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FULL LENGTH ARTICLE

Microbial assessment and prevalence of antibiotic resistance in polluted Oluwa River, Nigeria

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Abstract Antibiotics are emerging environmental contaminants, causing both short-term and long-term alterations of natural microbial communities due to their high biological activities. The antibiotic resistance pattern of bacteria from anthropogenic polluted Oluwa River, Nigeria was carried out. Microbial profiling and antibiotic sensitivity tests were carried out on water and sediment samples using 13 different antibiotics. Microorganisms isolated include those in the genera *Bacillus*, *Micrococcus*, *Pseudomonas*, *Streptococcus*, *Proteus* and *Staphylococcus*. The microbial count of isolates from water samples ranged between 94.10×10^2 Cfu/100 ml and 156.20×10^2 Cfu/100 ml while that of sediment samples ranged from 2.55×10^4 Cfu g^{-1} to 14.30×10^4 Cfu g^{-1}. From the water isolates, 100% resistance to antibiotics was found in *Micrococcus* spp. and *Pseudomonas* spp. while another *Micrococcus*, *Streptococcus*, *Staphylococcus* and *Bacillus* spp. showed between 40% and 90% resistances. From the sediment isolates, 100% resistance to antibiotics was found in a *Bacillus* spp. and *Pseudomonas* spp. while another *Bacillus*, *Micrococcus*, *Staphylococcus*, *Streptococcus* and *Proteus* spp. showed between 70% and 90% resistances. Multiple antibiotic resistance (MAR) was shown by all the isolates and *Bacillus*, *Micrococcus* and *Pseudomonas* spp. showed the highest resistances (100%) to all antibiotics. Thus, Oluwa River is not safe for public consumption.

Introduction

Antibiotics have over the decades been used for both human and animal disease treatment. They are however continuously found in the environment due to poor metabolism in the body. It is not yet clear and arguments among scientists increase daily about the involvement of man and his many anthropogenic activities in the spread of resistance elements in microorganisms. Several studies have reported lack of tangible relationship between anthropogenic activities and antibiotic resistance in bacteria and many believe that the elements that selects for resistance are naturally present within microbial genome (Davis and Anandan, 1970; Hughes and Datta, 1983; Barlow and Hall, 2002; Hall and Barlow, 2004; D'Costa et al., 2006, 2011; Wright, 2007, 2010; Baltz, 2008; Brown and Balkwill, 2009; Thaller et al., 2010; Toth et al., 2010; Bhullar et al., 2012; Cox and Wright, 2013). On the other

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hand, evidence abound that increased bacterial resistance to antibiotics and the transfer of resistance elements is a modern phenomenon having a strong link with anthropogenic activities (Knapp et al., 2010; Bhullar et al., 2012).

Besides the human health risks posed by the presence of antibiotic resistant bacteria in the environment, and the unwanted presence of antibiotics in water bodies, concern for the ecological fate and environmental threat of these drugs in the aquatic milieu is becoming a global phenomenon (Kümmerer, 2009). Bacterial resistance to antibiotics has been considered as a global public health menace. Different kinds of antibiotic resistant bacteria (ARB) are continuously detected in various environments ranging from aquatic to terrestrial ones. There is high possibility of resistance being spread by ARB from the environment to related human pathogenic microorganisms through numerous routes thereby suppressing the effectiveness of antibiotics (Threedeach et al., 2012).

A global strategy has been proposed by the World Health Organization to contain antibiotic resistance regarding its potential threat to both public and environment health (Pruden et al., 2006). Because of the high microbial biomass and abundant nutrients, as well as various antimicrobial agents, polluted water bodies represent a favorable habitat for both the survival of ARB and the transfer of antibiotic resistance, from where they spread resistant bacteria into subsequent aquatic and terrestrial environments (Bouki et al., 2013). Various ARB including multiple antibiotic resistant bacteria, have previously been encountered in a large number of water systems (Luczkiewicz et al., 2010).

Water pollution and reduction in quality is a major contributor to global freshwater scarcity, stressing the need for more integrated water management and monitoring (Dahunsi et al., 2014). Microbial and sediment pollution have been documented to be significant concern for rivers and streams and pathogens have also been known to impair or threaten more kilometers of water bodies than any other aquatic pollutant. In the same vein, bacterial pollution of water can result in unsafe drinking water, restrictions on recreation opportunities, and closures of shellfish beds (US EPA, 2010).

Sediment contamination has been reported to be the second leading cause of impairment to water bodies according to US EPA (2010), and this is because suspended sediment can directly impact aquatic organisms and can also increase water treatment costs in channel and reservoirs. Previous researchers have reported that several microbial contaminants are constantly adherent to sediment particles (Oliver et al., 2007), most of which are re-suspended from stream bottoms during the rising storm incidence. Besides, pathogens and sediment are usually both transported in water, either separately or adsorbed together.

Pollution of water by petroleum and allied products is a universal environmental phenomenon in places where there is exploration or processing of petroleum deposits (Abdelgawad et al., 2008). Bitumen is a sticky, highly viscous liquid or semi-solid usually found in most crude petroleum and in some natural deposits and therefore referred to as a pitch. It is composed of several high boiling point compounds and molecules with relatively low carbon to hydrogen C:H ratio (Yoon et al., 2009). A large deposit of natural bitumen occurs in the bitumen belt of South-western Nigeria. The toxicity of a material has been shown to be the most common measure of its potential environmental impact and this is applicable to bitumen whose impacts on the Nigerian physical environment especially communities in Ondo State are enormous as it contains heavy metals.

Due to the importance of Oluwa River as the major water source for drinking and other domestic usages within these communities, its sanitary level is of great concern. Thus in continuation of the few chemical toxicity studies on the environmental impacts of natural bitumen deposits and other contaminants in Oluwa River Ondo State, South Western Nigeria, the aim of this preliminary study was to evaluate the microbial population and the antibiotic resistant pattern of heterotrophic bacteria in the water and sediment of this polluted river as this will assist in the determination of the pollution impact on the bacterial isolates and the evaluation of the public health implications from the ARB.

Materials and methods

Description of collection site

Ondo State constitutes an economically significant part of South-western Nigeria and has one of the largest fresh and coastal areas in the country. It is located in the coordinate of Latitude 6° 35′ 19 N, Longitude 4° 50′ 3 E 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. A large deposit of natural bitumen occurs in the so called bitumen belt of South-western Nigeria. The seepage of the bitumen material exists especially during the dry season when temperature is above 37 °C where it occurs as a free flowing liquid. Oluwa is a major river of industrial, agricultural and environmental significance which winds through many communities within the State. The river receives continuous seepage from bitumen exploration apart from domestic and agricultural deposits besides other activities carried out along its course and from its many tributaries which in turn contribute to its pollution (Fig. 1).

Sample collection

180 water samples in this study were collected from 20 different sites (19 polluted sites and 1 unpolluted site that was used as control for the study) along the river course during the dry season of 2011 (n = 80) and wet season of 2012 (n = 100). These sites were selected after due consultation with local authorities and a water assessment monitoring group, who identified these sites as having poor water quality due to usage for domestic activities by the inhabitants of Agbabu community. All samples were collected during low tide and sample collection during the dry season was carried out when there was no rainfall for more than 2 months or when there was rainfall, not more than 2 mm for at least 15 days prior to sampling. In contrast, sample collection during the rainy season was done when the sampling sites had received more than 100 mm rainfall few days prior to sampling. From each site, grab water samples were collected into 5 L sterile plastic bottles with screw caps from 30 cm below the water surface and transported on ice to the laboratory for analysis within 6 h of collection. Sediment samples were collected from four different locations (A, B, C, D) and another one control at