

Supplementary Materials: Multidrug-Resistant Bacteria Isolated from Surface Water in Bassaseachic Falls National Park, Mexico

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Sample:	Sample size:	Inoculated and incubated overnight at:	To recovered:	Re-Inoculated and incubated overnight at:	Analysis of macroscopic and microscopic morphology:	To obtained purified colonies previous medium were re-inoculated in:
Raw water	10 mL of 1 L raw water	Selenite broth (37°C)	<i>Salmonella</i> & <i>Shigella</i> strains	SS Agar (37°C)		MacConkey Agar (37°C)
		Tetrathionate broth (37°C)	<i>Salmonella</i> & <i>Shigella</i> strains	SS Agar (37°C)	• Macroscopic: Shape, size, type of edge, transmitted or reflected light	MacConkey Agar (37°C)
		2% sodium chloride broth (37°C)	<i>Vibrio</i> sp.	TCBS Agar (37°C)		TCBS Agar (37°C)
		BBL buffered peptone water (37°C)	<i>Vibrio</i> sp.	TCBS Agar (37°C)	• Microscopic: Gram Stain	TCBS Agar (37°C)
Nutrient broth (42°C)	Pathogen enterobacteria (<i>Escherichia coli</i> sp.)	MacConkey Agar (37°C)	MacConkey Agar (37°C)			

Figure S1. Diagram of the microbiology analysis flux. Only samples that exceeded Mexican Standard (1000 MPN/100 mL) were processed as indicate. Isolates were preserve in trypticase soy broth with glycerol 30% vol:vol at -20 °C storage.

Table S1. Interpretative breakpoints.

Antimicrobial Agents	Bacteria	Susceptible ($\mu\text{g/mL}$)	Intermediate ($\mu\text{g/mL}$)	Resistance ($\mu\text{g/mL}$)
Amikacin	Enterobacteriaceae & <i>Vibrio cholerae</i>	≤ 16	32	≥ 64
Ampicillin		≤ 8	16	≥ 32
Ampicillin-sulbactam	Enterobacteriaceae & <i>Acinetobacter</i> spp.	$\leq 8/4$	16/8	$\geq 32/16$
Aztreonam	Enterobacteriaceae	≤ 8	16	≥ 32
Cefazolin		≤ 8	16	≥ 32
Cefepime		≤ 8	16	≥ 32
Cefotaxime		≤ 8	16–32	≥ 64
Cefotetan		≤ 16	32	≥ 64
Ceftazidime		≤ 8	16	≥ 32
Ceftriaxone		≤ 8	16–32	≥ 64
Cefuroxime		≤ 4	8–16	≥ 32
Ciprofloxacin		≤ 1	2	≥ 4
Gentamicin		≤ 4	8	≥ 16
Levofloxacin		≤ 2	4	≥ 8
Imipenem		≤ 4	8	≥ 16
Meropenem		≤ 4	8	≥ 16
Moxifloxacin		≤ 2	4	≥ 8
Piperacillin-tazobactam		Enterobacteriaceae <i>Pseudomonas aeruginosa</i> & Gram negative bacteria	$\leq 64/4$ $\leq 16/4$	32/4 64/4
Ticarcillin-clavulanic acid	<i>Pseudomonas aeruginosa</i> & Gram negative bacteria	$\leq 64/2$	32/2	$\geq 128/2$
Tobramycin		$\leq 16/2$	64/2	$\geq 128/2$
Trimetoprim- sulphamethoxazole		≤ 4	8	≥ 16

Table S2. Native and enterobacterial species/biotypes isolated from freshwater samples collected from the Bassaseachic National Park, México

Antimicrobial Category	Antibiotics Tested	Sample Number																								
		1	3	4	5	8	9	10	11	12	16 *	17 *	18	19	20	21 *	22 *	23	24	26	28	29	30	31	33 *	
Aminoglycosides	Tobramycin												I													
	Amikacin											R														
	Gentamicin																									
Carbapenems	Imipenem					I						I														
	Meropenem												R	R												
Non-extended spectrum Cephalosporin: 1st and 2nd generation	Cefazolin		R	R	R		R	R	R	R	I	R				R	R	I					R		R	
	Cefuroxime		R		I								R				I	R						I		R
Extended-spectrum Cephalosporin: 3rd and 4th generation	Cefotaxime											R			R	R			R							I
	Ceftriaxone		I							I																
	Ceftazidime											R														R
	Cefepime						R					R					I	R								R
Cephamycins	Cefotetan																									R
Fluoroquinolones	Ciprofloxacin												I	R	R											
	Moxifloxacin												I													
	Levofloxacin												I	R	R											
Sulfonamides	Trimetroprim-sulphamethoxazole	R													R	R	R									
Monobactams	Aztreonam										R	R														R
Penicillin	Ampicillin					R				I	R	R	R			R	R	R	R	R	R	R	R	R	R	R
Penicillin combinations	Ampicillin-sulbactam	R				R						R	R													R
	Piperacillin-tazobactam																									
	Ticarcillin-clavulanic acid																									R

Antimicrobial categories: R = resistant; I = Intermediate resistance.

	Biotype	Genera	Species		Biotype	Genera	Species		Biotype	Genera	Species		Biotype	Genera	Species
1	60010150	<i>Aeromonas</i>	<i>hydrophila</i>	10	77520002	<i>Citrobacter</i>	<i>freundii</i>	19	77115012	<i>Escherichia</i>	<i>coli</i>	28	40010010	<i>Shigella</i>	sp.
2	20424	<i>Acinetobacter</i>	<i>ivoffii</i>	11	76103172	<i>Enterobacter</i>	<i>cloacae</i>	20	77704370	<i>Klebsiella</i>	<i>pneumoniae</i>	29	60010101	<i>Vibrio</i>	<i>cholerae</i>
3	43005103	<i>Hafnia</i>	<i>alvei</i>	12	77103173	<i>Enterobacter</i>	<i>cloacae</i>	21 *	77714370	<i>Klebsiella</i>	<i>oxytoca</i>	30	41005007	<i>Vibrio</i>	<i>parahaemolyticus</i>
4	43005103	<i>Halnia</i>	<i>alvei</i>	13	77113010	<i>Escherichia</i>	<i>coli</i>	22 *	77714376	<i>Klebsiella</i>	<i>oxytoca</i>	31	40005007	<i>Vibrio</i>	<i>choleare</i>
5	43005103	<i>Hafnia</i>	<i>alvei</i>	14	53111010	<i>Escherichia</i>	<i>coli</i>	23	77714372	<i>Klebsiella</i>	<i>oxytoca</i>	32	30000002	<i>Yersinia</i>	<i>enterocolitica</i>
6	60010001	<i>Pasteurella</i>	<i>multocida</i>	15	53317010	<i>Escherichia</i>	<i>coli</i>	24	77714370	<i>Klebsiella</i>	<i>oxytoca</i>	33 *	76103176	<i>Enterobacter</i>	<i>cloacae</i>
7	60010001	<i>Pasteurella</i>	<i>multocida</i>	16 *	77113010	<i>Escherichia</i>	<i>coli</i>	25	53101002	<i>Salmonella</i>	<i>paratyphi A</i>				
8	60000110	<i>Tatumella</i>	sp.	17 *	77115010	<i>Escherichia</i>	<i>coli</i>	26	73101006	<i>Salmonella</i>	<i>paratyphi A</i>				
9	77100002	<i>Citrobacter</i>	<i>freundii</i>	18	77115010	<i>Escherichia</i>	<i>coli</i>	27	53505041	<i>Salmonella</i>	sp.				

* = Multidrug-resistance bacteria.