Resistance profile of *Staphylococcus* sp. isolates from tilapia fillet samples *Oreochromis niloticus* from the northwestern region of Paraná state, Brazil

Perfil de resistência de Staphylococcus sp. isolados de amostras de filete de tilápia Oreochromis niloticus da região noroeste do estado do Paraná, Brasil

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ABSTRACT

Staphylococcus sp. are responsible for several clinical problems in fish farming and humans. This microorganism has a high risk associated with its presence in large numbers, as they have the ability to produce, under certain conditions, thermostable enterotoxins, which, when ingested, cause poisoning and various foodborne diseases. Present in aquaculture environments, Staphylococcus sp, have great potential to acquire antibiotic resistance, due to the use of such substances in the indiscriminate treatment and prophylaxis of bacterial diseases. The presence of food resistant Staphylococcus sp is a current problem in food production and marketing and is a potential danger to human health and a risk to the world fish industry. One hundred tilapia fillet samples were used for isolation of Staphylococcus sp., obtained from fishes located in northwestern Paraná state. It was submitted to antimicrobial susceptibility testing with ten antibiotics. Regarding the 100 samples analyzed, all showed resistance: Tetracycline 18%, Penicillin 31%, Enrofloxacin 14%, Erythromycin 12%, Vancomycin 15%, Gentamicin 14%, Oxacillin 20%, Ampicillin 28% and Cephalothin 11%. The emergence of resistant microorganisms in food is worrying as it can cause serious infections, hospitalizations, and death.

Keywords: Staphylococcus sp, Tilapia, resistance, antibiotics.

RESUMO

Os Staphylococcus sp. são responsáveis por vários problemas clínicos na piscicultura e nos seres humanos. Este microrganismo tem um risco elevado associado à sua presença em grande número, pois tem a capacidade de produzir, sob certas condições, enterotoxinas termoestáveis, as quais, quando ingeridas, causam intoxicações e várias doenças de origem alimentar. Presente em ambientes aquícolas, o Staphylococcus sp, tem grande potencial para adquirir resistência aos antibióticos, devido à utilização de tais substâncias no tratamento indiscriminado e na profilaxia de doenças bacterianas. A presença de Staphylococcus sp resistente aos alimentos é um problema actual na produção e comercialização de alimentos e constitui um perigo potencial para a saúde humana e um risco para a indústria pesqueira mundial. Cem amostras de filetes de tilápia foram utilizadas para isolamento de Staphylococcus sp., obtidos de peixes localizados no noroeste do estado do Paraná. Foi submetida a testes de susceptibilidade antimicrobiana com dez antibióticos. Em relação às 100 amostras analisadas, todas mostraram resistência: Tetraciclina 18%, Penicilina 31%, Enrofloxacina 14%, Eritromicina 12%, Vancomicina 15%, Gentamicina 14%, Oxacilina 20%, Ampicilina 28% e Cefalotina 11%. O aparecimento de microrganismos resistentes nos alimentos é preocupante, pois pode causar infecções graves, hospitalizações e morte.

Palavras-chave: Staphylococcus sp, Tilápia, resistência, antibióticos.
INTRODUCTION

Fish is a food that stands out nutritionally as to the quantity and quality of its proteins, the presence of vitamins and minerals and mainly because it is a source of omega-3 eicosapentaenoic (EPA) and docosahexaenoic (DHA) essential fatty acids. The consumption of these lipids is associated with reduced risk of cardiovascular disease and important functions in the early stages of human development (Sartori & Amancio, 2012).

Due to its high water activity, chemical composition, oxidation-unsaturated fat content and, above all, its near neutral pH (Franco & Landgraf, 2008), the fish is affected by deteriorating and pathogenic microorganisms, among them *Staphylococcus* positive coagulase. This organism causes foodborne diseases, which can be infectious or intoxicating. (Forsythe, 2002).

Present in aquaculture environments, *Staphylococcus* sp, have great potential to acquire antibiotic resistance, due to the use of such substances in the treatment and prophylaxis of bacterial diseases, often indiscriminately. The *Staphylococcus Aureus* is the most frequently isolated microorganism in nosocomial infections worldwide with significant mortality and economic weight for the Health System. The situation has been aggravated in recent years by the appearance of community outbreaks of MRSA2 (CA-MRSA) and strains with intermediate resistance (VISA) or total vancomycin resistance (VRSA) (Guimarães; Momesso; Pupo, 2010). It is one of the most virulent microorganisms of its kind, and one of the most common agents of bacterial infections and food poisoning. Almost all isolates secrete coagulase, an enzyme that acts by binding blood plasma (Strohl; Rouse; Fisher, 2004).

The symptoms of staph poisoning appear quickly and include nausea, vomiting, and abdominal pain. In more severe cases headache, muscle aches and transient changes in blood pressure and pulse rate may occur. The symptom onset is usually rapid, occurring within hours of ingestion. The manifestation can be quite acute, depending on the individual's susceptibility to the toxin, the amount of food eaten and the overall health of the person. The disease is usually self-limiting and usually lasts 2 to 3 days. Severe cases last longer and may lead to death. (Alves, 2012).

The aim of this study was to investigate the resistance profile to ten antibiotics in *Staphylococcus* sp. isolates of tilapia fillets sold in 50 fish and pay lake, in northwestern Paraná State, to deepen knowledge about the epidemiology of infections/ colonization community by these pathogens and their correlations with population data of antimicrobial use.
2 MATERIAL AND METHODS

A total of 100 tilapia fillet samples were collected in 50 fish pay lake, two samples from each establishment, from northwestern Paraná State, from August 2017 to May 2018. The samples were packaged and sent to the University Paranaense laboratory where they were processed. It was weighed 25 g of the sample and placed in 225 mL of 0.85% peptone saline solution, resulting in a $10^{-1}$ dilution. From $10^{-1}$ dilution two further dilutions were performed $10^{-2}$ and $10^{-3}$, always inoculating 1 mL of the previous dilution into 9 mL of 0.85% peptone saline solution.

For isolation of Staphylococcus sp. strains, 0.1 mL of the decimal dilutions were inoculated on plates with Baird-Parker agar, specific medium for isolation of Staphylococcus sp. and incubated at 37 °C for 48 hours. After the incubation period, the plates were read and the characteristic colonies were selected. It was performed standardized bacterial suspension (approximately 108 CFU / mL) by McFarland’s scale. With a buckled platinum handle, a colony of Staphylococcus sp. was collected and inoculated into 4.5 mL of sterile saline solution. Along with the aid of a sterile swab, the suspension was seeded on the surface of Mueller Hinton agar plates. Also with the assistance of sterile tweezers were placed on the surface of the plate, discs containing antibiotics: Tetracycline, Penicillin, Enrofloxacin, Erythromycin, Vancomycin, Gentamicin, Oxacillin, Clindamycin, Ampicillin, Cephalothin. The plates were incubated in an oven at 37 °C for 24 hours after measuring the halos that formed around the discs. The halos were measured with the help of a ruler whose result is expressed in millimeters (mm) and a table for antimicrobial sensibility reading. Inhibition zone diameter measurements were defined in three strains categories: sensitive, intermediate and resistant. Inhibition halos were measured according to the National Committee for Clinical Laboratory Standards (CLSI, 2013).

3 RESULTS AND DISCUSSION

From the 100 samples analyzed, all showed resistance, such as: Tetracycline 18%, Penicillin 31%, Enofloxacin 14%, Erythromycin 12%, Vancomycin 15%, Gentamicin 14%, Oxacillin 15%, Clindamycin 20%, Ampicillin 28% and Cephalothin 11%. Regarding the susceptibility profile found for the isolated strains, we can see in table 1 the results of the antibiotics tested in Staphylococcus sp. isolated from tilapia fillet.
Table 1. Number of samples classified as resistant, intermediate or susceptible to 10 antibiotics tested in 100 samples of *Staphylococcus* sp. isolates of tilapia fillet from fish and pay lakes located in northwestern Paraná State.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>28%</td>
<td>5%</td>
<td>67%</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>11%</td>
<td>9%</td>
<td>80%</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>20%</td>
<td>13%</td>
<td>67%</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>14%</td>
<td>7%</td>
<td>79%</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>12%</td>
<td>30%</td>
<td>58%</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>14%</td>
<td>4%</td>
<td>82%</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>15%</td>
<td>32%</td>
<td>53%</td>
</tr>
<tr>
<td>Penicillin</td>
<td>31%</td>
<td>0%</td>
<td>69%</td>
</tr>
<tr>
<td>Tetracyclina</td>
<td>18%</td>
<td>18%</td>
<td>64%</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>15%</td>
<td>0%</td>
<td>85%</td>
</tr>
</tbody>
</table>

The results varied according to the resistance profile and bacteria sensitivity to the analyzed antibiotics. It was analyzed one hundred samples for the strain of *Staphylococcus* sp. the highest resistance profile for penicillin representing 31% of the samples and ampicillin with 28%. The lowest antibiotic resistance profile was found for cephalothin, where only 11% of the samples showed this profile.

Bacterial resistance to antimicrobials is a phenomenon that tends to grow a lot, either by using these drugs uncontrollably in hospitals or in the agricultural environment, or by using broad-spectrum drugs to fight infections empirically and impulsively, when smaller spectrum choices would be sufficient (Meireles, 2008).

Beta-lactams, including penicillins, act by preventing cell wall synthesis by binding and inhibiting penicillin-binding proteins (PBPs), which act as transpeptidases by incorporating peptide precursors into the forming wall. The resistance of *S. aureus* to oxacillin occurs through the production of beta-lactamases, which are predominantly extracellular enzymes that enable hydrolysis of the beta-lactam ring, not only in penicillin but also in other hydrolysis susceptible beta-lactam antimicrobials, including aminopenicillins. The production of beta-lactamases in *S. aureus* is encoded by the *blaZ* gene, which is part of a transposable element located in a plasmid (Meireles, 2008).

Costa et al. (2008), in Santa Maria RS, analyzing 51 isolates of jundiá (*Rhamdia quelen*), all (100%) were sensitive to gentamicin, (43,14%) were sensitive to erythromycin, 22 (43,14%) to ampicillin, and 5 isolates (3%) was sensitive to penicillin G, resistance to penicillin and ampicillin was more frequent, occurring in 10 isolates of different bacterial genera.

Previously Bordon (2014), analyzing 10 samples of tilapia fillets marketed in the city of São Paulo, SP, 90% of the samples showed at least 3 resistance genes to β-lactam and tetracyclines. In this study were found the tetracycline resistance genes *tetB, tetC, tetD,*
tetE, tetG, tetO, tetS and tetW, as well as in research in Australia the tetC gene was found in all freshwater trout samples (Ndí; Barton, 2012).

Jacobs & Chenia (2007), in South Africa with tilapia (Oreochromis niloticus) trout (Oncorhynchus mykiss) and carp (Cyprinus carps) detected tetB gene, tetracycline resistant gene, in 10.8% and tetE in 43.2% of isolates. Tamminen et al. (2011), in the Baltic Sea, with research on sediments from fish farms revealed a high number of tet genes, even after several years without the use of antibiotics in farms, suggesting the high resistance of these genes in the environment.

As tetracycline is highly used in the aquaculture environment, these results, together with that found in this study, which was 28%, it concerns because of the high resistance of genes in the environment demonstrates the great risk of environmental contamination worldwide.

Jiang et al. (2012), in China, a study with fish from aquaculture, was found that 53% of β-lactam resistant genes and 26% to ampicillin, which corroborate to this present work because the highest resistance found was for β-lactams and 28% ampicillin.

The genes blaLEN confer resistance to some antibiotics such as ampicillin, amoxicillin, among others (Chen et al., 2005). The Amoxylan is given in tilapia breeding to treat some infections (Lemos; Rodrigues; Lopes, 2006); however, regarding ampicillin use, although it is still used in fish farming, several studies show that bacteria commonly found in tilapia crops are resistant to this antimicrobial. (Jacobs & Chenia, 2007). In this study, 28% of 100 samples were ampicillin resistant and 5% with intermediate result.

In search of genetic improvement of tilapia, Brazilian producers have acquired species from Africa and Asian countries, which has had a tradition of cultivating these species for decades. However, by importing these tilapias, bacteria containing antimicrobial resistance may be introduced into Brazil due to the widespread administration of medicines in these countries. (Kubtiza, 2005).

Due to the characteristic of filtering fish, tilapia consume plankton that grows in the breeding ponds. However, in an attempt to increase the formation of this plankton and reducing costs, some producers use animal waste to feed them, bringing antibiotic residues and antimicrobial-resistant bacteria into the aquatic environment (Carneiro et al., 2007).

Cabello, et al. (2006), affirms that the wide use of antimicrobials in the prophylaxis of infections in aquaculture is increasing the possibility of contaminating the environment, animals, and humans with resistant bacteria. In this study, resistance to more than one antimicrobial was observed in Staphylococcus sp. where all samples showed resistance.
According to Rigatti (2010), the presence of oxacillin-resistant bacteria is worrying, however it is among the variant percentage in the country, emphasizing that it is not only a local but also a national issue, which has shown substantial growth of these bacteria in recent years. Being found in the tilapia fillets researched in this work 15% resistance and 32% intermediate result for oxacillin, a worrying and alarming fact for public health and a great risk to the consumer.

In Lavras (MG) Lima et al. (2006), in research with Nile tilapia, demonstrated by antibiograms high resistance to β-lactam antibiotics, mainly ampicillin in fresh and frozen fillets and in samples of intestinal content, fish surface, and feed, found significant values of isolates resistant to tetracycline, also in Lavras (MG), Carneiro et al. (2007), researching tilapia cultivation system in tanks, which did not previously receive or during the experiment any type of antibiotic. It was verified the presence of resistant bacteria in 3 culture systems, and ampicillin (β-lactam) and erythromycin (macrolide) showed the highest percentages (67 to 85%) and about 50% of the samples were resistant to tetracycline, suggesting that there are other sources of antibiotic contamination and not only through their administration in the tank culture system.

In South Africa, Jacobs & Chenia (2007) found resistance in isolates of Aeromonas bacteria from aquaculture fish, mainly in β-lactam antibiotics: ampicillin (86.5%), oxacillin (100%), amoxicillin (89.2%), augmentin (86.5%), cefuroxime (8.1%), ceftriaxone (2.7%) and tetracycline (78.3%) with the majority of isolates obtained from tilapia (Oreochromis niloticus). Demonstrating that antimicrobial resistance is expanding and affecting the entire aquaculture microbiota and not just Staphylococcus sp.

Grando et al. (2008) isolated strains of S. aureus from 29 food handlers being 42.10% from the hands and 57.90% from the nasal cavity of the evaluated individuals. The highest resistance index was found for penicillin G (78.95%), followed by tetracycline (26.31%), while the highest sensitivity index was observed for vancomycin (100%), followed by oxacillin (94.73). %) and ciprofloxacin (89.47%). Data that corroborate with this work because the highest resistance index was found for penicillin 31%, tetracycline 28%, and lowest index for vancomycin and oxacillin 15% followed by cephalothin 11%. In general, the strains showed low levels of resistance, however, the presence of S. aureus in the hands and nasal cavities of the handlers play an important role in the spread of the microorganism because it contaminates the food that will be consumed by the population, such as fish that are highly manipulated and minimally processed.
Even with the development of increasingly specific and broad-spectrum drugs, resistance remains a problem that requires constant consideration. The high occurrence of multiple antibiotic resistance poses a potential public health risk and may make it difficult to treat human and animal diseases, aggravating potentially curable clinical conditions. (Rodriguez & Vesga, 2005). Wistréich & Lechtman (1980) and Murray et al. (1992), they mention that due to the misuse of drugs, microorganisms (such as \textit{S. aureus}) become resistant to the agents and, consequently, more difficult to eliminate.

Padilha (2000), he stated that the extent and frequency of antimicrobial resistance make this issue significant for public health, has received attention from the World Health Organization (WHO) and the World Organization for Animal Health. The emergence and dispersal of resistant bacteria hinder clinical procedures, increases treatment costs, and human morbidity and mortality rates. In Brazil, there is no list of recommended antimicrobials for aquaculture and there is little information on the use of antibiotics, their concentrations and how they should be administered in aquaculture. Antimicrobials are indiscriminately used and released in large quantities into breeding tanks. Information on monitoring concentration and environmental impact, especially regarding acute toxicity, risk of contamination of farms and possible adverse effects is scarce (Carraschi, 2010). Regulating the use of antimicrobials in aquaculture is inefficient in Brazil and most developing countries (Carneiro et al., 2007).

The presence of resistant bacteria in the aquatic environment reflects significant changes in the population of other aquatic organisms, such as algae, thereby altering the ecological balance (Hernandez et al., 2005). Therefore, alternatives to planned antibiotic therapy, the adoption of sanitary management measures and the use of probiotics are very important to reduce risks to humans and the environment without financially damaging aquaculture production. (Cabello et al., 2006).

4 CONCLUSION

All isolates of \textit{Staphylococcus} sp. tested showed resistance, an alarming and worrying fact for public health as the consumption of resistant bacteria through contaminated food causes serious health problems and resistance to conventional medical treatments, increasing treatment costs and human morbidity and mortality rates, in addition to the large environmental contamination caused by these resistant bacterial genes.
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