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Effectiveness of Five Common Chemicals Against *Edwardsiella tarda* Isolated from Ornamental Fish

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Abstract

Edwardsiella tarda is a bacterial pathogen that causes edwardsiellosis in fish. The present study aimed to estimate the prevalence of *E. tarda* in diseased ornamental fish and to evaluate the antibacterial effect of five common aquaculture chemicals against *E. tarda*. A total of 27 diseased ornamental fish exhibiting red spots, ulcers, exophthalmia, and skin discolouration were collected. *E. tarda* was isolated only from ornamental pangasius (*Pangasianodon hypophthalmus*), while goldfish and Koi carp were negative. The prevalence of *E. tarda* in ornamental pangasius was 35.71%. The bacterial isolates were tested for antimicrobial resistance (AMR) against 13 classes of antimicrobials. Resistance was observed only to tetracycline. Tetracycline-resistant and intermediate-susceptible isolates harboured the *tet*A gene, as confirmed by PCR analysis. Five chemicals were tested for antibacterial activity against *E. tarda* at three concentrations: recommended dose, double dose, and half dose. The effect of the chemicals was assessed by measuring the reduction in total plate count (TPC). Acetic acid exhibited the strongest antibacterial activity, completely inhibiting *E. tarda* growth at ≥ 1000 ppm. Potassium permanganate ($KMnO_4$) demonstrated a 5.29 log reduction compared to the 3.96 log reduction with copper sulphate ($CuSO_4$) at the recommended dose of 2 ppm. However, povidone-iodine and $MgSO_4$ showed no reduction in TPC at any of the three doses. This study highlights the association between *E. tarda* and diseased ornamental pangasius, and demonstrates the effectiveness of acetic acid, $KMnO_4$, and $CuSO_4$ against *E. tarda*.

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Introduction

The trade in live ornamental fish as companion animals, along with related products, constitutes a globally significant economic sector. Ornamental fish farming and commerce constitute a dynamic international industry, involving nearly 1.5 billion fish representing around 5,000 different species. Nearly 98% of the ornamental fish in trade are farm-reared. India is one of the major producers of tropical ornamental fish, with the industry growing at about 20% each year (Girisha et al., 2021). Goldfish and Koi carp are among the most widely traded ornamental fish species in India due to their attractive colour variations, adaptability, and ease of maintenance in aquarium systems (Watson, Hill, & Pouder, 2004). Sutchi catfish, commonly known as ornamental pangasius or iridescent shark, is a freshwater catfish native to the Mekong basin (Singh & Lakra, 2011; Minh et al., 2022). In India, it is popular in the ornamental fish trade due to its shimmering body colouration and active swimming behaviour. The species attains a large size in natural habitats, but juveniles are widely reared as ornamental fish. Besides ornamental use, it is also important in aquaculture for food production across many Asian countries.

Since the ornamental fish trade is an emerging business in India, it is also at risk of infectious diseases, particularly those caused by bacterial pathogens (Larcombe, Alexander, Snellgrove, Henriquez, & Sloman, 2025). Species of the genus *Edwardsiella* are major bacterial pathogens in aquaculture, with five main species identified: *E. tarda, E. ictaluri, E. hoshinae, E. piscicida, and E. anguillarum* (Austin & Austin, 2016). Among them, *E. tarda* causes diseases in freshwater fish and also poses a threat to human health (Shetty, Maiti, Venugopal, &

Karunasagar, 2014; Davies et al., 2018). This bacterium can cause haemorrhagic septicaemia, skin lesions, and lesions in the internal organs, resulting in high mortality in many fish species (Park, Aoki, & Jung, 2012). *E. tarda* was recently reported in mass mortalities of Oscar (*Astronotus ocellatus*), Koi carp (*Cyprinus carpio* var. koi), and goldfish (*Carassius auratus*) farms in Kerala (Preena et al., 2020; Preena, Dharmaratnam, & Swaminathan, 2022; Vishnupriya, Swaminathan, Dharmarathnam, Sharma, & Preena, 2024). However, there are no reports of *E. tarda* infection in ornamental pangasius fish.

Ornamental fish farmers and hobbyists often rely on antibiotics to treat fish diseases. The widespread use of antimicrobials in ornamental fish culture has raised serious concerns about the emergence of antimicrobial resistance (AMR) in pathogens (Sicuro et al., 2020). In many cases, the improper use of antibiotics contributes to the development of resistant bacterial strains. When hobbyists discard overgrown fish from confined systems, AMR bacteria or resistance genes may enter the surrounding aquatic environment (Au-Yeung et al., 2025). Within these environments, AMR can spread among bacterial populations through mobile genetic elements. Hence, molecular detection and characterisation of AMR genes in pathogens associated with ornamental fish are crucial for understanding resistance mechanisms and their potential transmission (Verner-Jeffreys et al., 2009). The growing concerns about AMR limits the options.

In aquaculture, chemicals such as copper sulphate ($CuSO_4$), potassium permanganate ($KMnO_4$), povidone-iodine, magnesium sulphate ($MgSO_4$), and acetic acid are used as therapeutic and disinfecting agents for disease prevention or control. The recommended dose is 2 ppm for $CuSO_4$ and $KMnO_4$ (Darwish, Mitchell, & Straus, 2009; Farmer, Beck, & Straus, 2012), 100 ppm for povidone-iodine, 30,000 ppm for $MgSO_4$, and 1,000 ppm for acetic acid (FDA, 2011). These compounds are commonly applied for prophylaxis, water quality management, water disinfection, and the treatment of external parasites (Boyd & Tucker, 1998; Reverter, Bontemps, Lecchini, Banaigs, & Sasal, 2014). Copper sulphate and potassium permanganate act as strong oxidising agents, effective in reducing organic loads and controlling ectoparasites such as *Ichthyophthirius multifiliis* and *Trichodina* spp. (Straus, 1993; Noga, 2010). Povidone-iodine is a broad-spectrum antiseptic frequently used to disinfect fish eggs, equipment, and wounds (Stuart, Keller, & Drawbridge, 2010). Magnesium sulphate is occasionally applied as an osmotic agent to relieve stress and treat external parasitic infestations, while acetic acid serves as a mild disinfectant and antiparasitic bath treatment (Plumb & Hanson, 2011). Despite their extensive use in aquaculture, the antibacterial efficacy of these chemicals against *E. tarda* in ornamental fish diseases remains poorly understood. To date, few, if any, systematic studies have evaluated their direct antibacterial activity against *E. tarda*. Therefore, the present study focused on determining the prevalence of *E. tarda* in diseased ornamental fish collected from Visakhapatnam, Andhra Pradesh, India. Furthermore, the antibacterial activity of five chemicals against *E. tarda* was evaluated through an *in vitro* study.

Materials and Methods

A total of 27 morbid or diseased fish were collected from eight freshwater aquarium shops in Visakhapatnam (Fig. 1). Among these, 14 were ornamental pangasius, 8 goldfish, and 5 Koi carp. All the collected fish showed red spots, ulcers, exophthalmia, skin discolouration, dropsy, lethargy, and an unpleasant odour (Fig. 2).

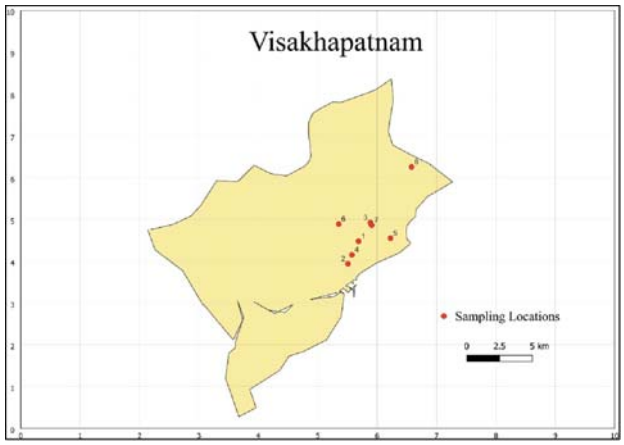

Fig. 1. The geographic map of Visakhapatnam showing the eight sampling sites

For targeted isolation of *E. tarda*, kidney swabs were aseptically collected on-site from the infected fish and brought to the laboratory on ice. The swabs were inoculated into tetrathionate broth (TTB) and incubated for 16 hours at 37 °C in a water bath. Bacteria from the broth were streaked onto Salmonella-Shigella (SS) agar (HiMedia, India) and incubated for 16 hours at 30 °C (Kumar et al., 2007).

Fig. 2. Clinical signs presumptive of infectious disease

Oxidase-negative, small black-centred colonies with translucent margins were picked and subcultured onto tryptone soya agar (TSA) (HiMedia, India) after overnight incubation at 37 ºC. The purified isolates were subjected to standard biochemical tests for preliminary identification of *E. tarda* as described by Collins, Lyne, Grange, and Falkinham (2004). Confirmatory bacterial identification was performed using the VITEK 2 (BioMerieux, France) compact automated system. *E. tarda* ATCC 15947 was used as a quality control strain in the study. The *E. tarda* isolates were preserved in 20% glycerol (HiMedia, India) and stored at -80 ºC.

The molecular identification of *E. tarda* was performed by PCR-based species-specific detection of *fim*D (Griffin et al., 2013) and *sod*B (Reichley et al., 2017) genes. The PCR reaction mixture (25 µL) contained 1 × PCR buffer, 200 µM dNTPs, 1.6 mM $MgCl_2$, 1.25 U Taq DNA polymerase (Takara Bio), 0.2–0.4 µM primers, and 50 ng template DNA. For *fim*D amplification, the PCR conditions included an initial denaturation at 94 ºC for 2 min, followed by 30 cycles of denaturation at 95 ºC for 20 s, annealing at 55 ºC for 20 s, and extension at 72 ºC for 1 min, with a final extension at 72 ºC for 7 min. For *sod*B amplification, the cycling conditions consisted of an initial denaturation at 94 ºC for 2 min, followed by 35 cycles of denaturation at 94 ºC for 30 s, annealing at 50 ºC for 30 s, and extension at 72 ºC for 30 s, followed by a final extension at 72 ºC for 7 min. The PCR products were analysed by electrophoresis on 1.5% agarose gel and visualised under UV light. Representative PCR amplicons of the *sod*B gene were sequenced. The *sod*B gene sequences in *Edwardsiella* sp. were downloaded, trimmed, compared, and aligned using the MUSCLE application of MEGA v10 along with the sequences generated in this study; a phylogenetic tree was constructed using the maximum likelihood method. Further, the percentage similarity matrix of *sod*B sequences between *Edwardsiella* species was determined using the BLASTN algorithm.

Antimicrobial susceptibility testing of all *E. tarda* isolates was conducted using the Kirby–Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2021) guidelines. The tested antibiotics belonged to 13 classes: penicillin [(ampicillin (10 µg)], β-lactamase inhibitors [amoxiclav (20 µg /10 µg)], cephem [cefoxitin (10 µg)], third-generation cephalosporins [ceftazidime (5 µg), ceftriaxone (30 µg), cefotaxime (10 µg)], fourth-generation cephalosporins [cefepime (30 µg)], carbapenems [imipenem (10 µg)], monobactams [aztreonam (30 µg)], aminoglycosides [amikacin (30 µg)], fluoroquinolones [enrofloxacin (5 µg)], quinolones [nalidixic acid (30 µg)], phenicols [chloramphenicol (30 µg)], tetracyclines [tetracycline (30 µg)], and sulphonamides [co-trimoxazole (1.25/23.75 µg)] (Hi Media, India). *E. coli* ATCC 25922 was used as a quality control strain to validate the results (Dwivedi et al., 2023). The plates were incubated at 37 ºC for 24 hours. The results were interpreted as per the 2021 CLSI breakpoints for *Enterobacteriaceae*.

The resistant phenotypes were screened for their corresponding ARGs by PCR. The screened ARGs were *tet*A, *tet*B, *tet*G, and *tet*G1 (Ng, Martin, Alfa, & Mulvey, 2001). The final volume of the PCR mixture was 25 µL containing 1 x PCR buffer, 200 µM dNTPs, 1.6 mM $MgCl_2$, 1.25 U Taq DNA polymerase (Takara Bio), 0.2-0.4 µM primers, and 50 ng template DNA. PCR amplification was carried out with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 ºC for 1 min, annealing at 55 ºC for 1 min, and extension at 72 ºC for 1.5 min. A final extension step was performed at 72 ºC for 7 min. The PCR products were analysed by electrophoresis on 1.2% (w/v) agarose gel.

Five different chemicals were tested for antibacterial activity against *E. tarda* isolates: $CuSO_4$, $KMnO_4$, povidone-iodine, $MgSO_4$ and acetic acid. The antibacterial effects of the chemicals were determined by the log reduction of *E. tarda* counts. Each chemical was tested at three concentration levels: recommended dose, half dose, and double dose. $CuSO_4$ and $KMnO_4$ were tested at concentrations of 1, 2, and 4 ppm. Povidone-iodine was tested at 50, 100, and 200 ppm; $MgSO_4$ at 15,000, 30,000 and 60,000 ppm; and acetic acid at 500, 1000 and 2000 ppm. Each bacterial isolate was revived on TSA and incubated at 30 °C for 18–24 hours. A standardised inoculum equivalent to 0.5 McFarland (approxi-

mately 10^8 CFU/mL) was prepared in sterile 0.85% saline and then diluted 100 times in tryptic soy broth (TSB) to achieve a final bacterial concentration of approximately 10^6 CFU/mL. Stock solutions of each chemical were prepared in sterile saline and diluted in TSB to reach the desired concentrations. For each treatment, 4.75 mL of the chemical solution diluted in TSB was inoculated with 250 µL of the bacterial suspension (final bacterial concentration of approximately 10^4 CFU/mL) in triplicate. The tubes were incubated overnight at 30 °C without shaking. The control group received only bacterial suspension without any chemicals. After incubation, tenfold serial dilutions (10^{-1} to 10^{-7}) were prepared in sterile saline, and 100 µL aliquots of the suitable dilutions were spread onto TSA plates. The plates were incubated at 30 °C for 24 hours, and the total plate count was determined after counting colonies on plates containing 30-300 colonies. The antibacterial efficacy of each chemical at different concentrations was evaluated by comparing the TPC values of the treated groups with those of the control, and the results were expressed as log TPC and log reduction in bacterial count relative to the control. The reduction of *E. tarda* was calculated using the following formula:

$$\text{Log reduction} = \log A_1 - \log A_2$$

where A_1 is the mean TPC of the control, and A_2 is the mean TPC of the treatment group.

The antibacterial activity of the tested chemicals was classified as poor (≤1 log reduction), moderate (≤3 log reduction), good (≤5 log reduction), and excellent (≥5 log reduction).

All statistical analyses were performed using IBM SPSS Statistics version 16. Data are presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) followed by Tukey's post hoc test was conducted to evaluate differences among treatment groups. Comparisons were performed among different dose levels within each chemical and between the chemicals at the same dose level. Variability between the isolates was also compared for each chemical. Statistical significance was set at $p < 0.05$.

Results and Discussion

The ornamental fish trade is a rapidly growing industry. Hobbyists make up 99% of the market, while public aquariums and other sectors account for the rest. In India, freshwater ornamental fish dominate, representing 80% of the market, with the remaining 20% comprising fish species from brackish and marine environments (Vishnupriya et al., 2024). The rapid expansion of the global ornamental fish trade has unintentionally facilitated the transboundary spread of pathogens. Ornamental fish are susceptible to a wide range of bacterial diseases, including columnaris disease caused by *Flavobacterium columnare*, edwardsiellosis caused by *Edwardsiella* spp., motile aeromonad septicaemia and Koi ulcerative disease associated with *Aeromonas* spp., pseudomoniasis caused by *Pseudomonas* spp., and vibriosis caused by *Vibrio* spp. These infections pose serious challenges to sustainable ornamental fish farming and aquarium management, leading to substantial economic losses (Larcombe et al., 2025). Few studies have reported the isolation of *E. tarda* from aquarium fish (Preena et al., 2022). In this context, the prevalence of *E. tarda* in diseased ornamental fish was determined, and the antibacterial activity of five chemicals against *E. tarda* was evaluated.

Out of 27 fish samples screened, 13 samples (1 Koi carp, 3 goldfish, and 9 ornamental pangasius) showed luxuriant growth in TTB. The positive samples were streaked onto SS agar. Small, translucent bacterial colonies with black centres were observed in five ornamental pangasius samples obtained from four aquarium shops. Bacterial identification of the isolates was carried out using a combination of biochemical tests, VITEK 2, and molecular methods. The presumptive *E. tarda* isolates were Gram-negative short rods, positive for catalase, methyl red, lysine, and ornithine decarboxylase, but negative for oxidase and arginine decarboxylase. VITEK 2 showed high accuracy (98.5–100%) in identifying *E. tarda*. Molecular confirmation of the isolates was performed by PCR amplification of the *fim*D and *sod*B genes. All five isolates were positive for PCR amplification of the *fim*D (445 bp) and *sod*B (500 bp) genes (Fig. 3).

The BLASTN analysis of the *sod*B sequences revealed 99.90% homology with the corresponding gene sequences of *E. tarda* in the GenBank database. The obtained nucleotide sequence was submitted to NCBI and assigned the accession number PQ035945. Phylogenetic analysis of the *sod*B gene showed a high degree of similarity with strains of *E. tarda* (Fig. 4).

The phylogenetic tree was generated using the maximum-likelihood method in MEGA 11 software.

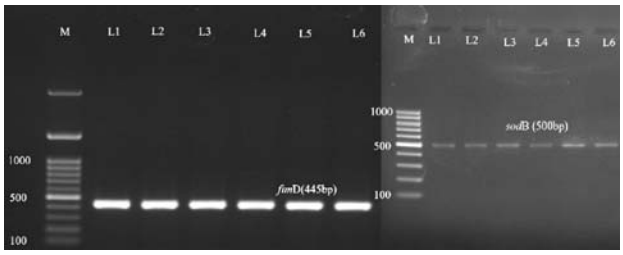

Fig. 3. PCR amplification of the *E. tarda*-specific *fim*D and *sod*B genes

M: 100bp Marker, L1: Positive Control, L2-L6: Five different bacterial isolates of *E. tarda*

Griffin et al. (2013) demonstrated the reliability of the *fimD* gene as an identification marker for *E. tarda*. To further differentiate *E. tarda* from closely related species, *sod*B gene sequencing was also employed, as suggested by Reichley et al. (2017). Our isolates showed > 99.75% similarity with *E. tarda*, while the closest matches were *E. hoshinae* (90.66%) and *E. piscicida* (88.44%). Previous research also supports the *sodB* gene as a robust marker for species-level discrimination within the genus *Edwardsiella* (Yamada & Wakabayashi, 1999; Elgendy, Sherif, Kenawy, & Abdelsalam, 2022).

Among the fish species, *E. tarda* was isolated only from ornamental pangasius, while *E. tarda* could not be isolated from any diseased goldfish or Koi carp. The failure to isolate *E. tarda* from goldfish and Koi carp in the present study can be due to differences in host immunity, bacterial load, environmental conditions, or strain virulence. A few earlier studies reported outbreaks of *E. tarda* infection in goldfish and Oscar farms in Kerala (Preena et al., 2022; Vishnupriya et al., 2024). In the present study,

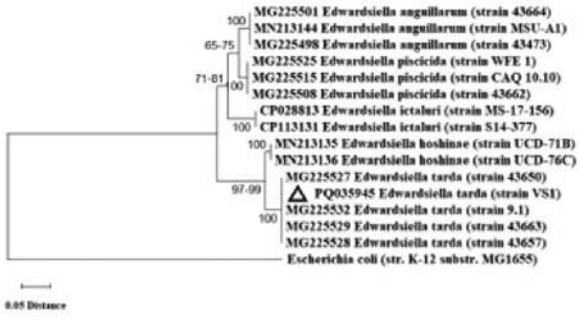

Fig. 4. Phylogenetic tree analysis of *E. tarda* isolates based on *sodB* nucleotide sequence.

The sequence generated in this study is marked by a triangle.

35.71% of ornamental pangasius were found to be affected by *E. tarda*. This finding suggests that pangasius might be more susceptible to *E. tarda* infection under captive aquarium conditions. A major outbreak of *E. tarda* infection in cultured pangasius was previously reported from Thrissur, Kerala (Joseph et al., 2019). To the best of our knowledge, this is the first report describing the prevalence of *E. tarda* in ornamental pangasius in India. This study could not determine the origin of the infected fish because retailers purchase fish from different ornamental fish dealers and often mix stocks at the time of sale. These dealers purchase fish from aquaculture farms located in different states. All *E. tarda* isolates were recovered from diseased aquarium fish, suggesting an association with disease in ornamental fish. However, the potential presence of commensal strains in the aquarium environment cannot be ruled out.

The antibiotic susceptibility test results indicated that among the five *E. tarda* isolates tested, only one was resistant to tetracycline, and three showed intermediate susceptibility. Only tetracycline resistance was observed among the *E. tarda* isolates, while remaining susceptible to 12 classes of antimicrobials. This finding is consistent with the documented use of tetracycline in ornamental fish culture for disease treatment (Vishnupriya et al., 2024). As tetracycline is the most common antimicrobial agent used in ornamental fish, its use may have contributed to the observed resistance. There are no strict rules and regulations for the use of antibiotics in ornamental fisheries, unlike in food fish aquaculture (Narendrakumar, Preena, & Swaminathan, 2023). The ornamental fish sector remains largely unregulated, particularly with respect to the prophylactic and therapeutic use of antimicrobials (Au-Yeung, Lam, Chan, & Mo, 2022; Au-Yeung et al., 2025). This regulatory gap has resulted in the widespread, unmonitored use of antibiotics, frequently at inappropriate dosages and durations, driven by the need to reduce mortality and maintain the aesthetic value of high-value species (Au-Yeung et al., 2025; Larcombe et al., 2025). Hobbyists, breeders, and even commercial traders often rely on empirical treatments without laboratory diagnosis, which not only reduces treatment efficacy but also contributes to the emergence and dissemination of resistant bacterial strains (Au-Yeung et al., 2025). These resistant microbes can persist in aquarium systems, be transferred to handlers, or enter natural ecosystems

Table 1. Total plate count of the *E. tarda* isolates treated with different chemicals

Chemical	Dose	Bacterial isolates (CFU/mL)					Mean
		VS1*	VS2*	VS3*	VS4*	VS5*	
$CuSO_4$	Half (1ppm)	$4.93\pm0.11^a \times 10^5$	$4.80\pm0.10^a \times 10^5$	$5.07\pm0.83^a \times 10^5$	$5.13\pm0.80^a \times 10^5$	$5.33\pm0.11^a \times 10^5$	$5.05\pm0.76^a \times 10^5$
	Recommended (2ppm)	$5.03\pm0.15^b \times 10^4$	$4.73\pm0.50^b \times 10^4$	$4.77\pm0.51^b \times 10^4$	$4.77\pm0.51^b \times 10^4$	$5.10\pm0.17^b \times 10^4$	$4.88\pm0.38^b \times 10^4$
	Double (4ppm)	$4.07\pm0.15^c \times 10^3$	$3.93\pm0.15^c \times 10^3$	$3.97\pm0.20^c \times 10^3$	$3.93\pm0.15^c \times 10^3$	$3.97\pm0.20^c \times 10^3$	$3.97\pm0.15^c \times 10^3$
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
$KMnO_4$	Half (1ppm)	$2.83\pm0.15^a \times 10^6$	$2.77\pm0.11^a \times 10^6$	$2.87\pm0.15^a \times 10^6$	$2.97\pm0.57^a \times 10^6$	$2.90\pm0.17^a \times 10^6$	$2.87\pm0.13^a \times 10^6$
	Recommended (2ppm)	$2.23\pm0.14^b \times 10^3$	$2.29\pm0.75^b \times 10^3$	$2.19\pm0.16^b \times 10^3$	$2.26\pm0.10^b \times 10^3$	$2.26\pm0.10^b \times 10^3$	$2.25\pm0.10^b \times 10^3$
	Double (4ppm)	$1.92\pm0.07^b \times 10^3$	$1.95\pm0.08^b \times 10^3$	$1.95\pm0.04^b \times 10^3$	$1.81\pm0.09^b \times 10^3$	$1.78\pm0.02^b \times 10^3$	$1.88\pm0.09^b \times 10^3$
	p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Povidone-iodine	Half (50ppm)	$3.63\pm0.30^a \times 10^8$	$3.93\pm0.15^a \times 10^8$	$3.70\pm0.30^a \times 10^8$	$3.50\pm0.52^a \times 10^8$	$3.60\pm0.34^a \times 10^8$	$3.67\pm0.32^a \times 10^8$
	Recommended (100ppm)	$3.37\pm0.46^a \times 10^8$	$3.60\pm0.34^a \times 10^8$	$3.77\pm0.11^a \times 10^8$	$3.60\pm0.10^a \times 10^8$	$3.60\pm0.26^a \times 10^8$	$3.59\pm0.28^a \times 10^8$
	Double (200ppm)	$2.84\pm0.25^a \times 10^8$	$3.67\pm0.51^a \times 10^8$	$3.12\pm0.05^a \times 10^8$	$2.78\pm0.14^a \times 10^8$	$2.74\pm0.04^a \times 10^8$	$3.03\pm0.42^a \times 10^8$
	p value	0.39	0.63	0.10	0.089	0.50	0.84
$MgSO_4$	Half (15000ppm)	$2.73\pm0.20^a \times 10^8$	$2.73\pm0.57^a \times 10^8$	$2.70\pm0.10^a \times 10^8$	$2.70\pm0.26^a \times 10^8$	$2.63\pm0.15^a \times 10^8$	$2.70\pm0.15^a \times 10^8$
	Recommended (30000ppm)	$2.63\pm0.57^a \times 10^8$	$2.73\pm0.11^a \times 10^8$	$2.63\pm0.11^a \times 10^8$	$2.60\pm0.10^a \times 10^8$	$2.63\pm0.57^a \times 10^8$	$2.65\pm0.91^a \times 10^8$
	Double (60000ppm)	$2.57\pm0.57^a \times 10^8$	$2.71\pm0.14^a \times 10^8$	$2.49\pm0.41^a \times 10^8$	$2.46\pm0.49^a \times 10^8$	$2.57\pm0.14^a \times 10^8$	$2.56\pm0.12^a \times 10^8$
	p value	0.76	0.99	0.71	0.68	0.97	0.72
Acetic acid	Half (500ppm)	$3.83\pm0.57 \times 10^8$	$3.90\pm0.17 \times 10^8$	$3.90\pm0.10 \times 10^8$	$3.77\pm0.20 \times 10^8$	$3.70\pm0.10 \times 10^8$	$3.82\pm0.14 \times 10^8$
	Recommended (1000ppm)	No growth	No growth	No growth	No growth	No growth	No growth
	Double (2000ppm)	No growth	No growth	No growth	No growth	No growth	No growth
	p value	-	-	-	-	-	-
Control		$4.08\pm0.14 \times 10^8$	$4.57\pm0.48 \times 10^8$	$4.22\pm0.19 \times 10^8$	$4.08\pm0.41 \times 10^8$	$4.43\pm0.65 \times 10^8$	$4.28\pm0.48 \times 10^8$

Values with different superscripts denote significant differences within column ($p < 0.05$).
*No significant difference was observed between the isolates for any chemicals

Table 2. Log reduction in the average total plate count of *E. tarda* isolate treated with different chemicals

Chemicals	Half dose	Recommended dose	Double dose
$CuSO_4$	2.95^{a}±0.07	3.96^{b}±0.07	5.05^{c}±0.06
Classification	Moderate	Good	Excellent
$KMnO_4$	2.19^{a}±0.06	5.29^{b}±0.17	5.37^{b}±0.12
Classification	Moderate	Excellent	Excellent
Povidone-iodine	0.05^{a}±0.03	0.07^{a}±0.06	0.13^{a}±0.04
Classification	Poor	Poor	Poor
MgSO4	0.2^{a}±0.05	0.19^{a}±0.02	0.22^{a}±0.05
Classification	Poor	Poor	Poor
Acetic acid	0.05^{a}±0.02	8.61^{b}±0.02	8.61^{b}±0.02
Classification	Poor	Excellent	Excellent

*Values with different superscripts denote significant differences within row ($p < 0.05$).

through the dumping of untreated water, posing a significant ecological and public health risk (Larcombe et al., 2025).

All the isolates that were resistant or showed intermediate resistance to tetracycline harboured the *tet*A gene. The resistance genes *tet*B, *tet*G, and *tet*G1 were not detected. This finding aligns with Lo, Lee, Wang, and Kuo (2014), who observed that most tetracycline-resistant isolates of *E. tarda* carried the *tet*A gene. Jun et al. (2004) identified *tet*A as the most widespread determinant of tetracycline resistance, which may be present on mobile plasmids and transferable by conjugation to other pathogens.

Chemicals such as potassium permanganate ($KMnO_4$), copper sulphate ($CuSO_4$), povidone-iodine, magnesium sulphate ($MgSO_4$), and acetic acid are widely used in aquaculture to control bacterial, fungal, and external parasitic (protozoan and metazoan) infections. However, their potential antibacterial effects have been poorly investigated. Potassium permanganate and copper sulphate have been granted "deferred regulatory status" by the FDA's Centre for Veterinary Medicine (CVM), allowing their use under specific conditions without full regulatory approval. The FDA has recognised povidone-iodine, magnesium sulphate, and acetic acid as Low Regulatory Priority (LRP) substances for use as therapeutics during disease (FDA, 2011). The proper application of these agents promotes fish health, reduces mortality, and minimises antibiotic use, thereby contributing to the mitigation of AMR (Larcombe et al., 2025). In the present study, the mean TPC of overnight cultures of *E. tarda* in the control group was $4.28 \pm 0.48 \times 10^8$ CFU/mL. In the 2 ppm $CuSO_4$ supplemented treatment, the mean count was $4.88 \pm 0.38 \times 10^4$ CFU/mL, indicating approximately a four-log reduction compared to the control. Moreover, all three concentrations of $CuSO_4$ significantly reduced the growth of *E. tarda* compared to the control. The half dose resulted in a 2.95 log reduction, whereas the recommended (2 ppm) and double dose (4 ppm) achieved 3.96 and 5.05 log reductions, respectively. Similarly, in $KMnO_4$ supplemented treatments, the mean TPC at 2 ppm was $2.25 \pm 0.10 \times 10^3$ CFU/mL. All three concentrations of $KMnO_4$ significantly reduced *E. tarda* growth relative to the control. The recommended dose (2 ppm) exhibited a 5.29 log reduction, compared to a 2.19 log reduction in the half-dose group. Treatments supplemented with povidone-iodine or $MgSO_4$ did not show any significant reduction in TPC. Among the five chemicals evaluated, acetic acid demonstrated the strongest antibacterial activity, completely inhibiting *E. tarda* growth at the recommended and higher doses (Table 1 and 2). The chemical-wise comparison of the effect of different doses on mean TPC of *E. tarda* is depicted in Fig. 5 (A, B and C).

Since no specific regulations or usage limits have been established for these chemicals, they are often freely applied in aquaculture systems (França, Paiva, Carvalho, Miashiro, & Lombardi, 2011). The present study demonstrated the differential antibacterial efficacy of these chemicals against *E. tarda*. Among the tested agents, $CuSO_4$ and $KMnO_4$ exhibited

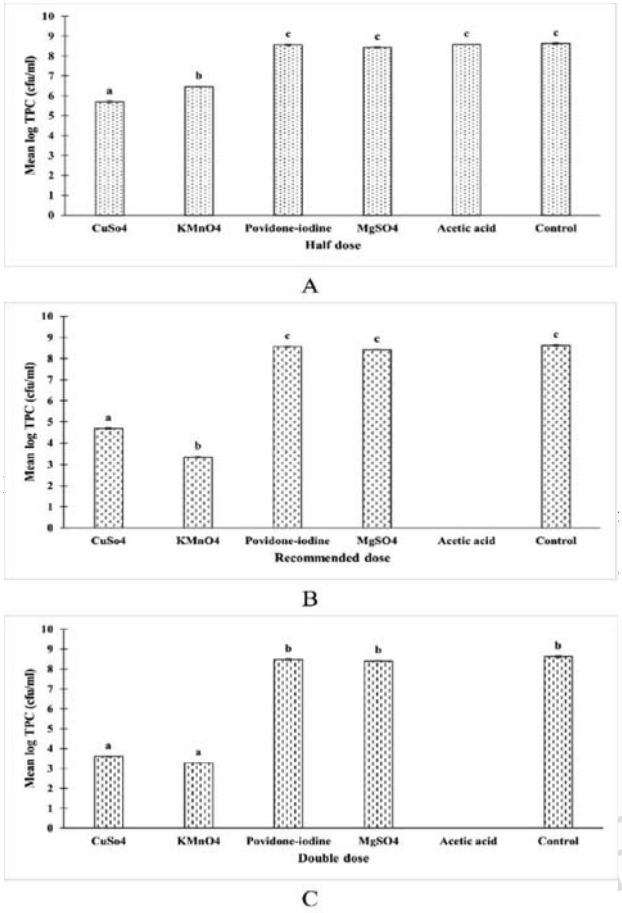

Fig. 5. Comparison of the mean total plate count of *E. tarda* supplemented with different chemicals at the (A) half dose of the recommended dose, (B) recommended dose, and (C) double dose of the recommended dose

moderate antibacterial effects, whereas Povidone-iodine and $MgSO_4$ showed negligible influence on bacterial growth. In contrast, acetic acid displayed the strongest anti-*E. tarda* activity, completely inhibiting bacterial growth at the recommended and higher doses. These findings indicate that the antibacterial potential of these chemicals varies considerably depending on the compound and applied concentration. A reduction in TPC of *E. tarda* was observed at the recommended and double doses of $CuSO_4$ and $KMnO_4$, but not with povidone-iodine and $MgSO_4$. $KMnO_4$, a strong oxidising agent, exerts bactericidal effects mainly through oxidative degradation of microbial cell components, including membrane lipids, proteins, and nucleic acids (Nonfodji et al., 2020; Banjare, Saha, Acharya, & Khan, 2024). At higher concentrations, $KMnO_4$ maintains a stronger and more sustained oxidative potential that may effectively inactivate resilient pathogens such as *E. tarda.* Conversely, $CuSO_4$ demonstrated significant antimicrobial activity even at the half dose due to the high toxicity of free cupric ions (Cu^{2+}) to bacteria. It interferes with bacterial enzymes and cellular respiration. These ions disrupt microbial metabolism by binding to proteins, damaging nucleic acids, and generating reactive oxygen species (ROS), leading to oxidative stress and cell death. The bioavailability of Cu^{2+} in water, particularly in water with low organic matter or low hardness, enhances its bactericidal action at low concentrations (Borkow & Gabby, 2005; Darwish, Mitchell, & Straus, 2012).

In a similar study, Darwish et al. (2012) also found that copper sulphate directly inhibited the growth of *Flavobacterium columnare*. On the other hand, the absence of a significant reduction in TPC in povidone-iodine and $MgSO_4$-treated groups suggests limited or transient antibacterial activity at the concentrations used. Acetic acid exhibited a pronounced inhibitory effect on *E. tarda*, leading to complete growth suppression at the recommended dose. This effect can be ascribed to its ability to penetrate the bacterial cell wall in its undissociated form, lower intracellular pH, and induce metabolic disruption (Trèek, Mira, & Jarboe, 2015). Similar bactericidal effects of acetic acid have been reported against *Salmonella* Typhimurium and *E. coli* isolated from food (Kim & Lee, 2021). These findings reinforce the potential of acetic acid as an effective antimicrobial agent in ornamental fish culture.

The comparative dose-wise analysis further highlighted that the efficacy of chemical treatments is both concentration- and compound-dependent. The superior inhibition by acetic acid, followed by $KMnO_4$ and $CuSO_4$, underscores its potential as a promising alternative for controlling the growth of *E. tarda*. The recommended doses of acetic acid and $KMnO_4$ were the most effective in reducing *E. tarda* growth. No major additional effect was observed at double the recommended dose, whereas the half dose showed significantly lower efficacy. Moreover, the use of higher doses of chemicals in the ponds may also adversely affect the environment and non-target aquatic organisms. Therefore, the results suggest using only the recommended doses. No variability in response among the isolates was observed, as all five isolates showed similar susceptibility profiles, suggesting no strain-specific resistance. However, environmental interactions and

the presence of organic load may influence the antibacterial effects. Further *in vivo* studies are warranted to establish optimal concentrations that ensure effective pathogen control while minimising potential toxicity in aquaculture systems.

Infectious diseases like edwardsiellosis can disrupt the livelihoods of ornamental fish farmers, exporters, distributors, wholesalers, and retailers involved in ornamental fish trading. Therefore, investigating new chemicals with antibacterial properties is crucial because the effectiveness of antimicrobials is becoming compromised by the emergence of AMR. This study found that $KMnO_4$ and $CuSO_4$ are effective against *E. tarda* at 2 ppm. These chemicals may therefore be useful for reducing the risk of edwardsiellosis and for prophylactic sanitation in ornamental fish culture. Additionally, acetic acid may serve as an effective antibacterial agent at concentrations of ≥1000 ppm, completely inhibiting the growth of *E. tarda*.

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