Journal of Water & Health

IWA PUBLISHING

Journal of Water and Health Vol 21 No 12, 1858 doi: 10.2166/wh.2023.206

Isolation and identification of antimicrobial susceptibility, biofilm formation, efflux pump activity, and virulence determinants in multi-drug resistant *Pseudomonas aeruginosa* isolated from freshwater fishes

Kummari Suresh[a], Devika Pillai [a,*], Manju Soman[b], Akula Sreenivas[c] and Robin Paul[b]

[a] Department of Aquatic Animal Health Management, Faculty of Fisheries Science, Kerala University of Fisheries and Ocean Studies, Kochi, Kerala, India
[b] Department of Animal Husbandry, Government of Kerala, Kochi, Kerala, India
[c] Agriculture Market Intelligence Centre, Professor Jayashankar Telangana State Agricultural University, Hyderabad, India
*Corresponding author. E-mail: devikamanoj.pillai@gmail.com

DP, 0000-0002-1702-4960

ABSTRACT

The present study was undertaken to evaluate the prevalence, underlying resistance mechanism, and virulence involved in *Pseudomonas aeruginosa* ($n = 35$) isolated from freshwater fishes in Andhra Pradesh, India. Antibiogram studies revealed that 68.5, 62.8, 37.1, 11.4, 8.5, 57.1, 54.2, and 48.5% of isolates had resistance to oxytetracycline, co-trimoxazole, doxycycline, enrofloxacin, ciprofloxacin, cefotaxime, ceftazidime, and ampicillin, respectively. The resistant isolates harboured the *tetA* (85.7%), *tetD* (71.4%), *tetM* (91.4%), *sul1* (80%), $bla_{CTX\text{-}M}$ (57.1%), bla_{TEM} (42.8%), and bla_{SHV} (48.5%) genes. In total, 50% of the isolates were altered as multi-drug resistant, and the multiple antibiotic resistance index was calculated as 0.4. Furthermore, 37.3, 48.5, and 14.2% of isolates were categorized as strong, moderate, and weak biofilm formers, possessing *pslA* (91.5%) and *pslD* (88.6%) biofilm encoding genes. In total, 82.8% of the isolates exhibited efflux pump activity and harboured the *mexA* (74.2%), *mexB* (77.1%), and *oprM* (37.1%) genes. Virulent genes *oprL*, *toxA*, *exoS*, and *phzM* were detected in 68.5, 68.5, 100, and 17.1% of isolates, respectively. The data suggested that *P. aeruginosa* harbours multiple resistance mechanisms and virulence factors that may contribute to antibiotic resistance and pathogenicity, and their distribution in fish culture facilities highlights the public health hazards of the food chain.

Key words: biofilm, efflux pump, fish, public health, virulence

HIGHLIGHTS

- Multi-drug-resistant *P. aeruginosa* strains were identified and characterized from freshwater fishes in Andhra Pradesh, India.
- Improper farm management and disease outbreaks are the major risk factors associated with antimicrobial use.
- Different antibiotic resistance mechanisms and virulence gene determinants were identified using the polymerase chain reaction.

INTRODUCTION

Aquaculture is a rapidly growing fisheries sector in India with an annual growth of 10.34%, and contributing about 1.24% to national gross value added (GVA) and 7.28% to agricultural GVA. Freshwater aquaculture contributes over 95% of the total aquaculture production (Mishra *et al.* 2017b). Techniques like polyculture, induced carp breeding, composite carp culture on Indian major carps, and exotic carps led to the development of freshwater aquaculture. Intensification of aquaculture has, however, increased the risk of disease outbreaks. Disease outbreaks are seen as significant barriers to aquaculture production and sustainability, and contribute between 10 and 15% of the cost of production (Mishra *et al.* 2017b). Bacterial pathogens are opportunistic in nature and closely related to physiologically unbalanced, nutritionally deficient conditions, poor water quality, and high stocking densities (Tavares-Dias & Martins 2017). *Aeromonas hydrophila*, *A. caviae*, *Edwardsiella tarda*, *Pseudomonas aeruginosa*, *P. putida*, *P. fluorescens*, *P. putrefaciens*, *Flexibacter columnar*, *Vibrio alginolyticus*, *Streptococcus iniae*, and *S. agalactiae* are the common fish bacterial pathogens responsible for disease outbreaks (Mishra *et al.* 2017a). This has led to a rise in the use of antimicrobials, which are now frequently utilized, sometimes excessively, and inappropriately in a wide range of farming areas (Page & Gautier 2012).

P. aeruginosa is a common bacterial pathogen that can survive in a variety of habitats in the environment. It is a common hospital-acquired pathogen that causes severe nosocomial infections, cystic fibrosis, respiratory and urinary tract infections, and wound and soft tissue infections in immune-compromised patients. Furthermore, *P. aeruginosa* is one of the primary global causes of septicaemia in both freshwater and marine water fishes with severe economic losses in fish farms (Algammal *et al.* 2020). It is a part of normal fish microbiota, but under stressful conditions, the bacteria may become pathogenic, which can lead to acute haemorrhagic septicaemia, gill necrosis, abdominal distension, splenomegaly, a friable liver, and a congested kidney (Ardura *et al.* 2013). There are reports of *P. aeruginosa* infections in *Labeo rohita*, *Catla catla*, and *Pangasianodon hypophthalmus* causing haemorrhages, eye opacity, tail and fin rot, abdominal dropsy, paleness, an enlarged liver, congested kidneys, and spleen (Yaseen *et al.* 2020; Beulah *et al.* 2022).

Antimicrobial resistance (AMR) is undoubtedly a public health concern due to antibiotic misuse and overuse in humans and farm animals, which has changed the natural bacterial population and increased AMR levels (Schar *et al.* 2020). High levels of antibiotic dependency in animal food production threaten both the food system and wildlife through AMR and the release of antibiotic-resistant bacteria (ARBs) and antibiotic-resistant genes into the environment (Lulijwa *et al.* 2020). ARBs accumulate in water, sediment, farm and wild animals, and in and around the farms, which limit the effective treatment options, undermining the sustainability of aquatic food production and animal welfare (Cabello *et al.* 2013). It is well known that *P. aeruginosa* displays resistance to a wide variety of antibiotics (Curran *et al.* 2018). Generally, the major mechanism of *P. aeruginosa* used to counter antibiotic attacks can be classified into intrinsic and acquired or adaptive resistance (Soto 2013). The intrinsic resistance of *P. aeruginosa* includes low outer membrane permeability, expression of efflux pumps that expel antibiotics out of the cell and production of antibiotic-inactivating enzymes. The acquired resistance of *P. aeruginosa* can be achieved by either horizontal transfer of resistance genes or mutational genes. The adoptive resistance of *P. aeruginosa* involves biofilm formation where the biofilm serves as a diffusion barrier to limit antibiotic access to the bacterial cell (Streeter & Katouli 2016). The prophylactic and therapeutic use of tetracycline and sulphonamides in aquaculture has led to the emergence of tetracycline and sulphonamides resistance in aquatic-borne bacteria (Aminov 2013). The resistance of *P. aeruginosa* to the β-lactam antibiotics is mainly attributed to the extended spectrum beta-lactamases (ESBLs). $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} are the most prevalent ESBL encoding genes and have been described in *P. aeruginosa* strains (Algammal *et al.* 2020). Besides, this bacterium possesses many virulence-related determinants, including cell-mediated and secreted virulence types. The cell-mediated types include pilli, flagella, and lipopolysaccharide, which are commonly involved in bacterial colonization and motility, the delivery of active proteins into the host cells, and the establishment of persistent infections. Likewise, the secreted virulence factors fortify the inflammatory processes, induce severe tissue damage, facilitate bacterial invasion and dissemination, and accelerate the progression of diseases (Mesquita *et al.* 2013).

There have been several reports on the AMR and virulence of *P. aeruginosa* isolated from fishes around the world (Algammal *et al.* 2020). Andhra Pradesh has become a real contender in fish and shrimp production, contributing 50% to the country's total production. Semi-intensive and intensive farming facilities are vulnerable to disease outbreaks. Based on the initial survey and interactions with local diagnostic laboratories and experts in the field, *P. aeruginosa* is opportunistically present in the aquatic environment, contributing to disease and AMR. Indeed, the identification of *P. aeruginosa* is essential for accurate diagnosis, outbreak prediction, and preventive and/or prophylactic measure implementation in aquaculture. Therefore, this study aimed to identify the prevalence, antibiogram, and underlying resistance mechanisms and virulence involved in the emergence and spread of multi-drug-resistant *P. aeruginosa* in freshwater finfish aquaculture.

MATERIALS AND METHODS

Sample collection

In the present study, data from 110 freshwater finfish farms were collected to better understand management practices adopted within the system for aquaculture production. A total of 310 fishes were collected, representing 140 *L. rohita* (Rohu), 109 *C. catla* (Catla), and 61 *P. hypophthalmus* (Pangasius) were randomly from 110 freshwater farms in Krishna (16 °36′21.22″N, 80 °42′56.39″E) and West Godavari (16 °53′55.65″N, 81 °18′9.30″E) districts of Andhra Pradesh, India, from June 2021 to October 2022 (Figure 1). A prescribed questionnaire under the All India Network Project on Fish Health was used to collect data regarding culture type, size of the farm, culture species, stocking densities, and aquaculture inputs for water, soil, feed, health management, and routine disease testing to better understand the daily practices followed in fish farms and to identify risks and challenges for farmers within the systems. Fresh specimens of apparently healthy fish

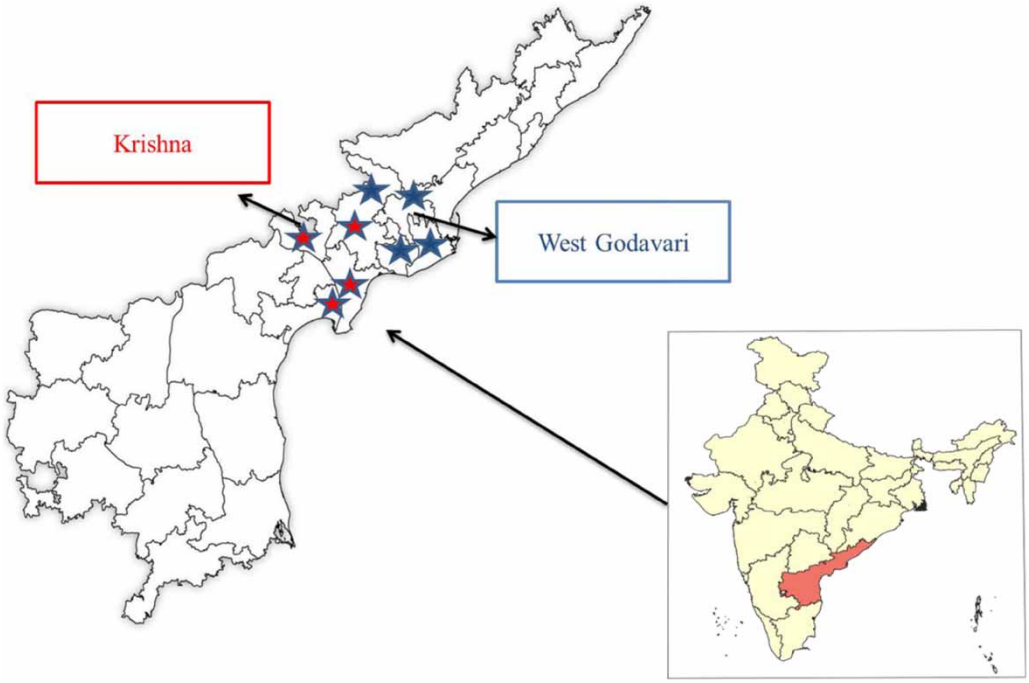

Figure 1 | Map of the study region showing the locations of sampling sites (Adobe® Photoshop® 7.0).

were transferred alive in aerated plastic bags to the microbiology laboratory, Department of Fisheries, Kaikaluru, Andhra Pradesh for further bacteriological examination. Samples from the gill, liver, kidney, and spleen were collected and processed aseptically following the protocols of Austin & Austin (2007). For selective enrichment, 1 g of sample was transferred into 9 ml of sterile malachite green broth (HiMedia, India) and incubated at 37°C for 18–24 h. To isolate the *P. aeruginosa* strains, a loopful of enriched culture was streaked onto cetrimide agar (HiMedia, India) and incubated at 37°C for 18–24 h. The yellowish-green colonies are suspected to be pseudomonads, as per Lamont & Martin (2003). The suspected *Pseudomonas* isolates were preserved in 20% glycerol-containing tryptic soy broth (HiMedia, India) and transported to the Department of Aquatic Animal Health Management, Faculty of Fisheries Science, Kerala University of Fisheries and Ocean Studies, Kerala, India, for further analysis.

Identification of *P. aeruginosa*

The BD Phoenix™ M50 automated system was used to identify *P. aeruginosa* isolated from fish samples (BD Diagnostics, USA). In this study, an ID-AST combo panel, NMIC/ID55, which is designed for bacterial identification and susceptibility testing, was used. The panels were only used for bacterial identification since the antibiotics selected for testing were not available on the panel's antibiotic list provided by manufacturers. All the steps were carried out in accordance with the manufacturer's instructions. Pure bacterial cultures were transferred into ID broth, and cells were adjusted by 0.5 McFarland with the help of a BD Phoenix Spec nephelometer. Then, the panels were sealed and put into the instrument for incubation at 35°C for 10–12 h. *P. aeruginosa* ATCC 27853 were used as quality control. The data management programme EpiCentre linked to the BD Phoenix system was used to analyse test results.

Antimicrobial susceptibility test

The antibiotic susceptibility testing (AST) was performed on Muller-Hinton agar (HiMedia, India) by the disc diffusion method in accordance with the Clinical and Laboratory Standards Institute guidelines (CLSI 2022). Antibiotic discs used for AST were based on the data obtained in the survey. The selected farms routinely used different antibacterial agents such as oxytetracycline, doxycycline, ciprofloxacin, enrofloxacin, and co-trimoxazole (combination of trimethoprim and sulphamethoxazole) for regular prophylactic and treatment protocol. The antibiotic discs used for AST included oxytetracycline (30 μg), doxycycline (30 μg), ciprofloxacin (5 μg), enrofloxacin (10 μg), co-trimoxazole (25 μg), imipenem (10 μg), meropenem

(10 μg), cefotaxime (30 μg), ceftazidime (30 μg), amikacin (30 μg), and ampicillin (10 μg). All the antibiotic discs were purchased from HiMedia, India. Exponential-phase bacterial cultures were adjusted to 0.5 McFarland standard, and 100 μL was plated onto MHA and incubated at 37°C for 18–24 h. *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as quality control isolates. The zones of inhibition were recorded and categorized as susceptible, intermediate, and total resistance according to the zone diameter interpretation standard described in the CLSI (2022). Isolates that exhibited resistance to three or more classes of antibiotics were categorized as multi-drug-resistant (Magiorakos *et al.* 2012). The multiple antibiotic resistance (MAR) index was calculated as per Krumpermann (1983). All the resistant *P. aeruginosa* isolates were examined for antibiotic resistance genes, biofilm development, efflux pump activity, and virulent profiles.

Phenotypic detection of biofilm formation and efflux pump activity

The biofilm formation assay was performed using the tissue culture plate method (Mathur *et al.* 2006). Individual colonies of *P. aeruginosa* isolates were inoculated into Brain Heart Infusion (BHI) broth (HiMedia, India) and supplemented with 2% sucrose. In total, 200 μL of bacterial suspension was added to a 96-well microtiter plate and incubated at 37°C for 18–24 h. As a negative control, 200 μL of sterile BHI broth was poured into a well. After incubation, the contents of each well were removed and washed three times with sterile deionized water to eliminate non-adherent bacteria. After 45 min of air drying, each well was filled with 200 μL of 0.2% (v/v) crystal violet (HiMedia, India) and incubated for 45 min at room temperature. The wells were then washed four times with sterile, deionized water. The dye was solubilized in each well with 200 μL of 33% glacial acetic acid (HiMedia, India), and the optical density (OD) was measured at 650 nm using the iMark™ Microplate Reader (Bio-Rad, USA). The mean OD values classified as strong (>0.108), moderate (0.108–0.083), and weak (0.083), and biofilm formers were used to quantify the biofilm-forming potential as per Hassan *et al.* (2011).

The efflux pump activities of isolates were assessed using the ethidium bromide agar cartwheel (EtBrCW) method (Martins *et al.* 2013). Bacterial suspensions having a 0.5 McFarland standard of turbidity were swabbed on tryptic soy agar with EtBr concentrations of 0, 0.5, 1, 1.5, and 2 mg/L and incubated at 37°C for 18–24 h. After incubation, the plates were examined for fluorescence under UV light in a gel documentation system (Bio-Rad, USA). Those plates lacking fluorescence indicated the presence of active efflux pumps, while those that fluoresced indicated the absence of efflux pumps.

Molecular characterization of biofilm formation, efflux pump activity, and virulence-encoding genes in *P. aeruginosa*

The genomic DNA of *P. aeruginosa* isolates was extracted according to the method of Kpoda *et al.* (2018). About three to four colonies of overnight-grown cultures were suspended in 200 μL of TE buffer and kept at 95°C for 10 min in a dry bath (IKA®, Dry Block Heater, India). The cell culture suspension was allowed to cool down and centrifuged at 12,000 rpm at 4°C for 10 min. The supernatant containing DNA was collected and kept at −20°C until use. The polymerase chain reaction (PCR) was carried out in a 25 μL reaction volume, targeting genes encoding for antibiotic resistance (*tetA*, *tetD*, *tetM*, *sul1*, $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV}), biofilm formation (*pslA*, *pslD*), efflux pump activity (*mexA*, *mexB*, and *oprM*), and virulence (*oprL*, *toxA*, *exoS*, and *phzM*) with specific primers using EmeraldAmp GT PCR Master Mix (Takara, India) in the Applied Biosystems™ Proflex™ (Thermo Fisher Scientific, USA) thermocycler. DNA template from *P. aeruginosa* ATCC 27853 was used as a positive control for *pslA*, *pslD*, *mexA*, *mexB*, *oprM*, *oprL*, *toxA*, *exoS*, and *phzM* genes, and nuclease-free water was used as a negative control. The primer sequences, PCR conditions, and expected amplicon size are depicted in Table 1. The PCR products were electrophoresed on 1.5% agarose at 80 V for 45 min, visualized, and photographed in a gel documentation system (Bio-Rad, USA).

RESULTS

Bacterial identification

The bacteriological examination revealed that the isolates were motile and Gram-negative bacilli, and arranged in double or short chains. The typical isolates of *P. aeruginosa* displayed large irregular colonies with a fruity odour and produced a yellowish-green fluorescent pigment on cetrimide agar (HiMedia, India). Among the 82 suspected isolates, 35 isolates were confirmed to be *P. aeruginosa* by the BD Phoenix™ M50 automated system. Only *P. aeruginosa* strains were included in this study. The total prevalence (Table 2) of *P. aeruginosa* was found to be 11.2% ($n = 35/310$), comprising *L. rohita* (9.56%; 11/115), *C. catla* (10%; 10/100), and *P. hypophthalmus* (14.7%; 14/95). Molecular characterization of virulence profiles showed that 68.5% (24/35), 68.5% (24/35), 100% (35/35), and 17.1% (6/35) of the isolates had *oprL*, *toxA*, *exoS*, and *phzM* virulence-encoding genes (Table 3).

Table 1 | List of primers, expected amplicon size, and annealing temperatures used in the present study

Target gene	Primer sequence (5′–3′)	Amplicon (bp)	PCR conditions					Reference
			Denaturation	Annealing	Extension	Cycles	Final Extension	
tetA	F- GGCGGTCTTCTTCATCATGC R-CGGCAGGCAGAGCAAGTAGA	502	94°C for 1 min	58°C for 30 s	72°C for 1 min	35	72°C for 7 min	Lanz *et al.* (2003)
tetD	F-GAGCGTACCGCCTGGTTC R- TCTGATCAGCAGACAGATTGC	780	94°C for 30 s	55°C for 30 s	72°C for 30 s	35	72°C for 5 min	Koo & Woo (2011)
tetM	ACACGCCAGGACATATGGAT ATTTCCGCAAAGTTCAGACG	536	96°C for 30 s	53°C for 30 s	72°C for 1 min	35	72°C for 5 min	Call *et al.* (2003)
Sul1	F-CGGCGTGGGCTACCTGAACG R-GCCGATCGCGTGAAGTTCCG	433	94°C for 15 s	69°C for 30 s	72°C for 1 min	30	72°C for 7 min	Karczmarczyk *et al.* (2011)
pslA	F-TGGGTCTTCAAGTTCCGCTC R-ATGCTGGTCTTGCGGATGAA	119	95°C for 30 s	57°C for 40 s	72°C for 1 min	30	72°C for 10 min	Maita & Boonbumrung (2014)
pslD	F-CTCATGAAACGCACCCTCCT R-TGCGACCGATGAACGGATAG	295						
mexA	F-ACCTACGAGGCCGACTACCAGA R-GTTGGTCACCAGGGCGCCTTC	179		59°C for 40 s	72°C for 1 min		72°C for 10 min	Pourakbari *et al.* (2016)
mexB	F-GTGTTCGGCTCGCAGTACTC R-AACCGTCGGGATTGACCTTG	244		58°C for 40 s				
oprM	F-CCATGAGCCGCCAACTGTC R-CCTGGAACGCCGTCTGGAT	205		59°C for 40 s				
oprL	F-ATGGAAATGCTGAAATTCGGC R-CTTCTTCAGCTCGACGCGACG	504	96°C for 1 min	58°C for 1 min	72°C for 1 min	40	72°C for 10 min	Algammal *et al.* (2020)
toxA	F-GACAACGCCCTCAGCATCACC R-AGCCGCTGGCCCATTCGCTCCAGCGCT	396	94°C for 1 min	59°C for 1 min		30		
exoS	F-GCGAGGTCAGCAGAGTATCGTTC R-GGCGTCACTGTGGATGC	118	94°C for 30 s	58°C for 30 s		36		
phzM	F-ATGGAGAGCGGGATCGACAGATG R- CGGGTTTCCATCGGCAG	875	94°C for 30 s	59°C for 30 s		30		
$bla_{CTX\text{-}M}$	F- CGCTTTGCGATGTGCAG R- ACCGCGATATCGTTGGT	550	94°C for 1 min	55°C for 30 s	72°C for 1 min	30	72°C for 7 min	
bla_{TEM}	F- ATAAAATTCTTGAAGACGAAA R- GACAGTTACCAATGCTTAATC	1,080	94°C for 1 min	50°C for 40 s	72°C for 1 min	32	72°C for 10 min	
bla_{SHV}	F- TTAACTCCCTGTTAGCCA R- GATTTGCTGATTTCGCCC	795	95°C for 1 min	52°C for 30 s	72°C for 1 min	32	72°C for 7 min	

Table 2 | Showing the sampling site, species collected, and prevalence of *P. aeruginosa*

Sampling site	Number of fishes	*L. rohita*	*C. catla*	*P. hypophthalmus*
Krishna	170	60	55	55
West Godavari	140	55	45	45
Number of *P. aeruginosa* isolates	35	11	10	14
Mean	155 ± 21	57.5 ± 3.5	50 ± 7.0	50 ± 7.0
Prevalence (%)	11.2	9.56	10	14.7

Antibiogram study

All ($n = 35$) isolates were screened for antibiogram, which displayed variable resistance patterns to the panel of antibiotics tested. Sixty-eight percent (24/35), 62.8% (22/35), 37.1% (13/35), 11.4% (4/35), 8.5% (3/35), 57.1% (20/35), 54.2% (19/35), and 48.5% (17/35) of isolates had resistance to oxytetracycline, co-trimoxazole, doxycycline, enrofloxacin, ciprofloxacin, cefotaxime, ceftazidime, and ampicillin, respectively. None of the isolates were found resistant to imipenem, amikacin, and meropenem. The isolates from *P. hypophthalmus* showed high resistance in comparison to the isolates from *C. catla* and *L. rohita*. In order to correlate AST findings with antibiotic-resistant determinants, the isolates were screened for specific antibiotic-resistant gene fragments harbouring *tetA*, *tetD*, *tetM*, *sul1*, $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} genes. They were prevalent in 85.7% (30/35), 71.4% (25/35), 91.4% (32/35), 80% (28/35), 57.1% (20/35), 42.8% (15/35), and 48.5% (17/35) of the *P. aeruginosa* isolates, respectively. Furthermore, 50% of the *P. aeruginosa* isolates were altered as multi-drug-resistant, having resistance to more than three antibacterial agents. The MAR index was found to be 0.4.

Biofilm formation

Pseudomonas aeruginosa strains were categorized as strong, moderate, and weak biofilm formers (WBF). Around 37.3% (13/35), 48.5% (17/35), and 14.2% (5/35) of biofilm formers were identified as strong biofilm formers (SBF), moderate biofilm formers (MBF), and WBF, respectively. All SBF isolates carried the *pslA* and *pslD* genes, whereas 62 and 79% of MBF carried *pslA* and *pslD*, respectively. Sixty-five percent of WBF possessed only the *pslA* gene, while none of the WBF carried the *pslD* gene. The overall prevalence of the *pslA* and *pslD* genes in all biofilm formers was 91.5% (32/35) and 88.6% (31/35), respectively.

Evaluation of efflux pump activity in *P. aeruginosa*

The efflux pump activity was detected in all concentrations of EtBr-coated agar plates. At EtBr concentrations of 1 and 2 mg/L, a unique efflux pump activity was detected (Figure 2). Overall, 82.8% (29/35) of *P. aeruginosa* isolates exhibited efflux pump activity, and 6 isolates did not. The isolates having active efflux pumps possessed *mexA* (74.2%; 26/35), *mexB* (77.1%; 27/35), and *oprM* (37.1%; 13/35) genes, which belong to the resistance-nodulation-division family. *MexA and mexB* were detected in 10 isolates, and *mexA* and *oprM* were detected in 13 isolates, while 16 isolates possessed all three genes (*mexA, mexB,* and *oprM*).

DISCUSSION

AMR is detrimental to human and animal health and has been widely acknowledged and addressed in recent years (Schar *et al.* 2020). Antibiotic-resistant organisms cause infections that are more challenging to treat, as they necessitate medications that are often more difficult to obtain, more expensive, and even more toxic (Lulijwa *et al.* 2020). Bacterial, mycotic, and parasitic diseases have been identified as substantial risk factors in fish farms in Andhra Pradesh, India (Mishra *et al.* 2017a). *P. aeruginosa* is a normal component of the fish microbiota, but under stressed conditions, the bacteria may become pathogenic to fish (Ardura *et al.* 2013). It is interesting to note that *P. aeruginosa* isolated from apparently healthy fish, indicating opportunistic distribution in the aquatic environment, can cause disease in stressed animals. In the present study, the total prevalence of *P. aeruginosa* was found to be 11.2%, comprising *L. rohita* (9.56%), *C. catla* (10%), and *P. hypophthalmus* (14.7%). This is in agreement with previous reports, where *P. aeruginosa* is highly prevalent in freshwater fishes (Ardura *et al.* 2013; Algammal *et al.* 2020). The prevalence of *P. aeruginosa* was found to be high in *P. hypophthalmus* due to the high-density culture with ineffective farm management. The dissimilarity of pathogen distribution in aquaculture is closely

Table 3 | AMR, biofilm formation, efflux pump activity, and virulence gene determinants of *P. aeruginosa* from fish samples

		AMR genes							Biofilm formation		Efflux pump genes			Virulence profiles			
Isolate	**Source**	***tetA***	***tetD***	***tetM***	***sul1***	***bla*$_{CTX-M}$**	***bla*$_{TEM}$**	***bla*$_{SHV}$**	***pslA***	***pslD***	***mexA***	***mexB***	***oprM***	***oprL***	***toxA***	***exoS***	***phzM***
PA01	Kidney	+	−	+	+	+	−	+	−	+	−	+	+	+	+	+	−
PA02	Spleen	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	−
PA03	Kidney	+	−	+	+	−	+	+	+	+	+	+	−	+	+	+	−
PA04	Gill	+	+	−	+	−	+	+	+	−	+	+	−	−	−	+	−
PA05	Kidney	+	−	+	+	+	−	+	+	+	−	−	+	+	+	+	−
PA06	Kidney	+	−	+	−	+	−	−	+	+	+	+	+	−	−	+	−
PA07	Kidney	−	+	+	+	+	−	+	+	+	−	−	+	−	+	+	−
PA08	Kidney	+	−	+	+	+	+	−	+	+	−	−	−	+	−	+	−
PA09	Liver	+	+	−	+	+	−	−	−	−	+	+	−	+	+	+	−
PA10	Liver	+	+	+	−	−	+	+	+	+	+	+	−	+	+	+	−
PA11	Spleen	−	−	+	+	−	−	−	+	+	+	+	−	+	+	+	−
PA12	Spleen	+	−	+	+	−	−	+	+	+	+	+	−	+	+	+	−
PA13	Liver	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+
PA14	Liver	+	+	+	−	−	+	+	+	+	+	+	+	−	−	+	+
PA15	Gill	+	+	+	+	+	−	−	+	+	−	−	+	+	+	+	+
PA16	Gill	−	+	+	+	+	−	−	+	+	+	+	−	+	+	+	−
PA17	Kidney	+	+	+	+	+	−	−	+	+	+	+	−	−	+	+	−
PA18	Kidney	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	−
PA19	Liver	+	+	+	+	−	−	−	+	+	−	−	+	+	+	+	−
PA20	Spleen	+	−	+	+	+	−	−	+	+	+	+	−	+	+	+	−
PA21	Spleen	+	+	+	−	+	+	+	+	+	+	+	−	+	+	+	−
PA22	Liver	+	+	+	+	−	−	−	+	+	−	−	+	+	+	+	−
PA23	Gill	+	−	+	+	+	+	+	+	+	−	−	−	−	−	+	−
PA24	Gill	−	+	+	+	−	−	−	+	+	−	−	−	+	−	+	−
PA25	Gill	−	+	+	−	−	+	+	+	+	+	+	+	−	+	+	−
PA26	Liver	+	−	+	+	+	−	−	+	+	+	+	−	+	+	+	−
PA27	Kidney	+	+	+	+	−	−	−	+	+	+	+	−	+	+	+	−
PA28	Liver	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−
PA29	Spleen	+	+	+	−	−	+	+	+	+	+	+	+	+	+	+	+
PA30	Liver	+	+	+	+	−	−	−	+	+	+	+	+	+	−	+	+

(*Continued.*)

Table 3 | Continued

		AMR genes							Biofilm formation		Efflux pump genes			Virulence profiles			
Isolate	**Source**	***tetA***	***tetD***	***tetM***	***sul1***	***bla$_{CTX-M}$***	***bla$_{TEM}$***	***bla$_{SHV}$***	***pslA***	***pslD***	***mexA***	***mexB***	***oprM***	***oprL***	***toxA***	***exoS***	***phzM***
PA31	Gill	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−
PA32	Liver	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	+
PA33	Kidney	+	+	+	+	−	+	+	+	−	+	+	−	−	+	+	−
PA34	Kidney	+	+	+	+	+	−	−	+	+	+	+	−	+	+	+	−
PA35	Spleen	+	+	−	+	+	−	−	−	−	+	+	−	−	−	+	−

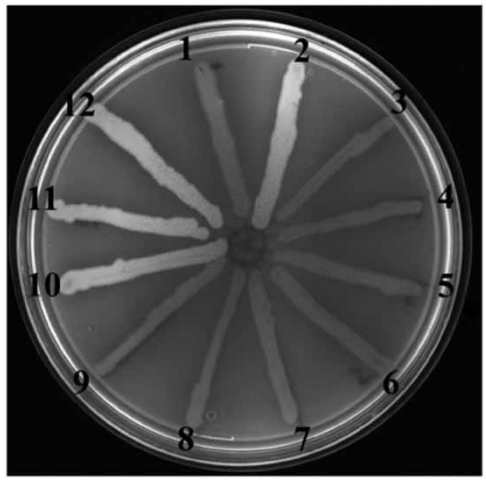

Figure 2 | Efflux pump activity of *P. aeruginosa* determined by the EtBrCW method (2 mg/L). Isolates 1, 3, 4, 5, 6, 7, 8, and 9 were positive for efflux pump activity as they did not fluoresce under UV light. Isolates 2, 10, 11, and 12 lack efflux pump activity and fluoresced because of EtBr retention.

associated with species, stocking density, and farming activities leading to stress (Mishra *et al.* 2017a; Lulijwa *et al.* 2020). According to the findings of the current study, the majority of fish farms hold high stocking densities (8,000–10,000 fish/acre) with ineffective farm management, poor biosecurity, and continuous cultures with partial harvesting. Furthermore, it was observed that all (100%) of the farms had been in operation for at least 3 years at the time of sampling. This likely indicates that several factors contribute to the emergence and spread of bacterial pathogens in the aquatic environment (Ali *et al.* 2020).

Diseases are substantial risk factors associated with morbidity and mortality, leading to economic losses. This may lead to the use of chemical and biological compounds for water quality management, pathogen prevention, and control (Mishra *et al.* 2017b). Imprudent use of antibiotics and the emergence of antibiotic resistance genes could result in the occurrence of multi-drug resistant (MDR) strains (Reverter *et al.* 2020). It is well known that *P. aeruginosa* has strong intrinsic and acquired resistance to a broad range of antibiotics (Horna *et al.* 2018). In the present study, 50% of isolates altered as MDR and were resistant to oxytetracycline, co-trimoxazole, doxycycline, enrofloxacin, ciprofloxacin, cefotaxime, ceftazidime, and ampicillin. The isolates possessed *tetA*, *tetD*, *tetM, sul1*, $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} genes. The MAR index was found to be high, indicating that all the isolates are originating from high-risk sources where the use of antibiotics is rampant. These findings closely resemble those of research on aquaculture-derived *Klebsiella pneumoniae* and *P. aeruginosa* from fish and shrimp farms in India (Das *et al.* 2018; Algammal *et al.* 2020). Previous studies reported that a strong relationship exists between trends in AMR isolates and antibiotic use, environmental factors, season, water quality, stocking density, pollution and global warming, and disease incidence (Mishra *et al.* 2017b; Reverter *et al.* 2020). Earlier studies investigated a link between pesticide use and AMR to commonly used antibiotics, which could be conferred by a plasmid that contributes to cross-resistance, namely, an unspecific organophosphorus hydrolase that degrades antibiotic derivatives (Rangasamy *et al.* 2017). Baseline data found during sampling indicate that pesticides (diclorvos, cypermethrin, amitraz, ivermectin, albendazole, emamectin benzoate, deltamethrin) and herbicides (glyphosate) were commonly used in fish culture. A sub-lethal effect of pesticides and herbicides is related to an adaptive MAR phenotype associated with an increased expression of efflux pumps and is responsible for MDR bacteria proliferation (Malagon-Rojas *et al.* 2020). This could be the situation with cross-resistance, where natural selection may be involved in multi-drug resistance resulting from horizontal gene transfer (Tincher *et al.* 2017; Malagon-Rojas *et al.* 2020). The use of cephalosporin antibiotics in aquaculture practices is unlikely. However, 57.1, 42.8, and 48.5% of the isolates possessed cephalosporin resistance genes $bla_{CTX\text{-}M}$, bla_{TEM}, and bla_{SHV} genes. Thus, our findings are in line with those of Malagon-Rojas *et al.* (2020) and Tincher *et al.* (2017) who opined that cross-resistance with other antimicrobials may be involved in the emergence and spread of MDR pathogens in aquaculture settings.

Biofilm development enhances antibiotic resistance and pathogenicity, resulting in persistent infections (Kamali *et al.* 2020). In the present study, all resistant isolates had the ability to produce biofilm with varying capabilities. Previous studies

have described that the association between antibiotic resistance and biofilm production in *P. aeruginosa* strongly supports our findings (Cepas *et al.* 2019). The polysaccharide synthesis locus (*psl*), which regulates the secretion of biofilm in *P. aeruginosa*, is largely composed of the *pslA* and *pslD* genes. In the present study, 91.5 and 88.6% of isolates were found to be positive *pslA* and *pslD* genes, respectively. Our research findings have been supported by Ugwuanyi *et al.* (2021) who reported the presence of *pslA* and *pslD* biofilm encoding genes in antibiotic-resistant *P. aeruginosa* from clinical isolates in Nigeria. In addition, active efflux pumps play a significant role in mediating bacterial multi-drug resistance. According to previous reports, *P. aeruginosa* has been found to express a high level of the *mexAB–OprM* efflux pump, which concurrently promotes resistance to multiple antibiotics (Pan *et al.* 2016). The prevalence of the *mexA*, *mexB*, and *oprM* genes was found to be 74.2, 77.1, and 37.1% of the isolates, respectively. Previous studies have reported that *mexAB-R* and *mexAB–oprM* efflux pumps were dominant in *P. aeruginosa* isolated from urinary tract infections in Egypt and Nigeria, and is lending support to our findings (Pan *et al.* 2016; Ugwuanyi *et al.* 2021). All active efflux pump-positive isolates were resistant to oxytetracycline and doxycycline and harboured the *tetA*, *tetD*, *tetM*, and *sul1* genes. Tetracycline resistance in bacteria is mediated by ribosomal protection and tetracycline-specific efflux via the presence of major facilitator superfamily transporters (Kobayashi *et al.* 2007; Aminov 2013). The findings of the current study clearly demonstrate that tetracycline resistance in aquatic foodborne bacteria is caused by major facilitator superfamily transporters.

P. aeruginosa can adapt to a hostile environment in the host by secreting a number of virulence factors that lead to infection and disease (Qin *et al.* 2022). In the present study, PCR results revealed that all the tested isolates were positive for *oprL*, *toxA*, *exoS*, and *phzM* genes in agreement with previous reports (Mesquita *et al.* 2013; Horna *et al.* 2018; Algammal *et al.* 2020). L-lipoproteins (*oprL*), which belong to outer membrane proteins present in *P. aeruginosa*, are responsible for antibiotic resistance and pathogenicity. These are highly prominent in *Pseudomonads*, so they would be a good target for identifying and determining the pathogenicity of clinical and environmental specimens (Remans *et al.* 2010). *ExotoxinA* is an extracellular substance that inhibits protein biosynthesis via the *toxA* gene on their chromosome (Lee *et al.* 2005). *ExoS* is a bifunctional toxin that activates both GTPases and ADP-ribosyltransferases. Similarly, pyocyanin is a major virulence factor expressed by the *phzM* gene, which aids in survival and colonization in hosts (Bradbury *et al.* 2010). Due to its zoonotic nature, *P. aeruginosa* has been identified as a possible hazard to both human and animal health, inevitably resulting in morbidity and mortality (Milivojevic *et al.* 2018). This could harm fish handlers' well-being and may have serious health consequences. Therefore, care must always be taken when handling the animals for regular health monitoring and disease diagnosis.

CONCLUSION

P. aeruginosa is a common bacterial pathogen that affects both humans and animals. The emergence of multi-drug-resistant pathogens in aquaculture settings in this region raises concerns about antibiotic misuse. Multiple factors play crucial roles in the AMR of *P. aeruginosa*. These findings highlight the need for a multifaceted approach from all the stakeholders in the sector to tackle the disease and AMR issues. Regular disease surveillance, identification of causative agents, and AST are all prerequisites to minimizing the emergence of antibiotic-resistant bacterial strains of potential public health concern. Also, routine surveillance and effective policies with proper guidelines have to be practiced to minimize the need for antibiotics. The presence of virulence genes in the isolates highlights the potential risk in causing diseases when prevailing conditions become favourable for them. Particularly, the higher prevalence of AMR *P. aeruginosa* in Pangasius farms where management practices are sub-optimal. As a result, maintaining biosecurity and good aquaculture practices (GAPs) addressing breed, seed, and feed selection are the only ways forward for disease control and prevention. If diseases are reduced through GAPs, then the use of antimicrobials and the resultant emergence of MDR bugs can be significantly controlled. Furthermore, the current study findings provide promising insights into the distribution of *P. aeruginosa* in freshwater aquaculture settings, resistance patterns, underlying mechanisms, and virulence profiles for future research.

ACKNOWLEDGEMENTS

The authors are grateful to the authorities of the Kerala University of Fisheries and Ocean Studies, Kochi, India, for providing all the necessary infrastructure facilities and scientific support for carrying out the Ph.D. research work. The authors are thankful to the Rajiv Gandhi Centre for Aquaculture, Tamilnadu, India, for providing laboratory facilities. The authors also acknowledge the fish farmers of Andhra Pradesh, India, for providing information and samples for this research.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

AUTHOR CONTRIBUTIONS

K.S. conceptualized the work, performed methodology, investigated the work, carried out data curation, and wrote the original draft. A.S. found resources and did data analysis. M.S. and R.P. reviewed and edited the manuscript. D.P. supervised and conceptualized the work, performed methodology, did critical review, and edited the original draft.

ETHICS APPROVAL

The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) registration number: 1174/ac/08/CPCSEA were properly followed in the execution of all the experiments.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

Algammal, A. M., Mabrok, M., Sivaramasamy, E., Youssef, F. M., Atwa, M. H., El-Kholy, A. W., Hetta, H. F. & Hozzein, W. N. 2020 Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor *opr*L and *tox*A virulence genes and bla_{TEM}, bla_{CTX-M}, and *tetA* antibiotic-resistance genes. *Scientific Reports* **10** (1), 1–12.

Ali, S. E., Jansen, M. D., Mohan, C. V., Delamare-Debouteville, J. & Charo-Karisa, H. 2020 Key risk factors, farming practices and economic losses associated with tilapia mortality in Egypt. *Aquaculture* **527**, 735438.

Aminov, R. 2013 Evolution in action: Dissemination of tet (X) into pathogenic microbiota. *Frontiers in Microbiology* **4**, 192.

Ardura, A., Linde, A. R. & Garcia-Vazquez, E. 2013 Genetic detection of *Pseudomonas* spp. in commercial Amazonian fish. *International Journal of Environmental Research and Public Health* **10** (9), 3954–3966.

Austin, B. & Austin, D. 2007 Characteristics of the pathogens: Gram-negative bacteria. In: *Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish* (Austin, B. & Austin, D. A., eds.). Springer Praxis Publishing, Berlin, pp. 81–150.

Beulah, G., Deepthimahanthi, D., Simhachalam, G., Chintagunta, A. D., Sravya, M. V. N. & Kumar, N. S. S. 2022 Purification and characterization of phytochemicals extracted from *Rhizophora mucronata*: Their efficacy against *Pseudomonas aeruginosa* infection in *Catla catla*. *Reviews in Analytical Chemistry* **41** (1), 275–286.

Bradbury, R. S., Roddam, L. F., Merritt, A., Reid, D. W. & Champion, A. C. 2010 Virulence gene distribution in clinical, nosocomial and environmental isolates of *Pseudomonas aeruginosa*. *Journal of Medical Microbiology* **59** (8), 881–890.

Cabello, F. C., Godfrey, H. P., Tomova, A., Ivanova, L., Dölz, H., Millanao, A. & Buschmann, A. H. 2013 Antimicrobial use in aquaculture re-examined: Its relevance to antimicrobial resistance and to animal and human health. *Environmental Microbiology* **15** (7), 1917–1942.

Call, D. R., Bakko, M. K., Krug, M. J. & Roberts, M. C. 2003 Identifying antimicrobial resistance genes with DNA microarrays. *Antimicrobial Agents and Chemotherapy* **47** (10), 3290–3295.

Cepas, V., Lopez, Y., Munoz, E., Rolo, D., Ardanuy, C., Marti, S. & Soto, S. M. 2019 Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. *Microbial Drug Resistance* **25** (1), 72–79.

CLSI. 2022 *Performance Standards for Antimicrobial Susceptibility Testing*, 32nd edn. CLSI Supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA.

Curran, C. S., Bolig, T. & Torabi-Parizi, P. 2018 Mechanisms and targeted therapies for *Pseudomonas aeruginosa* lung infection. *American Journal of Respiratory and Critical Care Medicine* **197** (6), 708–727.

Das, A., Acharya, S., Behera, B. K., Paria, P., Bhowmick, S., Parida, P. K. & Das, B. K. 2018 Isolation, identification and characterization of *Klebsiella pneumoniae* from infected farmed Indian Major Carp *Labeo rohita* (Hamilton 1822) in West Bengal, India. *Aquaculture* **482**, 111–116.

Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A. & Iqbal, M. 2011 Evaluation of different detection methods of biofilm formation in the clinical isolates. *Brazilian Journal of Infectious Diseases* **15**, 305–311.

Horna, G., Lopez, M., Guerra, H., Saenz, Y. & Ruiz, J. 2018 Interplay between MexAB-OprM and MexEF-OprN in clinical isolates of *Pseudomonas aeruginosa*. *Scientific Reports* **8** (1), 1–11.

Kamali, E., Jamali, A., Ardebili, A., Ezadi, F. & Mohebbi, A. 2020 Evaluation of antimicrobial resistance, biofilm forming potential, and the presence of biofilm-related genes among clinical isolates of *Pseudomonas aeruginosa*. *BMC Research Notes* **13** (1), 1–6.

Karczmarczyk, M., Abbott, Y., Walsh, C., Leonard, N. & Fanning, S. 2011 Characterization of multidrug-resistant *Escherichia coli* isolates from animals presenting at a university veterinary hospital. *Applied Environmental Microbiology* **77** (20), 7104–7112.

Kobayashi, T., Nonaka, L., Maruyama, F. & Suzuki, S. 2007 Molecular evidence for the ancient origin of the ribosomal protection protein that mediates tetracycline resistance in bacteria. *Journal of Molecular Evaluation* **65** (3), 228–235.

Koo, H. J. & Woo, G. J. 2011 Distribution and transferability of tetracycline resistance determinants in *Escherichia coli* isolated from meat and meat products. *International Journal of Food Microbiology* **145**, 407–413.

Kpoda, D. S., Ajayi, A., Somda, M., Traore, O., Guessennd, N., Ouattara, A. S. & Dosso, M. 2018 Distribution of resistance genes encoding ESBLs in Enterobacteriaceae isolated from biological samples in health centers in Ouagadougou, Burkina Faso. *BMC Research Notes* **11** (1), 1–5.

Krumpermann, P. H. 1983 Multiple antibiotic resistances indexing of *Escherichia coli* to identify high-risk sources of fecal contamination in foods. *Applied Environmental Microbiology* **46**, 165–170.

Lamont, I. L. & Martin, L. W. 2003 Identification and characterization of novel pyoverdine synthesis genes in *Pseudomonas aeruginosa*. *Microbiology* **149** (4), 833–842.

Lanz, R., Kuhnert, P. & Boerlin, P. 2003 Antimicrobial resistance and resistance gene determinants in clinical *Escherichia coli* from different animal species in Switzerland. *Veterinary Microbiology* **91** (1), 73–84.

Lee, V. T., Smith, R. S., Tummler, B. & Lory, S. 2005 Activities of *Pseudomonas aeruginosa* effectors secreted by the type III secretion system in vitro and during infection. *Infection and Immunity* **73** (3), 1695–1705.

Lulijwa, R., Rupia, E. J. & Alfaro, A. C. 2020 Antibiotic use in aquaculture, policies and regulation, health and environmental risks: A review of the top 15 major producers. *Reviews in Aquaculture* **12** (2), 640–663.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T. & Monnet, D. L. 2012 Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* **18** (3), 268–281.

Maita, P. & Boonbumrung, K. 2014 Association between biofilm formation of *Pseudomonas aeruginosa* clinical isolates versus antibiotic resistance and genes involved with biofilm. *Journal of Chemical and Pharmaceutical Research* **6** (5), 1022–1028.

Malagon-Rojas, J. N., Barrera, E. L. P. & Lagos, L. 2020 From environment to clinic: The role of pesticides in antimicrobial resistance. *Revista Panamericana de Salud Pública* **44**, 1–5.

Martins, M., McCusker, M. P., Viveiros, M., Couto, I., Fanning, S., Pagès, J. M. & Amaral, L. 2013 A simple method for assessment of MDR bacteria for over-expressed efflux pumps. *Open Microbiology* **7**, 72.

Mathur, T., Singhal, S., Khan, S., Upadhyay, D. J., Fatma, T. & Rattan, A. 2006 Detection of biofilm formation among the clinical isolates of staphylococci: An evaluation of three different screening methods. *Indian Journal of Medical Microbiology* **24** (1), 25–29.

Mesquita, C. S., Soares-Castro, P., Santos, P. M. & Mendez-Vilas, A. 2013 *Pseudomonas aeruginosa*: Phenotypic flexibility and antimicrobial resistance. *Microbial Pathogens and Strategies for Combating Them: Science, Technology and Education* **1**, 650–665.

Milivojevic, D., Šumonja, N., Medić, S., Pavic, A., Moric, I., Vasiljevic, B., Senerovic, L. & Nikodinovic-Runic, J. 2018 Biofilm-forming ability and infection potential of *Pseudomonas aeruginosa* strains isolated from animals and humans. *Pathogens and Disease* **76** (4), 41.

Mishra, S. S., Rakesh, D., Dhiman, M., Choudhary, P., Debbarma, J., Sahoo, S. N. & Mishra, C. K. 2017a Present status of fish disease management in freshwater aquaculture in India: State-of-the-art-review. *Journal of Aquaculture & Fisheries* **1** (3), 14.

Mishra, S. S., Das, R., Das, B. K., Choudhary, P., Rathod, R., Giri, B. S., Debbarma, J., Sahoo, S. N., Barua, A., Sahu, A., Patil, P. K. & Swain, P. 2017b Status of aqua-medicines, drugs and chemicals use in India: A survey report. *Journal of Aquaculture & Fisheries* **1** (4), 15.

Page, S. W. & Gautier, P. 2012 Use of antimicrobial agents in livestock. *Revue Scientifique et Technique-OIE* **31** (1), 145.

Pan, Y. P., Xu, Y. H., Wang, Z. X., Fang, Y. P. & Shen, J. L. 2016 Overexpression of MexAB-OprM efflux pumps in carbapenem-resistant *Pseudomonas aeruginosa*. *Archives of Microbiology* **198** (6), 565–571.

Pourakbari, B., Yaslianifard, S., Yaslianifard, S., Mahmoudi, S., Keshavarz-Valian, S. & Mamishi, S. 2016 Evaluation of efflux pumps gene expression in resistant *Pseudomonas aeruginosa* isolates in an Iranian referral hospital. *Iranian Journal of Microbiology* **8** (4), 249.

Qin, S., Xiao, W., Zhou, C., Pu, Q., Deng, X., Lan, L., Liang, H., Song, X. & Wu, M. 2022 *Pseudomonas aeruginosa*: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. *Signal Transduction and Targeted Therapy* **7** (1), 199.

Rangasamy, K., Athiappan, M., Devarajan, N. & Parray, J. A. 2017 Emergence of multi drug resistance among soil bacteria exposing to insecticides. *Microbial Pathogenesis* **105**, 153–165.

Remans, K., Vercammen, K., Bodilis, J. & Cornelis, P. 2010 Genome-wide analysis and literature-based survey of lipoproteins in *Pseudomonas aeruginosa*. *Microbiology* **156** (9), 2597–2607.

Reverter, M., Sarter, S., Caruso, D., Avarre, J. C., Combe, M., Pepey, E. & Gozlan, R. E. 2020 Aquaculture at the crossroads of global warming and antimicrobial resistance. *Nature Communications* **11** (1), 1–8.

Schar, D., Klein, E. Y., Laxminarayan, R., Gilbert, M. & Van Boeckel, T. P. 2020 Global trends in antimicrobial use in aquaculture. *Scientific Reports* **10** (1), 1–9.

Soto, S. M. 2013 Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. *Virulence* **4** (3), 223–229.

Streeter, K. & Katouli, M. 2016 *Pseudomonas aeruginosa*: A review of their pathogenesis and prevalence in clinical settings and the environment. *Infection Epidemiology and Microbiology* **2** (1), 25–32.

Tavares-Dias, M. & Martins, M. L. 2017 An overall estimation of losses caused by diseases in the Brazilian fish farms. *Journal of Parasitic Diseases* **41** (4), 913–918.

Tincher, C., Long, H., Behringer, M., Walker, N. & Lynch, M. 2017 The glyphosate-based herbicide roundup does not elevate genome-wide mutagenesis of *Escherichia coli*. *G3: Genes Genome Genetics* **7** (10), 3331–3335.

Ugwuanyi, F. C., Ajayi, A., Ojo, D. A., Adeleye, A. I. & Smith, S. I. 2021 Evaluation of efflux pump activity and biofilm formation in multidrug resistant clinical isolates of *Pseudomonas aeruginosa* isolated from a Federal Medical Center in Nigeria. *Annals of Clinical Microbiology and Antimicrobials* **20** (1), 1–7.

Yaseen, S. M., Abdelaziz, M., Abdel-moneam, A. D., Abd-Elhay, E., Wassif, I. M., Fawzy, M. & Moustafa, M. 2020 Phenotypic and genotypic characterization of the pathogenic *Pseudomonas aeruginosa* isolated from cultured *Pangasianodon hypophthalmus* in Egypt. *Egyptian Journal of Aquatic Biology and Fisheries* **24** (6), 453–467.

First received 14 July 2023; accepted in revised form 9 November 2023. Available online 20 November 2023