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Isolation, Identification, and Antibiotic Resistance Profiles of *Aeromonas hydrophila* Isolated from Sangkuriang Catfish (*Clarias gariepinus*) at Aqsa Sabar Subur Aquaculture Farm, Kupang City

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ABSTRACT

Aeromonas hydrophila (*A. hydrophila*) is an opportunistic Gram-negative bacterium commonly found in aquatic environments and is the principal causative agent of Motile Aeromonas Septicaemia (MAS) in cultured fish, including Sangkuriang catfish (*Clarias gariepinus*). Infection with *A. hydrophila* can cause substantial economic losses due to increased morbidity and mortality in cultured fish. Bacterial infections are commonly controlled using antibiotics; however, indiscriminate and repeated antibiotic use may induce antibiotic resistance, thereby reducing treatment efficacy. This study aimed to identify the presence of *A. hydrophila* and to determine its resistance status against ciprofloxacin, gentamicin, and tetracycline in Sangkuriang catfish (*C. gariepinus*) cultured at Aqsa Sabar Subur Farm, Kupang City. A descriptive method with purposive sampling was employed. A total of 30 kidney samples from apparently healthy Sangkuriang catfish were isolated on Rimler-Shotts Agar (RSA), followed by confirmatory testing through Gram staining and biochemical assays. Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA), with results interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints. Data were analysed descriptively and presented in tabular form. The results showed that 12 of 30 samples (40%) were confirmed positive for *A. hydrophila*. All isolates were fully susceptible to ciprofloxacin (100%) and predominantly susceptible to gentamicin (58.33% susceptible, 41.67% intermediate), whereas 25% of isolates were resistant to tetracycline, 16.67% intermediate, and 58.33% susceptible.

INTRODUCTION

Fish are a major source of animal protein widely consumed in Indonesia, offering high nutritional value at a relatively affordable price compared with other animal-derived products (Moniharapon *et al*., 2024; Damongilala, 2021). Fish products consistently dominate the animal-protein consumption pattern of the Indonesian population, including in East Nusa Tenggara (NTT) Province, where the total aquaculture production reached 1,415,498 tonnes for grow-out activities and 283,021 individuals for seed production in 2022 (BPS, 2022). Sangkuriang catfish (*Clarias gariepinus*) is the most widely cultured freshwater fish commodity in Indonesia (Iqbal, 2015). Its capacity to be cultured on limited land and water resources at relatively high stocking densities (Rini & Kolang, 2013), combined with rapid growth, high survival rate, and growing market demand, has made it a preferred commodity among freshwater fish farmers (Kordi, 2010).

Aqsa Sabar Subur is a household-scale freshwater aquaculture business that cultures *C. gariepinus* intensively for local consumption. Intensive rearing of *C. gariepinus* is commonly associated with disease outbreaks (Wahjuningrum et al., 2014), most of which are of bacterial origin (Muslikha *et al*., 2016). Among freshwater aquaculture pathogens, *Aeromonas hydrophila* is the most frequently reported causative agent of Motile Aeromonas Septicaemia (MAS) (Damayanti et al., 2024). *A. hydrophila* is an opportunistic pathogen that typically inhabits freshwater environments (Rejeki *et al*., 2016; Wang *et al.*, 2022) and can cause haemorrhagic septicaemia in *C. gariepinus*, particularly under conditions of stress (Anggraini *et al*., 2016). Outbreaks caused by this bacterium can be highly lethal, killing 80-100% of the population within one to two weeks (Nurruhwati *et al*., 2019). Similarly, Le *et al*. (2018) reported that MAS outbreaks in striped catfish could cause up to 80% mortality. Laith and Najiah (2014) reported that 73.3% of bacterial strains isolated from *Clarias* sp. were identified as *A. hydrophila*, a finding consistent with Hu *et al*. (2012), who recovered *A. hydrophila* from the kidneys of apparently healthy fish, indicating that the bacterium can be present subclinically and remains a dominant contributor to disease in aquaculture.

Antibiotics remain the primary means of preventing and treating *A. hydrophila* infections owing to their practicality and efficacy (Ghazy *et al*., 2024). However, widespread and inappropriate antibiotic use in aquaculture, whether for therapeutic or prophylactic purposes, has promoted the emergence of antibiotic-resistant bacteria (Prestinaci *et al*., 2015; Manyi-Loh *et al*., 2018; Adah *et al*., 2024), which in turn reduces treatment efficacy, increases the risk of infection spread, and raises mortality rates (Handayani *et al*., 2017). Recent studies have reported considerable resistance among *A. hydrophila* isolates from *C. gariepinus* against ciprofloxacin, gentamicin, and tetracycline (Fauzi *et al*., 2021). Abu Zied *et al*. (2025) reported resistance rates of 37.5% to ciprofloxacin and 12.5% to gentamicin, while Wulandari *et al*. (2019) reported high tetracycline resistance among isolates from Jambi. Similarly, Adah *et al*. (2023) reported that *Aeromonas* isolates from farmed Sangkuriang catfish (*C. gariepinus*) also exhibited resistance to several commonly used antibiotics. Uncontrolled antibiotic use may promote the emergence of resistance genes in *A. hydrophila*, turning aquatic environments into reservoirs of resistant bacteria and leaving residues in fish that pose risks to human health upon consumption.

Although research on *A. hydrophila* infection in cultured fish has been extensively conducted in Indonesia, no prior study has examined the isolation, identification, and antibiotic resistance profile of *A. hydrophila* in Sangkuriang catfish reared at the Aqsa Sabar Subur farm in Kupang City. Given the pathogenic and resistance-related risks posed by this

bacterium, the present study was conducted to identify the presence of *A. hydrophila* and to determine its resistance status against ciprofloxacin, gentamicin, and tetracycline in *C. gariepinus* cultured at this farm.

METHODS

Research Location and Time

This study employed a descriptive research method, which examines the current status of a group of people, an object, a set of conditions, a system of thought, or a class of events, with the aim of providing a systematic, factual, and accurate description of the facts, characteristics, and relationships among the phenomena under investigation (Kristiyanti, 2023). The data obtained were analysed descriptively and presented in the form of tables and figures. Sampling was carried out at the Aqsa Sabar Subur aquaculture farm, Kupang City, from April to June 2026, while necropsy and Laboratory examinations were conducted at the Laboratory of Animal Disease and Veterinary Public Health, Study Program of Veterinary Medicine, Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana.

Tools and Materials

The tools used in this study were writing instruments, a laptop, a camera, a stopwatch, a digital balance, a magnetic stirrer, a microwave, a laminar air flow (LAF) cabinet, a microscope, a Vernier caliper, an autoclave, a vortex mixer, a refrigerator, a water bath, an incubator, a cool box, a graduated cylinder, Durham tubes, test tubes, a test tube rack, centrifuge tubes, Petri dishes, forceps, straight inoculating loops, loop inoculating loops, sterile cotton swabs, microscope slides, a 5 mL syringe, a spirit lamp, matches, tissue paper, plastic clip bags, gloves, and face masks. The materials used in this study were Rimler Shotts Agar (RSA) (HiMedia), Triple Sugar Iron Agar (TSIA) (HiMedia), Sulfide Indole Motility (SIM) medium (HiMedia), Mueller Hinton Agar (MHA) (HiMedia), distilled water, methylated spirit, NaCl, crystal violet, Lugol's iodine, alcohol, safranin solution, 70% ethanol, Kovac's reagent, oxidase strips, a 0.5 McFarland standard, blank discs, 3% hydrogen peroxide (H_2O_2), immersion oil, antibiotic discs containing ciprofloxacin, gentamicin, and tetracycline, and kidney samples collected from apparently healthy cultured Sangkuriang catfish (*C. gariepinus*).

Sampling

Samples were collected using a purposive sampling technique. A total of 30 apparently healthy Sangkuriang catfish were collected from the culture ponds. Prior to necropsy, information on rearing management, feeding practices, pond-cleaning frequency, stocking density, and antibiotic-use history was obtained through direct observation and interviews with the farm owner. All procedures in this study were approved by the Animal Ethics Committee of the Faculty of Medicine and Veterinary Medicine, Universitas Nusa Cendana (ethical clearance number KEH/FKKH/NKEH/2026/008) and were conducted in strict accordance with animal welfare principles and established ethical standards for animal research.

Isolation and Macroscopic Examination

The body surface of each fish was aseptically disinfected with 70% alcohol-soaked cotton prior to necropsy (Bahariyanto *et al*., 2025). The kidney was selected for sampling because it filters metabolic waste from the blood and is commonly affected during systemic infection (Wahjuningrum *et al*., 2010). Kidney samples were placed in centrifuge tubes containing physiological NaCl solution, stored in a cool box, and transported to the laboratory. Samples were homogenised and streaked onto Rimler-Shotts Agar (RSA) supplemented with selective

supplement NO 5 (FD096) using the quadrant-streak technique, then incubated at 37 °C for 24 hours (Semwal *et al*., 2023). Colonies exhibiting characteristics typical of *A. hydrophila* were examined macroscopically for colony shape, colour, size, margin, and surface texture, and subsequently subjected to Gram staining.

Microscopic Examination and Biochemical Testing

Gram staining was performed following standard procedure using crystal violet, Lugol's iodine, 95% alcohol as a decoloriser, and safranin as a counterstain, and observed under a microscope at 100x magnification (Widyasari & Azizah, 2024). Confirmatory biochemical testing included Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM), the catalase test, and the oxidase test, performed following standard protocols (Gonçalves Pessoa *et al*., 2019; Chester, 2019; Bahariyanto *et al*., 2025).

Antibiotic Resistance Testing

Antibiotic susceptibility was assessed using the Kirby-Bauer disc diffusion method with reference to CLSI guidelines (Saleh *et al*., 2021). Confirmed isolates were suspended in 0.9% NaCl solution to a turbidity equivalent to 0.5 McFarland standard, then inoculated onto Mueller-Hinton Agar (MHA) using the spread-plate technique. Discs of ciprofloxacin (5 µg), gentamicin (10 µg), and tetracycline (30 µg) were placed on the inoculated medium, which was incubated at 37°C for 24 hours (Salam *et al*., 2023). Inhibition-zone diameters were measured with a calliper and interpreted as susceptible (S), intermediate (I), or resistant (R) according to CLSI (2015) breakpoints (Table 1).

Table 1. Interpretive Criteria for Antibacterial Inhibition Zones (CLSI, 2015)

Antibiotic	Disc Content (µg)	Susceptible (mm)	Intermediate (mm)	Resistant (mm)
Ciprofloxacin	5	≥21	16-20	≤15
Gentamicin	10	≥15	13-14	≤12
Tetracycline	30	≥15	12-14	≤11

Data Analysis

Data obtained from isolation, identification, and antibiotic susceptibility testing were analysed descriptively and presented in the form of tables.

RESULTS

Isolation and Macroscopic Identification of *Aeromonas hydrophila*

Isolation was performed on RSA medium supplemented with selective supplement NO 5 (FD096), which enhances the medium's selectivity for *A. hydrophila* by suppressing Gram-positive bacteria and Vibrio spp. of the 30 kidney samples examined, 12 samples (40%) exhibited bacterial growth on RSA with colony morphology characteristic of *A. hydrophila*, namely circular, small, smooth-margined, convex colonies with a bright yellow colour and no black precipitate, indicative of positive maltose fermentation and negative H_2S production (Figure 1). The results of *A. hydrophila* isolation are summarised in (Table 2). This morphology is consistent with the characteristics reported by Semwal *et al*. (2023) for *A. hydrophila* grown on RSA medium.



Figure 1. Isolation Results of *A. hydrophila* on RSA Medium

Table 2. Macroscopic Colony Characteristics of Presumptive *A. hydrophila* Isolates on RSA Medium

Characteristic	Observation
Shape	Circular
Colour	Bright yellow, without black precipitate
Size	Small
Margin	Entire (smooth)
Surface	Convex
Positive samples	12 of 30 (40%)

Microscopic Examination

Gram staining of the 12 presumptive isolates revealed short, red-stained, rod-shaped bacterial cells, consistent with a Gram-negative reaction (Figure 2). This finding corresponds to the description of *A. hydrophila* reported by Ahmed *et al*. (2018), who characterised the organism as a short, red, motile rod under Gram staining.

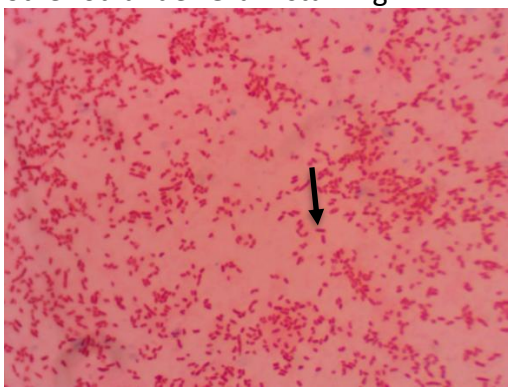

Figure 2. Gram Staining Results (100× Magnification)

Biochemical Characterisation

Confirmatory biochemical testing was performed on all 12 presumptive isolates. On TSIA medium, all isolates produced an acid/acid (A/A) reaction with negative H_2S production, indicating fermentation of glucose, lactose, and sucrose without hydrogen sulphide formation. On SIM medium, all isolates were H_2S-negative, indole-positive, and motile, consistent with the presence of flagella and tryptophanase activity. All isolates were catalase-positive, shown by gas-bubble formation upon exposure to 3% H_2O_2, and oxidase-positive, indicated by a colour change to purple on the oxidase strip (Figure 3). These combined

biochemical profiles are summarised in Table 3 and are consistent with the reference characteristics of *A. hydrophila* reported by Anggraini *et al*. (2016).

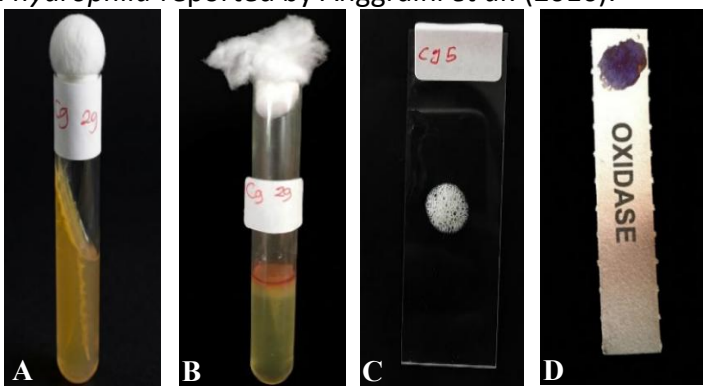

Figure 3. (A) TSIA Test, (B) SIM Test, (C) Catalase Test, (D) Oxidase Test

Table 3. Biochemical Characteristics of the 12 Confirmed *A. hydrophila* Isolates

Test	Result	Isolates with Result (n = 12)
TSIA (slant/butt)	Acid/Acid (A/A), H_2S negative	12 (100%)
SIM - H_2S	Negative	12 (100%)
SIM - Indole	Positive	12 (100%)
SIM - Motility	Positive (motile)	12 (100%)
Catalase	Positive	12 (100%)
Oxidase	Positive	12 (100%)

Based on the combined macroscopic, microscopic, and biochemical results, all 12 isolates obtained from the kidney of *C. gariepinus* reared at Aqsa Sabar Subur farm were confirmed as *A. hydrophila*, corresponding to an overall prevalence of 40% (12/30 samples).

Antibiotic Resistance of *Aeromonas hydrophila*

Antibiotic susceptibility testing of the 12 confirmed *A. hydrophila* isolates against ciprofloxacin (5 µg), gentamicin (10 µg), and tetracycline (30 µg) produced measurable inhibition zones around all discs, with a clear zone surrounding the blank disc absent in every replicate, confirming the validity of the assay and the absence of contamination. Individual inhibition-zone diameters and their interpretation are presented in Table 4.

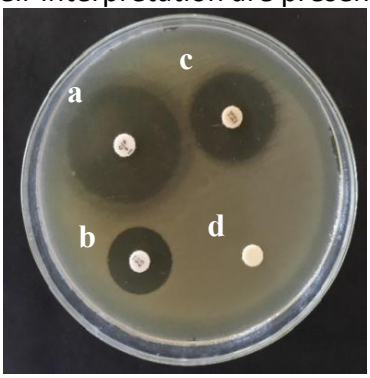

Figure 4. Diameter of the Inhibition Zone Formed on MHA Medium (a) Ciprofloxacin, (b) Gentamicin, (c) Tetracycline, (d) Blank Disk

Table 4. Inhibition Zone Diameters (mm) and Susceptibility Interpretation of *A. hydrophila* Isolates from the Kidney of *C. gariepinus*

Isolate Code	CIP (mm)	Interpretation	CN (mm)	Interpretation	TET (mm)	Interpretation
Cg5	34.10	S	19.85	S	24.91	S
Cg9	36.42	S	21.52	S	26.51	S
Cg10	25.31	S	20.52	S	10.47	R
Cg11	22.84	S	14.28	I	11.34	R
Cg13	33.57	S	18.08	S	25.15	S
Cg16	32.56	S	13.74	I	23.98	S
Cg17	24.03	S	13.18	I	23.84	S
Cg19	33.82	S	18.22	S	13.32	I
Cg20	34.76	S	14.12	I	14.11	I
Cg24	26.55	S	19.37	S	17.64	S
Cg28	31.70	S	13.74	I	21.72	S
Cg29	27.00	S	19.58	S	11.03	R

Note: CIP = ciprofloxacin; CN = gentamicin; TET = tetracycline; S = susceptible; I = intermediate; R = resistant.

Table 5. Susceptibility Distribution (%) of *A. hydrophila* Isolates (n = 12) Against Three Antibiotics

Antibiotic	Susceptible (%)	Intermediate (%)	Resistant (%)
Ciprofloxacin	100	0	0
Gentamicin	58.33	41.67	0
Tetracycline	58.33	16.67	25.00

Overall, the isolates were fully susceptible to ciprofloxacin (100%). Susceptibility to gentamicin was also favourable, with 58.33% of isolates susceptible and 41.67% intermediate, and no isolate classified as resistant. In contrast, tetracycline showed the lowest efficacy among the three antibiotics tested, with 58.33% of isolates susceptible, 16.67% intermediate, and 25% resistant.

DISCUSSION

The isolation of *A. hydrophila* from 12 of 30 (40%) kidney samples of apparently healthy *C. gariepinus* indicates that the bacterium can be present subclinically within the aquaculture population at Aqsa Sabar Subur farm. This finding is in agreement with Hu *et al*. (2012), who isolated *A. hydrophila* from the kidneys of clinically healthy fish in China, and with Rashid *et al*. (2013), who reported a similar occurrence in apparently healthy fish in Mymensingh, Bangladesh, demonstrating that *A. hydrophila* can persist as part of the resident microbiota without producing overt clinical signs.

The presence of *A. hydrophila* in the ponds is likely related to its ecological role as an opportunistic pathogen that can persist in water and sediment without causing disease in healthy fish, but which becomes pathogenic once fish experience physiological stress or a decline in environmental quality (Semwal *et al*., 2023; Hamid *et al*., 2026). Pathogenicity of *A. hydrophila* is mediated by multiple virulence factors, including flagella, fimbriae, lipopolysaccharide, and outer membrane proteins, which facilitate adhesion, colonisation, and tissue invasion (Rasmussen-Ivey *et al*., 2016). Accumulation of organic matter from

uneaten feed and faeces can further increase nutrient availability in the water column, thereby favouring bacterial proliferation (Zhang *et al*., 2020); at Aqsa Sabar Subur, pond cleaning is performed only once a month, a relatively low frequency that may allow organic sediment to accumulate and create conditions conducive to bacterial growth (Peatman *et al*., 2018). High stocking density, a common feature of intensive culture systems, may compound this risk by inducing stress, impairing host immune responses, and facilitating bacterial transmission through direct contact and shared water (Omeje & Kolndadacha, 2024).

The complete (100%) susceptibility of all isolates to ciprofloxacin indicates that this fluoroquinolone remains highly effective against *A. hydrophila* from this farm. This result is consistent with Saleh *et al*. (2021), who reported that 84.2% of *A. hydrophila* isolates from Nile tilapia remained e to ciprofloxacin, and with Ramadhani *et al*. (2025), who found ciprofloxacin to be more effective against *A. hydrophila* than other fluoroquinolones. Ciprofloxacin exerts a bactericidal effect by inhibiting DNA gyrase and topoisomerase IV, enzymes essential for bacterial DNA replication and transcription (Hooper & Jacoby, 2015), which likely explains its sustained efficacy despite widespread antibiotic use in aquaculture.

Gentamicin also demonstrated favourable activity, with 58.33% of isolates susceptible and 41.67% intermediate, and no isolate classified as resistant. This finding aligns with Rahmaniar *et al*. (2025), who reported that most *A. hydrophila* isolates from Nile tilapia and gourami remained susceptible to gentamicin. Gentamicin, an aminoglycoside, acts by binding to the bacterial 30S ribosomal subunit, thereby disrupting mRNA translation and protein synthesis (Chaves & Tadi, 2023). The occurrence of intermediate isolates may reflect an early stage of reduced susceptibility, potentially driven by aminoglycoside-modifying enzymes, altered membrane permeability, or increased efflux-pump activity (Fernández & Hancock, 2012), warranting continued surveillance despite the absence of overt resistance.

Tetracycline exhibited the lowest efficacy among the antibiotics tested, with 25% of isolates classified as resistant. A comparable pattern was reported by Saleh *et al*. (2021), who found 28.9% tetracycline resistance among *A. hydrophila* isolates, while Wulandari *et al*. (2024) reported an even higher resistance rate of up to 100% in isolates from Jambi. Conversely, Laith and Najiah (2014) found *A. hydrophila* isolates to remain fully susceptible to tetracycline, illustrating substantial geographic and management-related variability in resistance patterns. Tetracycline acts bacteriostatically by binding reversibly to the 30S ribosomal subunit, thereby blocking aminoacyl-tRNA attachment and halting polypeptide elongation (Chopra & Roberts, 2001). Resistance to tetracycline in *Aeromonas* spp. is commonly associated with tet genes, such as tetA and tetE, which encode efflux-pump proteins capable of expelling the drug from the bacterial cell before it reaches an inhibitory concentration (Piotrowska & Popowska, 2015).

The detection of tetracycline-resistant isolates in this study may be linked to prior use of this antibiotic at the Aqsa Sabar Subur farm as a disease-preventive measure. Repeated antibiotic exposure exerts a selective pressure that favours the survival and proliferation of resistance-gene carriers and promotes horizontal gene transfer among bacteria in the aquatic environment (Bello-López *et al*., 2019). This aligns with the observation of Jeamsripong *et al*. (2025) that extensive antibiotic use in the aquaculture sector is a key driver of rising resistance in *A. hydrophila*, with some isolates worldwide already exhibiting multidrug resistance (MDR) to three or more antibiotic classes.

Suboptimal pond management practices, including infrequent water-quality maintenance and potential feed over-supply leading to organic-matter accumulation, may further compound the emergence of resistant bacteria by supporting higher bacterial loads in

the rearing environment (Abdella *et al*., 2024). Environmental residues of antibiotics originating from aquaculture effluent may similarly act as a selective pressure that favours resistant bacterial populations (Jeamsripong *et al*., 2025), while contaminated water, sediment, and feed can serve as vehicles for horizontal transfer of resistance genes among bacteria (Done *et al*., 2015).

The finding of tetracycline-resistant *A. hydrophila* in this study underscores the relevance of a One Health approach to antimicrobial resistance in aquaculture. Because bacteria and resistance genes can move between animals, humans, and the environment, antimicrobial resistance in aquaculture is not confined to fish health alone but represents a broader public-health concern (Robinson *et al*., 2016). *A. hydrophila* has been identified as an important environmental reservoir of antibiotic-resistance genes in aquaculture systems (Jeamsripong *et al*., 2025). Accordingly, rational and evidence-based antibiotic use, guided by susceptibility testing, together with improved biosecurity and pond-water management, is recommended to curb further spread of resistance at the Aqsa Sabar Subur farm and similar small-scale aquaculture operations.

CONCLUSION

Aeromonas hydrophila was detected in the kidney of Sangkuriang catfish (*Clarias gariepinus*) cultured at the Aqsa Sabar Subur farm, Kupang City, with 12 of 30 examined samples (40%) confirmed positive based on macroscopic, microscopic, and biochemical characteristics. Antibiotic susceptibility testing revealed variable resistance profiles among the isolates: complete susceptibility (100%) to ciprofloxacin; 58.33% susceptibility and 41.67% intermediate susceptibility to gentamicin, with no resistant isolates; and 58.33% susceptibility, 16.67% intermediate susceptibility, and 25% resistance to tetracycline. These findings indicate that although ciprofloxacin and gentamicin remain effective therapeutic options, the emergence of tetracycline resistance warrants prudent antibiotic stewardship and continued resistance surveillance at this farm.

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