Innovative Academia Hub International
IAHI
EMPOWERING MINDS
INSPIRING EXCELLENCE
DRIVING INNOVATIONS

BSP ADVANCES IN SCIENCE,
ENGINEERING AND TECHNOLOGY
www.iahiservices.com

Aeromonas hydrophila: A Re-emerging Foodborne pathogen

Tersoo-Abiem, Evelyn Mnguchivir[1]*., Ariahu,
Charles Chukwuma[2] Igyor, Micheal Agba[3]
[1,2,3]*Department of Food Science and Technology,*
Joseph Sarwuan Tarka University, Makurdi, Nigeria.
**Corresponding author email: emtabiem@gmail.com*

Abstract

Aeromonas hydrophila is a ubiquitous bacterium found in terrestrial and aquatic environments and has been frequently isolated from food and water sources. It has been isolated from dairy products, meat and poultry, seafood, vegetables, ready-to-eat products, and a variety of refrigerated foods. *Aeromonas hydrophila* has been the focus of great attention recently mainly due to its fast distribution all over the world, increasing foodborne infections, the incidence of strains with antimicrobial resistance, and the increasing thermal resistance of some strains found in food. It is becoming recognized as a pathogen of serious public health concern that has been implicated in several infections but the exact incidence of its infection on a global basis is still unknown since many cases are not reported. Good hygiene practices, adequate heat processing of foods in combination with other hurdles, and preservation using multiple hurdles can be applied to control its growth and achieve safety and stability of food products.

Keywords: *Aeromonas hydrophila*, Food Safety, Re-emerging pathogen, D-value, Food infections, antimicrobial resistance.

1 Introduction

Foodborne illnesses are a long-standing public health concern worldwide [1]. The total global burden of foodborne disease is not known, mainly because of the lack of reports of all cases to public health authorities [2, 3]. It was estimated that at least 38 million cases of foodborne illness are caused by unspecific agents each year [3]. According to Puthucheary *et al*. [2], it is reasonable to assume that a great proportion of affected people do not seek appropriate medical care, hence the causative agents are not identified. Many agents including *Aeromonas* spp are known to cause illness, but their significance is difficult to estimate due to scanty data.

Several bacteria have been reported to cause foodborne illness. These include *A. hydrophila, Bacillus cereus, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens,* pathogenic *E. coli, Listeria monocytogenes, Salmonella* serovars, *Shigella* spp, *Staphylococcus aureus, Vibrio* spp., and *Yersinia enterocolitica* [4].

As stated by Oyarzabal [5] and Mahendra *et al.* [6], emerging or reemerging pathogens may appear due to series of circumstances that favor their spread. In the case of foodborne pathogens, the factors that play important roles include those related to the pathogen, the environment, food production processes, distribution chain, and the consumers The World Health Organization (WHO) associated the appearance of foodborne infections and outbreaks with factors that include changes in microorganisms, changes in human population and lifestyle, globalization of food supply, the inadvertent introduction of pathogens into new geographical areas, and exposure to unfamiliar foodborne hazards.

2 Genus *Aeromonas*

The genus *Aeromonas* are ubiquitous bacterium found in terrestrial and aquatic environments. They have been the focus of great attention recently mainly due to its fast distribution all over the world, ambiquity in correct identification and sorting of pathogenic *Aeromonas* species, the incidence of strains with antimicrobial resistance, and the ability of some strains to remain alive after the conventional wastewater treatments [7-9]. Initially, *Aeromonas* species was thought to be an opportunistic pathogen in immunocompromised people; but with the increasing number of cases of intestinal and extraintestinal diseases documented worldwide [9,10], it is becoming recognised as an enteric pathogen of serious public health concern. They grow well at higher temperatures and result in an increase in the number of infections during the warm season [11].

Aeromonas are divided into two large groups based on physiological properties and hosts: mesophiles and psychrophiles. The mesophilic aeromonads include mostly motile aeromonads, with *Aeromonas hydrophila* as a typical representative that causes infections mainly in humans while the psychrophillic group consists of primarily non-motile species, causing disease in fish [2, 11,12]. *A .hydrophila* has been identified as the most frequently isolated *Aeromonas* species [13].They have been implicated in several infections but the exact incidence of *Aeromonas* infection on a global basis is still unknown since many cases either go undetected or are not reported [2, 11].

Tomas [14], Hoel *et al*. [3] and Pessoa *et al*. [11] reported the ambiguity in the first classifications within the *Aeromonas* genus. The organisms were determined phenotypically based on their growth characteristics and biochemical tests, but for most environmental isolates of *Aeromonas* species, conventional biochemical test results sometimes did not necessarily correspond to results achieved by genetic methods. Therefore the use of molecular techniques has been employed to give a more refined and reliable taxonomy of the genus.

2.1 *Aeromonas hydrophila*

In the past few years, *Aeromonas hydrophila* has increasingly been recognized as a highly pathogenic emerging food borne bacteria of great concern to human health [15-18]. It is present in water, foods and the environment. *Aeromonas* spp. have been shown to form biofilms on various biotic and abiotic surfaces, which allow for the persistence of these pathogens in the water distribution system as well as in the human body [8, 16]. Its contamination of food can cause serious, long term foodborne illness [18], lead to economic losses to food industries and pose a critical threat to human's health [19]. Although the organism has also been described as a pathogen of fish [6, 20], it can be transmitted to human by ingestion of contaminated foods [11].

Aeromonas hydrophila is a Gram-negative motile, non-sporing rod, found in both salt water and fresh water environments. It is a facultative anaerobe but grows better in aerobic environment, with growth temperature ranging between 3 and 42- 45 °C, although often less than 40 °C, depending on the strain [8, 21], and optimum around 28 °C to 35 °C [21-23]. The microbe has a pH optimum of 7.0. It is unlikely to grow in foods below pH 6.0 stored at low temperatures [21]. It is neither salt (5 %) nor acid (minimum pH – 6.0) tolerant. According to Palumbo *et al.* [24], Kirov *et al.* [25] Devlieghere *et al*. [26] and Mantareva *et al*.[9], *A. hydrophila* has been shown to grow and multiply very rapidly at refrigeration temperatures as low as 5 °C, a temperature formerly thought adequate to keep food safe from foodborne pathogen hazards. Depending on the specific properties of foods, the growth /survival rate of *A.*

hydrophila can be significantly influenced [27].

2.2 Isolation of *Aeromonas hydrophila*

Starch Ampicillin agar has proved effective for the isolation of *Aeromonas* spp. from a range of food products. Starch incorporation into the medium serves as a differential agent since many Gram-negative bacteria associated with food fail to hydrolyse it; while ampicillin is used to inhibit coliforms and other Enterobacteriaceae [21]. Colonies of *Aeromonas* spp are 3-5 mm in diameter and are yellow to honey-coloured. Upon flooding with iodine, the colonies appear to have a clear zone around them due to starch hydrolysis. Other media commonly used for *A. hydrophila* isolation include Brilliant green bile agar, MacConkey agar and xylose desoxycholate citrate agar [21], blood agar, nutrient agar, thiosulphate citrate agar, ampicillin dextrin agar and Pyan's agar [6].

2.3 Molecular identification of *Aeromonas hydrophila*

Reports by Yano *et al*. [7] and Mohanty *et al*. [13] indicated that *Aeromonas* spp. have over 36 published species, where a number of them are pathogenic to humans, and most human clinical isolates belong to hybridization groups (HGs) HG-1, HG-4, HG-8, HG-9, HG-10, HG- 12, or HG-14. Among *Aeromonas* species, *Aeromonas hydrophila* has been the most commonly associated with human infections.

A number of molecular methods based on Restriction Fragment Length Polymorphisms (RFLP) and the 16S rDNA amplified by polymerase-chain-reaction (PCR) are being used to characterize aeromonads to species and strain level [14]. Polymerase chain reaction (PCR) methods have been developed by Wang *et al*. [28] and Hussain *et al.* [29] to detect the presence of *Aeromonas* species in a wide range of samples. According to Pessoa *et al*. [11], 16s rRNA gene analysis, housekeeping genes, genotyping techniques such as multilocus sequence typing (MLST), enterobacterial repetitive intergenic consensus-PCR (ERIC-PCR), and matrix assisted laser desorption-ionization time- of-flight mass spectrometry (MALDI-TOF) have been reported in molecular identification of *A. hydrophila*. In a recent study, Ma *et al.* [19] reported the use of DNAzyme-based sensor for detection of *A. hydrophila*.

2.4 Growth characteristics of *A. hydrophila*

Aeromonas hydrophila is a Gram-negative, rod shaped, motile, non-sporing and facultative anaerobic bacterium widely distributed in nature [6, 14]. As with other motile aeromonads, *A. hydrophila* may adhere to solid surfaces and form biofilms in aquatic environments [8, 30]. *A. hydrophila* generally produce circular, smooth, raised colonies on agar [13, 31]. Research by Cipriano [31], Pandove *et al*. [10] and Tersoo-Abiem *et al*. [32] demonstrated that strains of *A. hydrophila* display the following characteristics: they are positive to oxidase, indole, methyl red and Voges-Proskauer reaction, and positive to citrate utilization test. Urease, phenylalanine deamination, lysine decarboxylase, ornithine decarboxylase, and hydrogen sulfide are not produced. Sugar fermentation reaction shows acid as well as gas production from the following sugars: adonitol, arabinose, cellobiose, dextrose, fructose, galactose, inositol, lactose, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. Acid production from the carbohydrates arabinose, salicin, cellobiose, sucrose, and lactose may vary. It ferments glucose with or without the production of gas, and are insensitive to the vibriostatic agent (2, 4 diamino, 6, 7 -di-isopropyl pteridine).

Aeromonas species have been shown to resemble Enterobacteriacea both morphologically and biochemically [30]. *A. hydrophila* can be differentiated with the *Vibrio* spp and *Plesiomonas* spp using a positive oxidase reaction, resistance to the vibriostatic compound O/129, absence of ornithine decarboxylase activity and no growth in 6% NaCl [11].

3.0 Incidence of *A. hydrophila*

Aeromonas genus are ubiquitous, water-borne bacteria. The incidence of this pathogen has been observed to be higher in warm season than other seasons [23]. As reported by several researchers, they have been frequently isolated from marine waters, rivers, lakes, swamps, sediments, chlorinated and non-chlorinated drinking water supplies, water distribution systems (forming biofilms) [3, 15, 32, 33, 34]. Given its pathogenic qualities in immunocompromised individuals, the World Health Organization guidelines for drinking water quality recently 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. Most drinking water is monitored by the US Environmental Protection Agency, but owners of private wells must ensure their own drinking water is safe. The Centers for Disease Control and Prevention (CDC) recommends checking private wells yearly for the presence of bacteria or other contaminants [32, 36].

Due to the nature of its normal habitat, *A. hydrophila* has a high incidence in a variety of food products of both animal and plant origin. Its species have been isolated from dairy products, meat and poultry, seafood, vegetables and a variety of refrigerated foods, occurring in some foods at levels up to about 10^5 CFU/g or ml [11, 12, 22, 26, 37]. Table 1 and 2 show the occurrence of A.hydrophila in selected foods and water sources. A high incidence of *A.hydrophila* has been reported in ready-to eat products (8-52%) [38], vegetables (26—41 %), and meat and poultry (3—70 %), with the highest population in seafoods (31—72 %) [12]. Being a high moisture, highly nutritious low acidity fluid, with a pH ranging between 6.0 – 7.0, soymilk has also been reported to be a vehicle for transmission of *A.hydrophila*

Table 1: Incidence of *Aeromonas hydrophila* in Foods

Table 1: Incidence of *Aeromonas hydrophila* in Foods

Food	Occurrence (%)
Fresh fish	67
Smoked fish	70
Raw milk	85
vegetables	35
snails	29
Poultry	80
Meat	50
Ready-to eat foods	50

Source: Igbinosa *et al*. [15]

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(10)
Borehole	28	2(7.14)
Stream	5	1(20)

Values in parentheses denotes percentage

Source: Tersoo-Abiem *et al*. [32]

[32]. This raises concerns of the potential of *A. hydrophila* in food spoilage and foodborne infections.

The ability of *A. hydrophila* to grow in a variety of foods, and at refrigeration temperatures considered adequate for preventing growth of foodborne pathogens, raises the possibility that high numbers of the organism, and its preformed enterotoxins, may unknowingly be ingested from foods, especially when such foods are kept for appreciable times under refrigeration before consumption without proper handling and abused storage temperatures [11, 25, 26]. Although the bacterium is destroyed by heat, foods consumed without cooking or improperly cooked foods would most likely pose a threat to *A. hydrophila* contamination.

4.0 *A. hydrophila* infections

As reported by Daskalov [25], Adegoke and Ogunbanwo [35] and Mahendra *et al*. [6], the potential of *A. hydrophila* to cause human infections has been emphasized, especially with its psychrotrophic characteristics and wide distribution in the environment and foods. *Aeromonas* infections have been acquired through different contamination routes including ingestion of contaminated foods, trauma, injuries and exposure to untreated water.

A. hydrophila infection is usually characterised by symptoms which include abdominal pain, headache, nausea, chills, bacteremia, vomiting and fever. Other cases may involve acute self-limiting diarrhea with blood and mucus [39]; also, skin, bones, heart, lungs, eyes, kidney and other organs can be affected [11]. The incubation period is not known, but symptoms generally last from 1 to 7 days [21]. Both symptomatic and asymptomatic individuals habour *Aeromonas* species in their gastrointestinal tract. Studies have shown that the rates of faecal carriage in persons in the absence of disease in developed countries range from 0% to 4%, while the isolation rate from persons with diarrheal illness ranges from 0.8 to 7.4%.

Several *A. hydrophila* infection cases have been reported from hospitals in different regions of the world including India, Kenya, China, Spain, Mexico, Australia and Nigeria from different biological samples being tested (stool, eye, sputum, blood etc) [11]. Illnesses due to *A. hydrophila* range from mild diarrhea and mild cellulitis to life threatening cholera-like disease, necrotizing fasciitis and gas gangrene [13].

Epidemiological evidences have implicated the microbe as a cause of intestinal and extra-intestinal diseases in humans. Intestinal diseases such as bacteremia, gastroenteritis (being most common), septicaemia [13, 40]; extra-intestinal infections include wound infections, meningitis, endocarditis, cellulitis, respiratory infections, osteomyelitis, necrotizing fasciitis, ear infections, urinary tract infections and hepatobilliary infections among others [8, 13]. According to Hoel *et al*. [3], human *A. hydrophila* infections have occasionally been followed by life-threatening complication of hemolytic-uremic syndrome (HUS).

Necrotizing fasciitis and gas gangrene are diseases attributable to *A.hydrophila* which develop mainly in immunocompromised individuals requiring limb amputation as a life saving measure in many patients. Fatality rates from this condition can be as high as 30-40% when complicated with bacteremia [13]. Consequently, there is a critical need for early detection and appropriate targeted treatment of *A.hydrophila* infections.

According to Bradford *et al.* [41] and Pessoa *et al.* [11], the low level of isolations of *A. hydrophila* recorded in most clinical laboratories may not be a true reflection of prevalence or medical significance; this is supported by the fact that isolation rates frequently increase in laboratories that specifically target the microbe. Its detection in various diarrheal stool samples indicates they are not far from clinical routine as other enteric microbes.

The rate of recovery among children with diarrhea vary geographically, with food habits, level of sanitation, patient populations and isolation methods: 0.62 to 4% was reported in Malaysia; 2% in Sweden; 0.75% in Nigeria; 4.8% in Switzerland; 2.3% in Taiwan ; and 6.8% in Greece. Reports were made of aeromonads in 6.5% of all patients in India and in 6.9% of adult patients with acute diarrhea in Hong Kong. Previous reports on incidence rates from symptomatic patients ranged from 0.04% to 21% [15]. Recently, reports on human cases of aeromoniasis are increasing in Nigeria, as *A.hydrophila* has been isolated from children with diarrhea [8]. According to Reports by Bello *et al*. [42] exposure to water or food contaminated with *Aeromonas* spp. has been reported to precede some human *Aeromonas* infections which are particularly hazardous in patients with impaired immunity.

4.1 *A. hydrophila* Foodborne outbreaks

Food poisoning outbreaks involving *Aeromonas hydrophila* have been reported by several workers [33, 35, 37], in various parts of the world including Sweden in 1993, California in 1988 and a college in Xingyi city, China in 2012, in foods including Oysters, Shrimps, frozen fish, sashimi and edible land snails. Records from *Aeromonas* mediated outbreaks in Norway and Sweden suggested an infective dose in the range of 10^6 to 10^8 cells, although the infective dose in some cases were lower (10^3 to 10^4 cells) [3]. Although the numbers of food-borne outbreaks caused by *Aeromonas* spp. have been quite limited so far, the presence of *Aeromonas* spp. in the food chain cannot be ignored.

A. hydrophila has been associated with several food-borne outbreaks therefore, it has been considered as an organism of food safety concern. It has also been listed on the first and second Contaminant Candidate List (CCL) of potential waterborne pathogens in the United States [3, 6]. Hence, proper sanitary and processing procedures are essential in the prevention of the spread of *Aeromonas* infections [6, 12, 15].

4.2 Virulence factors of *Aeromonas hydrophila*

The virulence of *Aeromonas* is complex and involves multiple factors such as hemolysins, aerolysins, proteases, adhesins, relatively heat stable cholera-like enterotoxins and heat labile cytotoxic enterotoxins, phospholipase and lipases [15, 6]. In the reports by Igbinosa *et al*. [15] and Stratev and Odeyemi [12], these virulence factors have been linked with human diseases such as gastroenteritis, soft tissue, muscle infections, skin diseases, septicemia, meningitis, respiratory and hemolytic uremic syndrome. The virulence factors enable the bacteria to colonize, gain entry, establish, replicate, and cause damage in host tissues and to evade the host defence system and spread, eventually killing the host [11, 12].

As reported by Janda and Abbott [43] and Tsai *et al*. [44], virulence factors are present in two forms as cell-associated structures, and extracellular products. Among the cell-associated structures include pili, flagella, outer membrane proteins, lipopolysaccharide, and capsules. The major extracellular products include cytotoxic, cytolytic, hemolytic, and enterotoxic proteins. The pathogenicity of Aeromonads is attributed to these factors. Studies by Tomás [14] and Batra *et al.* [16] showed that *Aeromonas* spp possess all the virulence factors which help in the establishment of infection. The presence of fimbria, flagella, and capsule helps in the attachment of the bacteria to the host surface. They then derive iron from the host using various iron-binding proteins such as siderophores to enable survival of the pathogen within the host. Survival within the host is followed by the production of various exotoxins and enzymes such as proteases, elastases, lipases, and hemolysins to cause extensive cell and tissue destruction. Type II and III secretion systems in the bacteria enable them to evade the host immune response. Capsule, S-layer, lipopolysaccharides, and porin also enhance the pathogenic resistance mechanisms by compromising the host defense [43].

5.0 Antimicrobial resistance of *Aeromonas hydrophila*

Another important factor is the increasing incidence of multidrug resistance amongst *Aeromonas* spp worldwide [38, 45]. The emergence of multi-drug resistant bacteria such as *A. hydrophila* have made disease prevention a difficult task [9]. Reports by Ansari *et al*. [45] and Igbinosa *et al*. [15] indicated that the origins of antibiotic resistance in the environment is relevant to human health; this is because of the increasing importance of zoonotic diseases as well as the need for predicting emerging resistant pathogenic organisms. In addition to selection of antibiotic therapy in the clinical setting, antibiotic sensitivity patterns are sometimes useful as phenotypic characteristics for species identification, especially for clinical isolates.

The aeromonads have been regarded universally to exhibit resistance to the penicillins (penicillin, ampicillin, carbenecillin, and ticarcillin) for quite a long time. Most *Aeromonas* species show susceptibilities to aminoglycosides, tetracycline, chloramphenicol, trimethoprimsulfamethoxa-zole, and quinolones [15]. Several researchers [12, 15, 23, Daskalov, [23]; Igbinosa *et al*. [15]; Stratev and Odeyemi,[12] have reported resistance of *A.hydrophila* to trimethoprim (42%), pipemidic acid (67%), streptomycin (65%), cephalothin (93%), cefoxitin (56%), ticarcillin (87%), sulfamethoxazole (90%), naladixic acid (59%), ampicillin (99%), oxolinic acid (67%), and tetracycline (14%) in species isolated from different sources.

6.0 Heat Resistance of *A. hydrophila*

According to Smelt and Brul [46], Cebrian *et al*. [47] and Tersoo-Abiem *et al*. [32], depending on temperature and time of heating, heat injury in vegetative cells is multitargeted, resulting in denaturation of enzymes, injury to cell membrane, degradation of ribosomal RNA, protein unfolding, increased sensitivity of bacterial cells to compounds to which they are normally resistant, and eventually death. Earlier research had revealed low thermal resistance of *A. hydrophila*. Table 3 shows the thermal resistance of common foodborne pathogens in a variety of foods and buffers. Palumbo *et al.* [24] investigated the thermal resistance of five strains of *Aeromonas hydrophila* studied at 45 to 51°C in saline solution and raw milk. At 48°C, D-values for stationary phase cells heated in saline solution ranged from 3.49 to 6.64 min; for cells heated in raw milk, the D-values ranged from 3.20 to 6.23 min. At 48 °C, D-values for log-phase cells heated in saline solution ranged from 2.23 to 3.73 min, and z-values ranged from 5.22 to 7.69 °C. Schuman *et al*. [49] reported D-values for *Aeromonas hydrophila* at 48°C in egg suspension ranging from 3.62 to 9.43 min, and at 60 °C from 0.026 to 0.040 min; Guz and Sopinska [50] reported *Aeromonas hydrophila* D-value at 60 °C as 7 min in sterile distilled water.

Recently, in a study by Tersoo-Abiem *et al*. [48], D-values of *A. hydrophila* in soymilk ranged from 18.15 min at 50 °C (pH 7, 10% sucrose) to 0.57 min at 65 °C (pH 6.0, 10% sucrose).

Table 3: Range of D-values and z- values of different bacterial species in buffers and foods (pH range 5.5–7.0; a_w >0.98)

	Bacterial species	Temperature (^{o}C)	D-value (Minutes)	z (^{o}C)
Vegetative cells	*Aeromonas hydrophila*	60	1.14-7.0	9.5-9.7
	Campylobacter spp	60	<0.01-0.11	4.1-4.7
	Yersinia enterocolitica	60	0.07-0.8	4.0-5.8
	Salmonella typhimurium	60	0.60-1.60	4.6-5.96
	Escherichia coli	60	0.7-2.7	3.2-5.2
	Staphylococcus aureus	60	0.2-6.0	3.6-8.5
	Listeria monocytogenes	60	0.5-15	5.2-5.8

Source: [47, 48, 50, 57]

This showed an increase in the thermal resistance of the organism compared to earlier studies. Although the thermal resistance of *A. hydrophila* appears similar to that of other gram-negative bacteria associated with food, it must be noted that thermal resistance of microorganisms could be highly variable depending on the food or medium and microbial strain involved.

7.0 Control of *A. hydrophila* in foods

Various foods exhibit ecological parameters that significantly affect the behavior of their resident microflora. These include pH, moisture content or water activity (a_w), gas atmosphere composition (O_2, N_2, and CO_2), available nutrient content, antimicrobial constituents including competitive organisms, biological structure, and environmental temperature [3, 51]. Other factors include production hygiene (food contact surfaces, hygienic design, slicing equipment and utensils, bare hand contact), and cross contamination between ingredients [3].

According to Kim *et al*. [52], the generation time and lag phase of bacteria are greatly influenced by pH and temperature. Consequently, food-manufacturing processes that modify either or both the pH and storage temperature of foods are extensively used as mechanisms for preventing microbial growth in foods and to ensure food safety. These parameters mentioned above have either increased or decreased the rate of growth and strongly influence microbial survival either singly or in synergistic combination. Inadequate food processing allows survivors who are capable of repair and growth, to colonize the ecosystem again [53]. Several of these control factors can act in combination, usually at levels which would not be sufficient to control microbial growth singly [54]. When different parameters are combined in preservation, their effect becomes additive, which the target organisms fail to accommodate [22].

Numerous studies have reported a combination of hurdles to limit growth of *A. hydrophila*. Hoel *et al*. [3] studied the effect of pH, temperature and reduced water activity in brain heart infusion (BHI) medium. At 28 °C, *A. hydrophila* grew between pH 4.5 and 9.0 and 0–4% NaCl. However, the combined effect of pH 5.3, 1.5% NaCl and a temperature of 5 °C did not support the growth of *A. hydrophila*. Similarly, Chung *et al*. [54] reported the inhibitory effect of low temperature and pH, and 5% NaCl in a broth-based system. A combination of low temperature, low pH, and varied concentrations of salt and nitrite inhibited growth of *A. hydrophila* K144 [24]. Devlieghere *et al*. [26] reported significant influence of low temperature, low water activity and pH on growth of *A. hydrophila* in modified atmosphere packed cooked meat products. Park *et al*. [37] also demonstrated its inhibition at low temperature and humidity in fresh lettuce.

Based on reports by several researchers, growth of *A. hydrophila* is mainly influenced by temperature, pH and water activity. The organism is highly sensitive to adverse growth conditions, therefore it cannot vigorously grow when exposed to high temperatures or low refrigeration temperatures, acidic conditions or high water activity [3, 55, 56].

8.0 Conclusion

In conclusion, foods contaminated with food-borne pathogens such as *A. hydrophila* are of great food safety concern to consumers, manufacturers and regulatory agencies. Although the organism seems to exhibit increasing thermal resistance and resistance to antibiotics, good hygiene practices, adequate heat processing of foods in combination with other hurdles, and preservation using several hurdles can be applied to control its growth and achieve safety and stability of food products. *A. hydrophila* as a foodborne pathogen, may be captured in the National food safety policy to increase its awareness and state measures to control its incidence in processed foods. Future research can be carried out to determine the thermal resistance of *A. hydrophila* in other ready- to- eat foods. Also, there is a need to study the effect of antibiotic combination, to overcome antimicrobial resistance of the organism.

Conflict of interest

The authors declare that there is no conflict of interest.

REFERENCES

[1] O. Veys, S. Elias, I. Sampers, and E.C. Tondo, “Modelling the growth of *Salmonella* spp. and *Escherichia coli* O157 on lettuce,” Procedia *Food Science*, vol. 7, pp 168 – 172, 2019.

[2] S. D. Puthucheary, S. M. Puah, and K. H. Chua, “Molecular Characterization of Clinical Isolates of *Aeromonas* species from Malaysia,” *PLoS ONE*, vol. 7, no. 2, pp 1 – 7, 2012

[3] S. Hoel, O. Vadstein, and A, N. Jakobsen, “The Significance of Mesophilic *Aeromonas spp.* in Minimally Processed Ready-to-Eat Sea food,” *Microorganisms*, vol.7, no. 91, pp 1 – 25, 2019.

[4] G. V. Barbosa-Carnovas, M. D. Pierson, Q. H. Zhang, and D. Schaffner, “Kinetics of Microbial Inactivation for Alternative Food Processing Technology,” *Journal of Food Science Supplement*, vol. 65, no. 58, pp 16 – 40, 2011.

[5] O. A. Oyarzabal, “Emerging and Reemerging Foodborne Pathogens”. In: O. A. Oyarzabal, and S. Backert, “Microbial Food Safety: An Introduction, Food Science,” Text Series. Springer, Pp 1-5, (2012).

[6] P. Mahendra, Y. Ayele, and N. Durglishilvi, “Emerging role of *Aeromonas hydrophila* as a foodborne pathogen of public health concern,” *EC Microbiology*, vol. 16, no. 5, pp 55 – 58, 2020.

[7] Y. Yano, K. Hamano, I. Tsutsui, D. Aue-umneoy, and M. Ban, “Occurrence, molecular characterization, and antimicrobial susceptibility of A*eromonas* spp. in marine species of shrimps cultured at inland low salinity ponds,” *Food Microbiology*, vol. 47, pp 21-27, 2015

[8] P.T. Fowoyo, and F. Achimugu, “Virulence of *Aeromonas hydrophila* isolated from fresh water Catfish,” *Journal of Biosciences and Medicines*, vol. 7, pp 1-12, 2019.

[9] V.N. Mantareva, V. Kussovski, P. Orozova, L. Dimitrova, I. KuluAngelov, M. Durmus, and H. Najdenski, (2022). “Photodynamic Inactivation of Antibiotic-Resistant and Sensitive *Aeromonas hydrophila*

with peripheral Pd(II)- vs. Zn(II)-Phthalocyanines," *Biomedicines*, vol. 10, pp, 384. Doi: 10.3390/biomedicines10020384.

[10] G. Pandove, P.P. Sahota, V. Achal, and Y. Vikal, "Detection of *Aeromonas hydrophila* in water using PCR," *Journal of American Water Works Association*, vol. 103, no. 11, pp 59-65, 2011.

[11] R.F.G. Pessoa, W.F. Oliveria, M.T.S. Correla, A. Fontes, and L.C.B. Coelho, "*Aeromonas* and Human Health Disorders: Clinical Approaches," *Frontiers in Microbiology,* 13.868890. doi: 10.3389, 2022

[12] D. Stratev, and O.A. Odeyemi, "Antimicrobial resistance of *Aeromonas hydrophila* isolated from different food sources: A mini-review," *Journal of infection and public health*, vol. 9, pp 535-544, 2016.

[13] S. Mohanty, S.M. Ali, and P.K. Singh, "Necrotizing fasciitis and gas gangrene due to *Aeromonas hydrophila* in an immunocompetent host: A rare entity." *ID Cases*, 28, e01508, 2022.

[14] J. M. Tomas, "The main Aeromonads Pathogenic factors," *Microbiology*, pp 256-261. 2012.

[15] I. H. Igbinosa, E. Igumbor, F. Aghdasi, M. Tan, and A. I. Okoh, "Emerging "*Aeromonas* species infections and their significance in public health," The *scientific World Journal,* vol.62, no. 23, pp 1-13, 2012.

[16] P. Batra, P. Mathur, and M. C. Misra, "*Aeromonas* spp: An emerging Nosocomial pathogen," Journal *of Laboratory physicians*, vol.8, pp 1-4, 2016.

[17] H. M. Fadel, and M.M. El-lamine, "Vibriosis and *Aeromonas* infection in shrimp: Isolation, sequencing, and control," *International Journal of One Health*. Vol.5, pp 38-48, 2019.

[18] N. Umutoni, A.N. Jakobsen, K. Mukhatov, G.M. Thomassen, H. Kalsen, and L. Mehli, "Occurrence, diversity and temperature-dependent growth kinetics of *Aeromonas* spp. in lettuce," *International Journal of Food Microbiology*, vol. 335 (108852), pp 1-8, 2020.

[19] X. Ma, C. Wang, M. Qin, X. Tian, S. Fan, H. Zu, M. Lyu, and S. Wang, "Rapid Detection of *Aeromonas hydrophila* with a DNAzyme –based sensor," *Food Control*, vol. 123, pp 1-8, 2021.

[20] C. Dias, V. Mota, A. Martinez-Murcia, and M.J. Saavedra, "Antimicrobial resistance patterns of *Aeromonas* spp isolated from ornamental fish" Journal *of Aquaculture Research and Development,* vol. 3, pp 3, 2012.

[21] International Commission on Microbiological Specifications for Foods (ICMSF), "Microorganisms in Foods" Blackie Academic and Professional, 1996

[22] B. Ray, "Fundamental Food Microbiology," (3rd edition). CRC press pp 406 – 407, 2004

[23] H. Daskalov, "The importance of *Aeromonas hydrophila* in food safety," *Food Control*, vol. 17, pp 474-483, 2006.

[24] S.A. Palumbo, A.C. Williams, R.L. Buchanan, and J.G. Philips, "Thermal Resistance of *Aeromonas hydrophila,*" *Journal of Food Protection*, vol.50, no.9, pp761-764, 1987.

[25] S.M. Kirov, D. S. Hui, and J. L. Hayward, "Milk as a potential source of *Aeromonas* Gastrointestinal Infection," *Journal of Food Protection*, vol. 56. No.4, pp 306-312, 1993.

[26] F. Devlieghere, I. Lefevere, A. Magnin, and J. Debevere, "Growth of

Aeromonas hydrophila in modified-atmosphere-packed cooked meat products," *Food Microbiology*, vol. 17, pp 185-196, 2000.

[27] K. Neyts, G. Auys, M. Uyttendaele, J. Swings, and J. Debevere, "Incidence and identification of mesophilic *Aeromonas* spp. from retail foods," *Letters in Applied Microbiology,* vol. 31, pp 359-363, 2000.

[28] G. Wang, C. G. Clark, C. Liu, C. Pucknell, C.K. Munro, T.M. Kruk, R. Caldeira, D.L. Woodward, and F.G. Rodgers, (2003). "Detection and characterization of the hemolysin genes in *Aeromonas hydrophila* and *Aeromonas sobria* by multiplex PCR" *Journal of Clinical Microbiology*, vol. 41, pp 1048–1054, 2003.

[29] A.I. Hussain, G. Jeyasekaran, R. JeyaShakila, K.T. Raj, and E. Jeevithan, "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*, vol. 51, no.2, pp 401–407, 2014.

[30] J.V. Siergist, "Bacteremia. *Microbiology Focus*" vol. 3, no. 3, pp1-7, 2011.

[31] R.C. Cipriano, (2001). "*Aeromonas hydrophila* and motile aeromonad septicemias of fish," *Fish disease leaflet*, pp 28:1-7, 2001.

[32] E.M. Tersoo-Abiem, C.C. Ariahu, and M.A. Igyor, "Presence and Virulence Potential of *Aeromonas hydrophila* in Selected Water Sources for Household Consumption in Makurdi, Benue State. Microbiology," *Research Journal International,* vol. 31, no.3, pp 31-39, 2021.

[33] K. Krovacek, S. Dumontet, E. Eriksson, and S.B. Baloda, "Isolation and Virulence profiles of *Aeromonas hydrophila* implicated in an outbreak of food poisoning in Sweden," *Microbiology and Immunology*, vol.39, pp 655 – 661, 1995.

[34] J.H. Isonhood, P. Gerard, B. Leenanon, M. Drake, "Stress response of *Aeromonas hydrophila* to environmental challenges" *Food Microbiology* vol.19, no.4, pp 285-293, 2002.

[35] C.O. Adegoke, and S.T. Ogunbanwo, "Isolation and Characterisation of *Aeromonas* species isolated from Food and Diarrhoeagenic stool in Ibadan metropolis, Nigeria," *Food Science and Quality Management*, vol. 51, pp 20-31, 2016.

[36] M.J. Katz, N.M. Parrish, A. Belani, and M. Shah, "Recurrent *Aeromonas* Bacteremia Due to Contaminated Well Water," *Open Forum Infectious Diseases*, pp 1-3, 2015.

[37] S.H, Park, S. Choi, and S. Ha, "Predictive Modelling for the Growth of *Aeromonas hydrophila* on Lettuce as a Function of Combined Storage Temperature and Relative Humidity," *Foodborne pathogens and disease*, vol. 20, no. 20, pp 1-8, 2019.

[38] B. Lamy, S, Baron and O. Barraud, "*Aeromonas*: the multifaceted middleman in the One Health world," *Current Opinion in Microbiology*, vol. 65, pp24-32, 2022.

[39] Q. Zhang, G. Shi, G. Tang, Z. Zou, G. Yao, and G. Zeng, "A foodborne outbreak of *Aeromonas hydrophila* in a college, Xingyi city, Guizhou, China," *Western Pacific Surveillance and Response Journal*, vol.3, no.3, pp 1-4, 2012.

[40] E. Talagrand-Reboul, E. Jumas-Bilak, and B. Lamy, "The social life of *Aeromonas* through biofilm and quorum sensing systems," *Frontiers in Microbiology*, pp 8:37, 2017.

[41] A.K. Bradford, E.G. Cesar, and R.S. Bradly, "Media for the isolation of *Aeromonas hydrophila,"* *Journal of Clinical Microbiology*, pp 888-890, 1985.

[42] H.S. Bello, A. Mustapha, H.Y. Ismail, M.A. Isa, and H.K. Mangga, "Detection of *Aeromonas* species from water sources in North Eastern Nigeria," *International Journal of innovative Science, Engineering and Technology*, vol.3, no.2, pp 140-148, 2016.

[43] J.M. Janda, and S.L. Abbott, "The genus *Aeromonas*: taxonomy, pathogenicity, and infection," *Clinical Microbiology Reviews,* vol. *23,* no.1, pp 35-73, 2010.

[44] Y. Tsai, T. Huang, L. Kuo, P. Chuang, C. Hsiao, and K. Huang, "Comparison of Surgical Outcomes and Predictors in Patients with Monomicrobial Necrotizing Fasciitis and Sepsis caused by *Vibrio vulnificus*, *Aeromonas hydrophila* and *Aeromonas sobria*." *Surgical Infections*, vol. 23. Doi: 10.1089/sur.2021.337

[45] M. Ansari, E. Rahimi, and M. Raissy, "Antibiotic susceptibility and resistance of *Aeromonas* spp isolated from fish," *African Journal of Microbiology Research*, vol. 5, no. 31, pp 5772 – 5775, 2011.

[46] J.P. Smelt, and S. Brul, "Thermal Inactivation of Microorganisms" *Critical Reviews in Food Science and Nutrition,* vol. 54, no.10, pp 1371-1385, 2014

[47] G. Cebrian, S. Condon, and P. Manas, "Physiology of the Inactivation of Vegetative Bacteria by Thermal Treatments: Mode of Action, Influence of Environmental Factors and Inactivation Kinetics" *Foods,* vol. 6, no.107, pp 1-21, 2014.

[48] E.M. Tersoo-Abiem, C.C. Ariahu, and J.K. Ikya, "Thermal inactivation kinetics of *Aeromonas hydrophila* in soymilk of varying pH and sugar concentrations" *Journal of Food Processing and Preservation*, e17162 2022.

[49] J.D. Schuman, B. W. Sheldon, and P.M. Foegeding, "Thermal Resistance of *Aeromonas hydrophila* in Liquid Whole Egg," *Journal of Food Protection*, vol.6, pp 231-236, 1997.

[50] L. Guz, and A. Sopinska, "Influence of temperature on the growth, protease production, and heat resistance of *Aeromonas hydrophila* (HG-1), *A. bestiarum* (HG-2), and *A. salmonicida* (HG-3)," *Bulletin of Veterinary Institute in Pulawy*, vol. 52, pp 45-52, 2008.

[51] T.P. Labuza, B. Fu, and P.S. Taoukis, "Prediction for shelf-life and safety of minimally processed CAP/MAP chilled foods A Review," *Journal of Food Protection*, vol. 55, no. 9, pp 741-750, 1992.

[52] C. Kim, K. Wilkins, M. Bowers, C. Wynn, and E. Ndegwa, "Influence of pH and Temperature on Growth Characteristics of Leading Foodborne Pathogens in a Laboratory Medium and Selected Food Beverages," *Austin Food Sciences*, vol.3, no.1, pp 1031, 2018.

[53] T. Mcmeekin, J. Bowman, O. McQuestion, L. Mellefont, T. Ross, and M. Tamplin, "The future of predictive microbiology: Strategic research innovation applications and great expectations," *International Journal of Food Microbiology*, vol.128, pp 2-9, 2008.

[54] A.M. Gibson, N. Bratchell, and T.A. Roberts, "Predicting microbial growth: growth responses in a laboratory medium as affected by pH, sodium chloride and storage temperature," *International Journal of Food Microbiology*, vol. 6. Pp 155-178,

1988.

[55] M. Chung, B. Kim, S.Y. Park, and S. Ha, "Growth Kinetics and Predictive Model of *Aeromonas hydrophila* in a Broth-based System," *Food Science and Biotechnology*, vol. 21, no.1, pp 219-224, 2012.

[56] M. Sautour, P. Mary, N.E. Chihib, and J.P. Hornez, "The effects of temperature, water activity and pH on the growth of *Aeromonas hydrophila* and on its subsequent survival in microcosm water," *Journal of Applied Microbiology*, vol. 95, pp 807–813, 2003.

[57] M. M. Alves, C. Machado, C. Saraiva and J. A. Silva. Determination of D and z values for *Salmonella typhimurium* inoculated in an egg-based pastry, *Brazillian Journal of Food Technology*, 23, e2019122, 2020