fishes

MDPI

Article

Isolation and Identification of *Staphylococcus saprophyticus* from Diseased Hybrid Sturgeon

Yeying Wu [1,†], Yalan Feng [1,†], Mingyang Xue [2], Zidong Xiao [2], Lijuan Jin [3], Ren Gao [4], Yahong Chen [1], Tianwang Liang [1] and Yong Zhou [2,*]

[1] College of Life Sciences, Wuchang University of Technology, Wuhan 430200, China
[2] Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences, Wuhan 430223, China
[3] Wuhan Dynamic Life Science Co., Ltd., Wuhan 430223, China
[4] Zhaoqing Dahuanong Biology Medicine Co., Ltd., Zhaoqing 526238, China
* Correspondence: zhouy@yfi.ac.cn
† These authors contributed equally to this work.

Abstract: Hybrid sturgeon is an important economic fish species in China. In 2021, a bacterium was isolated from the liver and kidneys of freshwater-farmed hybrid sturgeon in Yichang City, Hubei Province, causing a disease with high mortality and surface bleeding. Through morphological observation, 16S rDNA gene sequence analysis, pathogenicity, an antimicrobial sensitivity test, as well as serum physiological and biochemical analysis, it was identified as *Staphylococcus saprophyticus* and named E702. The 16S rDNA gene sequence of E702 is highly homologous to *S. saprophyticus* in GenBank. Phylogenetic analysis showed that E702 and *S. saprophyticus* clustered into one clade. The 50% lethal dose of E702 was 2.14×10^5 CFU/g. The percentages of monocytes and eosinophils were markedly increased in the diseased sturgeon's blood, whereas the percentages of platelets and lymphocytes were decreased. The activity levels of aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in the diseased fish were significantly increased. The diseased fish suffered obvious damage to many tissues and organs, especially the liver and kidney, showing swelling, hyperemic and inflammatory cell infiltration. E702 was sensitive to antibiotics such as neomycin, cefazolin, norfloxacin, carbenicillin, gentamicin and ciprofloxacin. The study not only proved that *S. saprophyticus* was responsible for a great deal of hybrid sturgeon deaths, but also shed light on its potential risks in hybrid sturgeon farming. The research results provided the theoretical basis for the diagnosis as well as prevention of this disease.

Keywords: hybrid sturgeon; *Staphylococcus saprophyticus*; histopathology; hematological parameters; drug sensitivity

Key Contribution: A bacterial strain was isolated from artificially bred diseased hybrid sturgeon, an important commercial fish. The strain (E702) was identified as *Staphylococcus saprophyticus* by 16S rDNA sequencing and biochemical analysis. A hispathological analysis of the hybrid sturgeon infected with *S. saprophyticus* was carried out. An antimicrobial sensitivity test showed that the E702 was sensitive to various antibiotics. *S. saprophyticus* was the cause of lesions in multiple organs for the breeding hybrid sturgeon.

Citation: Wu, Y.; Feng, Y.; Xue, M.; Xiao, Z.; Jin, L.; Gao, R.; Chen, Y.; Liang, T.; Zhou, Y. Isolation and Identification of *Staphylococcus saprophyticus* from Diseased Hybrid Sturgeon. *Fishes* **2023**, *8*, 250. https://doi.org/10.3390/fishes8050250

Academic Editors: Jiong Chen and Li Lin

Received: 4 March 2023
Revised: 2 May 2023
Accepted: 6 May 2023
Published: 9 May 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Acipenseriformes is the only extant order of cartilaginous animals, represented by sturgeon and white sturgeon. It is the "living fossil" of the origin and evolution of fish and even vertebrates, so it is of great importance in science and research [1]. In addition, the sturgeon also has extremely high economic value. Caviar, which has been likened to "black soft gold", is usually made from lightly salted sturgeon roe

(http://language.chinadaily.com.cn/2011-11/01/content_14013902.htm, (accessed on 1 November 2011)) [2]. Traditional caviar comes from wild sturgeon [3]. However, due to the influence of human factors such as dams being built, environmental pollution and ecological destruction, wild sturgeons are gradually diminishing in number and are even endangered [4]. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) listed the all the world's existing sturgeons in Appendix II of the CITES in 1998 and implemented a wild sturgeon fishing and caviar export quota system. Owing to the decline in resources as well as in production year by year, the supply of caviar falls short [5,6]. It is an inevitable trend to produce caviar from farmed sturgeon instead of wild sturgeon. The hybrid sturgeon (*Acipenser baerii* ♀ × *Acipenser schrenckii* ♂) obtained from the hybridization between *Acipenser baerii* and *Acipenser schrenckii* is one of the main commercial breeding species in China, with significant economic benefits [7]. However, infectious diseases including bacterial [8], fungal [9] and viral [10] diseases have always limited the scale and quality of hybrid sturgeon farming.

In 2021, a mass of hybrid sturgeons died in Yichang, a city in Hubei province, China. This study aimed to investigate the etiology of this disease. The reason for the disease was clarified through a series of experiments such as morphological observation, biochemical identification, 16S rDNA gene sequence analysis and physiological and biochemical analysis of the serum. Finally, a strain was isolated from the liver and kidney of the diseased hybrid sturgeons. Further drug susceptibility testing was performed to determine the highly sensitive drugs for the treatment of the disease, so as to achieve better therapeutic effects and reduce the generation of drug-resistant strains. This study provided a theoretical basis for the follow-up pathogenesis research of this pathogenic bacteria and offered guidance for the prevention and treatment of diseases caused by this pathogen.

2. Materials and Methods

2.1. Fish

In 2021, a large number of breeding hybrid sturgeons died in a breeding base in Yichang, China, and diseased fish were collected for further experiments. The healthy hybrid sturgeons used for pathogenicity tests came from Yichang, and the fish length was (15 ± 2) cm. Healthy hybrid sturgeons (about 500 fish) were reared in 25 aquariums (0.8 m × 0.6 m × 0.6 m) at a temperature of 20 ± 1 °C and fed twice a day. Fish with similar growth and weight were selected for subsequent experiments. All experimental procedures were conducted according to the guidelines of the appropriate Animal Experimental Ethical Inspection of Laboratory Animal Centre, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences (ID: YFI2021-zhouyong-15).

2.2. Pathogen Isolation

The surface of the sick fish was sterilized with 75% ethanol and placed in a biological safety cabinet for dissection. Liver and kidney tissues were homogenized and spread on brain heart infusion (BHI) agar plates [11], and the plates were incubated at 28 °C for 36 h. Subsequently, individual colonies were selected by inoculation loops and streaked on new BHI agar plates for further isolation and purification. The purified strain E702 was inoculated into 800 μL of liquid BHI medium and cultured at 28 °C at 200 rpm, until the OD600 of the bacterial liquid was about 0.5. An amount of 100 μL of bacterial solution was used for Gram staining, and 600 μL of the bacterial solution was mixed with 300 μL of 50% glycerol solution and stored at −80 °C.

2.3. Morphological Observation

Bacteria were collected by centrifugation at 5000 rpm for 2 min and suspended in phosphate-buffered saline (PBS). The suspension was then spread onto glass slides to dry, followed by Gram staining, as described by Xiao [11]. In addition, the strain was observed with scanning electron microscopy and recorded.

2.4. 16S rDNA Sequence Analysis

The nucleic acid of strain E702 was extracted using the Bacterial Genomic DNA Kit (Axygen, Somerville, MA, USA), and the 16S rDNA gene of strain E702 was amplified using universal primers (F: 5′-AGAGTTTGATCATGGCTCAG-3′ and R: 5′-TACGGTTACCTTGTTACGACTT-3′) [12]. The amplification program was as follows: 30 s at 96 °C, 45 s at 57 °C, and 1 min at 72 °C for 32 cycles. The amplified products were identified by 1.0% agarose gel electrophoresis (Liuyi, China). The target bands were recovered and sequenced by Tsingke Bio-technology Co., Ltd. (Wuhan, China). The sequencing results were analyzed through the NCBI website (http://blast.ncbi.nlm.nih.gov, (accessed on 1 May 2022), and a phylogenetic tree was constructed with the software MEGA 4.0.

2.5. Biochemical Identification of Bacteria

The E702 strain was grown on Biolog Universal Anaerobe (BUA) agar medium at 30 °C for 24 h. In terms of the operating requirements of the Biolog bacterial identification kit (Biolog, Hayward, CA, USA), a single colony was selected and placed in the IF-inoculum (Biolog, USA). After the Biolog bacterial identification plate was inoculated with the inoculum containing E702 at a dose of 100 μL per well, it was placed in the Biolog automatic microorganism identification system for identification, and the results were recorded.

2.6. Toxicity Identification

Strain E702 was grown in liquid BHI medium for 20 h at 200 rpm with shaking at 30 °C. Bacteria were collected after centrifugation (5000 rpm, 2 min), and the pellet was rinsed twice with aseptic PBS (Cytiva, Washington, DC, USA). Bacteria suspensions of distinct concentrations (10^4, 10^5, 10^6 and 10^7 CFU/mL) were prepared with sterile PBS after the concentration was detected by a bacterial colony counter. The healthy hybrid sturgeons were randomly separated into 5 groups (4 groups for infection test, 1 group for control test), with 30 fish in each group. The infection test group was injected with 0.3 mL bacterial suspension with the above distinct concentrations, respectively, and the control group was injected with 0.3 mL aseptic PBS. After infection, the number of deaths in each group was observed and recorded for 14 consecutive days. The pathogenic bacteria were isolated and re-identified from the diseased fish. Experiments were performed in triplicate with biological replicates. The 50% lethal dose (LD50) value of the E702 strain was calculated with the method of Reed and Muench [13].

2.7. Histopathological Observation

Pathological tissues were sampled from four parts of each diseased fish, including the gills, liver, kidneys and intestines, and these were sectioned for observation. The significantly changed ones were selected for analysis. These collected organs and tissues were fixed with 4% paraformaldehyde. Histopathological section preparation included ethanol gradient dehydration (80%, 90%, 95% and 100%), paraffin embedding, sectioning (5 μm thickness, Leica, Wetzlar, Germany), hematoxylin-eosin staining (Solarbio, Beijing, China) and glass slides sealed with neutral resin [14]. Pathological changes in different tissues were observed by light microscopic examination of histopathological sections (Mount Olympus, Tokyo, Japan) [15].

2.8. Analysis of Blood Parameters

Blood was collected from the tail vein of the control (n = 6) and diseased hybrid sturgeon (n = 6) via sterile disposable syringes and used for smear preparation. By staining with Wright-Giemsa stain (Baso, Zhuhai, China), the cells on each blood smear were observed, classified and counted under the oil lens [16].

Blood was collected in a 2 mL centrifuge tube and left at 4 °C for 4 h before centrifugation at 5000 rpm for 15 min. The supernatant was analyzed with a biochemical analyzer (HITACHI, Hitachi City, Japan), and the data were analyzed with Excel software (Microsoft Office software 2013).

2.9. Antimicrobial Sensitivity Testing

The Kirby–Bauer disc diffusion method was adopted to perform antimicrobial sensitivity testing [17]. A suspension of strain E702 was spread evenly on the surface of Mueller–Hinton (MH) agar medium, with 100 μL of liquid bacterial suspension added to each plate, and 4 antimicrobial sensitivity test paper discs were then placed equally on the surface of the medium. Subsequently, the diameter of the inhibition zone was measured after 24 h of incubation at 28 °C. Vancomycin, furazolidone, streptomycin, chloramphenicol, gentamicin, neomycin, cefazolin, norfloxacin, carbenicillin and ciprofloxacin (Hangwei, Hefei, China) were selected for the antimicrobial sensitivity test.

2.10. Statistical Analyses

Statistical analyses were carried out using the SPSS software (version 13, SPSS, Chicago, IL, USA). Differences between means of treatments were performed with Duncan's test, with means considered significantly different at $p < 0.05$.

3. Results

3.1. Symptoms of Diseased Hybrid Sturgeons

The diseased fish were about 60 cm long (Figure 1A), and their mobility was reduced. The blood spots on both sides of the abdomen were evenly and symmetrically distributed (Figure 1A), and the cloacas were red or even purple (Figure 1A,B). The swim bladder, liver, kidneys, intestines and stomach all had varying degrees of hyperemia and swelling (Figure 1B). No parasites were found on the body surface, gills or in vivo.

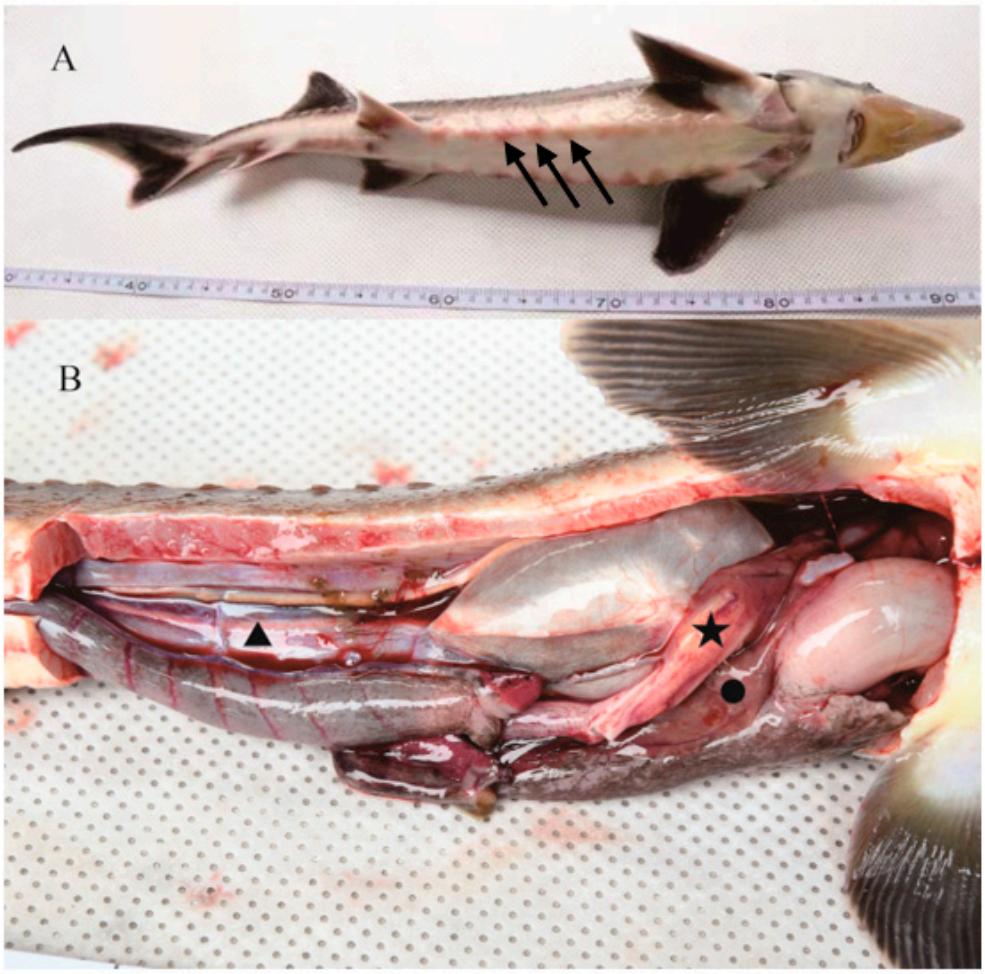

Figure 1. Clinical symptoms of E702-infected hybrid sturgeon. (**A**): The cloacas is red, and the abdomen is hyperemic (arrows). (**B**): Ascites (triangle), liver hemorrhage (dot), intestinal hyperemia (asterisk).

3.2. Bacterial Morphological Analysis

The bacteria isolated from diseased fish were shown as purple cocci after Gram staining (Figure 2A), indicating that they were Gram-positive bacteria. The bacteria were further observed with a scanning electron microscope, and it was found that the diameter of the bacteria was about 700 nm, the surface was not smooth, and there were flagella (Figure 2B).

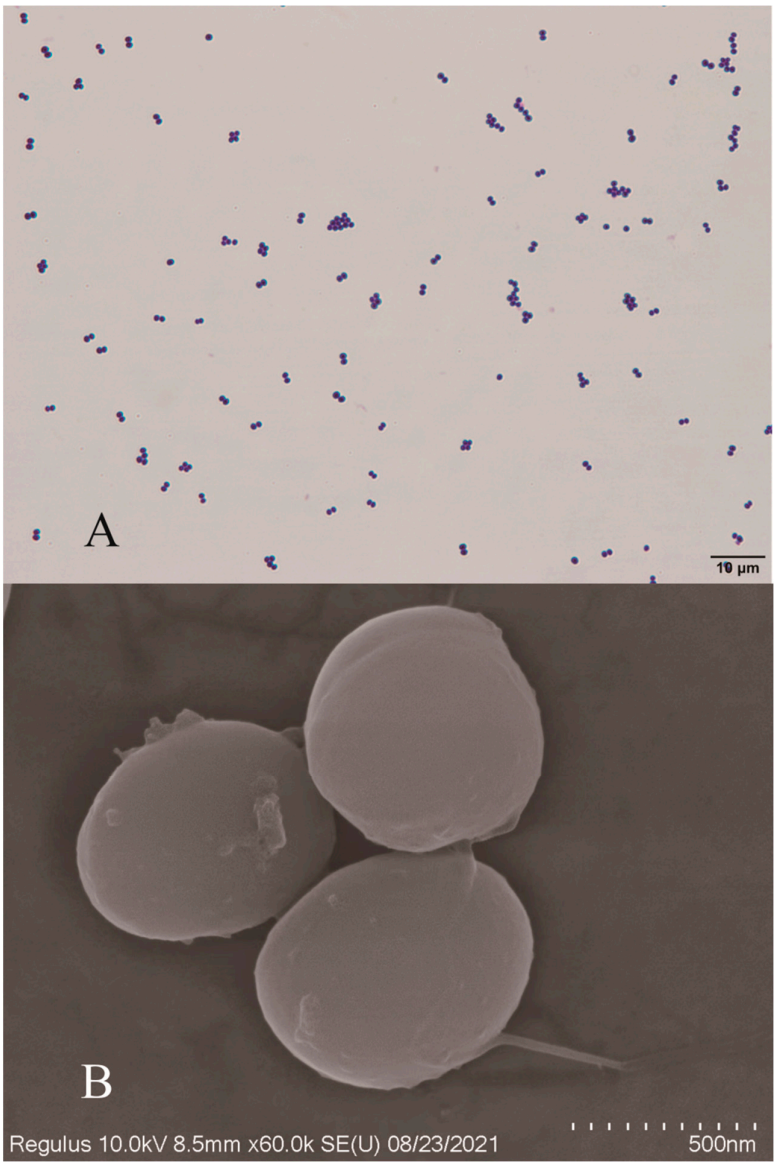

Figure 2. Gram staining and scanning electron microscopy of bacterium E702. (**A**): Gram staining (bar scale: 10 μm); (**B**): scanning electron microscopy (bar scale: 500 nm).

3.3. 16S rDNA Identification

The 16S rDNA of this strain was amplified and sequenced, and the full-length (1443 bp) fragment was subjected to NCBI BLAST analysis. The results revealed that this fragment had the greatest similarity to *Staphylococcus saprophyticus* and was clustered in the same clade with *S. saprophyticus* (GenBank accession numbers: MH396770.1, KJ958203.1 and MW228159.1) in the phylogenetic tree (Figure 3). These results indicated that this strain belongs to *S. saprophyticus*.

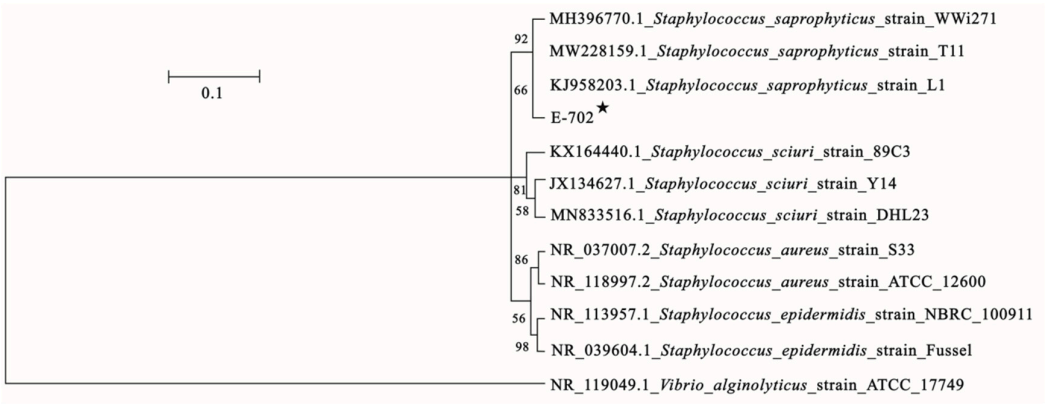

Figure 3. The values at the node indicate the percentage of trees in which this grouping occurred after bootstrapping the data (1000 replicates). The scale bar indicates the number of substitutions per site. The asterisk is bacterium E-702.

3.4. Biochemical Identification of Bacteria

To identify strain E702, biochemical analysis was performed using the Biolog microbial identification system, and the results were then matched to the database, identifying the strain as *S. saprophyticus* (Table 1).

Table 1. Biochemical identification results of strain E702.

Reagent	Result *	Reagent	Result
A1 Negative Control	N	E1 Gelatin	B
A2 Dextrin	P	E2 Glycyl-L-Proline	B
A3 D-Maltose	P	E3 L-Alanine	B
A4 D-Trehalose	P	E4 L-Arginine	P
A5 D-Cellobiose	B	E5 L-Aspartic Acid	B
A6 Gentiobiose	P	E6 L-Glutamic Acid	P
A7 Sucrose	P	E7 L-Histidine	P
A8 D-Turanose	P	E8 L-Pyroglutamic Acid	B
A9 Stachyose	N	E9 L-Serine	P
A10 Positive Control	P	E10 Lincomycin	N
A11 PH6	P	E11 Guanidine HCl	B
A12 PH5	N	E12 Niaproof 4	N
B1 D-Raffinose	N	F1 Pectin	P
B2 α-D-Lactose	P	F2 D-Galacturonic Acid	B
B3 D-Melibiose	B	F3 L-Galactonic Acid Lactone	B
B4 β-Methyl-D-Glucoside	P	F4 D-Gluconic Acid	P
B5 D-Salicin	B	F5 D-Glucuronic Acid	P
B6 N-Acetyl-D-Glucosamine	P	F6 Glucuronamide	P
B7 N-Acetyl-β-D-Mannosamine	P	F7 Mucic Acid	N
B8 N-Acetyl-D-Galactosamine	N	F8 Quinic Acid	N
B9 N-Acetyl Neuraminic Acid	B	F9 D-Saccharic Acid	N
B10 1% NaCl	P	F10 Vancomycin	N
B11 4% NaCl	P	F11 Tetrazolium Violet	P
B12 8% NaCl	P	F12 Tetrazolium Blue	P
C1 α-D-Glucose	P	G1 P-Hydroxy-Phenylacetic Acid	N
C2 D-Mannose	P	G2 Methyl Pyruvate	P
C3 D-Fructose	P	G3 D-Lactic Acid Methyl Ester	P
C4 D-Galactose	P	G4 L-Lactic Acid	P

C5 3-Methyl Glucose	B	G5 Citric Acid	P
C6 D-Glucose	B	G6 6α-Keto-Glutaric Acid	B
C7 L-Fucose	B	G7 D-Malic Acid	N
C8 L-Rhamnose	B	G8 L-Malic Acid	P
C9 Inosine	B	G9 Bromo-Succinic Acid	P
C10 1%Sodium Lactate	B	G10 Nalidixic Acid	P
C11 Fusidic Acid	N	G11 Lithium Chloride	P
C12 D-Serine	P	G12 Potassium Tellurite	P
D1 D-Sorbitol	P	H1 Tween 40	P
D2 D-Mannitol	P	H2 γ-Amino-Butyric Acid	P
D3 D-Arabitol	B	H3 α-Hydroxy-Butyric Acid	P
D4 Myo-lnositol	N	H4 β-Hydroxy-D, L-Butyric Acid	B
D5 Glycerol	P	H5 α-Keto-Butyric Acid	N
D6 D-Glucose-6-PO4	B	H6 Acetoacetic Acid	P
D7 D-Fructose-6-PO4	B	H7 Propionic Acid	N
D8 D-Aspartic Acid	N	H8 Acetic Acid	P
D9 D-Serine	N	H9 Formic Acid	P
D10 Troleandomycin	N	H10 Aztreonam	P
D11 Rifamycin SV	N	H11 Sodium Butyrate	P
D12 Minocycline	N	H12 Sodium Bromate	N

* Abbreviations: P, positive; N, negative; B, borderline.

3.5. Pathogenicity

The healthy hybrid sturgeon died after being injected with different concentrations of the E702 strain. According to the experimental results (Table 2), the LD50 of E702 was 2.14×10^5 CFU/g. *S. saprophyticus* was isolated from artificially infected hybrid sturgeons again.

Table 2. Mortality of *S. saprophyticus* infected with strain E702 at different concentrations.

Group	Bacteria Concentration/(CFU·mL^{-1})	Injection Dose/mL	Cumulative Death Number	Mortality
Test group	6.7×10^7	0.3	28	93.33%
	6.7×10^6	0.3	21	70.00%
	6.7×10^5	0.3	20	66.67%
	6.7×10^4	0.3	10	33.33%
Control	PBS	0.3	0	0%

3.6. Pathological Analysis

Organs of diseased hybrid sturgeon from farms were collected for histopathology observation. After isolating the pathogenic bacteria, they were verified using the Koch rule. The experimental group was used to detect its pathogenicity and artificially infected fish with this bacterium. The gill structure of the experimental group showed partial fusion, accompanied by infiltration of the inflammatory cells (Figure 4A). There was a large amount of inflammatory cell infiltration in the liver of diseased fish (Figure 4B). Renal cells infiltrated and merged with inflammatory cells (Figure 4C). A large number of fused cells were found in the intestinal section, some of which were accompanied by cell infiltration (Figure 4D).

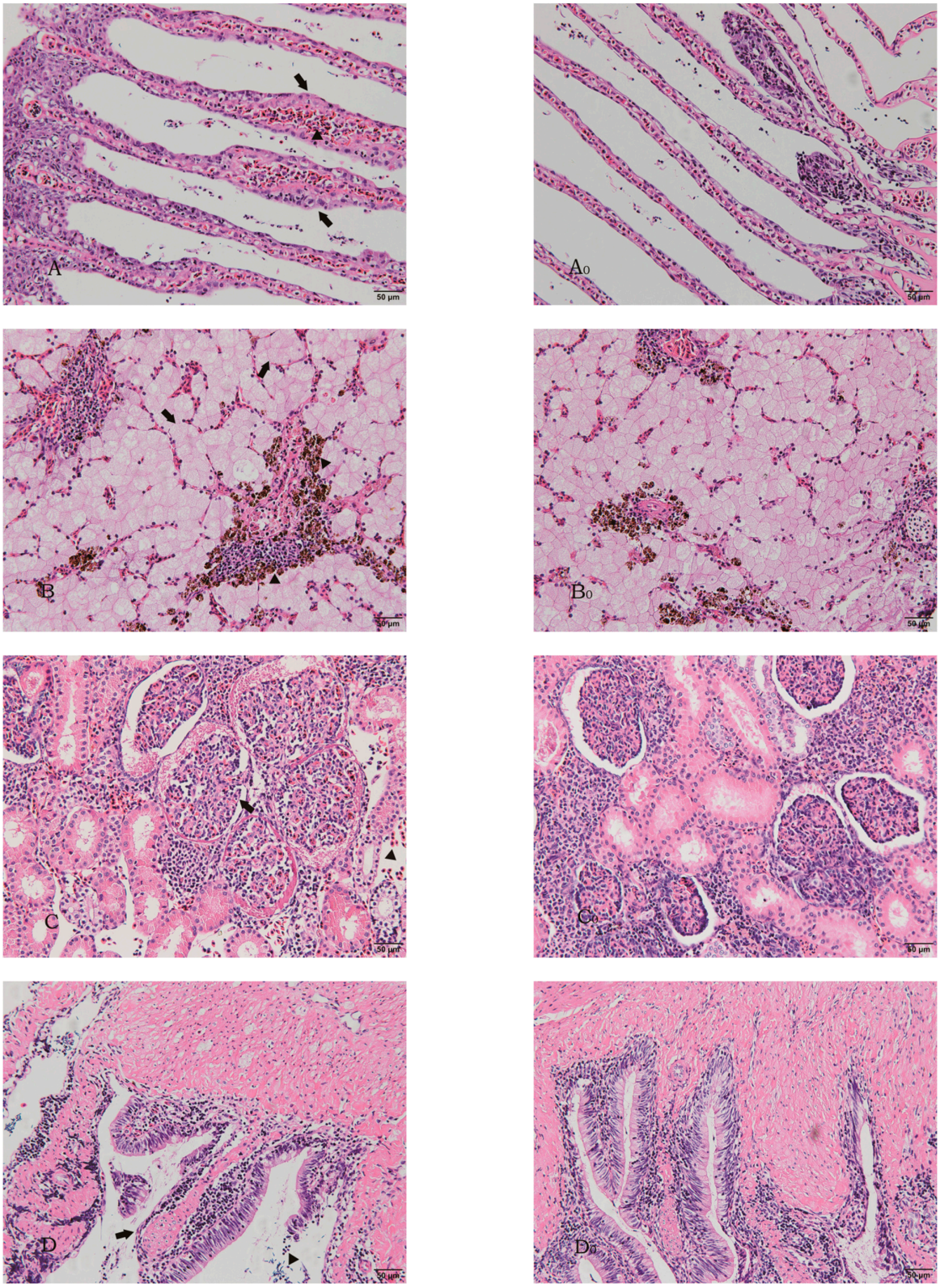

Figure 4. Histopathological observation of diseased hybrid sturgeon (scale: 50 μm). (**A**,**A**0) Gills: swollen gills, enlarged nuclei, hyperplasia of epithelial cells (arrows) and infiltration of inflammatory cells (triangles) in the diseased sturgeon. (**B**,**B**0) Liver: hepatocyte enlargement (arrow) with a large infiltration of inflammatory cells (triangles). (**C**,**C**0) Kidney: glomerular lesions

(arrows) with numerous inflammatory cells (triangles). (**D**,**D**$_0$) Intestine: Intestinal villi with structural damage, partial shedding of epidermal cells (arrows), with numerous inflammatory cells (triangles).

3.7. Blood Biochemical Analysis

By serum physiology and biochemical analysis, the glucose concentration of the diseased hybrid sturgeon was significantly reduced compared with the control, whereas the triglycerides (TG) and total cholesterol (TCHO) rose (Figure 5A). The glutamate-pyruvate transaminase (GPT), aspartate transaminase (GOT) and alkaline phosphatase (ALP) concentrations of the diseased hybrid sturgeons were significantly increased compared with the control (Figure 5B). The calculated p value was 0.0037.

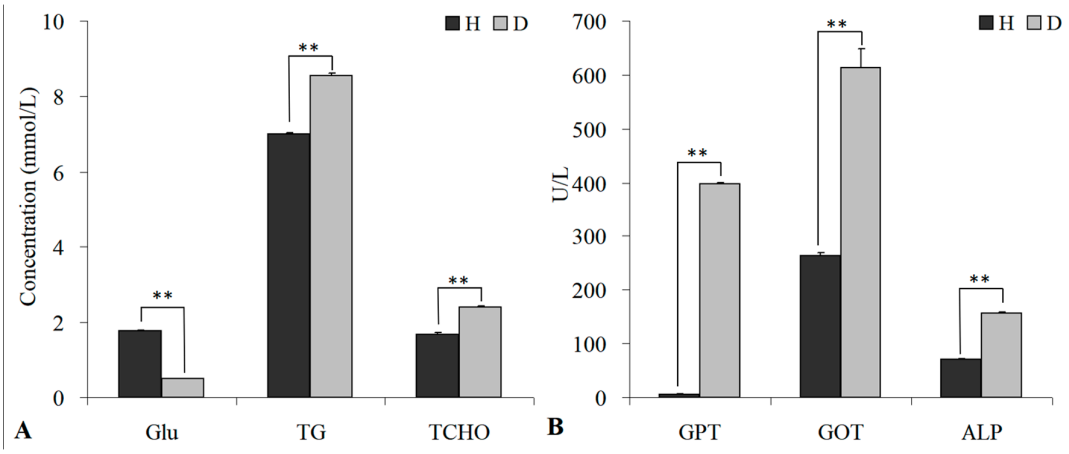

Figure 5. Blood biochemical analysis of hybrid sturgeon.(**A**) glucose (Glu), albumin (TG) and total cholesterol (TCHO). (**B**) Glutamate-pyruvate transaminase (GPT), aspartate transaminase (GOT), alkaline phosphatase (ALP); H, healthy hybrid sturgeon; D, diseased hybrid sturgeon; $p < 0.05$; ** $p < 0.01$).

3.8. Blood Count Analysis

In terms of leukocyte numbers (Figure 6), monocytes and lymphocytes were the most abundant cells in the diseased hybrid sturgeons, and the least abundant were eosinophils. The white blood cells of the control group were mostly platelets and lymphocytes, and a lower number were eosinophils. Compared with healthy hybrids, diseased hybrids had significantly higher proportions of monocytes ($p < 0.01$) and eosinophils ($p < 0.05$) and noticeably lower proportions of lymphocytes and platelets ($p < 0.01$).

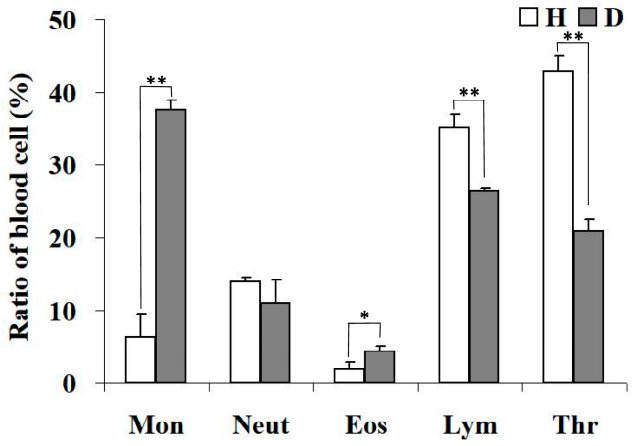

Figure 6. Blood cell count of hybrid sturgeon (Mon, monocytes; Neut, neutrophils; Eos, eosinophils; Lym, lymphocytes; Thr, thrombocytes; H, healthy hybrid sturgeon; D, diseased hybrid sturgeon; * $p < 0.05$; ** $p < 0.01$).

3.9. Drug Sensitivity

A number of drugs were used to test the antimicrobial sensitivity of the E702 strain, according to the diameter of the inhibition zone (Table 3). The results showed that the strain was highly sensitive to neomycin, cefazolin, norfloxacin, carbenicillin, gentamicin and ciprofloxacin, moderately sensitive to vancomycin, furazolidone and streptomycin and resistant to chloramphenicol.

Table 3. Detection of drug sensitivity of the E702 strain.

Medicine Name	Inhibition Zone (mm)	Sensitivity	Medicine Name	Inhibition Zone (mm)	Sensitivity
Vancomycin	15	I	Neomycin	21	S
Furazolidone	17	I	Cefazolin	34	S
Streptomycin	18	I	Norfloxacin	21	S
Chloramphenicol	9	R	Carbenicillin	22	S
Gentamicin	16	S	Ciprofloxacin	23	S

Abbreviations: S, susceptible; I, intermediate; R, resistant.

4. Discussion

S. saprophyticus isolated in this study is a common colonizing bacterium that can cause urinary tract infection [18], acute pyelonephritis, urethritis [19] and endocarditis [20,21] in humans. It has been found in the human gastrointestinal tract, rectum, blood [19,20,22] and intestines of some animals [23]. Previous studies have shown that *S. saprophyticus*, as the pathogenic bacteria of most aquatic products [24], reproduces rapidly, and its metabolites have a rancid smell [25], which seriously threatens the yield and quality of aquatic products and even causes water pollution. However, fish infected with *S. saprophyticus* have been rarely reported, and the pathogenesis of *S. saprophyticus* has been poorly understood so far. In the present investigation, the pathogenic strain E702 was isolated from the diseased hybrid sturgeon, which was Gram positive, and its surface was not smooth and had flagella as observed by scanning electron microscopy. The 16S rDNA of this strain was amplified, sequenced and compared, and the results showed that this fragment had the highest similarity with *S. saprophyticus*, which belongs to the Staphylococcus genus. The pathogenicity test clarified that the LD50 was 2.14×10^5 CFU/g, and the E702 strain had strong virulence to hybrid sturgeons. In addition, the results of reinfection experiments revealed that *Staphylococcus saprophyticus* was the pathogenic agent leading to the death of the hybrid sturgeons.

Lymphocytes are a crucial part of the immune system, exert a positive effect during the adaptive immune response [26] and can be used to assess the body's immune response after pathogen invasion. In the blood of the experimental group hybrid sturgeon in this study, the proportion of lymphocytes was significantly reduced, indicating a decline in immune function. Monocytes and granulocytes eliminate invading pathogens through phagocytosis, thereby participating in the immune response [27]. The percentage of monocytes and eosinophils in the blood of *S. saprophyticus*-infected hybrid sturgeons was dramatically increased, accordingly promoting phagocytosis. The function of platelets is mainly to promote hemostasis and accelerate coagulation, and the abnormal quantity and quality of platelets can cause bleeding diseases [28]. In addition, platelets have the function of phagocytosing viruses, bacteria and other particles [29–31]. As the line of defense against bacterial infections, a low number of platelets means that the body has a heavy infection. The diseased sturgeons in this study had markedly reduced platelets, which may be the main cause of systemic hemorrhage.

Aspartate aminotransferase (GOT) and alanine aminotransferase (ALT) are mainly distributed in liver cells and are usually less abundant in serum, but when liver cells are damaged, the cell membrane permeability increases, and the release of GOT and ALT into the blood will lead to a significant increase in enzyme activity in the serum [32]. Therefore, transaminases (especially ALT) are sensitive markers of hepatocyte damage. Alkaline phosphatase (ALP) is a critical regulatory enzyme in the biological metabolism of animals and is widely distributed in various tissues of the body, mostly in the liver [33,34]. Consequently, an abnormal ALP concentration can be used for the diagnosis and identification of liver diseases. The serum levels of GOT, ALT and ALP in the diseased hybrid sturgeons in this study were remarkably increased, indicating that the liver cells were damaged, and the liver function decreased.

Histopathologically, the lesions of E702-infected sturgeon had symptoms such as inflammatory cell infiltration, cell swelling, and cell fusion, which were similar to Bester sturgeon infected with *Lactococcus lactis subsp. Lactis* [35]. These results suggest that bacterial infection may lead to simultaneous lesions and reactions in multiple organs.

Staphylococci are very sensitive to antibiotics [36]. However, with the widespread use and abuse of antibiotics, *S. saprophyticus* has shown a trend of multiple drug resistance and has become a common pathogen of animal infection in recent years [37]. The E702 strain separated from the diseased hybrid sturgeon has a rough surface, as well as flagella, and is vulnerable to neomycin, cefazolin, norfloxacin, carbenicillin and ciprofloxacin, but moderately sensitive to vancomycin, furazolidone and streptomycin. In a previous study, a strain of *S. saprophyticus* named JY08 was isolated from *Acipenser schrenckii*, which has a smooth surface, is sensitive to doxycycline and is resistant to the compound sulfamethoxazole [38]. Differences in morphology and resistance may be related to environmental differences or their different origins. As a result, the results of the drug sensitivity test of pathogenic bacteria should first be referred to the diagnosis and treatment of bacterial diseases. In addition, it is also crucial to reasonably select drugs with good antibacterial treatment effects and to avoid multiple drug resistance caused by the abuse of antibiotics.

5. Conclusions

In this study, a germ strain was isolated from diseased hybrid sturgeon and identified as *S. saprophyticus*. The virulence test showed that the strain was pathogenic bacteria to sturgeon, and its LD50 was 2.14×10^5 CFU/g. The diseased hybrid sturgeon exhibited severe histopathological changes and marked changes in hematological parameters. This strain was sensitive to neomycin, cefazolin, norfloxacin, carbenicillin and ciprofloxacin. In sum, *S. saprophyticus* was the pathogen responsible for the mass mortality of hybrid sturgeon. The present research emphasizes its underlying ventures in hybrid sturgeon breeding and aquaculture.

Author Contributions: Conceptualization, Y.W., Y.F. and Y.Z.; Methodology, Y.F., T.L. and Y.C.; Software, T.L.; Validation, Y.W. and Y.Z.; Investigation, M.X. and Z.X.; Data Curation, L.J.; Writing—Original Draft Preparation, Y.W. and Y.F.; Writing—Review and Editing, Y.W., Y.F. and Y.Z.; Visualization, R.G.; Supervision, Y.Z.; Project Administration, Y.W.; Funding Acquisition, Y.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by grants from the Central Public Interest Scientific Institution Basal Research Fund, CAFS (2020TD44) and the National Freshwater Aquatic Germplasm Resource Center (FGRC18537).

Institutional Review Board Statement: All experimental procedures were conducted according to the guidelines of the appropriate Animal Experimental Ethical Inspection of Laboratory Animal Centre, Yangtze River Fisheries Research Institute, Chinese Academy of Fishery Sciences (ID Number: YFI2021-zhouyong-15).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We would like to thank Wuhan Donglikang Life Science Co., Ltd. for providing therapeutic products for the diseased sturgeons involved in this study.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Boschung, H.T.; Bailey, R.M. *Acipenseriformes: AccessScienc*e; McGraw Hill: New York, NY, USA, 2019. https://doi.org/10.1036/1097-8542.004900.
2. Caviar: Symbol of Luxury and Excess. 2011. Available online: http://language.chinadaily.com.cn/2011-11/01/content_14013902.htm (accessed on 1 November 2011).
3. Bledsoe, G.; Bledsoe, C.; Rasco, B. Caviars and fish roe products. *Crit. Rev. Food Sci.* **2003**, *43*, 317–356. https://doi.org/10.1201/b15995-181.
4. Bronzi, P.; Chebanov, M.; Michaels, J.; Wei, Q.; Rosenthal, H.; Gessner, J. Sturgeon meat and caviar production: Global update 2017. *J. Appl. Ichthyol.* **2019**, *3*, 257–266. https://doi.org/10.1111/jai.13870.
5. Ferreira, B.; Bertoncini, T. The IUCN Red List of Threatened Species. 2018. Available online: http://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T39303A46914065.en (accessed on 14 September 2018).
6. Jarić, I.; Bronzi, P.; Cvijanović, G.; Lenhardt, M.; Gessner, J. Paddlefish (*Polyodon spathula*) in Europe: An aquaculture species and a potential invader. *J. Appl. Ichthyol.* **2019**, *35*, 267–274. https://doi.org/10.1111/jai.13672.
7. Chen, X.; Li, C.; Yang, C.; Zhang, S.; Wu, J. Status and prospects of techniques in the sturgeon aquaculture industry in China. *Freshw. Fish.* **2017**, *47*, 108–112. https://doi.org/10.13721/j.cnki.dsyy.2017.06.016.
8. Pierezan, F.; Shahin, K.; Heckman, T.; Ang, J.; Soto, E. Outbreaks of severe myositis in cultured white sturgeon (*Acipenser transmontanus* L.) associated with *Streptococcus iniae*. *J. Fish Dis.* **2020**, *43*, 485–490. https://doi.org/10.1111/jfd.13145.
9. Soto, E.; Fast, M.; Purcell, S.; Coleman, D.; Yazdi, Z.; Kenelty, K.; Yun, S.; Camus, A. Expression of immune markers of white sturgeon (*Acipenser transmontanus*) during *Veronaea botryosa* infection at different temperatures. *Comp. Biochem. Physiol. Part D Genom. Proteom.* **2022**, *41*, 100950. https://doi.org/10.1016/j.cbd.2021.100950.
10. Mugetti, D.; Pastorino, P.; Menconi, V.; Pedron, C.; Prearo, M. The old and the new on viral diseases in sturgeon. *Pathogens* **2020**, *9*, 146. https://doi.org/10.3390/pathogens9020146.
11. Xiao, Z.; Xue, M.; Wu, X.; Zeng, L.; Zhu, Y.; Jiang, N.; Fan, Y.; Zhou, Y. Isolation and identification of *Staphylococcus warneri* from diseased *Coreius guichenoti*. *Aquacult. Rep.* **2022**, *22*, 100988. https://doi.org/10.1016/j.aqrep.2021.100988.
12. Lane, D.J. 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*; John Wiley & Sons Ltd.: New York, NY, USA, 1991; pp. 115–175. https://doi.org/10.4135/9781446279281.n7.
13. Reed, L.; Muench, H. A simple method of estimating fifty percent endpoints. *Am. J. Epidemiol.* **1938**, *27*, 493–497. https://doi.org/10.1093/oxfordjournals.aje.a118408.
14. Jiang, N.; Xu, J.; Ma, J.; Fan, Y.; Zhou, Y.; Liu, W.; Zeng, L. Histopathology and ultrastructural pathology of cyprinid herpesvirus II (CyHV-2) infection in gibel carp, *Carassius auratus gibelio*. *Wuhan Univ. J. Nat. Sci.* **2015**, *20*, 413–420. https://doi.org/10.1007/s11859-015-1114-9.
15. Xu, Y.; Li, Y.; Xue, M.; Xiao, Z.; Fan, Y.; Zeng, L.; Zhou, Y. Effects of dietary *Enterococcus faecalis* YFI-G720 on the growth, immunity, serum biochemical, intestinal morphology, intestinal microbiota, and disease resistance of crucian carp (*Carassius auratus*). *Fishes* **2022**, *7*, 18. https://doi.org/10.3390/.
16. Liu, W.; Xu, J.; Ma, J.; LaPatra, S.E.; Meng, Y.; Fan, Y.; Zhou, Y.; Yang, X.; Zeng, L. Immunological responses and protection in Chinese giant salamander *Andrias davidianus* immunized with inactivated iridovirus. *Vet. Microbiol.* **2014**, *174*, 382–390. https://doi.org/10.1016/j.vetmic.2014.10.028.
17. Barry, A.L.; Coyle, M.B.; Thornsberry, C.; Gerlach, E.H.; Hawkinson, R.W. Methods of measuring zones of inhibition with the Bauer-Kirby disk susceptibility test. *J. Clin. Microbiol.* **1979**, *10*, 885–889. https://doi.org/10.1128/JCM.10.6.885-889.1979.
18. Lawal, O.U.; Barata, M.; Fraqueza, M.J.; Worning, P.; Bartels, M.D.; Goncalves, Luisa; Paixão, Paulo ; Goncalves, Elsa; Toscano, Cristina; Empel, Joanna; Urbaś, Malgorzata; Domiìnguez, M.A.; Westh, Henrik; Lencastre, H. de; Lawal, O.U.; Miragaia, M. *Staphylococcus saprophyticus* from clinical and environmental origins have distinct biofilm composition. *Front. Microbiol.* **2021**, *7*, 663768. https://doi.org/10.3389/fmicb.2021.663768.
19. Hovelius, B.; Colleen, S.; Mardh, P.A. Urinary tract Infections in men caused by *Staphylococcus saprophyticus*. *Scand. J. Infect. Dis.* **1984**, *16*, 37–41. https://doi.org/10.3109/00365548409068407.
20. Choi, S.H.; Woo, J.H.; Jeong, J.Y.; Kim, N.J.; Kim, M.N.; Yang, S.K.; Ryu, J. Clinical significance of *Staphylococcus saprophyticus* identified on blood culture in a tertiary care hospital. *Diagn. Microbiol. Infect. Dis.* **2006**, *56*, 337–339. https://doi.org/10.1016/j.diagmicrobio.2006.08.012.
21. Garduño, E.; Quez, I.M.; Beteta, A.; Said, I.; Blanco, J.; Pineda, T.S. Staphylococcus saprophyticus causing native valve endocarditis. *Scand. J. Infect. Dis.* **2005**, *37*, 690–704. https://doi.org/10.1080/00365540510027200.
22. Rupp, M.E.; Soper, D.E.; Archer, G.L. Colonization of the female genital tract with *Staphylococcus saprophyticus*. *J. Clin. Microbiol.* **1992**, *30*, 2975–2979. https://doi.org/10.1016/0168-1656(92)90017-4.
23. Hedman, P.; Ringertz, O.; Lindström, M.; Olsson, K. The origin of Staphylococcus saprophyticus from cattle and pigs. *Scand. J. Infect. Dis.* **1993**, *25*, 57–60. https://doi.org/10.1080/00365549309169670.

24. Fu, L.; Wang, C.; Liu, N.; Ma, A.; Wang, Y. Quorum sensing system-regulated genes affect the spoilage potential of *Shewanella baltica*. *Food Res. Int.* **2018**, *107*, 1–9. https://doi.org/10.1016/j.foodres.2018.01.067.
25. Odeyemi, O.A.; Burke, C.M.; Bolch, C.; Stanley, R. Seafood spoilage microbiota and associated volatile organic compounds at different storage temperatures and packaging conditions. *Int. J. Food Microbiol.* **2018**, *280*, 87–99. https://doi.org/10.1016/j.ijfoodmicro.2017.12.029.
26. Scapigliati, G. Functional aspects of fish lymphocytes. *Dev. Comp. Immunol.* **2013**, *41*, 200–208. https://doi.org/10.1016/j.dci.2013.05.012.
27. Döring, M.; Stanchi, K.M.C.; Erbacher, A.; Haufe, S.; Schwarze, C.P.; Handgretinger, R.; Hofbeck, M.; Kerst, G. Phagocytic activity of monocytes, their subpopulations and granulocytes during post-transplant adverse events after hematopoietic stem cell transplantation. *Immunobiology* **2015**, *220*, 605–613. https://doi.org/10.1016/j.imbio.2014.12.002.
28. Yao, D.; Chai, Y.; Wang, Y.; Guo, K.; Hang, J. Microscopic structures of peripheral blood cells in chinese sturgeon (*Acipenser sinensis*). *J. Yangtze Univ. (Nat. Sci. Ed.)* **2015**, *12*, 22–26. https://doi.org/10.16772/j.cnki.1673-1409.2015.21.016.
29. Kullaya, V.I.; Mast, Q.D.; Ven, A.; Elmoussaoui, H.; Kibiki, G.; Simonetti, E.; Jonge, M.; Ferwerda, G. Platelets modulate innate immune response against human respiratory syncytial virus in vitro. *Viral Immunol.* **2017**, *30*, 576–581. https://doi.org/10.1089/vim.2016.0161.
30. Nording, H.M.; Seizer, P.; Langer, H.F. Platelets in inflammation and atherogenesis. *Front. Immunol.* **2015**, *6*, 98. https://doi.org/10.3389/fimmu.2015.00098.
31. Rossaint, J.; Kühne, K.; Skupski, J.; Aken, H.V.; Looney, M.R.; Hidalgo, A.; Zarbock, A. Directed transport of neutrophil-derived extracellular vesicles enables platelet-mediated innate immune response. *Nat. Commun.* **2016**, *7*, 13464. https://doi.org/10.1038/ncomms13464.
32. Chuang, C.; Tsai, C.; Lin, E.; Huang, C.; Lin, Y.; Lan, C.; Huang, C. Heat-killed Lactobacillus salivarius and Lactobacillus johnsonii reduce liver injury induced by alcohol in vitro and in vivo. *Molecules* **2016**, *21*, 1456. https://doi.org/10.3390/molecules21111456.
33. Whyte, M.P. Physiological role of alkaline phosphatase explored in hypophosphatasia. *Ann. N. Y. Acad. Sci.* **2010**, *1192*, 190–200. https://doi.org/10.1111/j.1749-6632.2010.05387.x.
34. Orimo, H. The mechanism of mineralization and the role of alkaline phosphatase in health and disease. *J. Nippon Med. Sch.* **2010**, *77*, 4–12. https://doi.org/10.1272/jnms.77.4.
35. Chen, M.; Hung, S.; Shyu, C.; Lin, C.; Liu, P.; Chang, C.; Shia, W.; Cheng, C.; Lin, S.; Tu, C. Lactococcus lactis subsp. lactis infection in Bester sturgeon, a cultured hybrid of Huso huso×Acipenser ruthenus, in Taiwan. *Res. Vet. Sci.* **2012**, *93*, 581–588. https://doi.org/10.1016/j.rvsc.2011.10.007.
36. Waisbren, B.A. The Sensitivity of Staphylococci to Antibiotics. *AMA Arch. Intern. Med.* **1958**, *101*, 397. https://doi.org/10.1001/archinte.1958.00260140229033.
37. De, S.; Souza, D.; Leif, S.; Paiva, P.R.; Fernandes, R.R.; Helena, C.E.; Soares, P.M.; Dos, S.; Natacha, M.; Nunes, B. *Staphylococcus saprophyticus* recovered from humans,food, and recreational waters in Rio de Janeiro, Brazil. *Int. J. Microbiol.* **2017**, *2017*, 4287547. https://doi.org/10.1155/2017/4287547.
38. Zhou, H. Isolation and identification of *Staphylococcus saprophyticus* from *Acipenser schrenckii* and its genomics analysis. *J. Fish. Res.* **2021**, *43*, 461–471. https://doi.org/10.14012/j.cnki.fjsc.2021.05.003.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.