



Prevalence and Pathogenicity of *Aeromonas* Species in Poultry

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Abstract

In this study 60 broiler chicken, 7 layer and 11 duck farms of different ages suffering from diarrhea and stunted growth were investigated for *Aeromonas* species, 9 broiler chicken farms yielded 14 *Aeromonas* isolates (7 *A. caviae*, 2 *A. hydrophila*, 3 *A. schubertii* and 2 *A. trota*), 1 layer farm yielded only one *A. trota* isolate and 2 duck farms produced 2 *A. hydrophila* isolates. Bacteriological examination of ration and water samples of 50 poultry farms revealed *A. caviae* (3 isolates), *A. hydrophila* (8 isolates) from ration and *A. caviae* (4 isolates), *A. hydrophila* (2 isolates) from water. The previous *Aeromonas* isolates from poultry farms were compared with standard *A. hydrophila* and *A. hydrophila*, *A. schubertii* of fish source when sensitivity, PCR identification and RFLP. Sensitivity test of poultry *A. hydrophila* was similar to that of standard but differed from that of the fish isolates, *A. hydrophila* isolates were more resistant to antibiotics than the other *Aeromonas* species followed by *A. caviae*, *A. trota* then *A. schubertii*. Also *A. hydrophila* isolates from fish and poultry were more resistant to antibiotics than those from water. Electrophoretic analysis of the PCR product (using specific primer 16S rRNA gene) revealed the specific amplification of 599 bp fragment for all selected *Aeromonas* isolates (identified poultry *Aeromonas*, standard and fish isolates). RFLP of 16S rRNA gene using Mbo1 and Alu1 restriction enzymes resulted in similarity among poultry, standard and fish isolates. 16S rRNA gene of *A. hydrophila*, *A. caviae* and *A. schubertii* was digested with Mbo1 only, while that of *A. trota* was digested with both Mbo1 and Alu1. Pathogenicity test for *A. hydrophila*, *A. trota*, *A. caviae* and *A. schubertii* were applied, mortality was 13.3% in *A. hydrophila*, 20% in *A. trota*, 13.3% in *A. caviae* and 6.7% in *A. schubertii* infected groups. *A. schubertii* infection induced marked effect on body weight than *A. caviae*, *A. trota* and *A. hydrophila*. Small intestine, liver, lung, spleen and kidney were collected from fresh ailing sacrificed infected chicks for histopathological examination.

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Introduction

Aeromonas species are widely distributed in the aquatic environment, including raw and processed drinking water [1].

Some *Aeromonas* species are pathogenic for fish and several cold-blooded animals (amphibians and reptiles) [2], among them, *Aeromonas hydrophila* has been widely studied, being responsible for a variety of fish pathological conditions, called "aeromonosis" [3].

Over the last few years, the interest in *Aeromonas* spp. has gone beyond the boundaries of fish pathology; this is due to aeromonads can play a secondary and a primary pathogen as well [4,5]. Motile aeromonads are considered to be emerging food-borne pathogens and it has been shown that some *Aeromonas* food isolates can produce different virulence factors, not only at optimal growth temperature, but also at refrigeration temperatures [6] and the increase of cases of human gastroenteritis, particularly in children younger than two years, the elderly and immunocompromised patients or in patients with chronic and weakening diseases [4].

Regarding mammals, the following clinical pictures have been sporadically observed: sepsis in dogs [7]; pneumonia and dermatitis in dolphins [8]; septicemia in seals [9] and rabbits [10]; abortion [11] and vesiculitis in cattle [12] and diarrhoea in piglets [13].

The studies related to the motile aeromonads of poultry are so limited. Isolation of motile aeromonads from the feces of turkey, pet and aviary birds has been reported in few occasions [14,15].

Recently, *A. hydrophila* has been isolated from an outbreak of diarrhoea in a flock and this agent has been implicated as a cause of enteritis in poultry [16].

The present work was aimed to study the prevalence of *Aeromonas* spp. in domestic birds, early diagnosis of *Aeromonas* spp. by using PCR, Examination of polymorphism and detection of genomic variation among the different isolated *Aeromonas* strains from birds, water and rations in comparison with standard and fish isolates, Application of sensitivity test for detection of drug of choice and the pathogenicity of isolated *Aeromonas* experimentally.

Material and methods

Field samples

Livers, intestines of 60 broiler chicken flocks and cloacal swabs of 7 layer and 11 duck flocks at Alexandria and El-Behera governorates were aseptically collected from ailing birds showing mortality and diarrhea. Also 25 grams ration and 10 ml water of 50 different poultry farms were collected.

Bacteriological examination

The collected samples were inoculated into Tryptic soya broth and incubated at 37°C for 24 hours then streaked onto the following media, nutrient agar, *Aeromonas* agar medium and RS (Rimler Shotts) medium.

All inoculated media were incubated at 37°C for 24-48 hours and the isolates were identified according to Carnahan et al., [17] and Abbott et al., [18].

PCR identification using 16S rRNA gene (Graf, 1999)[19].

Primer set No. Product size

Forward primer 5`-TCATGGCTCAGATTGAACGCT-3`

Reverse primer 5`-CGGGGCTTTCACATCTAACTTATC-3` 599 bp

Endonuclease restriction enzymes for RFLP (MboI & AluI)

In vitro susceptibility of isolated *Aeromonas* to various chemotherapeutic agents. The disc diffusion technique was adapted according to Finegold and Martin [20].

Experimental design

One hundred and sixty (160) one day old chicks, Avian breed were obtained from Fat hens Company. Five chicks were sacrificed and subjected to bacteriological examinations then divided into 5 groups to study the pathogenicity of isolated *Aeromonas* species and their effect on body weight, feed intake and feed conversion ratio. The chicks were weighed and feed intake was calculated weekly. The chickens were kept under observation for clinical signs and mortality till the end of experiment. Group 1: 30 birds were infected intra croup with 0.1 ml of *A. hydrophila* (1.5×10^9 cfu) at 5th day of age. Group 2: 30 birds were infected intra croup with 0.1 ml of *A. trota* (1.5×10^9 cfu) at 5th day of age. Group 3: 30 birds were infected intra croup with 0.1 ml of *A. caviae* (1.5×10^9 cfu) at 5th day of age. Group 4: 30 birds were infected intra croup with 0.1 ml of *A. schubertii* (1.5×10^9 cfu) at 5th day of age. Group 5: 40 birds non infected (control).

Three birds from each group were sacrificed at 2, 7, 14, 21st day post infection and subjected to post mortem examination. Liver, lung, intestine, spleen and kidney samples were collected for histopathological examination.

Histopathological examination

Autopsy samples were taken from the liver, kidney, lung, spleen and small intestine of birds in different groups and fixed in 10% formol saline for twenty-four hours. Washing was done in tap water then serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene and embedded in paraffin at 56 degree in hot air oven for 24 hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by hematoxylin & eosin stain for routine examination through the light electric microscope [21].

Results and discussion

Aeromonas species were considered as aquatic bacteria affecting fish, reptiles and -other species including poultry. Fifteen *Aeromonas* species were considered in the most recent classification, six of them have the public health importance including *A. hydrophila*, *A. caviae*, *A. veronii bv sobria*, *A. veronii bv veronii*, *A. jandaei* and *A. schubertii*. Other nine species are environmental including *A. salmonicida*, *A. encheilia*, *A. popoffi*, *A. media*, *A. eucrenophila*, *A. allosaccharophila*, *A. bestiarum*, *A. sobria* and *A. trota* [22]. In Egypt, during the last few years, only *Aeromonas hydrophila* was identified in poultry, so in this study *Aeromonas* species were isolated from natural clinical cases of poultry and identified by biochemical tests and PCR application. Also Restriction Fragment Length Polymorphism (RFLP) of 16S rDNA was applied to detect genomic variation among different *Aeromonas* strains of poultry when compared with standard and fish strains.

Sixty broiler chicken, 7 layer and 11 duck flocks of different ages suffered from diarrhea and stunted growth, were subjected to bacteriological isolation trials and *Aeromonas* species were recovered from 9 broiler chicken (15%), from 1 layer (14.3%) and from 2 duck flocks (18.2%) (Table 1). These results agreed with those reported by, Jindal et al., [23], who isolated *Aeromonas* species from 2 of 10 poultry faeces (20%), while Akan and Dikar [24], found *Aeromonas* species in 48 of 254 diarrhoeic chickens (18.8%) and Amal [25], in Upper Egypt isolated *A. hydrophila* from different ages of dead or sacrificed chickens, from ducks and turkeys with percentages of 15, 22.5 and 20% respectively. Mohamed FM and Mohamed MA [26], isolated *A. hydrophila* from 9 of 50 diarrhoeic broiler chickens (18%).

From 50 poultry farms, *Aeromonas* species (17 isolates) isolated from 11 ration (22%) and 6 water samples (11%) (Table 2) and identified as *A. caviae* (3 isolates), *A. hydrophila* (8 isolates) in ration and *A. caviae* (4 isolates), *A. hydrophila* (2 isolates) in water (Table 3). These results more or less agreed with World Health Organization (WHO) [27], that reported the isolation ratio of *A. hydrophila* from drinking water to be 1-27% and the infectious dose to be >1010 cfu, Jurin et al., [28], isolated *A. hydrophila* from 4 of 35 water samples (11.4%), Razzolini et al., [29] detected *Aeromonas* species in 12 of 200 drinking water samples (6%) and identified as *A. caviae* (41.7%), *A. hydrophila* (15.7%), *A. allosacharophila* (10.4%), *A. schubertii* (1%) and *A. spp.* (31.2%), Awaad et al., [30] showed that *A. hydrophila* persisted in chicken ration for 23 days, Donatella et al., [31] identified 27 *Aeromonas* species from surface water, 5 *A. hydrophila* (18.5%), 5 *A. caviae* (18.5%), 4 *A. veronii bv sobria* (14.8%), 1 *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%), Zaky et al., [32] isolated *Aeromonas* species (17 isolates) from different sites of Manzala lake and identified as *A. hydrophila* (11 isolates) and *A. sobria* (6 isolates) and also our result was disagreed with Mohamed FM and Mohamed MA [26] who isolated *A. hydrophila* from 18 of 37 fish meal (48.6%). Although the prevalence of *Aeromonas* species in poultry ration was found to be low, it was very high in fish meal. These data suggested that, motile aeromonads which originated from raw fish in fish meal will be reduced during feed processing.

On the other hand, from a total of 14 *Aeromonas* isolates, 7 *A. caviae* isolated from livers (3) and intestines (4), 2 *A. hydrophila* isolated from liver (1) and intestine (1), 3 *A. schubertii* isolated from liver (1) and intestines (2) and 2 *A. trota* isolated from liver (1) and intestine (1) (Table 4). These findings were in agreement with Ahmet Akkoc et al., [33] who found *A. hydrophila* in brain, lung, liver, kidney, and heart samples, França et al., [34] recovered *Aeromonas* species in pure culture from intestine, liver, lungs and trachea and Dashe et al., [35] isolated 11 *A. hydrophila* from livers (6), hearts (4) and bone marrow (1).

A polymerase chain reaction was standardized for the identification of biochemically identified *Aeromonas* isolates using specific primer 16S rRNA gene. Electrophoretic analysis of the PCR product revealed the specific amplification of 599 bp fragment for all selected *Aeromonas* isolates (Figure 1a,b). These findings are in confirmation with the results obtained by Gonzalez-Rodriguez et al., [36] who recorded 102 and 104 cell ml⁻¹ as the minimum detection level for 16S rRNA and aerolysin genes respectively. Porteen et al., [37] also identified *Aeromonas* species at 599 bp and 252 bp using 16S rRNA and aerolysin genes respectively.

Determination of *Aeromonas* spp., on the basis of phylogenetic relationships by RFLP of the PCR-amplified 16S rRNA

genes, carried out for twenty selected isolates. RFLP pattern of 200 and 300 bp was identical for standard, fish, ration, water and broiler *A. hydrophila* isolates, where 270 and 330 bp was identical for water and broiler *A. caviae* while 140 and 460 bp was identical for fish and broiler *A. schubertii* isolates when digested with Mbol but not digested with Alul (Figure 2a,b). On the other hand, *A. trota* of layer source has RFLP pattern of 180 and 390 bp with Mbol and while with Alul gave 70, 190 and 330 bp (Figure 2c). These results are in accordance with those of Martinez-Murcia et al., [38] who recorded that *Aeromonas* species exhibited very high levels of overall 16S rDNA sequence similarity to each other (ca 98-100%), RFLP method makes the identification of *Aeromonas* spp. possible, rapid, and reliable without the need for sequencing. Borrell et al., [39] described a recent protocol based on the RFLP patterns of the complete PCR-amplified 16S rDNA gene that enabled identification of most (10 species) *Aeromonas* spp. by using two endonucleases (Alul and Mbol) simultaneously, Figueras et al., [40] completed the previous protocol of Borrell et al., [39] to differentiate *A. salmonicida*, *A. bestiarum* and *A. popoffii* using endonucleases AlwNI and PstI. Also Lee et al., [41] reported the identification of *Aeromonas* species by sequence analysis corresponded to the identification by PCR-RFLP analysis.

In our work, fewer bands obtained because of using only one restriction endonuclease for each experiment rather than a combination of two enzymes. These results were supported by Ghatak et al., [42] who showed 3 bands for *A. caviae* with Mbol (834, 402 and 276 bp) and 2 bands for *A. hydrophila* with BstSNI (462 and 1104 bp) (Figure 3a,b,c,d,e).

Difference between our 16S rDNA RFLP patterns and those previously described indicated the restriction sites in known species are affected by intraspecies nucleotide diversity, i.e. differences between strains of the same species. These results were supported by Borrell et al., [39] who obtained a common pattern for the 9 new isolates of *A. popoffii* which differs from those previously reported because of either the digested sequence belongs to a new *Aeromonas* species or the restriction sites in known species are affected by intraspecies nucleotide diversity.

There were no digestion results with Alul for *A. hydrophila*, *A. caviae* and *A. schubertii*. This result in agreement with Figueras et al., [40] who reported further computer simulation on the 16S rDNA sequences of the type strains were carried out to confirm the endonuclease Alul produced species-specific patterns only for *A. sobria*, *A. jandaei*, *A. schubertii* and *A. veronii* (although the latter had a pattern identical to that of *Aeromonas* Group 501). On the contrary, Graf [19] indicated that the use of a single enzyme, Alul, can separate the species *A. veronii*, *A. caviae* and *A. hydrophila*. The main problem of Graf's method was that the enzymes were selected arbitrarily and not on the basis of a previous computerized analysis of the 16S rDNA gene sequences of the type strains of all species as described by Figueras et al., [40] (Figure 4a,b,c).

In our investigation, antibiotic sensitivity test is important to select the best drug of choice required to produce a therapeutic effect. The obtained results recorded in Table (5,6 and 7) revealed that, *A. hydrophila* standard strain was highly sensitive for gentamicin, norfloxacin, nitrofurantoin, amikacin and cefotaxime, while fish strain was highly sensitive for nitrofurantoin chloramphenicol, sulphamethoxazol & trimethoprim and Ampicillin-Sulbactam and poultry strains were highly sensitive for gentamicin and amikacin and sensitive to Ampicillin-

Sulbictam, norfloxacin and colistin-sulphate. *A. schubertii* fish strain was highly sensitive for gentamicin, chloramphenicol, norfloxacin, cephalothin and amikacin, while poultry strains were highly sensitive for gentamicin and amikacin and sensitive to Ampicillin-Sulbictam, norfloxacin, ciprofloxacin, nitrofurantoin and colistin-sulphate. Most isolated *A. caviae* strains were highly sensitive for gentamicin and amikacin and sensitive to Ampicillin-Sulbictam, norfloxacin, chloramphenicol, ciprofloxacin, nitrofurantoin and colistin-sulphate. Isolated *A. trota* strain was highly sensitive for gentamicin, cefotaxime and amikacin and sensitive to norfloxacin, nitrofurantoin and colistin-sulphate. These results matched with those of Kampfer et al., [43] who recorded that the most *A. hydrophila* isolates were highly susceptible to quinolones as ciprofloxacin and chloramphenicol. Amal [25] reported that gentamicin was the most effective drug (100%) while neomycin was moderately sensitive (80%). Awan et al., [44] recorded Most *Aeromonas* strains were resistant to penicillins, sulfamethoxazole, trimethoprim and macrolides but sensitive to tetracycline, chloramphenicol, nitrofurantoin, aminoglycosides, cephalosporins, quinolone, colistin sulphate and SXT (trimethoprim-sulfamethoxazole). Alam et al., [45] showed the drug sensitivity pattern of 100 *Aeromonas* isolates from poultry sources, none of the 100 isolates was found to be neither resistant to norfloxacin, nalidixic acid and gentamicin nor sensitive to erythromycin, neomycin, penicillin and ampicillin (Figure 5a,b).

A. hydrophila isolates are more resistant to antibiotics than the other *Aeromonas* species followed by *A. caviae*, *A. trota* then *A. schubertii* and *A. hydrophila* isolates from fish and poultry are also more resistant to antibiotics than those from water. Similar investigations reported by Kudinha et al., [46].

Finally, all isolates of *Aeromonas* examined in this study showed multiple resistances to at least 6-8 antibiotics. Similar findings on multiple drug resistance of *Aeromonas* strains have been reported from different parts of the world [47].

Experimental infection with the isolated *A. hydrophila*, *A. trota*, *A. caviae* and *A. schubertii* showed mortality of 13.3%, 20%, 13.3% and 6.7% respectively. Similar results were obtained by El-Khashab [48] who reported that the mortality rate ranged from 60 to 100% in experimentally infected 2 and 5 days old chicks with *A. hydrophila* organism via yolk sac, intramuscular, subcutaneous or oral inoculations, Mahmoud and Tanios [49] found that the mortality rate was relatively high (52.5%) after subcutaneous injection of a high dose (3.5×10^7) of the organism while it was 35% in the low dose 1.5×10^9 , While Awaad et al., [30] reported that hatched chicks from *A. hydrophila* infected eggs showed mortalities reached 13.3% and 1.7% during 1st and 2nd week post hatching respectively (Figure 6a,b).

Infected chicks of all groups showed depression, reluctant to move, ruffled feathers, inappetance, pasty vent and diarrhea. At necropsy, sacrificed chicks of all group revealed enteritis, unabsorbed yolk sac, distended gall bladder, generalized congestion, enlarged spleen & kidney, congested lungs and air sac turbidity. These results are in agreement with El-Khashab [48] who observed that infected chicks showed transitory period of depression characterized by ruffled feathers, pasty vent and generalized congestion of liver, spleen, lung, kidney and intestine (especially duodenum) with severe haemorrhagic enteritis, Ahmed [50] found the most predominant lesions findings were generalized venous congestion, petechial haemorrhages on the liver, omphalitis, enteritis and nephrosis. Mahmoud and Tanios [49] said that infected chickens showed depression, ruffled

feathers, congestion in most of internal organs, few cases of hepatic petechiae and severely congested and unabsorbed yolk sac. Awaad et al., [30] mentioned that survived infected chicks exhibited omphalitis, ruffled feathers, general weakness, inappetance, enteritis, unabsorbed yolk sac, distended gall bladder and congestion of liver and heart (Figure 7a,b).

Aeromonas species were re-isolated from most organs examined, these results matched with those of Awaad et al., [30] who recorded that the rate of *A. hydrophila* re-isolation from experimentally infected chicks was 95.6%, 26%, 8.7%, 4.4%, 2.2% and 4.3% from intestine, liver, heart, spleen, kidney and lung respectively. Zeinab et al., [51] re-isolated *A. hydrophila* from hatched chicks of experimentally infected eggs with a percentage of 44.4% and 66.6% from liver and yolk respectively, also re-isolated *A. caviae* from 50% and 62.6% of liver and yolk respectively.

Cloacal shedding of *A. hydrophila* and *A. trota* in experimentally infected chicks was persistent for up to 7 days post infection and 5 days with *A. caviae* and *A. schubertii*. Similarly, Ahmed [50] isolated *A. hydrophila* from the cloacal swabs of experimentally infected chicks for up to 16 days post infection.

On the other hand, the mean body weight showed non significant decrease till the end of experiment in group 1 (*A. hydrophila* infected chickens) and a significant decrease from 3rd to 5th week post infection in group 2 (*A. trota* infected chickens), group 3 (*A. caviae* infected chickens) and group 4 (*A. schubertii* infected chickens) as compared to the non infected control. Results showed that *A. schubertii* infection induced marked effect on body weight than *A. caviae*, *A. trota* and *A. hydrophila*. Similar results were recorded by Kutkat et al., [52] who observed retardation of growth in chicks infected with *A. hydrophila*. Ahmed [50] detected weight gain loss in *A. hydrophila* experimentally infected chicks when compared with control birds. In addition, Awaad et al., [30] revealed that the hatched chicks showed numerical difference in their weights between chicks taken from *A. hydrophila* infected eggs and these from non infected ones reached to 5, 30 and 103 grams at 1st, 2nd and 3rd week of age respectively (Figure 8a,b).

In this study, it was clear that *Aeromonas* species infection in chicks induced variable pathological lesions in different organs (intestine, liver, lung, kidney and spleen) at different ages post infection. These pathological lesions include small intestine of both *A. hydrophila* & *A. schubertii* infected groups showed oedema with inflammatory cells infiltration in lamina propria, while that of both *A. trota* & *A. caviae* infected groups showing focal desquamation associated with inflammatory cells infiltration and oedema in the lamina propria. Also there was fibrosis in lamina propria of *A. trota* infected group. Liver of *Aeromonas* species infected groups showed dilatation and congestion in portal vein, hepatic sinusoid and bile duct with inflammatory cells infiltration in the portal area and hepatic parenchyma, also *A. hydrophila* infected group showed focal haemorrhage and eosinophilic cells aggregation in the hepatic parenchyma. Lung of *Aeromonas* species infected groups showed congestion in stromal blood vessels with perivascular oedema and haemorrhage, also *A. hydrophila* infected group showed bronchiolar mucosal ulceration with inflammatory cells infiltration in the underlying tissue while bronchioles of *A. trota* and *A. schubertii* infected groups showed mucosal epithelial hypertrophy with peribronchiolar oedema and inflammatory cells infiltration. Kidney of *Aeromonas* species infected groups showed haemorrhage and inflammatory cells aggregation in between the degenerated tubules, also *A. caviae* infected group showed glomerular hy-

peritrophy. Finally spleen showed congestion in the red pulps with focal lymphoid proliferation and nodules formation in the white one in both *A. hydrophila* & *A. trota* infected groups, focal haemorrhage in the splenic parenchyma in both *A. hydrophila* & *A. schubertii* infected groups, lymphoid proliferation with nodules formation in the white pulps in *A. trota*, *A. caviae* & *A. schubertii* infected groups and focal necrosis in the parenchyma in both *A. caviae* & *A. schubertii* infected groups. These findings matched with those of Mahmoud and Tanios [49] who observed congestion of portal and sinusoidal blood vessels with focal area of hepatocellular necrosis and diffuse necrosis in the white pulp of the spleen, Awaad et al., [30] recorded haemorrhages in the intestinal villi, pulmonary oedema, alveolar congestion, subepithelial haemorrhage in the bronchioles, oedema with inflammatory cells aggregation in the cardiac muscles and hepatic coagulative necrosis and Zeinab et al., [51] histopathologically showed severe congestion of hepatic blood vessels and sinusoids with focal coagulative necrosis and hyperplasia of intestinal epithelium with slight congestion of blood vessels.

Table 1: Incidence of *Aeromonas* infection in poultry flocks.

poultry flocks	Total number	Positive for <i>Aeromonas</i>	Percent	Source of positive samples
Broilers	60	9	15%	liver & intestine
Layers	7	1	14.30%	Cloacal swabs
Ducks	11	2	18.20%	Cloacal swabs

Table 2: Incidence of *Aeromonas* infection in ration and water of poultry farms.

Samples	Total number	Positive for <i>Aeromonas</i>	Percent	Source of positive samples
Ration	50	11	22%	Feeders
Water	50	6	12%	Waterers

Table 3: Prevalence rate of *Aeromonas* isolates in ration and water of poultry farms.

<i>Aeromonas</i> isolates	Number of positive isolates				Total
	Ration		Water		
	No.	%	No.	%	
<i>A. caviae</i>	3	42.9	4	57.1	7
<i>A. hydrophila</i>	8	80	2	20	10
Total	11		6		17

Table 4: Prevalence rate of *Aeromonas* species recovered from different organs.

<i>Organs Bacteria</i>	Liver		Intestine		Total
	No.	%	No.	%	
<i>A. caviae</i>	3	42.9	4	57.1	7
<i>A. schubertii</i>	1	33.3	2	66.6	3
<i>A. hydrophila</i>	1	50	1	50	2
<i>A. trota</i>	1	50	1	50	2
Total	6		8		14

Table 5: Results of antibiotic sensitivity test of isolated *Aeromonas* species, standard and fish strain.

Antibiotic discs	Disc content	Inhibitory zone diameter (mm) R ≤ S ≥	Measured inhibitory zone of micro-organism - mm									
			<i>A. hydrophila</i> Standard	<i>A. hydrophila</i> Fish	<i>A. schubertii</i> Fish	<i>A. hydrophila</i> Ration	<i>A. hydrophila</i> Duck	<i>A. caviae</i> Water	<i>A. hydrophila</i> Water	<i>A. hydrophila</i> Ration	<i>A. hydrophila</i> Ration	<i>A. hydrophila</i> Broiler
Ampicillin (Oxoid)	10 µg	11-15	-	-	-	-	-	-	-	-	-	-
Chloramphenicol (Oxoid)	30 µg	12-18	22	25	26	10	8	18	8	8	20	8
Gentamicin (Oxoid)	10 µg	6-10	20	-	20	20	20	20	20	22	18	20
Colistin sulphate (El-Nasr)	10 µg	8-11	16	-	11	15	10	10	-	13	-	17
Nalidixic acid (Oxoid)	30 µg	13 - 19	-	-	-	15	18	25	7	10	20	-
Amoxy-Clavulanic (Oxoid)	30 µg (20 + 10)	13 - 18	8	-	16	-	-	15	8	-	-	-
Norfloxacin (Oxoid)	10 µg	12-16	25	15	25	22	20	30	8	25	20	8
Tetracycline (Oxoid)	30 µg	14 -19	-	-	-	-	-	-	-	-	-	-
Sulphamethoxazole & Trimethoprim (Oxoid)	25 µg (23.75 +1.25)	10-16	-	25	20	-	-	20	-	15	20	-
Ciprofloxacin (Oxoid)	5 µg	15-21	25	17	25	15	20	30	13	15	22	17
Cephalothin	30 µg	14-18	12	-	25	-	12	20	15	-	13	-
Nitrofurantoin	300 µg	14 -17	25	25	20	12	15	22	17	12	15	15
Amikacin	30 µg	14-17	23	15	25	23	23	27	24	22	18	27

Cefotaxime	30 µg	14-23	30	15	28	11	9	18	10	10	8	20
Ampicillin-Sulbictam	20 µg (10+10)	11-14	16	23	15	-	13	20	13	15	10	15
Ampicillin (Oxoid)	10 µg	11-15	-	-	-	-	-	-	-	-	-	-
Chloramphenicol (Oxoid)	30 µg	12-18	-	6	21	10	-	20	15	18	10	17
Gentamicin (Oxoid)	10 µg	6-10	20	20	22	25	-	18	20	25	20	20
Colistin sulphate (El-Nasr)	10 µg	8-11	15	15	17	10	16	14	15	13	15	12
Nalidixic acid (Oxoid)	30 µg	13-19	-	-	15	-	-	27	10	10	-	20
Amoxy-Clavulanic (Oxoid)	30 µg (20 + 10)	13-18	-	-	-	-	-	-	-	23	-	10
Norfloxacin (Oxoid)	10 µg	12-16	22	8	25	-	20	20	17	20	-	25
Tetracycline (Oxoid)	30 µg	14-19	-	-	-	-	-	-	-	-	-	-
Sulphamethoxazole & Trimethoprim (Oxoid)	25 µg (23.75 +1.25)	10-16	-	7	-	-	-	23	-	15	-	-
Ciprofloxacin (Oxoid)	5 µg	15-21	17	18	20	20	20	32	20	25	12	25
Cephalothin	30 µg	14-18	-	-	-	18	-	10	-	23	-	20
Nitrofurantoin	300 µg	14-17	20	17	20	18	20	25	20	23	15	28
Amikacin	30 µg	14-17	25	25	27	25	25	25	25	25	25	28
Cefotaxime	30 µg	14-23	30	13	20	20	-	30	28	27	20	25
Ampicillin-Sulbictam	20 µg (10 +10)	11-14	13	17	-	16	-	18	-	25	15	15

Table 6: Mortality associated with *Aeromonas* infection in 5 days old chicks.

Group No.	Infected bacteria #	No. of birds	Slaughtered birds	Mortality							Cumulative total (%)	Survivors
				1d	2d	1w	2w	3w	4w	5w		
1	<i>A. hydrophila</i>	30	15	0	0	0	0	0	2	2	13.3	11
2	<i>A. trota</i>	30	15	0	0	0	0	2	2	2	20	9
3	<i>A. caviae</i>	30	15	0	0	0	0	2	1	1	13.3	11
4	<i>A. schubrtii</i>	30	15	0	0	0	0	1	0	1	6.7	13
5	Control	40	15	0	0	2	0	0	2	0	10	21

#: 1.5 × 10⁹ CFU, Intra Croup at 5 days old; CFU: Colony Forming Unit; d: Day Post Infection w: Week Post Infection.

Table 7: Effect of infection on feed intake & body weight in broiler chicks.

Group No.	No. of birds	Infected bacteria#	Performance Parameters	Age / week post infection				
				1	2	3	4	5
1	30	<i>A. hydrophila</i>	B.Wt	257 ± 5.24	549.67 ± 13.68	1007 ± 29.19	1369.67 ± 39.51	1979.67 ± 59.72
			FI	269.6	345.5	607.8	619.3	1099.33
			FCR	1.73	1.18	1.33	1.71	1.8
2	30	<i>A. trota</i>	B.Wt	259.33 ± 5.24	532.67 ± 13.68	929.33 ± 29.19*	1362.33 ± 39.51	1794 ± 59.72**
			FI	262.5	336.7	530.6	562.9	1097.4
			FCR	1.65	1.23	1.34	1.3	2.54
3	30	<i>A. caviae</i>	B.Wt	261.67 ± 5.24	551.67 ± 13.68	940.33 ± 29.19*	1218.67 ± 39.51**	1609.33 ± 59.72**
			FI	265.6	374.3	510.3	520.7	1039.9
			FCR	1.67	1.29	1.31	1.87	2.66
4	30	<i>A. schubrtii</i>	B.Wt	256.33 ± 5.24	537.3 ± 13.68	901.67 ± 29.19**	1080.67 ± 39.51**	1471 ± 59.72**
			FI	258.7	326.9	546.9	559.3	1023.7
			FCR	1.69	1.16	1.5	3.12	2.62
5	40	control	B.Wt	267.2 ± 4.06	556.6 ± 10.59	1032.8 ± 22.61	1403 ± 30.61	2045.4 ± 46.26
			FI	276.91	291.25	488.5	448.3	1076
			FCR	1.66	1.01	1.03	1.21	1.67

IC: Intra Croup; CFU: Colony Forming Unit; B.Wt: Body Weight; FI: Feed Intake; FCR: Feed Conversion Rate = Feed Intake of a Certain Period ÷ Body Gain of the Same Period; *: Significant when Compared with Control (group 5) (P < 0.05); #: 1.5 × 10⁹ CFU, IC at 5 days old.

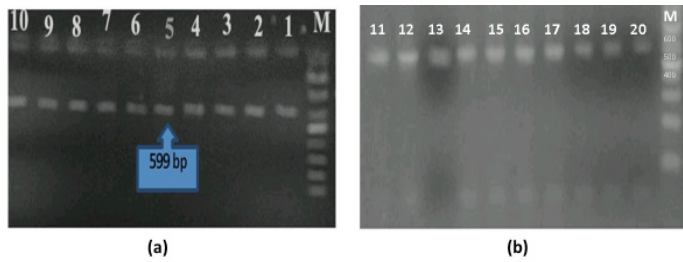


Figure 1: Electrophoretic pattern of 16S rRNA PCR product on 1.5% agarose gel electrophoresis. (a) Lane M: 100 bp ladder. Lane 1-10: Selected *Aeromonas* isolates 1-10. (b) Lane M: 100 bp ladder. Lane 11-20: Selected *Aeromonas* isolates 11-20.

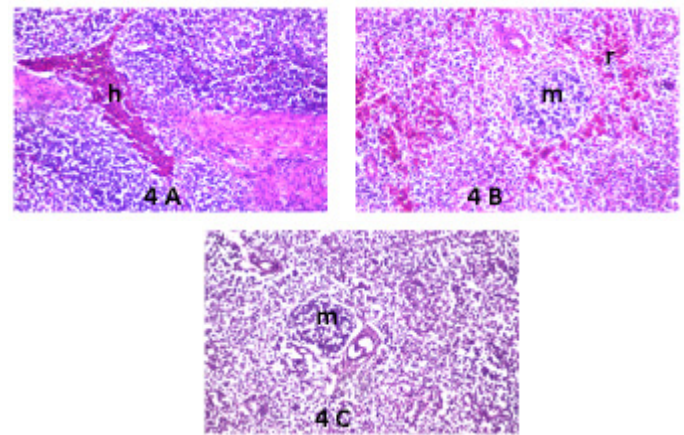


Figure 4a,b,c: Spleen showing histopathological alteration. (A) Spleen of both *A. hydrophila* & *A. schubertii* infected groups showing focal Haemorrhage in the splenic parenchyma (h). (B) Spleen of both *A. hydrophila* & *A. trota* infected groups showing congestion in the Red pulps (r) with focal lymphoid proliferation and nodules formation in the white one (m). (C) Spleen of *A. trota*, *A. caviae* & *A. schubertii* infected groups showing lymphoid proliferation with nodules formation in the white pulps (m).

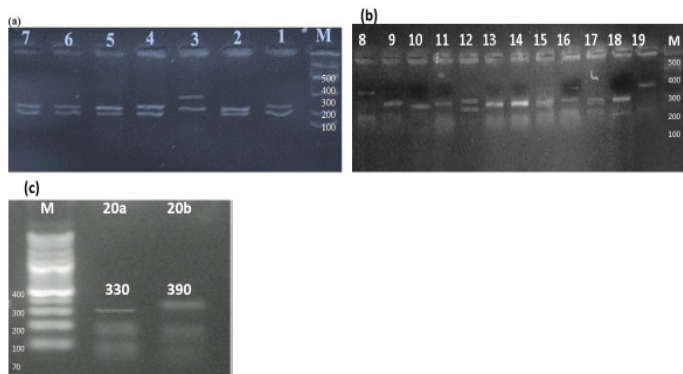


Figure 2a,b,c: Agarose gel showing the RFLP pattern obtained by restriction digestion of 16S rRNA PCR product with endonucleases *MboI* and *AluI*. (a) Lane M: 100 bp ladder. Lane 1-7: Selected *Aeromonas* isolates 1-7 digested with *MboI*. (b) Lane M: 100 bp ladder. Lane 8-19: Selected *Aeromonas* isolates 8-19 digested with *MboI*. (c) Lane M: 100 bp ladder. Lane 20 a: Isolated *A. trota* digested with *AluI*. Lane 20 b: Isolated *A. trota* digested with *MboI*.

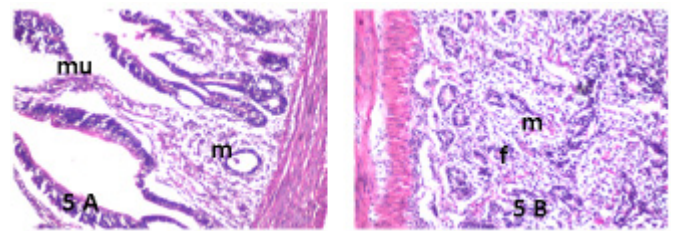


Figure 5a,b: Small intestine showing histopathological alteration. (A) The mucosal lining epithelium of both *A. trota* & *A. caviae* infected groups showing focal desquamation (mu) associated with inflammatory cells infiltration (m) and Oedema (o) in the lamina propria. (B) The lamina propria of the mucosal layer in *A. trota* infected group showing fibrosis (f) with inflammatory cells infiltration (m).

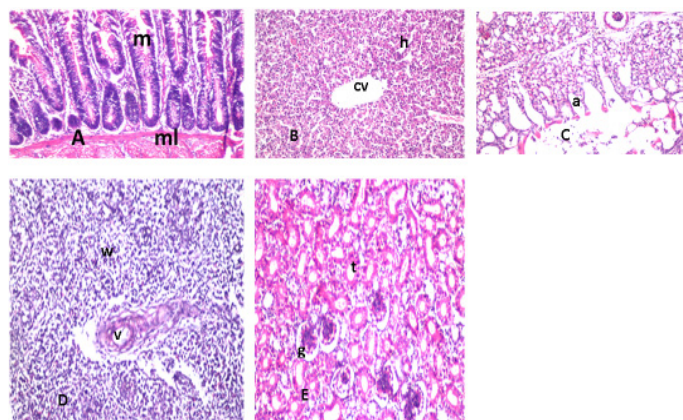


Figure 3a,b,c,d,e: Normal histological structure of different organs of non-infected control group without histopathological alteration. (A) Small intestine showing normal histological structure of the mucosal layer forming the villi (mu) with underlying submucosa and Muscularis (ml). (B) Liver showing normal histological structure of the Central Vein (cv) and surrounding Hepatocytes (h). (C) Lung showing normal histological structure of the lobules with lamellae and air Alveoli (a). (D) Spleen showing normal histological structure of the White pulp (w) with follicular blood Vessel (v) and surrounding red one. (E) Kidney showing normal histological structure of the Glomeruli (g) and Tubules (t).

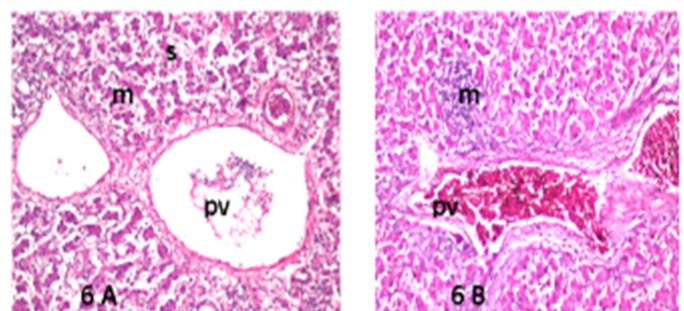


Figure 6a,b: Liver of *A. trota*, *A. caviae* & *A. schubertii* infected groups showing Severe dilatation and congestion in the Portal Vein (pv) and Sinusoids (s) associated with inflammatory cells infiltration in the hepatic parenchyma (m).

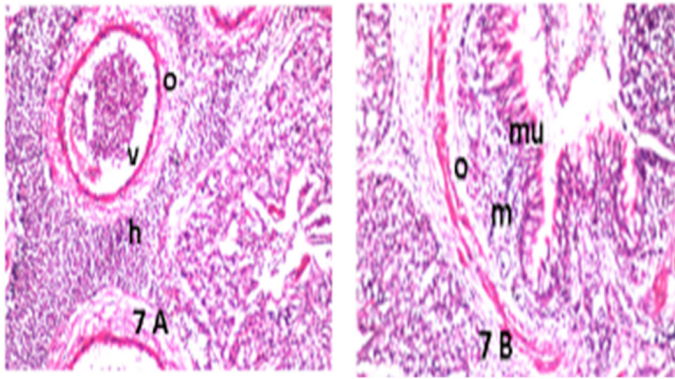


Figure 7a,b: Lung of both *A.trota* & *A.schubertii* infected groups showing histopathological alteration. (A) Lung showing Severe congestion in stromal blood Vessels (v) with perivascular Oedema (o) and Haemorrhage (h). (B) The bronchioles showing Mucosal epithelial hypertrophy (mu) with peribronchiolar Oedma (o) and inflammatory cells infiltration (m).

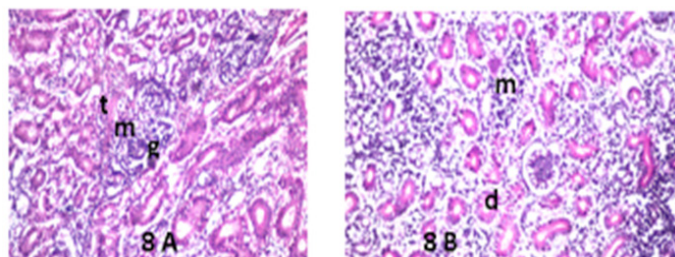


Figure 8a,b: Kidney of *A. trota* infected group showing histopathological alteration. (A) Kidney showing focal inflammatory cells infiltration (m) in between the degenerated Tubules (t) and Glomeruli (g). (B) Kidney showing diffuse inflammatory cells infiltration (m) in between the Degenerated tubules (d).

Conclusion

It was concluded that the isolation of *Aeromonas* species from broiler, layer and duck flocks in this study calls for re-examination of its roles in poultry although its lowest incidence. Their prevalence in drinking water reinforces the need to examine the public health risk of this water-borne pathogen. The drug resistance of *Aeromonas* isolated from poultry and poultry environments is alarming, which may pose a risk for both poultry and public health. Reduction of body weight explains the economic losses that may result from *Aeromonas* infection in chickens. Intestinal, hepatic, splenic, renal and pulmonary pathological lesions of *Aeromonas* infection in chickens revealed that this micro-organism is considered to be a causative agent for mortality problem in some chicken farms and/or trigger for other diseases as CCRD and mycotoxicosis. Finally, *Aeromonas* species can be early diagnosed in clinical cases by PCR and RFLP of 16S rRNA gene without biochemical tests, which may often have errors.

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