Virulence and antimicrobial susceptibility of clinical and environmental strains of *Aeromonas* spp. from Northeastern Brazil

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Abstract

This study aimed at isolating and identifying clinical and environmental strains of *Aeromonas* spp., through biochemical tests and the automated method VITEK 2, and by investigating the presence of the virulence genes cytotoxic enterotoxin (*act*), haemolysin (*asaI*) and type III secretion system (*ascV*) and the *in vitro* antimicrobial susceptibility. From the clinical isolates, 19 *A. hydrophila*, 3 *A. veronii* bv. *sobria* and 1 *A. caviae* were identified, while from the environmental strains, 11 *A. hydrophila*, 22 *A. veronii* bv. *sobria*, 1 *A. veronii* bv. *veronii* and 1 *A. caviae* were recovered. The gene *act* was detected in 69.5%, while *asaI* was detected in 8.6% and *ascV* in 34.7% of the clinical isolates. As for the environmental strains, the detection rates were 51.4%, 45.7% and 54.2% for the genes *act*, *asaI* and *ascV*, respectively. Resistance to amoxicillin-clavulanate and piperacillin-tazobactam was observed in 15 and 3 clinical strains, respectively, and resistance to ceftazidime, meropenem, imipenem, ciprofloxacin and trimethoprim-sulfamethoxazole was observed in one strain for each drug. Resistance to amoxicillin-clavulanate and piperacillin-tazobactam was detected in 17 and 1 environmental strain, respectively. Higher resistance percentages were observed in clinical strains, but environmental strains also showed this phenomenon and presented a higher detection rate of virulence genes. Thus, it is important to monitor the antimicrobial susceptibility and pathogenic potential of the environmental isolates.

Keywords: *Aeromonas* spp.; human infections; environments; virulence genes; antimicrobial resistance.

44 The species of the genus *Aeromonas* are facultative anaerobic, oxidase-positive,
45 glucose fermenters Gram-negative bacilli (Winn et al. 2006). They are more frequently found
46 in aquatic environments, such as public water supplies, rivers, lakes and estuaries. The
47 isolation of the microorganism from human food such as fish and shellfish has also been
48 described (Janda and Abbott 2010).

49 The pathogenic potential of *Aeromonas* spp. has been related to several virulence
50 factors that allow colonization, immune evasion and proliferation in the host. Among these
51 factors, researchers have mentioned the presence of cytotoxic enterotoxin, cytotoxic
52 enterotoxin, proteases, hemolysins and products of the type III secretion system (Beaz-
53 Hidalgo et al. 2013; Igbinosa et al. 2012). *Aeromonas* spp. infections usually occur after the
54 consumption of contaminated food and water or after traumatic lesions (Janda and Abbott
55 2010).

56 In general, most strains present *in vitro* susceptibility to aminoglycosides, third and
57 fourth generation cephalosporins, carbapenems, quinolones and sulfamethoxazole-
58 trimethoprim, but cases of resistance have been described (Chao et al. 2013, Chuang et al.
59 2011). In addition, *Aeromonas* spp. are intrinsically resistant to penicillins and present high
60 resistance levels to first and second generation cephalosporins (Janda and Abbott 2010;
61 Senderovich et al. 2012).

62 The wide number of sources from which *Aeromonas* can be recovered contribute to
63 the constant exposure of human beings to these bacteria, reinforcing the importance of
64 performing environmental monitoring. Thus, the present study aimed at isolating clinical and
65 environmental strains of *Aeromonas* spp. and investigating the presence of virulence genes
66 and the antimicrobial susceptibility of the tested strains.

67 The clinical strains used in this study were obtained from the collection of the clinical
68 microbiology laboratory of DASA, in Fortaleza, Ceará Brazil. The strains of *Aeromonas* spp

69 were recovered during 2012 and 2013, from clinical specimens obtained from patients with
70 different types of infections, such as sepsis, hepatobiliary diseases, skin and soft tissue
71 infection. These strains were kindly provided for our study by the trustee of DASA. This
72 period called our attention because of the higher prevalence of infections with this bacterial
73 genus, when compared to previous years. The number of cases diagnosed during the surveyed
74 years (2012-2013) was twice higher than the number of cases diagnosed during the years of
75 2010-2011.

76 Environmental strains were recovered from water samples collected from Sapiranga
77 Lake (3°48'46" S and 38°27'30.83"W), city of Fortaleza, Ceará, and from the digestive tracts
78 of prawns and fish collected in this lake. In addition, water samples from prawn
79 (*Macrobrachium amazonicum*) farming were also used for the recovery of *Aeromonas* sp.
80 These samples were collected weekly from tanks, during two consecutive hatchery cycles at
81 the Laboratory of Crustacean Culture of the State University of Ceará.

82 Environmental water samples (5mL) were collected from shallow areas of Sapiranga
83 Lake, while those from prawn farming tanks were collected from three areas: surface,
84 substrate and water filter. Two samples of lake water and eighteen samples of hatchery water
85 were processed in duplicate, in two tubes containing 2500 µL. The tubes were centrifuged at
86 3000 rpm, for 20 minutes. Then, 2000 µL of the supernatant were discarded and the remaining
87 volume of the tubes was homogenized, followed by the addition of sterile saline to reach a
88 final volume of 2000 µL. The solution was homogenized by vortexing, and allowed to settle
89 at 25 °C, for 30 minutes (Medeiros et al. 2008). Thereafter, 10-µL-aliquots of the supernatant
90 from each sample were seeded on the culture media.

91 The prawns and fish were collected in fish nets. To collect the digestive tracts, 10
92 prawns were pooled as a single sample, with a total of two samples. Three fish were collected
93 and each fish was considered as one single sample. The digestive tracts were removed by a

94 transversal cut, and the content was processed as described by (Brilhante et al. 2011).

95 The study was previously approved by the Chico Mendes Institute for Conservation of
96 Biodiversity/Biodiversity Authorization and Information System –SISBIO, under the number
97 28175-1.

98 For the primary isolation, the clinical samples were inoculated on blood agar and
99 Chromogenic chromID CPS medium (bioMérieux, França), which are commonly used in the
100 clinical microbiology laboratory. Environmental samples were plated on MacConkey agar
101 (Difco, USA). The plates were incubated at 35°C, for up to 48 hours. Subsequently, the
102 phenotypical characteristics of the colonies were observed and lactose negative colonies were
103 selected.

104 Gram-negative isolates that tested positive for cytochrome oxidase, glucose
105 fermentation, urease and indole production were classified as *Aeromonas* sp. (Winn et al.
106 2006). *Aeromonas* species were identified through the automated method Vitek 2 Compact®,
107 according to the manufacturer's protocol (bioMérieuxInc, Durham, NC, USA), and only
108 results with probability level above 93% were considered reliable (Tang et al. 2014). After
109 automated identification, the Voges-Proskauer test was included to differentiate between *A.*
110 *hydrophila* and *A. caviae*. For the quality control of the conventional biochemical tests,
111 *Escherichia coli* ATCC® 25922 and *Pseudomonas aeruginosa* ATCC® 27853 were included
112 in the assays.

113 DNA extraction was performed using the Wizard® Genomic DNA purification kit
114 (Promega USA). The concentration and purity of DNA were measured with a Qubit®
115 spectrophotometer (Invitrogen, Carlsbad, USA). Primers for the amplification of the assessed
116 virulence genes were designed based on previous studies. In order to detect the coding genes
117 for cytotoxic enterotoxin (*act*), hemolysin (*asa-1*) and type III secretion system (*ascV*), the
118 following primers were used: (5'–3') *act*_F- AGAAGGTGACCACCAAGAACA and *act*_R–

AACTGACATCGGCCTTGAAGCTC (Kingombe et al. 1999); *asa-1_F*-
TAAAGGGAAATAATGACGGCG and *asa-1_R*- GGCTGTAGGTATCGGTTTTTCG (Wang
et al. 2003); and *ascV_F*- CTCGAAGTGAAGAGCAGAATG and *ascV_R*-
GAACATCTGGCTCTCCTTCTCGATG (Martino et al. 2011). The reaction was conducted in
a final volume of 25µL, under the PCR conditions described by Martino et al. (2011).

The products for *act* (232bp; Kingombe et al. 1999), *asa-1* (249bp; Wang et al. 2003)
and *ascV* (577bp; Martino et al. 2011) were analyzed through electrophoresis in 1.8% agarose
gel, using an intercalating fluorescent loading dye, and visualized on an UV transilluminator
(Hu et al. 2012; Martino et al. 2011; Ottaviani et al. 2011). A previously identified strain of *A.*
hydrophila, which was positive for the presence of the three tested virulence genes, was used
as positive control in each of the performed PCR assays, during this research. Additionally the
mix without DNA was used as negative control of reaction.

The tested antimicrobial drugs and their respective concentration ranges were selected
based on the document M45-A2 from Clinical and Laboratory Standards Institute (CLSI,
2010) and the following drugs were included: amoxicillin-clavulanate (AMC), piperacillin-
tazobactam (PTZ), ceftazidime (CAZ), cefepime (FEP), meropenem (MEM), imipenem
(IPM), gentamicin (GN), ciprofloxacin (CIP) and trimethoprim-sulfamethoxazole (SXT)
(Sigma-Aldrich Corporation, USA). The tests were carried out by the broth microdilution
method as standardized by CLSI, document M07-A09 (CLSI 2012), in Mueller-Hinton broth
(Difco, USA) and final bacterial inocula of 5×10^5 UFC/mL were used (CLSI 2010).

The plates were read after 18-20h of incubation at 35°C. The minimum inhibitory
concentration (MIC) was defined as the lowest concentration able to inhibit 100% of growth,
except for trimethoprim-sulfamethoxazole, for which the MIC was defined as the lowest
concentration capable of inhibiting 80% of bacterial growth (CLSI 2012), when compared to
the growth of the control. The results were interpreted according to the document M45-A2

(CLSI 2010) and the interpretive criteria were: $S \leq 8/4$, $I = 16/8$ and $R \geq 32/16$ $\mu\text{g/mL}$ for AMC; $S \leq 16/4$, $I = 32/4$ – $64/4$, $R \geq 128/4$ $\mu\text{g/mL}$ for PTZ; $S \leq 4$, $I = 8$, $R \geq 16$ $\mu\text{g/mL}$ for CAZ; $S \leq 8$, $I = 16$, $R \geq 32$ $\mu\text{g/mL}$ for FEP; $S \leq 4$, $I = 8$, $R \geq 16$ $\mu\text{g/mL}$ for MEM; $S \leq 4$, $I = 8$, $R \geq 16$ $\mu\text{g/mL}$ for IPM; $S \leq 4$, $I = 8$, $R \geq 16$ $\mu\text{g/mL}$ for GN; $S \leq 1$, $I = 2$, $R \geq 4$ $\mu\text{g/mL}$ for CIP and $S \leq 2/38$ and $R \geq 4/76$ $\mu\text{g/mL}$ for SXT. *Escherichia coli* ATCC®-35218 and *E. coli* ATCC®-25922 were included as control strains (M100-S22, CLSI, 2012).

In order to analyze the MIC differences between species, origin (clinical or environmental strains), MIC values were submitted to Log2-transformation to analyze the number of dilutions, through a linear model. In addition, Pearson's Chi square test and Fisher's exact test were used in order to compare categorical data between species and origin and species and the presence of virulence genes. P-values lower than 0.05 indicated significant results.

Overall, 23 clinical isolates were obtained and identified as *A. hydrophila* (n=19), *A. veronii* bv. *sobria* (n=3) and *A. caviae* (n=1). The greatest number of isolates were obtained from blood samples (12/23), followed by liquid from the abdominal cavity (5/23), wounds (4/23), abdominal tissue (1/23) and urine (1/23). From the environmental samples, 35 isolates were obtained and identified as *A. hydrophila* (n=11), *A. veronii* bv. *sobria* (n=22), *A. veronii* bv. *veronii* (n=1) and *A. caviae* (n=1). These strains were recovered from the digestive tract of fish (3/35) and prawns (4/35), and from pond (7/35) and larviculture water (21/35; Table 1). *A. hydrophila* was the most prevalent species among clinical isolates, while *A. veronii* was more commonly observed among environmental sites ($P = 0.0004$).

The detection of a single virulence gene was found in 65.2% (15/23) of the clinical isolates, while more than one virulence gene was observed in 21.7% (5/23). The *act* gene was the most common in these isolates (11/15; 9 *A. hydrophila*, 1 *A. veronii* bv. *sobria* and 1 *A. caviae*) and the *ascV* gene alone was detected in four strains of *A. hydrophila*. The *act*

($P=0.0173$) and *ascV* ($P=0.001$) genes were more present in clinical strains of *A. hydrophila*, when compared to environmental isolates, and the combination of these genes was the most commonly observed combination among clinical strains (3/5 strains: 2 *A. hydrophila*, 1 *A. veronii* bv. *veronii*). Only one strain of *A. veronii* bv. *sobria* presented all the studied virulence genes (Table 1).

As for the environmental strains, the presence of only one virulence gene was observed in 25.7% (9/35) of the strains, whereas 51.4% (18/35) had more than one gene. The *act* gene alone was detected in three strains (2 *A. veronii* bv. *sobria*; 1 *A. hydrophila*), while the *ascV* gene was detected in six strains of *A. hydrophila*. In environmental strains, combinations were observed between *act* and *asa-1* (5/18), *asa-1* and *ascV* (3/18), *act* and *ascV* (2/18), only in *A. veronii* bv. *veronii*. In addition, eight strains showed all the virulence genes (6 *A. veronii* bv. *sobria*; 1 *A. caviae*; 1 *A. hydrophila*; Table 1).

Concerning the antimicrobial susceptibility, resistance to amoxicillin-clavulanate was observed in 35 isolates: 15/23 clinical strains (MIC=32/16 $\mu\text{g/mL}$) and 17/35 environmental strains (MIC $\geq$ 32/16 $\mu\text{g/mL}$). Piperacillin-tazobactam resistance was observed in three clinical strains (MIC $\geq$ 128/4 $\mu\text{g/mL}$) and one environmental strain (MIC=128/4 $\mu\text{g/mL}$). Resistance to ceftazidime (MIC=64 $\mu\text{g/mL}$), meropenem (MIC=16 $\mu\text{g/mL}$), imipenem (MIC=32 $\mu\text{g/mL}$), ciprofloxacin (MIC=64 $\mu\text{g/mL}$) and trimethoprim-sulfamethoxazole (MIC=16/304 $\mu\text{g/mL}$) was observed in one different clinical strain for each antimicrobial drug. Imipenem ($P=0.0001$), meropenem ($P=0.0381$) and ciprofloxacin ($P=0.0169$) had higher MICs against *A. hydrophila*, when compared to *A. veronii*. Furthermore, intermediate susceptibility to amoxicillin-clavulanate, piperacillin-tazobactam, imipenem and gentamicin was also observed among clinical and environmental strains (Table 2).

Finally, the MIC values of amoxicillin-clavulanate ($P=0.00149$), piperacillin-tazobactam ($P=0.0399$), imipenem ($P=0.00415$) and meropenem ($P=0.0399$) increased in the

194 presence of the genes *asa-1*, *act*, *ascV* and *ascV*, respectively.

195 *Aeromonas* spp. have been considered emerging infectious agents, particularly in
196 immunocompromised individuals (Parker and Shaw 2011). The prevalence of infections with
197 these microorganisms is most likely underestimated, as it is not a mandatory reportable
198 condition in most countries, and it varies greatly according to the geographic region and the
199 type of infection (Janda and Abbott, 2010). Bacteremia, for example, is a common type of
200 infection caused by these microorganisms, representing 30 to 55% of the cases (Chuang et al.
201 2011; Tang et al. 2014). Similar results were obtained in the present study, since 52% of the
202 clinical isolates were recovered from blood samples.

203 There are several pathways through which humans become colonized/infected with
204 *Aeromonas* species. *Aeromonas* spp. can be isolated from several sources, including aquatic
205 habitats, invertebrates (ticks and insects), fish, birds, and soils. The ingestion of contaminated
206 water and food is the main pathway of infection, but other routes include recreational
207 activities, such as diving, especially, if there is loss of skin integrity (Janda and Abbott 2010).
208 The risk of infection in people should also be considered when handling animals, mainly
209 prawns and fish (Kao and Kao 2012).

210 These data reinforce the importance of environmental monitoring to better
211 understand the pathogenic potential and antimicrobial susceptibility of *Aeromonas* strains.
212 The most frequent species were *A. veronii* bv. *sobria* (62.8%), followed by *A. hydrophila*
213 (31.4%). In a previous study, *A. hydrophila* and *A. veronii* bv *sobria* were among the most
214 frequently isolated species from water samples (Ottaviani et al. 2011), which have also been
215 described as important human pathogens (Chao et al. 2013; Tang et al. 2014).

216 All *Aeromonas* isolates recovered in the present study were identified through
217 manual phenotypical tests, for the initial screening, followed by automated analyses, through
218 the Vitek 2 system, which led to the identification of the species with a high probability

(above 93%). An additional test was performed (Voges Proskauer), in order to differentiate *A. hydrophila* from *A. caviae*. It is important to emphasize that commercial systems are capable of correctly identifying *A. hydrophila*, *A. veronii* and *A. caviae* (Janda and Abbott, 2010).

We also investigated the presence of virulence genes in clinical and environmental strains of *Aeromonas* spp. Combination of all genes was less frequently found in clinical strains (1/23), when compared to environmental ones (8/35), corroborating the findings of Ottaviani et al. (2011), who observed that environmental strains have a higher number of virulence genes than clinical strains. These data show that the distribution of virulence genes among *Aeromonas* is not uniform, demonstrating that virulence may vary among species of this genus and among strains of the same species (Aravena-Róman et al. 2014; Yano et al. 2015).

Antimicrobial resistance was more commonly observed among clinical isolates, when compared to environmental ones, as previously described (Aravena-Román et al. 2012). The clinical strains were resistant to seven of the nine tested drugs, while in the environmental strains resistance to two antimicrobials was observed. In addition, results of intermediate susceptibility observed in clinical and environmental strains are an alert for the potential emergence of antimicrobial resistant strains in *Aeromonas* spp. (Souza et al. 2010).

Amoxicillin-clavulanate and piperacillin-tazobactam presented a higher rate of resistance and were the only drugs to which both clinical and environmental strains were resistant. The production of β -lactamases has been described as the main cause of resistance among *Aeromonas* and some of these bacterial enzymes are not inhibited by β -lactamase inhibitors, such as clavulanic acid, tazobactam, and sulbactam (Chen et al. 2012; Senderovich et al. 2012).

Despite having observed a higher percentage of resistance in clinical strains, environmental strains also showed this phenomenon. In addition, virulence genes were

detected in strains of both origins. It is known that exposure to environmental sources (aquatic habitats, soils, animals), as well as the consumption of contaminated food and water, may represent a risk to human health. Hence, considering the ubiquity of *Aeromonas* spp., contributing to their constant interaction with humans, and the detection of decreased antimicrobial susceptibility and virulence genes among environmental strains, it is important to monitor the antimicrobial susceptibility and pathogenic potential of the environmental isolates.

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Table 1. *Aeromonas* spp. isolated from clinical and environmental samples and the frequency of virulence genes detection

ORIGIN	SITE	SPECIES	GENE							
			<i>act</i>	<i>asa-1</i>	<i>ascV</i>	<i>act+asa-1</i>	<i>act+ascV</i>	<i>asa-1+ascV</i>	<i>act+asa-1+ascV</i>	None
Clinical	Blood	<i>A. hydrophila</i> (n=10)	3	-	4	-	2	-	-	1
		<i>A. veronii</i> bv. <i>sobria</i> (n=1)	-	-	-	-	-	-	1	-
		<i>A. caviae</i> (n=1)	1	-	-	-	-	-	-	-
Clinical	Wound	<i>A. hydrophila</i> (n=2)	1	-	-	1	-	-	-	-
		<i>A. veronii</i> bv. <i>sobria</i> (n=2)	1	-	-	-	1	-	-	-
Clinical	Intra-abdominal liquid	<i>A. hydrophila</i> (n=5)	4	-	-	-	-	-	-	1
Clinical	Abdominal tissue	<i>A. hydrophila</i> (n=1)	1	-	-	-	-	-	-	-
Clinical	Urine	<i>A. hydrophila</i> (n=1)	-	-	-	-	-	-	-	1
Subtotal		n =23	11/23	0/23	4/23	1/23	3/23	0/23	1/23	3/23
Environmental	Larviculture water	<i>A. hydrophila</i> (n=10)	1	-	6	-	-	-	-	3
		<i>A. veronii</i> bv. <i>sobria</i> (n=9)	1	-	-	3	2	2	1	-
		<i>A. veronii</i> bv. <i>veronii</i> (n=1)	-	-	-	-	-	-	-	1
Environmental	Pondwater	<i>A. caviae</i> (n=1)	-	-	-	-	-	-	1	-
		<i>A. hydrophila</i> (n=1)	-	-	-	-	-	-	1	-
Environmental	Shrimpdigestivetract	<i>A. veronii</i> bv. <i>sobria</i> (n=6)	-	-	-	2	-	1	2	1
		<i>A. veronii</i> bv. <i>sobria</i> (n=4)	1	-	-	-	-	-	-	3
Environmental	Fish digestive tract	<i>A. veronii</i> bv. <i>sobria</i> (n=3)	-	-	-	-	-	-	3	-
Subtotal		n =35	3/35	0/35	6/35	5/35	2/35	3/35	8/35	8/35

(-) No gene amplification / *act*: cytotoxic enterotoxin / *asa-1*: hemolysin / *ascV*: type III secretion system

Table 2. Antimicrobial susceptibility of *Aeromonas* spp.

Drugs (µg mL ⁻¹)	Profile	Clinical strains			Environmental strains				MIC50	MIC90
		<i>A. hydrophila</i> (n=19)	<i>A. veronii</i> bv. <i>sobria</i> (n=3)	<i>A. caviae</i> (n=1)	<i>A. hydrophila</i> (n=11)	<i>A. veronii</i> bv. <i>sobria</i> (n=22)	<i>A. veronii</i> bv. <i>veronii</i> (n=1)	<i>A. caviae</i> (n=1)	<i>Aeromonas</i> spp. (n=58)	
AMC	S	-	-	-	4/2 - 8/4 (2)	0.5/0.25 - 8/4 (7)	4/2 (1)	-	32/16	32/16
	I	16/8 (5)	16/8 (2)	16/8 (1)	16/8 (1)	16/8 (7)	-	-		
	R	32/16 (14)	32/16 (1)	-	32/16 - 64/32 (8)	32/16 - 64/32 (8)	-	32/16 (1)		
PTZ	S	1/4 - 16/4 (17)	2/4 - 8/4 (2)	-	1/4 - 16/4 (11)	0.5/4 -16/4 (20)	0.5/4	16/4 (1)	4/4	64/4
	I	-	-	64/4 (1)	-	64/4 (1)	-	-		
	R	256/4 - 512/4 (2)	128/4 (1)	-	-	128/4 (1)	-	-		
CAZ	S	0.25 - 4 (18)	0.25 - 0.5 (3)	0.5 (1)	0.25 - 4 (11)	0.125 - 4 (22)	0.125 (1)	0.5 (1)	0.75	2.2
	I	-	-	-	-	-	-	-		
	R	64 (1)	-	-	-	-	-	-		
FEP	S	0.06 - 0.5 (19)	0.03 - 0.125 (3)	0.25 (1)	0.03 - 0.125 (11)	0.06 - 0.125 (22)	0.06 (1)	0.06 (1)	0.0925	0.1375
	I	-	-	-	-	-	-	-		
	R	-	-	-	-	-	-	-		
MEM	S	0.06 - 2 (18)	0.25 - 1 (3)	0.015 (1)	0.015 - 2 (11)	0.015 - 1 (22)	0.25(1)	1 (1)	0.25	1
	I	-	-	-	-	-	-	-		
	R	16 (1)	-	-	-	-	-	-		
IPM	S	2 - 4 (16)	1 - 2 (3)	0.25 (1)	0.125 - 2 (10)	0.06 - 2 (22)	2 (1)	1 (1)	2	4
	I	8 (2)	-	-	8 (1)	-	-	-		
	R	32 (1)	-	-	-	-	-	-		
GN	S	1 - 4 (18)	2 - 4 (3)	1 (1)	1 - 4 (10)	1 - 4 (18)	-	4 (1)	2	8
	I	8 (1)	-	-	8 (1)	8 (4)	8 (1)	-		
	R	-	-	-	-	-	-	-		
CIP	S	0.002 - 0.25 (18)	0.01 - 0.08 (3)	0.008 (1)	0.0005 - 0.008 (11)	0.00025 - 0.06 (22)	0.0005 (1)	0.001 (1)	0.004	0.015
	I	-	-	-	-	-	-	-		
	R	8 (1)	-	-	-	-	-	-		
SXT	S	0.06/1.18 - 0.25/4.75 (18)	0.125/2.3 - 0.25/4.75 (3)	0.25/4.75 (1)	0.03/0.59 - 0.125/2.3 (11)	0.06/1.18 - 0.25/4.75 (22)	0.06/1.18 (1)	0.06/1.18 (1)	0.125/2.375	0.25/4.75
	I	-	-	-	-	-	-	-		
	R	16/304 (1)	-	-	-	-	-	-		

(-) No strains / AMC: amoxicillin-clavulanate / PTZ: piperacillin-tazobactam / CAZ: ceftazidime / FEP: cefepime / MEM: meropenem / IPM: imipenem / CIP: ciprofloxacin / SXT: trimethoprim-sulfamethoxazole / MIC (n)