Phenotypic Antibiotic Resistance Pattern and Prevalence of *Aeromonas* Species Isolated from Fish in Suez Governorate

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Abstract:

Aeromonads are one of the emerging bacterial pathogens commonly found in fresh and brackish water environments, and they have been recognized as primary pathogens to fish for some time. In this study, 108 *Aeromonas* species were isolated from diseased Nile tilapia and *Mugil sehelii* with a total prevalence of 72and *M. sehelii* fish exhibited the highest percentage of *Aeromonas* infection (80%) while *O. niloticus* (62.9%). For *A. hydrophila*, The highest percent of infection was detected in isolates of *O. niloticus* with 45.45%, while in *Mugil* fish, the percentage of infection was 43.75%. For *A. sobria,* The highest percentage of infection was detected in isolates of Mugil fish. The percentage of infection with a percent of (56.25%) while in *O. niloticus* the percentage of infection with *A. sobria* was 54.54%. The multi-drug resistance(MDR)pattern of recovered isolates revealed 75.9% of the isolates showed resistance to cefaclor and erythromycin. 65.7% showed resistance to Tetracycline and Sulfisoxazole. Resistance to Trimethoprim was detected in 55.5% of the isolates, while resistance to Ciprofloxacin could be detected in 13.9% of the isolates.

Key words: *Aeromonas hydrophila*,*Aeromonas sobria*,MDR, *Mugil* fish and Nile tilapia

Introduction

Fish is a rich source of nutrients, is quickly digested, has high palatability, and has a good combination of high-quality protein, vitamins, and minerals, making it an important source of food for humans ***(Pal et al., 2018).*** On the other hand, fish can be a source of foodborne diseases like *Aeromonas* species, recognized as emerging foodborne pathogens that pose a serious threat to public health ***(Igbinosa et al., 2012).*** Bacterial illnesses are the most common and dangerous disease affecting fish production, accounting for 80 % of fish deaths ***(Zaki, 1991).*** Food poisoning and various human ailments are linked to *Aeromonas* species, including gastrointestinal infections and extra-intestinal infections such as skin and soft-tissue infections, traumatic wound infections, and the lower respiratory tract/urinary tract infections ***(Batra et al., 2016).*** *Aeromonas* species can be found in a variety of aquatic settings, including ground, surface, marine, drinking, and wastewater ***(Pandove et al., 2013).*** Aeromonas belongs to the Aeromonadaceae family, including Gram-negative oxidase-positive bacteria, facultatively anaerobic, glucose-fermenting bacteria, and motile rods ***(Pund and Theegarten, 2008).*** *Aeromonas hydrophila*, *Aeromonas caviae*, *Aeromonas veronii*, and *Aeromonas sobria* are among the 32 species and 12 subspecies of the *Aeromonas* genus ***(Janda and Abbott, 2010).*** *Aeromonas* pathogenicity is connected to the generation of virulence factors linked to exotoxin, cytotoxic, and hemolytic activities, which result in mucosal adhesion and colonization, followed by fluid buildup or epithelial changes, all of which are potential events leading to human disease ***(Daskalov, 2006).*** *Aeromonas* species have been linked to poor personal hygiene, a lack of sanitation, food and water-borne diseases worldwide, particularly in poorer nations.

Antibiotics, which were discovered decades ago, have aided in treating microbiological infections and diseases in humans and animals. Bacteria that were once sensitive to frequently used antibiotics are now becoming resistant to them. Some *Aeromonas* species, particularly clinical and environmental isolates, are among antibiotic-resistant bacteria. According to ***(Maria Jose 2012),*** antibiotic resistance of *Aeromonas* species to commercial antibiotics is becoming a public health issue ***(Dias et al., 2012).*** Multi-drug resistance has been increased worldwide, which is considered a public health threat. Several recent investigations reported the emergence of multidrug-resistant

bacterial pathogens from different origins, including humans, birds, cattle, and fish that increase the need for routine application of the antimicrobial susceptibility testing to detect the antibiotic of choice as well as the screening of the emerging MDR strains ***(Algammal et al., 2020; Algammal et al., 2021a; Algammal et al., 2021b; Algammal et al., 2021c)***

The purpose of this study was to detect the prevalence and antibiotic sensitivity of *Aeromonas* species in diseased Nile tilapia and *Mugil Sehelii* from different private farms in Shandora, Suez Governorate, Egypt.

Materials and methods

1 Fish samples Collection

A total of 150 natural fish samples represented by Nile tilapia (70 fish) and *Mugil Sehelii* (80 fish) with an average weight of 150- 250 gm and 50-70 gm respectively were collected from different private farms in Shandora, Suez Governorate, Egypt, between August and September 2019. Each fish sample was separately packed into a sterile plastic bag and then transferred in an icebox under aseptic conditions to a laboratory of Microbiology at the National Institute of Oceanography and Fisheries (NIOF), Suez, Egypt, and were subjected to the clinical and bacteriological isolation and identification.

2 Bacteriological examination

Aseptically loopful samples collected from livers and kidneys were directly streaked into Tryptic Soy broth **(**TSB, Oxoid, Hampshire, UK**)** according to ***(Villari et al., 2000, Monir et al., 2016).*** The overnight enriched broth was streaked onto *Aeromonas* isolation agar base media (Oxoid,UK)with rehydrated ampicillin and blood agar and incubated at 28°C for 18- 24 hrs. A single colony was further sub-cultured until a pure culture of bacteria was obtained. The isolated bacteria were identified morphologically (Gram staining) and biochemically using a variety of biochemical reactions ***(Sabur, 2006; Ahammed et al., 2016)***. Isolates were preserved in TSA slant and BHI broth (Lab M, UK), supplemented with 15% glycerol. ***(Buller, 2004).***

3 Biochemical identification

Biochemically identified *Aeromonas* isolates using the following tests; catalase, esculin hydrolysis, oxidase, arginine hydrolysis, indole, methyl red, Voges Proskauer, citrate utilization, urease, hydrogen sulfide production, nitrate reduction, gelatin liquefaction, oxidation- fermentation, and sugars fermentation according to ***(Rowland et al., 1994; Oxoid, 1998; Abbott et al., 2003; Monir et al., 2017 and Chen et al., 2019).***

4 Antibiogram

The antibiotic sensitivity of the bacterial isolates was determined against nine antimicrobials agents (Oxoid,UK) using the disc diffusion method ***(CLSI, 2020).*** The Mueller–Hinton plates were incubated for 24 hours at 30 °C. Finally, zones of inhibition were measured and compared to the antibiogram's reference data.

Results

1 External and Necropsy findings:

The clinical examination of Moribund and freshly dead fish displayed dark coloration of skin with detached scales, hemorrhage at the base of the fins with some erosion. The most common PM findings were hepatomegaly, friable liver with hemorrhagic patches, bloody ascitic fluid and congested kidney.

2 Morphological characters of the isolates:

The result revealed green colonies of *A. hydrophila* and *A. sobria* on *Aeromonas* isolation agar base with Ampicillin supplement and appeared whitish creamy colonies on TSA media. Smooth, convex, round pale white to grey color hemolytic (β-hemolysis) and non-hemolytic colonies on blood agar. The Gram staining of bacterial isolates showed that they are Gram-negative short rods motile, oxidase, and catalase-positive. These results were confirmed biochemically by a series of biochemical tests. The bacteria were able to grow in TSA containing 1%-4% NaCl at 24° C and 37° C and there is no growth detected at either 4° C or 40° C .*A.hydrophila* isolates were positive for oxidase, catalase, indole, methyl red, vogas Proskauer ,citrate utilization, gelatin liquefication, nitrate reduction, H2S production, bile esculin hydrolysis and fermentation of glucose ,mannose and sucrose and negative for urea hydrolysis. While *A.sobria* isolates were positive to oxidase, catalase, indole, methyl red , vogas Proskauer , citrate utilization, gelatin liquefication, nitrate reduction and fermentation of glucose, mannose and sucrose and negative for urea hydrolysis H2Sproduction and bile esculin hydrolysis

3 Frequency of infection:

Table (1) shows that 108 out of 150 isolates were identified as *Aeromonas species* with a total prevalence of 72%. *M. sehelii* fish exhibited the highest *Aeromonas* infection (80%) while *O. niloticus* (62.9%). For *A. hydrophila*, The highest percent of infection was detected in isolates of *O.niloticus* with 45.45%, while in *Mugil* fish, the percentage of infection was 43.75%. For *A. sobria,* The highest percentage of infection was

detected in isolates of Mugil fish. The percentage of infection with a percent of (56.25%) while in *O. niloticus* the percentage of infection with *A. sobria* was 54.54% as shown in Figure 1,2,3 . Statistically, there is a significant difference between the two fish species (P <0.05).

All the isolates were subjected to antimicrobial-resistant tests against nine commercially available antibiotics. The obtained result revealed a high level of multi antibiotic resistance among the isolates. 75.9% of the isolates showed resistance to cefaclor and erythromycin. 65.7% showed resistance to Tetracycline and Sulfisoxazole. Resistance to Trimethoprim was detected in 55.5% of the isolates, while resistance to Ciprofloxacin could be detected in 13.9% and resistance to piperacillin and tazobacteam (18.5%) could be detected in 13.9% .

Table 1. Prevalence of *Aeromonas species* in the examined fresh fish samples

Fish samples (n=150)	Positive samples (n=108)*	*Aeromonas hydrophila***	*Aeromonas sobria***
Nile tilapia	44 (62.9%)	20 (45.5%)	24 (54.54%)
Mugil sehelii	64 (80%)	28(43.8%)	36 (56.25%)

*Percentages were calculated according to the number of examined samples.

**Percentages were calculated according to the number of positive samples.

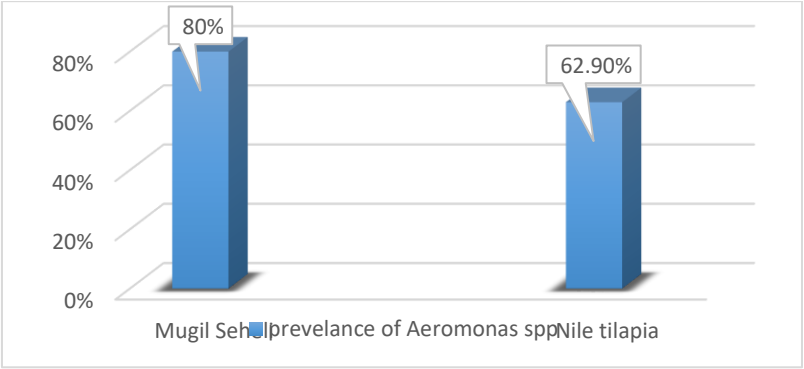

Fig (1): prevalence of *Aeromonas* infection among examined fish species

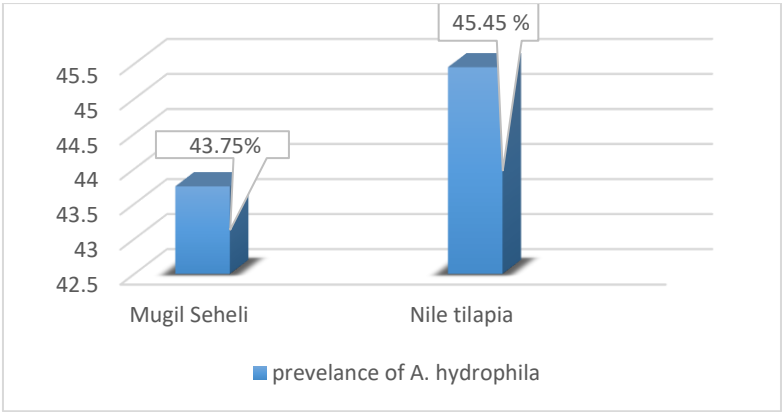

Fig (2): prevalence of *A. hydrophila* among examined fish species

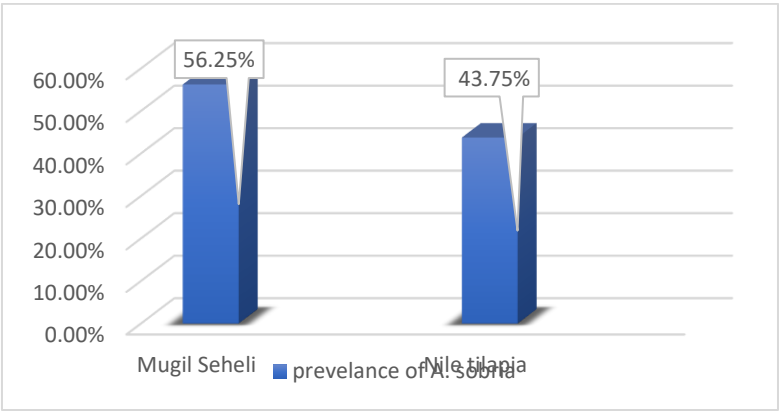

Fig (3): prevalence of *Aeromonas sobria* among examined fish species

4 Antimicrobial-resistant testing:

Table (3): Antibiogram profile of examined isolates

Antimicrobials	Number of resistance of isolates		
	***hydrophila* (n=48)**	***sobria* (n=60)**	**Total (n=108)**
Pipracillin-tazobacteam(100/10µg)	12	8	20(18.5%)
Tetracyclin (10 µg)	24	47	71 (65.7%)
Ciprofloxacin (5 µg)	3	12	15 (13.9%)
cefaclor (30 µg)	28	54	82 (75.9%)
erythromycin (15 µg)	34	48	82 (75.9%)
sulfisoxazole(300 µg)	28	43	71 (65.7%)
Trimethoprim (5 µg)	26	34	60 (55.5%)

Discussion

Bacterial infections are regarded as one of the most serious issues confronting the fish business, causing high mortality not just in cultured fish but also in the wild, and have had a negative impact on economics since the beginning of marine fish culture ***(Khalil and Abd El-Latif, 2013).*** *Aeromonas* belongs to the Aeromonadaceae family, which comprises a collection of Gram-negative bacteria, primarily motile rods with oxidase-positive, facultatively anaerobic, and glucose-fermenting properties ***(Pund and Theegarten, 2008).***

Aeromonas species is a major bacterial pathogen that causes hemorrhagic septicemia in many freshwater and marine fish, resulting in large economic losses due to the high mortality of particular fish species ***(Viji et al., 2011).*** *A. hydrophila* has been implicated in outbreaks of severe mortality in farmed fish ***(Aboyadak et al., 2015; Okasha et al., 2016).***

Clinical signs of diseased fish infected with *Aeromonas* species included cutaneous haemorrhage, gill congestion, hyphemia, haemorrhages in fins and operculum, abdominal congestion, abdominal edoema, hepatic portal redness, and intestinal swelling. Clinical symptoms generated by different *Aeromonas* strains were occasionally similar. However, there were situations when dissimilarities were detected. Even the same *Aeromonas* species might generate a variety of clinical signs in different fish ***(Chen et al., 2019).*** The results of naturally infected fish show that Nile Tilapia and *Mugil sehelii* both have illness concerns. Clinical manifestations ranged from dark skin pigmentation with detached scales to cutaneous hemorrhage, and hemorrhage at the base of the fins with some erosion which support the findings of (***Aboyadak et al., 2015; Okasha et al., 2016; Omar et al., 2016, and Abdel-moneam et al., 2021)*** in Tilapia fish.

Post mortem examination, the fish revealed apparent internal typical lesions. Internally there was congested friable enlarged liver with a congested kidney. These findings were agreed with those reported by ***Jagoda et al. (2014); El Deen et al. (2014); Aboyadak et al. (2015); Eissa et al. (2015); Okasha et al. (2016); Monir et al. (2017) and Abdel-moneam et al. (2021).*** All-over external and P.M findings can be attributed to bacterial invasion, multiplication, colonization, and toxins produced by invading microorganisms. ***(Miyazaki and Kaige, 1986 and Elsheshtawy et al., 2019).***

The identification result revealed that *Aeromonas* species appeared as green colonies on *Aeromonas*

isolation agar base with ampicillin as a supplement. This agreed with the finding of ***Maklad et al. (2019) and Shameena et al. (2019).*** On TSA, the bacteria showed creamy, round, convex, smooth edge and semi-translucent colonies and this agreed with the finding of ***Monir et al. (2017) and Fauzi et al. (2020).*** On blood agar, the bacteria were smooth, convex, round pale white to grey color hemolytic (β-hemolysis) and non-hemolytic colonies and this agreed with the finding of ***Ibrahiem et al. (2016) and Mzula et al. (2019).*** The Gram staining of bacterial isolates showed a Gram-negative rod shape. These findings were in line with results obtained by ***Monir et al. (2017) and Yazdanpanah-Goharrizi et al. (2020).***

The biochemical identification revealed that these isolates were presumptively identified as A. *hydrophila* and A.*sobria* and This result is agreed with reference strains in the System Identification Manual of Common Bacteria and Bergey's Manual of Systematic Bacteriology ***(Ran et al., 2018).*** In the present study, the optimal growth temperature of A. *hydrophila* and A. *sobria* isolated was 24˚C and 37˚C and had optimal growth at a concentration between 0-4% of sodium chloride **.** These findings were in line with ***Rahman and Hossain's (2010) and Nahar, et al. (2016)*** for growth temperature. For NaCl tolerance, the result disagrees with ***Nahar et al. (2016),*** who reported that *A. hydrophila* isolated from juvenile farmed pangasius can grow in 0-1% NaCl; however,, no growth was noted in 2-4% NaCl media.

In this study, out of 150 fish samples from different sources (*O. niloticus* and *Mugil sehelii*) 108 isolates were biochemically confirmed as *Aeromonas species* with a total prevalence of 72% Table (2). This result was higher than the result obtained by ***Matter et al. (2018)*** who reported that the total prevalence of *Aeromonas species* isolated from Tilapia and Mugil cephalus in Kafr El-Sheik and Ismailia Governorates was (55.27 %) and ***Arslan and Kucuksari* (*2015),*** who found that the total incidence of *Aeromonas species* isolated from freshwater fish and seawater fish were 45/74 (60.8%). On the other hand, some studies reported a higher percentage of total *Aeromonas species* isolated from fish as ***El-Ghareeb et al. (2019)*** found that the total prevalence of *Aeromonas species* was 90% (135/150) of examined fish samples

For Tilapia fish, the total prevalence of *A. hydrophila* was 45.5%. This result was higher than the result reported by ***Ebeed et al. (2017) and El-Gamal et al. (2018)*** who reported an incidence of *A.*

hydrophila as 25% and 23.3%, respectively. Also, our result is lower than those detected by ***Aboyadak et al., (2015),*** who detected a prevalence of 75%, reflecting the ubiquitous nature of *Aeromonas species* present in the gut flora of fish. In addition, the high water temperature may have increased the fish's susceptibility to *A. hydrophila* infection, as reported by ***Shayo et al. (2012).*** The prevalence of *A.sobria* in Tilapia fish was (54.54%), and this result is higher than observation recorded by ***John and Hatha (2013); Hassan et al. (2017); Ebeed et al. (2017) and Abd El-Tawab, et al. 2018*** who reported a prevalence of 40.57% 31.07%, 28% and 46.8% in Tilapia fish respectively.

For *Mugil seheli* the prevalence of *A.hydrophila* was 43.8% (n=28/64) **(**Table 2) and this result is higher than those detected by ***Ramadan et al. (2018),*** who reported that the infection rate of examined mullet with *A. hydrophila* was 37% (50/135). The prevalence of *A.sobria* in *Mugil seheli* was 56.25% **(Table 2).** This result was higher than those reported by ***Maklad et al. (2019),*** who recorded isolation of *A. sobria* from Mugil cephalus fish by 30.12%, respectively. A higher prevalence was reported by ***Abd El-Tawab et al. (2018)*** who reported that *A. sobria* was the most prevalent strain of aeromonad to other strains in common carp with 76.4%.

Aeromonads, particularly isolates from clinical and environmental samples, have been linked to a variety of diseases recently, including gastroenteritis ***(Igbinosa et al., 2012),*** bacteremia and septicemia ***(Papadakis et al., 2012).*** As a result, it is critical to conduct investigations into various aquatic sources for the existence of Aeromonas species and their resistance pattern to a variety of commercial antimicrobial treatments. The results of this investigation indicated that the isolates examined had a significant level of multi-drug resistance. 108 (100%) isolates were resistant to Bacitracin, Clindamycin, and Vancomycin. This was nearly identical to the results obtained by ***Vivekanandhan et al. (2002),*** who reported 99 % resistance to Bacitracin, and ***(Igbinosa and Okoh 2012),*** who detected 100% resistance to vancomycin, but higher than the results obtained by ***Igbinosa and Okoh (2012),*** who detected 66.7 % resistance to clindamycin.

In conclusion, phenotypic identification is a valuable epidemiological tool used to characterize *Aeromonas* spp. The routine application of antimicrobial susceptibility testing is essential to select the antibiotic

of choice due to the emergency of multi-drug resistance strains.

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نمط مقاومة المضادات الحيوية الظاهري وانتشار أنواع إيروموناس المعزولة من الأسماك في محافظة السويس

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الملخص العربي

تعتبر الأيروموناد أحد مسببات الأمراض البكتيرية الناشئة التي توجد عادة في بيئات المياه العذبة وشديدة الملوحة ، وقد تم التعرف عليها على أنها مسببات الأمراض الأولية التي تصيب الأسماك. في هذه الدراسة ، تم عزل 108 عزله من الايروموناس من البلطي النيلي والسهلية بنسبة إجمالية بلغ72 ٪ . .النتائج التي تم الحصول عليها تكشف عن نمط مقاومة الأدوية المتعددة بين العزلات ان جميع العزلات أظهرت مقاومة كاملة للباسيتراسين ، الكليندامايسين والفانكومايسين. 75.9٪ من العزلات أظهرت مقاومة ضد سيفاكلور والاريثروميسين و قد أظهر 65.7٪ مقاومة لمضادات التتراسيكلين والسلفيسوكسازول .تم الكشف عن مقاومة عقارترايموسبريم في55.5٪ من العزلات ، بينما تم الكشف عن مقاومة لعقار سيبروفلوكساسين في 13.9٪