Susceptibility of *Aeromonas Hydophila* Isolates to Antimicrobial Drugs

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Abstract

*Aeromonas hydrophila* is a microorganism widely distributed in nature: in water, soil, food. It is also part of the normal bacterial flora of many animals. As an opportune microorganism it is a secondary biological agent that contributes to the occurrence of a fish disease and its deterioration. Frequently, its presence is an indication of bad zoohygiene and zootechnical conditions in fish ponds. Reduced quality and quantity of feed, mechanical injuries, parasitosis, seasonal oscillation in temperature present some of the factors that produce favorable conditions for bacterial proliferation of aeromonas in fish ponds, so clinical symptoms of the disease occur. *Aeromonas* is almost always present in clinical isolates and may be unjustly accused for bad health of fish. Antibiotic therapy is applied even when the clinical findings are clear, what certainly affects the susceptibility to chemotherapeutics. The subject of our work was bacteriological examination of the material obtained from the carps with the observed skin changes and the carps without these changes. Also, antimicrobial susceptibility of *Aeromonas hydrophila* was tested. The aim of this research was to determined the presence of *Aeromonas hydrophilia* in the carp ponds and to test antibiotic susceptibility. The material consisted of the samples from the fish ponds where the carps were with and without changed skin. The method the isolation of *Aeromonas hydrophila* was used. The diffusion disk technique was used for testing antibiotic susceptibility. The isolates were tested for their susceptibility to Florephenikol, Flumequine, Olaqindox and Oxitetracycline. The obtained results point that antimicrobial susceptibility was the same regardless of the origin of the samples, i.e. the resistance was the same for both groups of samples (the strains isolated from the fish with skin changes and the strains from fish without changes on skin). The strains were highly resistant: 35% were resistant to flumequine and 40% to Oxitetracycline.

Keywords: *Aeromonas hydrophila*, fish, antimicrobial drugs

Introduction

*Aeromonas hydrophila* is a microorganism widely distributed in nature: in water, soil, food. It is also part of the normal bacterial flora of many animals. In humans Aeromonas causes different clinical symptoms as, for example, septicemia, infection of wounds and of gastrointestinal tract [1]. It is an opportune microorganism that may be primary or secondary biological agent and may be a causative agent in terrestrial and aquatic animals (fishes), but also humans [4,6]. The knowledge on the mechanism of pathogenesis is poor, but it is known that a number of different factors influence the development of the disease. If cell-surface proteins are present, like for example adhezin or extracellular quorum-sensing, protease, hemolysin and enterotoxin (acetilholinesterasisi) pathogenesis may develop [1,2,3]. Frequently, its presence is an indicator of bad zoohygiene and
zootechnical conditions in fish ponds [5]. Reduced quality and quantity of feed, mechanical injuries, parasitosis, seasonal oscillation in temperature present some of the factors that produce favorable conditions for bacterial proliferation of aeromonas in fish ponds, so clinical symptoms of the disease occur [5]. Aeromonas is almost always present in clinical isolates and may be unjustly accused for bad health of fish. Antibiotic therapy is applied even when the clinical findings are clear, what certainly effects the susceptibility to chemotherapeutics. This provides a possibility of change in susceptibility of Aeromonas towards hemotherapeutic agents. The subject of our research was bacteriological examination of the material obtained from the carps with observed skin changes and the carps without these changes. At the same time we wanted to examine sensitivity of Aeromonas hydrophila toward some chemotherapeutics. The objective of our paper was to determine the prevalence of Aeromonas hydrophilia in the carp in fish ponds and to test antimicrobial susceptibility and compare it to clinical finding in fish.

Material and methods

The material for this examination were the samples from a pond with fish without clinical changes of the skin of carp, and from a pond where changes on carp skin were diagnosed. Aeromonas was isolated by direct streaking on a media (Tryptose agar with 5% defibrinated sheep blood), and McCokney agar [14]. The media was incubated for 24 hours at 37°C. The identification of gram negative bacteria that formed complete or partial chemolisis was done by API strips. The sensitivity of Aeromonas to antimicrobial drugs was done on Muillerhinton agar using the method of disc diffusion [14, NCCLS]. The inhibition zone around every disc was measured, and the susceptibility was determined in correlation to the diameter of the inhibition zone around each disk of antimicrobials drugs. This was done according to the instruction [NCCLS]. The zone diameter smaller than 13 mm was interpreted as resistant (R), the zone diameter of 15-16 mm was intermediary (I), and zone diameter of 17 mm or more was considered sensitive (S). The sensitivity was measured for the following antimicrobial drugs: Florfenikol (30 µg), Flumequin (5µg), Olaquindox (5µg), Oxitetracyclin (30µg).

Results and discussion

The research has lasted for two years. A total of 75 samples was examined and in all the samples Aeromonas hydrophila was detected. Out of total, 62 samples were from the pond where fish had changes on the skin. The changes were several millimeters up to 2 cm large, without scales. The skin was inflamed, covered by ulcers filled with pus and bloody serous fluid. These changes were situated at the fin root and other parts of the skin. The other examined samples (13) originated from fish with no clinical changes. For susceptibility testing the method of disc diffusion was used. The antimicrobial drugs, usually applied in treating fish diseases, were used: Flumequin, Olaquindox, Oxitetracycline, Florefenicol. In Table 1 are displayed the results of bacteriological examination of fish with skin changes and the antimicrobial susceptibility testing of the isolates. In Table 2 are displayed the results of bacteriological examination of carp without skin change and susceptibility of the isolated Aeromonas.

Antimicrobial resistance of Aeromonas has been examined by many authors. Some of the authors [7] point out that Aeromonas hydrophila isolated from water, food and clinical samples was not susceptible to many antimicrobial drugs.

<table>
<thead>
<tr>
<th>Antimicrobial susceptibility testing of Aeromonas hydrophila isolated from skin changes of carp.</th>
<th>Carp with skin changes</th>
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<tbody>
<tr>
<td></td>
<td>O (%)</td>
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<tr>
<td>1 Flumequine</td>
<td>48.57</td>
</tr>
<tr>
<td>2 Olaquindox</td>
<td>49.13</td>
</tr>
<tr>
<td>3 Oxitetracycline</td>
<td>13.27</td>
</tr>
<tr>
<td>4 Florenikol</td>
<td>63.84</td>
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</table>
In 80 out of 238 isolates [8] antimicrobial resistance of *A. hydrophila* was observed. Our examination detected that the resistance to Flumequine and Oxitetracycline was higher than 35%, i.e. 40% of the isolated strains, regardless to their origin (from clinical material or from healthy fish). The results [9] show that 48% of the strains isolated from the aquatic environment (also *A. hydrophila*) were multiresistant, what may be the result of the spread of resistance genes among the isolated bacteria. Castro-Escarpulli et al. [10] report that the best antimicrobial effect is obtained by applying the first-generation quinolone and the second- and third-generation cephalosprins. Our research points out that a high percentage of the strains was resistant to Flumequine (over 35%) and Olaquindox (around 20%), as a representative of Quinolone. Multiple resistance was described by some authors [13] and it was reported that 100% of *A. hydrophila* isolates were resistant to methicilline and rifampicin, but 90 percent of strains were resistant to bacitracin and novobiocin. The resistance of *A. hydrophila* to flumequine and oxitetracycline has been observed over four years. In this period the number of the strains resistant to oxitetracycline increased form 21.7% to 77.2%, and to flumequine from 12.7% to 53.1%. Some authors [11], who analyzed the correlation between the producers of haemolysin of *A. hydrophila* strains from fish and multiresistence, warn that they may have an adverse effect on human health. Out of the total isolates, about 78.4% were the producers of haemolysin. The observation of hemoyisine in *A. hydrophila* strains is related not only to multiresistance, but also to the virulence of the bacterial strains [12]. The correlation of these features posses a problem not only for aquatic organisms, but also for humans.

### Table 2. Antimicrobial susceptibility of *Aeromonas hydrophila* isolated from the carp without changes on the skin.

<table>
<thead>
<tr>
<th>Antimicrobial drugs</th>
<th>Carp without skin changes</th>
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<tr>
<td></td>
<td>O (%)</td>
</tr>
<tr>
<td>1 Flumequin</td>
<td>50.2</td>
</tr>
<tr>
<td>2 Olaquindox</td>
<td>48.42</td>
</tr>
<tr>
<td>3 Oxitetracyclin</td>
<td>16.03</td>
</tr>
<tr>
<td>4 Florefenikol</td>
<td>66.43</td>
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</tbody>
</table>

**Conclusion**

This research points out that *Aeromonas hydrophila* was present in all the carp samples, regardless to their origin (both from the fish with clinical signs and the healthy fish). Antimicrobial susceptibility testing points on the resistance to Flumequine and Oxitetracycline (over 35%, i.e. 40%, respectively), and to Olaquindox (about 20% of the examined strains). Comparing the isolates (originating from fish with and without skin changes), it may be concluded that there is no difference in antimicrobial susceptibility. These results show that the strains in both sample groups were exposed to antimicrobial drugs and developed resistance. This means that antimicrobial drugs are used inappropriately and a further development of the resistance may be expected, so the number of effective antimicrobial drugs is diminishing. Since this is a microorganisms that may threaten human health, transmission of the reduced susceptibility may have negative consequences for humans.

### References


6. Tanmay Majumdar, Shubhamoy Ghosh, Joydeb Pal, Shibnath Mazumder; Possible role of a plasmid in the pathogenesis of a fish disease caused by Aeromonas hydrophila; Aquaculture 256 (2006) 95–104

7. Hristo Daskalov; The importance of Aeromonas hydrophila in food safety; Food Control 17 (2006) 474–483


11. Tha. Thayumanavan², G. Vivekanandhan², K. Savithamani², R. Subashkumar² and P. Lakshmanaperumalsamy; Incidence of haemolysin-positive and drug-resistant Aeromonas hydrophila in freshly caught finfish and prawn collected from major commercial fishes of coastal South India; FEMS Immunology and Medical Microbiology, Vol. 36, 2003, Pages 41-45

