Prevalence and Antimicrobial susceptibility of Pathogenic Bacteria in Tilapia zilli and Mugil capito

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Abstract

A total of 300 marine fishes of two different species represented as (150) tilapia Zilli and 150 mugil capito) were freshly collected randomly from the different markets in the Ismailia governorate, during different seasons. The samples were subjected to full clinical, postmortem, and bacteriological studies. The common clinical signs were darkness in the skin, hemorrhage in the base of fins, eyes & different parts of the body, abdominal distention, congestion in gills, and increase in mucous secretion. The postmortem finding showed white serous fluid tinged with blood in the abdominal cavity and pale or congested liver, kidney, and spleen. The result indicated that the prevalence of bacterial pathogens among naturally infected marine fishes were *Aeromonas hydrophila* 91 isolates (39.39%), *V. alginolyticus* 67 isolates (29%), *P. fluorescens* 42 isolates (18.18%), *V. fluvialis* 17 isolates (7.4%), *P.aeruginosa* 14 (6.06%). The result of the antibiogram revealed that *Aeromonas hydrophila* is highly sensitive to ciprofloxacin, while ciprofloxacin and rifampicin are more effective against *P. fluorescence*, also ciprofloxacin and amikacin against *P. aeruginosa*, while *V. alginolyticus* was highly sensitive to oxytetracycline and rifampicin. Ciprofloxacin and naldixic acid more effective against *V. fluvialis*

Key words: Tilapia zilli, Mugil capito, Aeromonas spp, Sensitivity test

Introduction

Bacteria play the main role in producing the disease in fish although incidence of bacterial diseases in fish generally do not develop simply as the result of exposing a host to an infectious agent ***Wedekind et al. (2010)***. Moreover, bacterial fish disease is a major problem facing fish farming industry, which is currently fast through a variety of growing with annual increase approximately 12%. ***FAO (2004)***.

The normal bacterial flora of fish is direct reflection of bacterial population water in which they swim and bacterial infections such as Vibriosis, Aeromonads, Pseudomonads, Photobacteria, Streptococci and Staphylococci were recorded in several fingerlings, juveniles, adults and brood stocks of some marine fish species ***(Toranzo et al., 2005).*** The prevalence of bacterial pathogens have been well documented in several cultured and wild fresh water fish species, however; only a few bacteriological surveys on the prevalence of bacterial pathogens responsible for outbreaks in marine fish, it is important to point out that diseases classically considered as typical of fresh water aquaculture, such as furunculosis (*Aeromonas salmonicida*), bacterial kidney disease (BKD) (Renibacterium salmoninarum) and some types of streptococcosis, are today important problems also in marine culture ***(Toranzo et al., 2005)***

Fish diseases are the result of the interaction among pathogen, host and environment. Consequently, only multidisciplinary studies involving the virulence factors of the pathogenic microorganisms, aspects of the biology and immunology of the fish, as well as a better understanding of the environmental conditions affecting fish cultures, will allow the application of adequate measures to control and prevent the microbial diseases limiting the production of marine fishes. ***Salah et al. (2012)***.

Therefore, the current investigation aimed to isolation and identification of bacterial pathogens from naturally infected marine fishes (Tilapia zilli and Mugil capito) with special reference to the best effective antibiotics for controlling the infection.

Material and methods

sampling:

A total of 300 marine fishes of two different spp. represented as (150 T.zilli and 150 M.capito) were freshly collected randomly from different markets in Suez Canal area, Ismailia governorate

during different seasons (from January 2019 to December 2019). In a rate 37 fish of each species were collected and examined seasonally, Clinical and post mortem examination were carried out using the methods described by ***Hudzicki (2009).***

Isolation of suspected bacteria

Samples from kidney liver, gills and spleen from examined fishes were cultured in general and selective media; tryptic soy broth, tryptic soy agar (Oxoid) supplemented with 2% NaCl (w/v), thiosulphate citrate bile salt agar (Oxoid), aeromonas base media supplemented with ampicillin and pseudomonas agar base media supplemented with 2% NaCl (w/v) and glycerin 2%. All inoculated media were incubated at 28℃ for 1- 2 days.

Identification of the isolated bacteria

Pure cultures of isolated bacteria were identified biochemically according to (***Brenner et al., 2005)*** and final confirmation of bacterial isolates was achieved by using the analytical profile index of API20E system ***(Buller, 2004).***

Antibiogram sensitivity test

This test was done following ***Hudzicki (2009)*** by using the disc diffusion method on Muller's Hinton agar medium and the following discs Oxytetracycline, Ampicillin, Amoxycillin, Lincomycin, Ciprofloxacin, Colistin sulphate, Nalidixic acid, Amikacin, Rifampcine and Erythromycin were kindly recorded. Susceptibility was defined as absence of growth on solid medium containing any of these antimicrobial agents. Presence of growth indicated non-susceptibility. The diameters of the inhibition zone appearing on the agar plate were measured and interpreted as susceptible (S), intermediate (I) or resistant (R).

Results

Clinical examination:

The examined fish showed darkness of external body surface, opacity of eye, increased in mucous secretion, exophthalmia and some fishes showed hemorrhage in eye, scales detachment and large irregular hemorrhagic areas into many parts of the body, in some cases, the gills were congested while in others appeared pale in color and swollen photo (1&2).

Postmortem examination

The examined fish showed white serous fluid in abdominal cavity, some tinged with blood. The liver surface and in some cases appeared congested while in some fish appeared to be normal with normal texture.

kidney congested and slightly enlarged. The intestine of some cases appeared normal. In some fish appeared hemorrhagic areas in the abdominal wall & on peritoneum and some fishes apparently healthy without any internal or external lesions.as shown in photo (3).

Bacteriological examination:

The result indicated that 150 naturally infected and 150 apparently healthy marine fishes were found to be infected with different types of bacteria.The samples were taken from liver, kidney, spleen, gills. The result demonstrated that the total number of bacterial isolates from (300) fish (69) isolate were negative with percentage of (23%) and 231 isolates were positive with percentage of (77%) from which 103 isolates from M.capito and 128 isolates belonging to T.zilli fish as shown in table (3), from the isolates there were 22 (14.29%) were oxidase positive bacterial isolates, Aeromonas (the most common bacterial pathogens) 158 (68.4%) (*A. hydrophila* 91(39.39),*V. alginolyticus* 67 (29%) , *P. fluorescens* 42(18.2%), *P. aeruginosa* 14 (6.1%), *V. fluvialis* 17 (7.4%) as shown in table (1).

the bacteriological examination revealed that the identification of 91 (39.39%) of *A. hydrophila* and the identification of 42 (18.2%) of *P. fluorescens* and 14 (6.1%) of *P. aeruginosa* and *V. fluvialis* isolates were identified as 17 (7.4%) depending on different tests applied to demonstrate the culture and biochemical characters as present in table (1).

Concerning the seasons, the highest prevalence of bacterial infection recorded in summer season (33.33%) followed by spring (24.42%), then autumn (21.97%), while minimal prevalence recorded in winter season (20.46%) as recorded in table (4) and figure (1).

Concerning the organs, the highest prevalence of *A. hydrophila* was recorded in liver (44.23%), followed by the kidney (36.54%) then spleen (11.54%). While the lowest prevalence recorded in gills (7.69%) and the highest prevalence of *Ps. fluorescens* was recorded in kidney (50%), followed by liver (25%), then gills (16.67%). While minimal prevalence was recorded in spleen (8. 33%). while the highest prevalence of *P. aeruginosa* was recorded in liver and kidney with the same percent (37.5%) and the minimal prevalence was recorded also equally in spleen and gills (12.5%). and the highest prevalence of *V. alginolyticus* was recorded in liver (36.84%), then kidney (31.58%), then spleen (23.68%). While the lowest prevalence

recorded in gills (7.89%) and the highest prevalence of *V. fluvialis* recorded in kidney (40%), then spleen (30%), followed by liver (20%). While the lowest prevalence was recorded in gills (10%) as shown in table (5) and figure (2).

Antibiogram sesitivity test

The result of antibiogram sesitivity test revealed that ciprofloxacin was more effective against *Aeromonas hydrophila* while ciprofloxacin and rifampicine more effective against *Pseudomonas fluorescens*, also ciprofloxacin and amikacin against *P. aeruginosa*, while *V. alginolyticus* was highly sensitive to rifampicine. Ciprofloxacin and naladixic acid more effective against *V. fluvialis* as shown in table (6).

Table (1): *The biochemical and morphological characters of isolated bacteria from examined marine fishes (T.zilli &M.capito)*

	A. hydrophila.	***P. fluorescens.***	***P. aeruginosa.***	***V. alginolyticus.***	***V. fluvialis***
1. Gram stain.	-	-	-	-	-
1. Shape.	Short rod.	Short rod	Short rod	Comma shape bacilli	Curved cell
2. Motility.	+	+	+	+	+
3. Cytochrome oxidase. (Ox).	+	+	+	+	+
4. Catalase Test.	+	+	+	+	+
5. B-Galactosidase production (OPNG).	+	-	-	-	+
6. Arginine hydrolase production (ADH)	-	+	+	-	-
7. Lysine decarboxylase production (LDC).	-	+	-	+	-
8.Ornithinedecarboxylase production (ODC).	+	+	-	+	+
9. Citrate utilization (CIT)	-	+	+	-	-
10. H_2S production (H_2S).	-	-	-	-	-
11. Urease production (URE)	-	-	+	-	-
12. Tryptophan deaminase production (TDA).	-	-	-	-	-

13. Indole production (IND).	+	-	-	+	+
14. Acetoin production (VP).	-	-	+	-	+
15. Gelatinase production (GEL)	+	-	+	+	+
16. Acid from glucose	+	-	-	+	**V**
17. Acid from manitole.	+	-	-	+	+
18. Acid from inositol.	+	-	-	-	-
19. Acid from sorbitol	+	-	-	-	-
20. Acid from rhaminos.	-	-	-	-	-
21. Acid from sacrose	+	-	-	+	+
22. Acid from melobiose	-	-	-	-	-
23. Acid from amylase	+	-	-	-	-

Table (2) *Prevalence of bacterial isolates recovered from examined marine fishes (T.zilli and M.capito) after oxidase +ve test*

A.hydrophila	***P. fluorescens***	***P. aeruginosa***	***V. alginolyticus***	***V.fluvialis***
91	42	14	67	17
39.39	18.18	6.06	29	7.4

Table (3) *Prevalence of bacterial species isolated from examined M.capito and T.zilli*

Fish spp. / **Bacterial spp.**	**M.capito**		**T.zilli**	
	No	**%**	**No**	**%**
A. hydrophila	40	39	72	56
P. fluorescens	14	13.55	26	20.55
P. aeruginosa	12	11.86	0	0
V. alginolyticus	7	6.77	23	17.80
V. fluvialis	30	28.8	7	5.48
Total	**103**	**44.70**	**128**	**55.30**

Table (4) *Seasonal prevalence of bacterial species in examined marine fishes*

Season. / Bacterial Isolates.	Winter		Spring		Summer		Autumn		Total	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
A. hydrophila	24	51.85	21	37.5	32	40.91	14	27.59	91	39.39
P.fluorescens	14	29.63	14	25	0	0	14	27.59	42	18.18
P.aeruginosa	0	0	7	12.5	7	9.090	0	0	14	6.06
V.alginolyticus	9	18.52	12	21.88	28	36.36	18	34.48	67	29
V.fluvialis	0	0	2	3.13	10	13.64	5	10.35	17	7.4
Total	**47**	**20.46**	**56**	**24.24**	**77**	**33.33**	**51**	**21.97**	**231**	**100**

Table (5) *Incidence of bacterial species isolated from examined organs of marine fishes*

organ	***A.hydropila***		***P. fluorescens***		***P. aeruginosa***		***V.alginolyticus***		***V. fluvialis***		**Total**	
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Liver	40	44.23	11	25	5	37.5	25	36.84	3	20	84	36.36
Kidney	33	36.54	21	50	5	37.5	21	31.58	7	40	87	37.84
Spleen	11	11.54	3	8.33	2	12.5	16	23.68	5	30	37	15.91
Gills	7	7.69	7	16.67	2	12.5	5	7.89	2	10	23	9.85
Total	**91**	**39.39**	**42**	**18.18**	**14**	**6.06**	**67**	**29**	**17**	**7.4**	**231**	**100**

Table (6) *Antibiogram of isolated bacterial species from examined marine fishes*

Antimicrobial disc	Isolated strains.							
	A. hydrophila		***P. fluorescens***		***P. aeruginosa***		***V. alginolyticus***	
	Inhb.z (mm)	**React.**	**Inhb.z (mm)**	**React.**	**Inhb.z (mm)**	**React.**	**Inhb.z (mm)**	**React.**
Ciprofloxacin	27	S	22	S	30	S	24	S
Colistine sulfate	7	R	10	M	10	M	10	M
Oxytetracycline	22	S	18	S		R	26	S
Erythromycin	16	M	16	R	12	R	15	R
Nalidixic acid	23	S	20	S	12	R		R
Amoxicillin		R	16	S	12	M	12	R
Rifampicine	15	R	21	S	14	R	26	S
Lincomycin		R		R		R		R
Ampicillin		R	12	R		R	12	R

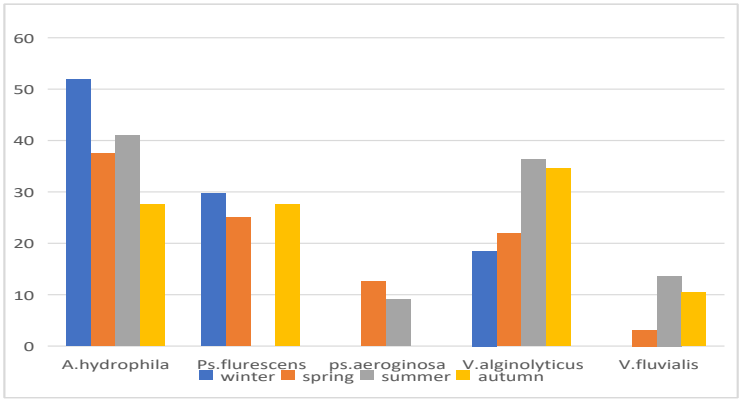

Figure (1) *Seasonal prevalence of bacterial species in examined marine fishes*

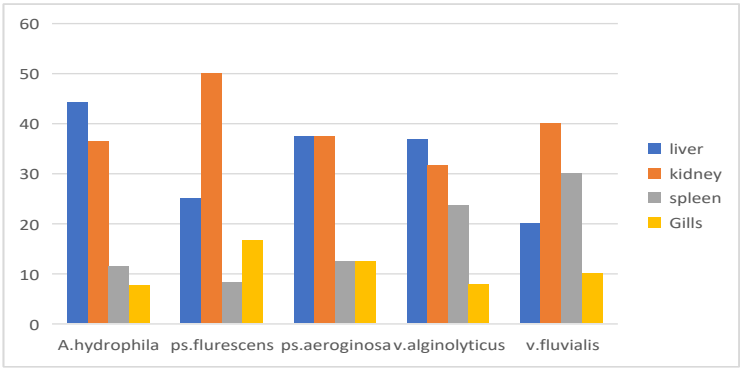

Figure (2) Incidence of bacterial species from examined organs of marine fishes

Photo (1) Diseased Capito with hemorrhage on body surface and on anal & ventral Fines

Photo (2) Diseased T. zilli showed hemorrhage around gill cover and on body surface

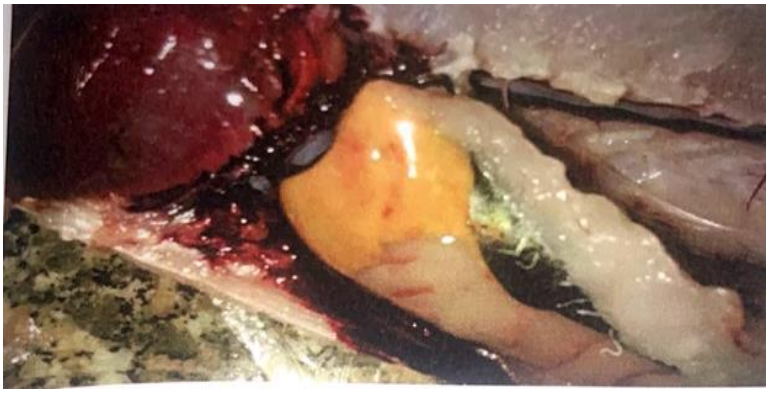

Photo (3) Diseased M. capito showed pale liver with hemorrhagic patches

Discussion

Fish are susceptible to a wide variety of bacterial pathogens. Many of these bacteria capable of causing disease are considered by some to be saprophytic in nature. These bacteria only become pathogen when fishes are physiologically unbalanced, nutritionally deficient, or there is other exophthalmia and some fish showed hemorrhages in eye, scales detachment and large stressor, i.e., poor water quality, overstocking, which allow opportunistic bacterial infection to proceed ***(Noga 2010)***.

Naturally infected marine fish (M.capito & T.zilii) were clinically examined and the examination revealed the presence of darkness of external body surface, opacity of eye, increased in musous secretion, irregular hemorrhagic areas into many parts of the body, at base of fins, on gills cover, at the

anal region, anal fin and caudal peduncle. There was abdominal distention observed in some fish and also fin & tail rot. This result supported by previous studies which revealed by ***Megahed (2000), Ahmed (2004), Toranzo et al. (2005), El-Ashram and Azza (2006)*** **and** ***Moustafa et al. (2010)*** who recorded these signs beside anal inflammation as well as prolapsed. Regarding bacterial examination of some marine fish (M.capito & T.zilli) present study detected that Tialpia spp. was the highest species infected with bacterial pathogens (55.30%) and Mugil spp.was the lowest one (44.70%) these may due to Mullet spp. was immunologically protected than Tilapia spp. This result agrees with ***El-Refaey (2013)*** who reported that regarding bacterial examination of fresh water fish, the study displayed that Tilapia spp was the highest spp. and the Mullets was the lowest one.

The present study showed that Aeromonas spp was isolated on Tryptic soya agar plate's colonies were creamy color, raised, rounded, shiny colonies. These results agree with ***Megahed (2000), Cipriano (2001), Ahmed (2004)*** **and** ***ElGendy (2007).***

The naturally infected marine fish was recorded in the summer season (33.33%), followed by the spring (24.24%), then autumn (21.97%), in contrast the lowest prevalence of infection recorded in winter (20.46%). This variation may due to the change in water environment as increase water temperature which help bacteria proliferation and decrease oxygen dissolved in waste and this considered as stress factor on fish and made them susceptible for infection and these results supported by those reported by ***Company et al. (1999) .***

In regards to the seasonal prevalence of *A. hydrophila* the result pointed out that the highest prevalence was recorded in winter (51.85%), followed by summer season (40.91%), then spring (37.5%), while the lowest recorded in autumn (27.59%). Several researches investigated that Aeromonads prevail during summer season. This may attribute to the activity during high temperature due to stress condition that decrease fish immunity rendering them susceptible to diseases. These results are in concordance with those obtained by ***Hayes (2000).***

In the present study *V. alginolyticus* recorded (29%) and are higher than those detected by ***Mustafa et al. (2010)***, and this higher prevalence of *V. alginolyticus* indicate its importance in marine culture as

supported by ***Zhu and Qian (2000).***

The highest prevalence of *V. fluvialis* was recorded in summer (13.64%), followed by autumn (10.35%) while minimal in spring (3.13%) and not isolated in winter.This result similar to ***Nam and Joh (2007)*** .

In regards to the total prevalence of *Pseudomonas*, the study recorded that (24.24%) of infected fish were positive for Pseudomonas infection. These results were higher than that reported by ***Hussain (2002)*** **and** ***Mustafa et al. (2010)***.

In regards to the seasonal prevalence of *P. fluorescens* our results detected that the highest prevalence of *P. fluorescens* was recorded in winter (29.63%), then autumn (27.59%) and spring (25%). While not recorded in summer season. This reveals that *Ps. fluorescens* has certain affinity to low temperature for propagation and spreading infection. This result agrees with ***Hoshino et al. (1997)***.

Concerning to the prevalence of bacterial isolates in various organs our investigation demonstrated that, prevalence of total bacterial isolates was (37.84%) in kidney so it is the most predominant site for isolation of bacterial pathogens that causing septicemia as it is considered as one of the main hematobiotic organs of fish, followed by liver (36.36%), then spleen (15.91%) and finally gills (9.85%). And this result goes along with ***El-Refaey (2013)***.

Results of antibiotic sensitivity revealed that *A. hydrophila* was highly sensitive to ciprofloxacin, nalidixic acid, oxytetracycline and also sensitive to amikacin. Intermediated to erythromycin but highly resistant to ampicillin, amoxicillin and linomycine and also rersistant to rifampicine and colistin sulfate. These result concur with ***El Ashram and Azza (2006),*** On the other hand disagree with ***ELAdawy (2002)*** who showed that aeromonas was highly sensitive to amikcacin.

Results of antibiotic sensitivity revealed that *Vibrio alginolyticus* was sensitive to ciprofloxacin, oxytetracycline and rifampicine. While intermediate to colistin sulfate. Otherwise resistant to ampicillin, erythromycin & lincomycine.These result assent with ***Wafeek et al. (2007)*** , and with ***Enany et al. (2011)*** .

Results showed that antibiotic sensitivity revealed that *P. fluorescens* was highly sensitive to ciprofloxacin, rifampicine & amikacin and also sensitive to oxytetracycline & nalidixic acid. Intermediated to colistin sulfate. While resistant to erythromycin, ampicillin.These results accede with ***Ahmed (2004), El-Ashram***

and Azza (2006) **and** ***Enany et al. (2011)*** .

Results of antibiotic sensitivity revealed that *P. aeruginosa* was highly sensitive to ciprofloxacin & amikacin. Intermediate for amokicillin & colisten sulfate and resistant to nalidixic acid, rifampicine and highly resistant to ampicillin, oxytetracycline, erythromycin & lincomycine. These result agree with ***Akinbowale et al. (2007)*** and ***Eissa et al. (2010)*****.**

Conclusion

It was concluded that the highest prevalence of bacterial isolates causing infection in marine fish were by *A.hydrophila* followed by *V. alginolyticus*, then *P. fluorescens* and the lowest prevalence was recorded for *P. aeruginosa* and *V. fluvialis* And the higher rate of infection recorded in summer season followed by the spring then autumn, in contrast the minimal incidence of infection were recorded in winter. Ciprofloxacin was considered the drug of choice for treatment and prevention of bacterial infection in marine fish either alone or combined with other antimicrobials.

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انتشار و مدى قابلية المضادات الحيوية للبكتريا الممرضة في أسماك الشبار الأخضر والطوبارة

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الملخص العربى

أجريت هذه الدراسة على عدد 300 سمكة من أسماك المياة المالحه الممثلة فى (أسماك الطوباره و الشبار الأخضر) و التي جمعت عشوائيا من مختلف أسواق مدينه الأسماعيليه موسميا . و لقد تم اجراء لها الفحوصات الظاهريه و التشريحيه والبكتريولوجيه .أظهرت هذه الدراسه ان العلامات المرضيه الرئيسيه في الأسماك المصابه كانت في صورة بقع داكنه علي سطح الجسم الخارجي ,نزف في أرجاء مختلفه من الجسم وخاصه في قاعدة الزعانف والعين وانتفاخ في المنطقه البطنيه ,احتقان في الخياشيم , وزياده في افراز مخاط أما الصفه التشريحيه فكانت علي هيئة احتقان مع تضخم في الكلية و الطحال مع ظهور الكبد أحيانا ما بين البني الداكن الي الأصفر الباهت مع وجود سائل شفاف و في بعض الأحيان مختلط بالدم في التجويف البطني لبعض الأسماك.

كما أوضحت النتائج عن مدي تواجد البكتريا الممرضه في أسماك المياه المالحه المصابه طبيعيا أن البكتريا السائده هي الأيروموناس هيدروفيلا (39.39%) يليها الفيبريوالجينوليتيكس (29%) يليها السودوموناس فلوريسنس (18.18%) ثم الفيبريوفلوفياليز (7.4) ثم السودوموناس ايريجنوزا (6.06%).

4.أظهرت الدراسات أن أعلي معدل اصابة سجلت في الصيف77 (33.33%) يليه الربيع 56(24.24%) يليها الخريف51 (21.97%) ثم سجلت أقل معدل أصابه ف الشتاء 47(20.46%).

...أظهرت النتائج أن معدل الأصابه بميكروب الأيروموناس هيدروفيلا كانت عالية في فصل الشتاء (51.85%) و أن ميكروب السيدوموناس فلوريسينس فأعلي نسبة اصابه سجلت كانت في فصل الشتاء (29.63%) ثم الخريف (27.59%) ويليها الربيع (25%) ولم يسجل نسب اصابة في فصل الصيف (0%) و بالنسبه للسيدوموناس ايريجنوزا أعلي نسبة اصابة سجلت كانت في فصل الربيع(12.5%) ثم فصل الصيف(9.09%) ولم يسجل اصابة في فصلي الشتاء والخريف بينما بكتريا الفيبريو الجينوليتيكس كانت نسبة الاصابة عالية في فصل الصيف (36.36%) يليه الخريف (34.48%) ثم الربيع (21.88%) وأقل معدل اصابة كان في فصل الشتاء (18.52%) و بالنسبه لبكتريا الفيبريوفلوفياليز كان معدل العزل أعلي في فصل الصيف (13.64%) ثم الخريف (10.35%) ويليه فصل الربيع (3.13%) ولم يتم العزل في فصل الشتاء.

أثبت اختبار الحساسيه أن السيبروفلوكساسين هو المضاد الحيوي الأكثر فاعليه بالنسبه للأيروموناس هيدروفيلا و بالنسبه للسودوموناس فلوريسنس وللسيدوموناس ايريجنوزا أيضا و كما أظهرت الفيبريوالجينوليتيكس حساسيه عاليه لمضاد الحيوى الاوكسي تتراسيكلين و الريفامبيسين والسيبروفلوكساسين و أيضا السيبروفلوكساين والنالدكسيك أسيد بالنسبه للفيبريوفلوفياليز