Antibacterial Resistance of *Aeromonas* Species Isolated from Fish and Water of Manzala Lake.

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Abstract
A total of 70 *Aeromonas* isolates isolated from 100 *Oreochromis niloticus*, 100 *Mugil cephalus* and 50 water samples from El Gamil region in Manzala Lake were investigated for antibiotic susceptibility test to 14 different antimicrobial agents using disc agar diffusion method. All strains showed (100%) sensitivity to norfloxacin and showed high sensitivity to cefotaxime (91.4%), gentamycin (90%), nalidixic acid (80%), amikacin (78.6%) and chloramphenicol (74.3%). On the other hand, all tested isolates were resistant to ampicillin, erythromycin and penicillin and they exhibited high resistance rate to vancomycin (94.3%) and doxycycline (91.4%). Multiple antimicrobial resistance (MAR) index values of the tested isolates were higher than 0.2. They were 0.38, 0.36, 0.36 and 0.37 for *A. hydrophila*, *A. sobria*, *A. caviae* and *A. schubertii*, respectively.

Key Words: *Aeromonas* spp., Antibiogram, Manzala Lake.

Introduction

Manzala Lake the biggest coastal lake in Egypt is a shallow brackish lake extending between the Damietta Nile River branch and the Suez Canal with a maximum length of 50 km along the Mediterranean coast ***(Ahmed et al., 2009)***. Domestic agricultural and industrial wastes are brought from urban centers along the lengths of main drains such as Bahr El Baqur drain through which more than 30% of the inflow passes to the lake ***(Hereher, 2014).***

Aeromonads are considered as example of emerging bacterial pathogens and broadly distributed in the environment in several natural habitats such as soil, fresh and brackish water and sewage ***(Garibay et al., 2006).***

The indiscriminate use of antimicrobials in aquaculture has been associated with increased levels of antibiotic resistance

causing unwanted drug residues in aquaculture products and in the environment ***(Rahman et al., 2009).*** The development of resistance to antimicrobial agents in bacterial pathogens is a global public health concern ***(Chugh, 2008).*** Ubiquitous bacteria, which are fit for colonizing diverse water types, are of specific interest to assessing potential forms of antimicrobial resistance dissemination. Given their ubiquity in water environment and patterns of gained antimicrobial resistance, members of the genus *Aeromonas* are good examples of such bacteria ***(Igbinosa and Okoh, 2012).***

Aeromonas spp. comprises an effective marker for monitoring antimicrobial resistance in aquatic environments **(Usui *et al.,* 2016)**. Increase antibiotic resistance among potentially pathogenic strains of Aeromonads, demonstrating an emerging potential health concern. ***(Amsaveni et al., 2014).*** Therefore, the present study aimed to investigate the resistance patterns of *Aeromonas* species isolated from Manzala Lake fish and water.

Material and Methods

Samples:

A total of 200 fish samples (100 *Oreochromis niloticus* and 100 *Mugil cephalus*) in addition to 50 water samples were collected from El Gamil region located in the eastern north corner of Manzala Lake during the period from June 2018 to November 2018. Samples were collected in a sterile container, labeled and transported in insulated ice-boxes with ice to Port Said laboratory for Food Hygiene, Bacteriology Unit for bacteriological examination.

Isolation and Identification of *Aeromonas* species from fish and water samples:

Samples were collected aseptically from fish and water for isolation of *Aeromonas* spp. according to ***APHA (1998)*** Fish and water samples were enriched in alkaline peptone water at 37°C for 24 hr. Enriched culture media were streaked on Aeromonas agar plates for Aeromonas isolation. Identification and biotyping of the isolates was carried out according to Aerokey II of ***Carnahan et al. (1991a)***.

Antimicrobial susceptibility tests of *Aeromonas* species isolates:

Isolated *Aeromonas* species were investigated for antibiotic susceptibility test to 14 different antimicrobial agents using disc agar diffusion method. Pure isolates were grown on nutrient agar plates for 18 h afterward 4–6 colonies were suspended in normal physiological saline and adjusted to turbidity of 0.5-M McFarland standard. Subsequently, the isolate suspension was spread onto Muller Hinton agar plates. Plates were allowed to dry and impregnated with the appropriate antibiotic disks. Plates were incubated at 36 °C for 24 h after which zones of inhibition were measured and

recorded ***(Igbinosa et al., 2013).*** The strains were characterized as sensitive, intermediate or resistive based on the diameter of the inhibition zones around the disc as described by ***NCCLS/CLSI (2007)***.The antibiotic discs used were: Amikacin (AK, 30 µg), Ampicillin (AM, 10µg), Cefotaxime (CTX, 30 µg),Chloramphenicol (C, 30µg), Doxycycline (DO, 30 µg), Erythromycin (E, 15µg), Gentamycin (CN, 10 µg), Nalidixic acid (NA, 30µg), Norfloxacin (NOR, 10µg), Oxytetracycline (T, 30µg), Penicillin G (P, 10u), Polymexin-B (PB, 300u), Trimethoprim + Sulphamethoxazole (SXT,1.25+23.75µg) and Vancomycin (VA, 30 µg).

Multiple Antibiotic Resistances (MAR) index:

Multiple antibiotic resistance index *(Sarter et al., 2007):*

The multiple antibiotic resistances (MAR) index of the bacterial isolates was calculated based on the following formula: MAR index = X / (Y × Z)

X = total antibiotic resistance cases.

Y = total antibiotic used in the study.

Z = total isolates.

Results

Table (1): *Identified Aeromonas species recovered from fish and water samples from Manzala lake (n=258):*

Identified isolates	No.	%
A. hydrophila	125	48.45
A. sobria	73	28.29
A. caviae	50	19.38
A. schubertii	10	3.88
Total isolates	258	100

% were calculated from the total number of isolates (n=258).

Table (2): *Antibiogram of Aeromonas species isolates recovered from Manzala lake fishes and water:*

Aeromonas species/ Antimicrobial discs	***A. hydrophila* (n=20)**			***A. sobria* n=(20)**			***A. caviae* n=(20)**			***A. schubertii* n=(10)**		
	S	I	R	S	I	R	S	I	R	S	I	R
	N. (%)	N . (%)	N. (%)	N. (%)	N. (%)	N. (%)	N. (%)	N. (%)	N. (%)	N. (%)	N . (%)	N. (%)
Amikacin (30 µg)	16 (80)	4 (20)	0 (0)	20 (100)	0 (0)	0 (0)	12 (60)	8 (40)	0 (0)	7 (70)	3 (30)	0 (0)
Ampicillin (10 µg)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	10 (100)
Cefotaxim (30 µg)	20 (100)	0 (0)	0 (0)	18 (90)	2 (10)	0 (0)	16 (80)	4 (20)	0 (0)	10 (100)	0 (0)	0 (0)
Chloramphnicol (30µg)	18 (90)	2 (10)	0 (0)	14 (70)	6 (30)	0 (0)	12 (60)	8 (40)	0 (0)	8 (80)	2 (20)	0 (0)
Doxycycline (30µg)	0 (0)	0 (0)	20 (100)	0 (0)	4 (20)	16 (80)	0 (0)	2 (10)	18 (90)	0 (0)	0 (0)	10 (100)
Erythromycin (15µg)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	10 (100)
Gentamycin (10µg)	18 (90)	2 (10)	0 (0)	20 (100)	0 (0)	0 (0)	16 (80)	4 (20)	0 (0)	9 (90)	1 (10)	0 (0)
Nalidixic acid (30 µg)	20 (100)	0 (0)	0 (0)	12 (60)	8 (40)	0 (0)	14 (70)	6 (30)	0 (0)	10 (100)	0 (0)	0 (0)
Norfloxacin (10µg)	20 (100)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	10 (100)	0 (0)	0 (0)
Oxytetracycline (30 µg)	12 (60)	6 (30)	2 (10)	8 (40)	8 (40)	4 (20)	12 (60)	4 (20)	4 (20)	5 (50)	3 (30)	2 (20)
Penicillin (10u)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	10 (100)
Polymixin-B (300u)	10 (50)	6 (30)	4 (20)	12 (60)	6 (30)	2 (10)	10 (50)	8 (40)	2 (10)	6 (60)	2 (20)	2 (20)
Trimethoprim + Sulfamethaxzole (1.25+23.75 µg)	14 (70)	6 (30)	0 (0)	16 (80)	4 (20)	0 (0)	14 (70)	6 (30)	0 (0)	5 (50)	5 (50)	0 (0)
Vancomycin (30µg)	0 (0)	0 (0)	20 (100)	0 (0)	0 (0)	20 (100)	0 (0)	2 (10)	18 (90)	0 (0)	2 (20)	8 (80)
Total resistance Cases			**106**			**102**			**102**			**52**

% is calculated according to the total number of isolates

S: Sensitive

I: Intermediate sensitive

R: Resistant

N: Number

Table (3): *Patterns of antimicrobial phenotype of total Aeromonas isolates recovered from Manzala lake fish and water (n= 70).*

Antimicrobial agents	Sensitive		Intermediate		Resistance	
	No	%	No	%	No	%
Amikacin (30 µg)	55	78.6	15	21.4	0	0
Ampicillin (10 µg)	0	0	0	0	70	100
Cefotaxim (30 µg)	64	91.4	6	8.6	0	0
Chloramphnicol(30µg)	52	74.3	18	25.7	0	0
Doxycycline (30µg)	0	0	6	8.6	64	91.4
Erythromycin (15µg)	0	0	0	0	70	100
Gentamycin (10µg)	63	90	7	10	0	0
Nalidixic acid (30 µg)	56	80	14	20	0	0
Norfloxacin (10µg)	70	100	0	0	0	0
Oxytetracycline (30 µg)	37	52.9	21	30	12	17.1
Penicillin (10u)	0	0	0	0	70	100
Polymixin-B (300u)	38	54.3	22	31.4	10	14.3
Trimethoprim+Sulfamethaxzole (1.25+23.75 µg)	49	70	21	30	0	0
Vancomycin (30µg)	0	0	4	5.7	66	94.3

% is calculated according to total number of isolates (n= 70)

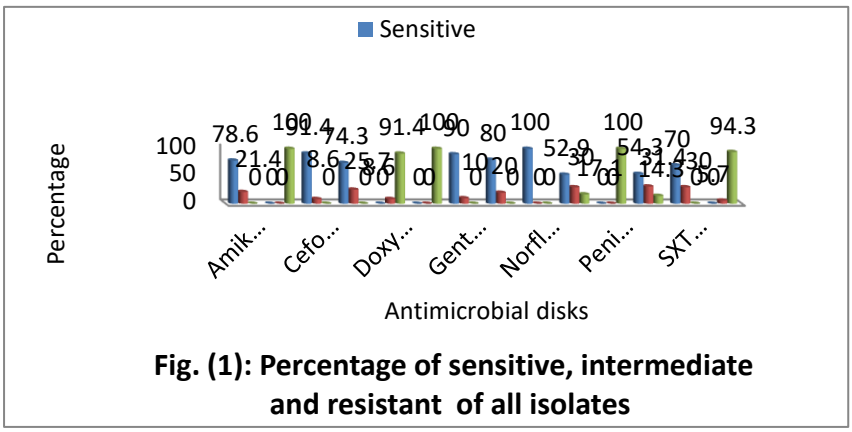

Fig. (1): Percentage of sensitive, intermediate and resistant of all isolates

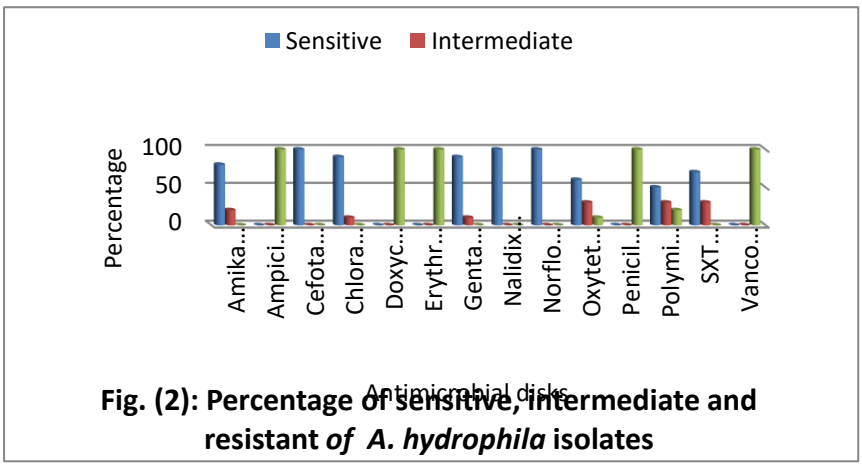

Fig. (2): Percentage of sensitive, intermediate and resistant *of A. hydrophila* isolates

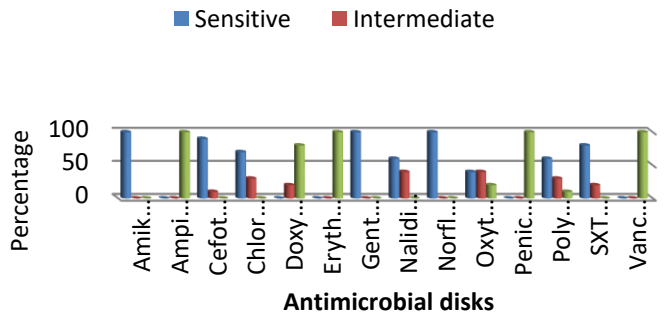

Fig. (3): Percentage of sensitive, intermediate and resistant of *A. sobria* isolates

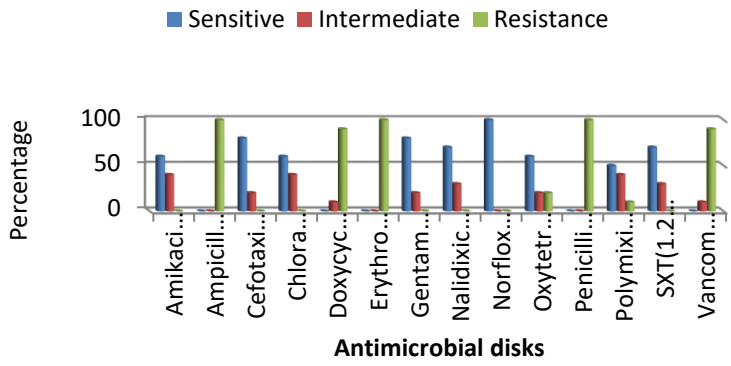

Fig. (4): Percentage of sensitive, intermediate and resistant of *A.Caviae* isolates

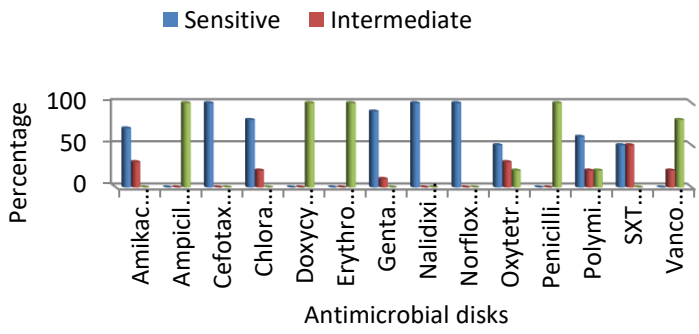

Fig.(5): Percentage of sensitive, intermediate and resistant of *A.schubertii* isolates

Table (4): MAR index and resistance patterns of the tested *Aeromonas spp.* isolates:

Aeromonas spp.	Total number of antibiotic resistance cases	MAR index
A. hydrophila	106	0.38
A. sobria	102	0.36
A. caviae	102	0.36
A. schubertii	52	0.37

Discussion

The present result in **Table (1)** revealed that a total number of 258 isolates belonging to *Aeromonas* spp. were recovered from fishes and lake water samples and they were biochemically identified into 4 species (*A. hydrophila*, *A. sobria*, *A. caviae* and *A. schubertii*). 70 isolated *Aeromonas* species were selected for Antimicrobial susceptibility test to 14 different antibacterial agents.

Antibiogram and antimicrobial profiles of *Aeromonas* species isolates recovered from Manzala Lake fish and water were summarized in **Table (2), (3)** and graphically represented in **Fig. (1)**.

The present study revealed that all strains showed (100%) sensitivity to Norfloxacin. Similar results were reported by ***Aravena et al. (2012)*** who found 100% sensitivity of *Aeromonas* spp. to norfloxacin. Also, high sensitivity to cefotaxime (91.4%), gentamycin (90%), nalidixic acid (80%) and amikacin (78.6%) were recorded. Furthermore, variable sensitivity of *Aeromonas* isolates to other antibiotics was observed which includes chloramphenicol (74.3%), trimethoprim + sulphamethoxazole (70%), polymexin-B (54.3%) and oxytetracycline (52.9%). In this concern, ***Ko et al. (2003)*** recorded that *Aeromonas* spp. are sensitive to cephalosporins, aminoglycosides, chloramphenicol, tetracycline, trimethoprim-sulfametoxazole and fluoroquinolones. However, ***Petersen and Dalsgaard (2003)*** found that most of *Aeromonas* strains were resistant to the commonly used antibiotics such as chloramphenicol, tetracycline and trimethoprim.

Absolute resistance of isolated *Aeromonas* spp. to ampicillin, and penicillin was observed in the present study which may be attributed to β- lactamase activity in the resistant isolates. The present findings agreed with ***Carnahan et al. (1991b)*** who mentioned that ampicillin resistance has been characteristic of genus *Aeromonas*. Additionally, ***Daood (2012)*** revealed that *Aeromonas* spp. were resistant to penicillins (penicillin, ampicillin, carbenicillin and ticarcillin), However, ***Stratev et al. (2013)*** found penicillin-sensitive strains. All tested strains showed (100%) resistance to Erythromycin. Similar findings reported by

Sreedharan et al. (2012) who reported that all *Aeromonas* isolates were resistant to erythromycin.

The present results indicated that there were a slight difference in antibiogram profiles and antimicrobial resistance pattern among *Aeromonas* species isolates as shown in **Table (2)**, **Fig. (2), (3), (4) and (5).**

Concerning *A. hydrophila* isolates, results in **Table (2)** and **Fig. (2)** showed that isolates exhibited (100%) sensitivity to cefotaxime, nalidixic acid and norfloxacin, also showed high sensitivity to chloramphenicol (90%), gentamycin (90%), amikacin (80%) and trimethoprim + sulphamethoxazole (70%). Meanwhile, they were sensitive to moderately sensitive to, oxytetracycline (60%) and polymexin-B (50%). Our results agreed with ***Vila et al. (2002)*** who stated that all *A. hydrophila* isolates were highly sensitive to cefotaxime 100%. They agreed with ***Kaskhedikar and Chhabra (2010)*** who reported that *A. hydrophila* showed 100% sensitivity to ciprofloxacin, cephotaxime, gentamycin and nalidixic acid, while 50% of the bacteria were susceptible to oxytetracycline. However, ***Guz and Kozinska (2004)*** found that all isolates of *A. hydrophila* were sensitive to trimethoprim-sulfamide. Contrariwise ***Rawal et al. (2016)*** who reported that all *A. hydrophila* strains were found resistant to polymyxin B, amikacin and trimethoprim.

The present results revealed that all *A. hydrophila* isolates were resistant to ampicillin (100%), doxycycline (100%), erythromycin (100%), penicillin G (100%) and vancomycin (100%). These results agreed with ***Awan et al. (2009)*** who indicated that *A. hydrophila* strains were 100% resist to ampicillin and vancomycin. Also, ***Vivekanandhan et al. (2002)*** observed resistance against erythromycin of more than 95% of *A. hydrophila* isolates. Meanwhile, ***Revina et al. (2017)*** found that *A. hydrophila* isolates were (50%) resistance to doxycycline, contra wise ***Popovic et al. (2000)*** who found that *A. hydrophila* strains were sensitive to erythromycin.

Concerning *A. sobria* isolates, results in ***Table*** **(2)** and **Fig. (3)** revealed that *A. sobria* isolates showed (100%) sensitivity to amikacin, gentamycin, and norfloxacin, also show high sensitivity to cefotaxime (90%), trimethoprim + sulphamethoxazole (80%) and chloramphenicol (70%). Meanwhile, they were sensitive to moderately sensitive to nalidixic acid (60%), polymexin-B (60%) and oxytetracycline (40%). This agreed with ***Awan et al. (2009)*** who demonstrated that *A. sobria* strains were sensitive to amikacin 100%, gentamicin 100% and cefotaxime 100% although ***Wang and Silva (1999)*** isolated *A. sobria*

strain sensitive to tetracycline (100%) and trimethoprim + sulphamethoxazole (100%). Contrariwise, ***Krovacek et al. (1992)*** who reported that *A. sobria* isolates were resistant to tetracycline and trimethoprim + sulphamethoxazole.

On the other hand, *A. sobria* isolates exhibited (100%) resistance to ampicillin (100%), erythromycin (100%), penicillin G (100%) and vancomycin (100%) and exhibit high resistance to doxycycline (80%). Our result agreed with ***Guz and Kozinska (2004)*** who reported that all *A. sobria* strains were resistant to ampicillin and penicillin, but less resistant to erythromycin (52%).

Concerning to *A. caviae*, results in **Table (2)** and **Fig. (4)** revealed that isolates were (100%) sensitive to norfloxacin, cefotaxime (80%), gentamycin (80%), nalidixic acid (70%) and trimethoprim + sulphamethoxazole (70%). Meanwhile, they were less sensitive to moderately sensitive to amikacin (60%), chloramphenicol (60%), oxytetracycline (60%) and polymexin-B (50%). Our results agreed with ***Vila et al. (2002)*** who revealed that *A. caviae* isolates were sensitive to nalidixic acid 74% trimethoprim/sulfamethoxazole 79%, but highly sensitive to cefotaxime 100%, gentamicin 100%, amikacin 100%. On the other hand, ***Yucel et al. (2005)*** stated that *A. caviae* strains were resistant to trimethoprim, but less resistant to chloramphenicol.

All *A. caviae* isolates showed resistance to ampicillin (100%), erythromycin (100%) and penicillin G (100%) and exhibited high resistance to doxycycline (90%) and vancomycin (90%). These results were confirmed also by ***Daood (2012)*** who demonstrated that all *A. caviae* were resistant to ampicillin and penicillin, but ***Awan et al. (2009)*** reported that *A. caviae* strains were resistant to vancomycin (100%), ampicillin (84.6%) and erythromycin (81.8%).

Concerning *A. schubertii* isolates, results in **Table (2)** and **Fig. (5)** revealed that all *A. schubertii* isolates (100%) were sensitive to cefotaxime, nalidixic acid, norfloxacin, gentamycin (90%), chloramphenicol (80%) and amikacin (70%). Meanwhile, they were less sensitive to moderately sensitive to polymexin-B (60%), oxytetracycline (50%) and trimethoprim + sulphamethoxazole (50%). In this concept, ***Awan et al. (2009)*** found that *A. schubertii* strains were (100%) sensitive to cefotaxim, gentamicin and (50%) to trimethoprimsulfamethox and ***Liu and Li (2012)*** found that all *A. schubertii* isolates were susceptible to chloramphenicol, gentamicin, norfloxacin, oxytetracycline, sulfamethoxazole/trimethoprim.

On the other hand, all isolates were resistant to ampicillin, doxycycline (100%), erythromycin (100%),

penicillin G (100%), vancomycin (80%), but showed less resistance (20%) to oxytetracycline and polymexin-B. These results agreed with ***Awan et al. (2009)*** who revealed that all strains of *A. schubertii* were resistant to ampicillin and erythromycin.

Results in **Table (4)** revealed that the MAR index values of all isolates higher than 0.2 as they were 0.38, 0.36, 0.36 and 0.37 for *A. hydrophila*, *A. sobria*, *A. caviae* and *A. schubertii*, respectively. These results agreed with ***Paul et al. (2015)*** who found the MAR index of *Aeromonas* spp. varied from 0.3 to 0.8 that indicated possible abuse of antibiotics. Also, ***Hossain et al. (2019)*** revealed that the MAR index values ranged from 0.19- 0.44 to 90.7% of *Aeromonas* isolates showed multidrug resistance.

MAR index exposes the spread of bacteria resistance in a given population. MAR index more than 0.2 indicates that the bacterial strain originates from an environment where many antibiotics are used **(*Ehinmidu, 2003)*** and thus posed health risk to human through the food chain. ***(Gwendelynne et al., 2005).*** In this study high incidence of multiple antibiotic resistances amongst *Aeromonas* species was detected suggesting presence of wastewater which acts as a reservoir of antibiotic resistance determinants. This become of particular importance especially with the increasing number of *Aeromonas* spp. infections and MDR strains that are spreading around the world ***(Batra et al., 2016).***

In conclusion Manzala Lake is exposed to high inputs of pollutants from industrial, domestic, and agricultural sources so regular monitoring the prevalence of *Aeromonas* and spread of antibiotic resistance is particularly important especially with the increasing utilization of lake water to cultivated and fatten fish of various species. There are need to ensure that discharged final effluents of wastewater treatment plants are adequately treated to remove such pathogens as *Aeromonas* species to prevent the dissemination of multidrug-resistant determinants into the receiving water bodies' environment.

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المقاومة للمضادات البكتيرية لأنواع من الأيروموناس المعزولة من أسماك ومياه بحيرة المنزلة

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الملخص العربى

أجريت هذه الدراسة علي عدد ٧٠ من معزولات الأيروموناس التى تم عزلها من ١٠٠ سمكة من نوع البلطي و ١٠٠ سمكة من نوع البوري، و ٥٠ عينة من مياة منطقة الجميل، الواقعة في الجانب الشمالي الشرقي لبحيرة المنزلة، والتي تم تصنيفها بالطريقة البيوكيميائية وتشمل الأيروموناس هيدروفيلا (٢٠) و الأيروموناس سوبريا (٢٠) و الأيروموناس كافيا (٢٠) و الأيروموناس شابرتي (١٠)، لمعرفة حساسية العترات ل ١٤ من المضادات الحيوية واسفرت النتائج ان العترات المعزولة التى تم إختبارها أظهرت بعض الاختلافات فى مقاومتها للمضادات الحيوية المختلفة مع وجود تباين فى حساسيتها وفى المجمل أظهرت العترات المعزولة حساسية عالية (١٠٠٪) للنوروفلوكساسين وكذلك سجلت معظم العترات حساسية جيدة لكل من السيفوتاكسيم (٩١,٤٪) والجينتاميسين (٩٠٪) والنالديكيسك اسيد (٨٠٪) والاميكاسين (٧٨,٦٪) والكلورمفينيكول (٧٤,٣٪) . بينما أظهرت العترات المعزولة بصفة عامة (١٠٠٪) مقاومة للمضادات الحيوية التالية الأمبسلين و الإيرثرومايسين والبنسيللن واظهرت مقاومة عالية للفانكوميسن (٩٤,٣٪) والدوكسي سيكلين (٩١,٤٪). هذا وقد وجد أن قيم مؤشر مقاومة مضادات الميكروبات المتعدد أعلى من ٠,٢ حيث كانت ٠,٣٨و ٠,٣٦و ٠,٣٦و ٠,٣٧ للأيروموناس هيدروفيلا والأيروموناس سوبريا والأيروموناس كافيا والأيروموناس شابرتي علي التوالي. مما يدل علي وجود مقاومة متعددة للعقاقير.