



Screening for Plasmid-Mediated Multidrug Resistant Bacteria in Ikpoba River Water Samples

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To cite this article:

Akpe Azuka Romanus, Okwu Grace Ifeoma, Esumeh Frederick Ikechukwu, Femi, Imah Justus. Screening for Plasmid-Mediated Multidrug Resistant Bacteria in Ikpoba River Water Samples. *International Journal of Microbiology and Biotechnology*.

Vol. 3, No. 2, 2018, pp. 31-35. doi: 10.11648/j.ijmb.20180302.11

Received: March 30, 2018; **Accepted:** April 16, 2018; **Published:** May 7, 2018

Abstract: The abuse and extensive use of antimicrobial agents by humans may increase resistant bacteria populations in the aquatic environment. The discharge of untreated wastewater into rivers and other non-point sources of pollution have led to the antibiotic resistant bacteria in the environment, particularly in surface waters. Studies on river water pollution and their implication to public health has been ongoing. Screening for multi-drug resistant bacterial status of Ikpoba River in Benin City, Nigeria was carried out using standard microbiological and physicochemical procedures. The bacteria isolated from the river water samples were *E. coli*, *Salmonella* sp, *Vibrio* sp, *Staphylococcus aureus*, and *Streptococcus faecalis*. The antibiotics susceptibility testing of the isolates revealed a multi-drug resistant status for *Staphylococcus aureus* and *Streptococcus faecalis*. The plasmid profile of these multi-drug resistant isolates was determined and results revealed that both isolates harboured plasmid of size 4.5kb. Antibiotic susceptibility of the isolates when cured of plasmid revealed loss of resistance to over 75% of the antibiotics they were originally resistant to. The microbial and physicochemical properties of the river showed that it is unfit for human consumption. The Plasmid mediated multidrug resistant status of some of the isolates is a threat to chemotherapy and is a cause for public health concern.

Keywords: River Water, Antibiotics, Pollution, Multi-drug Resistance, Plasmid

1. Introduction

Plasmid is a small DNA molecule located within a cell that is capable of independent replication. They are separated from the chromosomal DNA. Plasmid often carries genes that may benefit the survival of the host organism in antibiotic resistance. Chromosomes are big and contain all the essential genetic information for the survival of an organism under normal conditions. Plasmids, besides their small sizes, contain additional genes that are beneficial to the organisms under certain situations or conditions [1].

The abuse and extensive use of antimicrobial agents by humans may increase resistant bacteria populations in the aquatic environment. The discharge of untreated wastewater into rivers and other non-point sources of pollution have led to the antibiotic resistant bacteria in the environment, particularly in surface waters [2]. Water constitutes not only a way of dissemination of antibiotic resistant organisms among

human and animal population, but also the route by which resistance genes are introduced in natural bacterial ecosystem. Many of these bacteria harbour antibiotic resistance genes mainly inserted in plasmid, transposant or integron able to be transferred among water and soil bacteria community via horizontal gene transfer [3].

Due to indiscriminate use of broad spectrum antibiotic against infections in clinical setting, agriculture, and fish hatcheries, antibiotic resistance has dramatically increased among *Staphylococcus aureus* and *Streptococcus* species. In particular, *Staphylococcus aureus* has acquired resistance against the commonly abused broad spectrum antibiotic such as augmentin, ofloxacin, streptomycin, amoxicillin and ciprofloxacin that are used alone or in combination for treatment of common infections through self – medications. Some of these drugs are being excreted during treatment mostly as active compounds, therefore their biological action is not limited to the therapeutic site, but it is further moved into the environment. Water environment is the ideal medium

for the aggregation and dissemination of antibiotics, antibiotic – resistant bacteria and antibiotic resistance genes, which poses a serious threat to human health if ingested by the user [4].

Water is one of the most bacterial habitats on earth, a major way of disseminating microorganisms in nature and has been recognized as a significant reservoir of antibiotic resistant microorganisms [5, 6].

Numerous studies have demonstrated the importance of the environmental setting, e.g. water or soil on the cycling of antibiotic resistance in nature, either because antibiotic resistance mechanism can originate in environmental bacteria or because human and animal commensals and pathogens can contaminate the environment [7].

Ikpoba River is a fourth order stream located in Benin City, Edo State, Nigeria. Ikpoba River receives a variety of waste ranging from agriculture, domestic, industrial and from other sources and these wastes introduce foreign microorganisms into the river. Some of these organisms have serious medical and economic implications. The threat is further compounded by the increasing incidence of isolates with drug resistance [8]. The implication of the picture painted above is that, antibiotics resistance organisms are not only distributed in hospital wastewaters but also in animal production wastewaters, sewage, wastewater treatment plants, surface water, groundwater, and even drinking water [9]. This has further complicated the treatment of infections.

Other sources of pollution of Ikpoba River include wastes from abattoir, faecal matter from people living within the vicinity, pollution from laundry activity such as washing and bathing etc. all these are implicated in the introduction of antibiotic resistant isolates in Ikpoba River.

A lot of research works have been undertaken on Ikpoba River, some of which include the effects of brewery effluents on the water quality and rotifers of Ikpoba River [10]. The effects of brewery effluents on the microbiological quality of Ikpoba River, and the surrounding borehole water in Benin City have also been reported [11]. Also worked on was the influence of brewery discharge on the microbiological and physicochemical quality of Ikpoba River [12], but no work has been conducted on plasmid – mediated multidrug resistant bacteria in Ikpoba River water samples.

2. Methods

2.1. Sample Collection, Bacterial Isolation, Characterisation and Identification

Water samples were collected from the waste discharge point, upstream and down stream portions of Ikpoba River and inoculated in duplicates onto sterile MacConkey agar, nutrient agar and *Salmonella Shigella* agar plates. The plates were then incubated for 24-48 h at a temperature of 37°C. Thereafter emerging colonies were characterized and identified. The phenotypic and biochemical characteristics used to characterize and identify bacterial isolates included Gram staining, colonial appearance, motility, urease,

catalase, indole, oxidase, citrate, methyl red, voges proskaeur and sugar fermentation. These tests were performed and emerging colonies identified using standard methods [13].

2.2. Antibiotic Susceptibility Testing

Antibiotics susceptibility testing was carried out on the isolates using the disc diffusion method [14,15]. Mueller Hinton (MH) agar was prepared according to the instructions of the manufacturer. The commercially prepared antibiotic disc (Lab M) was used for the study. The disc consisted of the following antibiotics with their concentrations: pefloxacin, 10µg; gentamycin, 10µg, amoxicillin, 30µg; ciprofloxacin, 10µg; streptomycin, 30µg; cotrimoxazole, 30µg; ofloxacin, 30µg; chloramphenicol, 30µg, and augmentin, 25µg. The antibiotics discs were placed on MH agar plates earlier seeded with cell suspension of the isolates with a turbidity of 0.5 McFarland standards and incubated at 37°C for 24 h. The zones of inhibition were measured and recorded. The antibiotic sensitivity test was termed susceptible when the recorded zones of inhibition (mm) were ≥ 11 and resistant when the zones of inhibition to the antibiotic was ≤ 9 [16].

2.3. Plasmid Profile Analysis

Plasmid curing was carried out on the multi-drug resistant isolates using the methods earlier described by [17] and used by [18]. The curing agent used was sodium dodecyl sulphate (SDS). Physical evidence for the presence or loss of plasmid(s) in cured and non-cured isolates was obtained by alkaline phosphate method of rapid DNA isolation technique [19,20]. This involved four basic steps – cell harvest, lysis, deproteination and decontamination. The plasmids were characterized using agarose gel electrophoresis [21]. The molecular weights of plasmids were visualized using UV transilluminator (Alphamager TM 2200) at 302-365nm. Thereafter the susceptibility patterns of the plasmid cured isolates was performed and compared with the original isolates.

3. Results and Discussions

3.1. Bacterial Isolates and Their Antibiotics Susceptibility Patterns

The bacterial isolates from this study were *Escherichia coli*, *Salmonella* sp, *Vibrio* sp, *Streptococcus faecalis* and *Staphylococcus aureus*. *Escherichia coli* was the most predominant bacterial isolate followed by *Salmonella* sp. These and their corresponding antibiotics susceptibility patterns are shown in Table 1. It was observed that two of the isolates (*Streptococcus faecalis* and *Staphylococcus aureus*) were multi-drug resistant. These two isolates were resistant to the commonly abused broad spectrum antibiotics such as augmentin, gentamicin, pefloxacin, ofloxacin, streptomycin, amoxicillin, chloramphenicol used for treatment of common infections such as urinary tract infections (UTI), typhoid fever and other sexually transmitted diseases (STDs). The presence of multi-drug resistant bacteria agrees with the report [22] that

freshwater environment represent a potential reservoir of antibiotic resistant bacteria. The presence of antibiotic resistant bacterial isolates in environmental samples is attributed to the massive exposure of the environment to antibiotics through the use and misuse of antibiotics by human and in veterinary medicine [23]. This implies that in near future organisms would begin to acquire resistance against the newly synthesized antibiotics and it poses a serious threat to the future generation if no measures are put in place.

3.2. Plasmid Profiling

The plasmid profile analysis revealed that two of the isolates (*Streptococcus faecalis* and *Staphylococcus aureus*) had plasmids of the same size (4.5kb) as shown in Figure 1. The plasmid curing experiment was successful as shown in Figure 2. The success of this curing agrees with previous reports of elimination of plasmids using DNA intercalating agents -SDS, acridine orange, ethidium bromide and other agents [24, 25].

Table 2 showed the antibiotic susceptibility patterns of the two plasmid-cured multi-drug resistant isolates. It revealed loss of resistance to majority of the antibiotics.

The presence of plasmids aligns with earlier reports that

small DNA molecules known as plasmids are the key culprits in spreading the major global health threat of antibiotic resistance [26]. Plasmids are thought of as being important “vehicles” that transfer resistance genes between bacteria. Also, their number (plasmid copies) play a critical role in imparting resistance and other characteristics to the organisms. The post plasmid curing antibiotics susceptibility test conducted on the two multidrug resistant isolates revealed that resistance to most of the antibiotics was lost indicating that it was plasmid mediated. This consolidated the fact that plasmids play a significant role in antimicrobial resistance. The source of this is attributed to routine discharge of antibiotics and resistance genes from wastewater or runoff from livestock facilities and agriculture. Elevated levels of antibiotics resistance genes in aquatic environments are correlated to their proximity to anthropogenic activities.

Apart from resistance conferred by mutation, antibiotics resistance determinants in pathogenic bacteria could also originate from non-clinical environment [2]. This also agrees with the findings in this research carried out on Ikpoba River isolates.

Table 1. Antibigram of Bacterial Isolates from Ikpoba River Water Samples.

Isolates	Resistance To:	Susceptible To:
<i>Staphylococcus aureus</i>	Augmentin, gentamycin, pefloxacin, ofloxacin, streptomycin, amoxicillin, chloramphenicol and cotrimoxazole	Ciprofloxacin
<i>Streptococcus faecalis</i>	Chloramphenicol, cotrimoxazole, ofloxacin, pefloxacin, gentamycin, augmentin, sparfloxacin, amoxicillin	Ciprofloxacin and streptomycin
<i>Vibriosp</i>	Amoxicillin	Sparfloxacin, augmentin, gentamycin, pefloxacin, ofloxacin, cotrimoxazole, streptomycin, chloramphenicol, ciprofloxacin
<i>Salmonellasp</i>	Streptomycin and chloramphenicol	Ofloxacin, pefloxacin, amoxillin, augmentin, sparfloxacin, cotrimoxazole, ciprofloxacin
<i>Escherichia coli</i>	Ciprofloxacin	Sparfloxacin, augmentin, gentamycin, pefloxacin, cotrimoxazole, streptomycin, chloramphenicol, amoxicillin

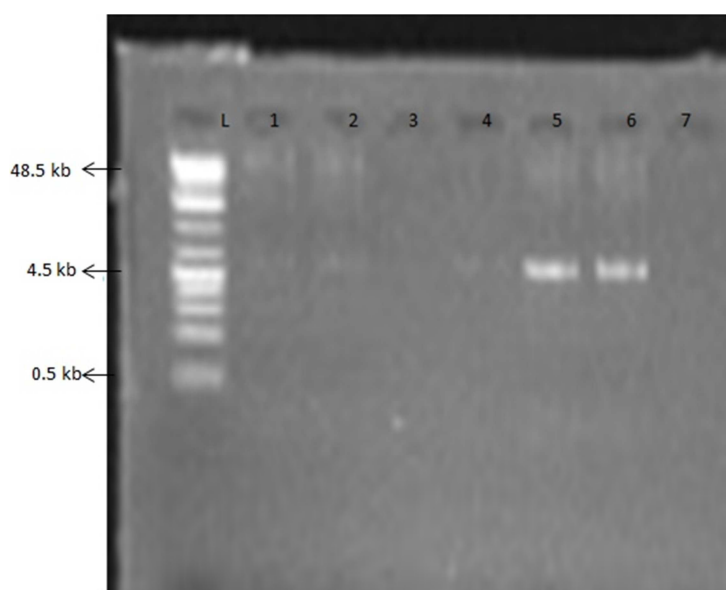


Figure 1. Plasmid profile of multi-drug resistant bacterial isolates analyzed with 0.8% agarose gel electrophoresis stained with ethidium bromide. L is 0.5kb-48.5kb DNA ladder. Samples 5 and 6 are positive for plasmid with bands at 4.5kb. Samples 1, 2, 3, 4 and 7 are no DNA template control.

Key

5= *Staphylococcus aureus*; 6= *Streptococcus faecalis*

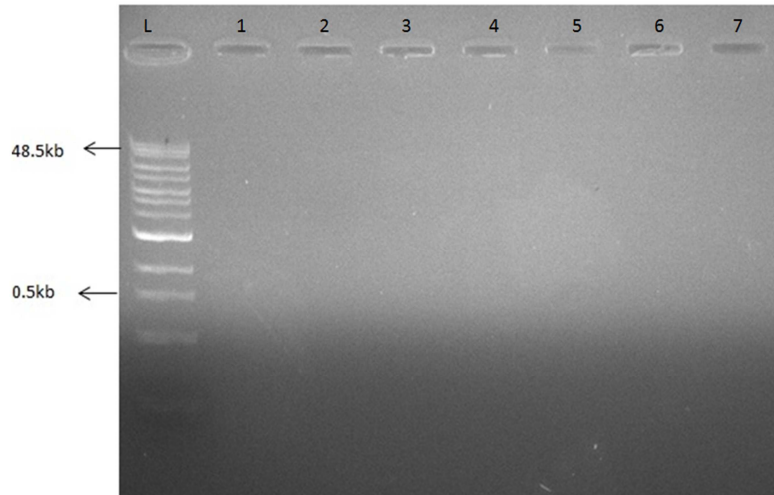


Figure 2. Plasmid profile (after curing) of multi-drug resistant bacterial isolates analyzed with 0.8% agarose gel electrophoresis stained with ethidium bromide. L is 0.5kb - 48.5kb DNA ladder (molecular marker). Samples 5 and 6 shows no plasmid after curing. Samples 1, 2, 3, 4 and 7 are no DNA template control.

Key
 5 = *Staphylococcus aureus*
 6 = *Streptococcus faecalis*

Table 2. Antibiogram of *Streptococcus faecalis* and *Staphylococcus aureus* pre-and post Plasmid Curing.

Isoltes	Resistance To:		Susceptible To:	
	BeforeCuring	AfterCuring	BeforeCuring	AfterCuring
<i>Staphylococcus aureus</i>	Augmentin, gentamycin, pefloxacin, ofloxacin, streptomycin, amoxicillin, chloramphenicol cotrimoxazole	Streptomycin and amoxicillin	Ciprofloxacin	Augmentin, gentamycin, pefloxacin, ofloxacin, Ciprofloxacin, chloramphenicol and cotrimoxazole
<i>Streptococcus faecalis</i>	Chloramphenicol, cotrimoxazole, ofloxacin, pefloxacin, gentamycin, augmentin, sparfloxacin, amoxicillin	Chloramphenicol	Ciprofloxacin and streptomycin	Ciprofloxacin, streptomycin cotrimoxazole, ofloxacin, pefloxacin, gentamycin, augmentin, sparfloxacin, and amoxicillin

4. Conclusion

The research has shown that some of the isolates of water samples collected from Ikpoba River, Benin City, Edo State, Nigeria had a multidrug resistant property necessitated by plasmid. This would hamper the successful use of antibiotics for treating infectious diseases. Though antibiotics are still effective for treating many bacterial infections, many strains are becoming increasingly difficult to treat and therapeutic options are getting fewer and this poses a serious health threat to the populace. The isolates could have acquired the resistance by horizontal gene transfer through conjugation because large scales of antibiotics are used in aquaculture and poultry farming. Some of these wastes are drained into river through runoff and all these predispose the environment to antibiotics thereby resulting in resistance of bacterial isolates.

Industries should endeavour to treat their wastewater before discharge as some of the disinfectant used in the factory could confer resistance to organisms. Also, the aquaculturists and poultry farmers that live within the vicinity of the rivers should avoid the abuse of antibiotics since the effect of some of these therapeutic agents do not end at the very site of action; the residual effect ends in the

environment. This could help to prevent the widespread of resistance found in bacteria and reduce the risk of treatment failures.

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