SVU-IJVS-8(2), 20-29 Print ISSN: 2535-1826 Online ISSN: 2535-1877
Research Article Open Access

Molecular Characterisation and Pathogenic Effects of Alcaligenes Faecalis Isolated from Infected Hatchery-Reared Clarias Gariepinus

Adebola O. Ajiboye*, Olayinka R. Anifowose[2] and Shadrack A. Mensah

[1]*Adebola O AJIBOYE (A.O. Ajiboye), B.Sc., M. Sc. Ph. D, Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria*

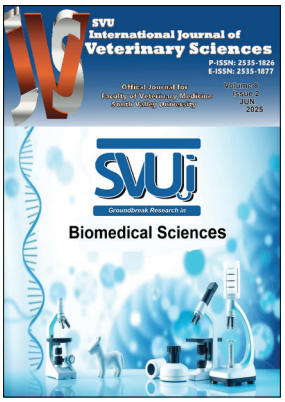
[2]*Olayinka R ANIFOWOSE (O.R. Anifowose), D.V.M, M. VSc., Ph.D. Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Ibadan, Nigeria, dranifowose@gmail.com,*

[1]*Shadrack Akotey MENSAH (S MENSAH), M. Sc. Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria, mshadrack49@gmail.com*

*Corresponding author: Adebola O. Ajiboy ; debron2005@yahoo.com

https://doi.org/10.21608/svu.2025.363354.1371

Submit Date; 2025_02_24, Revise Date; 2025_04_30, Accept Date; 2025_05_05



ABSTRACT

Bacterial pathogens are the causative agents of the most serious infectious disease in cultured fish causing mortalities and severe economic losses. This study evaluates molecular characterization and pathogenic effects of Alcaligenes faecalis isolated from hatchery-reared Clarias gariepinus juvenile. The bacterial isolates were characterized using biochemical tests, morphological traits, and *16*S rRNA gene. One hundred and twenty Clarias gariepinus juvenile were acclimatized and divided into two groups (infected and control) with three replicates (*20* fish per replicate). The fish were infected with A. faecalis isolates at a rate of *8.0* x *108* CFU/ml by immersion. The control group was not infected. The control and infected groups were observed for *14* days, mortalities, clinical signs, skin, and gross lesions were recorded. Haematology and histopathology of both control and infected group of fish were studied. Alcaligenes faecalis isolates showed evolutionary relationship with NCBI-reported isolates from India, Kenya, Pakistan, Italy, China, and Russia. The mortality rate was *0*% and *60.0*% in control and infected fish, respectively. Anaemia, heteropenia, leukocytopenia, and lymphocytopenia were observed in the infected group. The histopathological changes in the skin, gill and liver sections showed degeneration of surface epithelial cells, loose connective tissues collagen in the dermal layer, fused filament in the gill, and abnormal hepatocytes with severe glycogenic vacuolation on the cytoplasms. Alcaligenes faecalis was pathogenic and caused organ damages in infected African catfish. Proper diagnosis is a necessity for control of bacterial infections in Nigeria aquaculture industry.

Keywords: *antimicrobial resistance, pathology, biosecurity, isolates, bacteria*

INTRODUCTION

Aquaculture has emerged as the world's fastest-growing food production sector, contributing significantly to global food security and economic development. In 2014, aquaculture accounted for 73.8 million tons of aquatic animals, representing 44.1% of global fish production (FAO, 2016). The increasing demand for fish as a source of protein worldwide, fueled by both rapid population growth and technological advancements, is the driving force behind this growth (Ajiboye and Faniyi, 2014). As the world's food supply grows, aquaculture especially pond-based systems have developed more intensive techniques to produce larger yields (Alfred, 2020).

The industry is faced with several obstacles despite its achievements, chief among them

being the risk of infectious diseases. Nigeria stands as one of the leading contributors to Africa's aquaculture output, primarily through the cultivation of catfish (Clarias gariepinus). With an annual growth rate of 13.6% since 2000, Nigeria's aquaculture sector plays a pivotal role in food security and economic growth, contributing to the livelihoods of millions (Ogunji & Wuertz, 2023). However, despite considerable advancements, domestic fish production continues to fall short of demand, necessitating substantial fish imports (Ajiboye et al., 2013). This production shortfall is further exacerbated by disease outbreaks, which remain a significant barrier to sustainable aquaculture (Remilekun et al., 2021).

Bacterial diseases, in particular, present one of the most critical challenges to the aquaculture industry, resulting in high mortality rates, reduced growth, and significant financial losses (Tiamiyu et al., 2020). Opportunistic pathogens, such as Aeromonas, Edwardsiella, Vibrio, and Streptococcus species, have been identified as major culprits behind disease outbreaks in fish farms globally (Yanong et al., 2021). In Nigeria, bacterial infections continue to threaten the sustainability of C. gariepinus farming operations, leading to reduced production, economic losses, and adverse ecological impacts (Mishra et al., 2019).

Alcaligenes faecalis (A. faecalis), a Gram-negative, catalase-positive, obligate aerobic, oxidase-positive microorganism identified as non-fermenting bacterium (Huang, 2020). A. faecalis is the most frequently isolated member of family Alcaligenaceae in the clinical laboratory (Tena et al., 2015). It is usually found in soil and water (Pereira, 2000). It is an opportunistic, potential, and emerging pathogen causing infections in human and animals (Mordi et al., 2013). Alcaligenes faecalis has been reported to cause skin and soft tissue infections. Moreover, many infections have been associated with A. faecalis; urinary tract, otitis media, peritonitis, abscesses, pneumonia, endophthalmitis, and bacteremia (Huang, 2020, Mordi et al., 2013). Treatment of A. faecalis infection is usually problematic due to its high level of antibiotic resistance (Tena, 2015). Multi-drug resistance nature of A. faecalis with various classes of antibiotics such as aminoglycosides, carbapenems, quinolones, and penicillin has been reported. Although, a possible antibiotic therapy for A. faecalis infections has not been well established in literature (Majewski et al, 2020, Hasan et al., 2019, Al-Zakhari et al., 2020). The studies on isolation, identification and pathological effects of Alcaligenes faecalis in Clarias gariepinus are scarce in literature. Therefore, this study aims to isolate, identify and evaluate pathologic effect of A. faecalis isolated from infected hatchery-reared C. gariepinus juveniles.

MATERIALS AND METHODS

Sample Collection

Fresh moribund Clarias gariepinus juveniles (n=60) were sampled in nursery tanks of fish hatchery undergoing diseased outbreak, the hatchery was located in Ibadan, Oyo State, Nigeria. The dead fish were sampled between January and February 2024. The samples were transported with ice pack to the Aquatic Animal and Wildlife Medicine Laboratory, Department of Veterinary Medicine, University of Ibadan.

Isolation and Identification of Alcaligenes faecalis

The organs such as gill and liver were recovered from freshly moribund diseased fish. Kidney and liver samples were swabbed with sterile cotton swabs and inoculated in 10 ml buffered peptone water. The inoculum was cultured on Tryptic Soy, Blood, and MacConkey agar (HiMEDIA®, Mumbai, India), incubated at 320C for 24 hours. A single colony on TSA agar was subculture on TSA agar to obtain a pure colony. Alcaligenes faecalis isolates were identified by Gram staining, microscopic morphology, and conventional biochemical tests including catalase, oxidase, indole, H2S, gelatin hydrolysis, nitrate reduction, nitrite reduction, motility, urease, and sugar fermentation tests (Anifowose et al., 2024a). Moreover, the identification of A. faecalis was confirmed by PCR detection of the 16S rRNA gene as previously stated (Kyule et al., 2022), and gene sequencing was carried out.

16S rRNA Gene Sequencing and Phylogenetic Analyses

Amplification of the 16S rRNA gene was performed using PCR for all recovered A. faecalis isolates. The recovered A. faecalis strains showed similarity in

their phenotypic characteristics. Subsequently, the PCR products of four isolates picked at random were subjected to direct sequencing in both directions following purification using a QIAquick PCR-Product extraction kit (QIAGEN Sciences Inc., Germantown, MD, USA). The sequencing was performed using the Bigdye Terminator V3.1 cycle sequencing kit (Thermo Fisher Scientific, Waltham, MA, USA). The obtained sequence was placed in the GenBank with accession number PP790982.1. The consensus sequences were aligned with homologous sequences published in GenBank using Clustal W (Thompson et al., 1997) and BioEdit Sequence Alignment Editor (Hall, 1999). Phylogenetic trees were generated using Maximum Likelihood analysis based on the General Time Reversible model (Nei and Kumar, 2000) in MEGA 7 (Kumar et al., 2016) using 1000 bootstrap (Larkin et al., 2007; Anifowose et al., 2024a)

Experimental Infection Trial

African catfish juveniles (n=120) were collected from a reputable fish farm. The average weight and length of the fish were; 19.0 ± 0.81 g and 11.7 ± 0.6 cm. The fish were divided into two groups (infected and control) with three replicates (20 fish per replicate). Furthermore, a suspension of 1 ml of TSA broth culture of A. faecalis based on McFarland 0.5 was 1.6 x 108 CFU. Fish were infected by immersion in one liter of water containing (5 ml/L = 5 x 1.6 x 108) 8.0 x 108 CFU. The control groups were not infected. The fish were fed three percent body weight once daily starting from 96-hour post infection. Water contamination was prevented by changing 50% of the tank water daily. Ammonia, nitrite, dissolved oxygen, pH, and temperature of fish tanks water were observed and assessed daily. The fish were examined for skin lesions, clinical symptoms and mortalities for 14 days (Anifowose et al., 2024b).

Haematology

At the end of the experiment, blood samples (n=5) were collected for haematology. Fish were euthanized by transecting the spinal cord behind the skull for post- mortem examination. Fourteen days; of infection trial, fish were randomly sampled in each tank for blood collection. Four fish (n=5) were selected per tank. Blood was collected from caudal vein of five sampled fish using a 1-mL sterile hypodermal syringe affixed with a 24-gauge needle. The collected blood was placed in heparinized Eppendorf tubes. The blood samples were transported to the Clinical Laboratory of the Department Veterinary Medicine, University of Ibadan. Haematology was carried out according to the method described by Adeshina et al. (2020).

Histopathological Examination

Tissue specimens were obtained from skin, gill, and liver (based on post-mortem lesions) for

histopathological examination. Four fish each (n=4) were sampled from each infected and control groups. Tissues specimens from skin, gill, and liver were fixed with 10% neutral buffered formalin, dehydration, infiltration, embedment in paraffin, and stained with haematoxylin and Eosin according to Robers, (2004).

Statistical analysis

Statistical software SPSS version 23 was employed for statistical analysis of data. Mortality rate and haematology were subjected to Independent T-test. Differences were considered significant at $p < 0.05$ for all the datasets. Significant levels were determined using F-tests and P-values

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Animal Care and Use Research Ethics Committee, University of Ibadan (UI-ACUREC; approval number: UI-ACUREC/066-0625/12).

RESULT

Skin abrasion was observed in moribund diseased Clarias gariepinus juvenile (Figure 1).

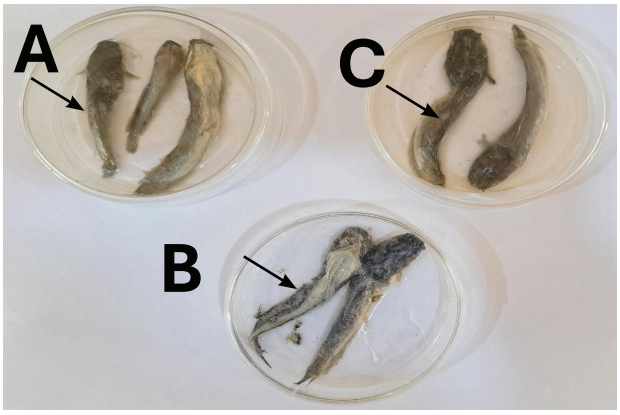


Figure 1: ***Skin ulceration (n A, B, C) of moribund diseased*** *Clarias gariepinus* ***juvenile***

All A. faecalis isolates were non-lactose fermenter, gram-negative, and coccobacilli under the microscope. The isolates showed negative reactions to indole, hydrogen sulfide, gelatin hydrolysis, nitrate reduction, and spore formation. Meanwhile, they give positive reactions to oxidase, catalase, motility, and nitrite reduction. The bacterial isolates were negative to all sugar fermentation tests: arabinose, glucose, sucrose, fructose, mannose, mannitol, sorbitol, and xylose (Table 1). The mortality rate observed were 60% and 0% in infected and control groups, respectively (Table 2). Phylogenetic tree showed evolutionary relationship of 16S rRNA gene of Alcaligenes faecalis observed in this study (PP790982.1) with strains from India, Kenya, Pakistan, China, Russia, and Italy (Figure 2). The hematological parameters measured in the infected group demonstrated significant variations compared to those of the control group, reflecting distinct responses to the infection. Notably, the infected group exhibited a marked reduction in packed cell volume (PCV) (17.00 ± 0.29) % relative to the control group (23.80 ± 0.41) %, indicating a diminished proportion of red blood cells within the total blood volume. This decrease in PCV coincided with a corresponding decline in hemoglobin count 5.18 ± 0.07 and red blood cell count 1.24 ± 0.01 in the infected group, signifying compromised oxygen transport capacity and erythropoietic activity (Table 3).

Moreover, the infected group demonstrated a notable decrease in white blood cell counts 13.00 ± 0.14 compared to the control group 15.38 ± 0.11, suggesting potential immunosuppressive effects or altered leukocyte kinetics associated with the infection. The differential leucocytic count showed significant increase in lymphocytes count 62.00 ± 0.76 in the infected group compared to the control group 69.38 ± 0.98, indicating a potential shift towards lymphocytic dominance in the immune response to the infection (Table 3).

The histopathological sections of infected fish skin showed poor epidermal layers with degeneration of surface epithelial cells; the dermal layer showed loose connective tissues collagen (Figure 3).

Meanwhile, control fish skin showed normal epidermal layers with mast cells, several reactive mucous goblet cells in the epidermis (Figure 4). Infected fish gill section showed fused filament (Figure 5), meanwhile, control fish gill showed normal filament, pilaster cells, chloride cells as well as epithelium, and cartilage (Figure 6). The infected fish liver section showed abnormal hepatocytes with severe glycogenic vacuolation on the cytoplasms (Figure 7). Meanwhile, control fish liver section showed normal central venule

Biochemical Tests	Alcaligenes faecalis
Gram Staining	Gram-negative
Shape	coccobacilli
Catalase	Positive
Citrate	Positive
Flagella	Positive
Capsule	Negative
Gelatin Hydrolysis	Negative
H_2S production	Negative
Indole	Negative
Nitrate reduction	Negative
Pigment	Negative
Oxidase	Positive
Motility	Positive
Nitrite reduction	Positive
Hemolysis	Positive
Urease	Negative
Spore	Negative
Hemolysis	Positive
Sugar Fermentation Test	Alcaligenes faecalis
Arabinose	Negative
Fructose	Negative
Glucose	Negative
Mannitol	Negative
Mannose	Negative
Sucrose	Negative
Sorbitol	Negative
Xylose	Negative

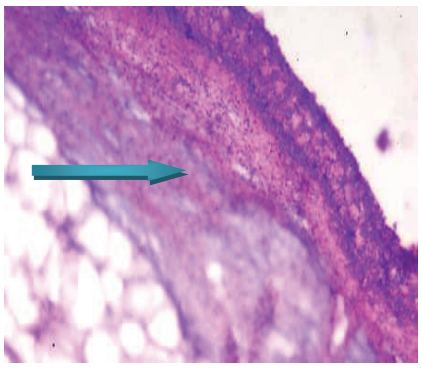
Table 1: *Alcaligenes faecalis* Isolates Biochemical Tests

Table 2: Mortality rate of *Clarias gariepinus* juveniles experimentally infected with

Tank	Control Fish	Infected Fish
Tank 1	0	14
Tank 2	0	10
Tank 3	0	12
Total	0^{a}	36^{b}
Percentage (%)	0	60%

Significance ($\alpha < 0.05$) based on independent T-test indicates different superscript along rows

Figure 2: Phylogenetic tree of 16S rRNA gene of Alcaligenes faecalis isolated from moribund diseased Clarias gariepinus in Ibadan, Oyo State using MEGA 7 software.



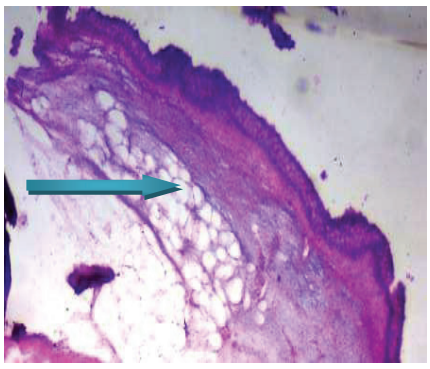


Figure 3: Photomicrograph of an infected Clarias gariepinus skin section stained by Haematoxylin and Eosin (x400), showing poor epidermal layers with degeneration of surface epithelial cells, the dermal layer (blue arrow) show loose connective tissues collagen.

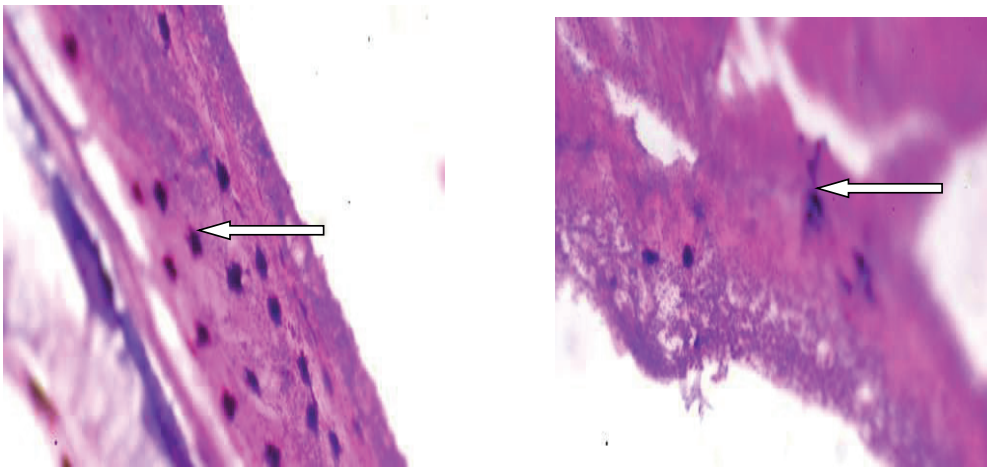


Figure 4: *Photomicrograph of a control* Clarias gariepinus *skin section stained by Haematoxylin and Eosin (x400), showing epidermal layers with mast cells (white arrow). there are several reactive mucous goblet cells in the epidermis include (white arrow), and normal connective tissue*

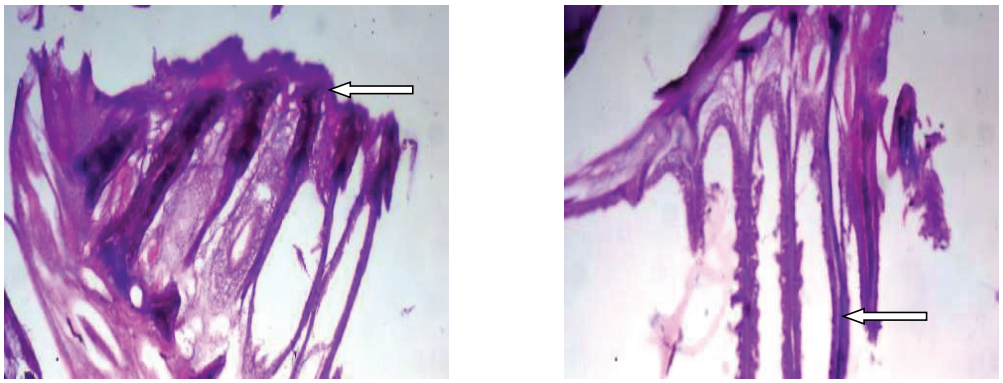


Figure 5: Photomicrograph of an infected Clarias gariepinus gill section stained by Haematoxylin and Eosin (x400), showing some normal Lamellae or filament (white arrow) as well as some fused filament (black arrow)

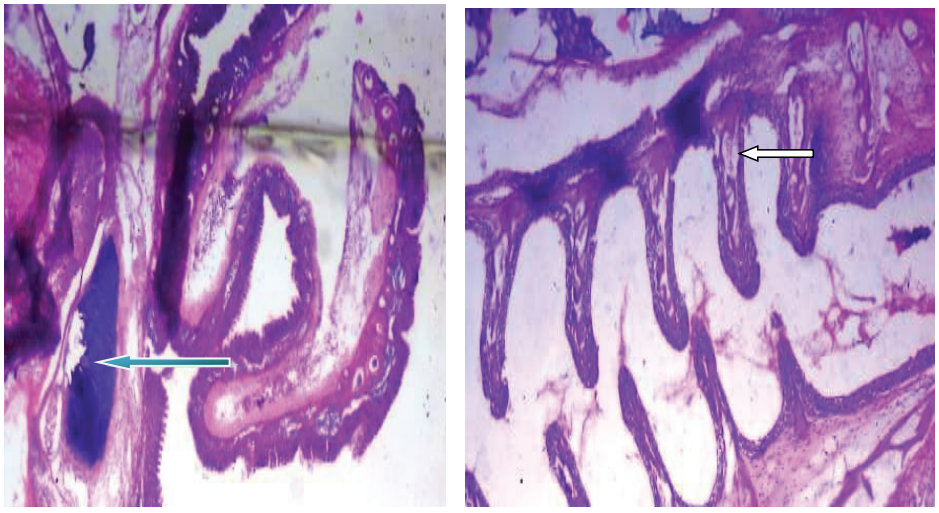


Figure 6: Photomicrograph of a control Clarias gariepinus gill section stained by Haematoxylin and Eosin (x400), showing normal gill arch and the Lamellae or filament (white arrow) containing normal pilaster cells, chloride cells as well as normal epithelium, normal cartilage (blue arrow) of the arch

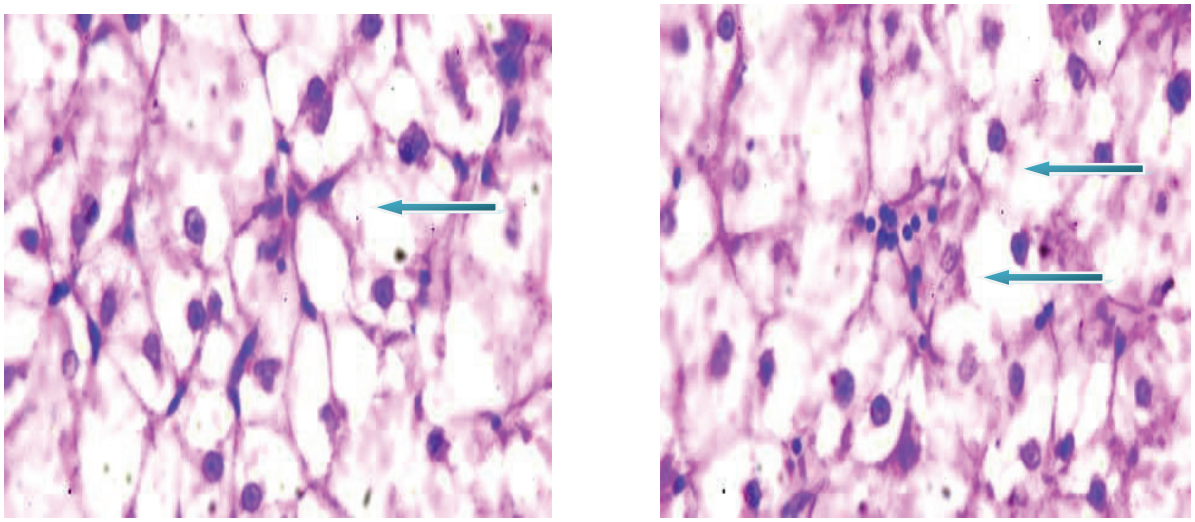


Figure 7: Photomicrograph of an infected Clarias gariepinus liver section stained by Haematoxylin and Eosin (x400), the morphology of the hepatocytes shows severe glycogenic vacuolation on the cytoplasms (blue arrow).

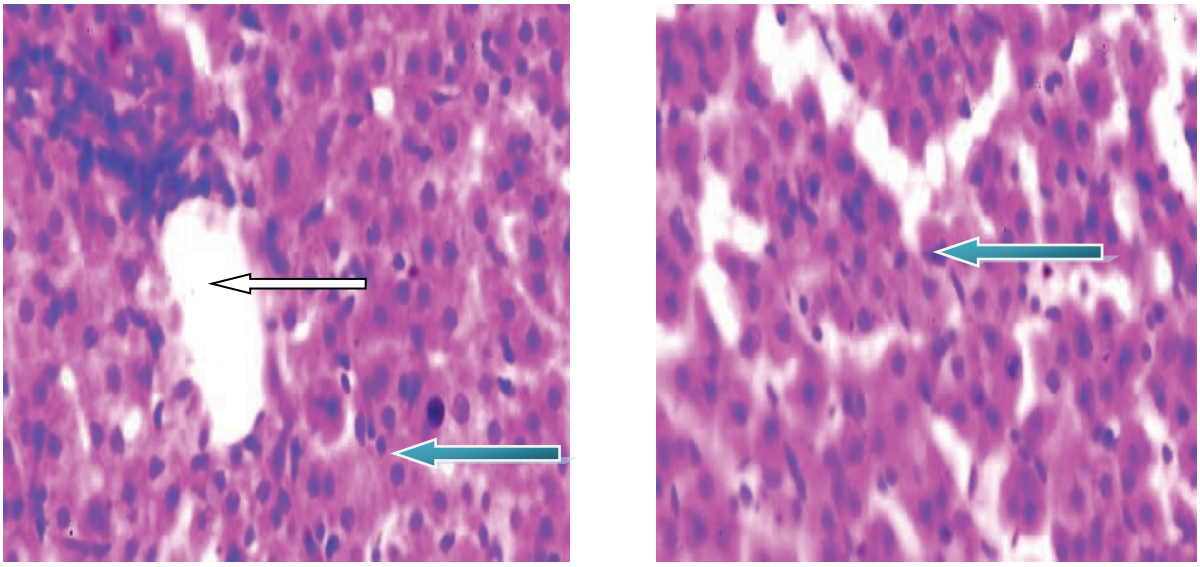


Figure 8: Photomicrograph of a control Clarias gariepinus liver section stained by Haematoxylin and Eosin (x400), showing normal central venule without congestion (white arrow), the morphology of the hepatocytes appears normal (blue arrow).

without congestion and the morphology of the hepatocytes appeared normal (Figure 8).

DISCUSSION

Clarias gariepinus is a widely cultivated fish throughout the world, therefore, identifying novel bacterial isolates is crucial for accurate diagnosis during disease outbreaks in fish hatcheries nursery and rearing ponds. The findings of this investigation identified bacterial isolates as A. faecalis. The biochemical and sugar fermentation tests of A. faecalis were similar to previous findings characterised A. faecalis as fish pathogens (Neelamegam et al., 2011).

Alcaligenes faecalis was pathogenic to C. gariepinus in this study. The mortality rate observed in infected fish was 60%, and this was similar to reports of Wang et al., (2020).

The hematological analysis revealed significant differences between the infected and control groups across various parameters. Specifically, the infected group exhibited lower packed cell volume, red blood cell, haemoglobin, white blood cells, increased lymphocytes count, and platelets compared to the control group indicating a lowered immune response.

Reduced red blood cell count, reduced haemoglobin, low packed cell volume below normal values are factors indicative of anemia, while a decreased red blood cell count suggests either circulating erythrocyte destruction or impairment of erythropoietic centers in infected fish (Achilike and Wusu, 2019).

Moreover, the platelet count was considerably lower in the infected group compared to the control group, potentially signifying impaired hemostasis. The result is in agreement with the findings of Olawale et al., (2015), who reported that the hematological findings revealed a significant decrease in white blood cell (WBC) count, packed cell volume, and platelet count in the experimental group compared to the controls ($P<0.05$). Throughout the study duration, WBC levels notably declined in rats exposed to E. faecalis, while remaining higher in the control groups. The markedly lower white blood cell counts in fish infected A. faecalis may stem from the cytotoxicity, tissue-destruction, depressive bone marrow, or inhibitory effects of the bacterial isolates (Anifowose et al., 2024c).

The severe leucopenia observed in infected fish was due to heteropenia and lymphopenia and this was statistically shown to be very significant ($p<0.05$). Such marked effect on leucocytes may be linked to the documented virulence of A. faecalis on white blood cells because of its production of Leucocidine, which specifically destroys white blood cells. This had been demonstrated with bovine and human white blood cells (Oladele et al., 2019). Such action of leucocidine points to its immunosuppressive effects.

The infected group exhibited degeneration of surface epithelium cells and poor epidermal layers. The dermal layer revealed loose connective tissues, collagen, and fat lobules. This suggests a disruption in the normal structure and function of the skin. In addition, the infected group fish gill slice stained showed some filament of the gill displayed fused.

In addition, the infected group fish liver section showed congested central vacuole with severe glycogenic vacuolation on the cytoplasms of the hepatocytes. The result of this study is similar to that of the result reported by Olawale et al., (2015) and (Zahran et al., (2019) who reported that histopathological examination revealed pronounced areas of hemorrhage, necrosis, and degeneration of liver tissues, with more severe manifestations observed in rat tissues infected with E. faecalis. The combination of cytolysin and Esp appeared to enhance the virulence of A. faecalis. The association between hemolysin production and bacterial virulence is well-documented, particularly in E. faecalis and A. faecalis (Muñoz-Atienza et al., (2013); Frank et al., (2013). Previous studies suggest that hemolysin contributes to virulence in experimental animal infections.

CONCLUSION

The study on novel strain of A. faecalis isolated from infected hatchery-reared C. gariepinus improved understanding on the molecular characteristics and pathogenic effect of the bacterial isolates in fish. Alcaligenes faecalis isolates were pathogenic to C. gariepinus and caused varying degree of damages in skin, gill and liver. This is the first study to report A. faecalis infection in C. gariepinus in Nigeria and West Africa.

ACKNOWLEDGEMENT

This research was supported by the German Academic Exchange Service (DAAD). The authors gratefully acknowledge this funding, which made the study possible

Competing Interest

This study has no competing interest to declare

Availability of Data

The data for this study is available on request

Consent for Publication

Consent is not applicable for this study

Authors Contribution to the study

The concept note was generated by Ajiboye, all the authors carried out the field work. Mensah and Anifowose generate data. Ajiboye and Mensah drafted the manuscript. Anifowose edited the drafted manuscript. All the authors approved the final manuscript

REFERENCES

Achilike NM, Wusu AD (2019). Hematological Profile of Clarias gariepinus Reared In Different Culture System. Journal of Agriculture and Environment,

15(1):165 – 177

Adeshina I, Emikpe BO, Jenyo-Oni A, Ajani EK, Abubakar MI (2020). Haematology, plasma biochemistry and serum of table size African catfish, Clarias gariepinus, naturally infected with Listeria species in Oyo State. Comparative Clinical Pathology, 29:69-73.

Ajiboye AO, Faniyi MA (2014). Growth Performance of Clarias gariepinus Fed Feeds Subjected to Different Storage Methods. International Journal of Agriculture and Food Science Technology, 5 (3):123-131

Ajiboye AO, Owoseni AA, Daramola OO (2013). Effects of Palm Oil on Microbial Load and Water Quality Parameters. International Journal of Lakes and Rivers, 6(1):19-27.

Alfred O, Shaahu A, Orban DA, Egwenomhe M. (2020). An Overview on Understanding the Basic Concept of Fish Diseases in Aquaculture. IRE Journals, 4(6):2456-8880.

Al-Zakhari R, Suhail M, Ataallah B, Aljammali S, Grigos A. (2020). Rare but fetal case of cavitary pneumonia caused by Alcaligenes Faecalis, Cureus, 12(6):e8934.

Anifowose OR, Oladosu GA, Omotosho OO (2024a). Occurrence and characterization of Proteus mirabilis from infected farmed African catfish in Ogun State, Nigeria. Molecular Biology Reports, 51(1):1-6

Anifowose OR, Ajiboye AO, Olakojo T. (2024b). Isolation, Phenotypic and Genotypic Characterization of multi-drug-resistant Enterobacter cloacae from Diseased African catfish in Lagos State. SVU-International Journal of Veterinary Sciences, 7(4):40-50

Anifowose OR, Obisesan OM, Adeoye BO. (2024c). Antimicrobial resistance and pathological impacts of Proteus mirabilis Infection in African Catfish (Clarias gariepinus) Juveniles. SVU-International Journal of Veterinary Sciences, 8(1):32-41.

Food and Agriculture Organization of the United Nations. (2016). The State of World Fisheries and Aquaculture 2016: Contributing to Food Security and Nutrition for All. FAO.

Frank KL, Guiton PS, Barnes AM, (2013). AhrC and Eep are biofilm infection-associated virulence factors in Enterococcus faecalis. Infection and Immunity, 81:1696–1708.

Hall TA. (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. In Nucleic Acids Symposium Series, 41:95-98.

Hasan MJ, Nizhu LN, Rabbani R (2019). Bloodstream infection with pandrug resistant Alcaligenes faecalis treated with double-dose of tigecycline. ID Cases, 18:e00600.

Huang C (2020). Extensively drug-resistant Alcaligenes faecalis infection. BMC infectious diseases, 20(1):833.

Kumar S, Stecher G, Tamura, K (2016). MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular biology and evolution, 33(7):1870-1874.

Kyule DN, Maingi JM, Njeru EM, Nyamache AK (2022). Molecular characterization and diversity of bacteria isolated from fish and fish products retailed in Kenyan markets, International Journal of Food Science, 2022(1), 2379323.

Larkin MA., Blackshields G, Brown NP., Chenna R, McGettigan PA, McWilliam H, Higgins DG (2007). Clustal W and Clustal X version 2.0. Bioinform, 23(21):2947-2948.

Majewski P, Majewska P, Gutowska A, Piszcz J, Sacha P, Wieczorek P, Tryniszewska E (2020). Molecular characterisation of clinical pandrug-resistant Alcaligenes faecalis strain MUB14. International of Antimicrobial Agents, 55(6):105939.

Mishra SS, Das R, Swain P (2019). Status of Fish Diseases in Aquaculture and assessment of economic loss due to disease. Contemporary Trends in Fisheries and Aquaculture, pp. 183–198.

Mordi RM, Yusuf EO, Onemu SO, Igeleke CL, Odjadjare EE. (2013). The prevalence of Alcaligenes faecalis in bacteremia, meningitis and wound sepsis in a tertiary health care institution in western part of Nigeria, The International Journal of Biotechnology, Conscientia Beam, 2(7):123-129.

Muñoz-Atienza E., Gómez-Sala B, Araújo C, Campanero C, Del Campo R, Hernández PE, Cintas LM (2013). Antimicrobial activity, antibiotic susceptibility and virulence factors of lactic acid bacteria of aquatic origin intended for use as probiotics in aquaculture. BMC microbiology, 13:1-22.

Neelamegam A, Kumar A, Saravanakumar A, Vijaylakshmi S, Thangavel B (2011). Characterization

of protease from Alcaligens faecalis and its antibacterial activity on fish pathogens, Journal of Environmental Biology, 32:781–786.

Nei M, Kumar S (2000). Molecular evolution and phylogenetics. Oxford university press.

Ogunji J, Wuertz S (2023). Aquaculture Development in Nigeria: The Second Biggest

Aquaculture Producer in Africa, Water, 15(24):4224.

Olawale O, Omokanye A, Akinyele B, Salawu O (2015). Histopathological findings in liver tissues of rats infected with Enterococcus faecalis strains, Journal of Infectious Diseases and Immunology, 7(4):45-52.

Oladele OO, Olufemi BE, Ajayi OA, Ameji SN, Ntiwunka UG (2019). Studies on Experimental Pseudomoniasis and Vibriosis in African Catfish (Clarias gariepinus) (Burchell, 1822). Vom Journal of Veterinary Science 14(1): 60 – 74.

Pereira M, Perilli M, Mantengoli E, Luzzaro F, Toniolo A, Rossolini GM. (2000). PER-1 extended-spectrum beta-lactamase production in an Alcaligenes faecalis clinical isolate resistant to expanded-spectrum cephalosporins and monobactams from a hospital in northern Italy, Microbial Drug Resistance, 6(1):85–90

Remilekun OA, Oladosu GA, Oladele OO. (2021). Causal factors of mass mortality of hatchery-reared Clarias gariepinus fry during exogenous feeding, International Journal of Fisheries and Aquatic Studies, 9(1):235-239.

Roberts, RJ, 2004. Fish Pathology. 3rd Ed., W.B. Saunders, USA

Tena D, Fernández C, Lago MR (2015). Alcaligenes faecalis: an unusual cause of skin and soft tissue infection, Japanese Journal of Infectious Diseases, 68(2):128-130.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic acids research, 25(24):4876-4882.

Tiamiyu OS, Oladosu GA, Anifowose OR, Ajayi OL (2020): Pathogenicity and Antibiotics Sensitivity Profile of Aeromonas Bestiarum used in Experimental Infection of Different Developmental Stages of Clarias gariepinus, Journal of Aquaculture, Marine Biology and Ecology: JAMBE-103

Wang M, Yi M, Lu M, Gao F, Liu Z, Huang Q, Li Q, Zhu D (2020). Effects of probiotics Bacillus cereus NY5 and Alcaligenes faecalis Y311 used as water additives on the microbiota and immune enzyme activities in three mucosal tissues in Nile tilapia Oreochromis niloticus reared in outdoor tanks, Aquaculture Reports, 17(2):100309.

Zahran E, Mahgoub HA, Abdelhamid F, Sadeyen JR, Risha E (2019). Experimental pathogenesis and host immune responses of Enterococcus faecalis infection in Nile tilapia (Oreochromis niloticus). Aquaculture, 512(2):734319.