zoonotic diseases

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Case Report

Recurrent Penicillin-Resistant Tonsillitis Due to *Lactococcus garvieae*, a New Zoonosis from Aquaculture

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Simple Summary: *L. garvieae* is a Gram-positive bacterium present in the aquaculture of freshwater and marine fish. Some cases caused by this pathogen have recently been reported in immunocompetent patients. This report presents the first two cases of recurrent tonsillitis due to *L. garvieae*, and their effective treatment by auto-vaccination with lysed bacteria.

Abstract: Zoonotic diseases are a group of infectious diseases that are transmitted naturally from animals to humans. *L. garvieae* is a Gram-positive bacterium which is present in the aquaculture of freshwater and marine fish. Some isolated cases of infection have been described, considering it an opportunistic agent in immunosuppressed patients. The most recent appearance of severe infections in immunocompetent patients or colonizing cardiac prostheses has set off the alarms. This manuscript presents the first two patients with recurrent tonsillitis due to *L. garvieae.* A 15-year-old male and an 8-year-old male had recurrent tonsillitis with more than three episodes per year. A culture of tonsillar exudate in both cases showed growth of *L. garvieae* with an antibiogram showing multi-resistance to antibiotics. Given the parents' wish not to carry out surgery, an autovaccine regimen with lysed bacteria was proposed with good evolution and remission of tonsillitis episodes in both cases. The oral autovaccine produces an immunomodulatory effect and could be a therapeutic weapon in the prevention of this zoonosis. Further studies are needed to determine the importance of foodborne transmission in human *L. garvieae* infections and to find suitable treatments for this wide range of infections.

Keywords: zoonotic diseases; microbiology; infectious diseases; tonsillitis; otolaryngology; pediatric; lactococcosis; oral vaccine; antibiotic resistance

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1. Introduction

L. garvieae appeared in 1983 in a research paper in which the bacterium was isolated as a causative agent of bovine mastitis. It is a Gram-positive, facultative anaerobic, non-motile bacterium that does not produce endospores, and it can be present in the aquaculture of freshwater and marine fish. It is a mesophilic bacterium of coccoid morphology with a diameter of 0.5–1.5 µm, immotile, non-spore-forming, and may be arranged in pairs or in short chains. It is a facultative anaerobe, has a homofermentative metabolism, and produces only the L (+) form of lactic acid. It has complex nutritional requirements and auxotrophy for various amino acids and vitamins. *L. garvieae* is able to grow at pH 9.6, in the presence of 6.5% sodium chloride, and between 10 and 42 °C; however, its optimum growth temperature is 30 °C.

The appearance of marine farms with freshwater species scattered along the coast and in interior territories, has become a common image in recent years due to the growing

demand for seafood that cannot be satisfied solely by wild fishing [1]. It is the food production sector with the highest growth in the world (almost an average of 10% per year since 1984), currently being a fundamental activity to supply food to the population and responsible for the production of 50% of food fish worldwide [1]. Although they have gained prominence over the last 25–30 years, infectious processes in fish caused by Gram-positive bacteria were first described in the late 1950s in Japan, where the first cases affecting rainbow trout occurred.

Infections in aquaculture fish caused by Gram-positive micro-organisms have spread to all important fish farming countries, especially those in the Mediterranean basin, with outbreaks reported in the UK, France, Italy, Spain, Israel, South Africa, Korea, Japan, Australia, Taiwan, and the USA. Their severity has worsened, causing average losses of up to 50–60% of the farmed fish in spite of medical treatments, and with the consequent increase in economic losses on the affected farms. The signs are typical of an acute infection, with lethargy, anorexia, melanosis, marked bilateral exophthalmos, visceral serositis and hemorrhaging, severe enteritis, or notable spleen enlargement [2].

Although information on the prevalence of infection with this pathogen in aquaculture is available, to date there is no spatial study assessing this zoonosis in specific areas. Developments in the Geographic Information System (GIS) during the last 30 years have provided more powerful and efficient tools to investigate spatial patterns, being a worthwhile tool in studying infectious diseases [3]. These analyses could help in understanding the role of environmental patterns in the conditioning of the *L. garvieae* pathosystem, such as the cardiac pathologies previously reported [4]. *L. garvieae*, ingested with aquaculture fish, has been identified as a component of the intestinal and oral microbiota in humans [5]. Some isolated cases of infection have been described, considering it an opportunistic agent in immunosuppressed patients [6]. The most recent appearance of severe infections in immunocompetent patients or colonizing cardiac prostheses has set off the alarms [4]. To date, there are no reports of infection in the ear, nose, or throat area caused by this pathogen. The aim of this manuscript is to present the first two cases reported in the literature of recurrent tonsillitis due to *L. garvieae*, and the proposed therapeutic management based on existing evidence.

2. Cases Description

We present a 15-year-old male with a history of recurrent tonsillitis (1–2/month) for more than 5 years, and an 8-year-old male with recurrent tonsillitis (>3 episodes/year) in the last 2 years. The CARE guidelines methodology was followed [7]. Both patients had no other medical history of interest. Their laboratory values were within the normal range. The vaccination schedule was correct and complete for the age of the patients. The mother of the 15-year-old male also had a history of recurrent tonsillitis during childhood that required tonsillectomy. The other parents had no history of tonsillar infection. In both cases, the parents did not wish for surgery. The main characteristics are summarized in Table 1.

Table 1. Main characteristics of the two patients reported.

	PATIENT 1	**PATIENT 2**
Age & gender	15-year-old male	8-year-old male
Number of episodes	1–2/month for more than 5 years	>3 episodes/year in the last 2 years
Family history	Yes	No
Laboratory values	Normal	
Vaccination schedule	Complete	
Clinical presentation	Fever (>38 °C), general malaise, odynophagia, dysphagia, headache, bilateral whitish tonsillar exudates with hypertrophy (grade III), and bilateral reactive neck lymphadenopathies	

All episodes of tonsillitis presented with fever (>38 °C), general malaise, odynophagia and dysphagia, headache, bilateral whitish tonsillar exudates with hypertrophy (grade III), and bilateral reactive neck lymphadenopathies. The infectious conditions meant frequent absenteeism from school, affecting the child's quality of life. All cases required antibiotic treatment for 7–10 days. Penicillin derivatives or second- and third-generation cephalosporins were used as a first choice. On multiple occasions, a second-line amoxicillin-clavulanic acid combination was necessary to obtain the cure. In addition, coadjuvant non-steroidal anti-inflammatory drugs, and sometimes short courses of oral corticosteroids, were necessary.

Given the continuous episodes of tonsillitis and the need of amoxicillin-clavulanic acid, a culture of tonsillar exudate was performed in order to offer a targeted antibiotic therapy. The result was the growth of *L. garvieae* with an antibiogram showing resistance to penicillin, cephalosporins, macrolides, and quinolones in both cases. It was only sensitive to high doses of amoxicillin or in combination with clavulanic acid.

Based on these results, a combination of amoxicillin-clavulanic acid during the episodes, with a sublingual bacterial autovaccine with an inactivated suspension of the pathogen (Apither SCIT—Asacpharma), was proposed as treatment (two sublingual pulses per day for 3 months). The bacterial suspension was 1 billion bacteria/mL inactivated with formaldehyde developed according to previous studies [8]. With a follow-up period of more than 3 years, none of the patients underwent tonsillectomy. The 15-year-old male obtained a 2-year period in which he presented only two tonsillitis per year that were successfully treated with the proposed antibiotic. After these two years, he decided to stop the administration of the autovaccine, and the frequency of episodes increased again. The 8-year-old male presented an inter-episode period of tonsillitis of more than 6 months up to the present date.

3. Discussion

Zoonotic diseases are a group of infectious diseases that are transmitted naturally from animals to humans. Globalization and the need to supply the population with food have caused the expansion of aquaculture. These changes in the way we eat can promote the appearance of new diseases such as lactococcosis. *L. garvieae* is a Gram-positive bacterium belonging to the family *Streptococcaceae*. The current taxonomy recognizes seven species within the genus *Lactococcus*: *Lactococcus lactis* (with subspecies *lactis*, *cremoris* and *hordniae*), *L. garvieae*, *L. piscium*, *Lactococcus plantarum*, *Lactococcus raffinolactis*, *Lactococcus chungangensis*, and *Lactococcus fujiensis*. They are very ubiquitous microorganisms, with their most common habitats being plants, animal skin, milk, and milk products. Some of the species are used in the food industry for the preservation of meat products, as well as starters in the production of cheeses and as flavor enhancers.

Given the ubiquitous distribution of *L. garvieae*, several authors highlight the increasing importance of this bacterium as a potential zoonotic agent, although its incidence in this regard has not yet been demonstrated and the isolation from faecal samples of healthy people suggests that not all strains are pathogenic to humans or that not all humans are susceptible. Initially, it was considered an opportunistic pathogen of low virulence in mammals capable of infecting only immunocompromised hosts. However, the subsequent emergence of new clinical cases demonstrated the ability of the bacterium to infect both immunocompromised and immunocompetent individuals [6]. To date, there have been reported cases of endocarditis (also in prosthesis), bacteriemia, peritonitis, liver abscess, osteomielitis, espondilodiscitis, urinary infection, meningitis, acalculous cholecystitis, and hip prosthetic infection.

Current knowledge on the mechanisms of pathogenicity of *L. garvieae* is still very limited despite the fact that it is a pathogenic microorganism of recognized importance in aquaculture and of increasing significance in veterinary and clinical medicine. A common feature of many pathogenic bacteria is the expression of adhesion factors or adhesins of a polysaccharide or protein nature. The expression of these factors is a key step in the

initiation of the infectious process as it allows the bacteria to bind to receptors in the different tissues of the host, thus making it difficult for the host to eliminate them [9]. A prerequisite for the spread and progression of *L. garvieae* infection is thought to be mucosal colonization, as the bacterium has a high capacity to attach to fish intestinal cells as well as liver and epithelial cells [10]. Furthermore, *L. garvieae* has been isolated from a surprising number of biological samples such as: vegetables, cheeses, meat, and raw cow's milk samples, processed meat products, fermented fish products, or cat and dog tonsils [11]. Currently, the manipulation or ingestion of contaminated raw fish and seafood are the most probable sources of infection. This was the most probable route of contact of *L. garvieae* with the patients presented, settling in the tonsillar crypts and forming biofilms. It has been determined that host immune genetic variations play a crucial role in sensitivity toward tonsillar diseases [12]. This explains why, despite the ubiquity of this pathogen, not all patients are colonized. Biofilms enable the bacteria to resist environmental stresses such as oxidative stress, pH change, antibacterial substances, and the host immune system, which would explain the repeated infections despite antibiotic treatment in our cases.

L. garvieae resistance to clindamycin, erythromicin, streptomycin, tetracycline, oxytetracycline, florfenicol, and some quinolones have previously been reported [13]. The exchange of antibiotic resistance genes from aquatic bacteria to human and animal pathogens has been demonstrated, and the industrial use of antibiotics in aquaculture affects negatively the antibiotic therapy of animal and human bacterial infections. There are various methods to eradicate the *L. garvieae* during fish breeding. Empiric antibiotic treatments are initiated after diagnosis of *L. garvieae* infections. These treatments commonly consist of high doses of β-lactams, such as ampicillin, amoxicillin, or ceftriaxone.

Moreover, different vaccination strategies are currently available as a control measure for lactococcosis. Due to its resistance to antibiotics, a bacterial autovaccine of *L. garvieae* is considered a good preventive therapy in aquaculture [14]. The autovaccine is an inactivated microbial suspension from a sample obtained from the patient. This sample is cultured to identify the pathogenic microorganisms, which are then isolated and inactivated using standardised procedures, thus guaranteeing their therapeutic activity. Candidates for this type of therapy are patients with an established and usually chronic or acute relapsing condition, in which antibiotic therapy or other biological preparations have failed to eliminate the causative agent. As a result, a specific and individualized vaccine is obtained for each patient. Bacterial immunotherapy enables the creation of a defensive state of the organism against microorganisms or their toxic products, mainly by inducing IgA, IgG, and IgM antibodies and also by stimulating the defensive cellular response through activation of the T-helper lymphocyte system and macrophages.

It has been observed that a *L. garvieae* oral biofilm vaccine can increase specific antibody titers, enhance phagocytosis, and induce pro-inflammatory gene expression in animals [14]. To date, there are no human studies assessing the *L. garvieae* autovaccine, but it is possible that this treatment could prevent infections [8]. In a retrospective observational study with similar autovaccine in 177 children aged 1–15 years with recurrent acute tonsillitis, a decrease in the frequency of episodes of acute tonsillitis was observed, and after a median of 9 months follow-up, no tonsillectomy was required in 50% of treated patients. The oral autovaccine produces an immunomodulatory effect, acting on the innate and adaptive branches of the immune system, reducing the risk of viral and bacterial infections, and controlling inflammation [15]. Based on these findings, this treatment was proposed in our patients, with a decrease in the number of acute tonsillitis.

4. Conclusions

This manuscript presents the first two cases in the literature of recurrent tonsillitis secondary to *L. garvieae*. *L. garvieae* can be acquired by the ingestion of contaminated foods and it can colonize the tonsillar crypts, producing chronic inflammation or infection with periods of exacerbation. Furthermore, the antibiogram results show the relevance of *L. garvieae* given its high number of resistances. As it is the case in aquaculture, autovaccine

therapy could be a therapeutic weapon in the prevention of this zoonosis. Further studies are needed to determine the importance of foodborne transmission in human *L. garvieae* infections and to corroborate the observed efficacy of this immunotherapy.

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