International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 11, 2018, pp.-1414-1417.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

PREVALENCE OF POTENTIAL PATHOGENIC AEROMONADS SPECIES IN SEA FISH, CRAB AND PRAWNS IN CHENNAI, INDIA

RONGSENSUSANG[1]*, DEEPAK S.J.[2], VINAYANANDA C.O.[1], ELANGO A.[2] AND PORTEEN K.[2]

[1]Department of Livestock Products Technology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, 600051 India
[2]Department of Veterinary Public Health and Epidemiology, Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, 600051 India
*Corresponding Author: Email - rongsen77@gmail.com

Received: November 01, 2018; Revised: November 26, 2018; Accepted: November 27, 2018; Published: November 30, 2018

Abstract- The study was undertaken to assess the prevalence of Aeromonads and their antimicrobial resistance and virulence genes potentials among isolated *Aeromonas* species from seafood such as fish, crab and prawns in Chennai, India. Total of 270 fresh sea foods samples - 30 samples of each sea foods *viz*., sea fish, prawn and crabs from each of 3 fish markets *i.e*., Marina beach, Chintadripet and Purasaiwakkam. Among 270 samples investigated, 172 samples showed positive by cultural method. The duplex PCR was performed to detect the 16S rRNA and aerolysin (aerA) gene in the isolates. Out of which, 160/172 samples were confirmed as *Aeromonas* species targeting 16S rRNA and 57/160 (35.53%) showed positive for aerolysin gene, a pathogenic marker. The antimicrobial resistance pattern of *Aeromonas* spp. isolates showed complete resistant against ampicillin, penicillin and cephalothin whereas sensitive against Enrofloxacin, gentamicin and tetracycline. The increased prevalence of aeromonads in sea food is attributed to cross contamination during post-harvest processing. The detection of potential pathogenic aeromonads and multiple antimicrobial resistances in *Aeromonas* species isolated raise serious public health concern worthy of further investigation.

Keywords- *Aeromonas spp., Antimicrobial resistance, Cross contamination, Potential pathogenic, Public Health crisis*

Citation: Rongsensusang, *et al*., (2018) Prevalence of Potential Pathogenic Aeromonads Species in Sea Fish, Crab and Prawns in Chennai, India. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 10, Issue 11, pp.-1414-1417.

Introduction

Aeromonads are Gram-negative, non-spore-forming, rod-shaped, facultative anaerobic bacteria that occur ubiquitously and autochthonous in aquatic environments [1,2]. *Aeromonas* species are widely distributed in nature and reported in water, soil and foods of animal origin [3,4]. They are part of the normal bacterial flora of many animals. Act as an opportunistic pathogen and/or secondary bacterial agent that contribute to the occurrence of a fish disease and its deterioration [5]. A variety of extracellular virulence factors such as enterotoxins, cytotoxins, haemolysins, aerolysins, proteases, haemagglutinins are produced by *Aeromonas* species, among these toxins most reported are aerolysin and hemolysin [6]. Aerolysin is also known as cytotoxic enterotoxin, which possess both hemolytic and enterotoxic properties [7]. Environmental strains containing aerolysin are potentially enterotoxigenic and can be passed from host to host, acquired directly from the environment but environmentally adapted strains are not much pathogenic [8]. *Aeromonas* spp. has been isolated from aquatic animals such as finfish, shellfish, crustaceans and amphibians. These organisms are pathogenic to many aquatic species and causes hemorrhagic septicemia (red sore disease) [9,10]. In recent times, *Aeromonas* have been associated with three types of human illnesses, including extra-intestinal, wound, and gastrointestinal infections. Extra-intestinal diseases are the most common with a mortality rate as high as 61% in septicemic patients, who are immunosuppressed. Wound infections caused by *Aeromonas* are linked circumstantially with injuries incurred during recreation or other activities in the aquatic environment. *Aeromonas* has also been implicated as causing food-associated gastroenteritis [11]. The major hurdle for antimicrobial treatment of bacterial disease is the development of multiple antimicrobial resistances. The increasing resistance to antimicrobial agents is a serious cause of concern, which warrants periodic monitoring of drug resistance of these organisms in different geographical areas.

The microorganism has the potential to be a foodborne pathogen of significant public health significance. In the present study, Aeromonads species were isolated from sea fishes, crabs and prawns collected from Chennai city and were further investigated for the prevalence of potential pathogenic genes and antimicrobial resistance.

Materials and Methods

Sample collection and bacterial isolation

Seafood like sea fishes, crabs and prawns were collected from 3 local markets of Chennai city namely, Marina beach, Chintadripet and Purasaiwakam fish markets. A total of 270 fresh sea foods samples were collected, which includes 30 samples of each of sea fish, prawn and crabs from 3 fish market separately. All samples were collected fresh in sterile polythene bags in hygienic conditions and transported under refrigeration temperature to laboratory and processed within 2 hours. The samples were enriched in alkaline peptone water (Himedia, Mumbai) at 1:9 dilutions of the samples and incubated at 37°C for 8 hours. After enrichment, they were plated on *Aeromonas* selective agar- Bile salts Irgasan brilliant green (BSIBG) agar (Himedia, Mumbai) and incubated at 37°C for 18-24 hours. The plates were then observed for the growth of typical translucent *Aeromonas* colonies. Further, typical presumptive colonies of *Aeromonas* were subjected to standard biochemical characterization and molecular confirmation.

Molecular Characterization of *Aeromonas* species

Extraction of DNA

Genomic DNA was extracted by snap chilled method as per Yousr *et al*. [12] with slight modification. In brief, two colonies of bacterial culture on selective media was suspended in 200 uL of nuclease free water and washed twice. The culture was again suspended in 200 uL of nuclease free water and kept in boiling water bath for 10 min.

It was transferred immediately to deep freezer (-20°C) for 15 min, followed by centrifugation at 5,000 rpm for 5 min and supernatant was transferred into a new Eppendorf tube for subsequent use as a DNA template.

PCR assay

The duplex PCR was performed to detect the 16S rRNA and aerA gene in the isolates [13]. The primers used in the present study were custom synthesized by Sigma-Aldrich and details of the primers are given in the [Table-1]. The 25µL PCR reaction mix was prepared using12.5µL of Taq DNA polymerase 2x Master Mix RED (Ampilqon, India) which provides Tris-HCl (pH 8.5), ammonium sulphate, 2 mM $MgCl_2$, 0.2% Tween 20, 0.4 mM (each) deoxyribonucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 2U of Taq DNA polymerase and 1µl of each oligonucleotide primer along with 3µl of template DNA. Also included a negative control to which no template DNA was added.

Table-1 Details of the primers used for detection of *Aeromonas* spp. and its cytotoxin [13].

Primer	Target Gene	Sequence	Product Size (bp)
16S rRNA	16S rRNA	(F)5'-TCA TGG CTC AGA TTG AAC GCT-3' (R)5'- CGG GGC TTT CAC ATC TAA CTT ATC-3'	599
Aerolysin	*aerA*	(F) 5'-GCA GAA CCC ATC TAT CCA G-3' (R) 5'-R TTT CTC CGG TAA CAG GAT TG-3'	252

The amplifications were carried out in a Thermocycler (Eppendorf Mastercycler Personal 5332, Italy). Temperature conditions consisted of an initial 94°C denaturation step for 2min followed by 35 cycles, with each cycle consisting of 1min at 94°C for denaturation, 1 min at 58°C for primer annealing, 0.5 min at 72°C for strand elongation and the final cycle at 72°C for 7min. The PCR products were electrophoresed on 1.5% agarose gel pre-stained with ethidium bromide (0.5ug/mL) and viewed under UV light using a UV trans illuminator with the DNA bands sized by extrapolation based on mobility of 100 bp DNA markers co-electrophoresed.

Antibiotic sensitivity test

Antimicrobial susceptibility was performed by the disc diffusion method on Mueller-Hinton Agar (Himedia, Mumbai, India) using antimicrobial discs (Himedia, Mumbai, India) according to the instructions of Clinical and Laboratory Standards Institute, 2015. Isolates were grown for 6 hours on alkaline peptone water (Himedia, Mumbai, India) and adjusted to 0.5 McFarland using sterile physiological saline, swabbed onto the Mueller-Hinton medium, and incubated with antimicrobial discs at 37°C for 18-24 hours. A total of 10 antibiotic discs including Ampicillin (AMP-10 mg), Azithromycin (AZM-30), Methicillin (MET-5), Enrofloxacin (EX-10mg), Cephalothin (CEP-30 mg), Streptomycin (S-10mg), Penicillin (P-2mg), Gentamicin (GEN-10 mg), Tetracycline (TE-30 mg) and Sulphadizine (SZ-30 mg) were used. The zone of inhibition was measured after incubation and compared with standard chart to determine the sensitivity and resistivity of the isolates to the test antibiotics used in the study.

Results and Discussion

Prevalence of Aeromonads

Majority of the studies showed the prevalence of *Aeromonas* in aquatic environments and living creatures and this genus is gaining importance because of its pathogenic effects on humans and fishes and the possible spoilage defects in food [14,15] sea foods like fish, crabs and prawns may also be a vehicle for pathogenic bacteria naturally occurring in aquatic environments referred to as indigenous or derived from polluted waters and or from post capture contamination, storage, transportation and handling [16]. Among 270 samples investigated, 172 samples showed positive by cultural method of which 160 samples were confirmed by PCR assay, targeting 16SrRNA, which includes 68, 42, and 50 samples of fishes, crabs and prawns, respectively. One of the valuable tools in identification of *Aeromonas* species is molecular identification by 16S rRNA sequence analysis [17,18] is employed in the present study [Fig-1]. The details of the prevalence of the *Aeromonas* are given in the [Table-2]. Occurrence of *Aeromonas* spp. was found to be significantly ($p<0.05$) higher in fish than crabs and prawn. There was no significant ($p>0.05$) difference among the samples collected from different locations. A previous research on the screening of *Aeromonas* in fish revealed that 22.3% of fishes were shown positive for *Aeromonas* [19]. Fish specimen from retail outlets of United Kingdom exhibited 19.4% incidence of *Aeromonas*. Hudson and De Lacy [20], reported 28 per cent of incidence of *Aeromonas* in fishes from retail outlets of New Zealand. Thayumanavan *et al*. [21] reported 89.80 % of incidence of *A. hydrophila* in marketed fish and Gobat and Jemmi [22] reported extremely high prevalence (95.06%) of *A. hydrophila* in fresh fishes sold in retail outlets of Switzerland, which are concurrence with our results. The results of phenotypic, biochemical and molecular characters of isolates are tabulated in the [Table-3]. All the isolates were Gram negative, catalase positive, oxidase positive, tolerate 3% NaCl, ornithine negative and fermented sucrose and glucose. The haemolytic ability of *Aeromonas* isolates on sheep blood agar was ascertained. They exhibited variations with regard to the other properties like Voges-Proskauer, utilization of arginine, esculin and citrate and sugar fermentation tests like mannitol, salicin and cellobiose. Haemolytic proteins are commonly isolated from pathogenic bacteria, and β-haemolysins are one of the important bacterial virulence factors [23]. Illanchezian *et al*. [24] reported a prevalence of 43.8% of *Aeromonas* from 5 major fish markets in Chennai, Tamil Nadu which exhibited β-haemolysin. From this, it is clear that the environmental aeromonads also possess pathogenic potential and may possibly be a potent contaminant of water supplies, fishes and seafood.

Potential Pathogenic Aeromonads

Aeromonads are considered as a potential foodborne pathogen when it carries aerolysin or hemolysin genes [25]. It is depicted from [Table-4], that highest prevalence aerolysin gene was reported in the isolates obtained from the fish samples (36.9%) and least prevalence rate was reported in the isolates obtained from the prawns (24.85%) with an overall prevalence of 35.53% among the *Aeromonas* spp. isolates. Gonzalez-Rodriguez *et al*., [26] reported duplex primer set targeted against aerolysin gene and species specific after analysing it for various species of *Aeromonas* to be efficient. Guerra *et al*. [27] revealed that the gene encoding aerolysin/hemolysin (cytotoxic enterotoxin) (aerA) was to be prevalent in 74.1% of his isolates from associated diarrhoeal diseases in South Brazil. Similarly, Miyagi and co-workers [28] reported prevalence of aerA gene in 44.7% of *Aeromonas* species investigated in Japan which concurred with our findings.

Antimicrobial resistance in Aeromonads

The antimicrobial resistance pattern of *Aeromonas* spp. isolates as follows Ampicillin (100%), Azithromycin (26.25%), Methicillin (92.5%), Enrofloxacin (8.75), Cephalothin (100%), Streptomycin (87.5%), Penicillin (100%), Gentamicin (56.25%), Tetracycline (42.5%) and Sulphadizine (78.12%), respectively. Similar results were observed by Castro-Escarpulli and workmates [29], in which they found complete resistant against ampicillin, penicillin, streptomycin and cephalothin. Similarly, Odeyemi and Ahmad [30] reported that, *Aeromonas* were resistant to Ampicillin, Sulphamethoxazole and Trimethoprim, but susceptible to Tetracycline (100%), Gentamicin (5.7%) and Oxytetracycline (24.5%). Jalal and co-worker [31] revealed that all the *Aeromonas* strains tested were 100% resistant to beta-lactam antibiotics and cephalosporins (cephalothin). According to Walsh *et al*. [32] *Aeromonas* spp. are not only capable of producing beta-lactamases, but also of expressing these enzymes in a coordinated manner, indicating that the expression of genes encoding beta-lactamases is controlled by a single mechanism, which may possibly be the reason for exhibiting complete resistance against them. *Aeromonas* strains are sensitive to quinolones although rarely, resistance to these drugs was reported. Cabello [33] reported that fish treated with antibiotics through various routes add up antibiotic in aquatic life. The non-consumed feed and antibiotic-containing feces make up sediment at river and sea basin. All this sum up for the antibiotic residues and alter the sediment microflora and selection of antibiotic-resistant bacteria.

The determinants of antibiotic resistance, emergence and selection in the aquatic environment can be transmitted through the horizontal gene transfer of microbial pathogens.

Table-2 Prevalence of *Aeromonas* spp. in samples obtained from different location (n=30, N=270)

Locations	Samples			Total
	Fish	Crab	Prawn	
Marina beach	22(73.33%)[a]	15(50.00%)[b]	16(53.33%)[b]	53(58.89%)
Chintadripet	21(70.00%)[a]	13(43.33%)[b]	16(53.33%)[b]	50(55.56%)
Purusaiwakkam	25(83.33%)[a]	14(46.67%)[a]	18(60.00%)[a]	57(63.33%)
Total	68(75.56%)[a]	42(46.67%)[b]	50(55.56%)[b]	160(59.26%)

Table-3 Phenotypic, Biochemical and Molecular characters of isolates

Test	Result	Percent Positive (N=160)
16s rRNA	Present	100
Aerolysin (aer) gene	Present	35.53
Gram staining	Gram Negative	100
oxidase test	+	100
catalase test	+	100
Haemolytic activity	+	32.5
VogesProskauer's test	-	32.5
Arginine utilization	+	80.25
Esculin	+	53.12
Citrate utilization	+	88.125
Ornithine utilization	-	0
NaCl 3%	+	100
Sugar fermentation tests		
Mannitol	+	94.37
Arabinose	+	71.25
Sucrose	+	100
Glucose	+	100
Salicin	+	61.25
Cellobiose	+	60.23
TCBS Agar plates	Yellow	75.625

Table-4 Prevalence of Aerolysin (aerA gene) in the *Aeromonas* isolates obtained from different location

Locations	Samples			Total
	Fish	Crab	Prawn	
Marina beach	12(54.54%)	7(46.66%)	6(37.5%)	25(46.23%)
Chintadripet	8(38.09%)	5(38.46%)	5(31.25%)	18(35.93%)
Purusaiwakkam	7(28%)	4(28.57%)	3(16.67%)	14(24.41%)
Total	27(36.90%)	16(34.42%)	14(24.85%)	57(35.53%)

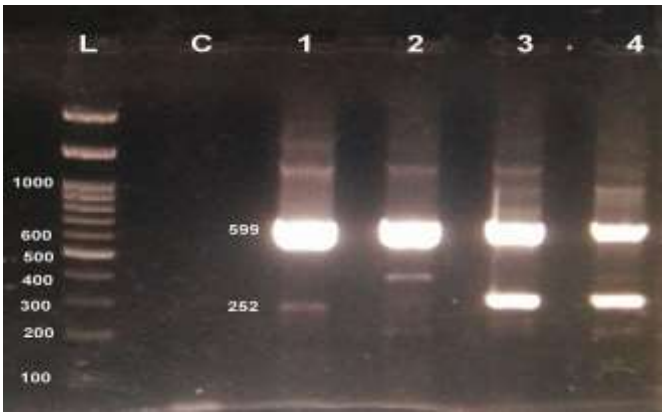

Fig-1 Duplex PCR results of *Aeromonas* spp. obtained from different samples (L: 100bp ladder, C: negative control, 1,2,3,4: samples positive for 16S rRNA-599bp. 1,3&4: samples positive for aerolysin (aer)-252bp)

Conclusion

The present study revealed the presence of potential pathogenic *Aeromonas* species in seafoods of Chennai. The prevalence of Aeromonads in seafood by PCR method is 160/270 (59.26%) and aerolysin (aerA) gene is 57/160 (35.53%). The isolates showed multiple antimicrobial resistance with complete resistant to ampicillin, penicillin and cephalothin. These seafoods serve as environmental reservoirs to support the survival, persistence and dissemination of *Aeromonas*. One of major reasons for high prevalence of Aeromonads could be cross contamination in post-harvest processing.

Application of research: Seafoods are one of the major foods stuff of this coastal area, monitoring the levels and species distribution of *Aeromonas* in seafoods and aqua culture environments will play an important role to prevent risk to human and aquatic animal health. Efforts are also required to reduce the level of *Aeromonas* in seafoods.

Research Category: Food Microbiology

Acknowledgement / Funding: Authors are thankful to Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Chennai, 600051, Tamil Nadu, India

***Research Guide or Chairperson of research: Professor Dr Elango A**
University: Tamil Nadu Veterinary and Animal Sciences University, Chennai, 600051
Research project name or number: Pilot Study

Author Contributions: All author equally contributed

Author statement: All authors read, reviewed, agree and approved the final manuscript

Conflict of Interest: None declared

Sample Collection: Total of 270 fresh sea foods samples - 30 samples of each sea foods *viz*., sea fish, prawn and crabs from each of 3 fish markets i.e., Marina beach, Chintadripet and Purasaiwakkam

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] Rey G., Fouillet A., Bessemoulin P., Frayssinet P. and Dufour A. (2009) *European Journal of Epidemiology*, 24, 495- 502.
[2] Abulhamd A. (2010) *International Journal of Chemical Engineering and Applications*, 1, 90–95.
[3] Agarwal R.K., Kapoor K.N. and Kumar A. (2000) *Indian Journal of Animal Science*, 70, 942-943.
[4] Bachhil V.N., Bhilegoankar K.N. and Agarwal R.K. (2002) *Indian Association of Veterinary Microbiologists, Immunologists and Specialists in Infectious Diseases*, 23, 81-82.
[5] Yu H.B., Rao P.S.S., Lee H.C., Vilches S. and Merino S. (2004) *Infection and Immunity*, 72, 1248-1256.
[6] Majeed K.N. and Macrae I.C. (1993) *Journal of Microbiology*, 73(297), 281-288.
[7] Chopra A.K., Peterson J.W., Xu X.J., Copenhaver D.H. and Houston C.W. (1996) *Microbial Pathogenesis*, 21, 357–377.
[8] Elmanama, A.A. and Ferwana, N. (2011) *Journal of Al Azhar University - Gaza, Humanities*, 13, 69-82.
[9] Yi S.W., You M.J., Cho H.S., Lee C.S., Kwon J.K. and Shin G.W. (2013) *Vet. Microbiol.*, 164, 195–200.
[10] Hussain I.A., Jeyasekaran G., Shakila R.J., Raj K.T. and Jeevithan E.(2014) *Journal of Food Science and Technology*, 51, 401–407.
[11] Abeyta C.J. and Wekell M. M. (1988) *Journal of Food Safety*, 9, I I-22.
[12] Yousr A.H., Napis S., Ali R., Rusul G. and Radu S.(2007) *ASEAN Food Journal*, 14(2), 115-122.
[13] Arora S., Agarwal R.K. and Bist B. (2006) *International Journal of Food Microbiology*, 106(2), 177-183.
[14] Thampuran N., Surendran P.K., Mukundan M.K. and Gopakumar K.

(1995) *Asian Fisheries Science*, 8, 103-111.
[15] Sinha S., Shimada T., Ramamurthy T., Bhattacharya S. K., Yamasaki S., Takeda Y. and Balakrish Nair G. (2004) *Journal of Medical Microbiology*, 53, 527–534.
[16] Ramasamy A., Muthusamy S. and Govindasami V. (2014) *Int. J. Pharm. PharmSci.*, 6(8),148-150
[17] Porteen K., Agarwal R.K. and Bhilegoankar K.N. (2007) *American Journal of Food Technology*, 2(1), 30-37.
[18] Joseph A.V., Sasidharan R.S., Nair H.P. and Bhat S.G. (2013) *Veterinary World*, 6(6), 300-306.
[19] Tsai G.J. and Chen T.W. (1996) *International Journal of Food Microbiology*, 31(1-3), 121-31.
[20] Hudson J.A. and De Lacy K.M. (1991) *Journal of Food Protection*, 54(9), 696-699.
[21] Thayumanavan Tha, Subashkumar R., Vivekanandhan G., Savithamani K. and Lakshmanaperumalsamy P. (2007) *American Journal of Food Technology*, 2(2),87-94.
[22] Gobat P.F. and Jemmi T. (1993) *International Journal of Food Microbiology*, 20(2), 117-20.
[23] Erova T. E., Sha J. and Horneman A.J. (2007) *FEMS Microbiology Letters*, 275(2), 301-311.
[24] Illanchezian S., Sathish Kumar J., Muthu Saravanan M. and Valsalam, S. (2010) *British Journal of Microbiology*, 41(4), 978-983.
[25] Cumberbatch N., Gurwith M.J., Langston C., Sack R.B. and Brunton J.L. (1993) *Infection and Immunity*, 23, 267-277.
[26] Gonzalez-Rodriguez M.N., Santos J.A., Otero A. and Garcia-Lopez M.L. (2002) *Journal of Applied Microbiology*, 93, 675– 680.
[27] Guerra I.M.F., Fadanelli R., Figueiro M., Schreiner F., Delamare A.P.L., Wollheim C., Costa S.O.P. and Echeverrigaray S. (2007) *Brazilian Journal of Microbiology*, 38, 638-643.
[28] Miyagi K., Hirai I. and Sano K. (2016) *Environ. Health Prev. Med*., 21, 287-294.
[29] Castro-Escarpulli G., Figuerasb M.J., Aguilera-Arreola G., Soler L., Ferna´ndez-Rendo´n E., Aparicio G.O., Guarro J. and Chaco´n M.R. (2003) *International Journal of Food Microbiology*, 84, 41– 49.
[30] Odeyemi O.A. and Ahmad A. (2017) *Saudi Journal of Biological Science*, 24, 65–70.
[31] Jalal A., Fatin N., MohdAshaari M., John A., Yunus K., Saad S., Omar M.N. (2010) *African Journal of Microbiology and Research*, 4, 640–645.
[32] Walsh T.R., Stunt R.A., Nabi J.A., MacGowan A.P., Bennett P.M. (1997) *Journal of Antimicrobial Chemotherapy*, 40, 171-178.
[33] Cabello F.C. (2006) *Environ. Microbiol*., 8, 1137-44.