Full Length Research Paper

Antibiotic susceptibility of *Lactococcus garvieae* isolated from rainbow trout (*Oncorhynchus mykiss*) in Iran fish farms

Mehdi Raissy¹* and Mahsa Ansari²

¹Department of Aquatic Animal Health, Faculty of Veterinary Medicine, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.
²Young Researchers Club, Islamic Azad University, Shahrekord Branch, Shahrekord, Iran.

Accepted 17 December, 2010

The rates of antibiotic susceptibility and resistance were investigated in *Lactococcus garvieae* isolates obtained from diseased rainbow trout. A total of 250 clinically suspected fishes were obtained from 10 rainbow trout fish farms in Chaharmahal and Bakhtyari Provinces Iran which is a major trout producing area. *L. garvieae* strains were isolated from fish tissues such as kidney, spleen and heart, and were identified by biochemical tests and polymerase chain reaction (PCR). All the isolates were found to be resistant to commonly used antibiotics. Resistance to different antibiotics ranged from 25 to 100% of all 52 isolates. These findings revealed a high rate of antibiotic resistance among the strains of *L. garvieae* which can be transferred to other bacteria.

Key words: Antibiotic susceptibility and resistance, *Lactococcus garvieae*, rainbow trout.

INTRODUCTION

*Lactococcus garvieae* is an emerging pathogen which has been isolated from cattle, Buffalos, economically important fish and freshwater shrimp (Elliott and Facklam, 1996; Bercovier et al., 1997; Alves et al., 2000; Chen et al., 2001; Diler et al., 2002). In addition, it has also been recovered from human (Elliott et al., 1991; Fefer et al., 1998) and therefore, the possibility of zoonosis should be considered. The bacterium is responsible for a serious problem in cultured marine and freshwater fish species such as yellowtail in Japan and rainbow trout in Europe, America and Australia (Elliott et al., 1991; Bercovier et al., 1997; Diler et al., 2002; Romalde et al., 2004). *L. garvieae* causes serious economic losses due to elevated rates of mortality (50 to 80% of the total production), decreasing growing rates and the appearance of these fish makes them unmarketable (Ghittino and Prearo, 1992). The septicemic processes caused by gram-positive coccus, commonly denominated as Streptococcosis, are not new; they were described for the first time at the end of the 50s in Japan, where the first cases were diagnosed in the intensive production of rainbow trout (Hoshina et al., 1958). The disease has been reported in rainbow trout in several countries such as Australia, South Africa, Japan, Taiwan, England, Turkey, countries of the Mediterranean area and Iran (Ghittino and Prearo, 1992; Palacios et al., 1993; Chen et al., 2001; 2002; Chang et al., 2002; Soltani and Tarahomi 2008). In Iran, Lactococcosis has appeared regularly in rainbow trout farms since 2002 (Soltani and Tarahomi, 2008) and it is becoming one of the most important risk factors in the trout industry especially during spring and summer months. The treatment of the disease is not successful in many cases because of development of the antibacterial resistance and recur-rent infection and it is clear that prevention of infection plays a major role. The aim of this study was to evaluate antimicrobial resistance of *L. garvieae*.

MATERIALS AND METHODS

Bacterial strains

A total of 250 fishes were obtained from 10 rainbow trout farms between September 2008 and August 2009. The samples of the
liver, kidneys and heart were placed on a 5% sheep blood agar (Oxoid) with 1% yeast extract agar (Merck) plates and then incubated at 24 and 37°C for 2 to 3 days under aerobic conditions. Standard physiological and biochemical tests recommended by Austin and Austin (1999) and Chang et al. (2002) were performed at 25°C. Identification of the L. garvieae isolates was confirmed by PCR assay as described by Zlotkin et al. (1998) and 52 isolates from 6 fish farms gave the expected 1100-bp PCR amplification product confirming the preliminary biochemical identification. Isolates were stored at -70°C in tryptic soy broth (TSB) containing 10% glycerol until further use.

Antibiotic susceptibility test

In preparation for antibiotic susceptibility tests, all 52 isolates were cultured for 24 h on sheep blood agar at 25°C in a CO₂ incubator (5% CO₂). Individual colonies of the bacteria were re-cultured on fresh blood agar to ensure purity of the isolates. Antibiotic susceptibility tests were performed using the disk diffusion method on Mueller-Hinton agar (Oxoid) according to the Clinical and Laboratory Standards Institute (2006). At least three colonies from the Columbia blood agar medium, incubated at 25°C for 24 h, were suspended in 2 ml of sterile saline to a density approximately equal to the McFarland opacity standard No. 0.5. A dry sterile swab was placed in the suspension and excess liquid was expressed into the tube. The bacterial suspension was inoculated onto Mueller-Hinton agar with the swab in such a way that the whole surface of the agar was covered.

Disks that contained the following antibiotics were used: penicillin G (10 U, Oxoid), ampicillin (10 µg, Oxoid), amoxicillin (25 µg, Oxoid), cloxacillin (25 µg, Oxoid), erythromycin (15 µg, Oxoid), clindamycin (2 µg, BBL), lincomycin (10 µg, Oxoid), gentamicin (10 µg, Oxoid), ciprofloxacin (5 µg, Oxoid), florfenicol (30 µg, Oxoid), oxytetracycline (30 µg, Oxoid), bacitracin (10 U, Oxoid), novobiocin (30 µg, Oxoid), sulphamethoxazole/trimethoprim (25 µg, Oxoid), ceftoxime (10 µg, Oxoid) and vancomycin (5 µg, Oxoid). The disks were dispensed on the surface of the medium and incubated aerobically at 25°C for 24 h. The results were recorded as resistant or susceptible by measurement of the inhibition of the zone diameter according to the interpretive standard of CLSI (2002, 2006).

RESULTS

Antibiotic susceptibilities of 52 L. garvieae isolates are given in Table 1. According to the results, L. garvieae isolates were completely resistant to lincomamide and poly-peptide class antibiotics and they also demonstrated strong resistance to other antibiotics, especially cloxacin and gentamycin, while the bacterial specimens were more susceptible to erythromycin and florfenicol than other antibiotics. On the basis of the results, L. garvieae isolates were highly resistant to the examined antibiotics as resistance to different antibiotics ranged from 25 to 100% for all 52 isolates. Antibacterial resistance results of bacterial isolates in different fish farms in Table 2 show that the isolates were resistant at least to one antibiotic and antibacterial resistance was seen in all fish farms. The lowest resistance to erythromycin, the choice drug for the bacteria, was observed in farm 1 (1 of 11 isolates), while the isolates of farm 6, 7, 9 and 10 were completely resistant to erythromycin.

Number of florfenicol and erythromycin sensitive and resistant isolates were (30, 13) and (20, 24), respectively. Non of isolates were sensitive to gentamicin, cloxacinil, bacitracin, clindamicin and lincomycin. According to the
Table 2. Antibacterial susceptibility and resistance of *L. garvieae* isolates in the studied fish farms.

<table>
<thead>
<tr>
<th>Farm</th>
<th>Number of isolates</th>
<th>Ampicillin</th>
<th>Amoxicillin</th>
<th>Cloxacillin</th>
<th>Erythromycin</th>
<th>Gentamicin</th>
<th>Novobiocin</th>
<th>Ciprofloxacin</th>
<th>Florfenicol</th>
<th>Oxytetracycline</th>
<th>Doxycycline</th>
<th>Bacitracin</th>
<th>Vancomycin</th>
<th>Clindamycin</th>
<th>Lincomycin</th>
<th>Cotrimoxazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>7</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td>R I S</td>
<td></td>
</tr>
</tbody>
</table>

results, florfenicol is recommended as the best choice for treatment of lactococcosis.

**DISCUSSION**

Antibiotic resistance surveillance programs are necessary to monitor the susceptibility of bacteria to commonly used antibiotics. Such information is important to discover the development of resistance and the choice of appropriate antibiotics. The results of this study provide useful information in the search for safe and efficient antibiotics. In addition, the results also gave some insight into the problems faced by trout culturists.

All bacterial strains studied in this research showed a high degree of resistance to all antibiotics used in both human medicine and those employed in aquaculture. The results of this study showed that more than 46% of the 52 *L. garvieae* strains were resistant to erythromycin and resistance to clindamycin and lincomycin was observed in all strains. It was reported that *L. garvieae* resistance to clindamycin is accepted as an identification marker (Elliott and Facklam, 1996), while resistance to erythromycin in 24/52 bacteria was not expected and should be considered seriously as this is the drug of choice for the bacteria. Alves d’Azevedo et al. (2000) unlike Diler et al. (2002) found that *L. garvieae* was resistant to erythromycin. Kav and Eganis (2008) reported that all *L. garvieae* isolates were sensitive to erythromycin. By comparison, the results indicate higher rates of erythromycin resistance among *L. garvieae* isolates than were previously reported for rainbow trout fish. These different results may be due to the differences of *L. garvieae* isolate and antibiotics usage in the area. Antibiotic treatment in trout culture is frequently applied on a long-term prophylactic basis in medicated feed. This method of treatment results in the rapid leaching of anti-biotics into the water and sediments allowing horizontal transfer of resistance (Inglis et al., 1993; Livesley et al., 1997; Guglielmetti et al., 2008). This may account for the high proportion of antibiotic-resistant *L. garvieae* isolates found in the farms. On the other hand, the horizontal transferability of antibiotic resistance between *L. garvieae* and *Listeria monocytogenes* was proved by Guglielmetti et al. (2008), demonstrating that a gene coding for
Clinical and Laboratory Standards Institute (CLSI) (2006). Methods for tetracycline resistance tet(S) can be transferred from a fish pathogen to a human pathogen by conjugation in vitro. R plasmids have been also found in other fish pathogens including *Yersinia ruckeri* (De Grandis and Stevenson, 1985 and Klein et al., 1996) and the genus *Aeromonas* (Sandaa et al., 1992; Starliper and Cooper, 1998).

These studies give some explanation as to why so many isolates were resistant to some antibiotics without ever having been in contact with this antibiotic.

The antibiotic resistance of *L. garvieae* has been related to excessive use of antibiotics. It may be suggested from the results that the application of antibiotics should be strictly controlled in fish farms to prevent the dissemination of antibiotic-resistant bacteria that may carry R-plasmids to fish diseases which would not respond to the usual antibiotic therapy and which may confer antibiotic resistance to otherwise antibiotic-sensitive bacterial species. It is significant that even now, farmers complain that antibiotics do not work as they used to.

**ACKNOWLEDGMENT**

The authors are grateful for the financial support of the Islamic Azad University-Shahrekord Branch.

**REFERENCES**


