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Review

A review on aeromoniasis in poultry: A bacterial disease of zoonotic nature

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Abstract

Aeromonas spp. are widely distributed in surface water, sewage, untreated and chlorinated, drinking water, as well as meats, fish, shellfish, poultry, and their products. A disease caused by *Aeromonas* spp. is designated as aeromoniasis. It can affect different aquatic animals, mammals, and birds in different geographic regions. Moreover, gastrointestinal and extra-intestinal disease conditions may be provoked in humans as a result of food poising with *Aeromonas* spp. Some *Aeromonas* spp. have been identified, however, *Aeromonas hydrophila (A. hydrophila*), *A. caviae*, and *A. veronii* bv sobria may be of public health significance. *Aeromonas* spp. are members of family *Aeromonadaceae* and genus *Aeromonas.* They are Gram-negative rod-shaped, facultative anaerobic, and oxidase and catalase-positive bacteria. The pathogenicity of *Aeromonas* in different hosts is mediated by several virulence factors such as endotoxins, cytotoxic enterotoxin, cytotoxins, hemolysins, adhesins, and extracellular enzymes such as proteases, amylases, lipases, ADP-ribosyltransferases, and DNases. Most avian species are susceptible to either natural or experimental infections with *Aeromonas* spp. Infection usually arises through feacal-oral route. Traveler's diarrhea as well as other systemic and local infections are the clinical picture of food poisoning associated with aeromoniasis in humans. Despite *Aeromonas* spp. being sensitive to various antimicrobials, multiple drug resistance has been commonly reported worldwide. Accordingly, this review highlights aeromoniasis in poultry regarding *Aeromonas* virulence factors epidemiology, pathogenicity, zoonosis, and antimicrobial resistance.

Key words: *Aeromonas*; health hazard; resistance; poultry; virulence.

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Introduction

Aeromonas spp. have a wide geographical distribution, being able to cause a wide spectrum of diseases in humans [1]. Aeromonads are considered as ubiquitous inhabitants of aquatic and terrestrial cold-blooded animals such as fish [2]. The clinical disease caused by *Aeromonas* spp. is referred to as aeromoniasis and is globally distributed. The disease in poultry has been reported in many parts of the world causing localized or systemic infections either alone or in combination with other infections [3,4]. *Aeromonas* spp. have the ability to adapt to different ecological sites in the host [5] and have astonishing characteristics that permit their capabilities to survive and flourish under diverse environmental conditions [6,7], thus allowing their cosmopolitan occurrence in nature. The droppings of living birds [8], poultry carcasses, and poultry plant processing water [9] are important sources of infection for the pathogenic types of *Aeromonas* spp.

Aeromonas spp. are Gram-negative, non-spore former, rod-shaped, facultative anaerobic, and oxidase, catalase, and indole-positive bacteria [10]. *Aeromonas* genus comprises more than 30 species, of which *Aeromonas hydrophila* (*A. hydrophila*), *A. caviae*, *A. media*, *A. veronii* bv. sobria, and *A. veronii* bv. veronii are of special clinical importance [11].

Aeromonads have been implicated in food-borne disease outbreaks, particularly in developing countries where hygiene is a challenge [12]. Strains of *A. hydrophila*, *A. sobria*, and *A. caviae* have been shown as emergent food-borne pathogens implicated in human gastroenteritis and extra-intestinal diseases [13]. However, the pathogenesis and virulence factors associated with aeromonads in different hosts are not fully understood [14]. *Aeromonas* spp. found in food can produce different exotoxins, some of which are enterotoxins [15].

Little information regarding aeromoniasis in poultry is available as most of the researches deal with the infection of aquatic animals and the presence of *Aeromonas* spp. in retail animals and poultry products. Based on currently available knowledge about aeromonads, this review article silhouettes at investigating aeromoniasis in poultry regarding *Aeromonas* virulence factors, epidemiology, pathogenicity, zoonosis, and antimicrobial resistance.

Etiology and virulence factors

Etiology

Aeromonas spp. are members of the family *Aeromonadaceae* and genus *Aeromonas*, Gram-negative and asporgenous short rod-shaped bacteria. They are facultative anaerobe microorganisms that can grow over a wide range of environmental conditions as pH values from 4.0 to 10.0 and salt concentrations up to 6.5% [16]. Setta [17] isolated *A. hydrophila* from cloacal samples of experimentally infected chicks for up to 16 days post-infection. The long persistence rate of *A. hydrophila* in the droppings explains its possible importance on health especially when it occurs in broilers associated with carcass contamination at processing [18]. Moreover, *A. hydrophila* could persist in chicken crates, droppings, ration, and sawdust and straw for 11, 9, 23, 22, and 17 days, respectively [18]. In addition, Kelley *et al.* [19] isolated *A. hydrophila* with other bacteria from litter during reutilization as a bedding supplement of growing broiler chickens.

The genus *Aeromonas* consists of two groups; one group is non-motile psychrophilic (*A. salmonicida*) and the other one comprising of three mesophilic motile *A. hydrophila* (*A. caviae* and *A. sobria*) [20]. Motile aeromonads are ubiquitous and autochthonous aquatic bacteria that are present in fresh, sewage, and brackish water [21] as well as in chlorinated and non-chlorinated drinking water [22,23]. The completely identified *Aeromonas* spp. are 15 types, 6 of them are of public health significance including *A. hydrophila*, *A. caviae*, *A. veronii* bv sobria, *A. veronii* bv veronii, *A. jandaei*, and *A. schubertii*. Other 9 species are environmental types including *A. salmonicida*, *A. enchelia*, *A. popoffi*, *A. media*, *A. eucrenophila*, *A. allosaccharophila*, *A. bestiarum*, *A. sobria*, and *A. trota* [24]. Based on the biochemical and physiological growth characteristics, *Aeromonas* spp. are belonging to 3 major groups; *A. caviae* group includes *A. caviae*, *A. eucrenophila*, and *A. media*; *A. hydrophila* group includes *A. hydrophila* and a motile biogroup of *A. salmonicida*; as well as *A. sobria* group includes *A. sobria* and *A. veronii*.

Most members of *Aeromonas* genus are mesophilic that can grow at an optimal growth temperature of 28 ºC, while some members can grow at temperatures ranging from 4 ºC to 42 ºC. They are non-lactose fermenter, but oxidase, catalase, indole, glucose fermenter, and nitrate reduction positive [25]. These organisms are able to grow on sheep blood agar and produce β hemolysis. According to the product information from the manufacturer, the Microbact™ 24E system (Oxoid) can identify the following species, *A. hydrophila*, *A. veronii* bv sobria, *A. veronii* bvveronii, and *A. caviae* [26].

Virulence factors

The pathology and virulence of *Aeromonas* spp. may result from stress responses and heat shock proteins [27]. Moreover, intestinal and systemic infections are mediated by numerous virulence factors [28,29] including endotoxins, cytotoxic enterotoxin (act), cytotoxins, hemolysins, adhesins, and extracellular enzymes such as proteases, amylases, lipases, ADP-ribosyltransferases, and DNases. The diversity in virulence among *Aeromonas* spp. isolates and the relationship of virulence markers reveal great variation according to the survival needs in the environment. The expression of *Aeromonas* virulence factors has been linked with gene regulation cascades associated with interactions of the pathogen with the environment [30].

Species of *Aeromonas* harbor different virulence genes such as haemolysin (*hly*A), aerolysin (*aer*A), and extracellular deoxyribonuclease (*exu*) [31]. Each of these genes has an essential role in the pathogenicity related to diarrheal diseases [32,33]. For instance, the *exu* gene codes for an extracellular DNase that blocks the antibacterial host defenses [34]. Its existence is correlated with the bacterial ability to invade, colonize, and survive in the host immune system [28]. This gene is prevalent in 96% of environmental *Aeromonas* spp. isolates worldwide [35]. Moreover, many toxin genes have been reported among *Aeromonas* spp. isolates [30,36]. In cases of intestinal infections, the heat-labile cytotonic enterotoxin (*alt*) gene has been associated with loose feces, *alt* plus heat-stable cytotonic enterotoxin (*ast*) with watery feces, while cytolytic enterotoxin (*act*) with bloody feces [37]. The *alt* gene causes excessive secretion of fluid inside the host's cell [30]. Besides, *aer*A gene is the major virulence contributor in pathogenic *Aeromonas* spp. [38]. It is a pore-forming toxin that binds to the receptors of the host's cell membrane. Following proteolytic activation, *aer*A gene causes pores that lead to the destruction of membrane permeability, osmotic lysis, and cell death [39]. It is important to note that *aerA* gene is the most prevalent in marine mammals and food sources [14]. Genes associated with *Aeromonas* virulence are not only present in clinical cases, but also they are present in different food sources such as water and fish [40]. Cytotonic enterotoxin is incriminated in the triggering of inflammatory response in host cells, disorders of the plasma membrane, and degeneration of the intestinal villi causing bloody diarrhea [33]. Biofilm formation is

another vital virulence factor and plays a potential role in the initial bacterial attachment, adhesion, and colonization of the host's surface epithelium and intestinal villi, as well as reduced susceptibility to antibiotics and recognition by the immunologic system [41,42]. The presence of biofilm-forming *Aeromonas* isolates in poultry and poultry workers was reported [9]. Igbinosa [43] assessed the presence of biofilm-forming *Aeromonas* isolates in chicken droppings and found that 42.1% (8/19), 31.6% (6/19), 10.5% (2/19), and 15.8% (3/19) were moderate, weak, non, and strong producers of biofilm, respectively. The biofilm genes may include polar flagellin (*fla*) and lateral flagellin (*laf*A).

Epidemiology

The incidence

The incidence of *Aeromonus* spp. in poultry flocks and environment of different countries (1990s-2020s) is presented in Table 1 [44-52].

Susceptibility and infection

Aeromonads infections were frequently reported in some avian spp. Aeromoniasis were represented as high mortality in chicks [17], septicaemia in turkey [53], salpingitis in ducks [54,55], epidemic deaths in Mallard ducks [56], watery diarrhea in waterfowls [27], necrotizing enteritis and septicemia in ostriches [49], conjunctivitis in pet parrots [57], haemorrhagic septicaemia in captive ground-hornbill [58], and

Table 1. The incidence of *Aeromonus* spp. in poultry flocks and environment of different countries (1990s-2020s).

Country	Source	Findings	Reference
Egypt	Broiler chickens, ducks, and turkeys	*A. hydrophila* was isolated from different ages of dead or euthanized chickens and from ducks and turkeys with percentages of 15%, 22.5%, and 20%, respectively.	[44]
	Broiler chickens	The isolation rate of *A. hydrophila* was 18% (9/50) from diarrheic cloacal samples, but 14% (7/50) from cloacal swabs of apparently healthy broilers.	[45]
	Broiler and layer chickens and broiler ducks	*Aeromonas* spp. were recovered from 9 out of 16 (15%) broiler chickens, from 1 out of 7 (14.3%) layer chickens, and from 2 out of 11 (18.2%) duck flocks suffering from diarrhea and stunted growth. Moreover, out of 14 *Aeromonas* isolates, 7 isolates were *A. caviae* (3 from liver and 4 from intestines); 2 were *A. hydrophila* (1 from liver and 1 from intestine); 3 were *A. schubertii* (1 from liver and 2 from intestines); and 2 were *A. trota* (1 from liver and 1 from intestine).	[4]
	Water surface	Seventeen isolates of *Aeromonas* spp. were isolated from Manzala Lake and identified as 11 *A. hydrophila* and 6 *A. sobria*	[46]
	Feed	*A. hydrophila* persisted in chicken's ration for 23 days	[18]
	Feed	*A. hydrophila* was found in 18 out of 37 (48.6%) in the fish meal.	[45]
	Feed and water	Seventeen *Aeromonas* spp. isolates were detected in 50 poultry flocks where 11 (22%) isolates were found in ration and 6 ones (11%) were isolated from water samples. The isolates were identified as 3 *A. caviae* and 8 *A. hydrophila* in ration as well as 4 *A. caviae* and 2 *A. hydrophila* in water.	[4]
Turkey	Broiler chickens	*Aeromonas* spp. were detected in 15 (29%) samples by the direct plating method and in 89 (17.5%) samples by enrichment method. Moreover, motile aeromonads were isolated from 48 (18.8%) of 254 diarrhoeic and from 41 (16.1%) of 254 normal chickens. Out of these isolates, 53 (59.6%), 14 (/5.7%), and 22 (24.7%) were identified as *A. hydrophila*, *A. sobria*, and *A. caviae*, respectively.	[47]
	African grey parrot	Both *Burkholderia cepacia* and *A. hydrophila* were detected in brain, lung, liver, kidney, and heart samples of birds showed neurological and respiratory signs and lesions.	[48]
California	Ostriches	*Aeromonas* spp. were identified microscopically from intestine, liver, lungs, and trachea of 10-years-old male ostrich had neurological signs, severe necrotizing enteritis, and septicemia. However, vitamin A deficiency might have predisposed the case to the *Aeromonas* infection.	[49]
Nigeria	Commercial chickens	Two thousand oro-pharyngeal swabs and samples from bone marrow, heart, liver, lung and spleen were collected from 400 apparently healthy and diseased chickens for isolation of *Aeromonas* spp. From the bone marrow, heart, and liver of the diseased chickens, a total 11 (0.5%) *A. hydrophila* isolates were identified. However, *Aeromonas* bacterium was not isolated at all from the apparently healthy chickens.	[50]
Brazil	Water surface	*Aeromonas* spp. were found in 12 of 200 (6%) drinking water samples and they were identified as *A. caviae* (41.7%), *A. hydrophila* (15.7%), *A. allosacharophila* (10.4%), *A. schubertii* (1%), and other *Aeromonas* spp. (31.2%).	[51]
Italy	Water surface	Twenty seven *Aeromonas* spp. were found in the surface water and represented as 5 *A. hydrophila* (18.5%), 5 *A. caviae* (18.5%), 4 *A. veronii* bv sobria (14.8%), *I A. salmonicida* (3.7%), 4 *A. eucrenophila* (14.8%), 1 *A. trota* (3.7%), 3 *A. media* (11.1%), 1 *A. bestiarum* (3.7%), 2 *A. sobria* (7.4%), and 1 *A. Jandaei* (3.7%)	[52]

droppings of raptors [59]. Moreover, diarrhea and weight loss were demonstrated in Japanese quails, canaries, cockatiels, and other psittacine and wild birds [60-63].

The horizontal mean of aeromonad's infection through fecal-oral route is common in birds [50]. Rosskopf and Woerpel [63] demonstrated that exposure of birds to *A. hydrophila* infection may occur via their food and transmission is primarily via oral routes and fecal shedding into the environment. This may reflect a disturbance in the intestinal ecology which has permitted the pathogen growth to high numbers [64]. Contaminated drinking water and un-hygienic contaminated feed particularly fish meal are sources of aeromonads infection in poultry. Fish and shellfish may harbor pathogenic *A. hydrophila*. For example, Farag [65] demonstrated that poorly processed fish or shellfish in poultry ration could lead to *Aeromonas* infection.

Some suitable environmental conditions such as increased humidity and temperature as well as poor hygienic conditions in hatcheries may provoke *A. hydrophila* infection via eggshell penetration [18,66]. Musgrove *et al.* [67] isolated *A. hydrophila* and other enterobacteria from the eggs shell of chickens. However, aeromoniasis is not a congenitally transferred disease, as the ovary and oviduct have no role in dissemination of *A. hydrophila* infection [18].

Pathogenicity

The pathogenicity of *Aeromonas* spp. is complex and multifactorial as it is associated with many virulence factors [68]. It has been documented that *A. hydrophila* either alone or in combination with other pathogens may cause localized and systemic infections in poultry [69]. Concomitant *Aeromonas* infections with other diseases such as salmonellosis [70] and fowl cholera [3] have been reported.

Depression, ruffled feathers, severe diarrhea, emaciation, and congested friable livers were observed 2 days post-experimental infection of Japanese quails with *A. hydrophila* [27]. Additionally, *A. hydrophila* challenge in 2 and 5-day-old chicks induced 60% to 100% mortality, gastrointestinal disturbance, and congestion of the liver, spleen, lungs, kidneys, and intestine [71]. Moreover, petechial haemorrhages on the liver, omphalitis, enteritis, and nephrosis have been reported following experimental inoculation of chicks with *A. hydrophila* [17]. Mahmoud and Tanios [72] detected a mortality rate of 52.5% following subcutaneous (S/C) injection of a dose of 3.5×10^7 of *A. hydrophila*, while the mortality was decreased to 35% following inoculation of 1.5×10^9 of *A. hydrophila*. Hatched chicks from *A. hydrophila* infected eggs showed mortalities reached 13.3% and 1.7% during 1st and 2nd week post-hatching, respectively, and the chicks exhibited omphalitis, enteritis, unabsorbed yolk sac, distended gall bladder, and congested liver and heart (18). In the study of Girh *et al.* [73], the results showed that S/C inoculation of 2-weeks-old Fayoumi chicks with *Aeromonas* spp. isolates induced mild pathogenicity with a long course of diarrhea and enteritis. The pathogenicity test for *A. hydrophila*, *A. trota*, *A. caviae*, and *A. schubertii* was reported in day-old chicks and the results revealed mortality rate of 13.3% in *A. hydrophila*, 20% in *A. trota*, 13.3% in *A. caviae*, and 6.7% in *A. schubertii* infected chicks [4]. Off-food, pasty vent, diarrhea, enteritis, unabsorbed yolk sac, distended gall bladder, generalized congestion, enlarged spleen and kidney, congested lungs, and air sac turbidity were also seen. Besides, *A. schubertii* infection induced more marked adverse effects on body weight than that of *A. caviae*, *A. trota*, and *A. hydrophila*.

Zonoosis

The risk of food-borne disease, due to *Aeromonas* infection has been increased due to the ability of the organism to grow at low temperatures and produce toxins [74]. Many predisposing factors are involved in human's aeomoniasis including ingestion of contaminated food and water [75], presence of other diseases conditions as diabetes [76], and immunosuppression and age of humans [77]. Depending on *Aeromonas* virulence and antibiotic resistance profiles, numerous spp. have been reported as important zoonotic pathogens [22]. Chickens carcasses, heart, and liver could be potential sources for the spread of *Aeromonas* infection and they present a possible threat to public health [78].

Aeromonads are identified as causative agents of diarrhea with a public health hazard importance [79]. Infants and the elderly are more severely affected by *Aeromonas*-diarrheal conditions than other ages [80]. In some areas, aeromonads have been regarded as emerging food-borne pathogens involved in human's gastroenteritis, ranging from mild diarrheal to cholera-like sickness [36,81]. Moreover, they are involved in human's extra-intestinal infections [82-84]. For example, *Aeromona*s spp. infections were reported to cause severe meningitis, cellulites, otitis, septicemia, endocarditis, osteomyelitis, peritonitis, bacteremia, septicaemia, and respiratory tract disease in humans [9,85]. Besides, the organisms were implicated as the

cause of traveler's diarrhea in 18 (2%) out of 863 patients [86].

Aquatic environment as well as different food including fish, seafood, and raw and cooked meat and chickens can be a potential vehicle for human's infections with aeromonads [15,87-91]. Examined 563 samples of fish, raw and cooked meat, and pre-prepared salads revealed the presence of mesophilic *Aeromonas* spp. in 287 samples as most of contaminated samples were offals (84.3%) and chickens (79.3%) [92]. *Aeromonas salmonicida* was isolated from paddlefish [93]. Moreover, *Aeromonas* spp. were isolated from frozen fish intended for human consumption in Mexico City [94]. Therefore, the presence of *Aeromonas* organisms in the raw meat samples can represent a serious potential risk for public health. In India, out of 154 food samples represented chickens, fish, and ready-to-eat sprouts, 22 (14.28%) isolates were *Aeromonas* spp. and the highest percentage of isolation was from chickens (28.6%), followed by fish (20%), and sprout (2.5%) [95]. Moreover, 53 (57.6%) and 27 (17%) of aeromonads isolates were characterized in 92 chickens and 158 minced meat samples, respectively. The isolation rate of aeromonads was significantly higher in chicken than in minced meat samples [96]. Mailafia *et al.* [97], in Nigeria, reported on isolation of *A. hydrophila* from diarrheic patients in a rate of 6.8%.

Antimicrobial resistance

Multiple drug resistance of *Aeromonas* spp. strains have been commonly reported worldwide due to frequent administration of antibiotics besides the classical resistance to β lactam group [98]. Considering the risk to human health, the incidence of antimicrobial resistance is alarming especially among *A. hydrophila*, *A. caviae*, and *A. sobria*, which are responsible for infections in both animals and humans [99]. Antibiotic resistance occurs either by carrying intrinsic genes or by acquiring resistance markers from other pathogens [15,74]. Aeromonads could possess integron that enables bacteria to acquire and transfer antibiotic resistance genes, giving rise to the risk of resistant bacterial infections [9,100]. Aeromonads were initially shown as susceptible to tetracycline, chloramphenicol, cephalosporins, aminoglycosides, and quinolones [22]. However, chromosomal inducible β lactamase class C has been detected as a major mechanism of *Aeromonas* spp. resistance to cephalosporins and cefoxitin [101]. Moreover, Sinha [102] found high levels of intrinsic resistance to antimicrobials among *Aeromonas* isolates due to *gyr* gene of chromosomal origin and *qnr* gene of plasmid origin. It has been reported that *Aeromonas* spp. revealed resistance to quinolones in domestic and free-living animals, hospital effluents, and wastewater [103-105]. Aeromonads may also become a reservoir of gene encoding resistance to some antimicrobials such as tetracycline. Stratev and Odeyemi [106] reported on the spread of tetracycline-resistant plasmids between *A. hydrophila* and *Escherichia coli* and between human and aquaculture in different locations. Besides, pathogenic *Aeromonas* spp. are capable of transferring the genes responsible for antimicrobial resistance to other pathogenic organisms in humans and throughout the food chain which is a risk to human and animal health [31]. Biofilm formation also enhances aeromonads to be more resistant to antimicrobial agents and host defenses. Bacteria may express more virulent phenotypes as a result of gene activation via bacterial communication (quorum sensing) or gene transfer [41]. It has been reported that *A. hydrophila* is the most resistant spp. to antimicrobials followed by *A. caviae*, *A. trota*, and then *A. schubertii* [4]. Moreover, *A. hydrophila* isolates of fish and poultry origins are more resistant to antibiotics than those of water origin [107].

Low resistance of *Aeromonas* isolates has been observed against tetracycline in different geographical regions [9,43,72,108]. The susceptibility of *Aeromonas* isolates to gentamycin has been also reported in samples collected from infected chickens [4,43], fresh and frozen chickens [108], and minced meat and chickens [109]. *Aeromonas* spp. showed variable resistance to cephalosporins. Igbinosa [43] found that cephalosporins (cefotaxime) were very potent against *Aeromonas* isolates when compared with (cephalothin) which showed an average effect. The resistance to the first generation of cephalosporins is expected due to the β lactamase activity of aeromonads and the expanded effect of metallo-β-lactamases [110]. Many studies showed resistance of *Aeromonas* isolates to penicillin [9,43,111-113], which may be attributed to the presence of intrinsic or chromosomally mediated resistance genes [4,114]. Antibiotics including aztreonam, cefotaxime, chloramphenicol, nalidixic acid, nitrofurantoin, and tobramycin showed excellent activity against all *Aeromonas* isolates [9,43,108]. Excellent sensitivity to chloramphenicol [99,112], nitrofurantoin, and tetracycline [108,115-118] have been also reported among *Aeromonas* isolates. Despite trimethoprim-sulfamethoxazole revealed good efficacy against *Aeromonas* isolates in many studies [9,43,113], it showed poor activity in another study [115].

Conclusions

Aeromonas spp. are frequently discovered in food, animals, and birds. The potential risk of *Aeromonas* gastro-enteric human's infections and the dissemination of the pathogen to animals, poultry, or humans with close contact and the wider community have been proven. Therefore, periodical regular screening of poultry flocks in different geographical locations is essential. Moreover, public health awareness and enlightenment of the hazard associated with *Aeromonas* infection is necessary.

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