*qacEΔ1–sul1)* were identified (6.0%). The variable region amplicons ranged from 791 to 447 bp, indicating different genetic arrangements into detected integrons. The criteria for defining the identity of the arrangements obtained were percentage match (> 75 %), length of match (> 100 bp), and probability of similarity. The sequences showed inserted cassettes corresponding to genes coding for adenylyl transferases (*aadA1, aadA2*) and dihydrofolate reductases (*dfrA17*). Three different single arrays were identified in the ten strains of *Aeromonas* spp. Empty integrons with no integrated cassettes arrangements between ends were found in ten strains. The first genetic array (*intI1-aadA1-qacEΔ1–Sul1)* was predominantly prevalent over the other genetic arrays identified (5/10). This array was found in several strains including (*A. hydrophila, A. caviae,* and *A. salmonicida)*. The second array was *intI1-aadA2-qacEΔ1–Sul1* (3/10) and was found in *A. caviae* and *A. salmonicida.* Finally, two strains of *A. caviae* showed an array with cassette *dfrA17* (Table 3). Integrons were not detected in plasmid preparations.

Discussion

Antimicrobial multiresistance represents an important public health problem worldwide, due to the emergence and dissemination of multiresistant strains in different environments, including the hospital. With the acquisition of infections associated with multidrug-

Figure 2. Antimicrobial resistance profile of Aeromonas spp., strains isolated from Mexican pediatric patients. β–lactam antibiotics tested: P, penicillin (10 U); AM, ampicillin (10 µg); OX, oxacillin (1 µg); CB, carbencillin (100 µg); AMC, amoxicillin-clavulanate (20-10 µg); CF, cephalothin (30 µg); CXM, cefuroxime (30 µg); CTX, cefotaxime (30 µg); CAZ, ceftazidime (30 µg); ATM, aztreonam (30 µg) and IMP, imipenem (10 µg). non β–lactam antibiotics: AN, amikacin (30 µg); GM, gentamicin (100 µg); K, Kanamycin (30 µg); S, streptomycin (10 µg); RA, rifampicin (5 µg); NA, nalidixic acid (30 µg); CIP, ciprofloxacin (5 µg); STX, trimethoprim-sulfamethoxazole (23.75 / 1.25 µg); CC, clindamycin (2 µg); PB, polymyxin B (300 U); E, erythromycin (15 µg); F / M, nitrofurantoin (300 µg); C, chloramphenicol (30 µg) and TE, tetracycline (30 µg).

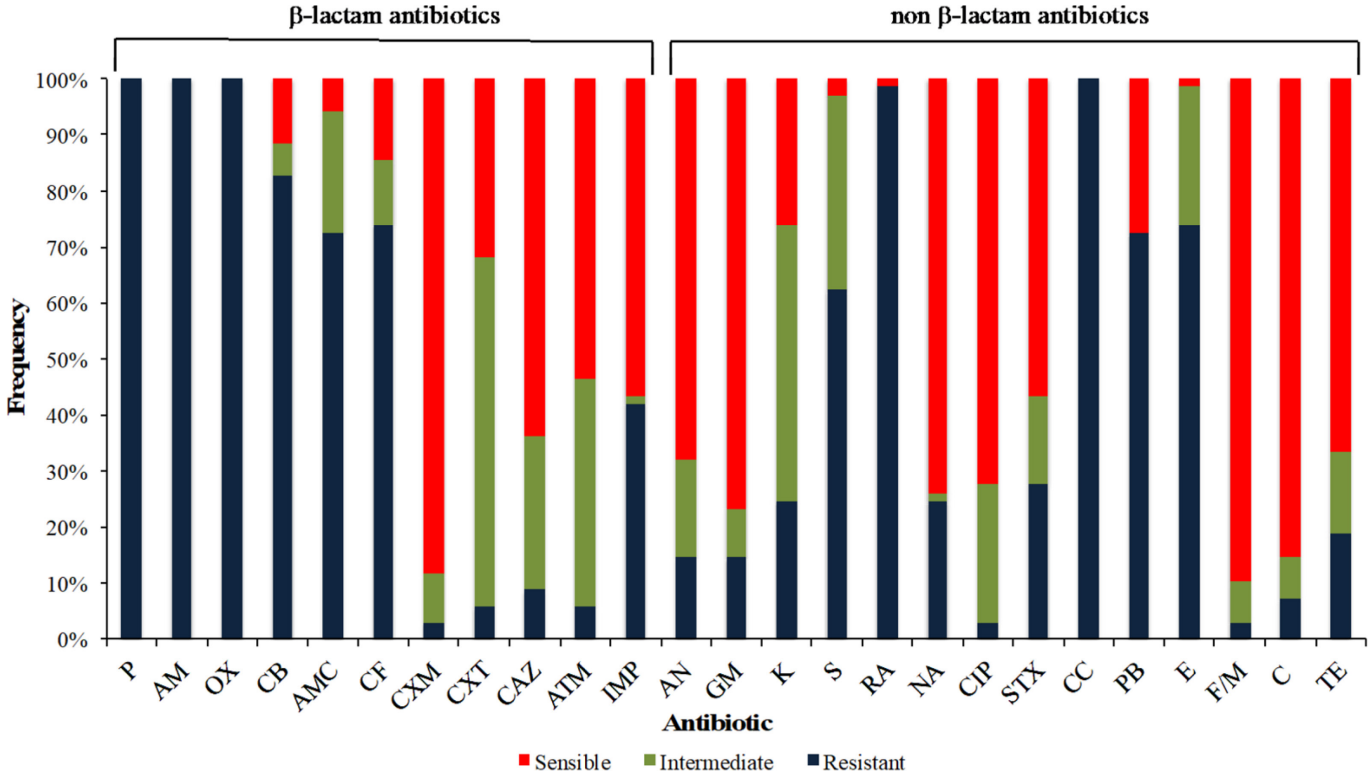


Table 3. Class 1 integrons identified in *Aeromonas* spp., strains isolated from Mexican pediatric patients.

Strain code	Specie	*Genetic array* 5´→3´	Variable region (pb)	Identity (%)	ID sequence GeneBank**	
402541	*A. caviae*	*intI1-aadA2-qacEΔ1-Sul1*	791	96	*S. maltophilia*	GQ896541.1
412573	*A. hydrophila*	*intI1-aadA1-qacEΔ1-Sul1*	779	98	*P. aeruginosa*	GQ853420.1
402562	*A. caviae*	*intI1-aadA1-qacEΔ1-Sul1*	779	98	*A. veronii*	FJ460183.1
3984441	*A. salmonicida*	*intI1-aadA1-qacEΔ1-Sul1*	779	95	*E. coli*	EU089666.1
422122	*A. salmonicida*	*intI1-aadA1-qacEΔ1-Sul1*	779	92	*A. veronii*	FJ460176.1
422122	*A. salmonicida*	*intI1-aadA2-qacEΔ1-Sul1*	791	96	*S.* Typhimurium	AJ879462.1
F-1724	*A. caviae*	*intI1-aadA1-qacEΔ1-Sul1*	779	92	*A. media*	FJ460183.1
448318	*A. caviae*	*intI1-dfrA17-qacEΔ1-Sul1*	447	93	*E. coli*	GQ896501.1
447261	*A. caviae*	*intI1-dfrA17-qacEΔ1-Sul1*	447	92	*E. coli*	GQ896501.1
449944	*A. caviae*	*intI1-aadA2-qacEΔ1-Sul1*	791	97	*E. aerogenes*	FJ004895.1

resistant pathogens, the spectrum of antibiotics of choice for treatment is increasingly reduced [33]. Therefore, it is important to know the evolution of the pathogen resistance profiles to offer a predictive value of the resistance phenotype and, consequently, to provide effective treatments [34]. Under this context, bacteria of the *Aeromonas* genus are pathogens that since the 80's have been considered causal agents of intestinal and extraintensinal infections in pediatric patients, where *A. hydrophila* and *A. caviae* are the species mostly identified as causative agents [35]. In this work, 66 strains of *Aeromonas* spp., in pediatric patients, being *A. caviae* the main causative agent of diarrheal infections. *Aeromonas caviae* cytotoxic has been previously recognized in outbreaks of gastroenteritis [36] and extraintestinal infections [37]. Furthermore, the identified species (*A. hydrophila, A. bestiarum,* and *A. encheleia*) of intestinal origin have already been previously isolated from children with gastroenteritis in smaller proportions (compared to *A. caviae)* [38]. To our knowledge, this is the first report that identifies *A. salmonicida* as a causative agent of gastroenteritis and present in peritoneal fluid. Few are the cases of the isolation of *A. salmonicida* from samples of clinical origin. *Aeromonas salmonicida* has been previously isolated from blood samples from patients with typical symptoms of bacterial sepsis [39] and postoperative endophthalmitis [40]. Even though the strains were mainly of intestinal origin, they were also isolated from patients with other diagnoses (other than gastroenteritis), such as septic conditions, renal failure, urinary tract infection, and tissue lesions. It is known that immunosuppression, due to chronic degenerative diseases, is an important factor for the acquisition of unusual pathogen infections. We speculate that some cases (patients with bacterial sepsis or renal failure) acquired the infection, due to the immunosuppression associated with their condition. Regarding the patterns of antimicrobial resistance, it has been reported that some of the species of the genus *Aeromonas* show a specific behavior of resistance to certain antibiotics [41], however, the resistance patterns identified in this study were heterogeneous. It has been shown that bacteria of the genus *Aeromonas*, as well as other phylogenetically distant bacteria, have several β-lactamases (chromosomal and plasmidic) that confer resistance to a large number of β-lactam antibiotics [42]. Carbapenems are antibiotics of the β-lactam family that have a broad spectrum of antibacterial activity and are resistant to hydrolysis by most β-lactamases, including ESBL and β-lactamases AmpC type [43]. These antibiotics are frequently used as a last resort in the treatment of infections caused by multiresistant Gram-negative bacilli. However, the increase in reports of strains resistant to carbapenems is increasing [44]. In this work a high percentage (42.42%) of imipenem resistant strains was identified, this data contrasts radically with the frequencies reported by Castanheira *et al.* (2009) [45], where only a frequency of 4.10% is reported in *Aeromonas* strains of extraintestinal origin. Future work is aimed at the identification of the *mcr* gene and its variants, or other mechanisms associated with resistance to this antibiotic, such as the production of *MexAB-OprM* efflux pumps or decreased expression of the OprD porin [46,47]. The frequency of isolation of new generation β-lactam antibiotic resistant strains in *Aeromonas* spp., is lower than that of enterobacteria or non-fermenting bacilli [48]. However, the increasingly frequent detection of strains resistant to antibiotics of extended spectrum that cause infections, shows the need to evaluate antibiotic resistance in *Aeromonas* strains of clinical origin. In this work, we identified *A. hydrophila* $bla_{\text{TEM-1}}$ gene, however, this molecular marker resistance having global dispersion, has been found in the *Enterobacteriaceae* family*, P. aeruginosa, H. influenzae,* and *Neisseria gonorrhoeae* [33,49]. Literature analysis showed that the $bla_{\text{TEM-24}}$ gene has

only been found in *Aeromonas* spp., strains [50]. Moreover, the MBL *bla-cphA* has been previously identified (at low frequencies) in strains of *A. hydrophila* isolated from estuarine water [29]. Although it was detected with very low frequencies in this work (6.06%), its detection becomes relevant due to its nature of inactivating a wide range of β-lactam antibiotics. Regarding the profiles of resistance to non β–lactam antibiotics, the high frequency of resistance to aminoglycosides contrasts with what was reported by [51], where they did not report resistance to gentamicin as reported by Pérez-Valdespino *et al.,* 2009 [52], where they reported a 9.75% resistance to streptomycin. Resistance to amikacin and kanamycin contrasts with the findings reported previously [9,53], where rates of 100% sensitivity to these two antibiotics were demonstrated, respectively.

In the literature there are reports of the presence of class 1 integrons in *Aeromonas* spp., strains of environmental origin and in food [29,54,55]. However, there are few reports on isolates of clinical origin. Strains carrier class 1 integrons were identified, with an incidence double to that reported by Lee *et al.,* 2008 [56], and lower reported by Pérez-Valdespino *et al.,* 2009 [52] in *Aeromonas* spp., strains. The genetic arrangements of cassettes identified were entirely monogenic. Variants of the *aadA* cassette have been reported in strains of *Pseudomonas*, *Enterobacterias*, *Acinetobacter* spp., and *Aeromonas* spp. [57-59]. The *drfA17* cassette (monogenic) has been described in *Aeromonas, E. coli,* and *S. enterica* [58,60]. Even when genetic arrangements were identified as monogenic nature, it draws attention to the high incidence of empty integrons. The detection of empty integrons or without cassette arrangements confirms the theory that indicates that the selection pressure plays a decisive role in the capture and maintenance of cassettes, and in the absence of this, there is a loss of cassettes. This dynamic release and capture cassettes, has been demonstrated in class 1 integrons identified in *Aeromonas* spp., strains of diarrheic origin [61]. The predominance of aminoglycoside cassettes and folate metabolism inhibitors in this and other works, could indicate that some cassettes (such as those aforementioned) are more stable with and without selection pressure. This study demonstrates the presence of *Aeromonas* spp., of clinical origin carrying resistance determinants for MBL, ESBL, and class 1 integrons, an important reservoir of resistance cassettes. The evidences shown in this work mark the importance of the inclusion of diagnostic protocols aimed at the search of *Aeromonas* of different clinical origins. This is because *Aeromonas,* due to its heterogeneity in its virulence and resistance to multiple drugs, have been identified as the causative agent of intestinal and extraintestinal infections, mainly in a vulnerable group, such as pediatric patients.

Conclusion

β-lactamases genes bla_{TEM}, $bla_{ImiS\text{-}cphA}$, and genetic cassette *drfA17* in class 1 integron were observed in *Aeromonas* strains isolated from diarrheal and extra-intestinal samples collected from pediatric patients. The production of ESBL and class 1 integrons, in *Aeromonas* collected from pediatric patients, determines a major detection challenge for the clinical microbiology laboratory and represents a remarkable epidemiological risk of nosocomial spread of multidrug-resistant determinants.

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