Presence and Virulence Potential of *Aeromonas hydrophila* in Selected Water Sources for Household Consumption in Makurdi, Benue State

Tersoo-Abiem Evelyn Mnguchivir¹*, Ariahu Charles Chukwuma¹ and Igyor Micheal Agba¹

¹Department of Food Science and Technology, Federal University of Agriculture, Makurdi, Benue State, Nigeria.

Authors’ contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

This study was conducted to investigate the prevalence of *Aeromonas hydrophila* in selected drinking water sources in Makurdi. A total of 100 water samples (Tap, river, stream, well, pond and borehole water) were collected from different locations in Makurdi. Isolation and identification of the organism was performed using standard microbiological techniques. Further confirmation of the isolates as *Aeromonas hydrophila* was carried out using the Microbact 24E detection kit and polymerase chain reaction (PCR). *A. hydrophila* was detected in 12 (12%) out of the 100 samples; 6.67%, 8.82%, 7.14%, 25%, 30% and 20% of tap, well, borehole, river, pond and stream water samples respectively. The highest isolation rate of *A. hydrophila* (30%) was from pond water. All *A. hydrophila* isolated exhibited haemolysin, protease and lipase activity. The findings of this study revealed that treated and untreated drinking water sources in Makurdi are contaminated with potentially virulent *A. hydrophila* strains which may pose a health risk to consumers. Therefore, basic water treatment should be applied to drinking water to reduce public health threat posed by this finding.
Keywords: Aeromonas hydrophila; drinking water; virulence factors.

1. INTRODUCTION

Aeromonas hydrophila and related species are Gram-negative short rods, facultatively anaerobic, non-sporing, oxidase positive bacteria [1]. Due to its ubiquitous nature, A. hydrophila is found in many foods and have been isolated from dairy products, meat and poultry, seafood, vegetables and fresh water [2,3]. Aeromonas hydrophila has been incriminated as the main cause of Aeromonas associated human diseases beside A. sobria and A. caviae [4]. It has been reported to be responsible for intestinal and extra intestinal diseases ranging from relatively mild illnesses such as gastroenteritis and wound infections to life-threatening conditions such as septicemia, hemolytic-uremic syndrome and necrotizing fasciitis in humans [5]; also peritonitis and pneumonia [6]. Reports show that a greater risk of infection occurs in young children, elderly people and immune compromised patients [7]. Virulence in A. hydrophila is multifactorial, with disease resulting from the production of various virulent factors including haemolytic, cytotoxic and enterotoxigenic properties [8].

A. hydrophila has received much attention both as an emerging human pathogen and as an indicator of pollution associated with several aquatic environments including lakes, rivers, well, pond and chlorinated water sources that are considered to significantly impact public health [9,10]. In a study reported by Figueras and Ashbolt [11], Aeromonas was more prevalent than Salmonella or E.coli in patients with diarrhea in Nigeria. Globally, there is a problem in detection and monitoring of microbial pathogens in drinking water. Some developing countries have low drinking water quality due to inefficient common surveillance tools for waterborne pathogens [9,12]. According to Krovcak et al. [13], outbreaks of food –borne infections caused by Aeromonas are infrequently reported. However, a large part of the unidentified food borne infections and outbreaks may probably be caused by organisms such as A. hydrophila which are not routinely tested for identifying the etiology of food poisoning. Aeromonas hydrophila has been reported to be implicated in several foodborne outbreaks [3,9,10,13,14]. Reports indicate that the most common source of Aeromonas outbreaks has been water supplies, with A. hydrophila frequently isolated from surface and subterranean waters and also water distribution systems of treated and untreated drinking water, exposing the consumer to health risks [13,15]. Although the frequency of Aeromonas diarrhea is about 1.62% infections per million people, with high mortality in children [11], its occurrence in water and foods should not be neglected.

Given its health risks on individuals, the World Health Organization guidelines for drinking water quality have added Aeromonas to the list of potential human pathogens, and public water systems are now required to report the presence of Aeromonas through the Consumer Confidence Report Rule [16]. With the majority of virulent Aeromonas strains belonging to A. hydrophila HG1, regular monitoring of drinking water sources and a reliable identification of this strains is necessary to establish the risk associated with its presence in water sources [9,17].

With the wide spread nature of virulent and antibiotic resistant strains of A. hydrophila in drinking water sources, which exposes consumers to health risks, [2,7], it is important to assess the prevalence of A. hydrophila in drinking water sources in Makurdi, especially untreated water sources consumed by low income earners and rural dwellers.

The main objective of this study was to determine the presence of A. hydrophila in selected drinking water sources in Makurdi, and its virulence potential.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 100 drinking water samples of 10 ml each were randomly collected aseptically in sterile Bijou bottles from borehole, river, tap, pond, stream and well in selected locations in Makurdi, Benue State, Nigeria (7.7322°N, 8.5391°E). These were conveyed to the laboratory within 3 h for analyses.

2.2 Isolation of Aeromonas spp. from Water

An aliquot of 1ml of each water sample was added in 9 ml Nutrient broth for enrichment and incubated at 37°C for 24 h. A loopful from the enrichment broth was then plated on starch-ampicillin agar (SA agar) and incubated at 37°C for 24 h. After the incubation period, plates were observed for growth consistent with Aeromonas...
2.4 Molecular Identification of Aeromonas hydrophila

2.4.1 Aeromonas hydrophila DNA extraction

Presumptive A. hydrophila cultures were grown in trypticase soy broth (TSB) at 37°C for 18 h to obtain young culture prior to the extraction of genomic DNA. DNA extraction was carried out according to the procedure described by Hussain et al. [22]. Briefly, 1 ml of the cell suspension was centrifuged at 10,000 × g for 10 min at 4 °C and the cell pellet mixed with 600 μl of guanidine hydrochloride buffer (pH 8.0), incubated at room temperature for 30 min and again centrifuged at 10,000 × g for 10 min at 4°C. A 500 μl portion of the supernatant was transferred into another tube and mixed with 100% ice cold ethanol and centrifuged at 13,000 × g for 20 min at 4 °C. The supernatant was discarded and the pellet washed with 95%, and then 90% ethanol followed by centrifugation at 10,000 × g for 10 min at 4 °C. The pellet was then resuspended in 50 μl of molecular grade water, quantified in a Biophotometer (Eppendorf AG, Hamburg, Germany) and then stored at −20 °C to be used as PCR template.

2.4.2 PCR amplification

The amplification of the A. hydrophila specific gene was employed for the confirmation of A. hydrophila isolates. The sequences of the pairs of primer (AHH1) were forward 5'-GCC GAG CGC CCA GAA GGT GAG TT-3' and reverse 5'-GAG CGG CTG GAT GCG GTT GT-3' with the estimated amplicon size of 130 bp. The reaction mixture (25 μl) for the PCR amplification consisted of 1 μl of the genomic DNA, 10 μM each of the forward and reverse primers (1 μl), 12.5 μl of 2x PCR BIO Taq Mix Red (PCR Biosystems Ltd, London, UK) and 9.5 μl ultra pure water. Amplification was done in a thermal cycler (Kyratec SuperCycler Thermal Cycler, Australia). The PCR conditions for the A. hydrophila AHH1 gene identification included initial denaturation at 94°C for 3 mins, then the 45 cycles consisting of denaturation at 94°C for 60 s, annealing at 57°C for 60 s and an extension at 72°C for 90 s. The final step of extension was at 72°C for 3 mins and held at 4°C until collection [22,23].

2.4.3 Agarose gel electrophoresis for visualization of the PCR products

The PCR products were analyzed using agarose gel electrophoresis (Bio-Rad Powerpac 300) in 1.5% agarose pre-stained with 0.5 mg of ethidium bromide per ml and were visualized and recorded by Gel Doc (GMV20-Model). The amplicon sizes of the products were measured using Gelpilot 100bp DNA ladder (Qiagen, Hilden, Germany).

2.5 Phenotypical Characterisation of Isolates

Presumptive Aeromonas colonies were identified by standard physiological and biochemical tests according to Harrigan and McCance [19], Cowan [20], and Bergey’s Manual of Systematic Bacteriology [21]. The isolates were differentiated on the basis of their cultural and morphological characteristics such as growth size and shape, elevation and pigmentation. They were then subjected to various biochemical tests including Gram reaction, Indole, Vogues Proskauer and Methyl red test, Oxidase, Catalase, motility test, citrate test, nitrate reduction, ammonia production tests, fermentation of sugars, gelatin hydrolysis and hydrogen sulphide production. Growth in different conditions such as pH, NaCl concentration and temperature was also tested. The isolates were further characterised using the Microbact 24E.

(yellow coloured colonies) and four colonies were randomly selected from each plate. The isolates were sub-cultured on Starch agar and incubated at 37°C for 24 h repeatedly to obtain pure cultures [18]. These were then stored on nutrient agar slants in the refrigerator for further identification.

2.3 Phenotypical Characterisation of Isolates

Presumptive Aeromonas colonies were identified by standard physiological and biochemical tests according to Harrigan and McCance [19], Cowan [20] and Bergey’s Manual of Systematic Bacteriology [21]. The isolates were differentiated on the basis of their cultural and morphological characteristics such as growth size and shape, elevation and pigmentation. They were then subjected to various biochemical tests including Gram reaction, Indole, Vogues Proskauer and Methyl red test, Oxidase, Catalase, motility test, citrate test, nitrate reduction, ammonia production tests, fermentation of sugars, gelatin hydrolysis and hydrogen sulphide production. Growth in different conditions such as pH, NaCl concentration and temperature was also tested. The isolates were further characterised using the Microbact 24E.
2.6 Molecular Identification of Aeromonas hydrophila

2.6.1 Aeromonas hydrophila DNA extraction

Presumptive A. hydrophila cultures were grown in trypticase soy broth (TSB) at 37°C for 18 h to obtain young culture prior to the extraction of genomic DNA. DNA extraction was carried out according to the procedure described by Hussain et al. [22]. Briefly, 1 ml of the cell suspension was centrifuged at 10,000 × g for 10 min at 4 °C and the cell pellet mixed with 600 μl of guanidine hydrochloride buffer (pH 8.0), incubated at room temperature for 30 min and again centrifuged at 10,000 × g for 10 min at 4 °C. A 500 μl portion of the supernatant was transferred into another tube and mixed with 100% ice cold ethanol and centrifuged at 13,000 × g for 20 min at 4 °C. The supernatant was discarded and the pellet washed with 95%, and then 90% ethanol followed by centrifugation at 10,000 × g for 10 min at 4 °C. The pellet was then resuspended in 50 μl of molecular grade water, quantified in a Biophotometer (Eppendorf AG, Hamburg, Germany) and then stored at −20 °C to be used as PCR template.

2.6.2 PCR amplification

The amplification of the A. hydrophila specific gene was employed for the confirmation of A. hydrophila isolates. The sequences of the pairs of primer (AHH1) were forward 5'-GCC GAG CGC CCA GAA GGT GAG TT-3' and reverse 5'-GAG CGG CTG GAT GCG GTT GT-3' with the estimated amplicon size of 130 bp. The reaction mixture (25 µl) for the PCR amplification consisted of 1 µl of the genomic DNA, 10 μM each of the forward and reverse primers (1 µl), 12.5 µl of 2x PCR BIO Taq Mix Red (PCR Biosystems Ltd, London, UK) and 9.5 µl ultra pure water. Amplification was done in a thermal cycler (Kyratec SuperCycler Thermal Cycler, Australia). The PCR conditions for the A. hydrophila AHH1 gene identification included initial denaturation at 94°C for 3 mins, then the 45 cycles consisting of denaturation at 94°C for 60 s, annealing at 57°C for 60 s and an extension at 72°C for 90 s. The final step of extension was at 72°C for 3 mins and held at 4 °C until collection [22,23].

2.6.3 Agarose gel electrophoresis for visualization of the PCR products

The PCR products were analyzed using agarose gel electrophoresis (Bio-Rad Powerpac 300) in 1.5% agarose pre-stained with 0.5 mg of ethidium bromide per ml and were visualized and recorded by Gel Doc (GMV20-Model). The amplicon sizes of the products were measured using Gelpilot 100bp DNA ladder (Qiagen, Hilden, Germany).

2.7 Detection of Virulence

2.7.1 Detection of lipase production

The presence of extracellular lipases was determined using the method described by Abd-El-Malek [4]. Each serially diluted isolate was plated on phenol red agar (prepared by incorporating phenol red (0.01% w/v), olive oil (0.1% v/v), CaCl2 (0.1% w/v) and agar (2% w/v), and incubated at 37°C for 24 h. Positive lipase activity was confirmed by observing the formation of a precipitate with yellow colouration around the colonies. An uninoculated plate served as a control.

2.7.2 Detection of protease production

The skimmed milk agar was used for this test. It was prepared by adding 1% (w/v) skimmed milk to the appropriate agar as described by Harrigan and McCance [19]. The media was sterilized by autoclaving at 121°C for 15 mins. The warm media was dispensed in sterile Petri dishes and allowed to solidify. The plates were inoculated by streaking the respective isolates across the plates. Uninoculated plates served as control. At the end of the incubation, a clear zone around the line of streaking indicated casein hydrolysis due to the activity of the protease enzyme.

2.7.3 Detection of haemolysin production

Haemolytic activity was detected by the plate method. Each isolate was streaked onto 5% sheep blood agar plates and incubated at 37°C for 24 h. The presence of a clear zone around the colonies was taken as positive for haemolysin production [10].

3. RESULTS AND DISCUSSION

3.1 Isolation and Characterisation of Aeromonas hydrophila from Selected Water Sources for Household Consumption

Twelve presumptive A. hydrophila strains isolated from fresh water sources in Makurdi were identified to species level using their
Aeromonas hydrophila showed yellow to honey coloured colonies of 2-3mm diameter. Flooding the plates with lugol’s iodine showed a clear zone of hydrolysed starch surrounding the colonies against a black agar background [7,18,24].

Table 1 shows the morphological and biochemical test results for identification of presumptive A. hydrophila isolated from selected drinking water sources in Makurdi. All isolates were Gram negative, rod shaped, oxidase and catalase positive. The biochemical tests result (Table 1) obtained from Microbact 24E was used to identify the isolates with reference to Microbact data base. These characteristics were in conformity with the documentations of Adegoke and Ogunbanwo [10], Cowan [20] and Bergey’s manual [21]. Reports from previous researchers have shown that Aeromonas identification is not always conclusive without molecular identification as some species display heterogenous biochemical characteristics [25]. Therefore, the isolates were further confirmed using Polymerase Chain Reaction (PCR) technique. Twelve A. hydrophila were identified.

Fig. 1 shows amplified Polymerase Chain Reaction products of A. hydrophila strains on Ethidium bromide stained agarose gel (1.5%). PCR identification gives a reliable identification of A. hydrophila isolates [26]. PCR products of 130 bp amplicon size were obtained from the reaction synonymous with identification of A. hydrophila. This result agrees with biochemical tests using Microbact 24E. Similar PCR products were reported for A. hydrophila isolated from various natural and treated water sources [27,28,29].

3.2 Prevalence of Aeromonas hydrophila in Selected Water Sources for Household Consumption in Makurdi

The results on Table 2 show the presence of A. hydrophila in selected water sources for household consumption in Makurdi. Aeromonas hydrophila was found in at least one water sample from all the water sources assessed with higher rates of occurrence in stream, river and pond water. These are drinking water sources used mostly by rural dwellers without treatment. This may have a potential risk on residents of these areas as most of the people living in this areas use this water for domestic activities and for drinking especially during the dry season. Similar reports were made by earlier researchers in other parts of the world [17,30,31,32,33], that A. hydrophila is a pathogen associated with water from ponds, rivers, lakes, borehole or groundwater, surface water and chlorinated water. The presence of this pathogenic organism can pose severe health risks to consumers, children and immune compromised individuals in particular, being a primary pathogen in cases of acute diarrhea [22,34].

The lowest occurrence of the organism was observed in tap water. Koksal et al. [35] and Scoaris et al. [36] reported similar low presence of A. hydrophila in tap water sources. This is likely due to treatment given to the water, especially chlorination, which can reduce the number of microorganisms in water. A. hydrophila in tap water may occur due to low levels of residual chlorine, contamination through broken water pipes, stagnant piped water and presence of organic matter [11,37]. The presence of A. hydrophila in tap water confirms its possibility as a vehicle for transmission of the organism.

3.3 Virulence Factors of A. hydrophila from Selected Water Sources for Household Consumption in Makurdi

The identified A. hydrophila strains were tested for various virulence factors including haemolysin, proteases and lipase production. Results on Table 3 revealed the virulence of A. hydrophila strains from the selected water sources. All the A. hydrophila strains exhibited haemolytic, protease and lipase activity which has been reported as the most common virulence factors in motile aeromonads [32]. Similar virulent factors in A. hydrophila were reported by other researchers [10,15,26]. Haemolysins are a group of multifunctional enzymes that play a vital role in A. hydrophila pathogenesis. Ahh1 is the most abundant of the strains of A. hydrophila that produce many widely distributed haemolysins. The presence of this gene is a strong evidence of pathogenic potential of A. hydrophila isolates [22,23,38]. The production of these virulence properties have been known to contribute to pathogenesis and disease in humans [8,38]. The pathogenesis is that of a toxico-infection with symptoms including fever, diarrhoea and abdominal pain [39]. Senderovich et al. [34] reported similar virulence in A. hydrophila isolated from diarrhea patients. More attention is required to ensure safety of consumers of water from such sources because a high rate of virulent A. hydrophila occurrence can lead to waterborne outbreak [40].
Fig. 1. Ethidium bromide stained agarose gel (1.5%) showing amplified PCR products of *Aeromonas hydrophila* strains

Lane M- 100bp DNA ladder (Gelpilot, Qiagen); Lane P- Negative reference strain (E. coli ATCC 35401); Lane P1- Positive reference strain (A. hydrophila ATCC 7966); Lanes 1 to 12 - A. hydrophila isolates: M39a, M39b, MK1, M22, A1E, A2A, A2C, A2E, BC1, B22, MX and MC

Table 1. Morphological and biochemical tests for identification of presumptive *Aeromonas hydrophila*

| Characteristics | M39a | M39b | MK1 | M22 | A1E | A2A | A2C | A2E | BC1 | B22 | MX | MC |
|-----------------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|----|----|
| Gram reaction   | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| Shape           | rod  | rod  | rod | rod | rod | rod | rod | Rod | rod | rod | rod | rod|
| Oxidase         | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Catalase        | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Motility        | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Nitrate         | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Lysine          | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| Ornithine       | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| H₂S             | +    | -    | +   | +   | -   | -   | +   | +   | +   | +   | +  | +  |
| Indole          | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Urease          | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| V-P             | +    | +    | -   | +   | +   | -   | +   | +   | +   | +   | +  | +  |
| Methyl red      | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Citrate         | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Gelatin         | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Sucrose         | +    | -    | +   | -   | +   | -   | +   | -   | -   | -   | +  | +  |
| Lactose         | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| Arabinose       | +    | +    | -   | +   | +   | -   | +   | +   | -   | -   | +  | +  |
| Inositol        | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| Adonitol        | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| Raffinose       | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| Salicin         | -    | +    | -   | +   | -   | +   | -   | +   | -   | -   | -  | -  |
| Glucose         | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Mannitol        | -    | +    | -   | +   | +   | -   | +   | +   | +   | +   | +  | +  |
| Starch          | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Xylose          | -    | +    | -   | +   | -   | +   | +   | +   | -   | -   | -  | -  |
| ONPG            | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Sorbitol        | +    | +    | -   | +   | -   | +   | -   | +   | -   | -   | -  | -  |
| Growth at 37°C  | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| 6.0% NaCl       | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |
| pH 4            | -    | -    | -   | -   | -   | -   | -   | -   | -   | -   | -  | -  |

Key: + = positive, – = negative, G = gas
Table 2. Prevalence of Aeromonas hydrophila in selected water sources for household consumption in Makurdi

| Water source | Number of samples collected | Number of samples positive for A. hydrophila |
|--------------|-----------------------------|---------------------------------------------|
| Tap          | 15                          | 1(6.67)                                     |
| Well         | 34                          | 3(8.82)                                     |
| River        | 8                           | 2(25)                                       |
| Pond         | 10                          | 3(30)                                       |
| Borehole     | 28                          | 2(7.14)                                     |
| Stream       | 5                           | 1(20)                                       |

Values in parentheses denotes percentage

Table 3. Virulence characteristics of Aeromonas hydrophila from selected household water sources in Makurdi

| Isolates | M39a | M39b | MK1 | M22 | A1E | A2A | A2C | A2E | BC1 | B22 | MX | MC |
|----------|------|------|-----|-----|-----|-----|-----|-----|-----|-----|----|----|
| Virulence test                                      | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Haemolysin activity                                 | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Protease activity                                   | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Lipase activity                                     | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |
| Starch hydrolysis                                   | +    | +    | +   | +   | +   | +   | +   | +   | +   | +   | +  | +  |

Source of A. hydrophila: M39a and M39b- River water; MK1 and MX– Borehole water; MC- Tap water; BC1- Stream water; M22, A1E and B22- Well water; A2A, A2C and A2E- pond water

4. CONCLUSION

This study indicates the presence of pathogenic A. hydrophila with virulence potential in stream, pond, river, well, borehole and tap water sources evaluated in Makurdi. This poses a major threat to public health since these sources of water are used for drinking and household chores. Therefore, there is a need to advocate for adequate basic water treatment/ purification such as boiling of water from these sources before drinking to reduce the health risks associated with A. hydrophila infections, especially in susceptible populations such as the immunosuppressed, children and elderly people.

DISCLAIMER

The research was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Daskalov H. The importance of Aeromonas hydrophila in food safety. Food Control. 2006;17:474-483.
2. Stratev D, Odeyemi OA. Antimicrobial resistance of Aeromonas hydrophila isolated from different food sources: A mini-review. Journal of Infection and Public Health. 2016;9:535-544.
3. Park SH, Choi S, Ha S. Predictive modeling for the growth of Aeromonas hydrophila on lettuce as a function of combined storage temperature and relative humidity. Food borne Pathogens and Disease. 2019;20(20):1-8
4. Abd-El-Malek AM. Incidence and virulence characteristics of Aeromonas spp in fish. Veterinary World. 2017;10(1):34-37.
5. Yano Y, Hamano K, Tsutsui I, Aue-umneoy D, Ban M. Occurrence, molecular characterization, and antimicrobial susceptibility of Aeromonas spp in marine species of shrimps cultured at inland low salinity ponds. Food Microbiology. 2015;47:21-27
6. Poffe R, Op de Beeck E. Enumeration of Aeromonas hydrophila from domestic wastewater treatment plants and surface waters. Journal of Applied Bacteriology. 1991;71(366-370).
7. Ansari M, Rahimi E, Raissy M. Antibiotic susceptibility and resistance of Aeromonas spp isolated from fish. African Journal of Microbiology Research. 2011;5(31):5772 – 5775
8. Fadel HM, El-lamine MMM. Vibriosis and Aeromonas infection in shrimp: Isolation,
sequencing, and control. International Journal of One Health. 2019;5:38-48.

9. Igbinosa IH, Igumbor EU, Aghdasi F, Tan M, Okoh AI. Emerging Aeromonas species infections and their significance in public health. The scientific World Journal. 2102:1-13.

10. Adegoke CO, Ogunbanwo ST. Isolation and characterisation of Aeromonas species isolated from food and Diarrhoeagenic stool in Ibadan metropolis, Nigeria. Food Science and Quality Management. 2016;51:20-31

11. Figueras Salvat MJ, Ashboll N. Aeromonas. In: J.B. Rose and B. Jiménez-Cisneros, (eds) Global Water Pathogen Project; 2019.

12. Pandove G, Sahota PP, Achal V, Vikal Y. Detection of Aeromonas hydrophila in water using PCR. Journal of American Water Works Association. 2011;103(11):59-65.

13. Krovacek K, Dumontet S, Eriksson E, Baloda SB. Isolation and Virulence profiles of Aeromonas hydrophila implicated in an outbreak of food poisoning in Sweden. Microbiology and Immunology. 1995;39:655 – 661.

14. Zhang Q, Shi G, Tang G, Zou Z, Yao G, Zeng G. A foodborne outbreak of Aeromonas hydrophila in a college, Xingyi city, Guizhou, China. Western Pacific Surveillance and Response Journal. 2012;3(3):1-4

15. Bhowmik P, Bag PK, Hajra TK, Sarkar RP, Ramamurthy T. Pathogenic potential of Aeromonas hydrophila isolated from surface waters in Kolkata, India. Journal of Medical Microbiology. 2009;58:1549-1558.

16. Katz MJ, Parrish NM, Belani A, Shah M. Recurrent Aeromonas bacteremia due to contaminated well water. Open Forum Infectious Diseases. 2015;1-3.

17. Khor WC, Puhah SM, Tan JAMA, Puthucheary SD, Chua KH. Phenotypic and genetic diversity of Aeromonas species isolated from fresh water lakes in Malaysia, PLoS ONE. 2015;10(12):1-13

18. Palumbo SA, Moragan DR, Buchanan RL. Influence of temperature, NaCl, and pH on the growth of Aeromonas hydrophila. Journal of Food Science. 1985;50 (5):1417–1421.

19. Harrigan WF, McCance ME. Laboratory methods in food and dairy microbiology. Academic press; 1976.

20. Cowan ST. Cowan and steel’s manual for the identification of medical bacteria, 3rd edition, Cambridge University Press; 1986.

21. Holt JG. The shorter berger’s manual of determinative bacteriology. 8th Edition. The Williams and Wilkins Company, Baltimore USA; 1981.

22. Hussain IA, Jeyasekaran G, JeyaShakila R, Raj KT, Jeevithan E. Detection of hemolytic strains of Aeromonas hydrophila and A. sobria along with other Aeromonas spp. from fish and fishery products by multiplex PCR. Journal of Food Science and Technology. 2014;51(2):401–407.

23. Wang G, Clark CG, Liu C, Pucknell C, Munro CK, Kruk TMAC, Caldeira R, Woodward DL. F.G. Rodgers, Detection and characterization of the hemolysin genes in Aeromonas hydrophila and Aeromonas sobria by multiplex PCR. Journal of Clinical Microbiology. 2003;41:1048–1054.

24. Corry JEL, Curtis GDW, Baird RM. (Eds.) Starch Ampicillin Agar (SAA). Pharmacopeia of culture media. In: Handbook of culture media for food microbiology, 2nd edition. Progress in Industrial Microbiology. 2003;37:600-602.

25. Persson S, Al-Shuweli S, Yapici S, Jensen JN, Olsen KEP. Identification of clinical Aeromonas species by rpoB and gyrB sequencing and development of a multiplex PCR method for detection of Aeromonas hydrophila, A. caviae, A. veronii, and A. media. Journal of Clinical Microbiology. 2015;53(2):653–656.

26. Cagatay IT, Sen EB. Detection of pathogenic Aeromonas hydrophila from rainbow trout (Onchorhyncus mykiss) farms in Turkey. International Journal of Agriculture and Biology. 2014;16:435-438.

27. Casco’n A, Anguita J, Hernanz C, Sa’nchez M, Ferna’ndez M, Naharro G. Identification of Aeromonas hydrophila hybridization group 1 by PCR assays. Applied and Environmental Microbiology. 1996;62(4):1167-1170.

28. Sarkar A, Saha M, Roy P. Identification and Typing of Aeromonas hydrophila through 16S rDNA-PCR Fingerprinting. J. Aqua. Res. Dev. 2012;3(6):1-4

29. Othman RM, Al-Thahe FS, Faaz1 RA and Jassim HY. Molecular detection of Aeromonas hydrophila isolated from infected carp Cyprinus carpio breeding in aquafarming in Basra, Iraq. Life Science Archives, 2017;3(2):974 – 980.
30. Fuzihara TO, Pisani B, Simoes M, Brigido BM, Leopoldoeslva C, Vannucci L, Arioshi K. The occurrence of Aeromonas spp in drinking water. Rev Inst Adolfo Lutz. 2005;64(1):122-7.

31. Kivanc M, Yilmaz M, Demir F. The occurrence of Aeromonas in drinking water, Tap water and porsuk river. Brazilian Journal of Microbiology. 2011;42:126-131

32. Zaky MM, Salem M, Persson KM, Eslamian S. Incidence of Aeromonas species isolated from water and fish sources from Lake Manzala (Egypt). International Journal of Hydrology Science and Technology. 2011;1(3):312-414.

33. Bello HS, Mustapha A, Ismail HY, Isa MA, Mangga HK, Detection of Aeromonas species from water sources in North Eastern Nigeria. International Journal of innovative Science, Engineering and Technology. 2016;3(2):140-148.

34. Senderovich Y, Ken-Dror S, Vainblat I, Blau D, Izhaki I, Halpern M. A molecular study on the prevalence and virulence potential of Aeromonas spp. recovered from patients suffering from diarrhea in Israel; 2012.

35. Koksal F, Oguzkurt N, Samasti M, Atlas K. Prevalence and antimicrobial resistance patterns of Aeromonas strains isolated from drinking water samples in Istanbul, Turkey. Chemotherapy. 2007;53:30-35

36. Scoaris DO, Bizerra FC, Yamada-ogatta SF, Filho BAA, Ueda-Nakamura T, Nakamura CV, Filho BPD. The occurrence of Aeromonas spp. in the bottled mineral water, well water and tap water from the Municipal supplies. Brazilian Archives of Biology and Technology. 2008;51(5):1049-1055.

37. Villarruel-Lopez EA, Fernandez-Rendon L, Mota-de-la-Garza J, Ortigoza-Ferado. Presence of Aeromonas spp in water from drinking-water- and wastewater-treatment plants in Mexico city. Water Environment Research. 2005;77(7):3074-3079.

38. Rasmussen-Ivey CR, Figueras MJ, McGarey D, Liles MR. Virulence Factors of Aeromonas hydrophila: In the wake of Reclassification. Frontiers in Microbiology. 2016;7(1337):1-10.

39. Stratev D, Vashin I, Rusev V. Prevalence and survival of Aeromonas spp in foods – A review. Revue Méd. Vét. 2012;163(10):486-494

40. Pablos M, Remacha MA, Rodriguez-Calleja JM, Santos JA, Otero A, Garcia Lopez ML. Identity, virulence genes, and clonal relatedness of Aeromonas isolates from patients with diarrhea and drinking water. European Journal of Clinical Microbiology and Infectious Diseases. 2010;29:1163-1172.

© 2021 Mnguchivir et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/67457