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Microbial flora of six freshwater fish species from Asa river, Ilorin, Nigeria.

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Resumen: Se analizó la flora microbiana de piel, pulmones y tracto gastrointestinal en 75 peces de seis géneros. Predominaron los Enterobacteriaceae (*Escherichia coli, Enterobacter aeogenes, Klebsiella pneumoniae y Edwardsiella tarda*), *Aeromonas hydrophila* y *Acinetobacter sp.* Entre las bacterias Gram-positivas las más comunes fueron *Staphylococcus* y *Micrococcus.* Los hongos incluyeron *Achyla colorata, Saprolegna* sp., *Mucor* sp., *Rhizopus* sp. y *Aspergillus* sp. Todas las bacterias mostraron factores de resistencia por lo menos a un antibiótico.

Key words: African fishes, fish endoparasites, bacteriae, fungi, Nigeria.

The aquatic medium is extremely vulnerable to pollution that could result from domestic, industrial, and agricultural discharges, contamination from soil and airborne infections. Hence, fishes and other aquatic life are prone to all environmental hazards which may be favourable or otherwise. Although infection as a result of microbial contamination does not usually result in disease, environmental stresses may upset the balance between the potential pathogens and their hosts. A number of diseases involving normal flora of gastrointestinal tract have been a documented. Some of these microflora may not be pathogenic for fishes but when consumed by man, a desease condition ensues *e.g.* typhoid fever and bacterial food poisonings.

The public health significance of fish contamination lies not only in their ability to cause disease but also in their possible role in the transfer of antibiotic resistant strains to other pathogens. Cross-contamination of household utensils and other foods by such fishes could also spread infection at home. Their microflora, especially from the gastrointestinal tract, can be of economic importance because it is capable of effecting rapid post-mortem deterioration; in aquaculture, it has a growth-depressing effect.

The information about the microbial populations of homeotherms, like fishes, has been concerned for most part therefore with their role in contamination and spilage of catches and with the spread of faecal pollution indicator organisms (Geldrich and Clarke 1966). This study was undertaken to investigate the microflora of the gastrointestinal tract, skin and gills of fishes randomly caught in the Asa River, which has been much abused by human activities. The potentials of the isolates for pathogenicity, their role in the epidemiology of some human infections and in fish spoilage were appraised.

Sampling site: The Asa River runs through the Ilorin urban area wiht a catchment region of about 104 km^2 (8^o 36'- 8^o 24' N; 4^o 36' - 4^o 10'E). It is fed in the urban fringe area with street drains, sewer out-drains, industrial discharges and similarly subjected to indescriminate dumping of garbage and human faecal matter.

Notwithstanding, the river is used for fishing, laundry and irrigation of vegetable and cereal farms.

Sample collection: A total of 75 live fishes randomly caught at different points on the river and belonging to six genera were examined over a period of five months. All samples were

TABLE 1

Frequency of isolation of bacterial species from different anatomical sites

Bacterial Species	Anatomical Sites Skin	Gills	S. Intestine	Total	%
Escherichia coli	23	36	45	104	19.0
Enterobacter aerogenes	12	3	41	56	10.2
Klebsiella pneumoniae	-	-	7	7	1.2
Shigella flexneri	-	-	6	6	1.1
Edwardsiella tarda	11	7	13	31	5.6
Proteus	2	5	19	26	4.7
Salmonella typhimurium	-	-	7	7	1.2
Serratia sp.	-	-	4	4	0.7
Aeromonas hydrophila	5	11	17	33	6.0
Acinetobacter sp.	6	9	29	44	8.0
Pseudomonas alcaligenes	11	7	18	36	6.6
Staphylococcus aureus	29	27	14	70	12.8
Staphylococcus epidermidis	31	17	10	58	10.6
Micrococcus luteus	5	21	32	58	10.6
Glostridium perfringes	-	-	77	77	1.2

transported immediately to the laboratory in sterile plastic containers.

Postmorterm and microbiological analysis: The fishes were killed in the laboratory and processed immediately. Each body was swabbed with a sterile cotton swab which was then used to seed plates of Nutrient Agar, MacConkey Agar and Malt Extract Agar (Oxoid). To obtain the gut specimen, the ventral surface was thoroughly washed with cotton wool, dipped in alcohol to disinfect it. The area was opened aseptically and one gram of the small intestine was sectioned and macerated in 9 ml of sterile distilled water. This was serially diluted to 10^{-3} and 1 ml was plated using the media as for the skin specimen. The same procedure was repeated for the fills after aseptically opening the operculum.

The baiting technique of Alabi (1967) was also used to supplement the isolation of fungi in addition. For this purpose, separate portions of the gills and scrappings of the skin were put into petri dishes containing sterile disttilled water and boiled seeds of *Crotolaria retusa.* The plates were observed after 96 hours at room temperature for mycelial growth. Duplicate platings were made for all specimens.

Representatives of most numerous colonial types from all sources were selected from plates containing the highest countable dilution. The isolates were purified and characterized presumptively by colonial morphology, pigmentation, and staining. In addition, the ability of the isolates to produce oxidase and catalase, ferment lactose and metabolise glucose fermentatively or oxidatively was tested. For the identification of Gram positive species, sucrose and mannitol fermentations and nitrate reductase determination were used. Gram negative isolates were further separated on the basis of carbohydrate utilization, production of urease and the indole, methyl red, Voges-Proskaeur and citrate utilization reactions. These tests and final identification were done according to Cowan and Steele (1974).

The vegetative and reproductive structures of the fungal isolates were examined for identification purposes.

The fishes examined were identified as *Tillapia zilli, Synodontis senegalensis, Clarias obscura, Bargrus bayad, Auchenoglanis occidentalis* and *Clarotes laticeps.* All were positive for microorganisms. Sixteen bacterial species and five of fungi were identified from the three different anatomical sets (Table 1). The gut specimens accounted for 48.6% of the total bacteria isolated while 26.6 % and 24.5 % were recorded for the gill and the skin, respectively. Of the bacterial species, the members of the family Enterobacteriaceae *(Escherichia coli, Enterobacter aerogenes, Shigella flexneri, Edwardsiella tarda, Klebsiella pheumoniae, Proteus vulgaris, Salmonella typhimurium* and *Serratia* sp.) predominated (45.3 %). Other Gram negative rods included *Aeromonas hydrophila, Acinetobacter* sp. and *Pseudomonas alcaligenes.* Prominent among the Gram positive bacterial genera were *Staphylococcus* and *Micrococcus.*

All the above species were isolatable from the three defferent specimens except *K. pneumoniae, S. flexneri, S. typhirmurium* and *Serratia* sp. which were restricted to the gastrointestinal tract. *Flavobacterium brevi* was isolated only from the skin. Generally no significant differences were shown in the frequency of isolation of the other species from the three sites.

The fungal species were identified as *Mucor* sp., *Rhizopus* sp., *Aspergillus* sp., *Achyla colorata* and *Saprolegnia* sp. All were common to the three anatomical setes except *Saprolegnia* sp. which was not recovered from the skin.

The findings here confirm that fishes can be infected with a variety of microbial species, especially those of bacteria in the freshwater environment. It has also been establised that microflora of fishes relates to that on the environment, as indicated by the similarities between the isolates and typical fresh water bacteria.

Some of the isolates are derived from external sources like soil and sediments, *e.g. Pseudomonas, Clostridium* and *Proteus*. Hence the fishes are transient carriers of such microbes. The isolates from the gills and the skin can be accounted for in part by the filtering effect of the former or the slime layer of the latter and partly as a result of the active bacterial multiplication and adaptation. However, most of the isolates identified as members of Enterobacteriaceae, particularly the coliforms, are associated with faecal contamination and are also indicative of the possible presence of enteric pathogens. Therefore, the isolates have serious consequences to their host (fishes), to the animals that feed on them and finally to man.

The microbial population constitutes a significant burden throughout the lifespan of fishes as they have a role in nutrition, growth and disease susceptibility. For example, many outbreaks of salmonellosis have been attributed to livestock feeds and though fish products prepared for human consumption appear to be less of a problem than animal feeds, they are certainly of more immediate public health concern. *Aeromonas hydrophila* is an opportunistic pathogen causing Motile Aeromonas Septicaemia (MAS), red-nose decease and furunculosis of aquatic animals including fish (Hazen *et. al.* 1978). As observed by Fijan (1972) these diceases may result in massive mortalities if allowed to go unchecked. *Shigella* infections often occur through ingestion. On the economic side, *Pseudomonas* and *Flavobacterium* have frequently been implicated in spoilage of fish. Among the fungal isolates are species of *Aspergillus* and *Saprolegnia* which have been associated with outbreaks of diseases in fish.

It is therefore apparent that greater attention must be given to the microflora of fishes, especially that of the gastrointestinal tract, and to the natural body of water holding them. The concern for public health risk regarding the beneficial uses of Asa river becomes more grave as long as the indigenous Ilorin population continues to use the river for fishing, irrigation and recreation, in spite of its gross pollutional status.

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