

Isolation and Identification of *Aeromonas spp.* from fish and their handlers

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ABSTRACT: The present study was conducted to evaluate the prevalence of *Aeromonas spp.* in Sharkia Governorate, Egypt. The samples comprised of tilapia (*Oreochromis niloticus*, n=160), mugil (*Mugil cephalus*, n=105), human stool (n=27) and fish sellers hand swabs (n=51). A total of 15(4.3) *Aeromonas spp.* isolates were recovered and biochemically confirmed using biochemical test such as oxidase, triple sugar iron, indole production, Methyl Red, Voges-Proskauer, urease, of which, 4 were from tilapia viscera and muscles, 8 from mugil viscera and muscles and 3 from hand swabs. No isolates were recovered from stool samples. This study revealed that the prevalence of *Aeromonas spp.* in *Mugil cephalus* were more than in *Oreochromis niloticus*.

KEYWORDS: *Aeromonas spp.*; isolation; biochemical test.

Date of Submission: 24-12-2022

Date of Accepted : 15-01-2023

I. INTRODUCTION

Aeromonas species are facultative anaerobic Gram-negative bacteria that belong to the family Aeromonadaceae. These bacteria have a broad host spectrum, with both cold- and warm-blooded animals, including humans and are known as psychrophilic and mesophilic (Tomás, 2012). In fish, these bacteria cause hemorrhagic septicemia, fin rot, soft tissue rot and furunculosis. Many species have been implicated in fish disease, including *A. hydrophila*, *A. veronii*, a biovar, *A. sobria*, and *Aeromonas salmonicida* (Beaz-Hidalgo and Figueras, 2013). *Aeromoniasis* symptoms include: skin hemorrhages, body ulceration, red sores, off-appetite, lethargy, popeyes, dropsy and fins rot (Hu et al., 2012). *Aeromonas spp.* belong to family Aeromonadaceae, it is facultative anaerobic, non-spore forming Gram-negative bacterium, motile, bacilli or coccobacilli (Carnahan and Altwegg, 1996). Gastroenteritis is the most prevalent human infection caused by *Aeromonas spp.*, although other severe illnesses, such as systemic infections, are less frequent (Janda and Abbott, 1998).

The pathogenicity of *Aeromonas spp.* has been related to a number of possible virulence factors as hemolysin, aerolysin, proteases, lipases and DNases. These virulence factors play a major role in the development of diseases either in fish or in humans (Umeha et al., 1992).

The antimicrobial resistance between enteric bacteria including *Aeromonas* species is considered a serious problem worldwide. This problem may be attributed to the wide overuse or misuse of these agents in the treatment of different bacterial infections or their incorporation in animal feeds as growth promoters in sub therapeutic doses (Vivekanandhan et al., 2002). The random use of antimicrobials increases clonal selection, which allows the distribution of multidrug resistant (MAR) bacteria (Carvalho et al., 2012).

The ability of bacterial cell to settle on abiotic or biotic surface and encased in a hydrated matrix of exopolymeric substances, polysaccharides, proteins and nucleic acids called biofilm formation (Costerton, 2001). *Aeromonas spp.* have the ability to attach on different surfaces forming biofilms (Elhariry, 2011). which is regarded as a public health hazard especially for those who inhabit the coastal area (Odeyemi and Ahmad, 2017b).

II. METHODS

Samples

A total of 265 fish samples comprised 160 tilapia (*Oreochromis niloticus*) and 105 mugil (*Mugil cephalus*) were collected from marketed fish in Sharkia Governorate, Egypt. Fish samples were collected in sterilized polyethylene bags and transferred to the lab. Viscera and muscles were sampled separately from each individual fish under complete aseptic conditions; samples were collected from each fish after sterilization of the surface by hot spatula according to the international commissions on microbiological specifications for foods (ICMSF, 1998).

Human samples; 27 stool and 51 hand swabs were collected and directly immersed in sterile alkaline pepton water (APW, Oxoid CM1028). Stool samples were collected from from Al Mabarrah, Al Ahrar and The University hospitals in Zagazig city, Sharkia Governorate, Egypt while, hand swabs were collected from fish sellers.

Isolation and identification

Each sample (fish viscera, fish muscles, human stool and hand swabs) were pre enriched in APW (Alkaline pepton water) at 37°C for 24 h, then a loopful of the incubated broth was streaked onto Aeromonas agar (LAB 167) and the plates were incubated at 37°C for 24 h under aerobic conditions. Suspected colonies were purified by plating on nutrient agar plates and were then subjected to biochemical identification according to Bergey's Manual of Determinative Bacteriology (Austin and Austin, 1999). The colonies were examined for morphological characterizations such as shape, Gram stain and motility test. Biochemical characterization was carried out using oxidase, triple sugar iron, indole production, Methyl Red, Voges-Proskauer, urease (MacFaddin, 2000).

III. RESULTS AND DISCUSSION

A total of 343 samples collected from Sharkia(Zagazig,Minia-elkamh and Hehyamarkets) governorate, Egypt, were examined for *Aeromonas* spp. contamination. Table (1) illustrates that a total of 15 (4.3%) isolates were identified by morphological and biochemical examination.

The isolated bacteria were purified and identified according to their morphological characteristics on *Aeromonas* selective agar (LAB 167)as a selective and diagnostic medium for isolation of *Aeromonas* spp.The isolates appeared are yellow, shiny and circular on *Aeromonas* selective agar media).The suspected colonies were subjected to Gram staining and biochemical examination by different tests Table (2).Microscopic examination showed Gram Negative bacilli and coccobacilli. Figure (3 A, B, C) demonstrate the result of some biochemical tests.

Table (1): Prevalence of *Aeromonas* spp.in fish and human samples according to their sources:

| Types of samples | | No. of examined samples | No. of infected samples | No. of biochemically confirmed samples |
|---------------------|---------|-------------------------|-------------------------|--|
| Tilapia: (n=160) | Viscera | 114 | 14(8.7%) | 3(1.9%) |
| | Muscles | 46 | 2(1.25%) | 1(0.62%) |
| Mugil: (n=105) | Viscera | 63 | 9(8.5%) | 5(4.7%) |
| | Muscles | 42 | 5(4.7%) | 3(2.8%) |

| | | | |
|-------------------------------|-----|-----------|----------|
| Total | 265 | 30(11.3%) | 12(4.5%) |
| Fish seller:Hand swabs (n=51) | 51 | 12(23.5%) | 3(5.8%) |
| Patient:Stool (n=27) | 27 | 0 | 0 |
| Total | 343 | 42(12.2) | 15(4.3%) |

Table (2) Biochemical tests for identification of Aeromonas species:

| Test | Result |
|-------------------------|-----------------------------------|
| Gram stain | Negative bacilli and coccobacilli |
| Motility | Motile |
| Oxidase | + |
| Urease | - |
| Triple Sugar Iron (TSI) | A/K |
| Methyl red | V |
| Voges Proskauer | V |
| Indole | + |

+:positive, -:negative,A/K: acid butt /alkaline slant and V: variable

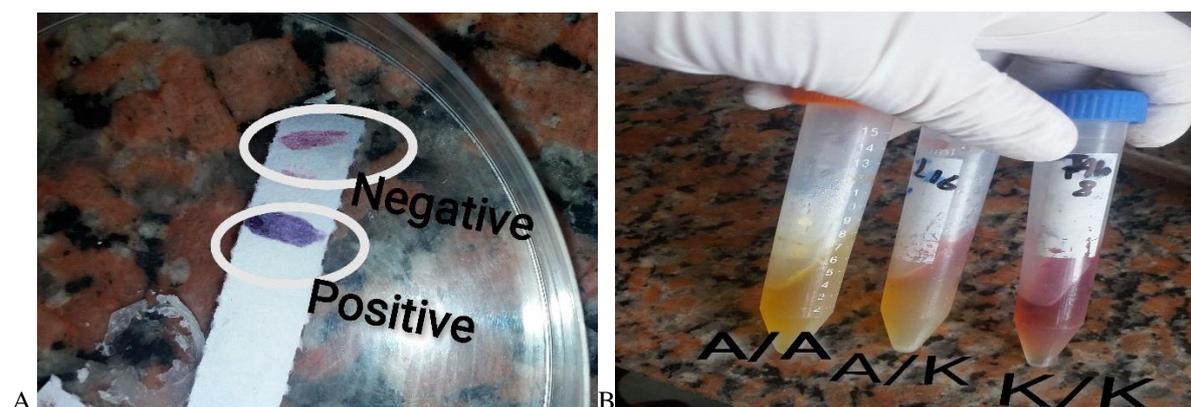




Figure (3): The biochemical tests for identification of *Aeromonas* spp. isolates. A: Oxidase test, B: TSI test where A/A:acid butt/ acid slant ,A/K: acid butt /alkaline slant, K/K:alkaline butt/alkaline slant and C:Urease test.

Aeromonas species play important roles to cause diseases in fish, human, and other animals (Kimet al.,2017). This study was investigated the prevalence of *Aeromonas* spp. in tilapia, mugil, stool and hand swabs in Egypt. *Aeromonas* spp. was isolated from 1.9% Tilapia viscera, 0.6% tilapia muscles, 3.8% mugil viscera, 1.0% mugil muscles, 3.9% hand swabs indicating that fish in retail in the study area are regarded as potential source for infection of human consumers.

In this study the contamination of the examined fish by *Aeromonas* spp. was detected 12.2%. This is higher than the result recorded by Mohamed et al. (2019) who surveyed 379 sample from fish and their handlers and reported isolation rate of 6.3%. While, Castro-Escarpulli et al. (2003) Recorded a prevalence of 32.8% of *Aeromonas* spp. isolated from frozen fish. Abd-El-Malek et al. (2017) reported higher isolation rate 36% in cultured Nile tilapia. In our study the isolation rate of *A. hydrophilus* was 1.2%. while, Ibrahim et al. (2008) recorded 3.1% isolation rate of *A. hydrophila* from tilapia viscera in Giza and Sharkia, Egypt. On the other hand, Ashiru et al. (2011) in Nigeria failed to isolate the bacteria from tilapia viscera.

The present study showed that there was no *Aeromonas* spp. isolates from human stool this may be due to follow the personal hygiene procedures which lead to elimination of *Aeromonas* spp. and because of all samples were from adults without diarrhoea. In accordance, the isolation rate of *Aeromonas* spp. in adults was less (Goodwin et al.,1983) than in children (Gracey et al.,1982). On the other hand, Borchardt et al. (2003) reported a lower occurrence of *Aeromonas* species 0.7% in stool samples in Castro-Escarpulliet al. (2003) isolated 82 strain of *Aeromonas* species from 250 samples of frozen tilapia (*Oreochromis niloticus*) collected from fish markets in Mexico City. Molecular identification revealed a higher contamination rate with *A. salmonicida* (67.5%), followed by *A. bestiarum*(20.9%), *A. veronii*(5.2%), *A. encheleia*(3.9%) and *A. hydrophila*(2.6%). Finally, this study concluded that the consumed fish considered as an important reservoir for *Aeromonas* spp. of public health significance. El-Gohary et al. (2011) examined 36 freshwater fish samples comprised of *Oreochromis niloticus* and *Mugil cephalus* (18 samples, each) obtained from different fish market in Dakahlia Governorate Egypt the result showed that the overall percentage of *Aeromonas* spp. was 27.8 of *O. niloticus* and 16.6% of *M. cephalus*. while, *A. hydrophilus* was identified with percentages of 27.7 and 5.5 in *O. niloticus* and *M. cephalus* respectively. Abd-Ellah et al. (2014) studied the prevalence of two *Aeromonas* spp.; *A. hydrophila* and *A. caviae* in 486 fish samples (intestinal contents and surface swabs) collected from El-Abbasa and El-Warwary fish farms in Egypt. the result showed the presence of *A. hydrophilus* with percentages of 25.9 and 23 in intestinal contents and surface swabs, respectively. while, the respective percentages of *A. caviae* were 37 and 21 in intestinal contents and surface swabs. El-bouhy et al. (2015) collected hemorrhagic diseased fish (312 tilapia and 158 mullet) from fish farm at Sahl El-Housinia, Sharkia, Egypt. Then, the bacteriological examination of samples was carried out from gills, kidney, intestine, liver and spleen. The prevalence of *A. sobria* in tilapia was (35.8 %) and (20.8%) in mullet fish. Vila et al. (2003) determine the prevalence of *Aeromonas* spp. associated with traveler's diarrhea. a total of 863 stool samples of diarrheic patients isolated from Tropical Medicine Unit of the Hospital Clinic of Barcelona, Spain revealed the presence of 2% (18/863) of *Aeromonas* species. *A. hydrophila* was isolated in 5.5% (n=1), also *A. jandaei* was 5.5% (n=1), *A. veronii* biotype *sobria* were 50% (n=9) and *A. caviae* were 38.8% (n=7). El-Gohary et al. (2011) examined 85 human samples consists of; 30 hand swabs, 20 stool swabs from fish sellers and 35 stool swabs from diarrheic patients (20 adult and 15 children) for the presence of *Aeromonas* spp. The samples were collected from Dakahlia Governorate, Egypt. There is no *Aeromonas* spp. isolated from fish sellers' stool samples, while it was present by 16.6% in fish sellers hand swabs, 6.6 % in diarrheic children and 15% in, diarrheic adults. However, *A. hydrophila* present in fish sellers' hand swabs, adults stool swabs and children stool swabs by percentage

(60%, 15%, 0%), respectively. While, *A. caviae* was present in (40%, 0%, 6.6%) of fish sellers' hand swabs, adults stool swabs and children stool swabs, respectively.

V –CONCLUION

The genus *Aeromonas* is widely distributed in aquatic environment and increasingly reported as a primary pathogen of fish and human. This study indicates the presence of *Aeromonas* spp.in fresh and RTE fish may be a major threat to public health. Consequently, the public should be aware of the danger that may accompany handling fresh fish or consumption of improperly cooked fish (either grilled or fried).

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