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Antimicrobial resistance determinants of *Escherichia coli* isolates recovered from some rivers in Osun State, South-Western Nigeria: Implications for public health

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HIGHLIGHTS

- *Escherichia coli* isolates were recovered from some rivers in Southwestern Nigeria.
- The antibiogram of the isolates were evaluated including relevant resistance genes.
- β-lactams and tetracyclines resistance determinants were more frequent.
- Phenicols and aminoglycosides resistance determinant were less frequent.
- Significant positive associations existed among *ampC*, bla_{TEM}, bla_Z and *tetA* genes.

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ABSTRACT

The inevitable development of resistance has sunk the great success achieved in the discovery of antimicrobial agents and dashed the hope of man in the recovery from infections and illnesses, as diseases and disease agents that were once thought to be controlled by antimicrobials are now re-emerging in new leagues resistance to therapy. A total of 300 PCR confirmed *Escherichia coli* isolates recovered from different river sources in Osun State, Nigeria were evaluated for their antibiogram profiling by the disc diffusion method and the resistant isolates were further profiled for their genotypic antimicrobial resistance determinants by polymerase chain reaction assays. Among the 20 antimicrobials selected from 10 families, resistance among sulfonamides, β-lactams and tetracyclines were found to be most frequent than phenicols and aminoglycosides with a noticeable increase in the number of multi-drug resistance ranging from three to nine antimicrobials. A total of 19 resistance determinants were assessed with their prevalence and distributions obtained as follows; [sulfonamides *sulI* (8%), *sulII* (41%)], [β-lactams; *ampC* 22%; $bla_{TEM,}$ (21%), and bla_Z (18%),], [tetracyclines *tetA* (24%), *tetB* (23%), *tetC* (18%), *tetD* (78%), *tetK* (15%), *and tetM*, (10%)], [phenicols; *catI* (37%), *catII* (28%), and *cmlA1* (19%)] and [aminoglycosides; *aacC2* (8%), *aphA1* (80%), *aphA2* (80%), *aadA* (79%) and *strA* (38%)]. The Pearson chi-square exact test revealed many strong significant associations among *ampC*, bla_{TEM}, bla_Z and *tetA* genes with some determinants screened. The findings signify high increase in the prevalence of multidrug resistant *E. coli* isolates and resistance determinants indicating increased public health risks associated with the ingestion of waters from untreated sources. Hence, a necessity for safe water supply, provision of proper sanitation facilities and good surveillance programmes to monitor antimicrobial resistance patterns in water bodies.

1. Introduction

Antimicrobial agents are widely used in human and veterinary medicine to control bacterial infections. Their usage for livestock can be for therapeutic, prophylactic, metaphylactic or growth promotion purposes. About 90% of the antimicrobials used in animal husbandry are for growth promotion and prophylaxis (Sarmah et al., 2006; Pitout and Laupland, 2008). With the majority of them being excreted unchanged into the environment, concerns about their potential impact on the aquatic environments keep increasing over time (Sarmah et al., 2006; Wright, 2007; Kemper, 2008).

Antimicrobial resistance has an important impact on health policies and involves an increasing number of bacterial species and resistance mechanisms. It has been observed that the highest prevalence of

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antimicrobial-resistant bacteria occur in those countries where they are extensively used for prevention and treatment of microbial infections in humans as well as animals (Kummerer, 2004; Junco-Diaz et al., 2006). Although antimicrobial therapy is a significant tool for the treatment of these infections, its widespread resistance has become a cause of great concern in veterinary medicine (Monroe and Polk, 2000; Teshager et al., 2000; Schoevaerdts et al., 2011). Indeed, a close association and direct correlation exist between the use of antimicrobial agents for the treatment of infections in animals and the levels of resistance observed (Schwarz and Chaslus-Dancla, 2001; Mellon et al., 2001; Bibbal et al., 2009). Antimicrobial resistance among pathogens has become an emerging threat to human and veterinary medicine due to the excessive and indiscriminate use for treatment, prophylaxis or growth promotion, with increased multidrug resistant *Escherichia coli* now becoming a major public health issue both in the developed and developing countries (Ajamaluddin et al., 2000; Okeke et al., 2005; Chandran et al., 2008).

Contamination of water sources with faecal indicator bacteria like *E. coli* is a serious challenge due to its ability to transmit diseases. The risk associated with these bacteria further increases with a corresponding increase in their resistance to antimicrobial therapy (Da Silva and Mendonca, 2012). These bacteria could be transmitted from environment to human via direct or indirect route (Iversen et al., 2004; Kim et al., 2005; Rodríguez et al., 2006). *E. coli* has been the foremost indicator of faecal contamination and possible incidence of water-borne diseases that are most injurious to health and a significant reservoir of genes coding for antimicrobial resistance and therefore is a useful indicator for resistance in bacterial communities (Bucknell et al., 1997; WHO, 2010). However, recovery of indicator bacteria may depend upon the extent of contamination in a particular water source (Warner et al., 2008). *E. coli* has been reported to acquire and transfer virulence and antimicrobial resistance genes to enteric pathogenic and normal flora bacteria in the environment through horizontal transfer of resistance (R) plasmids, transposons and integrons (Platt et al., 1986; Pang et al., 1994; Schwarz and Chaslus-Dancla, 2001; Nordmann and Poirel, 2005; Leverstein-van Hall et al., 2002; Pruden et al., 2006; Tenover, 2006; Ozgumus et al., 2007), although the resistance they carry may not be a problem as such but the transfer of resistance factors to zoonotic pathogens inhabiting the gut has stern implications for animal and human health (Ranjana et al., 2008).

In surface water, it is difficult to find an area where antibiotics cannot be detected, except for the pristine spot in the mountains before the rivers or streams go through urban or agricultural areas (Yang and Carlson, 2003). Apart from chemical pollution caused by antibiotics themselves, their use may also accelerate the development of antimicrobial resistance determinants and bacteria, which further shade health risks to humans and animals (Kemper, 2008).

Antimicrobial resistance determinants (ARDs) from hospital wastewaters, animal husbandry and aquaculture areas directly exposed to the environment are eventually carried to nearby streams, rivers, lakes, or other aquatic bodies or even leach downward through the soil during rainfall. Several reports have indicated that bacteria harbouring ARDs can be released from sewage treatment plants (STPs) into surface waters (Tennstedt et al., 2005; Chen et al., 2007; Auerbach et al., 2007) and the genes in surface and ground waters can transfer antibiotic resistance to the bacteria in drinking water or the food chain (Chee-Sanford et al., 2001).

Scores of ARDs have been found in the isolates or microbial communities in the natural waters, which were not or slightly polluted (Jacobs and Chenia, 2007; Mohapatra et al., 2008; Rahman et al., 2008). Several types of aminoglycoside resistance genes have been detected in the microorganisms isolated from surface water, including *aac* (Lee et al., 1998), *aad* (Park et al., 2003; Mukherjee and Chakraborty, 2006), *aph* (Poppe et al., 2006) and *str* (Mohapatra et al., 2008). Sulfonamide resistance genes including *sulI*, *II*, *III* and *A* were detected in the microorganisms of river water and sediments (Lin and Biyela, 2005; Pei et al., 2006; Poppe et al., 2006; Mohapatra et al., 2008). The presence of ARGs, such as *tet*, *van* and *sul* has been reported in wastewater, surface water and sediments (Schwartz et al., 2003; Pei et al., 2006; Ram et al., 2007). Similarly, beta lactams *ampC* and *bla* genes have been detected in surface water biofilms and estuarine water (Schwartz et al., 2003; Henriques et al., 2006).

Globally, antimicrobial resistant bacteria and their associated determinants have become an important environmental contamination issue which is currently receiving an increased attention (Kummerer, 2004; Pruden et al., 2006; Sapkota et al., 2007). Thus, adequate information on the prevalence of antimicrobial resistance in pathogens would serve as basis for proper selection of optimal treatment when necessary (Okeke et al., 2005). Several waterborne outbreaks attributed to multidrug resistant *E. coli* have been reported worldwide and high prevalence of multidrug resistance indicates a serious need for antibiotic surveillance and planning of effective interventions to reduce multidrug resistance in such pathogens (Olayinka et al., 2004). The monitoring and surveillance of both antibiotic usage and multiple antibiotic resistances especially in nosocomial infections is necessary to setting up of effective containment programmes and audit of such programmes (Mthembu, 2008; Kamat et al., 2008). The spread of ARDs into environments where antibiotics are not used remains a postulation yet to be thoroughly investigated; although it has been hypothesized that water could disseminate AMR (WRC, 2001). Modern food animal production which depends on the use of large amounts of antibiotics for disease control and agricultural purposes, particularly for growth enhancement provides favourable conditions for selection, spread and persistence of antimicrobial-resistant bacteria capable of causing infections in animals and humans through food chains (ETAG, 2005; Mathew et al., 2007). As a result, when water bodies are contaminated with faecal materials containing antibiotic resistant bacteria, they could serve as a source and/or pool of antibiotic resistant genetic elements that could possibly be transferred to other bacterial species and make the water quality unpleasant to health (Biyela and Bezuidenhout, 2004) and also create a favourable condition for the spread of antibiotic resistant genetic elements from place to place (APUA, 1999).

Unfortunately, in Nigeria and particularly Osun State, previous studies have only reported high levels of microbial contamination and phenotypic antimicrobial susceptibility profiling of water sources used by the population (Lateef et al., 2003; Olowe et al., 2008). To the best of our knowledge, there is dearth of information regarding the genotypic characteristics of antibiotic resistance determinants of *E. coli* isolates recovered from aquatic environments in South Western Nigeria and it is in the light of this that the study aimed at investigating the prevalence and distribution of antimicrobial resistance determinants of *E. coli* isolates from some selected rivers of Osun State, South Western Nigeria, as the first of its kind in the region.

2. Materials and methods

2.1. Description of study area and sampling sites

Water samples were collected from ten rivers at different locations in Osun State, South-Western Nigeria. The sites were selected after due consultation with the State Ministries of Environment, Water Resources, Lands and Housing for proper mapping of the state rivers. Table 1 shows the description of the sampling sites. For convenience, the sampling locations were coded as follows: R1: Erinle-Ede; R2: Ido-Osun; R3: Osun-Osogbo, R4: Oba-Iwo; R5: Ejigbo; R6: Ilobu-Okinni; R7: Asejire-Ikire; R8: Shasha; R9 and Ila-Oke Ila, R10: Inisha-Okuku and illustrated on the map found below (Fig. 1).

2.2. Sampling and isolation of presumptive E. coli

Water samples were aseptically collected monthly over a period of one year from September 2011 to August 2012. All samples were collected in 1.5 L sterile bottles and transported on ice to the laboratory