

Prevalence of common bacterial pathogens in marine fishes

Elsayed, M.E*.; Essawy, A.M**.; Abou El-Atta, M.E*** and EL-Banna, N.I****.

*Professor of Microbiology, Faculty of Veterinary Medicine, Suez Canal University.

**Head Research of Microbiology, Animal Health Research Institute.

***Senior Research, Fish health dept. Central laboratory for Aquaculture, Research.

****Researcher of Microbiology, Animal Health Research Institute.

Abstract:

A total of 200 marine fish of two different species represented as (100 *Tilapia zillii* & 100 *Mugi Capito*) were freshly collected randomly from different markets in EL-Mansoura city, during different seasons. And subjected to clinical, post mortem and bacteriological studies. The common clinical signs were darkness in skin, hemorrhage in base of fins, eyes & different parts on the body, and abdominal distention, congestion in gills and increase in mucous secretion. The Post Mortem finding showed white serous fluid in abdominal cavity and sometime tinged with blood, pale or congested liver, kidney and spleen. The result indicated that the prevalence of bacterial pathogens among naturally infected marine fish were *A. hydrophila* 52 isolates (39.39%), *V. alginolyticus* 38 isolates (28.79%), *Ps. fluorescens* 24 isolates (18.18%), *V. cholerae* 10 isolates (7.58%) and *Ps. aeruginosa* 8 isolates (6.06%). And among 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%). The results of pathogenicity declared that the *A. hydrophila* was highly pathogenic to *T. zillii* causing 100% mortality within 4 days followed by *V. alginolyticus* causing (90%) mortality, then *Ps. Fluorescens* (80%), *Ps. aeruginosa* (60%) while *V. cholerae* was non pathogenic strain. The results of antibiogram revealed that, ciprofloxacin and naladixic acid were more effective against *A. hydrophila*, while ciprofloxacin and rifampicine more effective against *Ps. fluorescens* also ciprofloxacin and amikacine against *Ps. aeruginosa*. While *V. alginolyticus* was highly sensitive to ciprofloxacin.

Keywords: Marine fish, Bacterial diseases, Isolation, Seasonal variation, Antibiotic sensitivity.

Introduction

Aquaculture is considered an important source of high nutritive value, cheap animal proteins and it becomes an important economic activity in many countries. In this way the Egyptian Government within the strategy of the food, paid special interest to fish meat and within this strategy fish aquaculture and fish industry were considered as one of the main principal sources of the national income in Egypt. On the long run water resources will be the most limiting factor to be considered in aquaculture development in Egypt, especially freshwater aquaculture. Therefore, marine water is the immediate alternative sources for water needed for mariculture system.

According to *Toranzo et al (2005)* several aspects would be raised regarding the infectious diseases caused by bacteria in marine fish: (i) Only a relatively small number of pathogenic bacteria are responsible for important and significant economic losses in cultured fish;

(ii) Several classical diseases considered as typical of fresh water aquaculture are today important problems in marine culture;

(iii) Clinical signs (external and internal) provoked by each pathogen depend on the host species, fish age and stage of the disease; (iv) There is no correlation between external and internal signs of the disease; and (v) The severity of the disease and the mortality are

higher in cultured fish than that in wild fish populations, due to the lack of the stressful conditions that usually occur in the culture facilities.

The present work was planned to isolate and identify the most common pathogenic bacteria in some marine fish and evaluate the seasonal variation, prevalence in different organs of isolated bacteria and antibiotic sensitivity of pathogenic one among examined fish.

2. Material and methods

2.1. Sampling:

A total of 200 marine fish of two different spp. represented as (*100 T.zillii* and *100 M.Capito*) were freshly collected randomly from different markets in EL-Mansoura city, during different seasons. In a rate 25 fish of each species were collected and examined seasonally. Clinical and P.M examination were carried out using the methods described by (*Schäperclaus et al, 1992*).

2.2. Isolation of suspected bacteria:

Samples from liver, kidney, spleen and gills from examined fish were cultured on general and selective media; tryptic soy broth, tryptic soy agar (oxid) supplemented with 2% NaCl (w/v), thiosulphate citrate bile salt agar (oxid), 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°C for 1-2 days.

2.3. Identification of isolated bacteria:

Pure cultures of isolated bacteria were identified by gram stain and biochemically as described in the (*Bergey's manual of systemic bacteriology*, 2005). Final confirmation of bacterial isolates was achieved by using the analytical profile index of API20 E system (*Buller, 2004*).

2.4. Experimental infection:

A total number of 70 *T.zillii* fish were collected alive and apparently healthy from EL Abbassa fish farm, EL sharkia, Egypt with an average body weight 20±5 g and were used for experimental infection with *A. hydrophila*, *Ps.fluorescens*, *Ps.aeruginosa*, *V.alginolyticus* and *V.cholerae* isolated from naturally infected marine fish. The Fish were maintained in glass aquaria supplied with well aerated dechlorinated tap water with addition of salt to be acclimated. All experimental fish were feed with commercial ration at rate of 5% body weight per day.

Fish were divided into seven groups (10 fish of each) and the inocula of bacterial isolates were prepared for I/P injections, according to (*Austin & Austin, 1999*). Five groups were consistently inoculated I/P with bacterial suspension of (*Aeromonas hydrophila*, *Ps. fluorescence*, *Ps.aeruginosa*, *V.alginolyticus* and *V. cholerae*) at dose of 0.2 ml of (3 X 10⁷ CFU) using spectrophotometer to adjust dose.

The sixth group were injected I/P with 0.2 ml of saline containing *V. cholerae* (2.5x10⁸CFU /ml) (*Austin and Austin, 2007*). The last group was left as control and injected I/P with 0.2 ml sterile saline. As shown in (Table 5). All experimentally injected fish observed daily for 1-2 weeks to record any clinical signs and mortalities. Postmortem examination was done on freshly dead fish to record gross lesions. And re-isolation of injected pathogenic bacteria from dead and scarified fish.

2.5. Antibiotogram sensitivity:

Were done according to (*Schaperclaus et al (1992)*), using the disc diffusion method on Muller's Hinton agar medium and following discs oxytetracycline, ampicillin, amoxycillin, lincomycin, Ciprofloxacin, colistin sulphate, nalidixic acid, amikacin, rifampicin and erythromycin, that were kindly recorded.

3. Result

3.1. Results of Clinical and P.M examination:

Symptoms detected in the naturally diseased fish included darkness of external body surface, opacity of eye, increased in mucous secretion, exophthalmia and some fish showed hemorrhages in eye, and large irregular hemorrhagic areas into many parts of the body, at base of fins, on gills cover, at the anal region, anal fin. There were abdominal distention observed in

some fish. The Post mortem examination showed white serous fluid in abdominal cavity, some tinged with blood. The liver appeared pale anemic, friable with some hemorrhagic patches and pinpoint hemorrhagic on liver surface. Kidney were congested and slightly enlarged. The intestine of some fish were hemorrhagic, inflamed with sever congestion. Spleen enlarged and congested and in some cases appeared pale and in other cases appeared normal. In some fish appear hemorrhagic areas in the abdominal wall & on peritoneum. (Photo1).

3.2. Results of bacteriological examination:

The results of bacteriological examination of examined fish revealed the isolation of 132 bacterial isolates of gram negative bacteria which identified according to morphological, biochemical and API20 into *A. hydrophila*, *Ps. fluorescens*, *Ps. aeruginosa*, *V. alginolyticus* and *V. cholerae* as shown in (Table 1). The result indicated that 99 naturally infected and 101 apparently healthy marine fish were found to be infected with different types of bacteria. The results shown that from total number of collected gram negative bacterial isolates were 22(14.29%) isolates oxidase -ve and 132(85.71%) were oxidase positive isolates. From oxidase positive bacterial isolates the higher percentage was recorded to *A. hydrophila* 52(39.39%), followed

by *V. alginolyticus* 38(28.79%), *Ps. fluorescens* 24 (18.18%) and the minimal prevalence was recorded for *V. cholerae* 10(7.58%) and *Ps. aeruginosa* 8 (6.06%). (Table 2).

The highest prevalence of bacterial pathogens among 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 minimal prevalence of infection recorded in winter(20.46%) (Table 3). The distribution of different bacterial isolates in different organs and tissues of examined marine fish as shown in (Table 4). The highest rate of *A. hydrophila* isolation was recorded in liver (44.23%), while the minimal prevalence recorded in gills (7.69%). *Ps. fluorescens* was isolated in high percentage from kidney (50%), followed by liver (25%), and lowest percentage was recorded from spleen (8.33%). The highest prevalence of *Ps. 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%). *V. alginolyticus* was recorded in higher rate in liver (36.84%), while the lower rate recorded in gills (7.89%). The highest prevalence of *V. cholerae* was recorded in kidney (40%), while the lowest prevalence was recorded in gills (10%).

3.3. Result of experimental infection:

Result of pathogenicity test (Table 5) showed that clinical signs of the

disease were seen after 24 hrs post injection of fish with different pathogenic bacterial isolates (*A. hydrophila*, *Ps. fluorescens*, *Ps. aeruginosa* and *V. alginolyticus*) and this signs included inflammatory changes at site of injection, hemorrhages all over the body, bilateral distention of abdomen, congested liver, kidney and spleen. Re-isolation of the pathogenic bacterial isolates were obtained from all freshly dead and scarified experimentally infected fish. Mortality rate recorded for pathogenic bacteria was (100%, 80%, 60% and 90%) respectively.

While the I/P infected fish groups with *V. cholerae* of two concentration showed no clinical signs or no mortalities which proved that these strains were non pathogenic. The results of antibiogram revealed that, ciprofloxacin and naladixic acid were more effective against *A. hydrophila*, while ciprofloxacin and rifampicine more effective against *Ps. fluorescens* also ciprofloxacin and amikacine against *Ps. aeruginosa*. While *V. alginolyticus* was highly sensitive to ciprofloxacin.

Table (1): The biochemical and morphological characters of isolated bacteria from examined marine fish.

	<i>A. hydrophila.</i>	<i>Ps. fluorescens.</i>	<i>Ps. aeruginosa.</i>	<i>V. alginolyticus.</i>	<i>V. Cholerae.</i>
1. Gram stain.	-	-	-	-	-
1. Shape.	Short rod.	Short rod	Short rod	vebrionic bacilli	vebrionic bacilli
2. Motility.	+	+	+	+	+
3. Cytochrome oxidase. (Ox).	+	+	+	+	+
4. Catalase Test.	+	+	+	+	+
5. B-Galactosidase production (OPNG).	+	-	-	-	+
6. Arginine dihydrolase production (ADH)	-	+	+	-	-
7. Lysine decarboxylase production (LDC) .	-	+	-	+	-
8. Ornithine decarboxylase production (ODC).	+	+	-	+	+
9. Citrate utilization (CIT)	-	+	+	-	-
10. H ₂ S production (H ₂ S).	-	-	-	-	-
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 G-ve, oxidase positive bacterial isolates from examined marine fish.

No.of oxidase +ve	<i>A. hydrophila</i>	<i>Ps. fluorescens</i>	<i>Ps.aeruginosa</i>	<i>V.alginolyticus</i>	<i>V. cholerae</i>
132	52	24	8	38	10
85.71	39.39	18.18	6.06	28.79	7.58

Table (3) Seasonal prevalence of G-ve bacterial species in examined marine fish.

Season. Bacterial Isolates.	Winter		Spring		Summer		Autumn		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
<i>A. hydrophila</i>	14	51.85	12	37.5	18	40.91	8	27.59	52	39.39
<i>Ps.fluorescens</i>	8	29.63	8	25	0	0	8	27.59	24	18.18
<i>Ps.aeruginosa</i>	0	0	4	12.5	4	9.090	0	0	8	6.06
<i>V.alginolyticus</i>	5	18.52	7	21.88	16	36.36	10	34.48	38	28.79
<i>V. cholerae</i>	0	0	1	3.13	6	13.64	3	10.35	10	7.59
Total	27	20.45	32	24.24	44	33.33	29	21.97	132	100

Table (4) Incidence of G-ve bacterial species from examined tissues and organs of marine fish.

Organ	<i>A. hydrophila</i>		<i>Ps. fluorescens</i>		<i>Ps. aeruginosa</i>		<i>V. alginolyticus.</i>		<i>V. Cholerae.</i>		Total	
	No.	%	No.	%	No.	%	No.	%	No	%	No.	%
Liver	23	44.23	6	25	3	37.5	14	36.84	2	20	48	36.36
Kidney	19	36.54	12	50	3	37.5	12	31.58	4	40	50	37.84
Spleen	6	11.54	2	8.33	1	12.5	9	23.68	3	30	21	15.91
Gills	4	7.69	4	16.67	1	12.5	3	7.89	1	10	13	9.85
Total	52	39.39	24	18.18	8	6.06	38	28.79	10	7.58	132	100

Table (5) Mortality rate of experimentally infected fish with *A. hydrophila*, *Ps. fluorescens*, *Ps. aeruginosa*, *V. alginolyticus* and *V. cholerae*.

Group.	Dose per fish.	Fish No.	Dead fish during 7 days after Injection of pathogenic bacteria.							No. of dead fish.	MR%
			1	2	3	4	5	6	7		
1	<i>A. hydrophila</i> 0.2 ml of 3×10^7 CFU	10	3	3	3	1	-	-	-	10	100%
2	<i>Ps. fluorescens</i> 0.2 ml of 3×10^7 CFU	10	2	2	1	2	1	-	-	8	80%
3	<i>Ps. aeruginosa</i> 0.2 ml of 3×10^7 CFU	10	0	2	2	2	-	-	-	6	60%
4	<i>V. alginolyticus</i> 0.2 ml of 3×10^7 CFU	10	2	3	3	1	-	-	-	9	90%
5	<i>V. cholerae</i> 0.2 ml of 3×10^7 CFU	10	-	-	-	-	-	-	-	-	0%
6	<i>V. cholerae</i> 0.2ml of 2.5×10^8 CFU	10	-	-	-	-	-	-	-	-	0%
7	Control group 0.2ml of sterile saline	10	-	-	-	-	-	-	-	-	0%



Photo (1) Diseased *M. capito* showed hemorrhages in peritoneum.

4. Discussion

Fish are susceptible to a wide variety of bacterial pathogens.

Naturally infected marine fish (*M. capito* & *T. zillii*) were clinically examined and found darkness of external body surface, opacity of

eye, exophthalmia and some fish showed hemorrhages in eye, scales detachment and large irregular hemorrhagic areas into many parts of the body, at base of fins, anal fin and caudal peduncle. This results supported with that mentioned by **Toranzo et al (2005)**, **El-Ashram and Azza(2006)**, and **Moustafa et al (2010)**. Post mortem finding of most examined fish pointed out that liver appeared to be pale anemic, friable with some hemorrhagic patches on its surface and in some cases appear congested. Kidney and spleen were congested and slightly enlarged, and in some cases pale. In some fish appeared hemorrhagic areas in the abdominal wall. This results are in accordance with those reported by **Toranzo et al (2005)**, **El-Ashram and Azza (2006)**, **Moustafa et al (2010)** and **El-Refaey (2013)**.

In this work many types of bacterial strains were isolated from marine fish including *A. hydrophila*, *Ps.fluorescens*, *Ps.aeruginosa*, *V.alginolyticus* and *V. cholerae*. And this results agree with **Kannapiran et al (2009)** who declared that *Vibriosis* was one of the most prevalent fish diseases caused by bacteria belonging to the genus *Vibrio*. *V.alginolyticus*, *V.cholerae (non-O1)*, *V. vulnificus (Biotype 2)*, *V. anguillarum*, were considered to be predominant fish pathogens. Similar to **Hossain et al (2011)** who suggested that *Aeromonas* and *Pseudomonas* were the important bacterial pathogens

frequently isolated from the diseased fish throughout the world. Regarding prevalence of bacterial isolates in this work, the high prevalence of bacterial isolates was recorded to *A. hydrophila* (39.39%), followed by *V.alginolyticus* (28.79%), *Ps. fluorescens* (18.18%). And this results in accordance with **Zorrilla et al (2003)** who showed that the most frequently isolated microorganisms were identified as *Vibrio* (69.90%). Other microorganisms belonging to *Pseudomonas spp.*, and *Aeromonas spp.*, in which *V.alginolyticus* was the most frequently species isolated (21.35%), and with **Ullmann et al (2005)** who demonstrated that a total of 134 *Aeromonas* strains were classified as *A. hydrophila* (67%), *A.caviae*(26.1%), and *A.sobria* (6.0%) by biotyping. Also **Ahmed (2004)** who isolated *ps. fluorescens* from naturally infected *Mugil species* with percentage 21.33%. In regards to the seasonal prevalence of *A. hydrophila* the result pointed out that the highest prevalence of *A. hydrophila* 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 researchers investigated that motile *aeromonas spp.* These results are in concordance with **Hayes (2000)** who concluded that outbreaks of *A. hydrophila* were usually associated with change in environmental conditions. Stressors including

overcrowding, high temperature, sudden change in temperature, poor nutritional state, and fungal or parasitic infection that made stress on fish and increase its susceptibility to the infection. On the other hand *Maalej et al (2003)* noticed that *Aeromonas* dynamics exhibited a seasonal prevalence in natural seawater, *Aeromonads* decreased very rapidly in sea water during the cold period from the end November to April.

In regards to the seasonal prevalence of *Ps.fluorescens* our results detected that the highest prevalence of *Ps.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. These results are supported by *Hoshino et al (1997)* who illustrated that the proteinase activity of *Pseudomonas spp.* increase in low temperature. And *Iqbal et al (1999)* and *Golomazou et al (2006)* who demonstrated that *Pseudomonas* were isolated mainly in cold months of winter .while these results disagree with *Hoda et al (1999)* who revealed that the prevalence of *Pseudomonas* were lower in winter than summer. On contrast this result was not in accordance with *Eissa et al (2010)* who isolate *Pseudomonas spp.* from and revealed significant difference among four seasons, it was 43.33% (April 2008), 24.44%

(August 2008), 21.11% (November 2008) and 17.77%(January 2009). And mentioned that the high prevalence of *Ps.fluorescens* in winter season may due to the high affinity of this bacteria to low water temperature as concluded by *El-Moghazy (2004)*.

In regards to the seasonal prevalence of *Ps.aeruginosa* these results showed that the highest prevalence of *Ps.aeruginosa* was recorded in spring (12.5%), followed by summer (9.09%). But not recorded in winter and autumn. This result agree with *Eissa et al (2010)* who isolated *Ps.aeruginosa* 12 isolates of total 80 *Pseudomonas spp.* isolates (15%)from marine fish.

In regards to the seasonal prevalence of *V.alginolyticus* the results were demonstrated that the highest prevalence of *V.alginolyticus* was recorded in summer (36.36%), then autumn(34.48%), followed by spring(21.88%), while minimal recorded in winter (18.52%).The obtained results of seasonal prevalence of *V.alginolyticus* were higher than that reported by *Mustafa et al (2010)* who mentioned that the highest prevalence of *V.alginolyticus* in summer (8.57%), autumn (5.30%), spring (2.04%) and (0.81%) in winter .On the other hand, *Golomazou et al (2006)* reported that *V.alginolyticus* were not associated with a particular season.

Concerning to rate of recovery 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 nearly agree with *El-Refaey (2013)* who concluded that most of bacterial infections affect haemobiotic system mainly liver, kidney and spleen.

From the results of antibiogram sensitivity test, it cleared that ciprofloxacin and naladixic acid were more effective against *A. hydrophila*, and this results agree with *El Ashram and Azza (2006)*, *Abou El Atta and El Tantawy (2008)* and *Enany et al (2011)*. While ciprofloxacin and rifampicine more effective against *Pseudomonas spp.* and these results similar to that detected by *Akinbowale et al (2007)* and *Enany et al (2011)*. While *V. alginolyticus* was highly sensitive to ciprofloxacin, and this results supported with that obtained by *Wafeek et al (2007)* and *Enany et al (2011)*.

It was concluded that the highest prevalence of bacterial isolates causing infection in marine fish were by *A. hydrophila* followed by *V. alginolyticus*, then *Ps. fluorescens* and the minimal prevalence was

recorded for *Ps. aeruginosa* and *V. cholerae*. 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 antibiotics.

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مدى تواجد البكتيريا الممرضة الشائعة في أسماك المياه المالحة

محمود عزت السيد*، أبو الخير محمد إبراهيم عيسوي*، محمد السيد أبو العطا** ونهى إبراهيم البنا***
 * قسم الميكروبيولوجيا- كلية الطب البيطري- جامعه قناة السويس- **معهد بحوث صحة الحيوان -
 *** المعمل المركزي لبحوث الثروة السمكية العباسية شرقية - ***معهد بحوث صحة الحيوان.

تم اجراء هذه الدراسة على عدد ٢٠٠ سمكه من أسماك المياه المالحة ممثله فى (أسماك الطوباره و الشبار الأخضر) و التى جمعت عشوائيا من مختلف أسواق مدينة المنصورة موسميا ما بين حيه و حديثه النفوق. و لقد تم اجراء لها الفحوصات الظاهريه و التشريحيه و البكتريولوجيه.

أظهرت هذه الدراسه أن العلامات المرضيه الرئيسيه فى الأسماك المصابه كانت فى صورة بقع داكنه على سطح الجسم الخارجى، نزف فى ارجاء مختلفه من الجسم وخاصة فى قاعده الزعانف، و العين و انتفاخ فى المنطقه البطنيه، احتقان فى الخياشيم، و زياده فى افراز مخاط. أما الصفة التشريحيه فكانت على هيئة احتقان مع تضخم فى الكليه و الطحال، مع ظهور الكبد احيانا ما بين البنى الداكن الى الأصفر الباهت، مع وجود سائل شفاف و فى بعض الأحيان مختلط بالدم فى التجويف البطنى لبعض الأسماك. كما أوضحت النتائج عن مدى تواجد البكتريا الممرضة فى أسماك المياه المالحة المصابه طبيعيا أن البكتريا السائده هى الايرومونات هيدروفيليا (٣٩،٩%) يليها الفبريوالجينوليتكس (٢٨،٧٩%) يليها السيدومونات فلورسينس (١٨،١٨%) ثم الفبريو كوليرا (٧،٥٨%) ثم السيدومونات ايرجينوزا (٦،٠٦%). كما أثبتت الدراسه أن أعلى معدل اصابه سجلت فى الصيف (٣٣،٣٣%) يليه الربيع (٢٤،٤٢%) يليها الخريف (٢١،٩٧%) ثم سجلت أقل معدل اصابه فى الشتاء (٢٠،٤٦%).

أوضحت العدوى الصناعيه أن الايرومونات هيدروفيليا أكثر البكتريا المعزوله ضراوه حيث سببت ١٠٠% نفوق خلال ٤ ايام ثم يليها الفبريوالجينوليتكس حيث سببت ٩٠% ثم يليها السيدومونات فلورسينس ٨٠% و السيدومونات ايرجينوزا ٦٠% و وجد أن سلالات ميكروب الفبريو كوليرا المعزوله ليست ممرضه.

أثبت اختبار الحساسيه أن السبروفلوكساسين و النالد كسيك أسيد هما المضادان الحيويان الأكثر

فاعليه بالنسبه للايرومونات هيدروفيليا بينما أن السبروفلوكساسين و الريفاميسين بالنسبه

للسيدومونات فلورسينس و السبروفلوكساسين و الاميكاسين بالنسبه للسيدومونات بيرجينوزا، وكما

أضهرت الفبريوالجينوليتكس حساسيه عاليه لمضاد الحيوى السبيروفلوكساسين.