Studies on Pseudomonas Septicemia in Some Tilapia in Ismailia


*Animal Health Research Institute, Ismailia branch, Egypt **Department of Fish disease and management, Faculty of Vet. Med., Suez Canal University, Ismailia, Egypt

Abstract

A total of 200 fish, 100 Oreochromis niloticus weighing (60-300 g) and 100 Tilapia zillii weighing (40-70 g) were collected randomly from different sites in Ismailia Governorate Egypt, during the period from October 2015 to September 2016. The clinical signs and postmortem lesions in naturally infected fishes were represented as hemorrhages on external body surface, hemorrhagic ulcer and congestion in most internal organs. The total prevalence of isolated Ps. aeruginosa in O. niloticus was 40% with the highest prevalence in winter 60% while the lowest prevalence was in summer 24%. The highest intensity was from liver 75% while the lowest was from gills 20%. On the other hand, prevalence in T. zillii was 22% with the highest in winter 36% while the lowest prevalence was in summer 8%. The highest intensity was from liver 72.7% while the lowest was from gills 13.6%. PCR is sensitive, rapid and specific method to detect resistant genes (MexA at 293 bp and MexB at 244 bp) and 16S rRNA gene at 530 bp in the selected isolates of Ps. aeruginosa. The antibiogramme of Ps. aeruginosa isolates showed high sensitivity to ampicillin, cefalexin and trimethoprim/sulfamethoxazole while they were resistant to amoxicillin and tobramycin. Challenge test revealed that the mortality rate in O. niloticus and T. zillii with Ps. aeruginosa by I/P route representing 80 and 50% of the total fishes, respectively. It was concluded that, O. niloticus is more susceptible to Pseudomonas septicemia than T. zillii in both natural and experimental infection.

Introduction

Fish has become an important resource in Egypt to meet the food and nutrition security needs of a rapidly expanding human population. Tilapia were mild, white flesh that is appealing to consumers, easy to rise and harvest, making them a good aquaculture species (Khalil et al., 2010). Bacterial fish diseases were the major problems in aquaculture as it found naturally in the fish environment and under certain stress conditions caused severe economic losses (Olsson et al., 1998). Pseudomonades were
opportunistic Gram negative pathogens, causes outbreak when the normal environmental conditions changed (Roberts, 1989). *Ps. fluorescens*, *Ps. angulliseptica*, *Ps. aeruginosa* and *Ps. putida* were identified in various species of fish as causative agents of pseudomonas septicemia (El-Nagar, 2010). Among DNA marker, the Polymerase Chain Reaction (PCR) was highly sensitive specific and rapid method which improved the detection of *Ps. aeruginosae* specially when using species-specific primer (Xu et al., 2004). Therefore, the aim of this work was to determine the clinical signs and postmortum lesions in naturally and experimentally infected Tilapias in addition to, isolation and identification of the causative agents of pseudomonas septicemia. Besides, studying total and seasonal prevalence, intensity in different organs of the infected fishes, the antibiogramme of the isolated strain and polymerase Chain Reaction (PCR) for confirmation of identification and for detection of antibiotic resistant genes (*mexA* and *mexB*).

**Material and Methods**

**Collection of Fish Sample:** A total of 200 fish, 100 *O. niloticus* (60-300 g) and 100 *T. zillii* (40-70 g) were collected randomly from different sites in Ismailia Governorate Egypt, during the period from October, 2015 to September, 2016. Live fish samples were placed in plastic bags and brought to the Animal Health Research Institute, Ismailia branch under standard measures of transportation. Upon arrival, the fishes were subjected to clinical and bacteriological examination (Plumb and Bowser, 1982), observed signs and detected lesions were recorded. Then, they were subjected to bacteriological examination.

**Bacterial isolation and identification:** Specimens from gills, liver, kidneys, intestine and spleen were collected under complete aseptic conditions. They were cultured directly onto plates of Pseudomonas isolation agar (Buller, 2004), 5% sheep blood agar and nutrient agar. The plates were incubated at 37 °C for 24-48 hrs. After the recommended incubation period for each type of media; each type colony was picked up and re-streaked on a new plate of its original culture media and re-incubated at the same temperature and period. When the pure colonies were grown; a loopful of each pure culture was inoculated into a nutrient slope agar as a stock culture for biochemical identification of isolates by Vitek2 compact system (bioMe'rieux, Marcy 1’ Etoile, France) (Barry et al., 2003) and for preservation of the microorganism.

**Pathogensity Test:** A total number of 40 apparently healthy fish, (20) *O. niloticus* weighing (70 ± 5 g) and (20) *T. zillii* weighing (40 ± 5 g) were reared in well prepared
aquaria. They were allowed to acclimate to lab conditions for one week at 25 ± 1°C and fed with commercial pelleted ration at 3% of bodyweight. They were divided into two groups A (treated group n=10) were injected intraperitonially with dose 0.2 ml of 24 hrs trypticase soya broth culture (3×10^7 living bacterial cell \ ml) according to Reed and Muench (1938) after matching with McFarland tube and B (control group n=10) were injected intraperitonially with dose 0.2 ml of sterile trypticase soya broth. Injected fishes were reared for 14 days and mortalities and clinical signs were recorded.

**Molecular typing:** The procedures of DNA extraction were carried out according to the methods described by Touihri et al., (2009). Oligonucleotide primers used for detection of *P. aeruginosa* have specific sequence and amplify a specific product as shown in table (1).

**Table (1): Oligonucleotide primers sequences:**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Product size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA</td>
<td>ATGGAAATGCTGAAAATTCCGC-CTTCTTCAGCTGACGCGACG</td>
<td>530 bp</td>
<td>(Pirnay et al., 2000)</td>
</tr>
<tr>
<td>MexA</td>
<td>CGA CCA GGC CGT GAG CAA GCA GC-GGA GAC CTTCGCCGC GTT GTC GC</td>
<td>293 bp</td>
<td>(Xavier et al., 2010)</td>
</tr>
<tr>
<td>MexB</td>
<td>GTGTTCG-GCTCGCAGTACTC-AACCGTCGGGATTGACCTTG</td>
<td>244 bp</td>
<td>(Xavier et al., 2010)</td>
</tr>
</tbody>
</table>

**Results**

**Clinical picture:** The clinical signs and postmortem lesions in naturally infected fishes showed hemorrhages on external body surface, hemorrhagic ulcers, ascatitis, detached tail and congestion in all internal organs with septicemic fluid in the abdomen (Plate 1, 2).

**Bacteriological examination:** The isolated bacteria were positive for oxidase, catalase, urease, citrate and gelatin liquefaction. While, they were negative for indole, V.P., methyl red and triple sugar iron agar (TSI). It hadn't the ability to produce acid from mannitol, glucose, sorbitol and sucrose. PCR is sensitive, rapid and specific method to detect resistant genes (*MexA* at 293 bp & *MexB* at 244 bp) and 16S rRNA gene at 530 bp in the selected isolates of *Ps. aeruginosa* figure (1, 2).

**Total and seasonal prevalence:** The total prevalence of *Ps. aeruginosa* in *O. niloticus* was 40% with the highest prevalence in winter 60% while the lowest prevalence was in summer 24%.
The highest intensity was from liver 75% while the lowest was from gills 20%. The total prevalence of *Ps. aeruginosa* in *T. zillii* was 22% with the highest prevalence in winter 36% while the lowest prevalence was in summer 8%. The highest intensity was from liver 72.7% while the lowest was from gills 13.6%.

**Antibiogramme test:** *Ps. aeruginosa* isolates were sensitive to ampicillin, cefalexin, cefpodoxime, ceftiofur, ceftiraxone, imipenem, amikacin, gentamicin and trimethoprim/sulfamethoxazole. In addition to, they were intermediate resistant to enrofloxacin, marbofloxacin and tetracycline while, they were resistant to amoxicillin, pipracillin, tobramycin and nitrofurantoin.

**Experimental infection:** The challenge test revealed that the mortality rate in experimentally infected fishes (*O. niloticus* and *T. zillii*) with *Ps. aeruginosa* by I/P route representing 80% and 50% of the total fishes, respectively.

**Plate (1): Clinical signs of infected fishes (O. niloticus and T. zillii)**
- **A:** *O. niloticus* showing hemorrhagic ulcer.
- **B:** *T. zillii* showing skin darkening, hemorrhage on gill cove, abdomen and tail erosion.

**Plate (2): Post mortem lesions of infected fishes (O. niloticus and T. zillii)**
- **A:** *O. niloticus* showing congested liver and gill erosion.
- **B:** *O. niloticus* showing enlarged gallbladder.
- **C:** *O. niloticus* showing septicemic fluid in the abdomen.
Figure (1): Detection of *Pseudomonas aeruginosa* 16s rRNA (530bp) gene by PCR. Lanes 0: negative control; lanes 1-7: *Pseudomonas aeruginosa* showing bands at 530bp.

Figure (2): Detection of *Pseudomonas aeruginosa* resistant genes MexA (293bp) and MexB (244bp) by PCR. Lanes 0: negative control; lanes 1-7: *Pseudomonas aeruginosa* showing MexA and MexB bands at 293bp and 244bp respectively.

**Discussion**

The most observed clinical signs of infected tilapia were large irregular hemorrhages on body surface, in addition to exophthalmia, eye cloudiness and scales detachment, darkening of the skin, congested gills, ulceration of the skin and abdominal distention (ascites due to serohemorrhagic fluids). These results agreed with (Altinok et al., 2006; Austin and Austin, 2007; Eissa et al., 2010; Janga et al., 2014; Enany et al., 2016). These signs may be attributed to the action of the extracellular enzymes and degrading toxins (Todar, 2010).

The post mortem examination were varied lesions among the affected fishes as the liver was pale and congested in some fishes and congested with necrotic patches in other fishes, spleen and kidney were congested and enlarged in addition to the intestine was hyperemic and contained yellow mucous. Some other fishes showed signs of septicemia in all internal organs. These results in agreement with (Blanco et al., 2002; Altinok et al., 2007; Saleh and Azza, 2012; Enany et al., 2016).

Selective amplification of *Pseudomonas* 16S rRNA gene by PCR has been used to detect
differentiate Pseudomonas species. It was also used for genus or species level identification of *Ps. aeruginosa* ([Drancourt et al., 2000; Porteous et al., 2002]). MexA and MexB resistant genes were found in *Ps. aeruginosa* by using specific primer designed by ([Xavier et al., 2010]). The electrophoresis of MexA and MexB genes PCR product was noticed with specific bands at 293 and 244 base pair respectively in agreement with ([Lister et al., 2009]) who studied MexAB-OprM (coding gene: MexA, MexB and OprM) in *P. aeruginosa* which were genes for efflux pumps responsible for multidrug resistance as it pump antibiotics out of the cell.

The total prevalence of *Ps. aeruginosa* in naturally infected *O. niloticus* and *T. zilli* was 40% and 22% respectively. The obtained result of *T. zilli* was similar to [El-Nagar (2010)]. But, lower prevalence was recorded by [Masbouba (2004)] who demonstrated that the prevalence of infection by *Ps. aureginosa* was (29.1%). In addition to [Eissa et al. (2010)] who concluded that *ps septicemia* was found in 30.83% of the 480 examined *O. niloticus* in fish farms in Egypt. On the other side, lower observation of *O. niloticus* infection by pseudomonas species was recorded by [Zorrilla et al. (2003)] who detected that low infection rates of pseudomonas among the examined marine fish (15.27 %). These differences may be due to fish species, age, nature of water, time and place of research. The highest prevalence of *Ps. aeruginosa* infection in examined *O. niloticosa* and *T. zilli* in different seasons was in winter (60%, 36%) followed by autumn (44%, 28%) then spring (32%, 16%) while the lowest prevalence was in summer (24%, 8%) respectively. These findings were in agreement with ([El-Sayyad et al., 2010; Mastan, 2013] and [Toranzo et al. (2005)]. While, disagreed with [Eissa et al. (2011)] who found that Pseudomonas species prevalence of infection was 43.33% (April 2008), 24.44% (August 2008), 21.11% (November 2008) and 17.77% (January 2009). These findings may be attributed to stress, including a lowered water temperature, may trigger outbreaks of pseudomonas septicemia Markovic et al. (1996) and Azza et al. (2002). The differences between the two types of fish in seasonal prevalence may be explained by the resistance of marine fish (*T. zilli*) to infection due to high water salinity. *Ps. aeruginos* were isolated from *O. niloticus* and *T. zilli* organs (liver, kidneys, spleen, intestine and gills) and the highest intensity was from liver followed by kidneys, spleen, intestine and the lowest was from gills. These results might be due to the organ most associated with the detoxification and biotransformation process is the liver and due its function, position and blood supply, it is also one of
the organs most affected by contaminants in the water (Camargo and Martinez, 2007). These findings agreed with (Eissa et al., 2010; El-Nagar, 2010).

_Ps. aeruginosa_ isolates were sensitive to ampicillin, cefalexin and trimethoprim/sulfamethoxazole while they were resistant to amoxicillin and tobramycin. These results in agreement with (Eissa et al., 2010; Khalil et al., 2010; López et al., 2012b; Abdullahi et al., 2013; Hanna et al., 2014). On the other hand, these results disagreed with Mastan (2013) who mentioned that amikacin did not show any effect against _Ps. aeruginosa_ isolates. This might be explained by _Ps. aeruginosa_ was able to develop mutational resistance and characterized by the biofilm mode of growth, which protects bacteria against antibiotics and the innate and adoptive defense mechanism (Fux et al., 2005).

The mortality rates in experimentally infected _O. niloticus_ and _T. zillii_ with _Ps. aeruginosa_ by intra-peritoneal route were (80% and 50%) respectively. These results agreed with Enany et al. (2016). While, this findings disagreed with Austin and Stobie (1992) who stated that pseudomonads cause 100% mortalities within 7 days by I.P. or I.M. routes into rainbow trout. In addition to, Hossain et al. (2006) who reported that _Ps. aeruginosa_ produced 30% mortality in _O. niloticus_. Besides, Hossian and Rahman (2011) who stated that Pseudomonas species cause 50% mortality in the experimental fishes. This may be attributed to differences in fish species, age and virulence of strain.

In conclusion, _O. niloticus_ is more susceptible to Pseudomonas septicemia than _T. zillii_ in both natural and experimental infection.

References


Austin, B., Stobie, M. (1992): Recovery of Serratiaplymuthica and presumptive _Pseudomonas pseudoalcaligenes_ from skin lesions in rainbow trout, Oncorhynchusmykiss (Walbaum), otherwise infected with enteric
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الملخص العربي

أجريت هذه الدراسة على عدد 200 سمكة من نوعين مختلفين من الأسماك ممثلين كالتالي: 100 سمكة من أسماك الحلمي الملاح في و 100 سمكة من أسماك الشبار الأخضر تم تجميعهم شمسة و موسمياً وفحصهما مباشرة من أماكن مختلفة من محافظة الإسماعلية إبداءاً من شهر أكتوبر 2015 وحتى شهر سبتمبر 2016. وذلك لعزل وتصنيف المسبب المرضي لمرض التسمم الدموي السدوموناسي في أسماك البلطي البني والشبار الأخضر خلال المواسم المختلفة عن طريق استخدام الطرق التقليدية والتقنيات الحديثة مثل (الإيوه بي أي 20 - جهاز الفينك 2 و فعالية البوليميريز المتسلسل). بالإضافة إلى دراسة مدى حساسية الميكروبا المعزول لمضادات الحيوية.

وقد أوضحت النتائج مايلي:

1- أهم العلامات التشريحية للأسماك المصابة (البلطي البني والشبار الأخضر) هي: بقع نزيح على الجسم و حفظ و غيوم في العين، بروز فتحة الإخراج، تساقط للقولون، تغير لون الجلد واستفاقة في البطن.

2- أهم العلامات التشريحية للأسماك المصابة تثبيت بين شحوب في اللون، احتقان و زيادة في الحجم للkid - الطحال - الكلي - الامعاء والحوصلة المرارية مع وجود سوائل بجوف البطن.

3- كانت نسبة الإصابة الكلية والسدوموناسي اريجينوزا في أسماك البلطي البني 40% بحيث كانت أعلى نسبة إصابة في موسم الشتاء (60%) بينما سجل موسم الصيف أقل نسبة إصابة (24%). كما سجل الكبد أعلى نسبة إصابة (75%) بينما سجلت الخياشيم أقل نسبة إصابة (20%).

4- كانت نسبة الإصابة الكلية والسدوموناسي اريجينوزا في أسماك الشبار الأخضر 22% بحيث كانت أعلى نسبة إصابة في موسم الشتاء (36%) بينما سجل موسم الصيف أقل نسبة إصابة (8%). كما سجل الكبد أعلى نسبة إصابة (72.7%) بينما سجلت الخياشيم أقل نسبة إصابة (13.6%).

5- أوضح اختبار الحساسية السدوموناسي اريجينوزا أنها حساسة للاميبسين و سيفالكسين والتراميبيريم/ السلفاميثوكساليين. بينما انه مقاوم للكلوكسيدين والتوبراميسين.

6- أوضح اختبار العدوي علامات مرضية شبيهة للعلامات المرضية في الأسماك المصابة طبيعياً وكانت نسبة النتائج في الأسماك (البلطي البني والشبار الأخضر) المصابة بالعدوي المعتمدة بواسطة الحقن البريتوني 80 و 50% على التوالي من مجموع الأسماك المعرضة للعدوي.