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ORIGINAL ARTICLE

Microbial evaluation and occurrence of antidrug multi-resistant organisms among the indigenous *Clarias species* in River Oluwa, Nigeria

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Abstract Fish may harbor pathogens on or inside its body when in contaminated environment. *Clarias gariepinus* and *Clarias buthopogon* were analyzed to evaluate the likely impact of pollution on the antidrug resistance pattern of their microbial isolates. Different bacterial and fungal counts were observed on the fish organs (skin, muscles and gills). The highest bacterial count was 1,040,000 Cfu/mL while the lowest was 101 Cfu/mL. The highest fungal count obtained was 344,000 Cfu/mL while the lowest was 65 Cfu/mL. Bacterial isolates belonging to genera *Bacillus*, *Clostridium*, *Alcaligenes*, *Flavobacterium*, *Enterobacter* and *Corynebacterium* were obtained from the organs. Also, fungal isolates belonging to the genera *Penicillium*, *Aspergillus*, *Rhizopus*, *Monila* and *Fusarium* were isolated. The resistance of isolates from *C. gariepinus* to drugs was between 50% and 90% with *Bacillus species* showing the highest resistance. For isolates from *C. buthopogon*, 40–90% resistance was observed with *Alcaligenes faecalis* showing highest resistance. Five patterns of multiple drug resistance were observed among the bacterial isolates with antibiotics ranging from 4 to 9. Also, result of fungal isolates showed susceptibility to ketoconazole and resistant to fluconazole and griseofulvin. The public health implications of consuming these fishes are discussed.

1. Introduction

The aquatic ecosystem is usually a reservoir of toxic substances which may be introduced deliberately or accidentally. When such pollutants are present, they impair the quality of water and make it unsuitable for aquatic life (Ayandiran and Dahunsi, 2016; Dahunsi et al., 2011). In Nigeria, pollution of the surface waters by oil and solid wastes is widespread over the last decades and is rendering most of them unsuitable for human use (Bakare et al., 2003). Such toxic substances

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discharged into water bodies are not only accumulated through the food chain (Dahunsi et al., 2012a), but may also either limit the number of species or lead to high microbial load being formed (Okafor, 1985). It is known that contamination of a particular water body can always be linked to industrial processes or sewage/effluent disposal as reported (Sathware et al., 2007).

In the past, it was thought that fish in open waters (marine and fresh) were generally safe due to lack or paucity of epidemiological evidence of fish-borne diseases. However, recent reports from studies in the areas of environmental pollution, fish and water preservation/management suggest otherwise (Obasohan et al., 2010). Fish are usually found at the top of the food chain and as such can accumulate large quantities of pollutants in their muscle and organs (Dahunsi et al., 2012b). Pathogenic microbes are known to cause many diseases in both wild and cultured fish and they may range from primary to opportunistic pathogens (Inglis et al., 1994). Fish may also harbor pathogens on or inside its body after exposure to contaminated water or food but the study of diseases of fish is hindered by the lack of knowledge of numerous interactions existing between pathogens and their fish hosts (Nyaku et al., 2007). Several researchers have reported the isolation of bacteria belonging to different genera from fish in different polluted water bodies (Adewoye and Lateef, 2004; Al-Harbi, 2003; Hamed et al., 2013; Kolawole et al., 2011).

Bacterial resistance in the environment is a growing phenomenon among environmentalists and several reports have failed to establish a reliable relationship between anthropogenic activities and antibiotic resistance in bacteria as many believe that resistance elements are naturally innate in the microbial genome (Baltz, 2008; Brown and Balkwill, 2009; Bhullar et al., 2012; Cox and Wright, 2013; D'Costa et al., 2011; Thaller et al., 2010; Toth et al., 2010; Wright, 2007, 2010). However, other reports have documented increased antibiotic resistance in bacteria and also established tangible relationship between the transfer of resistance elements and anthropogenic activities (Ayandiran et al., 2014; Bhullar et al., 2012; Knapp et al., 2010). To this effect, bacterial resistance to antibiotics has been considered a global public health menace. Besides the human health risks posed by the presence of antibiotic resistant organisms (bacteria and fungi) in the environment, and the unwanted presence of antibiotics in water bodies, concern for the ecological fate and environmental risk of antibiotics in the aquatic environment is on the increase Kümmerer, 2009.

Ondo State constitutes an economically significant part of South-Western Nigeria and has one of the largest fresh and coastal water areas in the country (Ayandiran and Dahunsi, 2016). It is located in the coordinates 6°35′19N, 4°50′3E and altitude 61 m. This is where bitumen was first spotted in Nigeria in 1910 and two bitumen observatory wells were dug in the State in the 60 s during the early explorative activity of Nigerian natural bitumen. The seepage of the bitumen material exists especially during the dry season when temperature is above 37 °C during which it occurs as a free flowing liquid. Oluwa is a major river of economic, agricultural and environmental significance flowing through many communities within the State. The major pollutant of the river is bitumen runoff (Olajire et al., 2007) besides other domestic and agricultural activities carried out along it course and from its many tributaries thereby contributing to its pollution.

Clarias species constitutes the major fauna population of River Oluwa (Ayandiran and Dahunsi, 2016), and is usually found in abundance especially during the rainy season. It is also a major source of livelihood for the populace who are majorly farmers and fishermen. Prior to this research, the microbiological quality and antibiotic resistance pattern of the microbial species in the fish from this river is yet to be established and so, their public health impact is not ascertained. The present work is therefore necessary to bridge the lack of understanding about the relationship between anthropogenic activities such as the presence of bitumen seepage and other domestic and agricultural pollutants and emergence of antibiotic resistance pattern in different bacteria inhabiting the fish species in the Oluwa River.

2. Materials and methods

2.1. Sample collection

Adult sizes between 4 and 5 month old, (weight 0.6 ± 0.2 kg; length 24 ± 2 cm) of *Clarias gariepinus* and *Clarias buthopogon* were collected twice during the same rainy season (early and peak) of a year from the different polluted portions (up and down streams) of River Oluwa while control samples were collected from the unpolluted portion of the river and this was done from year 2011 to 2013. In all, one hundred and eighty (180) fish samples (60 each from upstream, downstream and control sites) were collected for each of *C. gariepinus* and *C. buthopogon*. Fishing was done during late night with the help of professional local fishermen. Gill nets about 12.192 m long and 1.828 m wide with a cork line at the top rope and metal line with the ground rope made locally of nylon were used for fishing. Two fishermen with the help of a boat helped in the collection of fish samples into sterilized plastic bucket and were aseptically transported to the laboratory.

2.2. Sample preparation

After arrival at the laboratory, fish samples were dissected and the various organs separated. Twenty (20) gram of each part was homogenized separately in 250 mL of 0.1% (w/v) peptone water before serial dilution using the method of Odoli (2006). Sterilized mortar and pestle and other glass wares were used for the homogenization.

2.3. Isolation, characterization and identification of bacterial isolates

Microbial isolation was carried out on the samples (skin, muscles and gills) via serial dilution and pour plate method already described (Adewoye and Lateef, 2004) using nutrient agar, MacConkey agar, Mannitol salt agar and Potato Dextrose agar (Oxoid). One milliliter from each prepared fish organ was serially diluted, and then plated in duplicate on nutrient agar, MacConkey agar, Mannitol salt agar and Salmonella–Shigella agar. All plates for aerobic organisms were incubated appropriately at 37 °C for 24–48 h. For the isolation of *Clostridium* spp., samples were first cultured on Reinforced Clostridia Medium (RCM) and then sub-cultured on blood agar incubated in an anaerobic jar (Oxoid) containing a