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Short Communication

IDENTIFICATION AND SCREENING OF EXTRACELLULAR VIRULENCE FACTORS OF AEROMONAS SPP. IN ENVIRONMENTAL WATER SAMPLES FROM TAMILNADU, SOUTH INDIA

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ABSTRACT

Objective: *Aeromonas* species (spp.) are emerging pathogen that is present in drinking water supplies which are a major reason for public health concern. Factors contributing to virulence include toxins, haemolysins, adhesins and various hydrolytic enzymes. The present study aimed at investigating the incidence of *Aeromonas* spp. and its virulence factors from environmental water bodies in Coimbatore.

Methods: A total of 180 water samples were collected and screened for the presence of pathogenic *Aeromonas* spp. using starch ampicillin agar. The conventional method of identification might lead to misidentification of *Aeromonas* spp. thus molecular based identification using 16S rRNA gene was carried out followed by the identification of production of extracellular virulence factors like haemolysin, gelatinase, caseinase, lipase and DNase.

Results: Incidence of *Aeromonas* in water sample was found to be 55% (100 positive isolates) which was also confirmed with the molecular identification. The isolates showed 60% of haemolytic activity,, 34% of gelatinase activity, 77% of caseinase, 52% of lipase and 48% of DNase activity.

Conclusion: This study, thus, provides valuable information for the existence of pathogenic *Aeromonas* spp. in the environmental water bodies. Use of such water for domestic and commercial purpose might lead to the transfer of the pathogenic *Aeromonas* spp.

Keywords: *Aeromonas* spp, Haemolysis, Caseinase, DNase, Gelatinase and lipase.

Water is the best source of microbial load that causes infections, among which *Aeromonas* spp. cause wound infections and septicaemia and have been associated with diarrhoeal illness [1, 2]. The prevalence of *Aeromonas* in the water distribution system varies depending on the geographic region. Motile aeromonads have been recognized as occasional pathogens and the most common bacteria in freshwater habitats throughout the world. The pathogenesis of *Aeromonas* infection is complex and multifactorial, as aeromonads produce a wide variety of virulence factors which include aerolysin/hemolysin, cytotoxins, enterotoxins, proteases, lipases, DNases and adheins [3-6]. The detection of virulence factors in *Aeromonas* is a key element in the determination of potential pathogenicity because more than two virulence factors act multi-functionally and multifactorially. Thus, it is necessary to continue surveying the distribution of known virulence determinants in currently circulating *Aeromonas* strains and hence the current study is carried out on that aspect among the environmental water bodies in Coimbatore.

Hundred and eighty water samples were processed for the detection of *Aeromonas* spp. using starch ampicillin agar plates (SAA) by spread plate technique followed by Gram staining, oxidase and catalase test. Genomic DNA isolated from the positive isolates were subjected to polymerase chain reaction (PCR) with 16S rRNA gene primer *Aeromonas* sp. (1050 base pair (bp)) (F-5′ CAGAAGAAG CACCGGCTAAC 3′ and 16S rRNA R-3′ TTACCTTATTACGACTTCAC 5′) with initial denaturation at 95 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 sec, annealing at 52 °C for 30 sec and extension at 72 °C for 1 min, and final extension at 75 °C for 5 min. The PCR product of one of the isolate was submitted for sequencing at Chromous Biotech, Bangalore. Production of extracellular virulence factors such as haemolysin, gelatinase, lipase, DNase and caseinase was detected as per the protocol from John and Hatha [7].

Environmental water samples revealed 55% incidence of *Aeromonas* spp. (100/180 isolates) in SAA plate with yellow to honey coloured colonies and were Gram-negative, oxidase and catalase positive showing intense purple colour in 5-10 seconds and effervescence respectively. Evidence for the existence of *Aeromonas* sp. in drinking water, lake water and the Mediterranean Sea were observed in previous studies [8, 9, 10]. Molecular confirmation using 16Sr RNA primer resulted in expected amplicon at 1050 bp (fig. 1) authenticating 55% incidence of *Aeromonas* spp. Sequenced PCR product was submitted in NCBI (Gen Bank Accession: KT279069).

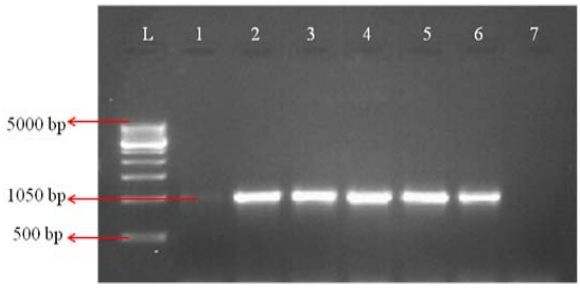

Fig. 1: Amplification of 16S rRNA gene in *Aeromonas* spp. Lane L-DNA Ladder 500-5000 bp, Lane 1 to 7-Distinct bands at 1050 bp amplicon of *Aeromonas* spp. 16Sr RNA gene

Environmental isolates of the current study showed 60% of haemolytic activity out of which 24% showed α-haemolysis, 36% showed β-haemolysis and 40% were non-haemolytic (γ-haemolytic). Haemolytic activity is an virulent importance factor in which the protein degrades the RBCs of the host [11]. The difference in the percentage of the activity was observed in various studies [11, 12-14] which might be due to environmental conditions of multiplication, which alters the genes that decode haemolysin.

Extracellular enzyme detection among the isolates of the current study recorded with 34% producers of gelatinase, 48% with DNase activity, 77% with caseinase production and 52% of the isolates

producing lipases. These results were contrary to the results of the previous studies [7, 15, 16]. This difference in the production of extracellular enzymes might be due to the external environmental conditions and the sources of the isolates. The pathogenic nature of *Aeromonas* spp. is in part, associated with the production of exoenzymes, such as proteases and lipases [17]. Caseinolytic and gelatinolytic activity encountered among the motile aeromonads are considered as pathogenicity markers [18].

In conclusion, the study reveals the existence of *Aeromonas* spp. in environmental water samples which carries various virulence determinants proving its pathogenicity. Poor water management practices could trigger infections caused by these potential pathogens/opportunists and might lead to disease outbreaks through cross contamination which poses a serious health concern.

CONFLICT OF INTERESTS

Declared none

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