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ORIGINAL ARTICLE

Molecular identification of bacteria isolated from Tilapia (*Oreochromis* spp.) from the State of Hidalgo, Mexico. A potential source of foodborne diseases

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KEYWORDS
Identification of bacteria;
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Abstract Tilapia production is considered an alternative to increasing the income of the rural population, and in recent years has surpassed traditional agricultural and livestock activities, playing a fundamental role in food production at the national and global level. The State of Hidalgo has significant aquaculture production; however, there are no previous records related to the microorganisms present. Therefore, this study aimed to identify bacteria that cause foodborne diseases or are associated with human diseases, present in Tilapia in the State of Hidalgo. Sixty-nine isolates were obtained from a collection recovered from a sampling previously conducted in different municipalities of Hidalgo and from different Tilapia organs. Once the isolates were activated, DNA extraction was obtained and molecular identification was performed using the *rpoD* or *16S* rRNA genes, and later sequenced by the Sanger method. Twelve genera and 19 species were identified: *Aeromonas* (40.6%), *Shewanella* (14.5%), *Acinetobacter* (8.7%), *Citrobacter*, *Comamonas*, *Plesiomonas*, *Pseudomonas*, and *Vibrio* (5.8%), *Kosakonia* (2.9%) and *Exiguobacterium*, *Glutamicibacter* and *Pantoea* (1.4%). In this study, various isolates of health importance were identified, because 66.7% of the bacteria found have been associated with foodborne diseases, which can mainly affect immunosuppressed or immunocompetent individuals. This study reveals the presence of pathogens in a highly consumed product; therefore, it is necessary to implement strategies to reduce the presence of these pathogens of public health importance.

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PALABRAS CLAVE
Identificación de bacterias;
Enfermedades transmitidas por alimentos;
Tilapia

Identificación molecular de bacterias aisladas de Tilapia (*Oreochromis* spp.) del estado de Hidalgo. Una fuente potencial de enfermedades transmitidas por alimentos

Resumen La producción de tilapia ha permitido incrementar los ingresos de la población rural en ciertas áreas de México y, en los últimos años, ha superado a las actividades agrícolas y pecuarias tradicionales, jugando un papel fundamental en la producción de alimentos a nivel nacional y mundial. En el estado de Hidalgo (México) existe una importante producción acuícola; sin embargo, no hay registros relacionados con la presencia de microorganismos en este producto. Por lo tanto, este estudio tuvo como objetivo identificar bacterias causantes de enfermedades transmitidas por alimentos, o asociadas a enfermedades en humanos, en ejemplares de tilapia en el estado de Hidalgo. Se obtuvieron 69 aislamientos de una colección recuperada de un muestreo realizado con anterioridad en diferentes municipios de dicho estado y a partir de diferentes órganos de tilapia. Una vez activados los aislamientos, se extrajo el ADN y se realizó la identificación molecular utilizando los genes *rpoD* o *16s* ARNr; posteriormente se secuenciaron por el método de Sanger. Se identificaron 19 especies distribuidas en los siguientes 12 géneros: *Aeromonas* (40,6%), *Shewanella* (14,5%), *Acinetobacter* (8,7%); *Citrobacter*, *Comamonas*, *Plesiomonas*, *Pseudomonas* y *Vibrio* (5,8%); *Kosakonia* (2,9%) y *Exiguobacterium*, *Glutamicibacter* y *Pantoea* (1,4%). El 66,7% de las bacterias encontradas se han asociado a enfermedades de transmisión alimentaria, que pueden afectar principalmente a personas inmunodeprimidas, aunque también a inmunocompetentes. Este estudio revela la presencia de patógenos en un producto muy consumido, por lo que es necesario implementar estrategias para reducir la carga de patógenos de importancia sanitaria en este producto.

Introduction

Aquaculture is one of the economic activities with the most significant development and growth in recent decades. Reports from the FAO indicate that there has been an increase in the consumption of foods of aquaculture origin, estimated at 20.7 kg per capita, pointing to an increase in apparent consumption of 3% between 1961 and 2021[47]. Tilapia (*Oreochromis* spp.) enjoys great popularity due to its rapid growth and adaptability to different environments and food sources. However, high concentrations of animals per pond undermine the health status of Tilapia, causing stress, reducing their growth, risking their immune system, and making them susceptible to pathogens present in the environment[19]. The State of Hidalgo has an active population of 3357 fishermen and about 432 aquaculture production units, producing Tilapia, channel catfish (*Ictalurus punctatus*), rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*); among these, Tilapia rank first, accounting for 60.22% of the total production, resulting in 274 tons in live weight for the year 2022[10]. This is marketed through the production units and directly, in local markets, by intermediaries in regional markets and nearby states that demand weight and size, but are less demanding regarding the sanitary quality of the product[46]. Foodborne diseases (FBDs) are caused by food contamination at any stage of the production, supply, and consumption chain. Their origin is attributed to environmental contamination, storage, and transformation of food, involving any process, from breeding to processing and consumption[9], and have been noted as a priority in worldwide public health[11]. The bacteria that cause diseases are diverse and the genera associated with infections in aquaculture and their derivatives are *Aeromonas* spp., *Streptococcus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Plesiomonas* spp. and *Vibrio* spp.[37]. Furthermore, the genera *Pseudomonas* spp., *Aeromonas* spp., and *Shewanella* spp., have been isolated from fillets[3,15], and have been identified as causing FBDs. The conditions caused by these genera that have been observed in humans are diverse, ranging from weakness, fever, headache, and mild diarrhea to more serious conditions in immunocompromised individuals or in people suffering from chronic diseases, such as gastroenteritis, septicemia, necrotizing fasciitis and meningoencephalitis[18,32]. For these reasons and due to the possible risks that they represent for public health, this study aimed to identify pathogenic bacteria with the potential to cause FBDs, isolated from Tilapia raised in municipalities of aquaculture importance in the State of Hidalgo using molecular techniques.

Materials and methods

Origin of the bacterial isolates

Tilapias were captured by the producer in aquaculture production units located in the municipalities of Tezontepec de Aldama (2004 m.a.s.l.), Ixmiquilpan (1680 m.a.s.l.), Progreso de Obregón (1985 m.a.s.l.), Chilcuautla (1864 m.a.s.l.) and Huasca de Ocampo (2089 m.a.s.l.) (Fig. 1). The producer provided 30 specimens, each was assigned a number in progressive sequence and samples were taken from the dorsal, pectoral, ventral, anal and caudal fins,

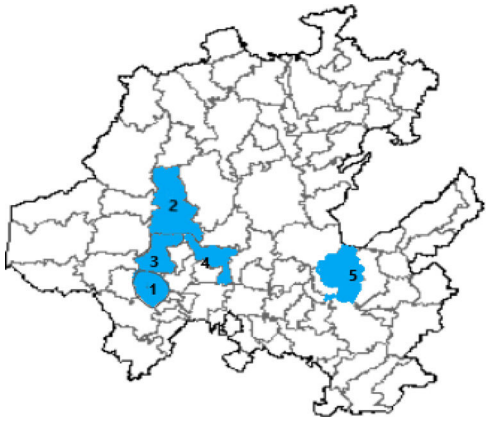

Figure 1 Municipalities where the fish sampled came from. In numerical order: 1. Tezontepec de Aldama, 2. Ixmiquilpan, 3. Chilcuautla, 4. Progreso de Obregón, 5. Huasca de Ocampo.

as well as from the intestine, liver, spleen, and kidneys (Tables 1 and 2). For bacteriological isolation, each sample was plated on Trypto-Casein Soy Agar (TSA) (MCD LAB, Tultitlán de Mariano Escobedo, Mexico) and ampicillin-dextrin agar media (HiMedia, India) incubated at 30 °C for 24 h. Circular, convex, yellow colonies, with 1–3 mm in diameter were selected as presumptive *Aeromonas* spp.[11,41]. The bacteria used were preserved at −20 °C in cryovials containing trypticase soy broth (Difco, BD), with 10% glycerol (PROMEGA), and form part of the strain collection isolated from Tilapia in 2021.

Extraction and quantification of DNA

The strains were subjected to thermal shock following the methodology of Reyes-Rodríguez[41]. A bacterial colony was suspended in 1 ml of sterile distilled water and washed by centrifugation. Subsequently, the bacterial pellet was suspended in 200 μl of sterile distilled water, incubated at 100 °C for 5 min and frozen in a block of ice for 5 min, repeating the cycle three times. Finally, they were centrifuged for 10 min at 10 000 rpm. The supernatant was quantified using a spectrophotometer (Nanodrop ND-100, Thermo Fisher Scientific Inc., US) and stored at −20 °C until use.

Enterobacterial repetitive intergenic consensus-PCR

For the ERIC-PCR test, the primers used were ERIC-1R (5′-ATGTAAGCTCCTGGGGATT CAC-3′) and ERIC-2 (5′-AAGTAAGTGACTGGG GTGAGCG-3′)[48]. The technique was performed using a mix adjusted for a final volume of 50 μl. Master Mix 2X (Promega, Wisconsin, USA) (1×) Taq (0.625 U), dNTPs (200 μM) and $MgCl_2$ (1.5 mM), primer forward and reverse (10 μM) and DNA (15 ng) were used, it was optimized with $MgCl_2$ (1 mM) and Taq Polymerase (GoTaq, Gentaq) (0.375 U). The amplification cycles used were: an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation (1 min at 94 °C), hybridization (1 min at 52 °C), extension (6 min at 74 °C), and final extension at 74 °C for 6 min. Ten microliters of 5X Green Go Taq (Promega, USA) were added to the amplified products and observed on a 1.5% agarose gel (Invitrogen) previously prepared with 4 μl of ethidium bromide. Fourteen wells were prepared in each gel. The 100 bp DNA ladder marker occupied two wells, and a positive control was included. Samples were loaded accordingly, and electrophoresis was performed in a THERMO CE chamber (Massachusetts, USA) for 120 min at 80 V and 400 mA. Gel images were captured for subsequent analysis using GelJ software. The isolates were clustered using the unweighted pair group method with arithmetic mean (UPGMA) analysis and the dice similarity coefficient.

Sequencing identification

To identify the isolates, sequences of the *rpoD* and *16S rRNA* genes were analyzed based on the similarity percentage of the type strains for each gene and the position of the sequences in the phylogenetic analysis. The *rpoD* gene was amplified with the primers reported by Reyes-Rodríguez[41]. The PCR reaction included 5 μl of 10× PCR Go-Taq reaction mix (50 mM KCl, 75 mM Tris–HCl, pH 9.0, 2 mM $MgCl_2$; Promega), 1 μl of 10 mM dNTP mix, 0.5 μl of Taq DNA polymerase (5 U/μL; Promega), 1 μl of 10 mM of each primer, and 5 μl of DNA, adjusted to a final volume of 50 μl. The conditions were as follows: 95 °C for 5 min followed by two cycles at 94 °C for 1 min, 63 °C for 1 min (hybridization), 72 °C for 1 min, two cycles at 94 °C for 1 min, 61 °C for 1 min, 72 °C for 1 min, two cycles at 94 °C for 1 min, 59 °C for 1 min, 72 °C for 1 min, 30 cycles at 94 °C for 1 min, 58 °C for 1 min, 72 °C for 1 min[44]. The amplified products (830 bp) were observed on a 1.5% agarose gel (Invitrogen), placed in an electrophoresis chamber under the following conditions: 80 V, 400 mA for 50 min; those that tested negative were subjected to PCR amplification of 16S ribosomal RNA.

Fragments of the 16S *rRNA* gene were amplified using the universal primers described by Borrell[2]. The PCR reaction included 10 μl 5X GoTaq® Flexi Reaction Buffer (Promega, Wisconsin, USA), 1 μl dNTP mix, 1.25 U (0.25 μl) Taq DNA polymerase, 35.75 μl nuclease-free water, 1 μl of each primer and 1 μl of DNA, adjusted at a final volume of 50 μl, the presence of a 1503 bp amplicon was verified on 1.5% agarose gels under conditions similar to those described above and time was reduced to 45 min. The conditions in the thermocycler were: initial denaturation for 3 min at 93 °C, followed by 35 cycles of denaturation for 1 min at 95 °C, annealing for 1 min at 56 °C, and extension for 1 min at 72 °C. Final extension for 10 min at 72 °C for 10 min. The amplified products (1503 bp) were observed a 1.5% agarose gel placed in an electrophoresis chamber, under the conditions described previously.

The amplified products were purified using the Wizard® SV Gel and PCR Clean-Up System kit (Promega, #A9281, Wisconsin, US). They were sequenced by the Sanger method at the National Laboratory of Genomics for Biodiversity of the Center for Research and Advanced Studies of the National Polytechnic Institute, Mexico. The sequences obtained were assembled using the MEGA 11 software (https://www.megasoftware.net) and compared in the National Center for Biotechnology Information (NCBI) database using the BLAST tool. The nucleotide sequences of the strains identified were aligned using the ClustalW program including the published sequences for the type strains.

Table 1 Molecular identification of bacteria isolated from Tilapia (*Oreochromis* spp.) using the *rpoD* gene.

Molecular identification	Id fish	Id strain	Source	Origin	% identity to type strain	GenBank nucleotide accession code
Acinetobacter johnsonii	6	2.6.2	Gills	Huasca	99.13	CP037424.1
	7	3.1.3.1 Sn	Gills	Ixmiquilpan	97.72	CP028574.2
Acinetobacter pittii	14	5.2.2 Sn	Gills	Ixmiquilpan	99.13	CP028574.2
	15	5.3.6 Sn	Spleen	Ixmiquilpan	97.85	CP028574.2
	15	5.3.7	Kidneys	Ixmiquilpan	98.10	CP028574.2
Aeromonas dhakensis	8	3.2.3 A	Gills	Ixmiquilpan	99.48	LC547062.1
Aeromonas hydrophila	8	3.2.2 A	Gills	Ixmiquilpan	97.71	KC601666.1
	22	8.1.4 Sn	Intestine	Tezontepec	97.39	MW838164.1
	23	8.2.4	Intestine	Tezontepec	97.02	LC550442.1
Aeromonas sp.	10	4.1.2 TCBS Y	Gills	Ixmiquilpan	98.20	JQ403281.1
Aeromonas veronii	1	2.1.3 Sn	Gills	Huasca	97.29	AY987683.1
	2	2.2.2	Gills	Huasca	97.29	CP121846.1
	4	2.4.4	Intestine	Huasca	97.68	CP014774.1
	6	2.6.5	Liver	Huasca	98.79	MW838148.1
	14	5.2.1 Sn	Fins	Ixmiquilpan	98.86	MW838131.1
	16	6.1.1	Fins	Chilcuautla	97.61	MW838152.1
	16	6.1.6	Spleen	Chilcuautla	97.51	MW838148.1
	17	6.2.3	Gills	Chilcuautla	98.61	MW838153.1
	18	6.3.2 TCBS	Gills	Chilcuautla	97.96	FR682788.1
	23	8.2.1 Sn	Fins	Tezontepec	99.07	CP014774.1
	24	8.3.7.1 A	Kidneys	Tezontepec	97.81	DQ411512.1
Aeromonas veronii bv. sobria	20	7.2.3.2	Gills	Tezontepec	97.90	LC547084.1
Citrobacter freundii	3	2.3.4	Intestine	Huasca	99.50	CP042534.1
	24	8.3.1	Fins	Tezontepec	98.54	CP033744.1
Comamonas aquatica	9	3.3.7 Sn	Kidneys	Ixmiquilpan	97.63	CP016603.1
Comamonas thiooxydans	5	2.5.6	Spleen	Huasca	98.65	AP026738.1
Kosakonia cowanii	1	2.1.3	Gills	Huasca	97.47	CP107077.1
Pantoea sp.	4	2.4.3	Gills	Huasca	98.04	CP061083.1
Plesiomonas shigelloides	1	2.1.1 Sn	Fins	Huasca	98.29	CP050969.1
	16	6.1.4	Intestine	Chilcuautla	97.89	CP050969.1
	20	7.2.5	Liver	Tezontepec	98.80	MZ668584.1
Pseudomonas chengduensis	19	7g	Fins	Tezontepec	97.67	JX042517.1
Pseudomonas fulva	2	2.2.3	Gills	Huasca	99.38	CP014025.1
Shewanella xiamenensis	16	6.1.2	Gills	Chilcuautla	98.86	CP092630.1
	18	6.3.3	Gills	Chilcuautla	99.11	CP092630.1
	27	9.3.4.2	Intestine	Progreso	97.30	CP091833.1
Vibrio cholerae	14	5.2.4 A	Intestine	Ixmiquilpan	97.19	CP053806.1

Genetic distances were obtained using the Kimura's two-parameter model[26]. Phylogenetic trees were constructed using the neighbor-joining method[43] using the MEGA 11 software.

Results and discussion

In this study, 69 bacteria isolated from Tilapia (*Oreochromis* spp.) from the State of Hidalgo were identified at distinct developmental stages and in unaltered organs. Genera identified were *Aeromonas* 40.6% (28/69), *Shewanella* 14.5% (10/69); *Acinetobacter* 8.7% (6/69), *Citrobacter*, *Comamonas*, *Plesiomonas*, *Pseudomonas* and *Vibrio* 5.8% each (4/69), *Kosakonia* 2.9% (2/69); *Exiguobacterium*, *Glutamicibacter* and *Pantoea* 1.4% each (1/69). The organs with the highest percentage of isolations were the gills 39.1% (27/69), followed by the intestine 21.7% (15/69), fins 17.4% (12/69), liver 10.1% (7/69), spleen 7.3% (5/69) and kidneys 4.4% (3/69) (Tables 1 and 2).

The ERIC-PCR test allowed us to make a first filter among the strains, as we obtained 6 clusters (A, B, C, D, E and F), from which we selected a strain from each one, considered to be the ''representative'' strain. Following the molecular identification, we found that each cluster corresponded to different strains of *Aeromonas* sp., *Aeromonas veronii*, *Shewanella xiamenensis*, *Plesiomonas shigelloides*, and *Acinetobacter johnsonii* (supplementary material).

The genus with the highest presence was *Aeromonas*, specifically *A. veronii* 64.3% (18/28) *Aeromonas* sp., and *A. hydrophila* 14.3% each (4/28), *A. dhakensis* and *A. veronii bv. sobria*. It is a Gram-negative pathogen with coccobacillary morphology, usually found in aquatic envi-

Table 2 Molecular identification of bacteria isolated from Tilapia using the *16S* rRNA gene.

Molecular identification	Id fish	Id strain	Source	Origin	% identity to type strain	GenBank nucleotide accession code
Acinetobacter sp.	14	5.2.5 Sn	Liver	Ixmiquilpan	98.25	OP990183.1
Aeromonas hydrophila	26	9.2.6	Spleen	Progreso	99.78	MF457586.1
	14	5.1.4.2	Intestine	Ixmiquilpan	98.51	MN646994.1
Aeromonas sp.	21	7.3.3	Gills	Tezontepec	99.71	EF491850.1
	23	8.2.2	Gills	Tezontepec	97.50	EF491849.1
	6	2.6.3	Gills	Huasca	99.85	MT226399.1
	16	6.1.3	Gills	Chilcuautla	99.44	JF490068.1
	16	6.1.4	Intestine	Chilcuautla	97.84	JF490067.1
Aeromonas veronii	16	6.1.4 A	Intestine	Chilcuautla	99.20	MT226399.1
	21	7.3.4	Intestine	Tezontepec	98.80	JF490067.1
	23	8.2.3 Sn	Gills	Tezontepec	99.62	JF490067.1
	24	8.3.1	Fins	Tezontepec	98.83	MT226399.1
Citrobacter braakii	25	9.1.3 Sn	Gills	Progreso	97.73	HQ812975.1
Citrobacter freundii	25	9.1.Est	Intestine	Progreso	99.65	OP788133.1
Comamonas	27	9.3.1	Fins	Progreso	98.45	MT527532.1
aquatica	10	4.1.3 a	Gills	Ixmiquilpan	98.49	MT527532.1
Exiguobacterium mexicanum	27	9.3.2 TCBS	Gills	Progreso	99.84	MT585191.1
Glutamicibacter soli	26	9.2.1 TCBS	Fins	Progreso	99.88	MK253330.1
Kosakonia cowanii	6	2.6.4 A	Intestine	Huasca	97.32	MZ501320.1
Plesiomonas shigelloides	27	9.3.5.2	Liver	Progreso	97.29	MT573123.1
Pseudomonas	25	9.1.2 TCBS	Gills	Progreso	99.18	MH185879.1
anguilliseptica	25	9.1.2.2	Gills	Progreso	98.36	MH185879.1
Shewanella putrefaciens	25	9.2 L	Fins	Progreso	97.70	CP046329.1
Shewanella sp.	13	5.1.4 Sn	Intestine	Ixmiquilpan	98.25	MG930082.1
	11	4.2.1	Fins	Ixmiquilpan	97.58	MN005116.1
Shewanella	12	4.3.5	Liver	Ixmiquilpan	97.40	MG428836.1
xiamenensis	13	5.1.3 A	Gills	Ixmiquilpan	97.61	MG428898.1
	13	5.1.5 Sn	Liver	Ixmiquilpan	99.78	MG428836.1
	27	9.3.1.1	Fins	Progreso	97.01	MN005116.1
	20	7.2.4 Sn	Intestine	Tezontepec	97.05	CP053796.1
Vibrio cholerae	21	7.3.5 Sn	Liver	Tezontepec	98.22	MG062858.1
	21	7.3.5	Liver	Tezontepec	99.06	CP053796.1

ronments, capable of affecting a wide variety of species, domestic animals, frogs, reptiles, freshwater, and saltwater fish, and considered an opportunistic human pathogen capable of affecting immunocompromised and immunocompetent individuals[21]. It has also been isolated from different food sources. Therefore, it is regarded as an emerging pathogen and a public health risk as a result of the multiple reports of its presence in raw, lightly processed foods on display and ready for consumption[23], due to its ability to survive and multiply at low temperatures[15]. Members of the *Aeromonas* genus are known pathogens in global aquaculture; *A. hydrophila*, *A. veronii*, *A. sobria*, *A. dhakensis*, *A. jandei* have been found in both healthy and sick fish, spreading due to stressful breeding and handling conditions that compromise the immune system[8], allowing the passage of bacteria to internal organs. *A. caviae*, *A. piscicola*, *A. veronii*, *A. dhakensis*, *A. hydrophila*, *A. schubertii*, and *A. trota* are also reported as disease-causing species in humans[11], accounting for 96% of gastroenteritis cases. *A. veronii* is a public health risk and several outbreaks associated with food of aquatic origin have been reported, for example, disease was reported from consumed fish in China[31], while a high prevalence of *A. veronii* was observed in adult diarrhea cases in a hospital in Taiwan[7]. This genus causes gastroenteritis, septicemia, hepatobiliary tract and pleuropulmonary infections, meningitis, peritonitis, and hemolytic uremic syndrome by food consumption or by direct contact with immunocompromised persons[41]. The presence of this genus in Tilapia (*Oreochromis* spp.) represents a problem for human health.

Isolates of *Shewanella* sp., *S. xiamenensis* and *S. putrefaciens* were also identified. They were found in the fins, intestines, liver, and gills. The *Shewanella* genus, which are Gram-negative bacilli are widely distributed in aquatic environments, sediments, and soils[6]. *S. putrefaciens* is a halophilic bacterium of great relevance in the food industry, due to its ability to survive refrigeration temperatures, causing putrefaction of meat from aquaculture, poultry, or bovine sources[37]. Reports indicate that *S. putrefaciens*, among *S. xiamenensis*, and *S. algae* are a growing threat to human health, causing skin and soft tissue infections, bacteremia, otitis, and hepatobiliary infections. *S. putrefa-*

ciens can adapt to different niches, thereby facilitating the acquisition of a wide variety of mobile elements; thus, it is considered a reservoir of resistance genes because it participates in the dissemination of antimicrobial resistance[6]. This pathogen can be a threat because it was identified from tilapias intended for human consumption, and inadequate management can lead to possible consequences for health.

The genus *Acinetobacter* consists of Gram-negative coccobacilli, ubiquitous in nature and present in soils, bodies of water, drinking water, various foods, and nosocomial environments[5]. In this study we identified six isolates of this genus. *A. pitti*, which causes diseases in fish and humans, is highly prevalent in nosocomial environments[30], while *A. johnsonii*, an emerging pathogen in aquaculture, has been associated with cases of bacteremia in humans[28].

Pseudomonas is an opportunistic freshwater and saltwater fish pathogen and a serious threat to the aquaculture industry[38]. The genus is widely distributed in soils, plants, and animals due to its low survival and adaptability requirements. In this study, *P. chengduensis*, *P. fulva* and *P. anguilliseptica* were identified. *P. fulva* has been isolated from wetlands, rice plantations, plants, and oil soils[40]; however, cases of infection in humans are rare, typical of nosocomial origin, and associated with trauma, ventricular drainage, or transfusions using contaminated equipment[27].

Vibriosis is a disease caused by different members of the genus *Vibrio*, Gram-negative bacilli resulting in high mortality rates and significant losses in the aquaculture sector, most members of this genus are opportunistic pathogens[22]. *Vibrio cholerae* can be isolated from aquatic environments, especially in warm months[16]. The consumption of raw or poorly cooked seafood and fillets causes in humans profound dehydration, dysentery, and in some cases extraintestinal diseases such as meningitis, septicemia, acute diarrhea, and in particular cases, death[50]; however, only the strains of serogroups O1 and O139 cause cholera epidemics, although some non-O1/O139 strains can produce heat stable toxins and cause septic infections[17,32]. In this study four isolates were identified; nonetheless, in Mexico the regulations specify that it should be absent in this type of products, considering that it is regarded as a worldwide threat to public health.

Plesiomonas shigelloides is the only species of the genera, in which only four isolates were identified. This genus is a Gram-negative bacillus with a wide distribution in water and has been isolated from terrestrial and aquatic mammals, fish, mollusks, crustaceans, amphibians, reptiles, and birds[24]. Infection in humans is mainly associated with the consumption of raw or undercooked fish and shellfish and causes gastrointestinal and extraintestinal illnesses, usually causing chills, fever, abdominal pain, diarrhea, vomiting, cellulitis, meningitis, urinary tract infections, and, in severe cases, profuse diarrhea and septicemia[12].

Comamonas spp. comprises species of Gram-negative bacilli[49]; *Citrobacter* is a genus belonging to the *Enterobacteriaceae* family, comprising Gram-negative bacilli[20]. Four isolates were identified in each of the two genera, and both are usually found in soils, bodies of water, water used in airplanes, plants, and as commensals in the digestive system of pets, birds, and domestic animals, in addition to the respiratory and urinary tracts of humans[29]. They are considered opportunistic pathogens[33]. *Comamonas kerstersii*, *Comamonas aquatica*, *Comamonas thiooxydans*, and *Comamonas terrigena* have been reported as causing infection in immunocompromised individuals[42].

Kosakonia cowanii (previously known as *Enterobacter cowanii*) and *Pantoea* sp. are pathogens isolated mainly from plants, soils, and rarely in infant formulas and healthcare facilities. Few cases of infections in humans have been reported, mainly occurring in newborns or in individuals with chronic diseases[1,34]. The genus *Exiguobacterium* has variable morphology (ranging from small bacilli to cocci), is Gram negative, and can be isolated from various environments such as soils, sediments, permafrost, glaciers, industrial waters, and hydrothermal vents, it is not considered pathogenic[25]. *Exiguobacterium mexicanum* was isolated for the first time from *Artemia* spp. larvae[35] and has shown no pathological potential. *Glutamicibacter soli* belongs to the genus *Glutamicibacter*. It has been isolated from wastewater, soil, human urine, roots, and from the fly *Protophormia terraenovae*[4].

All identified genera are usually found in water, sediments, or soil near aquaculture farms; however, *Aeromonas*, *Vibrio*, *Plesiomonas*, *Pseudomonas*, and *Shewanella* are not only pathogenic for fish but also considered threats to human health[6,11,12]. Their presence in the skin, gills, intestines, spleen, and kidneys facilitates the subsequent colonization of the fillet since the muscle is considered sterile until the death of the fish. These bacteria can migrate due to ulcerative lesions, potentially resulting in septicemic processes, both at the time of the fish death and during the handling processes to obtain the fillet[3]. If ingested raw, lightly, or inadequately cooked, it can cause FBDs[9]. This agrees with the findings reported in our study, hence the bacteria identified with the highest prevalence were found in fins and gills, followed by the intestine. Foysal[19] demonstrated high prevalence of pathogens such as *Vibrio cholerae*, *Aeromonas* spp., *Acinetobacter* spp., *Staphylococcus* spp., *Streptococcus* spp., *Mycobacterium*, and *Escherichia-Shigella* found in both the skin and intestine of fish intended for human consumption sold in local markets and in shopping malls in Bangladesh. Sripradite[45] also reported the presence of *Aeromonas hydrophila*, *Salmonella* sp. and *Vibrio cholerae* in fillet, intestine, liver, and kidneys of Tilapia sold fresh, displayed on ice and shopping centers. These microorganisms can persist in undercooked or raw preparations, as reported by Park[36], who demonstrated the prevalence of *A. hydrophila* in sushi samples (20–51.4%) obtained from shopping centers and restaurants. Li[32] showed the presence of *V. cholerae* (3.8%) among other pathogens, in different fish purchased from farms, restaurants, supermarkets, and online stores; Díaz-Cárdenas[14] found the genera *Acinetobacter*, *Pseudomonas*, *Vibrio*, *Aeromonas* and *Citrobacter* in ceviche samples, showing that these bacteria are capable of surviving different preparation processes. In addition, it should be considered that many of the gastrointestinal conditions caused by these bacteria can go unnoticed and may not be accurately diagnosed, particularly when appropriate follow-up is lacking, a situation that is further aggravated when vulnerable populations are involved, and where dehydration could trigger serious clinical conditions.

Surveillance, prevention, and management measures play an essential role, as some of the bacteria identified in this study have multiple characteristics that could

affect more than one sector, and cause various types of diseases. As mentioned previously, all the identified genera are usually found in diverse environments. *Aeromonas*, *Shewanella*, *Vibrio*, and *Pseudomonas* are classified as food spoilage organisms affecting food quality[13]; *Aeromonas*, *Plesiomonas*, and *Vibrio* are considered bacteria with zoonotic potential worldwide[39,50]. Furthermore, there is growing evidence pointing to *Aeromonas*, *Pseudomonas*, *Plesiomonas*, *Acinetobacter*, *Vibrio*, and *Citrobacter* as genera exhibiting antimicrobial resistance and potentially serving as reservoirs of antibiotic resistance genes[18,27,38,39,45].

In Mexico, Tilapia is consumed primarily cooked or fried, which significantly reduces its microbial load. However, several studies have documented the presence of pathogenic bacteria in aquaculture production systems, which represents a potential risk to public health[12,31,32]. Microbiological analyses performed on non-edible tissues, such as skin, gills, intestines, and culture water, have identified microorganisms such as *Salmonella* spp., *Aeromonas* spp., *Vibrio* spp., *Plesiomonas* spp. and *Escherichia coli*, which are recognized foodborne pathogens[9,11,12,14,21,32]. These bacteria can be transferred to the edible muscle of fish due to poor hygiene practices during the capture, filleting, transportation or storage stages, as well as due to injuries to the fish or unhealthy conditions of the culture water[15,37]. Furthermore, subsequent inadequate practices, such as poor refrigeration, cross-contamination, or the preparation of semi-raw dishes, such as *ceviches* or pickles, can compromise the safety of the final product, especially in vulnerable populations such as immunocompromised individuals, young children, and older adults[12,21]. Therefore, the detection of pathogens in Tilapia external matrices constitutes a significant health alert regarding hygiene and sanitation deficiencies in production systems, which must be addressed to reduce the risk of contamination of the final product intended for human consumption. Although studies that do not include muscle analysis do not allow direct estimation of consumer risk, they provide sufficient evidence to justify corrective measures in production and handling practices.

Conclusion

This study demonstrated the presence of microorganisms, such as *Aeromonas*, *Shewanella*, *Acinetobacter*, *Pseudomonas*, *Vibrio*, and *Plesiomonas*, among others, obtained from Tilapia cultured in the State of Hidalgo. The possible presence of these microorganisms is attributed to their ubiquity and wide distribution in the environment, as well as to the biological composition of fish, which makes them susceptible to contamination. These microorganisms can affect various species and be transmitted throughout the production process, causing various diseases. For all these reasons, further research is needed to design and implement surveillance, control, and other measures to prevent or mitigate risks to public health.

Conflict of interest

The authors have no conflict of interest to declare.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version available at https://doi.org/10.1016/j.ram.2025.09.003.

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