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Prevalence of *Pseudomonas* sp. in Fin Fishes and their Antibiotic Susceptibility

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The Gram-negative bacteria *Pseudomonas* sp. were enumerated in seven fresh fin fish species and their antibiotic susceptibility to 12 antibiotics assessed. Among the seven fish species, pathogenic *Pseudomonas* sp. were observed only in 4 species of fish and 5 isolates were confirmed as *Pseudomonas* sp. through biochemical tests. These five *Pseudomonas* strains were tested against 12 antibiotics, viz. ampicillin, gentamycin, amoxycillin, tobramycin, cotrimoxazole, cefotaxime, netillin, nalidixic acid, ceftazidime, ciprofloxacin, amikacin and nitrofurantoin. All the isolated *Pseudomonas* sp. were resistant to amoxycillin. Intermediate resistance was recorded against ampicillin, ceftazidime and nitrofurantoin. All the isolates were sensitive to 6 of the 12 antibiotics tested, i.e. gentamycin, tobramycin, cefotaxime, netillin, ciprofloxacin and amikacin. Antibiotic susceptibility studies revealed that fresh seafoods from Tuticorin have *Pseudomonas* contamination and that some strains may have antibiotic-resistant genes.

Key words: *Pseudomonas* sp., Fin fishes, Antibiotic resistance, Seafood, Food-borne pathogens.

Pseudomonas sp. are aerobic, Gram-negative, rod-shaped bacteria belonging to the family Pseudomonadaceae and class g-proteobacteria[1]. The genus *Pseudomonas* includes species of varied economic and ecological importance and is comprised of fluorescent species such as *Pseudomonas aeruginosa*, *P. fluorescens* and *P. putida*, and non-fluorescent species like *P. pseudoalcaligens*, *P. cepacia*, *P. maltophilia* and *P. stutzeri*. These species show dissimilarities and are frequently isolated from aquatic, clinical and agricultural environments[2-5]. *Pseudomonas aeruginosa, P. cepacia, P. putida* and *P. stutzeri* are human pathogens, generally isolated from contaminated environments. Some species like *P. aeruginosa* and *P. putida* are fish pathogens[6]. *Pseudomonas* sp. have been isolated from shellfish and finfish culture environments[7,8] and are also associated with spoilage of seafood[9]. *P. syringae* is a plant pathogen[10] and *P. aeruginosa* is an opportunistic pathogen known to infect eyes, ears, burns and wounds, and to cause nosocomial infections[8]. *Pseudomonas* sp. is capable of growing on substrates with unusual carbon sources such as soap residues and adhesives from contaminated environments and even on substrates with certain antiseptics. Their resistance to most antibiotics has also been a source of medical concern. This resistance is probably related to cell-wall porins, which control the entry of molecules through the cell wall[11].

Seafood is a major vehicle for the transmission of several bacterial diseases[12]. Even

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though fishery products are a good source of nutrition for humans they can also be a source of food-borne pathogens[13]. Microbial quality of the fresh fish depends, besides other factors, on the quality of water from where the fish are caught and the sanitary conditions of the landing centres. Contamination of fishes with pathogenic bacteria may occur from their natural marine environment or through unhygienic handling, ice, containers, soil, wash water, etc.[14]. Even if the fish catch is landed in prime condition, possible contamination at insanitary landing sites eventually renders the catch microbiologically unsuitable for consumption. Letting out of untreated sewage into sea right at the sites where fishing crafts offload their catch is yet another major source of *Pseudomonas* contamination[15]. Kumar and Surendran[16] reported that seafood is more susceptible to *Pseudomonas* infection. This may pose a threat to consumers and presence of such pathogens needs to be researched. This study was intended to estimate the presence of *Pseudomonas* sp. in seafood samples of Tuticorin and to test their susceptibility to 12 antibiotics.

MATERIALS AND METHODS

Seven species of fresh fish samples such as *Leiognathus dussumieri*, *Auxis thazard*, *Istiophorus platypterus*, *Scomberoides lysan*, *Carangoides malabaricus*, *Lethrinus rubrioperculates* and *Sphyraena acutipinnis* were collected from the fish-landing centre in Tuticorin and brought to the laboratory, packed in ice. Bacterial counts of the fish samples were enumerated by methods described in FDA BAM[17]. Fish samples (10 g) were homogenized with 90 ml of diluent; this volume was further diluted by transferring 1 ml sample to 9 ml of diluent. Then 0.1 ml of sample was plated on a plate count agar by the spread plate method.

Cetrimide agar base was the specific medium used to isolate *Pseudomonas* sp. Isolated and purified cultures were streaked on trypticase soy agar slants for further identification using *Bergey's Manual of Systematic Bacteriology*[18]. Key biochemical characteristics like pigment formation, oxidative/fermentative reactions, arginine hydrolysis, gelatin liquefaction, indole formation, oxidase and catalase reactions, and utilization of amino acids (arginine, lysine, alanine, valine) and sugars (glucose, arabinose, xylose) were tested for the confirmation of *Pseudomonas* species.

Biochemically confirmed *Pseudomonas* sp. were assayed for antibiotic susceptibility on Mueller Hinton agar (Hi Media, Mumbai, India) by using the method of Bauer *et al.* [19]. The isolates were tested using antibiotic discs (Hi Media) for their susceptibility to a set of 12 antibiotics: ampicillin (10 mcg), gentamycin (10 mcg), amoxycillin (30 mcg), tobramycin (10 mcg), cotrimoxazole (75 mcg), cefotaxime (30 mcg), netillin (30 mcg), nalidixic acid (30 mcg), ceftazidime (30 mcg), ciprofloxacin (5 mcg), amikacin (30 mcg) and nitrofurantoin (300 mcg). The results were recorded on the basis of the inhibition zone from the zone size interpretative chart supplied by Hi Media [20].

RESULTS AND DISCUSSION

The results of total plate count of all the seven fish species are presented in Table 1. The highest plate count was observed in *Sphyraena acutipinnis*, followed by *Auxis thazard* and *Istiophorus platypterus*, and the lowest in *Scomberoides lysan*. Iyer *et al.*[21] reported that samples with a bacterial load of $<1\times10^6$ CFU/g could be considered as 'acceptable' but all the fishes exceeded the limit. The bacterial counts of all the seven fish species were above the acceptable limit of 5×10^5 CFU/g [22].

Table 1. Total plate count (TPC) of the fish species

Fishes	TPC (CFU/g)
Scomberoides lysan	7.2×10^5
Auxis thazard	3.45×10^6
Sphyraena acutipinnis	4.0×10^6
Istiophorus platypterus	1.83×10^6
Carangoides malabaricus	1.62×10^6
Leiognathus dussumieri	1.5×10^6
Lethrinus rubrioperculates	1.60×10^6

High bacterial load in fresh fish with no visible signs of spoilage is an indication of poor hygienic standards at the landing centre and poor landing practices of the fish handlers. Our results agreed with those of Nambiar and Iyer[23]. The TPC indicates the freshness and the potential shelf-life

of the product[8]. Bacterial load is assessed to check the quality of a product. The lesser the quantity of bacteria, the higher the quality of the food and vice versa. Our results show that the quality of fresh fin fishes was poor at the time of collecting from the landing centre. Sukumar[15] reported that the heterotrophic bacterial count of *Sardinella* sp. bought from a fish-landing site in Tuticorin was 6.9×10^6 CFU/g, which agreed with the values of the present study. However, it is believed that the numbers of bacteria in fish from unpolluted waters are at the lower end of the ranges and that the higher numbers result from the poor hygienic standards onboard the fishing crafts during initial handling[24]. So the high counts obtained in this work must be due to the unhygienic handling and poor sanitary status of the landing centre. Conditions at some of the landing sites in Tuticorin were poor as reported by Sukumar[15].

Ghasemi *et al.*[25] reported that the muscle sample of *Scomberomorus juttatus* and *Otolithes ruber* had low bacterial counts of 10^2 and 5.5×10^2 CFU/g, respectively; these values are much below the acceptable limit (5×10^5 CFU/g). The total viable count for six frozen fish species ranged from $2.0\text{x}10^3$ to 7.4×10^3 CFU/g [26]. It is generally believed that newly caught healthy fish are sterile but bacteria are found in variable numbers in three parts of the fish: the slime coat, gills and intestine. Numbers in skin have been reported to range from 10^3 to 10^5 CFU/cm^2, in gills from 10^3 to 10^4 CFU/g of tissue and in intestines from 10^2 to 10^9 CFU/ml contents[27].

According to the sensitivity of the five *Pseudomonas* strains isolated from four of the seven fish species to 12 antibiotics, they were classified as sensitive, intermediately sensitive and resistant (Table 2).

Table 2. Antibiotic susceptibility of the *Pseudomonas* isolates

Antibiotics	Antibiotic disc concentration	Standard chart inhibition zone (mm) (Hi Media)			Culture codes Inhibition zone (mm)				
		Resistant	Intermediate	Sensitive	MP3	MS1	MI1	MB5	MP5
Ampicillin	10 mcg	13	14–16	17	0	20	12	10	15
Gentamycin	10 mcg	12	13–14	15	27	25	27	27	27
Amoxicillin	30 mcg	13	14–17	18	0	13	11	0	11
Tobramycin	10 mcg	12	13–14	15	35	21	25	20	25
Cotrimoxazole	25 mcg	10	11–15	16	0	7	17	17	17
Cefotaxime	30 mcg	14	15–22	21	30	21	23	22	27
Netillin	30 mcg	12	13–14	15	30	21	23	28	26
Nalidixic acid	30 mcg	13	14–18	19	24	0	27	29	30
Ceftazidime	30 mcg	14	15–17	18	20	0	0	21	16
Ciprofloxacin	5 mcg	15	16–20	21	33	21	30	30	35
Amikacin	30 mcg	14	15–16	17	24	23	28	29	28
Nitrofurantoin	300 mcg	14	15–16	17	9	16	19	21	22

A total of 32 strains were isolated from the seven fish species; based on biochemical confirmation, five of the 32 isolates were found to be *Pseudomonas* sp. The five *Pseudomonas* isolates were coded as MP3, MP5, MS1, MI1 and MB5. Isolates MP3 and MP5 were isolated from *C. malabaricus*, MS1 from *L. dussumieri*, MI1 from *I. platyperus* and MB5 from *S. acutipinnis.* MP3 and MS1 exhibited maximum resistance to four antibiotics. MP3 showed resistance towards ampicillin, amoxycillin, cotrimoxazole and nitrofurantoin, while MS1 showed resistance towards amoxycillin, cotrimoxazole, nalidixic acid and ceftazidime. MI1 was found to be resistant to three antibiotics, ampicillin, amoxycillin and ceftazidime. MB5 was resistant to ampicillin and amoxycillin. MP5 was resistant to amoxicillin. All the isolates were found to be resistant to amoxycillin. MP5 showed intermediate susceptibility against ampicillin and ceftazidime, and MSI showed intermediate susceptibility towards nitrofurantoin. Six antibiotics –

gentamycin, tobramycin, cefotaxime, netillin, ciprofloxacin and amikacin – were capable of inhibiting all the five *Pseudomonas* strains. In another study comparing clinical and environmental strains of *P. aeruginosa*, amikacin was found to be the most effective drug against both the strains[28]. In yet another study on *P. aeruginosa* amikacin was again found to be the most potent antibiotic and ciprofloxacin the least potent[29].

Prevalence of *Pseudomonas* sp. in fish and their antibiogram need to be studied because there are pathogenic *Pseudomonas* sp. other than *P. aeruginosa. P. putida*, *P. stutzeri* and *P. cepacia* are also pathogenic to humans and are generally isolated from hospital environments[6]. *P. mallei* causes infection in animals and humans and *P. paucimobillis* has been isolated from clinical specimens[18]. Such non-pathogenic *Pseudomonas* sp. are a problem because of their haemolytic activity. Also presence of *Pseudomonas* sp. in fishes cannot be neglected because strains of *P. aeruginosa* from the environment were found to be more resistant to antibiotics than clinical isolates and their virulence was found to be the same as that of clinical strains[28,30]. In this work only one strain of *Pseudomonas* sp. isolated from marine fin fishes showed resistance to the tested antibiotics. The study has also revealed that seafoods sold in Tuticorin are contaminated with *Pseudomonas* strains. Improving the sanitary conditions of the fish-landing centres and inculcating the need for good hygienic practices among fishermen, fish workers and fish vendors are urgently needed.

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