Prevalence and Antimicrobial Resistance Profiles of *Pseudomonas aeruginosa* Isolated from *Oreochromis niloticus, Tilapia zilli,* and *Clarias gariepinus*

Mahmoud Ezzat[1], Amal emam[2], Mohamed Abou El-Atta[3], Fatma yousseff[4], Ali Wahdan[1*]

[1]Department of Bacteriology, Immunology and Mycology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt. [2]Central laboratory for Aquaculture Research (CLAR), Abbassa, Sharkia, Egypt. [3]Veterinarian., and [4]Animal Health Research Institute, pathology department, Ismailia, Egypt.

Corresponding author*: Ali Wahdan, dr_aly_w@yahoo.com, 01032155346

Abstract

Pseudomonades, which causes ulcerative syndrome, is one of the most serious aquaculture diseases. A total of 150 *Oreochromis niloticus*, *tilapia zilli*, and *clarias gariepinus* with evidence of septicemia were randomly taken from El-Temsah Lake and fish farms in Ismailia governorate for isolation of *P. aeruginosa* and detection of its sensitivity to various antimicrobial drugs. The high rate of isolation was in *Clarias gariepinus* (42.5 %), *Oreochromis niloticus* (34.5 %), and then *Tilapia zillii* (26 %) were the most common fish species for *P. aeruginosa*. The highest prevalence was found in the liver (38.35 %), followed by kidney (30.58 %), spleen (19.9 %), and gills (11.17 %). According to antimicrobial resistance patterns, *P. aeruginosa* isolates were extremely resistant to Tetracycline and Oxytetracycline, Amoxicillin clavulanic acid, Ampicillin/ Sulbactam, Tobramycin, Cefotaxime, and Ceftriaxone, but very sensitive to Colistin (100 %). The isolated multidrug resistant *P. aeruginosa* should be followed up to control the disease in aquaculture industry.

Key words: *P. aeruginosa,* Fish, Prevalence, Antimicrobial resistance

Introduction

Pseudomonas in fish exhibited petechial hemorrhage, detached scales, darkening of the skin, exophthalmia, and abdominal ascites, and were thought to be the main cause of high mortalities and economic losses among fish and fish farms. ***(Austin, Austin et al. 2007).***

Pseudomonas species are frequently found in natural water

sources and are one of the leading causes of septicemia in both marine and fresh water fish around the world, resulting in significant financial losses and decreased fish farm efficiency. ***(Olsson, Jöborn et al. 1998).***

Pseudomonas aeruginosa is motile (contains a single flagella), rod-shaped, with a size range of 0.6 to 2 mm, and can grow in a variety of culture mediums. Young bacteria on nutrient agar have a light green hue and a fruity odor, but older bacteria might become blue and brown when they die. The bacteria in nutritional broth have a green tint on the surface***. (Monsen, Lövgren et al. 2009).***

Pseudomonas can cause a problem for human consumers too, generally caused by *P. aeruginosa* cause healthcare associated illnesses ***(Bagshaw and Laupland 2006).*** The virulence factors of *P. aeruginosa* are generally involved in colonization and chronic infection, while extracellular factors, extremely toxic, are associated with acute infection ***(Bricha, Ounine et al. 2009).***

Antibiotic susceptibility of bacteria must be understood in order to effectively manage the illness they induce. Antibiotic usage in aquaculture has been observed around the world, as well as the possible spread of resistant pathogens between land and aquatic habitats. ***(Cabello 2006) (Makharita, El-Kholy et al. 2020).*** As a result, there is a scarcity of knowledge about the antimicrobial drug susceptibility of the aquatic environment, which is a critical aspect of AMR pathogenesis. **(*Biyela, Lin et al. 2004) (Wahdan, Fadel et al. 1930, Algammal, Hashem et al. 2021).*** So the aim of this investigation was the detection of the prevalence and antimicrobial resistance pattern of *P. aeruginosa* isolated from different fish species.

Materials and methods

Samples:

A total of 150 *Oreochromis niloticus, tilapia zilli and clarias gariepinus*, 50 from each type of fish showed signs of septicemia and others apparently healthy were collected randomly from El-Temsah Lake and from fish farms in Ismailia governorate. The collected fish were transferred alive in a plastic container to the bacteriological lab in the faculty of veterinary Medicine at Suez Canal university and

External examination

Clinical examination was performed as ***(Austin and Austin 2007)*** to identify clinical abnormalities in fish that indicate infection External hemorrhages, erosions, and ulcers in any part of the body, stomach distension, and

loss of reflex erythema of fins and scales were all noted in the fish.

Internal examination:

Examination of the internal organs was done according to the method described by ***(Austin and Austin 2007).***

Isolation and identification of *pseudomonas* spp.

A loopful of incubated broth streaked on pseudomonas agar base media, on MacConkey's agar media. The plates were incubated at 37°C for 24hr ***(APHA, 1992).*** Bacterial smears were produced from presumed pure cultures, stained with Gram's stain, and inspected microscopically through an oil immersion lens to assess their morphological properties. The structure, surface, edge, color, and opacity pigmentation development of colonial appearance were investigated. Pseudomonas colonies were circular, smooth, moist, convex, 1-2 mm in diameter, shiny, and pigmented in some cases. Biochemical tests for bacterial recognition performed out through techniques specified by ***(MacFaddin 2000).***

Antimicrobial sensitivity testing of the recovered *P. aeruginosa* isolates

The sensitivity of examined *P. aeruginosa* to 11 different antimicrobial drugs was investigated using the disc agar diffusion method, as stated by ***(Quinn et al., 2002).*** Based on the diameter of the inhibitory zones around the disc, the isolates were classified as sensitive, moderate, or resistance. The findings were interpreted according to the NCCLS/CLSI (2018) guidelines ***(Wayne 2011).***

Results

A total of 206 isolates showed character of *P. aeruginosa*, 69 isolates were obtained from *Oreochromis niloticus* with prevalence (34.5%), 52 were obtained from *Tilapia zilli* with prevalence (26%) and 85 were obtained from *Clarias gariepinus* with prevalence (42.5%) here colonies on pseudomonas base agar showed light green pigmentation after incubation at 37 ºC for 24 hrs as shown in Table 1.

The present results in Table 2 showed that the distribution of *P. aeruginosa* in infected *Oreochromis niloticus* was high in liver 26 samples with prevalence (37.68%) followed by the kidney 20 samples with prevalence (28.98%), then spleen 15 samples with prevalence (21.73%) then gills 8 samples with prevalence (11.59%). The distribution of *P. aeruginosa* in infected *Tilapia zilli* was high in liver 18 samples with prevalence (34.61%) followed by the kidney 16 samples with prevalence (30.7%), then spleen 13 samples with prevalence (25%)

then gills 5 samples with prevalence (9.61%). The distribution of *P. aeruginosa* in infected *Clarias gariepinus* was high in liver 35 samples with prevalence (41.18%) followed by the kidney 27 samples with prevalence (31.76%), then spleen 13 samples with prevalence (15.3%) then gills 10 samples with prevalence (11.76%). The distribution of *P. aeruginosa* in different organs and tissues in infected *Tilapia zilli, Oreochromis niloticus and Clarias gariepinus* was high in liver 79 samples with prevalence (38.35%) followed by the kidney 63 samples with prevalence (30.58%), then spleen 41 samples with prevalence (19.9%) then gills 23 samples with prevalence (11.17%).

Pseudomonas aeruginosa isolates were highly sensitive (100%) to Colistin (10µg). and were intermediate sensitive (68.3%) to Ciprofloxacin(5µg) and (66.7%) to sulfa-trimethoprim (STX) while exhibited remarkable resistance (90.0%) to both Tetracycline (TE30) and Oxytetracycline (T30), (85.0%) to Amoxicillin clavulanic acid (Amc30), (83.3%) to Ampicillin/ Sulbactam (A/S), (80.0%) to Tobramycin (10µg), (78.3%) to Cefotaxime (CTX30) and (70.0 %) to Cefatriaxone (CTR 30µg).

Table 1: *The prevelance of P. aeruginosa isolated from different fish species.*

Fish species	No of fish	No of samples	positive sample of pseudomonas	
			No	%
Oreochromis niloticus	50	200	69	34.5 %
Tilapia zilli	50	200	52	26 %
Clarias gariepinus	50	200	85	42.5 %
total	150	600	206	34.33 %

Table 2: *Distribution of P.aeruginosa in different organs*

Fish species	No. of isolates	organs							
		liver		Kidney		spleen		gills	
		No	%	No	%	No	%	No	%
Oreochromis niloticus	69	26	37.68	20	28.98	15	21.73	8	11.59
Tilapia zilli	52	18	34.61	16	30.7	13	25	5	9.61
Clarias gariepinus	85	35	41.18	27	31.76	13	15.3	10	11.76
Total isolates	206	79	38.35	63	30.58	41	19.9	23	11.17

Table 3: *Antimicrobial sensitivity of P. aeruginosa.*

Antimicrobial agent	*Group*	***P. aeruginosa* of fish source**					
		No.= 60					
		Sensitive		**Intermediate**		**Resistant**	
		No.	**%**	**No.**	**%**	**No.**	**%**
Tetracycline (TE30)	**Tetracycline**	0	0.0	6	10.0	54	90.0
Oxytetracycline (T30)	**Tetracycline**	0	0.0	6	10.0	54	90.0
Amikacine (AK30)	**aminoglycoside**	2	3.3	18	30.0	40	66.7
Tobramycin(10µg)	**aminoglycoside**	0	0.0	12	20.0	48	80.0
Cefotaxime (CTX30)	**Cephalosporins**	0	0.0	13	21.7	47	78.3
Cefatriaxone (CTR 30µg)	**Cephalosporins**	0	0.0	18	30.0	42	70.0
Amoxicillin clavulanic acid (Amc30)	**Penicillins**	0	0.0	9	15.0	51	85.0
Ampicillin/ Sulbactam (A/S)	**Penicillins**	0	0.0	10	16.7	50	83.3
sulfa-trimethoprim (STX)	**Sulfonamides**	3	5.0	40	66.7	17	28.3
Ciprofloxacin(5µg)	**quinolones**	9	15.0	41	68.3	10	16.7
Colistin (10µg)	**polymyxin**	60	100.0	0	0.0	0	0.0

Discussion

Antibiotic-resistant bacteria in fish can pose a serious concern to public health since they can be passed on to other bacteria with clinical importance in humans. Antibiotic options for treating common infectious diseases in humans are becoming increasingly restricted, expensive, and inefficient as antibiotic-resistant bacteria appear. ***(Zaky and Ibrahim 2017)***.

The present results showed the common clinical signs of *P. aeurginosa* in naturally infected *Oreochromis niloticus, Tilapia zilli* and *Clarias gariepinus* were distributed hemorrhage on the pectoral region, caudal fins, and gill cover, as well as detached scales and hemorrhagic ulcers on the skin in some fish. Internal examination revealed congestion throughout the body, as well as septicemic fluid in the abdomen. This is a similar result to ***(Austin and Austin 2007, Algammal, Mabrok et al. 2020)***

The present results in Table 1 revealed that a total of 228 (33.4%) isolates out of 683 investigated samples were found positive for *P. aeruginosa*. Among different sources, the prevalence of *P. aeruginosa* was 34.33%, 28.3% and 21.7% in all examined fish, human and water samples respectively. This result was nearly similar to ***(Enany et al., 2016)*** who detected that the prevalence of *P. aeruginosa* was 36.36% in fish samples and 25% in human samples. And that result was less than that obtained by (***Elshafiee, Nader et al. 2019)*** *P. aeruginosa* was isolated from humans working on farms in the Giza Governorate with a 20% incidence. Fish can acquire harmful bacteria from the natural aquatic environment because their bacterial load reflects the water quality in which they were captured. ***(Alawy, El-Tras et al. 2015, Ismail, Wahdan et al. 2019).***

The obtained results showed the prevalence of *P. aeruginosa* isolated from different fish species and the result revealed that a total 206 isolates showed characteristics of *P. aeruginosa,* the most predominant fish species for *P. aeruginosa was Clarias gariepinus* with prevalence (42.5%) followed by *Oreochromis niloticus* with prevalence (34.5%) then *Tilapia zilli* with prevalence (26%). That was similar to ***(Enany et al., 2016)*** who found that the prevalence of *P. aeruginosa in Clarias gariepinus* (52%) was more than in *Tilapia zilli* (23.3%). And also similar to ***(Ismail and El Lamei 2017*)** who found that the occurrence of *P. aeruginosa* in *Oreochromis niloticus* (40%) was more than in *Tilapia zilli* (22%). And that result was different to

***(Algammal, Mabrok et al. 2020*)** who found that the high prevalence was in *Oreochromis niloticus* (32.73%) then in *Clarias gariepinus* (30%). This difference in the prevalence of *P. aeruginosa* isolated from different fish species may be due to different seasons and location of isolation.

As regards to Table 2, the total distribution of *P. aeruginosa* in different organs and tissues in infected fish was high in liver (38.35%) followed by kidney (30.58%) then spleen (19.9%) then gills (11.17%). This result agrees with ***(Eissa, Abou El-Ghiet et al. 2010*)** who recorded that the organism was mainly isolated from liver (35%) followed by kidneys, spleen and gills (30%, 21.25% and 13.75% respectively).

The present result revealed that the highest prevalence of *P. aeruginosa* was in liver and the lowest was in gills. Similar result was obtained by ***(Abd El Tawab, Maarouf et al. 2016)*** who found that among organs, the highest incidence of *P. aeruginosa* was found in liver (33.3%) and the lowest was found in gills (16.7%). The presence of *P. aeruginosa* in at least one organ of the fish was considered positive for the bacterium.

Regarding to table 3 the antimicrobial pattern of *P. aeruginosa* was agree with ***(Nasreen, Sarker et al. 2015*)** who recorded the resistance of *P. aeruginosa* to tetracycline and gentamycin. and agree with ***(Magdy, El-Hady et al. 2014*)** who mentioned that all examined *P. aeruginosa* isolates were resistant to Amoxicillin, Cephalothin, Erythromycin, Lincomycine and Nitrofurantoin. And ***(Eid, El Tabiy et al. 2016*)** which their findings revealed that all Pseudomonas isolates tested positive for Ampicillin/sulbactam, Penicillin, and Amoxicillin resistance. The overuse and abuse of antibiotics is the cause of the high levels of resistance that are increasing year after year. So, prior to an antibiotic therapy for an infectious disease caused by bacteria, susceptibility testing is critical to avoid antibiotic resistance and ensure efficient treatment, although antibiotics should be used only when absolutely necessary.

Conclusion:

The high prevalence rate of P. aeruginosa was in *Clarias gariepinus* (42.5 %), followed by *Oreochromis niloticus* (34.5 %), and then *Tilapia zillii* (26 %). According to antimicrobial resistance patterns, isolates were extremely resistant to Tetracycline and Oxytetracycline, Amoxicillin clavulanic acid, Ampicillin/ Sulbactam, Tobramycin, Cefotaxime, and Ceftriaxone, but very sensitive to Colistin (100 %).

References

Abd El Tawab, A. A., A. A. Maarouf and N. M. Ahmed (2016). "Detection of Virulence factors of Pseudomonas species isolated from fresh water fish by PCR." Benha Veterinary Medical Journal **30**(1): 199-207.

Alawy, A. E., W. F. El-Tras, H. R. El Raiy and D. F. Khater (2015). "Impact of industrial wastewater on water and fish quality of Nile River in Kafr El-Zayat, Egypt." Benha Veterinary Medical Journal **28**(1): 78-87.

Algammal, A. M., H. R. Hashem, K. J. Alfifi, H. F. Hetta, N. S. Sheraba, H. Ramadan and R. M. El-Tarabili (2021). "atp D gene sequencing, multidrug resistance traits, virulence-determinants, and antimicrobial resistance genes of emerging XDR and MDR-Proteus mirabilis." Scientific reports **11**(1): 1-15.

Algammal, A. M., M. Mabrok, E. Sivaramasamy, F. M. Youssef, M. H. Atwa, A. W. El-Kholy, H. F. Hetta and W. N. Hozzein (2020). "Emerging MDR-Pseudomonas aeruginosa in fish commonly harbor opr L and tox A virulence genes and bla TEM, bla CTX-M, and tet A antibiotic-resistance genes." Scientific Reports **10**(1): 1-12.

Austin, B. and D. Austin (2007). "Characteristics of the pathogens: Gram-negative bacteria." Bacterial Fish Pathogens: Diseases of Farmed and Wild Fish: 81-150.

Austin, B., D. A. Austin and C. Munn (2007). Bacterial fish pathogens: disease of farmed and wild fish, Springer.

Bagshaw, S. M. and K. B. Laupland (2006). "Epidemiology of intensive care unit-acquired urinary tract infections." Current opinion in infectious diseases **19**(1): 67-71.

Biyela, P., J. Lin and C. Bezuidenhout (2004). "The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes." Water Science and Technology **50**(1): 45-50.

Bricha, S., K. Ounine, S. Oulkheir, N. Haloui and B. Attarassi (2009). "FACTEURS DE VIRULENCE ET EPIDEMIOLOGIE LIES AU PSEUDOMONAS AERUGINOSA VIRULENCE FACTORS AND EPIDEMIOLOGY RELATED TO PSEUDOMONAS AERUGINOSA." Revue Tunisienne d'Infectiologie-Oct **2**: 7-14.

Cabello, F. C. (2006). "Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment."

Environmental microbiology **8**(7): 1137-1144.

Eid, H., A. El Tabiy and S. Fathy (2016). "Prevalence and Molecular Characterization of Pseudomonas Species Isolated from Fish Markets in Port-Said." Suez Canal Veterinary Medical Journal. SCVMJ **21**(1): 1-12.

Eissa, N., E. Abou El-Ghiet, A. Shaheen and A. Abbass (2010). "Characterization of Pseudomonas species isolated from tilapia "Oreochromis niloticus" in Qaroun and Wadi-El-Rayan lakes, Egypt." Global Veterinaria **5**(2): 116-121.

Elshafiee, E. A., S. M. Nader, S. M. Dorgham and D. A. Hamza (2019). "Carbapenem-resistant Pseudomonas aeruginosa originating from farm animals and people in Egypt." Journal of Veterinary Research **63**(3): 333-337.

Ismail, M. and M. El Lamei (2017). "Studies on Pseudomonas Septicemia in Some Tilapia in Ismailia." Suez Canal Veterinary Medical Journal. SCVMJ **22**(1): 107-117.

Ismail, M., A. Wahdan, M. Yusuf, S, E. Metwally and M. Mabrok (2019). "Effect of dietary supplementation with a synbiotic (Lacto Forte) on growth performance, haematological and histological profiles, the innate immune response and resistance to bacterial disease in Oreochromis niloticus." Aquaculture Research **50**(9): 2545-2562.

MacFaddin, J. F. (2000). "Biochemical tests for identification of medical bacteria."

Magdy, I., M. El-Hady, H. Ahmed, S. Elmeadawy and A. Kenwy (2014). "A contribution on Pseudomonas aeruginosa infection in African catfish (Clarias gariepinus)." Research Journal of Pharmaceutical, Biological and Chemical Sciences **5**(5): 575-588.

Makharita, R. R., I. El-Kholy, H. F. Hetta, M. H. Abdelaziz, F. I. Hagagy, A. A. Ahmed and A. M. Algammal (2020). "Antibiogram and genetic characterization of carbapenem-resistant gram-negative pathogens incriminated in healthcare-associated infections." Infection and drug resistance **13**: 3991.

Monsen, T., E. Lövgren, M. Widerström and L. Wallinder (2009). "In vitro effect of ultrasound on bacteria and suggested protocol for sonication and diagnosis of prosthetic infections." Journal of clinical microbiology **47**(8): 2496-2501.

Nasreen, M., A. Sarker, M. Malek, M. Ansaruzzaman and M. Rahman (2015). "Prevalence and resistance pattern of Pseudomonas aeruginosa isolated from surface water." Advances in Microbiology **5**(01): 74.

Olsson, J., A. Jöborn, A. Westerdahl, L. Blomberg, S. Kjelleberg and P. Conway (1998). "Survival, persistence and proliferation of Vibrio anguillarum in juvenile turbot, Scophthalmus maximus (L.), intestine and faeces." Journal of Fish Diseases **21**(1): 1-9.

Wahdan, A., A. Fadel and M. Mabrok (1930). "New insights into the effect of Origanum extracts on the gene expression profiles of multidrug-resistant isolates of Pseudomonas aeruginosa retrieved from Oreochromis niloticus." Turkish Journal of Fisheries and Aquatic Sciences **20**(7): 507-519.

Wayne, P. (2011). "Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing."

Zaky, M. M. and M. E. Ibrahim (2017). "Screening of bacterial and fungal biota associated with Oreochromis niloticus in Lake Manzala and its impact on human health." Health **9**(04): 697.

انتشار ونمط مقاومة مضادات الميكروبات لميكروب السيدوموناس ارجينوزا المعزولة من أنواع مختلفة من الأسماك

محمود عزت[1] - امل امام[2]- محمد ابوالعطا[3]- فاطمه يوسف[4]- علي وهدان[1]

1 قسم البكتيريولوجيا والمناعة والفطريات كلية الطب البيطرى جامعة قناة السويس الاسماعيلية- مصر. 2بمركز بحوث الثروة السمكية بالعباسة- العباسة - أبوحماد – شرقية. 3 طبيبه بيطرية 4 معهد بحوث صحة الحيوان ، قسم الباثولوجى ، الإسماعيلية ، مصر.

تعد السيدوموناس ، التي تسبب متلازمة التقرح ، من أخطر الأمراض التى تصيب الأحياء المائية. تم أخذ مجموعه 150 سمكة من البلطى النيلى و البلطى الاخضر و القرامبط ظهرت عليها علامات تسمم الدم بشكل عشوائي من بحيرة التمساح والمزارع السمكية في محافظة الإسماعيلية لعزل السيدوموناس ارجينوزا والكشف عن حساسيتها لمختلف الأدوية المضادة للميكروبات. كانت القرامبط(42.5%) يليها البلطى النيلى (34.5%) ثم البلطى الاخضر(26%) أكثر أنواع الأسماك شيوعاً في السيدوموناس ارجينوزا. كان أعلى انتشار في الكبد (38.35٪) ، يليه الكلى (30.58٪) ، الطحال (19.9٪) ، والخياشيم (11.17٪). وفقًا لأنماط مقاومة مضادات الميكروبات ، كانت عزلات السيدوموناس ارجينوزا شديدة المقاومة لمضادات التتراسيكلين والأوكسي تتراسيكلين ، وحمض أموكسيسيلين كلافولانيك ، والأمبيسيلين / سالباكتام ، وتوبراميسين ، وسيفوتاكسيم ، وسفترياكسون ، لكنها شديدة الحساسية للكوليستين (100٪).