Streptococcus agalactiae Serotype Ia ST7 CC1 in Farmed Nile Tilapia in Latin America: Age-Dependent Disease and Antimicrobial Susceptibility of an Emerging Clonal Lineage

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Article

Streptococcus agalactiae Serotype Ia ST7 CC1 in Farmed Nile Tilapia in Latin America: Age-Dependent Disease and Antimicrobial Susceptibility of an Emerging Clonal Lineage

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Abstract

Recently, a strain of *Streptococcus agalactiae* serotype Ia sequence type 7 clonal complex 1 (SaIa ST7 CC1) has emerged in Latin American tilapia aquaculture as an international threat. This study evaluated outbreaks of acute streptococcosis occurring between 2021 and 2025 on commercial Nile tilapia (*Oreochromis niloticus*) farms located in six Latin American countries, with an aim to combine molecular, clinical, pathological and environmental data. In total, 360 moribund or recently dead fish at various production stages (larvae/fry, pre-grow-out and grow-out) were examined, and 25 S. agalactiae isolates were serotyped, subjected to real time PCR analysis multilocus sequence typing (MLST), virulence and antimicrobial resistance gene profiling and antimicrobial susceptibility testing. All isolates belonged to SaIa and had the same ST7 CC1 MLST profile, which created a highly homogeneous cluster that grouped with reference SaIa ST7 CC1 strains previously isolated from tilapia farms in Asia. These results are consistent with the regional spread of a single clonal line. At larval and fry stage, SaIa ST7 CC1 was associated with hyperacute septicemia, gastrointestinal hemorrhage and frequent intestinal intussusception; while in pre-grow-out and grow-out fish neurological signs were more prominent followed by ocular signs, systemic hemorrhages and coelomic lesions. Histopathological examination showed profuse colonization of brain, spleen, liver, and intestine by Gram-positive cocci accompanied by significant acute circulatory and inflammatory lesions and few chronic granulomatous responses consistent with a fast-progressing high aggressive infectious process. All outbreaks occurred during extended episodes of hot water (>32°C) with large day–night thermal gradients and reduced dissolved oxygen, suggesting that thermal stress may exacerbate disease expression in systems affected. All SaIa ST7 CC1 strains exhibited phenotypic susceptibility to florfenicol and amoxicillin, but 84% (21/25) and 100% (25/25) of them exhibited intermediate susceptibility to oxytetracycline and enrofloxacin, respectively. Five of the 21 isolates (23,8%) with intermediate susceptibility to oxytetracycline carried tetracycline resistance genes (tetM, tetO). These findings identify SaIa ST7 CC1 as a clinically relevant threat of emerging thermally facilitated and geographically expanded streptococcosis for tilapia production in Latin America. Immediate priorities include screening of imported broodstock using MLST or whole-genome sequencing, harmonized regional molecular surveillance, climate-adaptive farm management practices, prudent antimicrobial use and serotype-matched vaccination and breeding strategies that improve both disease- as well as heat-resilience.

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1. Introduction

Global farmed tilapia (*Oreochromis* spp.) production is approximately 7 million metric tons in 2024, representing an annual increase of about 4–5% versus 2023 and an economic valuation of around US$ 15–15.5 billion with forecasts up to US$ 21 billion by the year 2035 at a compound yearly growth rate close to 3.1% [1,2]. While North America (NAM), Central America (CAM) and South America (SAM) individually produce approximately 900,000 metric tons of tilapia per year (about 13% of global production), they are the backbone of the tilapia industry in Latin America (LAM) [3].

This continuous growth is mainly due to the species' great adaptability to different environmental conditions and rapid growth that allowed it most widespread adoption in various production systems and climatic zones [4]. Nevertheless, production intensification was implemented without risk-based internal and external biosecurity plans as well as heterogeneous livestock practices among the LAM-producing countries. This is within the context of increasing environmental variability related to climate change, which predisposes the sector to the emergence, re-emergence and/or recurrent outbreaks of bacterial and viral disease having important productive, economic and social impact.

In the past two decades, another factor has taken center stage as a key driver in redefining host–pathogen–environment interactions for aquaculture systems and other crop production systems—global warming [5,6]. Increasing water temperatures stimulate pathogen replication, virulency, life cycle dynamics, and the spread of disease in fish, weaken biosecurity measures that were usually developed under less extreme climatic conditions. Tilapia, often characterized as tolerant to environmental variations and even climate change [4,7], is now increasingly subjected to both elevated and variable temperatures, decreased levels of dissolved oxygen (DO), and changes in pH and salinity associated with modified precipitation patterns along with extreme weather events [8–10].

In this context, experimental and field data show that *Streptococcus agalactiae* (group B Streptococcus, GBS), the etiological agent of piscine streptococcosis in tilapia, is classified into 10 serotypes (Ia, Ib, II–IX) on the basis of capsular polysaccharide antigens (cps) [11], that reveal dramatic virulence changes driven by temperature variation [12,13]. In Papua, Negara Bagian Papuaba (Indonesia), strains that were CAMP test negative (deficient in the cfb gene) in 2013 became CAMP test positive within a decade by 2023, alluding to adaptive shifts of virulence traits under warmer conditions [14]. In tandem, temperature induced decreases in oxygen solubility also cause much lower dissolved oxygen levels at higher temperatures (e.g., 32°C vs. 22°C), and together with changes to salinity and pH escalate physiological stress and impair disease resistance [9,10,15,16].

Five years of dynamic shift in the epidemiology of *S. agalactiae* in farmed Nile tilapia across LAM [17,18]. Historically, the burden of disease has been ascribed to *S. agalactiae* serotype Ib (SaIb), including a range of distinct sequence types (ST 103, ST 260, ST 261, ST 552, ST 553, and ST 927) [19]. However, since 2021 S. agalactiae serotype Ia (SaIa), sequence type 7 (ST7), clonal complex 1 (CC1) has spread in several LAM tilapia producing countries [17,18,20]. Phylogenetic analyses so far indicate that this lineage originated from a close ancestor of isolates found in tilapia farmed in Asian countries [17]. Comparative studies from Asia have demonstrated that β-hemolytic bacteria including SaIa or *S. agalactiae* serotype III (SaIII) are more pathogenic to farmed tilapia than non-hemolytic variants e.g. SaIb [21–26].

Given the circumstances, the objective of this study was to describe and characterize the SaIa ST7 CC1 strain associated with outbreaks of acute streptococcosis in commercially farmed Nile tilapia in six Latin American countries. We specifically (i) elucidated the clinical and pathological manifestations of disease across different stages of production: larvae/fry (FRY), pre-grow-out (PGO), grow-out (GO); (ii) identified serotype, sequence type and phylogenetic relationships between isolates using real-time PCR assays and multilocus sequence typing (MLST); (iii) profiled key virulence and antimicrobial resistance genes as well as derived antimicrobial susceptibility to therapeutics regularly employed in regional tilapia aquaculture.This work contributes to the understanding of the molecular epidemiology and pathogenesis of SaIa ST7 CC1, integrated with

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clinical, pathological, and environmental data, and serves as a guide for designing climate-adapted surveillance, prevention, and control strategies for streptococcosis in tilapia production systems in Latin America.

2. Materials and Methods

2.1. Ethical Statement

Pathovet Labs is accredited by the National Council for the Control of Animal Experimentation (CONCEA) of the Ministry of Science, Technology, and Innovation (MCTI) and has its own Institutional Committee for the Care and Use of Animals (CEUA), established in accordance with Brazilian federal legislation and CONCEA/MCTI Resolution No. 50/2021. This study was conducted under fully field conditions at commercial Nile tilapia farms in the context of active streptococcosis outbreaks. Sampling, clinical examination, euthanasia, and transport procedures strictly adhered to institutional standard operating protocols for field disease investigation and diagnostic sampling.

At the time of study design and implementation, the research team did not consider prior ethics committee approval necessary, as the study involved no experimental manipulation beyond routine diagnostic procedures and all fish were handled under standard commercial farm conditions. These criteria align with those outlined by Bennett et al. [27], who recognize that field collection and diagnostic studies conducted under commercial conditions are frequently exempt from formal Animal Ethics Committee (AEC) review, as they fall outside the scope of procedures typically regulated by such bodies. However, the study was reviewed by the CEUA-Pathovet Labs Ethics Committee in March 2026, which confirmed that the procedures were conducted in accordance with the ethical standards applicable to field studies in farmed fish (Protocol No. 04/2026).

Fish were euthanized by overdose of tricaine methanesulfonate (MS-222) in accordance with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals and institutional animal care protocols. Antimicrobial susceptibility testing was performed following Clinical and Laboratory Standards Institute (CLSI) guidelines.

2.2. Field Fish Sampling

Between 2021 and 2025, acute outbreaks of a presumably bacterial systemic disease occurred in different age ranges, FRY (~0.5 to 5 g), PGO (~5 to 80 g), GO (~100 g to harvest), in different commercial Nile tilapia farms in six countries (C1-C6) in North America (NAM), Central America (CAM), and South America (SAM) (Table 1). Therefore, this study was conducted using only tilapia farmed on commercial farms, and no animals were used under experimental conditions.

All sampled farms operated semi-intensive production systems with variable stocking densities depending on the production system: 0.2–2.0 kg/m^3 in FRY, 3.0–5.0 kg/m^3 in PGO, and 10–20 kg/m^3 in GO. Farms in the FRY phase used earthen pond systems, fiberglass, or geomembrane tanks (PVC/HDPE), or concrete tanks, with water supplied from rivers, lakes, reservoirs, or groundwater (C1, C3, C5). The PGO and GO phases included net cage systems in rivers, lakes, or reservoirs (C2, C4, C6), or earthen ponds fed by river diversions (C1, C3, C4, C5).

A total of 360 moribund and recently dead specimens (FRY n= 180; PGO/GO n = 180) were intentionally sampled in six countries (C1-C6) (Table 1), selecting one commercial farm per country (due to the collaborative network). All fish were subjected to anatomopathological analysis and 92 were selected for histopathological characterization (Table 1). From this total number of fish, 25 isolates were selected for study due to logistical and budgetary reasons (Table 1): 5 isolates each from C1, C2, C4, and C6; 3 isolates from C3; and 2 isolates from C5.

In all farms where the first outbreak of piscine streptococcosis was observed, periods of time of 3-4 weeks were recorded with average water temperatures >32°C, with peaks of 34°C and a thermal differential >5°C between day and night (Table 1). At the same time, oxygen concentrations were recorded in a range between countries of 1.0 to 1.8 mg/L (night) and 5.5 to 6.5 mg/L (day), and

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between 20 and 25% (night) and 70% and 80% (day) of saturation. No significant differences were observed between farms/countries in these environmental indicators.

2.3. Clinical Signs and Gross Pathology

Moribund and recently dead specimens were selected through intentional sampling from six (06) commercial farms in each country. Prior to necropsy, behavioral changes, clinical symptoms, and external macroscopic signs were recorded on all sampled fish. External inspection included systematic examination of the skin, scales, fins (including pectoral fin insertion sites), eyes, nasal cavities, mouth and oropharyngeal cavity, anus, and gills according to standardized ichthyopathological protocols.

Fish were euthanized by overdose of tricaine methanesulfonate (MS-222) in accordance with AVMA guidelines and institutional animal care protocols. Following surface disinfection with 70% ethanol, fish were systematically necropsied with sterilized instruments. The abdominal cavity of each specimen was opened using aseptic technique to expose the internal organs and perform gross anatomopathological examination. Organs examined included the liver, spleen, kidneys, pericardial cavity, heart, digestive tract, gills, and gonads. The skull was carefully opened to expose the cranial cavity and brain. Finally, tissue samples were collected from multiple target organs selected based on the suspected systemic bacterial pathology following standardized guidelines.

2.4. Bacteriological Culture and Serotyping

The basic bacteriological process was carried out in situ in the laboratories available on each farm and consisted of the aseptic plating of different organs of each fish according to Table 1 on 5% blood agar (Hardy Diagnostics, Santa Maria, CA, USA) and incubation at 28°C for 24 to 48 hours. The bacterial colonies suspected of being *S. agalactiae* (e.g., gray-white, flat, and mucoid) were confirmed as Gram-positive cocci or chains of cocci by Gram staining and then reseeded on Brain Heart Infusion (BHI) agar supplemented with 5% defibrinated sheep blood (Heel do Brasil Biomedica Ltd.a., São José dos Pinhais, PR, Brazil). The same 25 isolates were subsequently used to continue molecular and phenotypic characterization.

Then, 25 isolates were selected for serotyping using a commercial latex agglutination kit for GBS type Ia, Ib, and III (SaIII) (ImmuLex™, SSI Diagnostica S/A, Copenhagen, Denmark) following the manufacturer's instructions. Concisely, 10 µL of each kit reagent was added to a single GBS colony suspended in 10 µL of saline solution. The reaction was deemed positive if agglutination was observed after 30 seconds. All isolates that tested positive for *Streptococcus* serotype Ia were considered for further analysis.

2.5. Histopathological Examination

Tissue samples of ~0.5–1 cm^3 in volume (n = 92) were collected from each fish from the brain, heart, hepatopancreas, mid-kidney, spleen, stomach, and intestines. These samples were fixed in 10% neutral buffered formalin (1:10 *v/v* sample to fixative ratio) for 24 hours at room temperature, then transferred to 70% ethanol for long-term preservation. Following fixation, the samples were dehydrated using a graded alcohol series and processed according to standard histological protocols. Sections 4 µm thick were cut from each tissue block and stained with Hematoxylin and Eosin (H&E) and Gram for microscopic analysis. The stained sections were examined using a Leica DM-2000 optical microscope (Leica, Hamburg, Germany). Images were captured with a Leica DFC-295 digital camera (Leica, Hamburg, Germany) and analyzed using the Leica Application Suite Software (LAS), Image Analysis (Leica, Hamburg, Germany).

2.6. Real Time PCR-Based Serotyping

Genomic DNA from 25 pure bacterial culture colonies was fragmented with magnetic beads using the L-Beader 24 tissue disruptor (Loccus do Brasil Ltda, Cotia, SP, Brazil), according to the

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manufacturer's instructions. Then, bacterial genomic DNA was semi-automatically extracted using Maxwell® RSC instrument for the RSC Genomic DNA Kit (Promega Corporation, Madison, WI, USA), according to the manufacturer's protocol. A volume of 100 µL of supernatant from each sample was dissolved in 1 ml of PBS (pH 7.4) contained in a tube with 200 µL of lysis buffer and 20 µL of proteinase K.

Specific primers were used to identify serotypes Ia, Ib, and III of *S. agalactiae* using the primers described previously [27] (Supplementary Table S1). The qPCR was performed using a total volume of 10 µL for each sample, containing 2X GoTaq qPCR Master Mix (5 µL) (Promega Corporation, Madison, WI, USA), 300 nM of each primer (1.4 µL), 1.5 µL DNA of each sample (duplicate). The qPCRs were carried out in the QuantStudio 3 Real-Time PCR System (Applied Biosystems, Life Technologies, Carlsbad, CA, USA) using the following parameters: 95°C for 2 min for initial denaturation, 95°C for 15 s, and 60°C for 60 s for 40 cycles. A positive control (DNA o *S. agalactiae* serotypes Ia, Ib, and III), a negative control without DNA (UPW) and negative extraction control were also included in every run.

Table 1. Summary of *S. agalactiae* isolates recovered from acute outbreaks of a systemic disease in Nile tilapia of different age ranges on different commercial farms in six countries of LAM. Average cumulative mortality rates on each farm during the outbreaks and recorded water temperature ranges are detailed. L: liver; B: brain; I: intestine; S: spleen, K: kidneys. GP: gross pathology; HP: histopathology.

Region	Country	Date	Stage	# fish GP/# fish HP	Weight (g)	Organs	Main change	# isolates (ID)	Water temperature (°C)	Cumulative mortality (%)
CAM	C1	July 2023	FRY	60/06	5,0	Organs pool	Whirling, mortality	C1-A1	32-33°C	48%
			FRY		5,0			C1-A2		
			PGO	20/07	30,0	I	Intussusception	C1-A3	>32°C	55%
			PGO		50,0	B, L, K	Hemorrhagic septicemia	C1-A4		
			GO	10/06	600,0	L, K, I	Hemorrhagic septicemia; intussusception	C1-A5		
	C2	April 2023	FRY	60/06	2,0	Organs pool	Whirling, mortality	C2-A1	32-34°C	52%
			FRY		2,0			C2-A2		
			PGO	20/07	10,0	I	Intussusception	C2-A3	>32°C	57%
			PGO		30,0	B, L, K	Hemorrhagic septicemia	C2-A4		
			GO	10/06	400,0	B, K, I	Hemorrhagic septicemia; intussusception	C2-A5		
SAM	C3	April 2025	PGO	10/05	150,0	L, S, B	Hemorrhagic septicemia; intussusception	C3-A1	>32°C	55%
			GO	20/06	180,0			C3-A2		
			GO		300,0			C3-A3		
	C4	November 2023	FRY	60/06	3,0	Organs pool	Whirling, mortality	C4-A1	32-34°C	50%
			FRY		3,0			C4-A2		
			PGO	20/07	40,0	B, L	Hemorrhagic septicemia; intussusception	C4-A3	>32°C	47%
			PGO		60,0			C4-A4		

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			GO	10/06	500,0		Hemorrhagic septicemia	C4-A5		
	C5	April 2025	PGO	10/05	100,0	B, L, S	Hemorrhagic septicemia	C5-A1	>32°C	50%
			GO	10/05	400,0			C5-A2		
NAM	C6	August 2021	PGO	20/07	30,0	B, K, I	Hemorrhagic septicemia	C6-A1	>32°C	54%
			PGO		80,0			C6-A2		
			PGO		90,0			C6-A3		
			GO	20/07	500,0			C6-A4		
			GO		800,0			C6-A5		

All qPCR runs were accompanied by the expression of the Nile tilapia reference gene as an endogenous extraction control (β-actin) [28]. Cycling threshold (Ct) values were manually set and recorded up to a maximum of 40 Ct, checking that the threshold remained constant between runs. Fish samples were considered positive at Ct levels below 35 and negative between Ct 35-40 or in samples with no Ct (NoCt).

2.7. Multilocus Sequence Typing and Phylogenetic Analysis

Genomic DNA for MLST (multilocus sequence typing) analysis was extracted from pure culture colonies, as described above. Fragments (459 to 519 bp) of the reference genes were amplified by PCR using the primers described previously [29] (Supplementary Table S1). The amplified products were purified and sequenced in both directions using the Sanger method with Big Dye 3.1 reagent and a 3500xL capillary sequencer (Applied Biosystems, Life Technologies, Carlsbad, CA, USA). The sequences obtained were aligned using ClustalW and BioEdit software [30]. After aligning the sequences from the seven amplicons from each bacterial isolate with the *S. agalactiae* PubMLST database (https://pubmlst.org/organisms/streptococcus-agalactiae), a sequence type (ST) and clonal complex (CC) were assigned to each strain.

A phylogenetic analysis was performed based on the concatenated complete nucleotide sequence of the seven housekeeping genes derived from the MLST analysis of each isolate. Evolutionary relationships among 41 *S. agalactiae* isolates (25 LAM ST7 CC1 from this study; 16 reference strains from Asia and earlier LAM isolates from GenBank) were inferred using the Neighbor-Joining method with Maximum Composite Likelihood distances [31]. Sequences spanning 3,455 aligned positions (1st, 2nd, 3rd codon positions, noncoding sites; gaps removed by complete deletion) were analyzed in MEGA11 [32]. Nodal support was evaluated with 1,000 bootstrap pseudoreplicates; values ≥70% are displayed. Trees were drawn to scale with branch lengths representing evolutionary distance (substitutions per site). Isolates were classified into serotype groups (SaIa ST7, SaIb, SaIII) based on phylogenetic clustering with reference strains. All MLST sequences obtained were submitted to GenBank and assigned accession numbers PZ024150 to PZ024324.

2.8. Detection of Virulence and Antimicrobial Resistance Genes

Genomic DNA for the detection of virulence (VGs) and antimicrobial resistance genes (ARGs) was extracted from pure culture colonies, as described above. PCR was used to amplify virulence genes (VGs), including adhesins, invasins, and immune evasion genes (Supplementary Table S1), in PCR assays using primers and conditions that have been published previously using protocols described previously [33,34]. In addition, PCR amplification of antimicrobial resistance genes (ARGs) (Table 2) was performed using the protocols previously described [35,36]. Briefly, PCR was performed following the conditions described by the authors using a total volume of 25 µL for each sample but using the GoTaq® Green Master Mix (12.5 µL) (Promega Corporation, Madison, WI, USA). The reaction was carried out in a T100 thermocycler (Bio-Rad Laboratories, Hercules, CA, USA) with the following program: initial denaturation at 95 °C for 1 minute, followed by 25 cycles of 95°C for 5 seconds and 60°C for 4 minutes. The amplification products were analyzed by electrophoresis on 1.5% agarose gel with ethidium bromide dye.

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2.9. Disk Diffusion Susceptibility Testing

Disk diffusion was performed following CLSI M100 procedures [37], with adaptations for fish isolates. Fresh overnight cultures were suspended in sterile saline 0.85 % and adjusted to a 0.5 McFarland standard. A sterile swab was used to inoculate Mueller–Hinton agar (MHA) plates. Commercial antimicrobial disks corresponding to the agents evaluated in the MIC assay (oxytetracycline, OTC, 30 µg; florfenicol, FFC, 30 µg; amoxicillin, AMX, 10 µg; enrofloxacin, ENR, 5 µg) were aseptically placed onto the agar surface. Plates were incubated at 28 ± 2 °C for 18–24 h in ambient air. After incubation, inhibition zone diameters were measured in millimeters [38].

2.10. Minimum Inhibitory Concentration (MIC) Determination

Antimicrobial susceptibility testing of *S. agalactiae* fish isolates was performed using the broth microdilution reference method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines with adaptations. MICs were determined following CLSI M07 procedures [39]. Cation-adjusted Mueller–Hinton broth (CAMHB), was used as the test medium. Antimicrobials tested (OTC, FFC, ENR, AMX) were prepared as stock solutions diluted in sterile saline 0,85 % immediately prior to panel preparation. Two-fold serial dilutions were prepared in microdilution in 96-well microplates to cover ten different concentrations. The inoculum was standardized to a 0.5 McFarland turbidity and diluted to yield a final inoculum of 5×10^5 CFU/mL in each well. Plates were incubated at 28 ± 2 °C for 16–20 h in ambient air. MICs were recorded as having the lowest concentration with no visible growth. Quality control (QC) testing was performed in parallel using *Staphylococcus aureus* ATCC 29213, following the QC ranges specified in CLSI M07/M100 and VET01. Interpretive categories (susceptible, intermediate, resistant), when available, were assigned using the most recent CLSI breakpoints for veterinary-specific criteria (VET01). When no breakpoints existed for the antimicrobial and fish pathogen combination, MICs were reported without categorical interpretation.

3. Results

3.1. SaIa ST7 CC1 is the Same Clone Circulating in Farmed Tilapia in LAM

All strains obtained from tilapia farms in the six LAM countries were phenotypically characterized as Gram-positive, beta-hemolytic cocci and identified as SaIa by agglutination test and PCR (Figure 1). Each of the 25 isolates were confirmed as positive by real time qPCR for SaIa (average Ct = 16.92, SD = 1.44) (Supplementary Table S2). Furthermore, MSLT analysis of each of these isolates showed the same combination of alleles *adhP*(10), *pheS*(1), *atr*(2), *glnA*(1), *sdhA*(3), *glcK*(2) and *tkt*(2) distinctive of ST7, which belongs to CC1 (Supplementary Table S2). Moreover, phylogenetic analysis showed that SaIa strains isolated from tilapia farmed in NAM, CAM, and SAM were closely related to each other (99% identity) (Figure 2), but also to SaIa strains previously obtained from tilapia farmed in China, Thailand, the Philippines, and Vietnam (99%) (Figure 2). SaIa ST7 CC1 isolates caused similar mortality rates in the field, regardless the productive stage (FRY = 48-52%; PGO/GO = 47-57%) (Table 1). In all field outbreaks from which the SaIa ST7 CC1 clone was isolated, water temperatures >32°C were recorded (Table 1).

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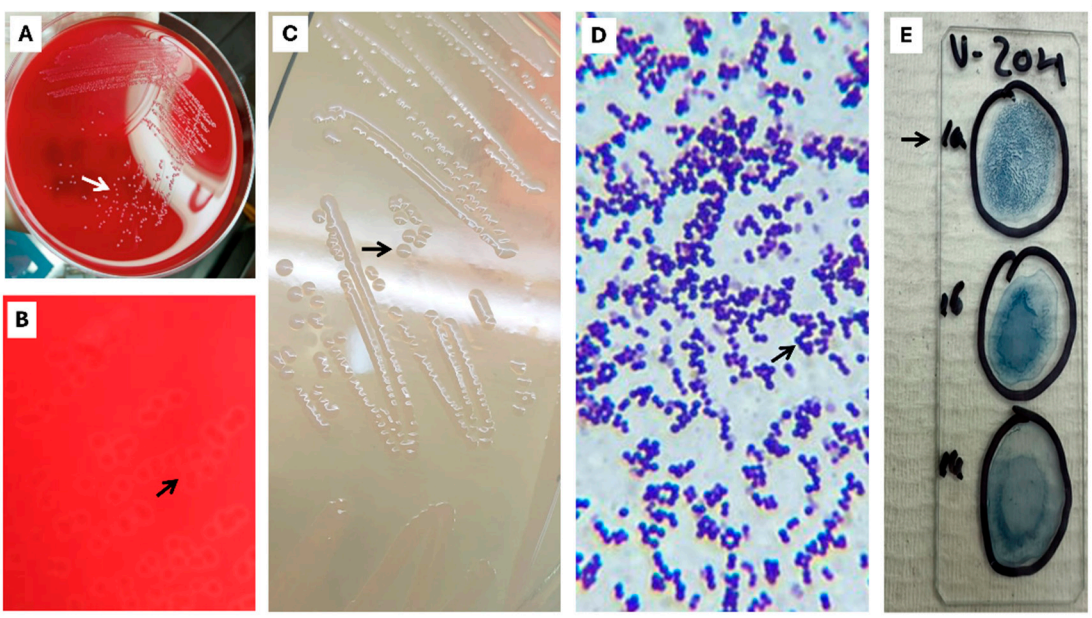

Figure 1. Bacteriological culture and serotyping. (A) Inoculation on 5% blood agar plates in field laboratory; (B) β-hemolytic colonies on 5% blood agar plates; (C) Suspected Streptococcus colonies on HBI agar; (D) Gram-positive cocci confirmed from pure colonies; (E) An agglutination test for S. agalactiae serotypes Ia, Ib, and III showing agglutination only with Ia means the bacterial suspension contains the Ia capsular polysaccharide, indicating it belongs to serotype Ia.

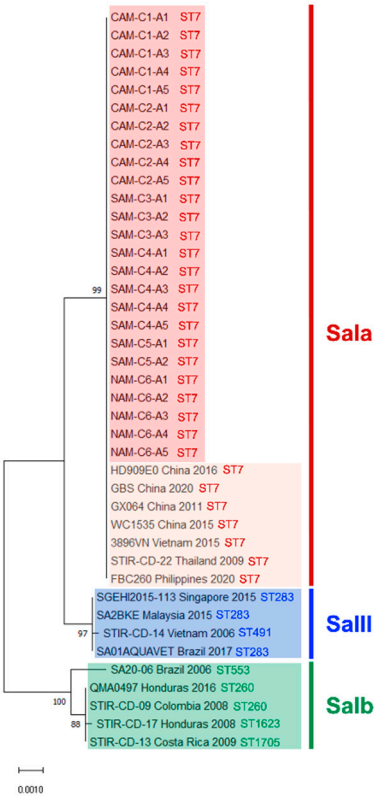

Figure 2. Phylogenetic tree of SaIa ST7 CC1 isolates from LAM and reference strains constructed with concatenated sequences of seven MLST housekeeping genes. Sequences were retrieved from the GenBank and PubMLST databases. Neighbor-Joining tree of 41 S. agalactiae nucleotide sequences showing monophyletic

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clustering of 25 ST7 CC1 LAM isolates (this study; red box) with Asian and Southeast Asian reference strains (orange box), indicating a single clonal introduction followed by regional dissemination (SaIa ST7). Nine reference isolates of SaIII and SaIb strains from Asia (blue box) and LAM (green box) form distinct basal clades. Numbers at nodes represent bootstrap support (%) from 1,000 replicates; only values ≥70% displayed. Scale bar = 0.0010 substitutions per site. Tree inferred using Maximum Composite Likelihood distances in MEGA11.

3.2. The Clinical Disease Caused by SaIa ST7 CC1 is Similar and Age-Dependent Throughout LAM.

3.2.1. Clinical Signs and Gross Pathology

Outbreaks of piscine streptococcosis caused by *SaIa* ST7 CC1 was reported in all age ranges or farming phases of tilapia in LAM (FRY, PGO, GO) (Figure 3). In larvae/fry, fish streptococcosis was mainly characterized by anorexia (75.0%), lethargy (83.9%), erratic swimming and whirling (81.7%), cerebral hemorrhage (18.6%), ascites (21.1%), stomach/intestinal hemorrhage (74.4%), intussusception (43.3%) (Figure 4) and, in several cases, only by hyperacute mortality (Table 2).

In larger fish, both in the PGO and GO, the piscine streptococcosis was characterized primarily by anorexia (86.7%), lethargy (79.4%), erratic swimming and whirling (87.8%), darkening of the skin (66.1%), unilateral/bilateral exophthalmos (73.9%), and corneal opacity (71.7%). Less frequent were cases in which fecal strings protruding from the anus and/or floating in the water were observed (25.6%), C-shaped spinal curvature (24.4%), and hemorrhagic-purulent skin ulcers in the perianal region (Table 2) (Figure 3).

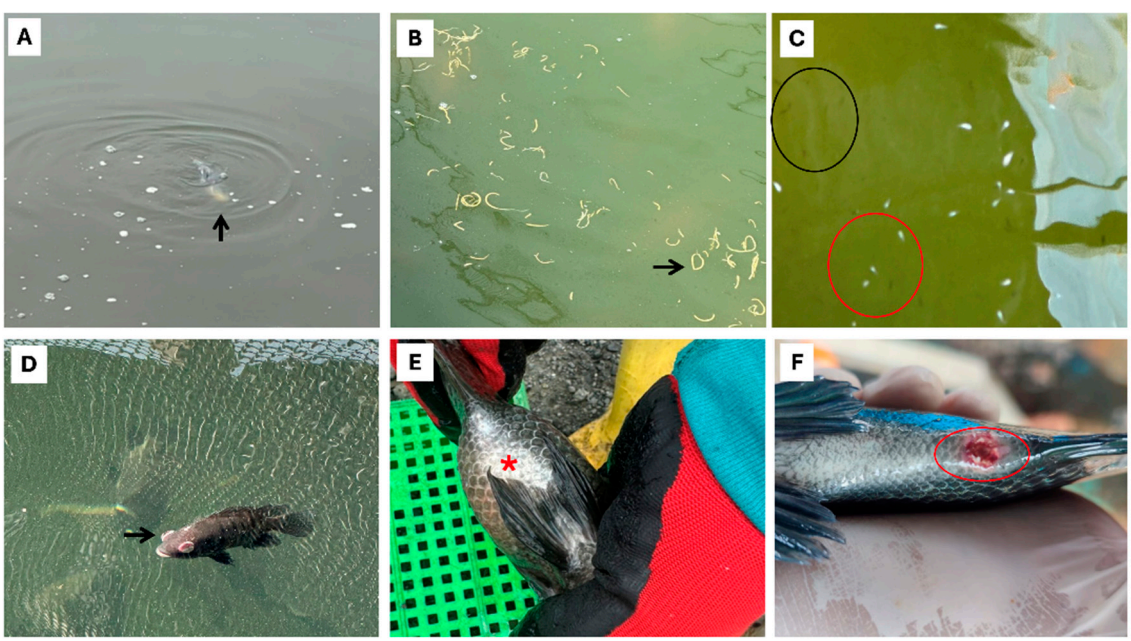

Figure 3. External signs and systemic manifestations in farmed tilapia at different stages of production in six LAM countries. (A) Erratic swimming, whirling, and lateral or vertical buoyancy in PGO/GO fish; (B) Erratic swimming and whirling in fry; (C) Several fecal strings/mucoid fecal casts floating in the water; (D) Bilateral exophthalmos (bulging eyes), corneal opacity (whitish eyes), and hemorrhage; (E) Darkening of the skin and abdominal distension; (F) Hemorrhagic-purulent skin ulcers in the perianal region.

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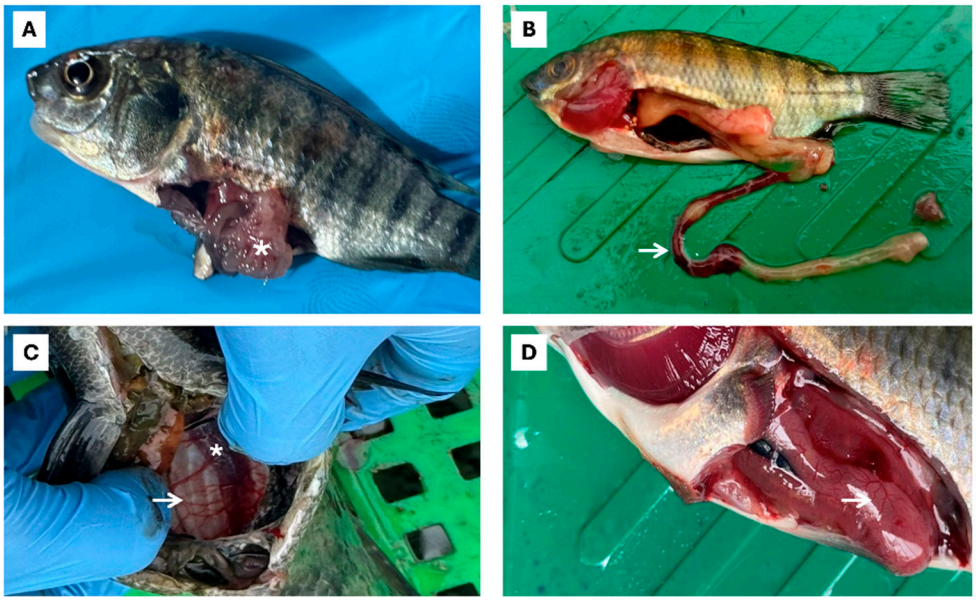

Figure 4. Internal macroscopic lesions in farmed tilapia at different stages of production in six LAM countries. (A) Thinned wall with enteritis and serosal hemorrhage, and intestinal intussuseption (white asterik); (B) Hemorrhagic areas and infarcts due to intestinal intussusception (white arrow); (C) Congested swim bladder (white arrow) and renomegaly (white asterik); (D) Hepatic hemorrhage and congestion (white arrow).

Table 2. Frequency of clinical and pathological findings in farmed tilapia in different age ranges and production stages in six LAM countries.

Clinical and pathological findings	FRY (<5 g; n=180)	PGO/GO (≥5 g; n=180)
Clinical signs		
Loss of appetite or anorexia	75,0%	86,7%
Lethargy and dying individuals on the shores	83,9%	79,4%
Erratic swimming, spiraling or uncoordinated movements, loss of buoyancy control	81,7%	87,8%
Abdominal distension	21,7%	68,3%
C-shaped spinal curvature	15.6%	24.4%
Fecal strings (protruded from the anus and/or in varying quantities in the water)	9.4%	25.6%
External inspection		
Head & eyes		
Unilateral or bilateral exophthalmos (pop-eyes)	0,0%	73,9%
Corneal opacity (whitish or opaque eyes)	9,4%	71,7%
Bleeding in the eyes	7,2%	25,6%
Abscesses in the jaw or head region	0,0%	14,4%
Skin & fins		
Hemorrhages at the base of the fins and tail	0,0%	22,8%
Hemorrhagic-purulent skin ulcers in the perianal region	0,0%	20,6%
Fin erosion	12,8%	34,3%
Darkening of the skin	18,9%	66,1%
Petechiae on body surface and operculum	10,6%	24,4%
Gills		
Gill pallor	17,2%	31,7%
Whitish areas on the gill surface	6,1%	37,7%

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Internal inspection		
Coelomic cavity		
Serosanguineous ascites	21,1%	51,7%
Multiple abdominal adhesions	0,0%	37,8%
Purulent-appearing material	0,0%	15,6%
Heart		
Pericarditis or whitish discoloration of the heart	0,0%	62,2%
Presence of purulent material in the pericardial sac, and/or epicardium	0,0%	67,8%
Brain cavity		
Cerebral edema	18,3%	68.3%
Cerebral hemorrhage	20,6%	66,1%
Presence of yellowish purulent material and opacity of the meninges	22,8%	62,8%
Stomach & Intestines		
Hemorrhage and congestion	74,4%	81,1%
Intussusception	43,3%	17,2%
Hepatopancreas		
Irregular appearance and coloration; with pale and congested areas	12,8%	74,4%
Fibrinous adhesions	0,0%	18,3%
Abscesses	0,0%	11,7%
Spleen		
Splenomegaly	9,4%	57,8%
Presence of pale areas	0,0%	9,4%
Kidneys and swim bladder		
Renomegaly	0,0%	7,2%
Pallor	0,0%	63,9%
Gas accumulation in the swim bladder and congestion	18,3%	53,3%
Skeletal muscle		
Abscesses (calcified or not)	0,0%	15,6%

Abdominal distension due to gas accumulation in the swim bladder or bloody fluid in the coelomic cavity (68.3%), pericarditis or whitish discoloration of the heart (62.2%), and the presence of yellowish purulent material and opacity of the meninges (62.8%) (Figure 5) were also observed (Table 2) Some interesting findings were the irregular appearance and coloration in the liver mixing pale and congested areas (68.3%) (Figure 4) and the presence of abscesses with purulent material and melanosis in the skeletal muscle recorded from fish weighing 100 g up to harvest weight (15.6%) (Figure 5).

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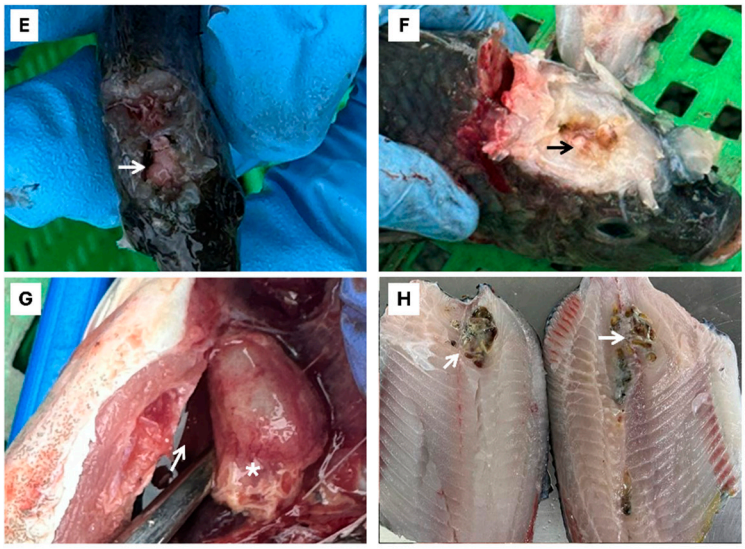

Figure 5. Internal macroscopic lesions in farmed tilapia at different stages of production in six LAM countries. (A) Meningeal hemorrhage (white arrow); (B) Thickening and opacity of the meninges and brain and accumulation of fibrinopurulent exudate in the brain cavity (white arrow); (C) Thickening and opacity of the epicardium/pericardium with the presence of adherent fibrous deposits (whitish/grayish fibrin) (white asterisk), and accumulation of fibrinopurulent exudate in the pericardial cavity (white arrow). (D) Multiple foci with cavitation in skeletal muscle consistent with bacterial abscesses containing bloody, brownish-gray or yellowish green fibrino-purulent exudate, melanin, and dystrophic mineralization.

3.2.2. Histopathology

Histopathological examination of infected fish revealed marked microscopical changes (Supplementary Figure S1), including an intestinal mucosal hyperemia containing numerous coccoid bacteria, with mild to severe hemorrhage and mild to moderate mixed inflammatory reaction (89,3%), gastric mucosal hyperemia containing numerous coccoid bacteria (Figure 6), with epithelial ulceration and mild mixed inflammatory reaction (67,9%), acute mixed diffuse meningitis with moderate, multifocal, necrotizing mixed encephalitis (42,9%) (Figure 6), and severe diffuse splenic capillary dilation containing coccoid bacteria (Figure 7) and hyperplasia of melanomacrophage centers (42,9%). According to the macroscopic pathology in the liver of larger fish, the microscopic hepatic changes were characterized by the presence of intravascular and perivascular coccoid bacteria in the hepatic parenchyma with acute perivascular histiocytic inflammatory reaction and hyperemia (37.0%) (Figure 7), moderate hyperplasia of the bile ducts in the hepatic parenchyma (21.4%) and moderate centrilobular vacuolar hepatopathy (10.7%).

Overall, most fish showed intense colonization by Gram-positive coccal bacteria (Figure 6 and 7), especially distributed in the blood and various tissues/organs (stomach, intestines, brain, heart, spleen, liver), confirming the systemic nature of the infection and that bacterial invasion occurs through the bloodstream. The presence of granulomatous lesions, common in cases of chronic streptococcal infection, was not frequently observed in the tissues, suggesting a hyperacute course of the infection. The minimal inflammatory cell reaction is noteworthy; however, phagocytic cells containing bacteria were observed in the spleen and in the meninges/brain.

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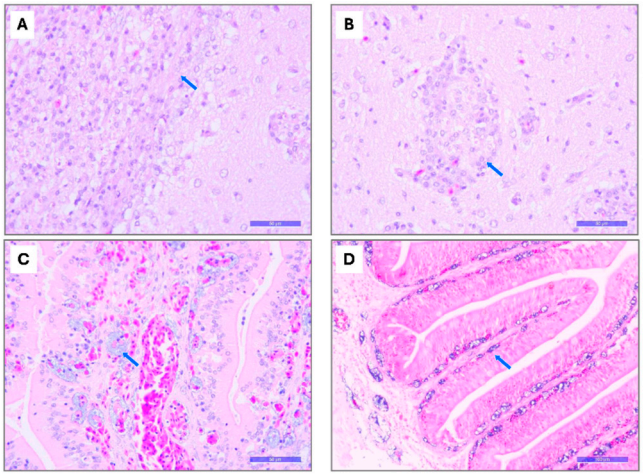

Figure 6. Main microscopic changes in tissue from farmed tilapia at different stages of production in six LAM countries. (A) Brain (H&E) Thickening of the meninges with mononuclear inflammatory cells (blue arrow) (Bar 50 um). (B) Brain (H&E) Histiocytic perivascular cuff (blue arrow) (Bar 50 um). (C) Intestine (H&E) Hyperemia and presence of cocci bacteria in the vascular lumen of the chorion (blue arrow) (Bar 50 um). (D) Intestine (Gram). Gram-positive cocci bacteria in the submucosa and lamina propria of the villi (blue arrow) (Bar 50 um).

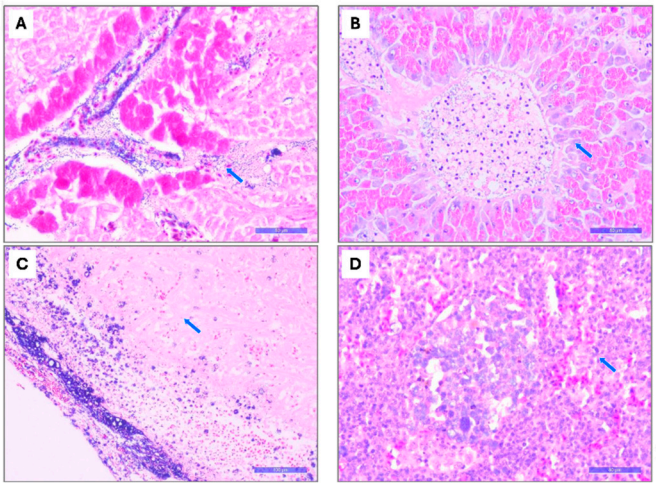

Figure 7. Main microscopic changes in tissue from farmed tilapia at different stages of production in six LAM countries. (A) Liver (Gram) Presence of Gram-positive cocci bacteria in the lumen and perivacular space of the hepatopancreas (blue arrow) (Bar 50 um). (B) Liver (H&E) Vacuolar depletion in the hepatic parenchyma with the presence of coccobacteria in the vascular luminal space (blue arrow) (Bar 50 um). (C) Heart (Gram). Thickening of the epicardium and presence of numerous gram-positive cocci (blue arrow) (Bar 100 um). (D) Spleen (H&E) Severe diffuse splenic capillary dilation with inclusion of coccal bacteria (blue arrow) (Bar 50 um).

3.3. LAM SaIa ST7 CC1 Shows a Uniform Virulence Profile

Although the LAM isolates of SaIa ST7 CC1 showed remarkable uniformity in the presence of the virulence genes (VGs) analyzed, slight differences grouped them into 2 profiles (arbitrarily named in this study as A, and B) (Table 3). The predominant VGs profile was *spb1-bca-cfb-bac-dtIR* (A) (Supplementary Figure S2), present in 60% (15/25) of the strains, followed by the *spb1-bca-cfb-dtIR* (B) profile in 40% (10/25). Each isolate from C1, C3, C4, and C5 showed profile A (4 out of 6 countries), while each isolate from C2 and C6 showed only profile B. All isolates from all countries showed no presence of *sodA* or *scpB*. Supplementary Table S3 systematically compares the virulence profiles obtained in this study with SaIa isolates described in China, Thailand/Vietnam, Indonesia, and Egypt.

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3.4. LAM SaIa ST7 CC1 Exhibits a Similar Pattern of Antimicrobial Susceptibility

One hundred percent (100%) of the SaIa ST7 CC1 isolates analyzed did not show expression of antibiotic resistance genes (ARGs) for erythromycin, phenicols or lincomycin (*ermB, ermTR, mefA, linB, fexA, fexB*) (data are not shown in Table 4). However, 20% (5/25) of the isolates, recovered from fish in 3 of the 6 countries, showed ARGs for OTC (*tetM* or *tetO*) (Table 4) (Supplementary Figure S3). The isolates exhibited susceptibility (16%, 4/25) and intermediate susceptibility (84%, 21/25) to OTC (Table 4). The 23.8% (4/21) of the isolated strains that showed intermediate susceptibility to OTC carried *tetM* or *tetO* (Table 4). The four susceptible isolates showed an MIC of 0.5 µm/mL for OTC, while the 21 isolates with intermediate susceptibility (I) showed an MIC between 1 and 4 µm/mL for OTC (Table 4).

No SaIa isolate showed ARGs for FFC (*fexA, fexB*), and all isolates showed susceptibility on the antibiogram with an inhibition zone between 30 and 36 mm (Table 4). The 84% (21/25) of the isolates had an MIC <1 µm/mL and 16% (4/25) had an MIC of 2 µm/mL for FFC. Similarly, all isolates (25/25) showed susceptibility to AMX with an inhibition zone between 30 and 46 mm and an MIC ≤0.5 µm/mL (Table 4). Finally, all isolates (25/25) showed intermediate sensitivity to ENR with an inhibition zone between 21 and 26 mm and an MIC of 4 µm/mL (Table 4). Overall, the phenotypic susceptibility data suggest that the ST7 CC1 clone currently circulating in LAM has not yet acquired clinically relevant antimicrobial resistance, though continued surveillance is warranted.

Table 3. Description of virulence gene expression profiles obtained from the 25 SaIa isolates recovered from the six LAM countries (arbitrarily named in this study as A, B and C).

Gene	Product	Main function	C1 (n=5)	C2 (n=5)	C3 (n=3)	C4 (n=5)	C5 (n=2)	C6 (n=5)	Total (n=25)
spb1	Spb1 surface protein	Invasion of epithelial cells	+	+	+	+	+	+	100%
bca	αC protein (α antigen)	Adherence, invasion, resistance to phagocytosis	+	+	+	+	+	+	100%
cfb	CAMP factor	Pore-forming cytolysin	+	+	+	+	+	+	100%
dltR	D-alanine regulator	Resistance to antimicrobial peptides	+	+	+	+	+	+	100%
bac	βC protein (β antigen)	IgA binding, factor H; immune evasion	+	-	+	+	+	-	60%
sodA	Superoxide dismutase A	Protection against oxidative stress	-	-	-	-	-	-	100%
scpB	C5a peptidase	Evasion of neutrophil recruitment	-	-	-	-	-	-	100%
Virulence gene profiles (n=25)			**A**	**B**	**A**	**A**	**A**	**B**	

4. Discussion

4.1. Molecular Epidemiology

The high genetic homogeneity finding among SaIa ST7 CC1 isolates reflects the recent clonal expansion occurring in LAM tilapia production from a molecular epidemiological perspective

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[17,22,34,40]. MLST is useful for initial molecular epidemiology, but it offers limited resolution when it comes to reconstructing routes of introduction, microevolution, or the number of independent dissemination events; therefore, whole-genome sequencing is strongly recommended for future studies. The close phylogenetic relationship of these new isolates to strains of Asian SaIa ST7 CC1 suggests an epidemiological connection between the two regions consistent with a history of movements of live tilapia broodstock and fingerlings connecting them [41]. Though the data available do not suffice to reconstruct direct transmission lines, introduction by trade in live fish retains plausibility among scenarios that should be investigated in import risk analysis.

4.2. Pathological and Clinical Disease

Clinically, SaIa ST7 CC1 led to a consistent but stage-modulated disease pattern on all farms assessed [42]. Hyperacute septicemia with anorexia, lethargy, erratic swimming behavior (swimming in circles), severe gastrointestinal hemorrhage and frequent intestinal intussusception were dominant in FRY with rapid development to high cumulative mortality during short timespans [43]. This rapid systemic dissemination often concludes with the death of the fish within 48 hours, preventing the development of distinctive neurological signs or generalized hemorrhage [44–48]. Intestinal intussusception, although rarely documented in piscine streptococcosis, could be observed in parasitic, viral and bacterial enteritis [49]. The detection of ST7 in larvae up to 0.5 g is consistent with early horizontal transmission in the hatchery or vertical transmission, although this study did not aim to obtain direct evidence of parental infection. Vertical transmission of S. agalactiae from parents to fry has been demonstrated in tilapia, even in the gonads of asymptomatic broodstock [45–47,50].

Neurological and ocular signs (erratic swimming, loss of buoyancy control, exophthalmos, and corneal opacity) were common alongside generalized hemorrhagic lesions and changes to the coelome in PGO and GO fish [34,44–48,51]. In adult fish, undigested fecal matter expelled externally through the anus (25.6%) was recognized as a clinically relevant manifestation of streptococcosis in tilapia. Although not pathognomonic, fecal strings of a similar nature have been detected in 20-40% of experimentally infected fish [52]. In addition, progressive abscesses of the skeletal muscle with dystrophic mineralization, such as cavitations (15.6%), especially in the caudal peduncle region, have significant consequences for fillet quality, carcass condemnation, and the potential risk of zoonosis [21,33,53].

The ubiquity of outbreaks across all production phases—from larvae to harvest-size fish—underscores the pathogen's capacity to evade host defenses regardless of age-related immunocompetence factors [46]. However, recent outbreaks of strains like ST283 have been documented in fish of all sizes, from fry to harvest-size adults, and even in broodstock, confirming its ability to affect multiple life stages and potentially cross species barriers as a zoonotic pathogen [26,42,54,55].

Histopathological analyses showed heavy Gram-positive cocci colonization of tissues in brain, spleen, liver and intestine, with severe acute circulatory and inflammatory damage associated to the organs in absence of significant chronic granulomatous responses. The rapid systemic spread, strong neurotropism, and very-low-degree chronic inflammation is indicative of a highly virulent hyperacute pathogenesis associated with *S. agalactiae* lineages to sensitive tilapia hosts. Comparative pathogenicity studies have revealed that ST7 consistently produces more severe histopathological lesions in the brain, liver, and spleen compared to ST283, suggesting differences in tissue tropism and pathogenic mechanisms [34]. The microscopical changes described for SaIa are consistent with the results of this study, which also showed a high frequency of gram-positive cocci in the brain, demonstrating the ability of SaIa ST7 CC1 to cross the blood-brain barrier (BBB) and establish severe meningoencephalitis in farmed juvenile and adult tilapia.

The repeated temporal link between outbreaks of SaIa ST7 CC1 and extended durations of elevated water temperature indicates that thermal stress could be a key component in amplifying disease within the relevant production systems [41,56]. All affected farms sustained temperatures exceeding 32°C for significant periods with pronounced day–night thermal differentials and low

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dissolved oxygen, or conditions known to have detrimental effects on tilapia physiological resilience and immune performance. While similar environmental data from non-afflicted farms were unavailable to quantify the relative risk, the concurrent timing of outbreaks with thermal anomalies, combined with experimental data, lends support to a model in which high temperature and the associated stressors lower the threshold for expression of SaIa ST7 CC1 disease.

Genetically improved lines, including the widely disseminated GIFT strain and commercial derivatives, were selected for rapid growth [57–61] and feed efficiency [62,63] at optimal aquaculture temperatures (27-30°C) [64–66]. Genetically improved farmed tilapia exhibit significantly lower heat tolerance compared to native and locally-adapted Nile tilapia strains, with thermal tolerance differences ranging from 3.6°C to 4.0°C, at lethal temperature thresholds [61,67–69]. Concurrent research indicates that heat stress compromises tilapia immune function [70,71], and facilitates the expression of pathogen virulence factors [13,61,71].

At the same time, biosecurity protocols designed for stable temperatures fail when thermal stress pushes systems to >32°C [6,14,72,73]. The El Niño phenomenon (2023-2024) generated significant environmental changes on the continent [74], primarily water scarcity and high temperatures. These findings highlight the importance of combining temperature observations into imperatives for streptococcosis risk assessments and adapting farm management practices accordingly to reduce climate-induced spillover of disease.

4.3. Virulence Gene Architecture and Host-Adapted Pathogenesis

The highly conserved VG repertoire in SaIa ST7 CC1 isolated from different environments in six LAM countries suggests that the clone has successfully established itself in a stable set of determinants that provide a substantial selective advantage in tilapia, regardless of local environmental conditions [33,75]. In contrast, ST7 isolates from non-tilapia hosts are more heterogeneous suggesting adaption to tilapia was mediated by host-specific genomic specialization [33,75,76].

The predominant VG profile detected in LAM isolates (*spb1-bca-cfb-dltR*), which is defined by the universal absence of *sodA* and *scpB*, however differed from the ten profiles found in seminal Chinese tilapia isolates, where a *dltR-bca-sodA-spib-cfb-bac* combination was common [33]. The regional divergence highlights genomic plasticity for *S. agalactiae* and suggests a model of local adaptation that incorporates acquisition as well as loss of specific determinants [19,23,33,77].

The conserved presence of *cfb* (CAMP factor) in all isolates in this study—despite its variable frequency in human and bovine SaIa ST7 isolates [33,34,78–80], suggests that CAMP-mediated cytotoxicity is a critical determinant of pathogenesis in tilapia, where phagocyte lysis is a central immune evasion mechanism. CAMP-test negative (lacking the *cfb* gene) *S. agalactiae* strains in 2013 were reported as CAMP-test positive by 2023, suggesting adaptive shifts in virulence traits under warmer conditions [14]. In contrast, the pervasive absence of *sodA* and *scpB* suggests that these strains have transitioned from a paradigm based on oxidative stress-resistance and complement inactivation to one more rapid and cytolytic in nature, mediated by CAMP factor and capsular C antigen (*bca*), supplemented with adhesion/invasion (*spb1*), resistance to antimicrobial peptide killing (*dltR*) and evasion of innate immunity (*bac*).

This profile is mechanistically consistent with the observed pattern of rapid bacteremia, hemorrhagic septicemia and meningoencephalitis. Additional virulence factors, including *cylE*, *lmb* and *hylB* which are common to other piscine CC1 lineages [23,34,40,81] were not investigated in this study but should be prioritized in future genomic work to fully account for the neurotropic phenotype. These findings have immediate implications for vaccine design [17,20]; they lend support to strategies aimed at targeting conserved SaIa ST7 CC1 antigens and suggest that prime-boost regimens rather than single injectable doses may be needed to elicit effective protection.

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4.4. Antimicrobial Susceptibility and Early Resistance Alarms

All SaIa ST7 CC1 isolates were phenotypically susceptible to FFC and AMX, according to the interpretive criteria applied suggesting that this emergent clone has not yet developed robust resistance to key aquaculture therapeutics deployed in LAM [82]. However, 84% and 100% of the isolated strains showed intermediate susceptibility to OTC and enrofloxacin ENR, respectively. This pattern contrasts with reports from other geographic regions, where *S. agalactiae* and related fish-pathogenic streptococci exhibit reduced susceptibility or established resistance to commonly used antimicrobials [40,83,84].

The intermediate susceptibility to OTC may be preliminarily linked to the sporadic detection of *tetM* and *tetO* genes in ST7 CC1 isolates; but, since this study assessed only the presence or absence of these genes on mobile genetic elements, without evaluating functional expression, the clinical and epidemiological significance of this finding remains uncertain. To characterize the trajectory and stability of resistance in this clone, more detailed genomic analyses such as whole-genome sequencing to elucidate the genetic context of ARGs and functional studies on gene expression under different antimicrobial and environmental conditions will be needed.

4.5. Implications for Surveillance, Biosecurity and Future Research

Integrated evidence shows that SaIa ST7 CC1 constitutes an emerging threat for tilapia aquaculture streptococcosis in LAM, with high virulence across production stages and associated to a geographically dispersion, thermal stress, and early acquisition of ARGs. Professional control will need integrated, diversely scaled intervention systems rather than farm level solutions. Priority measures include the introduction of molecular screening (MLST or whole-genome sequencing) of imported broodstock and fingerlings to screen against entry and re-entry of high-risk clones; the establishment of harmonized regional outbreak reporting and molecular surveillance networks to monitor SaIa ST7 CC1 and related lineages; and incorporation of climate-adaptive management strategies such as temperature- and dissolved oxygen monitoring, stocking density adjustments, and movement restrictions during intervals associated with increased thermal risk. The design and deployment of serotype-matched vaccines working against SaIa ST7 CC1, placed in the context of wider biosecurity plans, will ultimately be key to reducing dependence on antimicrobials and increasing tilapia production system resilience.

In future studies, whole-genome characterization of SaIa ST7 CC1 isolates should target multiple countries and time points to investigate virulence determinants, antimicrobial resistance gene content, and microevolutionary patterns under regional selective pressures. Controlled infection experiments in tilapia with distinct genetic backgrounds under the same temperature and environmental regimes would help unravel the relative roles of pathogen genotype, host lineage, and thermal stress on clinical disease outcomes and survival. These efforts, followed by longitudinal field studies integrating environmental monitoring with production data and pathogen genomics will provide the basis to improve risk models, inform selective breeding programs for disease and heat tolerance, as well as guide evidence-based policies of antimicrobial use and fish movement in the LAM tilapia industry.

Table 4. Comparative characterization of ARGs detection, antibiotic susceptibility testing results, and MIC values for OTC, FFC, AMX, and ENR obtained for the SaIa ST7 CC1 clone isolated from Nile tilapia raised in six different Latin American countries.

Region	Country	Bacterial ID	Gene AMR		OTC		FFC		AMX		ENR	
			tetM	*tetO*	mm	µm/mL	mm	µm/mL	mm	µm/mL	mm	µm/mL
CAM	C1	C1-A1			S (30)	0,5	S (30)	0,5	S (35)	≤0,5	I (25)	4,0

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		C1-A2		S (30)	0,5	S (34)	0,5	S (34)	≤0,5	I (25)	4,0
		C1-A3		S (30)	0,5	S (30)	0,5	S (32)	≤0,5	I (25)	4,0
		C1-A4		S (30)	0,5	S (30)	0,5	S (30)	≤0,5	I (25)	4,0
		C1-A5	+	I (26)	1,0	S (30)	0,5	S (30)	≤0,5	I (24)	4,0
	C2	C2-A1	+	I (22)	4,0	S (32)	1,0	S (35)	≤0,5	I (23)	4,0
		C2-A2	+	I (21)	4,0	S (31)	2,0	S (36)	≤0,5	I (23)	4,0
		C2-A3		I (28)	4,0	S (36)	1,0	S (40)	≤0,5	I (21)	4,0
		C2-A4		I (21)	4,0	S (33)	2,0	S (38)	≤0,5	I (21)	4,0
		C2-A5		I (21)	4,0	S (32)	1,0	S (35)	≤0,5	I (24)	4,0
SAM	C3	C3-A1	+	I (28)	4,0	S (35)	1,0	S (46)	≤0,5	I (22)	4,0
		C3-A2	+	I (28)	4,0	S (36)	<0,5	S (40)	≤0,5	I (21)	4,0
		C3-A3		I (27)	2,0	S (36)	1,0	S (41)	≤0,5	I (22)	4,0
	C4	C4-A1		I (23)	2,0	S (30)	1,0	S (35)	≤0,5	I (21)	4,0
		C4-A2		I (25)	2,0	S (30)	2,0	S (35)	≤0,5	I (21)	4,0
		C4-A3		I (23)	1,0	S (30)	1,0	S (34)	≤0,5	I (23)	4,0
		C4-A4		I (24)	2,0	S (31)	1,0	S (35)	≤0,5	I (21)	4,0
		C4-A5		I (24)	1,0	S (30)	1,0	S (33)	≤0,5	I (21)	4,0
	C5	C5-A1		S (30)	0,5	S (31)	1,0	S (40)	≤0,5	I (25)	4,0
		C5-A2		S (30)	0,5	S (33)	1,0	S (41)	≤0,5	I (23)	4,0
NAM	C6	C6-A1		I (26)	2,0	S (31)	1,0	S (39)	≤0,5	I (26)	4,0
		C6-A2		I (27)	2,0	S (31)	1,0	S (36)	≤0,5	I (24)	4,0
		C6-A3		I (26)	4	S (30)	2,0	S (34)	≤0,5	I (23)	4,0
		C6-A4		I (25)	2	S (31)	1,0	S (35)	≤0,5	I (23)	4,0
		C6-A5		I (24)	2	S (32)	1,0	S (46)	≤0,5	I (24)	4,0

5. Conclusions

The cluster of strains identified as SaIa ST7 CC1, associated with acute streptococcosis outbreaks in Nile tilapia across six countries in LAM, reflects the recent emergence of a clonal lineage exhibiting

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uniform yet age-dependent disease expression, both clinically and pathologically. In larvae and fingerlings, hyperacute septicemia with severe gastrointestinal involvement predominates, whereas in pre-grow-out (PGO) and grow-out (GO) fish, a systemic neurotropic disease with significant ocular and coelomic lesions is observed.

All field outbreaks coincided with intervals of high-water temperature exceeding 32°C and low dissolved oxygen, reinforcing the role of thermal and environmental stress as key edifiers of disease expression in intensively farmed tilapia under anthropogenic climate warming. Although SaIa ST7 CC1 retains phenotypic susceptibility to the critical aquaculture antimicrobials, its recent detection of *tetM* and *tetO* in a subpopulation of isolates suggests that tetracycline resistance determinants are already present and could emerge through inappropriate use of drugs.

These findings characterize SaIa ST7 CC1 as a threat to LAM tilapia production that warrants regional coordination. It is essential that these measures include molecular screening (MLST and, where possible, whole genome sequencing) of imported broodstock and fingerlings; harmonization of outbreak reporting and molecular surveillance networks; incorporation of temperature and dissolved oxygen control into risk-based farm management and movement control policies. At the same time, vaccination programs with compatible serotypes must be developed and implemented, along with prudent use of antimicrobials and continuous monitoring of susceptibility. All of this is aimed at maximizing mortality reduction, maintaining the effectiveness of antimicrobials, and improving the resilience of tilapia production systems against the introduction of pathogens and climate-related stress.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/doi/s1, Table S1: Primers used to determine virulence (VGs) and antibiotic resistance genes (ARGs) in 25 strains of S. agalactiae Ia isolated between 2021 and 2025 from larvae/fry, juveniles (PGO), and adults (GO) of Nile tilapia experiencing outbreaks of piscine streptococcosis in six Latin American countries.; .Table S2: qPCR (CTs) and MLST results for the 25 isolates from each Latin American country; Table S3: Systematic comparative analysis of the virulence profiles obtained in this study with the Sa1a isolates described in China, Thailand/Vietnam, Indonesia and Egypt; Figure S1: Frequency of microscopic changes observed in different tissues of fish undergoing outbreaks of streptococcosis caused by SaIa ST7 CC1 in all productive stages of tilapia farming in six Latin American countries; Figure S2: Results of conventional PCR amplification showing the expected band size for detection of the target virulence gene in the samples analyzed. The amplification products were analyzed by electrophoresis on 1.5% agarose gel with ethidium bromide dye. L1: molecular weight marker; L2-L8: sample amplicons for the respective target gene; C: control. The colored lines show amplicons of the isolates C6-A1 (white), C2-A3 (red), C2-A2 (yellow), and C2-A3 (turquoise); Figure S3: Results of conventional PCR amplification showing the expected band sizes for the detection of antimicrobial resistance genes in the samples analyzed. The amplification products were analyzed by electrophoresis on 1.5% agarose gel with ethidium bromide dye. L1: molecular weight marker; L2-L10: sample amplicons for the respective target gene; C: control. Amplicons for the tetO gene in isolates C2-A3 (red), C2-A2 (yellow), and C2-A3 (turquoise).

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