Microbiota characterization of the catfish (*Cathorops agassizii and Genidens genidens)* sting venom

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Abstract

This work aimed to study the bacterial contamination of the catfishes stings *Genidens genidens* (Valenciennes, 1839); and *Cathorops agassizii* (Agassiz, 1829) found in the estuary-bay complex of Santos and São Vicente (São Paulo State). Fish samples for bacteriological analyses were obtained and constituted of a group of 50 specimens, being 25 of *Cathorops agassizii* and 25 of *Genidens genidens*,. The bacteriological analyses showed that there was contamination of the stings by 13 different strains of Enterobacteriaceae with *Klebsiella pneumoniae* (26,80%) as the most frequent bacteria and lower percentual frequencies for *Enterobacter* sp and *Escherichia coli* (16,27%) and *Serratia marcescens*, *Serratia* sp. and *Proteus mirabilis* (1,16%). Gram positive bacteria, as well fungi species were not detected in the samples. In basis of the Gram negative species characterized , is possible to consider the bacterial strains are representative of the environmental public health conditions, as well as, accidents with these fish stings are able to develop significative acute secondary infections in humans.

Key-words:
Microbiota.
Brazilian catfishes.
Secondary infection.
Accident.

Introduction

Marine Catfishes are widely distributed along the Brazilian coast, playing an economical role in the south coast by its great contribution to fishermen that seek them along rivers, estuaries and lagoons areas at the spawning time[12].

Due to its abundance in the coastal area and its frequent capture in stocking-waters or in bottoms, stings can penetrate deeply and break inside human skin and soft muscle tissues[7] mostly in consequence of manipulation of live fish, being the symptoms an effect of the action of glands and toxins soon after the immediate wound contamination occurs[13].

However, little is known about the secretory glands and pharmacological action of poisons of most brazilian fishes. Although few species are able to cause fatal accidents in humans, many of them can produce serious poisonings characterized by intense and potential pain, as well threatens to the life according Church and Hodgson, 2002[4].

Signs and symptoms of these accidents may be defined as: punctiform or deep wounds, erythema, edema, local pain, pain irradiation for the root member, cold sweating, indisposition, fever, nausea/vomits, psychomotor agitation and secondary infection[5].

Likewise, accidents provoked by stings of *Plotosus* fish (*Plotosus canius*) produces intense pain, swelling and, gangrene in the affected areas. Some authors[6] also showed that the Arabian catfish produces a poison in glands close to the pectoral sting, which has not been referred in the literature in relation to the Brazilian Catfishes. Studies on its chemical composition and proteic components distribution[7], have shown that this poison has high concentration of proteins, though small of lipids, carbohydrates and nucleic acids as well as a capability to induce similar enzymatic activities as the alkaline phosphatase, esterases and hemolysins[3].

Toxicity of the proteic soluble fraction

extract from the skin of catfish *Arius bilineatus* (previously identified as *Arius thalassinus*), was also examined in rabbits, and when intravenously injected it caused death in the tested animals[8]

Material and Methods

Sampling Area

The sampling area comprised a region of the complex bay- estuary of Santos and São Vicente (23º52'30" S and 46º22'30" W), which is characterized as a important industrial, commercial, tourist and fishing pole at São Paulo State, Brazil.

This area is also exposed to a strong environmental impact by fecal coliforms, heavy metals, hydrocarbonates, detergents and surfactants compounds, since it receives residues and industrial wastes from Cubatão, Santos, Vicente de Carvalho, São Vicente and even from the Great São Paulo City area [9].

Fish and Stings Collection

Specimens and stings of *Cathorops agassizii* (Agassiz, 1829), and *Genidens genidens* (Valenciennes, 1839), popular named "bagre amarelo and bagre urutu", respectively, were obtained by fishing procedures. As soon as collected, pectoral and dorsal stings (n=3) were sectioned with sterilized haemostatic tweezers and pliers and individually conditioned in tubes containing Stuart transport Medium (Oxoid). This procedure was performed in 50 specimens of catfish, *C. agassizii* (N=25) and *G. genidens* (N=25), weekly collected from November (2002) to January (2003).

Microbiological Analysis

All procedures here described were carried out at the São Vicente Laboratory, UNESP. Samples were *homogeneized* for 1 min, then seeded in Petri dishes containing Brucella Agar (Oxoid) added with 5% of defibrinated sheep blood, Chocolate Agar, Phenylethylalcohol Agar Médium (Oxoid) supplemented with 5% of sheep blood, *Pseudomonas* Selective Agar (Oxoid), M-*Enterococcus* Agar (Difco), Mac Conkey Agar (Oxoid), Manitol Salt Agar (Oxoid), Mycosel Agar (Oxoid) and Sabouraud Dextrose Agar (Oxoid) added with chloramphenicol, Mycosel Agar and Sabouraud Dextrose Agar, all incubated in conventional atmosphere at 37º C / 24-48 h for the aerobic and facultative bacteria isolation.

For fungi isolation, inoculated plates of Mycosel Agar and Sabouraud Dextrose Agar were incubated at 37º C and observed for 14 days. Concomitantly, Petri dishes containing Brucella Agar supplemented with 5% of lysed sheep blood, 1% of vitamin K1 (1 g/mL) and hemine (5 g/mL), 100 μg of gentamycin and 7,5 μg of vancomycin and Clostrisel Agar (Merck), after incubation at 24-48 h to 37º C in GASPAK jars, were used for the strict anaerobic bacteria isolation. Microbial identification was done according standard methods described by Koneman *et al.* (1985); Baron *et al.* (1994) and Murray *et al.* (1995)[3,8,11].

Results and Discussion

According the data shown in the figure 1, the most frequent bacterial species were K. *pneumoniae*, followed by *Enterobacter* sp and *Escherichia coli*. The characterized flora was exclusively represented by *Enterobacteriaceae*.

The sting contamination by *Enterobacteriaceae*, mainly represented by coliforms organisms, expresses a low environmental quality. These bacteria inhabiting the gut of humans and animals can cause serious infections when found in organic sites other than the bowel.

Regarding to these aspects, Murphey, Septimus and Wagner[10] referred the sting contamination by *Enterobacteriaceae Aeromonas hydrophila* and *Vibrio* spp. In this study, notwithstanding the use of an extended methodology, it was only observed the presence of *Enterobacteriaceae* in the stings, which does not agrees with the findings of Murphey, Murphey, Septimus and Wagner[10].

On the other hand, studies that show the biochemical characterization, as well as the physiopathological and immunological

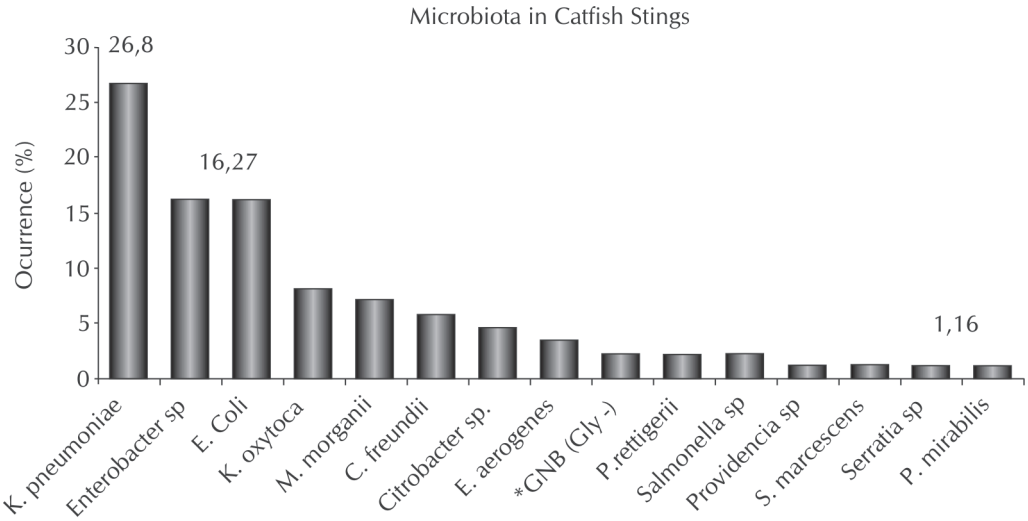

Figure 1 - Distribution of bacterial strains in 50 samples of Catfishes

*Gram Negative Bacilli (Glycose)

effects of these catfish venoms are in progress.

Some authors, as Thulesius et al. [11], describe for *Arius thallasinus* the production of venom in the skin and in glands close to pectoral stings with similar pharmacological properties. Al Hassan et al. [6], in this species observed that these fishes are able to secrete under stress or when wounded, a proteic gel through the skin surface by unicellular glands named "club cells" and some toxic proteins with soluble and insoluble fractions from the skin mucus, that ranged among 15 kDa to 45 kDa. The authors also *emphasize* the absence of studies about the biochemical structure of the toxic proteins found in the stings.

Conclusion

Taking in consideration the results obtained with the characterization of a Gram negative bacterial flora contaminating the sting of Catfish, it is plausible to infere that in basis of a close relationship between cause and effect, the aggravation of the lesions produced in first degree by the poison physiopathological action, possibly induces the emergence of deep wound infections which mostly demands immediate nosocomial internment of the victims.

Caracterização microbiológica do veneno do ferrão do Bagre (*Cathorops agassizii and Genidens genidens)*

Resumo

Este trabalho teve por objetivo o estudo da contaminação bacteriana do veneno do ferrão dos bagres *Genidens genidens* (Valenciennes, 1839); e *Cathorops agassizii* (Agassiz, 1829) encontrados no Complexo Baia-Estuário de Santos e de São Vicente (Estado de São Paulo). Foram obtidas amostras dos peixes para análises bacteriológicas que constituíam de um grupo de 50 espécimes sendo, 25 *Cathorops agassizii* e 25 de *Genidens genidens*. As análises bacteriológicas mostraram que havia contaminação nos ferrões por 13 diferentes linhagens de *Enterobacteriaceae*, sendo a *Klebsiella pneumoniae* (26,80%) a bactéria mais

Palavras-chave:
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Bagre brasileiro.
Infecção secundária.
Acidente.

freqüente enquanto que as *Enterobacter* sp e *Escherichia coli* (16,27%), *Serratia marcescens*, *Serratia* sp e *Proteus mirabilis* (1,16%) apresentaram os mais baixos percentuais de contaminação. Nas amostras que apresentaram Bactérias Gram positivas não foram detectadas espécies de fungos. Enquanto que nas amostras que apresentaram bactérias Gram negativas, foi possível considerar alta contaminação bacteriana representando periculosidade em relação aos aspectos ambientais voltados á saúde pública. Destaca-se ainda que acidentes ocorridos por ferimentos causados em função do ferrão do bagre podem desenvolver significativas infecções secundárias agudas em humanos.

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