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Molecular Characterization and Antibiogram of *Pseudomonas aeruginosa* Isolated from Fish Sold in Markets of Tirupati, India

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Abstract

Pseudomonas aeruginosa is an emerging opportunistic pathogen and is one of the pathogens in the acronym ESKAPE representing a global threat to human health associated with antimicrobial resistance. The present study aimed for molecular characterization, virulence gene profiling and antimicrobial resistance studies of *P. aeruginosa* isolated from 200 fish samples collected from different fish markets in Tirupati, Andhra Pradesh, India. Of the 200 fish samples screened, 30.5% were confirmed as *P. aeruginosa* by PCR, targeting the 16S rRNA gene. Among the positive isolates, it was found that 70.4% and 75.4% of the isolates harbored *toxA* and *exoS* virulence markers, respectively. All 61 *P. aeruginosa* isolates recovered were phenotypically characterized for antimicrobial resistance patterns by the disc diffusion method. The study has found 100% resistance against erythromycin followed by 98.4% resistance to amoxicillin, 91.8% resistance against tetracycline, 88.5% resistance to vancomycin and cotrimoxazole, 45.9% resistance to ciprofloxacin, 44.2% resistance against cefepime and 9.9% resistance to gentamicin. The MAR indices were in the range from 0.5 to 1.0. The prevalence of multiple antibiotic resistance of *P. aeruginosa* harboring virulence genes in fish can pose a serious threat and hazard to public health through the consumption of raw or undercooked fish.

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Introduction

Pseudomonas aeruginosa is an encapsulated, gram-negative, aerobic, non-lactose fermentative, rod-shaped bacterium measuring 0.5 to 0.8 ìm in size that belongs to the family, Pseudomonadaceae. *P. aeruginosa* is a common environmental microorganism and is a natural inhabitant of water, soil, skin and mucous membranes of fish and animals, in a variety of foods and most man-made environments throughout the world. This organism is also able to thrive in adverse atmospheric conditions (e.g. low oxygen levels, high temperatures up to 42^0C, and minimal nutritional conditions) owing to its diverse genetic composition (Lambert, 2002).

P. aeruginosa enters the fish either through the oral route or through broken or abraded skin and damaged gills (Algammal et al., 2020). The infected fish can spread the infection to other healthy fishes as well as to humans. In humans, consumption of fish and its byproducts infected with *P. aeruginosa* may cause gastroenteritis (Novotny, Dvorska, Lorencova, Beran, & Pavlik, 2004) and entry of these through skin or wounds may result in dermatitis, bacteremia, urinary tract infections, respiratory tract infections, soft tissue infections, bone and joint infections and a variety of systemic infections.

The six bacterial pathogens viz., *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp. present in the acronym ESKAPE is

commonly associated with antimicrobial resistance, representing a global threat to human health. Due to its high morbidity and mortality infection rates in healthcare settings especially among immunocompromised individuals and other highly vulnerable patients, *P. aerugionsa* is being recognized as an emerging gram-negative opportunistic pathogen (Chen et al., 2018). This organism is also categorized under the critical category of the WHO Global Priority Pathogen List for which new antibiotics are urgently needed. Multidrug resistant *P. aeruginosa* is a new threat to animals and humans, which involves frequent treatment failures for the infections caused by this bacterium (Pereira, Rosa, & Cardoso, 2015).

Out of several virulence factors associated with *P. aerugionsa*, two extracellular toxins namely exotoxin A and exoenzyme S are important for the pathogenic activity of *P. aeruginosa*. Exotoxin A, the most important virulence factor, is an extracellular product encoded by the *tox*A gene, which causes direct tissue damage and necrosis by inhibiting protein-biosynthesis in the host cell, that resembles the action of diphtheria toxin (Sadikot et al., 2005). Exoenzyme S is a major cytotoxin and bi-functional protein encoded by the *exo*S gene, which is involved in the stages of colonization, invasion and dissemination of infection (Bradbury, Roddam, Merritt, Reid, & Champion, 2010). The gene *ExoS* targets the small Ras-like proteins, inhibiting internalization and DNA synthesis and inducing apoptosis (Vance et al., 2005).

There are limited studies to identify the prevalence of *P. aeruginosa* among fish samples in India (Suresh et al., 2023; Ghosh et al., 2024; Carol, Jeyasanta, Mani, & Patterson, 2013). Hence, the present investigation was designed to assess the presence of *P. aeruginosa* among raw fish samples by both conventional and molecular methods and also to study antimicrobial resistance patterns of the isolates by phenotypic characterization.

Materials and Methods

A total of 200 fish samples of different varieties *viz.,* catla (*Catla catla*), rohu (*Labeo rohita*) and murrel (*Channa striata*) were collected aseptically in sterile zip lock covers from different fish markets in and around Tirupati, Andhra Pradesh, India (Fig. 1). These samples were immediately transferred to the Department of Veterinary Public Health and Epidemiology, College of Veterinary Science, Tirupati in an ice box for further processing.

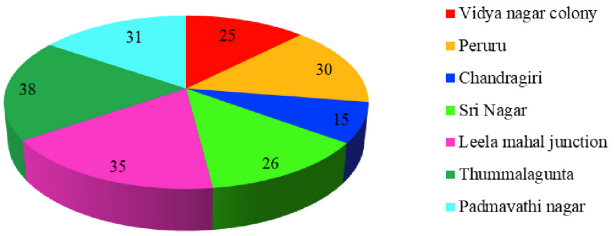


Fig. 1. Sampling frame (Tirupati Rural)

Twenty-five grams each of 200 fish samples, especially gill portions were collected, homogenized and inoculated in 225 ml of alkaline peptone water. After incubation at 37^0C for 24 h, a loopful of enriched broth inoculum was streaked on Cetrimide agar and the plates were incubated for 24 h at 37^0C. The greenish-blue colour colonies with a characteristic fruity grape like odour were considered as presumptive *Pseudomonas* spp. colonies (Algammal et al., 2020).

All the presumptive colonies were further confirmed to genus specific by biochemical tests *viz.*, IMViC tests, Catalase test, Oxidase test, Mannitol motility test, aesculin hydrolysis test, gelatin liquefaction test, TSI agar test and nitrate reduction test to confirm *aeruginosa* isolates (Markey, Leonard, Archambault, Cullinane, & Maguire, 2013). The organisms that showed positive for catalase, oxidase test, motility test and variable haemolysis on blood agar were identified as *P. aeruginosa* isolates and further confirmed by molecular method.

DNA was extracted from all biochemically confirmed *P. aeruginosa* isolates by boiling and snap chilling method as described by El-Aziz (2015). Further, these DNA extracts were subjected to PCR, targeting the 16S rRNA gene for molecular confirmation of these isolates as *P. aeruginosa*. The primers used in the present study are listed in Table 1 and the amplification conditions are represented in Fig. 2.

A duplex PCR was carried out to detect virulence genes *viz., tox*A and *exo*S genes as per the studies of Khalifa, Khallaf, and Hashem (2016) with slight modifications. The primers employed in the present study are listed in Table 1 and the amplification conditions are represented in Fig. 3.

All the *aeruginosa* isolates were studied for their

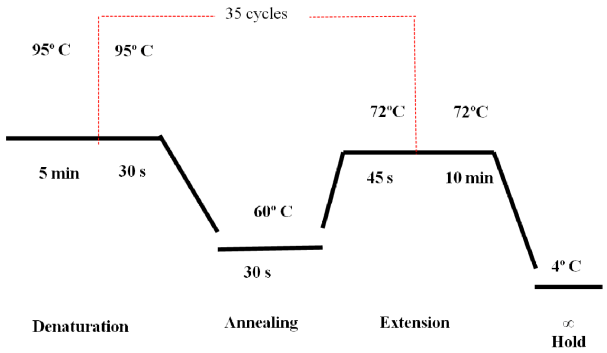


Fig. 2. PCR cycling conditions for species-specific identification of *P. aeruginosa* by 16S rRNA

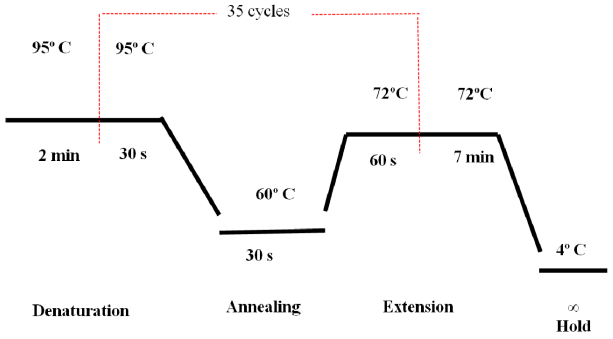


Fig. 3. Cycling conditions used for Virulence profiling of *P. aeruginosa* (toxA and exoS genes)

phenotypic antimicrobial resistance patterns against commonly used antibiotics used in human and veterinary therapy *viz.*, amoxicillin, cefepime, tetracycline, cotrimoxazole, gentamicin, vancomycin, ciprofloxacin and erythromycin by Kirby-Bauer method (Bauer, Kirby, Sherris, & Turck, 1966). Initially the test was performed by inoculating the test colonies in nutrient broth and incubating at 37°C for 24h and the concentration of the culture was adjusted to 0.5 McFarland standards (1-2x10^8CFU/ml). Later, Muller Hinton agar plates were swabbed with nutrient broth culture using sterile swab and antibiotic discs were placed carefully on swabbed surface of plates. These plates were incubated at 37°C for 24h and zones of inhibition for different antibiotics were measured to the nearest mm and the results were tabulated. The values were interpreted as Resistant (R), Intermediate (I) and Susceptible (S) using CLSI guidelines (CLSI, 2010).

MAR index was calculated for all *P. aeruginosa* isolates as per the procedure given by Krumperman (1983), employing the formula a/b, where 'a' represents the number of antibiotics to which the isolate is resistant and 'b' the number of antibiotics to which the isolate was tested.

Results and Discussion

Out of 200 fish samples screened for *P. aeruginosa* which were collected from different areas of the Tirupati rural region, only 63 isolates (31.5%) were identified as *P. aeruginosa* by conventional method which is similar to the findings of Algammal et al. (2020) from Egypt (31.57%). These findings were also in concordance with the studies of Boss, Overesch, and Baumgartner (2016) from India (34.7%) and Magdy, El-Handy, Ahmed, Elmeadawy and Kenwy (2014) from Egypt (32.3%). On contrary, higher prevalence rates of 63% and 62% than in the present study were reported in fish samples by Rani, Chelladurai, and Jayanthi (2016) from India and Yagoub and Ahmed (2009) from Sudan. In contrast to this study, lower incidence rates of *P.aeruginosa* in fish samples were reported by Wamala *et al.* (2018) from Uganda (4.2%), Abd El Tawab, Maarouf, and Ahmed (2016) from Egypt (12%), Eissa, El-Ghiet, Shaheen, and Abbass (2010) from Egypt (2.5%) and Rani and Manavalan (2015) from India (15.38%). Kumar and Surendran (2005) from India also reported a low prevalence rate of 20% in brackish water, fresh water, farm sediments, fish and

Table 1. Details of oligonucleotide primers used in this study

Targetgene	Primer sequence (5^1 -3^1)	Ampliconsize (bp)	Reference
16S rRNA	F- CTACGGGAGGCAGCAGTGG R- TCGGTAACGTCAAAACAGCAAAGT	150	El-Aziz (2015)
toxA	F- CTGCGCGGGTCTATGTGCC R-GATGCTGGACGGGTCGAG	270	Khalifa et al., 2016
exoS	F- CTTGAAGGGACTCGACAAGG R-TTCAGGTCCGCGTAGTGAAT	504	Benie et al. 2017

prawn samples from aquaculture farms of Ernakulam than the present study.

Further, molecular characterization of these 63 presumptive isolates by PCR targeting 16S rRNA gene confirmed 61 isolates (30.5%) as *P. aeruginosa.* Similarly, Benie et al. (2017) from West Africa also reported a 30.4% prevalence in fresh and smoked fish samples by PCR targeting 16S *r*RNA gene. Globally, several researchers reported similar prevalence rates of *P. aeruginosa* isolates in fish samples by PCR - Algammal et al. (2020) from Egypt (31.57%), Eid, Tabiy, and Fathy (2016) from Egypt (27.06%), Magdy et al. (2014) from Iraq (32.3%). Qasim (2019) from Iraq also reported similar prevalence rate (30%) of *P. aeruginosa* in minced meat samples by targeting 16S rRNA gene. In contrast to the present study, the higher prevalence rate of *P. aeruginosa* isolates (100%) was reported by El-Aziz (2015) in fish samples by PCR targeting 16S *r*RNA gene. The excessive contamination of the fish with *P. aeruginosa* may be attributed to poor storage conditions, unhygienic transportation and cross contamination with the water and ice used during the storage and transportation (Mhenni, Alberghini, Giaccone, Traunt, & Catellani, 2023). *The* variations in prevalence rates of *P. aeruginosa* globally could be related to the geographical distribution, environmental factors, host susceptibility and the season of sample collection.

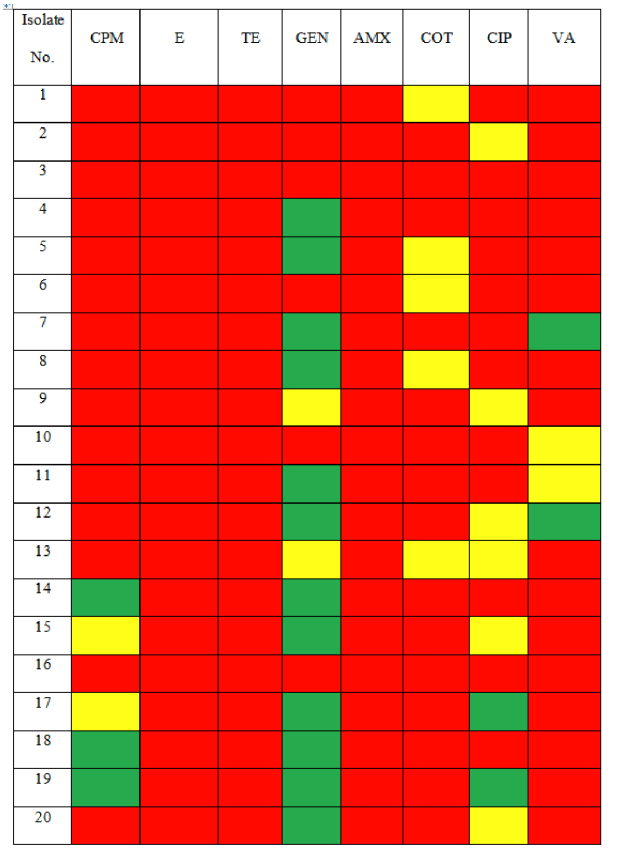
On virulence gene profiling, out of 61 *P. aeruginosa* isolates, it was found that 43 (70.4%) and 46 (75.4%) isolates harbored *toxA* and *exoS* virulence markers respectively. In contrast to this, El-Gamal and Rashed (2019) and Algammal et al. (2020) reported that all the *P. aeruginosa* isolates obtained in their studies from fish samples harbored *tox*A gene only (100%). Similarly, Lanotte et al. (2004) have found that all the *P. aeruginosa* isolates isolated from cystic fibrosis patients and swabs collected from burns and wounds harboured *tox*A virulence marker. On contrary, Abd El Tawab et al. (2016) from fish samples and Khalifa et al. (2016) from fish samples have identified 66.7% and 27.9% of *P. aerugionsa* isolates harbored *tox*A gene.

While, Benie et al. (2017) has identified 67.3% and 78.8% of *P.aerugionsa* isolates obtained from fresh fish and smoked fish samples harbored *exo*S gene. In contrast, Lanotte et al. (2004) and Banerjee et al. (2017) have found 84.5% and 31.5% of *P. aeruginosa* isolates recovered from cystic fibrosis patients and bovine subclinical mastitis samples harbored *exo*S gene. Whereas, Algammal et al. (2020) has identified that none of *P. aerugionsa* isolate obtained from fish samples harbored *exo*S gene.

The *P. aeruginosa* isolates recovered from fish samples were characterized phenotypically to study antimicrobial resistance patterns by disc diffusion

Table 2. Antimicrobial resistance patterns of *P. aeruginosa* isolates obtained in this study

Antimicrobial agent tested	Number of *P. aeruginosa* isolates (%)		
	Susceptible (mm)	Intermediate (mm)	Resistant (mm)
Amoxicillin	0	1.6	98.4
Cefepime	27.9	27.9	44.2
Ciprofloxacin	32.8	21.3	45.9
Cotrimoxazole	1.6	9.8	88.6
Erythromycin	0	0	100
Gentamicin	77	13.1	9.9
Tetracycline	4.9	3.3	91.8
Vancomycin	6.6	4.9	88.5



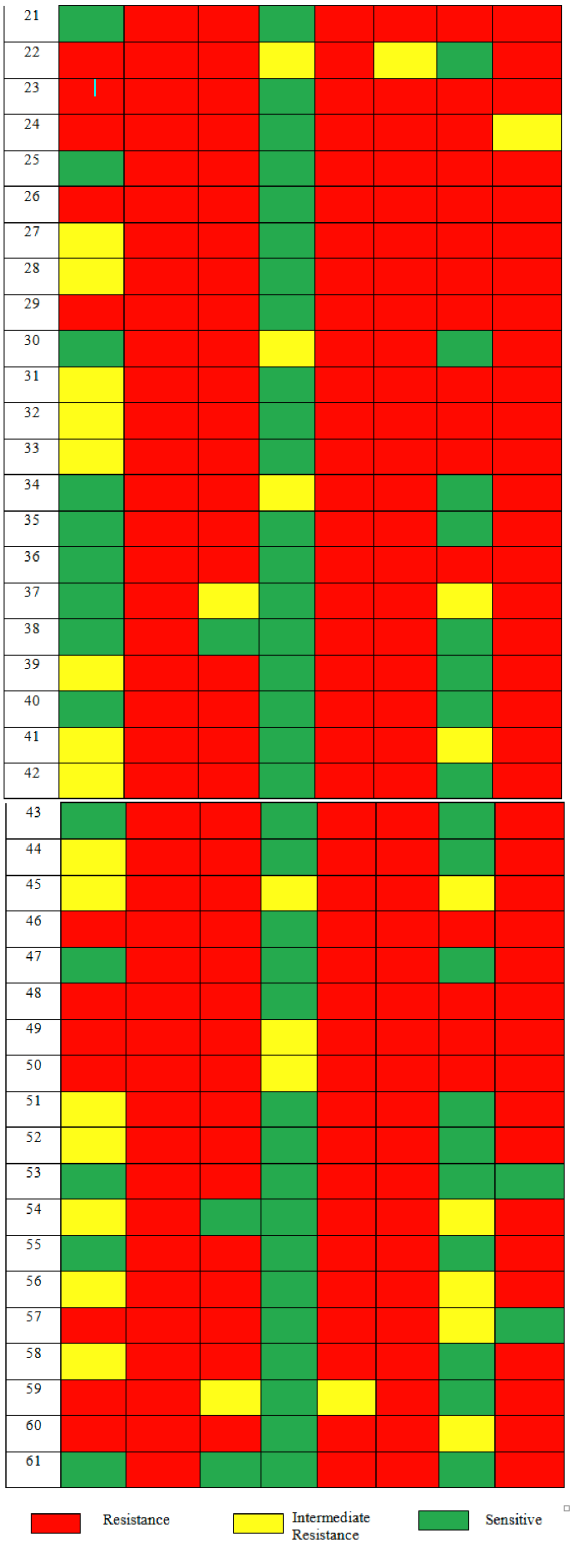


Fig. 4. Heat map of antibiotic resistance detected in *P. aeruginosa* isolates

CPM- Cefepime; E- Erythromycin; TE- Tetracycline; GEN- Gentamicin;

AMX - Amoxicillin; COT- Cotrimoxazole; CIP - Ciprofloxacin; VA- Vancomycin

method. The variable antimicrobial resistance patterns of these isolates were given in Table 2 and also depicted in the heat map representation (Fig. 4). All these 63 *P. aeruginosa* isolates have shown eleven different antimicrobial resistance patterns ranging from one to twelve with the antibiotics used in the present study (Table 4). In this study, it was found that all the 61 isolates were resistant to erythromycin (100%), 60 isolates were resistant to amoxicillin (98.4%), 56 isolates to tetracycline (91.8%), 54 isolates against vancomycin and cotrimoxazole (88.5%), 28 isolates resistant to ciprofloxacin (45.9%), 27 isolates against cefepime (44.2%) and 6 isolates resistant to gentamicin (9.9%). *P. aeruginosa* can develop resistance to antibiotics through a number of mechanisms *viz.,* intrinsic resistance, acquisition of resistance genes and mutational processes.

Similar to the present study, Abdullahi et al. (2013) and Eid et al. (2016) have also reported that 100% of *P. aeruginosa* isolates obtained from fish samples were resistance against erythromycin. On contrary, Abd El Tawab et al. (2016) reported a lower resistance (68.8%) to erythromycin in fresh water fish samples.

In the present study, *P. aeruginosa* isolates showed 98.4% resistance to amoxicillin. In contrast, Algammal et al. (2020) and Akani, Hakam, and Sampson (2019) have reported lower resistance rates against amoxicillin from fish samples (83.3%) and from West African mud creeper samples (81.3%) respectively.

The resistance noticed to tetracycline was 91.8% in the present study. Nasreen, Sarker, Malek, Ansaruzzaman and Rahman (2015) have also reported similar resistance pattern for *P. aeruginosa* isolates obtained from surface water samples against tetracycline (93.7%) while Kumar and Surendran (2005) has reported 100% resistance from fish samples towards oxytetracycline. On contrary, Algammal et al. (2020) from fish samples (75.6%) and Swetha et al. (2017) from milk samples (78.9%) have found a lower resistance against tetracycline than the present study.

Similarly *P. aeruginosa* isolates showed 88.5% resistance to vancomycin and cotrimoxazole. Similar to the present study, Swetha et al. (2017) has also reported 89.47% resistance against vancomycin by the isolates obtained from milk samples. Whereas, Patel, Kumar, Suthar, Desai, and Kalyani (2019)

Table 3. MAR indices of *P. aeruginosa* isolates obtained in the present study

Isolate No.	MARindex	Antibiotics that were resistant
1	1.00	CPM, E, TE, GEN, AMX, COT, CIP,VA
2	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
3	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
4	0.88	CPM, E, TE, AMX, COT, CIP, VA
5	0.88	CPM, E, TE, AMX, COT, CIP, VA
6	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
7	0.75	CPM, E, TE, AMX, COT, CIP
8	0.88	CPM, E, TE, AMX, COT, CIP, VA
9	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
10	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
11	0.88	CPM, E, TE, AMX, COT, CIP, VA
12	0.75	CPM, E, TE, AMX, COT, CIP
13	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
14	0.75	E, TE, AMX, COT, CIP, VA
15	0.88	CPM, E, TE, AMX, COT, CIP, VA
16	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
17	0.75	CPM, E, TE, AMX, COT, VA
18	0.75	E, TE, AMX, COT, CIP, VA
19	0.63	E, TE, AMX, COT, VA
20	0.88	CPM, E, TE, AMX, COT, CIP, VA
21	0.75	E, TE, AMX, COT, CIP, VA
22	0.88	CPM, E, TE, GEN, AMX, COT, VA
23	0.88	CPM, E, TE, AMX, COT, CIP, VA
24	0.88	CPM, E, TE, AMX, COT, CIP, VA
25	0.75	E, TE, AMX, COT, CIP, VA
26	0.88	CPM, E, TE, AMX, COT, CIP, VA
27	0.88	CPM, E, TE, AMX, COT, CIP, VA
28	0.88	CPM, E, TE, AMX, COT, CIP, VA
29	0.88	CPM, E, TE, AMX, COT, CIP, VA
30	0.75	E, TE, GEN, AMX, COT, VA
31	0.88	CPM, E, TE, AMX, COT, CIP, VA
32	0.88	CPM, E, TE, AMX, COT, CIP, VA
33	0.88	CPM, E, TE, AMX, COT, CIP, VA
34	0.75	E, TE, GEN, AMX, COT, VA
35	0.63	E, TE, AMX, COT, VA
36	0.75	E, TE, AMX, COT, CIP, VA
37	0.75	E, TE, AMX, COT, CIP, VA
38	0.50	E, AMX, COT, VA
39	0.75	CPM, E, TE, AMX, COT, VA
40	0.63	E, TE, AMX, COT, VA
41	0.88	CPM, E, TE, AMX, COT, CIP, VA
42	0.75	CPM, E, TE, AMX, COT, VA
43	0.63	E, TE, AMX, COT, VA
44	0.75	CPM, E, TE, AMX, COT, VA
45	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
46	0.88	CPM, E, TE, AMX, COT, CIP, VA
47	0.63	E, TE, AMX, COT, VA
48	0.88	CPM, E, TE, AMX, COT, CIP, VA
49	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
50	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
51	0.75	CPM, E, TE, AMX, COT, VA
52	0.75	CPM, E, TE, AMX, COT, VA
53	0.50	E, TE, AMX, COT
54	0.75	CPM, E, AMX, COT, CIP, VA
55	0.63	E, TE, AMX, COT, VA
56	0.88	CPM, E, TE, AMX, COT, CIP, VA
57	0.75	CPM, E, TE, AMX, COT, CIP
58	0.75	CPM, E, TE, AMX, COT, VA
59	0.75	CPM, E, TE, AMX, COT, VA
60	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA
61	0.50	E, AMX, COT, VA

reported a 100% resistance to vancomycin by their isolates recovered from milk samples. In contrast to cotrimoxazole resistance patterns in the present study, a higher and lower resistance patterns were reported by Carol et al. (2013) from fishes (100%) and Swetha et al. (2017) from milk samples (78.9%) respectively.

For ciprofloxacin, *P. aeruginosa* isolates in the present study have shown 45.9% resistance and is similar to the findings of Akani et al. (2019) who identified 43.8% ciprofloxacin resistant isolates from West African mud creeper samples. While, Benie et al. (2017) have reported that 33.2% and 32.2% of their isolates obtained from fresh fish and smoke fish samples were ciprofloxacin resistance. Whereas, lower resistance for ciprofloxacin was found in the findings of Swetha et al. (2017) who reported 36.8% resistance by the isolates of *P. aeruginosa* recovered from milk samples. On contrary, Eid et al. (2016) and Nasreen et al. (2015) reported 100% susceptibility towards ciprofloxacin by *P. aeruginosa* isolates isolated from fish and surface water samples respectively.

The *P. aeruginosa* isolates in the present study have shown 44.2% resistance against cefepime. On contrary to this, Benie et al. (2017) had reported 10% and 23.7% resistance prevalence against cefepime by the isolates obtained from fresh fish and smoke fish samples respectively. Hosu, Vasaikar, Okuthe and Apalata (2021) also identified a lower resistance (36.1%) to cefipime by the isolates recovered from aquatic environmental and abattoir wastewater samples.

In the present study, 9.9% *P. aerugionsa* isolates have shown resistance to gentamicin. In contrast to these findings, higher resistance to gentamicin among *P. aeruginosa* isolates was observed by Algammal et al. (2020) from fish samples (67.6%), Swetha et al. (2017) from milk samples (42.1%) and Abdullahi et al. (2013) from aquaculture environmental samples (23.08%) respectively.

The variations in the antibiotic resistance results might be due to difference in the drug usage in different parts of the world and due to the several mechanisms that have been reported for *P. aeruginosa*, including reduced expression or loss of OprD porin causing reduced antibiotic permeability, over expression of MexAB-OprM pump which increases antibiotic efflux, Production of β-lactams and aminoglycosides inactivating enzymes, mutations of gyrases and topoisomerases which cause fluoroquinolone resistance. These mechanisms in combination lead to multiple drug resistance (Zeng, Wang, Huang, Shi, & Wang, 2014).

The MAR indices were calculated for all the 61 *P. aeruginosa* isolates and found that the index ranges from 0.5 to 1.0 (Table 3). The isolates with intermediate resistance were also considered resistant while calculating the MAR index (Krumperman, 1983). The MAR value higher than 0.2 (Gufe et al., 2019) indicates that the isolates might have originated from high-risk sources where several antibi-

Table 4. Anti-drug profile of *P. aeruginosa* isolates obtained in the present study

S.No.	MARindex	Resistant antibiotics	Antibioticsresistant profile
1	1.00	CPM, E, TE, GEN, AMX, COT, CIP, VA	12
2	0.88	CPM, E, TE, AMX, COT, CIP, VA	19
3	0.88	CPM, E, TE, GEN, AMX, COT, VA	1
4	0.75	CPM, E, TE, AMX, COT, CIP	3
5	0.75	E, TE, AMX, COT, CIP, VA	6
6	0.75	CPM, E, TE, AMX, COT, VA	8
7	0.75	E, TE, GEN, AMX, COT, VA	2
8	0.75	CPM, E, AMX, COT, CIP, VA	1
9	0.63	E, TE, AMX, COT, VA	6
10	0.50	E, AMX, COT, VA	2
11	0.50	E, TE, AMX, COT	1

otics have been used and has high risk potential while MAR index value less than 0.2 indicates that the isolates has originated from sources where antibiotics are seldom used and has low risk potential. Eleven different antibiotic resistance patterns were identified in this study, which is similar to the studies of Odjadjare et al. (2012), who reported five to eleven antibiotic patterns exhibited by their isolates. While, Lateef (2004) reported two to seven antibiotic patterns. On contrary, lower MAR index ranges were reported by Abdullahi et al. (2013) (0.2 to 0.6) with four antibiotic resistance patterns and Hosu *et al*. (2021) (0.69 to 0.8) respectively.

MAR indexing method is a simplified rapid method of distinguishing organisms from different origins either from high-risk sources of contamination where antibiotics are frequently used or from low-risk sources (Algammal et al., 2020). In the present study, all the 63 *P. aeruginosa* isolates had a MAR index of above 0.2 which clearly indicates that all these isolates were from high-risk sources with high-risk potential and favors the spread of antimicrobial resistance pathogens in both animals and humans.

The present study on antimicrobial resistance patterns reveals the importance and necessity of monitoring and surveillance on antimicrobial resistance and resistance patterns of *P. aeruginosa* at both local and regional levels and the implementation of the One Health approach. These studies also suggest that there is a need for judicious and restricted use of antibiotics at different levels to prevent the emergence of resistance to commonly used antibiotics in the human and veterinary therapy. This can also be achieved by implementing good aquaculture practices, pre-treating of sewage from hospitals and strengthening the research capacity at individual and organizational levels.

Conclusion

The present investigation confirmed the presence of pathogenic *P. aeruginosa* in fish samples, harbouring the most virulence genes such as *toxA* and *exoS*. It could pose a serious threat and hazard to susceptible people through the consumption of raw or under cooked fish. The present study also revealed the multiple antibiotic resistance of *P. aeruginosa* isolates and suggests that there is need for judicious and restricted use of antibiotics at various levels to overcome antimicrobial resistance problem and to prevent the emergence of new resistant strains. Our study also recommends the processing of seafood with clean and pure water and thoroughly cooked to lower the risk of food poisoning in humans.

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