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Plasmid and antibiotics susceptibilities profile of *Enterobacteriaceae* isolated from communal water sources in Ogun State Nigeria

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Abstract

The increasing role of Enterobacteriaceae in antibiotic resistance, spread and reservoir is a pressing public health concern. This study was carried out to determine the plasmid profile and the antibiotic resistant patterns of water-borne Enterobacteriaceae recovered from some selected drinking water sources in 6 towns in Ogun State (Nigeria). Sixty-eight Enterobacteriaceae consisting mainly of Enterobacter spp, Escherichia coli, Klebsiella spp, Salmonella spp, Citrobacter freundii, Serratia spp were recovered and identified presumptively using standard microbiological and biochemical methods. The susceptibility of the isolates to 11 antibiotics were carried out by disk diffusion method while the plasmid analysis was by alkaline lysis method. The susceptibility result showed that >90% of the isolates were resistant to 9 antibiotics while all the isolates were susceptible to imipenem and meropenem among the other antibiotics investigated. Out of 40 Enterobacteriaceae investigated for the presence of plasmids, 18 isolates were positive for the presence of plasmids, E. coli (4), Enterobacter aerogenes (7), K. oxytoca (3), K. pneumoniae (4) with sizes range of 33.5 – >33.5kb. Presence of bacteria with resistance plasmids in drinkable water is a cause for concern due to the possible health risks to humans and animals.

Keywords: Plasmids; Enterobacteriaceae; Antibiotics resistance

Introduction

Antimicrobial resistance (AMR) among microorganisms is a global problem. Increasing rate of resistance among pathogenic bacteria responsible for both community and hospital acquired infections has resulted in high morbidity and mortality (Andersson and Levin, 1999). Additional implication of this worrisome phenomenon is the high economic burden associated with long hospital stay and treatment cost due to multidrug resistant (MDR) pathogenic bacteria. In the Europe and United States, at least 1.5 billion euros and 20 billion dollars are spent annually respectively on massive extra health care cost as a result of AMR in hospitals (ECDC, 2009; Robert *et al.,* 2009). Top on the list of Gram negative bacteria implicated in AMR includes *Escherichia coli*, *Enterobacter* spp, *Kebsiella pneumoniae* and *Pseudomonas aeruginosa*.

The threat to life caused by many of these pathogens has been attributed to indiscriminate use of antibiotics either in form of intentional drug abuse of prescribed drugs, over- the- counter sales, therapeutic purposes and at sub-therapeutic doses for human treatment and growth promotion in livestock farming (Voss *et al.,* 2005; Lessenger and Feinberg, 2008; Davis and Davis, 2010). Countless reports have established hospital environment as a principal reservoir for AMR genes. There are also reports indicating food processing animals such as poultry, cattle and pigs as well as domestic animals and pets being reservoirs for AMR bacteria capable of transmission to human population via consumptions, interactions and body fluids contacts (Guardabassi *et al.,* 2004; Faldynova *et al.,* 2013; Ayeni *et al*., 2016).

The aquatic system remains one of the viable media on which many people depend for several activities such as fishing, farming via irrigation, drinking and washing. Contamination of water by human activities and environmental factors such as discharges from municipal, industrial sewages, run off from agricultural land and from spills of chemical waste and human faeces may lead to increase in aquatic flora. Consequently, one or more of these contaminants could harbour non-resident bacteria serving as pathogens in water or as a vehicle for infection and transmission of diseases (Fawell and Nieuwenhuijsen, 2003). These pathogens have been reported widely as responsible for various water-borne infections such as cholera, typhoid fever, dysentery, which account for more than 50% of mortality and morbidity worldwide especially among children (Fenwick, 2006). Various studies in Nigeria have shown the microbial quality of communal water sources harbouring pathogenic bacteria (Agbabiaka and Sule, 2010; Oyedeji *et al*., 2011; Akpoveta *et al.,* 201; Onwughara *et al*., 2013, Odumosu *et al.,* 2014,). Water has been established as reservoir for pathogenic bacteria harbouring transferable genes responsible for various antimicrobial resistances (Lin *et al.,* 2004; Soge *et al.,* 2009; Adesoji *et al*., 2015). In bacteria, transfer of resistance genes is mostly carried out via mobile genetic elements, especially among the Gram negative species (Odumosu *et al.,* 2013; Diene and Rolain, 2014). Many of these resistant genes are either chromosomally encoded or via mobile genetic elements such as plasmids, integrons and transposons (Poirel *et al.,* 2009). Beta-lactam drugs such as cephalosporins and carbapenems are antibiotics widely used for the treatment of infections caused by Gram negative bacteria because they are less toxic and effective. However, resistance to beta-lactam antibiotics by bacteria is usually by the production of enzymes called *β*-lactamases such as extended-spectrum β-lactamases (ESBL) which are mostly encoded and disseminated via plasmids and are commonly reported among the family Enterobacteriaceae (Carattoli, 2009). Carbapenem remains the last option for non-toxic therapy for drug resistant bacteria especially among Gram negative bacteria. Control of the spread of antimicrobial resistance among Gram negative bacteria via plasmids and other mobile genetic element is important in the management and treatment of infections especially among hospitalized and critically ill patients. This will ensure reduction in the resistance genes such as cabapenamase which is currently a global threat. This study was carried out to access the antimicrobial susceptibilities of isolates to two common carbapenem (imipenem and meropenem) and ascertain the plasmid profiles of bacteria isolated from various communal water supply in Ogun state.

Materials and methods

Sample collection and bacterial isolates

The description of the bacterial isolates recovered from sixty drinking water samples that were collected from 6 towns in the southern part of Ogun state was in line with Odumosu and Akintimehin, (2014) Biochemical identification of these isolates was carried out by standard guidelines described previously (Cheesbrough, 2006) and by Microbact 24E.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) for amoxycillin/clavulanic acid, nitrofurantoin, ciprofloxacin, ceftazidime, cefotaxime, cefuroxime, ofloxacin, gentamicin and cefixime performed by disc diffusion technique and interpreted according to Clinical and Laboratory Standard Institute guidelines has been previously reported in our earlier study (Odumosu and Akintimehin, 2014) while additional AST to imipenem and

meropenem for resistance to beta-lactam was included in this present study solely for the investigation of carbapenamase resistance among the resistant isolates

Plasmid extraction and quantification

Forty isolates were selected randomly according to their resistance profile for the presence of plasmid. The extraction was carried out by alkaline lysis protocol as previously described by Sambrook *et al.* (1989) with slight modifications. A 2mL of an 8 hour culture of each isolates, shaken at 200 rpm was centrifuged at 13, 000 rpm for 2mins at 4ºC. The washed pellet were suspended in 150 µL cold alkaline solution I (50mM Tris pH 8.0 with HcL, 10 mM EDTA, 100 µg/mL RNase stored at 4°C), lysed with 300 µL alkaline solution II (200 mM NaOH, 1% SDS) and complete lysis with 150 µL of alkaline solution III (3.0M Potassium acetate, pH 5.5), inverted several times and incubated on ice for 5 mins. Bacterial lysate was removed via centrifuge at 13, 000 rpm for 10 mins at 4ºC, using equal volume of phenol: chloroform solution for centrifuging supernatant, plasmids were precipitated using ice cold 70% ethanol and electrophoresed on 0.8% agarose gel

Results

Antimicrobial susceptibilities

All the isolates (100%) were susceptible to imipenem and meropenem. The susceptibility result for other classes showed that > 90% of the isolates were resistant to 9 antibiotics as interpreted according to CLSI (2014).

Plasmid profiling:

Out of 40 *Enterobacteriaceae* investigated for the presence of plasmids, 18 isolates were found harbouring plasmids by alkaline lysis method in the following distribution *Enterobacter aerogenes* (7), *E. coli* (4), *K. pneumoniae* (4), *K. oxytoca* (3),. Of the organisms, 67.5% carried various sizes of plasmid as shown in Fig 1 below. Plasmids estimates by quantification using the DNA super mix revealed sizes ranging from 33.5 – >33.5kb.

Discussion

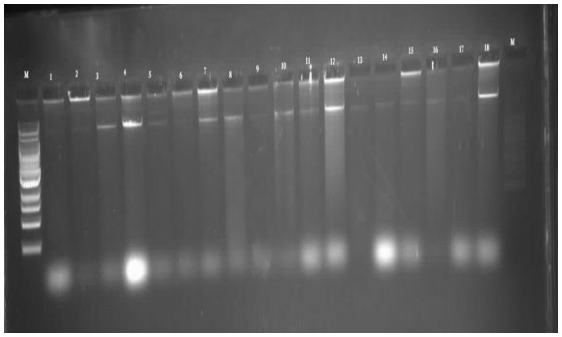
Fecal contamination of drinking water remain a notable cause of diseases such as typhoid and cholera in the developing world (Fawell and Nieuwenhuijsen, 2003). Bacteria harboring resistant genes are worrisome because they constitute clinical failure once they are implicated in infectious diseases (Tanwar *et al* 2014). In this present study, Gram negative bacteria isolated from various drinking water have shown high level of resistance to commonly used antibiotics as reported in our previous study (Odumosu and Akintimehin, 2014). Additional investigation of their susceptibilities to carbapenem class of antibiotics reveals all the isolates were susceptible to imipenem and meropenem. This confirms carbapenem are still potent antibiotics for the treatment of multidrug resistant Gram negative bacteria.

There was an observed carriage of plasmids among the investigated bacteria in this study. Out of 40 randomly selected bacteria, 18 (45%) of the isolates were harboring at least a copy of plasmids. The sizes ranged from 33.5 – >33.5kb among all the investigated bacteria isolates suggesting a possibility of similar source of dissemination of mobile genetic elements among these pathogens. *Enterobacter aerogenes* dominated the population with 7 strains positive for carriage of plasmids and two among the isolates having 3 copies of plasmids each. This is followed by *E. coli* and *K. pneumoniae* having 3 strains each positive for plasmids. This is in agreement with previous studies on bacteria isolated from drinking water from Pakistan, Nigeria, Egypt and Uganda harboring plasmids of varying copies and sizes (Diab *et al.,* 2002; Bello *et al*., 2012; AbdelRahim *et al* 2015). Plasmids has been reported to be responsible for antimicrobial resistance in bacteria especially in members of Enterobacteriaceae (Carattoli, 2009).

The incidence of plasmids among bacteria isolated from drinking water is troubling because plasmids often allow development and spread of resistance in niches irrespective of previous exposure to antibiotics and they also have high capacity for transference among bacteria of urelated genus and communities (Dionisio *et al*., 2002). The presence of pathogenic bacteria such as isolated in this study necessitate constant routine microbiological analysis to include the detection of pathogenic bacteria and not only fecal contaminants because safe water also demands that water is free from pathogenic bacteria.

Raw sewage overflow, septic tanks and leaking sewer lines as well poor wastewater and solid waste management in close proximity to human activities and water reservoir have a role to play in the contamination of drinking water which may lead to public health hazards (Efuntoye and Apanpa, 2010; Graham and Polizzotto, 2013). The result of this study reveals the investigated drinking water does not meet the WHO specification that states; *E. coli* or thermo-tolerant bacteria must not be detected in any 100ml sample of a drinking water (WHO, 2011). *E.coli* and other coliforms were detected in this study at high prevalence. Additionally, the presence of mobile genetic materials i.e. plasmids among 45% of the resistance isolates suggests the drinking water might serve as reservoir for antibiotic resistance. Although this present study did not investigate the presence of genes contained in the extracted plasmids among the isolates and their

transferability, however various studies have shown plasmids to have the capacity of



Lane M = DNA supermix of fragments ranging from 500bp to 33.5kb
Lane 1 – 18 Shows ≥ 1 No of plasmids each of sizes 33.5 – >33.5kb sizes each
Lane 3, 4, 8, 9, 10, 11, 12 and 18 has 2 copies of plasmid each
Lane 5, 7 and 15 has 3 copies of plasmid each

Fig 1: Plasmid profile of *Enterobacteriaceae* isolates from communal water supply harboring resistance genes and are transferable via conjugation.

Conclusion.

Drinking water containing resistant bacteria harboring resistant mechanisms and genes has been shown in this study and this underscores the need for quality assessment of drinking water to prevent upsurge of multidrug resistant bacteria disseminated via community outbreaks of infection. Situation of water for consumption in proximity to septic tanks and public waste disposal areas should be discouraged to prevent quick access of microorganism into water.

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