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A N I N T E R N A T I O N A L J O U R N A L

Bacterial Community Associated with Fish and Water from Congonhas River, Sertaneja, Paraná, Brazil

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ABSTRACT

A bacteriological study was conducted on fish and water from Congonhas River, Sertaneja (22º58' S; 50º58' W), Paraná State, Brazil. From 44% of the analysed fish, bacteria belonging to Pseudomonas, Acinetobacter, Aeromonas, *Enterobacteriaceae*, Micrococcus, Bacillus *and* Lactobacillus *were isolated. The group most frequently isolated from fish was* Aeromonas. *In the water, the bacterial groups detected were* Pseudomonas, Acinetobacter, Aeromonas, *Enterobacteriaceae*, Bacillus *and* Flavobacterium, *from which* Flavobacterium *and* Acinetobacter *were the most abundant. The numbers of Colony Forming Units per millilitre of water varied from* $3.1x10^2$ *to* 1.0×10^3. *Although a clear pattern was not detected in the susceptibilities/resistances of the isolated strains to nine antimicrobial substances, Gram negative aerobic bacteria were more resistant than the other strains. A simultaneous resistance to furazolidone, oxolinic acid and norfloxacin, particularly in the bacteria isolated from fish, as well as in the aerobic strains isolated from water was observed. The antimicrobial substances to which less resistances were found were oxytetracycline in the strains isolated from water, and trimethoprim-sulphamethoxazole, oxytetracycline and chloramphenicol in those isolated from fish.*

Key Words: Bacterial community, fish, Congonhas River, *Aeromonas*, *Pseudomonas*, resistance

INTRODUCTION

Although there is no certainty about the existence of strictly aquatic bacteria, the most widespread opinion among the various authors is that the majority of the bacteria found in aquatic environments are of soil origin and carried into the water due to rain or to accidental introduction, whether they are natural or a direct consequence of human activity. However, every water mass has its bacterial community, although these communities may vary greatly in both the present groups and the number of cells (Sousa, 1996).

Among the bacteria better adapted to life in soil or water are strains belonging to *Bacillus* and *Pseudomonas* (e.g., *P. aeruginosa*). However, other groups have been referred to be present on several occasions in freshwater, including Enterobacteriaceae, *Flavobacterium*, *Acinetobacter*, *Moraxella*, *Aeromonas*, *Micrococcus*, *Staphylococcus*, *Streptococcus* and anaerobic bacteria (e.g., *Clostridium*) (Toranzo *et al.*, 1989; Sousa, 1996).

Concerning the bacterial load of water, the numbers found varied greatly, usually increasing from the water-springs to the river mouths and

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coastal waters, where 10^5 colony forming units per millilitre (CFU/ml) are often detected (Sousa, 1996).

The internal organs of apparently healthy fish should be sterile, but isolations of bacteria from kidney, liver and spleen have frequently been reported (Nieto *et al.*, 1984; Lindsay, 1986; Cahill, 1990; Sousa, 1996). The presence of microrganisms in internal fish organs could indicate the breakdown of immunological defense mechanisms (Cahill, 1990), but, as McVicar (1997) pointed out, the occurrence of an infection in a fish could not be necessarily an abnormal event nor it would lead to a disease situation. Moreover, under natural conditions, most infectious agents coexist with their host without causing significant disease. However, stress factors such as overcrowding found in aquaculture facilities are frequently blamed for the dissemination of infections and also for the occurrence of many disease outbreaks (McVicar, 1997). According to this author, there is much evidence of infections transfer from wild into farmed fish populations, as it is generally concluded that most of the fish farm diseases have its origin in the endemic occurrence of a range of pathogens in the surrounding area. Therefore, the knowledge of the infectious agents that exist in an aquatic ecosystem is of great importance if one is to, for instance, install an aquaculture facility. The knowledge of the pathology of species potentially important in aquaculture contributes to the determination and implementation of adequate prophylactic measures. Obviously, these include all the actions intended to prevent the introduction of bacteria considered as obligate pathogens such as, for instance, *Aeromonas salmonicida*, *Yersinia ruckeri* or *Renibacterium salmoninarum*.

Taking into account the scarcity of bacteriological work conducted on fish in Brazil (Sousa *et al*., 1996a), the present study was done in Congonhas River, close to its mouth in Tibagi River, Sertaneja, Paraná State. This was the first bacteriological study conducted in a natural freshwater fish population and, simultaneously, in their environment, in Brazil.

MATERIALS AND METHODS

In this study, a bacteriological analysis was conducted of fish and water from Congonhas River, near its mouth in Tibagi River, Sertaneja (22º58' S; 50º58' W), Paraná State, Brazil. Fifty-nine fish belonging to fourteen species (Table 1) were captured with gill nets and immediately transported to the laboratory where they were sacrificed. After careful dissection, kidney and liver samples of each fish were aseptically streaked onto Tryptic Soy Agar (TSA; Difco) and incubated for 48-72 hours at 22ºC.

Water samples were collected at margins and in the middle of the river, about 15cm down the water surface in 100ml sterilised bottles. These samples were immediately transported to the laboratory and subjected to serial ten-fold dilutions in sterile distilled water. From each of these dilutions, 1ml was plated onto TSA. Plates were also incubated for 48-72 hours at 22ºC.

After incubation, the total numbers of CFU/ml as well as the CFU/ml corresponding to each of the different morphological types present in each plate were determined using only the plates with 30-300 colonies. Representative colonies of different morphological types present in each plate were isolated and re-streaked in new plates containing TSA until purity was attained.

Pure cultures of the isolated bacteria were subjected to standard morphological, physiological and biochemical tests and the taxonomic position of those isolated were determined mainly following the schemes of Holt *et al.* (1994) and Thoesen (1994). API 20E and API 20NE systems (bioMérieux) were also employed, and the results were recorded after 48 hours at 22ºC. The resistance/susceptibility patterns of those isolated were determined by the disc diffusion method on Müeller-Hinton agar (MHA; Difco) for the following antimicrobial substances: furazolidone (50μg), oxolinic acid (2μg), norfloxacin (10μg), oxytetracycline (30μg), tetracycline (30μg), chloramphenicol (30μg), ampicillin (10μg), streptomycin (10μg) and trimethoprim-sulphamethoxazole (23.75μg -1.25μg).

RESULTS

The results obtained are summarised in Tables 1 to 4. Twenty-six (44%) of the analysed fish had at least one bacterial strain in their internal organs (Table 1). The bacterial groups detected in these fishes, as well as the number of strains isolated, whether in pure or mixed cultures, are indicated in Table 2. *Aeromonas* was the most abundant group,

from which 13 strains were isolated (10 in pure culture), followed by *Pseudomonas* with 6 strains isolated and Enterobacteriaceae and *Bacillus* with 3 isolates from each group. *Acinetobacter*, *Micrococcus* and *Lactobacillus* were also detected. Four strains of Gram-positive bacilli were not identifiable.

Table 3 shows the qualitative and quantitative composition of the water bacterial community. Most of the groups found in these environmental samples were also represented in the internal fish organs. Thus, *Pseudomonas*, *Acinetobacter*, *Aeromonas*, Enterobacteriaceae and *Bacillus* were detected in both types of samples, while *Flavobacterium* was only isolated from the water and *Micrococcus* and *Lactobacillus* were present only in fish. However, 9% of the strains isolated from the water were not assigned to any bacterial group.

Table 1 - Taxonomic status of the analysed fish and numbers of individuals with at least one bacterial strain in their internal organs

SUPERORDER	ORDER	FAMILY	SUBFAMILY	SPECIES	Number of fish analysed	Number of fish with bacteria
Ostariophysi	Characiformes	Characidae	Tetragonopterinae	*Astyanax altiparanae*	8	1
			Triportheinae	*Triportheus angulatus*	6	3
		Acestrorhynchidae		*Acestrorhynchus lacustris*	3	3
				Schizodon intermedius	5	2
		Anostomidae		*Leporinus elongatus*	1	0
				Leporinus friderici	1	0
		Curimatidae		*Steindachnerina insculpta*	16	9
		Serrasalmidae		*Serrasalmus spilopleura*	2	1
				Pimelodus maculatus	2	2
	Siluriformes	Pimelodidae	Pimelodinae	*Iheringichthys labrosus*	7	4
			Luciopimelodinae	*Pinirampus pirinampu*	2	0
				Crenicichla sp. 1	2	0
Acanthopterygii	Perciformes	Cichlidae		*Crenicichla* sp. 2	1	0
		Sciaenidae		*Plagioscion squamosissimus*	3	1
				Total	59 (100%)	26 (44%)

The numbers of CFU/ml of water varied from 3.1 x 10^2 in the middle of the river to 1.0 x 10^3 on its left margin. Although some asymmetry was observed in the abundance of the diverse bacterial groups detected in the three water -sampling sites, the most significant fact was the clear predominance of aerobic over facultative anaerobic bacteria. In fact, 70% of the isolated strains belonged to *Flavobacterium* (31%), *Acinetobacter* (26%) and *Pseudomonas* (13%). *Aeromonas* was the only group of facultative anaerobic bacteria that accounted for more than 10% of the strains isolated from the water. However, one must take into account that the methodology employed prevents the isolation of both anaerobic and microaerophilic bacteria.

Table 2 - Bacterial strains isolated, in pure (P) or mixed (M) cultures, from the fish used in this study.

	Pseudomonas		***Acinetobacter***		***Aeromonas***		**Enterobacte-riaceae**		***Micrococcus***		***Bacillus***		***Lactoba-cillus***		**Other Gram+ bacilli**		**Not identified**		**Total**	
Culture	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M	P	M
Astyanax altiparanae	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-
Acestrorhynchus lacustris	-	-	-	-	2	-	-	-	-	-	-	-	-	-	1	-	-	-	3	-
Triportheus angulatus	-	-	-	-	-	-	-	-	1	-	1	-	-	-	-	-	1	-	3	-
Schizodon intermedius	-	1	-	-	1	-	-	-	-	-	-	1	-	-	-	-	-	-	1	2
Steindachnerina insculpta	3	2	-	1	3	1	1	1	-	-	-	-	-	-	-	2	-	-	7	7
Serrasalmus spilopleura	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	1	-	-	-	2
Pimelodus maculatus	-	-	-	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-	2	-
Iheringichthys labrosus	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	1	-	4	-
Plagioscion squamosissimus	-	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-	-	-	-	2
Total	3	3	-	1	10	3	1	2	1	-	2	1	1	-	1	3	2	-	21	13

Table 3 - Bacterial groups detected in the water (temperature = 19ºC) of Congonhas River. CFU/ml (colony forming units detected per millilitre of water).

	CFU/ml	*Pseudomonas*	*Flavobacte-rium*	*Acinetobacter*	*Aeromonas*	Enterobacte-riaceae	*Bacillus*	Not identified
Right Margin	4.3×10^2	28%	32%	-	9%	7%	12%	12%
Centre	3.1×10^2	-	-	-	62%	3%	3%	32%
Left Margin	1.0×10^3	11%	40%	46%	-	3%	-	-
Average	5.8×10^2	13%	31%	26%	13%	4%	4%	9%

Table 4 - Susceptibility/Resistance profiles of bacteria isolated from fish used in the present study. R-resistant; S-susceptible; FUR 50, furazolidone (50µg); OA 2, oxolinic acid (2µg); NOR 10, norfloxacin (10µg); OT 30, oxytetracycline (30µg); T 30, tetracycline (30µg); C 30, chloramphenicol (30µg); AMP 10, ampicillin (10µg); S 10, streptomycin (10µg); SXT 25, trimethoprim-sulphamethoxazole (23,75µg-1,25µg)

	FUR 50	OA 2	NOR 10	OT 30	T 30	C 30	AMP 10	S 10	SXT 25	Number of strains with profile
Pseudomonas	R	R	R	S	S	S	S	R	S	4
	R	R	R	S	S	S	S	S	S	1
	R	R	R	S	R	S	S	R	S	1
Flavobacterium	-	-	-	-	-	-	-	-	-	-
Acinetobacter	R	R	R	S	S	S	S	S	S	1
Aeromonas	S	S	S	S	S	S	R	R	S	12
	S	S	S	S	S	S	R	S	S	1
Enterobacteriaceae	R	R	R	S	S	S	S	S	S	1
	S	S	S	S	S	S	R	R	S	1
	S	S	S	R	R	R	R	S	S	1
Micrococcus	R	R	S	S	S	S	S	S	S	1
Bacillus	S	S	S	S	S	S	S	S	S	1
	S	R	R	S	S	S	S	S	S	1
	R	R	R	S	S	S	S	S	S	1
Lactobacillus	R	R	R	S	S	S	S	S	S	1
Other Gram+ bacilli	R	R	R	S	S	S	S	S	S	3
	R	R	R	S	R	S	S	S	S	1

Table 5 - Susceptibility/Resistance profiles of the bacteria isolated from the water of Congonhas River. R - resistant; S - susceptible; FUR 50, furazolidone (50µg); OA 2, oxolinic acid (2µg); NOR 10, norfloxacin (10µg); OT 30, oxytetracycline (30µg); T 30, tetracycline (30µg); C 30, chloramphenicol (30µg); AMP 10, ampicillin (10µg); S 10, streptomycin (10µg); SXT 25, trimethoprim-sulphamethoxazole (23,75µg -1,25µg)

	FUR 50	**OA 2**	**NOR 10**	**OT 30**	**T 30**	**C 30**	**AMP 10**	**S 10**	**SXT 25**	**Percentage of strains with profile**
Pseudomonas	R	S	S	S	S	S	R	R	S	50
	R	R	R	S	R	R	R	S	R	33
	R	R	S	S	S	S	S	S	S	11
	S	S	S	S	S	S	R	R	S	6
Flavobacterium	R	R	R	S	S	S	R	S	S	61
	S	S	S	S	R	R	R	R	S	16
	S	S	S	S	S	S	S	R	S	16
	R	R	R	S	S	S	S	R	R	7
Acinetobacter	R	S	S	S	S	R	R	S	R	73
	R	S	S	S	S	S	S	R	S	18
	R	S	S	S	S	R	S	S	R	9
Aeromonas	S	S	S	S	S	S	R	R	S	100
Enterobacteriaceae	S	S	S	S	S	S	S	S	S	50
	R	S	S	S	S	S	R	R	S	33
	R	S	S	S	S	S	S	S	S	17
Micrococcus	-	-	-	-	-	-	-	-	-	-
Bacillus	S	S	S	S	S	S	S	S	S	83
	S	R	R	S	S	S	S	S	R	17
Lactobacillus	-	-	-	-	-	-	-	-	-	-
Other Gram+ bacilli	-	-	-	-	-	-	-	-	-	-

The susceptibility/resistance profiles of the isolated bacteria to nine antimicrobial substances are shown in Tables 4 and 5. The aerobic Gram-negative bacteria showed, in general, more resistances than the other groups. This was particularly apparent in *Pseudomonas* isolated from fish (Table 4) and, mostly, in the three groups of aerobic Gram-negative bacteria (*Pseudomonas, Flavobacterium* and *Acinetobacter*) isolated from water (Table 5), in which many multirresistances were observed. Particularly significant was the fact that 33% of the *Pseudomonas* isolated from water were resistant to seven out of the nine antimicrobial substances used in this study, being susceptible only to oxytetracycline and streptomycin.

Strains susceptible to all the antimicrobial substances used were found only in *Bacillus* and Enterobacteriaceae. The majority of the bacteria from these two groups that were isolated from water showed no resistance at all. However, the group which showed the least resistances was *Aeromonas*. All strains belonging to this genus were resistant to ampicillin and all but one were resistant also to streptomycin. It should be noted, however, that the resistance to ampicillin is a well-known feature of the majority of *Aeromonas*. Concerning the strains isolated from fish, except for Enterobacteriaceae and, mostly, *Aeromonas*, the antimicrobial substances to which more resistances were detected were furazolidone, oxolinic acid and norfloxacin. On the other hand, only one strain belonging to Enterobacteriaceae was resistant to oxytetracycline and to chloramphenicol, while no resistance at all was detected to the potentiated sulphonamide. Little resistance was also found to tetracycline and to ampicillin. To ampicillin, only the *Aeromonas* and two strains of Enterobacteriaceae were resistant. Among the strains isolated from water, many resistances were found, particularly to furazolidone, ampicillin and streptomycin. Curiously, in these bacteria, no resistance was detected to oxytetracycline. Little resistance was also found to tetracycline and, except for *Acinetobacter*, to trimethoprim-sulphamethoxazole and to chloramphenicol.

DISCUSSION

From the internal organs of the fish included in this study, strains belonging to *Pseudomonas*, *Acinetobacter*, *Aeromonas*, Enterobacteriaceae, *Micrococcus*, *Bacillus* and *Lactobacillus* were isolated. From these groups, *Micrococcus* and *Lactobacillus* were not detected in the water, of which, however, *Flavobacterium* strains were also isolated. The presence of these bacteria both in the aquatic environment and in the internal fish organs have been often observed (Sugita *et al.*, 1985; Sakata, 1989; Toranzo *et al.*, 1985, 1993; Cahill, 1990). *Aeromonas* was the most abundant group in the fish, while, in global terms, the Gram-negative aerobic bacteria (specially *Flavobacterium* and *Acinetobacter*) were predominant in the water of the Congonhas River. Several of these bacteria are opportunistic pathogens and may cause mortalities when the fish are under stress. Among these were many strains of *Aeromonas*, which have been the cause of economic losses frequently associated to elevated levels of water pollution (Sousa *et al.*, 1996b). The number of CFU/ml obtained in this research was in the ranges referred by other authors (Horsley, 1973; Sousa, 1996).

Although a clear pattern of susceptibility/resistance to the various antimicrobial substances was not detected, the aerobic Gram-negative bacteria in general showed more profiles of susceptibility/resistance and more resistances. Among these bacteria resistance to more antibiotics was found (five antibiotics in *Flavobacterium* and seven in *Pseudomonas*). The difficulty of treating septicaemias caused by *Pseudomonas* with the drugs normally available in aquaculture has been already referred by other authors (Toranzo *et al.*, 1992). On the contrary, the Gram-negative facultative anaerobic bacteria (specially *Aeromonas*) showed less resistance. Only the groups Enterobacteriaceae and *Bacillus* strains were susceptible to the nine antibiotics used, while the *Aeromonas* was resistant only to ampicillin (which is a characteristic feature of this group) and, with the exception of only one strain to streptomycin. Curiously, a simultaneous resistance to furazolidone, oxolinic acid and norfloxacin was frequently observed, mainly among the bacteria isolated from fish and among the aerobic bacteria isolated from water. The antimicrobial substances to which less resistance was detected were oxytetracycline for the bacteria isolated from the water, and, for those isolated

from fish, trimethoprim-sulphamethoxazole, oxytetracycline and chloramphenicol.

Analysis of Tables 4 and 5 showed that the antibiotic most indicated for the treatment of outbreak caused by any of these bacteria was oxytetracycline as only one strain (belonging to the Enterobacteriaceae group) was resistant to this drug. Trimethoprim-sulphamethoxazole could also be a good choice, as no resistance to this compound was found among the bacteria isolated from fish and with the exception of *Acinetobacter*, also among those isolated from water little resistances were detected to this sulphonamide. Another option for treatment could be tetracycline, as only three strains isolated from fish (one belonging to *Pseudomonas*, one to Enterobacteriaceae and the third being a Gram-positive bacillus which was not identifiable), as well as some strains of *Pseudomonas* (33%) and of *Flavobacterium* (16%) isolated from water were resistant to this substance.

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RESUMO

Foi realizado um estudo da comunidade bacteriana de peixes e da água do rio Congonhas, próximo à sua foz no rio Tibagi, município de Sertaneja, Paraná, Brasil. De 44% dos peixes analisados, foram isoladas estirpes de *Pseudomonas*, *Acinetobacter*, *Aeromonas*, Enterobacteriaceae, *Micrococcus*, *Bacillus* e *Lactobacillus*. Destas, a mais abundante foi *Aeromonas*. Na água do rio Congonhas foram detectados os grupos *Pseudomonas*, *Acinetobacter*, *Aeromonas*, Enterobacteriaceae, *Bacillus* e *Flavobacterium*, dos quais os predominantes foram *Flavobacterium* e *Acinetobacter*. Os números de unidades formadoras de colônias por mililitro de água variaram entre $3{,}1x10^2$ e $1{,}0x10^3$. Embora não tenha sido detectado um padrão claro nas resistências das diferentes estirpes a nove substâncias antimicrobianas, tais resistências foram observadas em maior número entre as bactérias Gram negativas aeróbias. Foi observada muito freqüentemente uma resistência simultânea à furazolidona, ao ácido oxolínico e à norfloxacina, sobretudo nas bactérias isoladas dos peixes e nas aeróbias isoladas da água. Os antibióticos aos quais foram observadas menos resistências foram a oxitetraciclina, nas bactérias isoladas da água, e o trimetoprim-sulfametoxazol, a oxitetraciclina e o cloranfenicol, nas estirpes isoladas dos peixes.

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