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Antibiotic Susceptibility Profile of Bacteria Isolates from Some Fishponds in Niger Delta Region of Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author OEN designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed literature searches. Authors OKA and AAI managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the antibiotic susceptibility profile of bacterial isolates from concrete and earthen fish ponds.
Study Design: Comparative analysis.
Place and Duration of Study: The African Regional Aquaculture Center Allu (ARAC), Port Harcourt River State, from August 2013-Jauary 2014.
Methodology: Water samples from both concrete and earthen fish ponds were collected and analyzed at the environmental microbiology laboratory of the University of Port Harcourt. The bacterial isolates present in water samples were isolated using the spread plate technique, and identified using biochemical characteristics while the antibiotic susceptibility pattern of the isolates was obtained using the disc-diffusion method.
Results: A total of 94 bacterial isolates (47 from concrete and 47 from earthen ponds) comprising of the following genera *Escherichia coli, Staphylococcus* sp., *Aeromonas* sp., *Salmonella* sp.,

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Shigella sp., *Pseudomonas* sp., *Vibrio* sp., *Proteus* sp., *Enterobacter* sp., *Serattia* sp., *Streptococcus* sp. and *Klebsiella* sp., were identified. Data showed that all the isolates from both concrete and earthen ponds exhibited multiple drug resistance (MDR). All the isolates were 100% resistant to ceftazidime, cefuroxime, cefixime and cloxacillin in both ponds. The highest susceptibility of 100% was recorded with erythromycin in concrete ponds and 0% in earthen pond, ofloxacin 85% in concrete pond and 75% in earthen followed by gentamicine with 91% in concrete pond and 50% in earthen ponds. Among the isolates, *Enterobacter* species were resistance to all antibiotics tested (100%), followed by *Proteus* sp., *Vibrio* sp. and *E. coli* with 87.5% respectively while the least resistance was found in *Pseudomonas* sp. (50%).

Conclusion: In conclusion, the prevalence of antimicrobial resistance in the pond water is a serious health concern since the microbial flora of a fish is the reflection of its aqueous environment. This implies that antibiotic resistant bacteria present in the pond water will also be present in the cultivated fish and fish product. The multiple antibiotic resistances among the pond water pathogens may further complicate the clinical management of diseases caused by the consumption of fish cultivated in these ponds.

Keywords: Antibiotic; aquaculture; bacteria isolates; susceptibility; fishpond.

1. INTRODUCTION

Fisheries and aquaculture supply the world with about 110 million metric tons of food fish per year [1]. 47% of this supply is derived from aquaculture production. However, this production is hampered by unpredictable mortalities that may be due to negative interactions between fish and pathogenic bacteria [2]. There are many factors, biological and economical, which affects the feasibility of fish farming, but one factor above all others which can only destroy the economic viability of fish farming is infectious disease [3]. Factors affecting disease outbreak in fish farm includes overcrowding, organic pollution, hypoxia, stress, higher temperature and low dissolve oxygen [4]. In fish farming, the potential for disease problems associated with intensive fish farming would increase the probability of the use of a number of antimicrobial drugs in their management. Antimicrobial agents are used as tools to maintain health and in the prevention of disease of cultured animals [5]. However, overuse of antimicrobial agents can potentially result in antibiotic resistance in pathogenic bacteria, subsequently making them less responsive to antibiotic. In aquaculture, antibiotics at therapeutic levels are frequently administered for short periods of time via the oral route to groups of fish that share tanks or cages. The most common route for the delivery of antibiotics to fish occurs through mixing the antibiotic with specially formulated feeds. However, fish do not effectively metabolize antibiotics and will pass them largely unused back into the environment as feces. It has been estimated that 75 percent of the antibiotics fed to fish are excreted into the water [6]. The long standing practice of using low doses of antibiotics for a long period of time for growth promotion, and arbitrary use of antibiotics in animal husbandry is a strong contributor to the development of the antibiotic resistant bacteria in the environment. Integrated fish farming is another practice that contributes to the rise in the number of antibiotic resistance in fish ponds. Animal manure which contains antimicrobial residues from the feed fed to the livestock is directly shaded in to the fish pond to increase the phytoplankton production. This practice also introduces a lot of pathogenic organisms into the pond environment [7].

Antibiotics routinely used for the treatment of human infections are also used in animal rearing, either for therapeutic or prophylactic purposes or for growth promotion, which has contributed to development of antibiotic resistance. Thus, the public health hazards related to antimicrobial use in aquaculture include the development and spread of antimicrobial resistant bacteria and resistance genes, and the occurrence of antimicrobial residues in aquaculture products. The greatest potential risk to public health associated with antimicrobial use in aquaculture is thought to be the development of a reservoir of transferable resistance genes in aquatic bacteria, from which such genes can be disseminated by horizontal gene transfer to other bacteria and ultimately reach human pathogens [8]. All drugs legally used in aquaculture must be approved by the government agency responsible for veterinary medicine. These regulatory agencies set rules for antibiotic use, including permissible routes of delivery, dose forms, withdrawal times, tolerances, and use by species, including dose

rates and limitations. In most countries with an important aquaculture industry, government agencies exert some controlling actions. It is therefore the aim of this study to examine the susceptibility of bacterial isolates from concrete and earthen fish ponds within Niger Delta to commonly used antibiotics.

2. MATERIALS AND METHODS

2.1 Sampling Site and Sample Collection

The sampling site (The African Regional Aquaculture Center) lies between 4-6ºN and longitude 5-8ºE within the Niger delta region of Nigeria .The region is characterized by high biodiversity, water ways, vast flood plains, characteristic swamps, and mangrove forest areas. Pond water samples were aseptically taken from the ponds using sterile screw cap bottles. Composite samples were obtained by collection at different sampling points and depths of 15 cm below the water surface. The water samples were transported to the laboratory in an ice-packed container for microbiological analysis within 8 hours of collection.

2.2 Isolation of Total Heterotrophic Bacteria (THB)

Samples of the pond water were serially diluted in ten folds using physiological saline. Total viable heterotrophic bacteria counts were determined by plaiting in duplicate 0.1 ml aliquots of the different dilutions on already prepared nutrient agar plates using spread plate technique. Colony counts were taken after incubation at 37ºC for 24 h. Discrete colonies were picked and sub- cultured to obtain pure cultures which were stored at 4ºC for microscopic and biochemical identification.

2.3 Isolation of Enteric Bacteria

One tenth milliliter of serially diluted pond water samples were plated on MacConkey agar using the spread plate technique to screen for coliform bacteria. Typical colonies of lactose and non lactose fermenting colonies were purified. Enrichment of the water samples were also made on alkaline peptone water and spread plated on thioglycholate citrate bile salt sucrose (TCBS) agar for the isolation of *Vibrio* species. Isolates were purified on nutrient agar and stored on agar slants at 4ºC for characterization and identification [9].

2.4 Characterization and Identification of Bacterial Isolates

The purified bacterial isolates were characterized and identified using series of biochemical reactions as described by [9].

2.5 Antibiotic Resistance Testing

Antibiogram of the bacterial isolates from the pond water samples were ascertained in triplicate on Mueller-Hinton, agar using the standardized disc diffusion method of [10], and the zone of inhibition interpretation was used for the in-vitro determination of the bacterial sensitivity to various antibiotics selected. Selection was based on the drugs most commonly used in the treatment of human and animal infections. The antibiotics and their concentration includes Ceftazidim (30ug), cefuroxime (30 µg), gentamicin (10 µg), cefixime (5 µg), ofloxacin (5 µg), augmentin (30 µg), nitrofurantoin (300 µg), ciprofloxacin (5 µg) Cefraxone (30 µg), erythromycin (5 µg), and cloxacillin (5 µg). Commercially prepared paper discs with the various antibiotics were mounted on Muller-Hinton agar plates containing pure cultures of the various isolates. Results obtained from the test were classified as resistance or sensitive after measuring the zones of inhibition.

3. RESULTS AND DISCUSSION

3.1 Results

A total of 94 isolates from earthen pond and concrete ponds belonging to the following genera *Escherichia, Salmonella, Pseudomonas, Shigella, Aeromonas, Klebsiella, Proteus, Enterobacter, Staphylococcus, Streptococcus* and *Vibrio* species were isolated from both ponds, while *Serratia* was isolated only in concrete pond. The bacterial isolates and their frequency of isolation is presented in Fig. 1. *Aeromonas* and *Staphylococcus* species were the dominate species followed by *E. coli*.

The individual resistance of organisms to all the antibiotics tested is presented in Fig. 2. *Enterobacter* showed 100% resistance to all antibiotic tested in both ponds, *Salmonella*, *Shigella, Aeromonas*, *Klebsiella,* and *Staphylococcus* showed 60% resistance to all antibiotics tested in both concrete and earthen ponds, while *E. coli*, *Vibrio,* and *Proteus species* had 85.5% resistance in earthen ponds, and 45%

in concrete ponds respectively. *Streptococcus* species had 60% in concrete pond and 50% in earthen ponds, while *Pseudomonas* recorded 50% in both ponds.

The percentage susceptibility of the test antibiotic is presented in Fig. 3. The cephalosporins, which include Cetazidime had 10% susceptibility in concrete pond, and 0% in earthen pond. Ceuroxime, cefixime and cloxacillin had 0% susceptibility in both ponds. Gentamicin had (90% and 50%) in ponds, ofloxacin (80% and 78%), augmentin (8.5% and 8%), nitrofurantoin (55% and 50%), and ciprofloxacin (79% and 50%) in both concrete and earthen pond respectively. Erythromycin had 100% susceptibility in concrete pond and 0% in earthen pond while ceftrazone had (50%) susceptibility in both concrete and earthen ponds.

3.2 Discussion

A total of 94 identified isolates belonging to the following genera, *E. coli, Staphylococcus* and *Streptococcus, Aeromonas, Klebsiella, Salmonella, Shigella*, *Proteus*, *Pseudomonas*, *Enterobacter*, *Vibrio* and *Serratia* specie, from concrete and earthen fish ponds were subjected

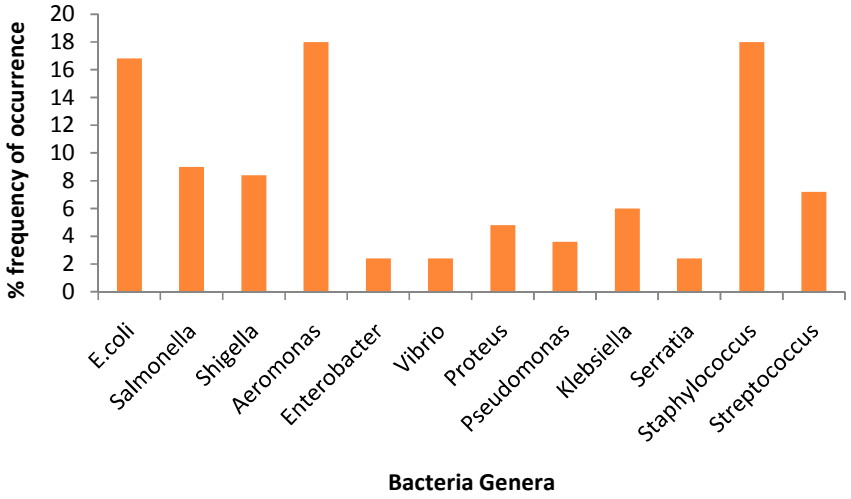

Fig. 1. Percentage frequency of occurrence of bacterial isolates

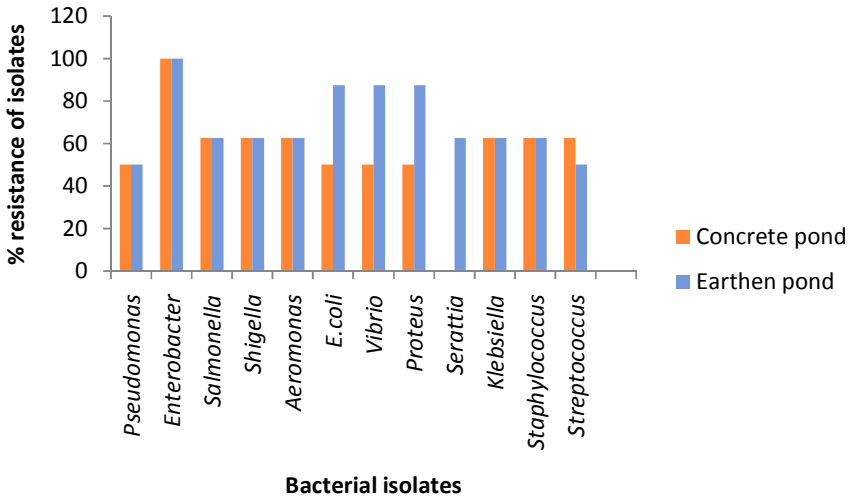

Fig. 2. Percentage resistance of bacterial isolates to test antibiotic

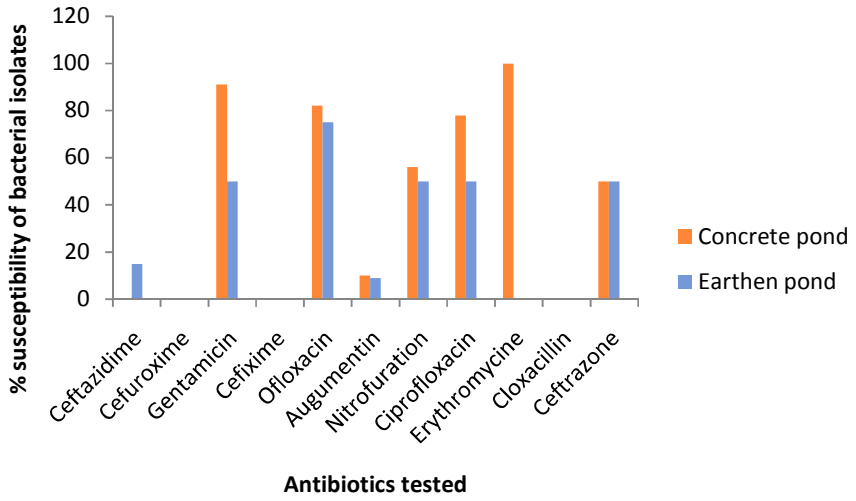

Fig. 3. Percentage effect of antibiotics on bacterial isolates

to antibiotic sensitivity testing. The antibiotic sensitivity test conducted on the bacteria isolates, to ascertain their level of susceptibility to commonly used antibiotics, revealed multiple drug resistance of four to six antibiotics. The relatively high level of resistance to antimicrobial agents is a reflection of misuse or abuse of these agents in the environment [11]. The present study reports a higher percentage of antibiotic resistant in isolates from earthen ponds, when compared to concrete pond. Multiple drug resistance is an extremely serious public health problem and it has been found to be associated with the outbreak of major epidemics throughout the world [12]. Thus, the multiple drug resistance shown by these bacterial isolate is worrisome, because of the public health implications. Handling of fish materials and consumption of fish can expose the handler to certain risks, either from the water or from the fish themselves.

The high rate of antibiotic-resistant bacteria from all the fish ponds has ecological and public health implications, which could become a problem associated with the transfer of resistance determinants to human pathogenic bacteria that could enter the human population through fish consumption [13].

The cephalosporin's, (ceftazidime, cefuroxime, cefixime, and ceftrazone) and penicillin (cloxacillin) are beta-lactam antibiotics that act by disrupting the synthesis of peptideglycan layer of bacterial cell wall. According to [14], there is an association between cephalosporin's usage, and emergence of multiple antibiotic resistances in organisms. Beta-lactamases are the major determinants to resistance to beta-lactam antibiotics, since all gram negative bacteria are chromosomally mediate B-lactamase enzymes which leads to resistance to cephalosporin antibiotics. The remarkable resistance to the entire cephalosporin's drugs shows that resistance to one antimicrobial agent in a class can confer resistance to other members of the same group. This also implies that resistance to cephalosporin can confer resistance to other beta- lactam drugs like penicillin [15].

The quinolines (ofloxacin and ciprofloxacin) act by inhibiting DNA gyrase thereby interfering with DNA replication. The susceptibility of the bacterial isolates to ofloxacin and ciprofloxacin (80%, 75%) and (78%, 50%) for concrete and earthen ponds respectively shows their potency in diseases control. This could be attributed to the newness of the drug as they have not been used in aquaculture. This result corroborates with the result of [7,16] who reported highest susceptibility in ofloxacin and ciprofloxacin. Similarly, [17] recorded susceptibility of (93.3%) for ofloxacin and ciprofloxacin respectively, during their study on the pollution status of New Calabar River.

Gentamicine an amino-glycoside antibiotics that acts by binding to the 30S subunit of the bacterial chromosome, thereby interrupting protein synthesis inhibited (90% and 50%) of bacterial isolates in both concrete and earthen ponds. Similar to this result, [10,18] reported higher

susceptibility to getamicine antibiotics, while (17) recorded 100% susceptibility in their study.

Augmentin is a combination of (Amoxicillin and clavulante). A penicillin-class antibacterial and beta-lactamase inhibitor that combines the mode of action of both antibiotics had a susceptibility of (10%) in both ponds. This could be attributed to the resistance of the organisms to penicillin, which is a beta-lactam antibiotic.

The resistance of bacterial isolates to Erythromycin, Cloxacillin and Augmentin in this study has also been previously reported by [19], who worked on antibiotic resistance in enteric bacteria from fish media. Likewise, [20,21]. Also, [10] recognized *S. marcescens* as an opportunistic pathogen and strains of it are now resistant to commonly used antibiotics. In the same vein, the resistance of *Enterobacte*r species to all antibiotics tested implies that infection caused by *Enterobacter* would be difficult to cure [22]. In Nigeria, several studies have reported high levels of multiple drug resistance in bacterial isolates such as *E. coli*, *S. aureus, P. vulgaris,* S. *pyogenes*, *B. cerus, Klebsiella* sp., *Micrococcus* sp. *P*. *aeuroginosa, Enterobacter* sp, *S. epidermis* from diverse environmental samples like hospitals [16], rivers [23], and drinking water. These studies showed steady rise in the resistance of various isolates to different antibiotics.

Overall, ofloxacin, ciprofloxacin and gentamicin were successfully found to inhibit more than 70% of the present bacterial isolates. The susceptibility of bacterial isolates to ofloxacin may be due to the newness of the drug. Therefore, it is suggested that ofloxacin, gentamincin and ciprofloxacin should be the antibiotics of choice in combating diseases associated with the consumption fish and fish products.

4. CONCLUSION

The prevalence of antimicrobial resistance in the pond water is a serious health concern. Since the microbial flora of a fish is a reflection of its aquatic environment, this implies that antibiotic resistant bacteria present in the pond water will also be present in the cultivated fish and fish product. Considering the fact that many antimicrobial agents used in human medicine are also used in aquaculture, the resistance of human pathogenic bacteria to these antimicrobials severely limits the therapeutic option in human infection. The multiple antibiotic resistances among the pond water pathogens may further complicate the clinical management of diseases caused by the consumption of fish cultivated in these ponds.

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COMPETING INTEREST

Authors have declared that no competing interests exist.

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