2.5 Isolation of bacteria/ bacterial counts

One gram (1 g) of gills, intestine and liver sample of C. gariepinus were separately macerated and put into a sterile capped test tube containing 9 mL of sterilized alkaline peptone water and 1 ml of experimental pond water was dispensed into 9 mL of sterilized peptone water (Bello et al., 2012; Bello, 2014). The pond water was also enriched in alkaline peptone water for the isolation of the presumptive *Plesiomonas shigelloides*. Serial dilution was carried out and 0.1 mL each from 10^{-4} and 10^{-5} dilution factor was dispensed into Petri dishes that were appropriately labelled and the molten sterilized medium was poured aseptically into a Petri dish. The plates were swirled gently for even distribution of inoculums and allowed to set/gel and then incubated at 37 ℃ for 24 h. The organism grew into visible different colonies after 24 h. Total viable counts were determined and the results were expressed in $\log_{10}$ CFU/mL for pond water and $\log_{10}$ CFU/g for fish tissues. Also, 3~5 colonies of presumptive *P. shigelloides* were picked, purified and stocked on nutrient agar slant for further study.

2.6 Identification of isolates

Identification of the isolates was based on the procedures described by Mohammed et al. (2026). After observing cultural growth indices, the positive culture was subjected to Gram staining to study staining properties and cellular morphology under a 100X objective of a light microscope. Mixed colonies and Gram-negative bacteria were subcultured on both broth and nutrient agar (Oxoid, UK) and further incubated aerobically for 24 h. Pure culture of single colony type from (both broth and) nutrient agar were transferred onto nutrient slant for a biochemical test including catalase, oxidase, urease test, motility test, indole reaction test and fermentative/oxidative tests, hemolysis on blood agar and Gram staining techniques as described by Quinn et al. (2002) and Medical Research Council (MRC, 2017).

2.7 Gram staining technique

Young growing cultures of 18~24 h of the test isolates were used to prepare smears on clean grease-free microscopic slides. This was done by first cleaning the glass slides with cotton wool soaked with ethanol. A distinct colony of the isolates was picked with a sterile wire loop and emulsified with distilled water to form a smear and fixed. The smear was then stained with aqueous crystal violet for 1 min and was rinsed off gently with water, 95% Lugol iodine was added. The smear was decolourized with acetone until there was no violet colour on the slide. This was then rinsed off gently with water again, counter-stained with safranin for about 30 seconds, and then rinsed with water. The slide was carefully dried and examined under an oil immersion microscope with a 100x objective (MRC, 2017).

2.8 Antibiotic susceptibility

The antibiotic susceptibility profile of presumptive *P. shigelloides* was determined by using the disc diffusion technique as described by Kirby-Bauer with some modified disc diffusion techniques using 12 antibiotic discs (Biotec Lab. the United Kingdom) corresponding to the drugs containing most used in the treatment of human and animal infections caused by bacteria. The antibiotic sensitivity results for presumptive P. shigelloides were interpreted using the recommended guidelines by the Clinical Laboratory Standard Institute (CLSI, 2020). The antibiotics include; Cotrimoxazole (COT) 25 µg, Cefuroxime (CRX) 30 µg, Tetracycline (TET) 10 µg, Gentamicin (GEN) 10 µg, Ceftazidime (CPZ) 30 µg, Chloramphenicol (CHL) 10 µg, Ceftriaxone (CTR) 30 µg, Ciprofloxacin (CPR) 5 µg, Cefotaxime (CTX) 30 µg, Vancomycin (VAN) 30 µg, Amikacin (AMK) 30 µg and Meropenem (MEM) 10 µg. An 18~24 h old culture of all the isolates was prepared, after which the standardized broth culture of the inoculum was used to inoculate solidified pre-sterilized Mueller Hinton Agar plates. The antibiotics disc containing a specific concentration of antibiotics was placed on the Mueller Hinton Agar using sterile forceps and incubated at 32 oC for 24 h. The diameter of zones of inhibition was measured in millimetres and interpreted using CLSI (2020) standard and classified as sensitive, intermediate sensitive and resistant (MRC, 2017).