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Distribution and antibiogram of bacterial species in effluents from abattoirs in Nigeria

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ABSTRACT

The study investigated the bacteriological effluent qualities of abattoirs in Abakaliki, Southeast Nigeria between June and September 2015. Wastewater samples were collected from two abattoirs, from their point of discharge into surface water bodies with sterile sample bottles and transported to the Laboratory for bacteriological analyses. Bacteria species were isolated, characterized, and identified using standard microbiological and biochemical techniques. Antibiotic susceptibility study was carried out using Kirby-Bauer disc diffusion method. The result of the total heterotrophic bacteria count obtained from Nkwo–Ezzangbo abattoir ranges from 7.00×10^6 Colony Forming Unit (CFU)/ml to 7.90×10^6 CFU/ml, while that of Abakaliki abattoir ranges from 5.50×10^6 CFU/ml to 6.95×10^6 CFU/ml. Antibiotic studies showed that majority of the gram-negative isolates were sensitive to the antibiotics. Resistance was obtained against augmentin, nalidixic acid (NAL) for *P. aeruginosa*. *E. coli* had resistance against ceporex, septrin, and NAL, while the gram-positive streptococci had resistance against cetriaxone and ampicillin. The presence of these multi-drug resistant strains of the isolated organism in abattoir effluents could act as a vehicle to disseminate antibiotic resistance to other bacteria. This emphasizes the need for proper treatment and safe disposal of abattoir effluents in the study area.

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Abattoir effluents; antibiotics resistance; Abakaliki; Bacteria species

Introduction

Abattoir is a slaughter house derived from the French word "abattre" meaning "to strike down" [1]. It is a place where animals such as cattle, goats, and other animals are dressed, killed, and distributed for consumption. All over the world, abattoirs have being generally studied to pollute the environment either directly or indirectly from their various processes [2].

In Nigeria, adequate abattoir waste management is lacking in all public abattoirs such that large solid wastes and untreated effluents are common sites [3,4] unlike in developed countries where these facilities are adequately provided [4]. Many abattoirs in Nigeria dispose their effluents directly into streams and rivers without any form of treatment [1]. Reports have also shown that indiscriminate disposal of abattoir effluents may introduce enteric pathogens into surface and ground water and the pathogens isolated from abattoir wastewaters can survive in the environment and pose danger to humans and animals. Disposed abattoir effluents into water bodies without any prior treatment of the effluent may impair the water bodies; thus, polluting the environment directly or indirectly [5].

According to Landhausser et al. [6] abattoir effluents could increase levels of nitrogen, phosphorus, total solids in receiving water bodies considerably. When organic matter exceeds the capacity of the micro-organisms in water that breakdown and recycle the organic matter, it encourages rapid growth or blooms of algae leading to eutrophication [6]. Bacteria from abattoir waste discharged into water bodies can be absorbed to sediments and when the bottom stream is disturbed, the sediments release the bacteria back into the water columns presenting long-term health hazards [7].

Wastewater or effluent generated from the abattoir is characterized by the presence of many

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pathogenic microorganisms such as *Salmonella, Escherichia coli* (including serotype 0157:H7), *Shigella*, parasite eggs, and amoebic cysts [4,8] which are of public health importance. Also, several pathogenic bacteria and fungi species have been isolated from abattoir wastewater and surface water; including *Staphylococcus*, *Escherichia coli*, *Streptococcus*, *Salmonella*, *Aspergillus*, *Mucor*, *Saccharomyces*, and *Penicillium* species [4,9,10]. These pathogens might threaten public health by migrating into ground or surface water.

In view of the huge cost of treatment failure as a result of antibiotics resistance in our environment, this work was aimed at identifying bacteria and their susceptibility patterns to antimicrobial drugs from abattoir wastes and its receiving waters in Abakaliki, Nigeria.

Materials and Methods

Description of study area

Nkwo–Ezzangbo is one of the largest and most populous markets in Ohaukwu local government area of Ebonyi State. It is bounded by Ishielu local government on the north, Ezza south local government to the south, Izzi local government on the east, and Benue State on the west. The Nkwo abattoir is located along Enugu-Abakaliki expressway. It is a major market for trading donkeys down to south east of Nigeria. It has an abattoir, and several donkeys are slaughtered in this abattoir. On the average, the abattoir produces about 2,071 donkey heads per day. Close to the slaughtering slab is a heap where paunch materials are dumped and have accumulated over the years. The waste materials from the abattoir are washed through drainage, which links the abattoir and the Nkwo stream which is some 450 meters away. Nkwo stream is a tributary to Ogbagu River. While, Abakpa abattoir is located in Abakaliki, which is the capital city of Ebonyi State in South-eastern Nigeria. Abakpa is the largest market in Ebonyi State and several animals are slaughtered in the abattoir like cows, chickens, goats etc. The waste materials from the abattoir are washed through a drainage which links the abattoir and the lyiokwu stream.

Sample collection

A total of 20 abattoir effluents used in this research work were collected aseptically with the aid of a sterilized sample container between June and September 2015. The samples were collected in the morning during the peak activities between 7.00 and 8.00 AM. Study was carried out during the rainy season. Four effluent sampling points was sampled and the sampled points depict different activities within and outside the abattoirs. Sampling points A–C were located within the abattoir, while D was located in the stream. Samples were transported to Microbiology Laboratory of Ebonyi State University, Abakaliki in ice jackets and were processed within 4 hours of collection.

Microbiological analysis

Isolation and identification of isolates from the abattoir effluent samples were aseptically carried out using standard microbiology techniques as described by Cheesbrough [11]. The abattoir effluent samples were aseptically inoculated into culture media namely: Pseudomonas cetrimide selective agar for the isolation of *Pseudomonas aeruginosa*, MacConkey agar for the isolation of *Streptococcus* spp., and Eosin methylene blue agar for the isolation of *Escherichia coli.* The agar plates were incubated at 37°C for 18–24 hours. The pure isolates were maintained on agar slants for further characterization and identification.

Total heterotrophic bacterial counts

This was determined with the nutrient agar using the spread plate technique as described by Coker et al. [9]. Here 0.1 ml of the serially diluted samples each was inoculated into different sterile nutrient agar plates in triplicates. The plates were incubated for 24 hours at 37°C. After incubation, colonies that appeared on the plates were counted and the mean expressed as Colony Forming Unit (CFU)/ml.

Antibiotic susceptibility test

Bacteria isolates were subjected to *in-vitro* susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute (CLSI) [12]. In brief, by taking pure isolated colony, bacterial suspension was adjusted to 0.5 McFarland turbidity standards. The diluted bacterial suspension was then transferred to Mueller–Hinton agar plate using a sterile cotton swab and the plate was seeded uniformly by rubbing the swab against the entire agar surface followed by 24 hours incubation. After the inoculums were dried, antibiotic impregnated discs were applied to the surface of the inoculated plates using sterile forceps. The plates were then

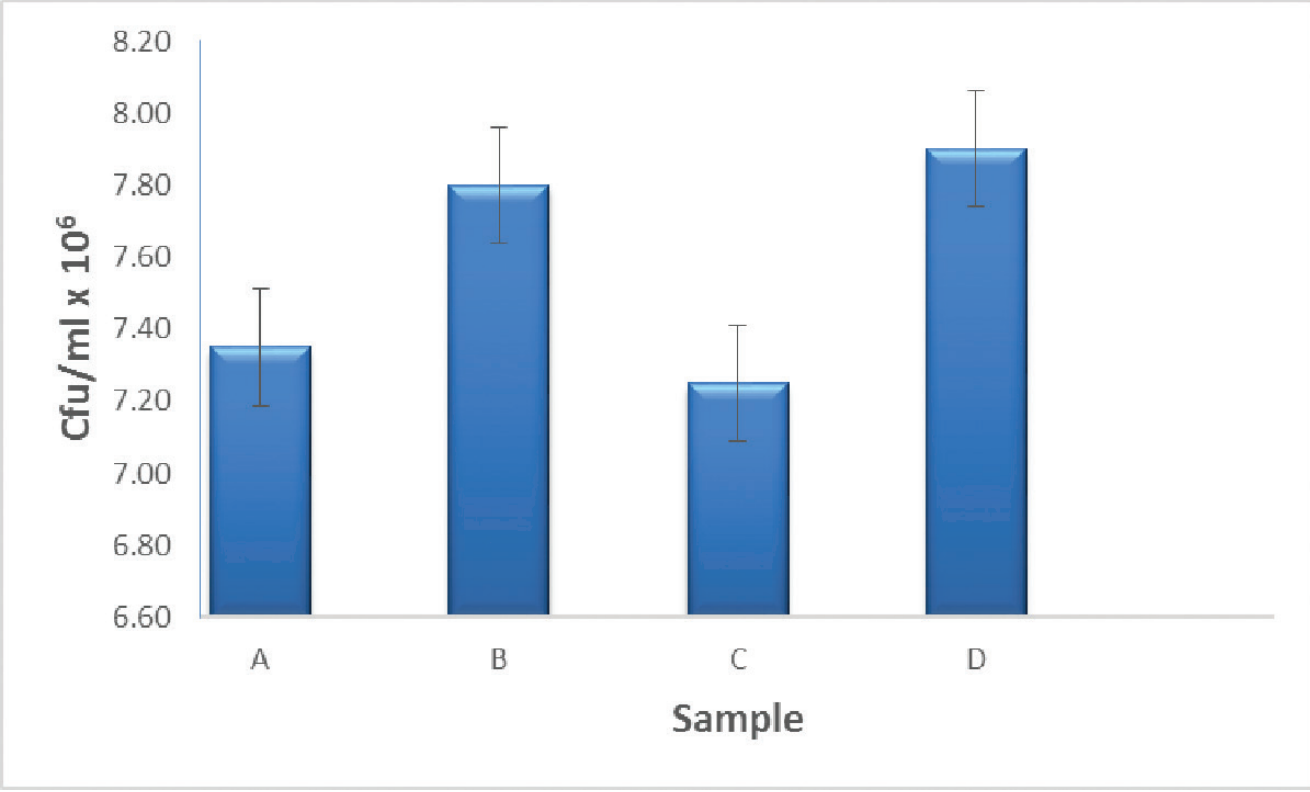

Figure 1. Total heterotrophic bacterial load from Nkwo–Ezzangbo abattoir. A = sampling point A (Slab), B = sampling point B (Channel), C = sampling point C (Butchering), and D = sampling point D (River).

incubated aerobically at 37°C for 24 hours. Finally, the zone of inhibition was measured including the disk diameter. The susceptible, intermediate, and resistant categories were assigned on the basis of the critical points recommended by the CLSI, [12] and according to the manufacturer's leaflet attached to the discs. The standard antibiotic discs (Oxoid, England) and their concentrations used against the gram-negative isolates were ofloxacin (OFX) (10 µg), perfloxacin (10 µg), ciprofloxacin (5 µg), augmentin (AUG) (10 µg), gentamicin (GEN) (10 µg), streptomycin (STR) (10 µg), ceporex (CEP) (10 µg), septrin (SXT) (10 µg), ampicillin (AMP) (5 µg), and nalidixic acid (NAL) (10 µg), while the gram-positive discs and their concentrations used against the gram-positive isolates were levofloxacin (LEV) (5 µg), ciprofloxacin (5 µg), norfloxacin (NOR) (10 µg), GEN (10 µg), OFX (10 µg), erythromycin (ETM) (10 µg), clindamycin (10 µg), ceftriaxone (30 µg), cefixime (5 µg), and AMP (5 µg). These antibiotics were chosen because they are either used in both human medicine and animal veterinary practice or because previous studies have reported microbial resistance to them.

Analysis of data

Data obtained were presented as mean ± standard error. Significance of difference between different treatment groups was tested using one-way analysis of variance (ANOVA) and significant results were compared with Duncan's multiple range tests using SPSS window 7 version 1.6 software. For all the tests, the significance was determined at the level of $P < 0.05$.

Results

The result of the total heterotrophic bacteria count obtained from Nkwo–Ezzangbo abattoir ranges from 7.00×10^6 CFU/ml to 7.90×10^6 CFU/ml, the highest bacterial load was obtained from sampling point D, while the least count was obtained from sampling point C (Fig. 1). Statistical analysis using ANOVA revealed that there was no significant difference ($P < 0.05$) in the total heterotrophic bacterial count from the different sampling points.

The result for the total bacteria counts from Abakaliki abattoir ranges from 5.50×10^6 CFU/ml to 6.95×10^6 CFU/ml. Sample point A had the least

bacterial count, while sample point C had the highest bacterial count (Fig. 2).

A total of 25 bacterial species comprising (*Escherichiacoli, Pseudomonas,* and *Streptococcus* species) were targeted and isolated from the abattoir effluent samples. *Escherichia coli* had the highest occurrence (31.4%), followed by *P*. *aeruginosa* (22.9%), while *Streptococcus* spp. had the lowest occurrence (17.1%) (data not shown). The results of the antibiotic studies of the gram-negative isolates (*E. coli* and *P. aeruginosa*) recovered from Ezzangbo abattoir showed that *E. coli* was sensitive to nine out of the 10 antibiotics used, but was resistant only to NAL (Fig. 4). While the *E. coli* isolates recovered from Abakaliki abattoir was also sensitive to seven of the antibiotics, while it was resistant to NAL, SXT, and CEP (Fig. 3). *Pseudomonas aeruginosa* recovered from the two abattoirs was resistant to AUG and NAL (Figs. 3 and 4). The result of the antibiotic studies from the gram-positive *Streptococcus* species recovered from Ezzangbo abattoir was resistant to ceftriaxone, AMP, and cefixime (Fig. 5), while the isolate from Abakaliki abattoir was resistant to AMP and ceftriaxone (Fig. 6).

Discussion

The total bacterial counts of abattoir effluents from both Abakaliki and Ezzangbo abattoirs during the study period as illustrated in (Figs. 1 and 2) indicate very high microbial loads. These values are higher than the WHO accepted limit for microbial contamination for any surface sample which should not exceed 1.20×10^6 CFU/ml [13]. The result also revealed that Nkwo–Ezzangbo abattoir had a higher count of bacteria when compared to that of Abakpa abattoir (Figs. 1 and 2). The low bacterial count as observed from Abakpa abattoir may be due to reduced human activities and also, as a result of some level of hygienic habit by the urban dwellers, as they are aware of the health implications of poor cleanliness. While possible reasons for the high bacteria counts obtained from Nkwo–Ezzangbo abattoir might be related to poor sanitary condition of the abattoir. Observations made from the study location showed that, wastewater from the slaughtering and dressing slabs in both abattoirs is washed into open drainage without any form of treatment. The wastewater could eventually percolate into surrounding surface and ground waters which poses danger to those working in the abattoirs and those living around them as available water sources close to them become contaminated by the effluents. The result obtained from this study is consistent with previous studies done by other authors such as Adesemoye et al. [10] and Rabah et al. [14], who reported similar high count in the range of (×107 CFU/g) bacteria from soil samples contaminated with wastewater at Agege and Ojo in Lagos and also, in Sokoto abattoir, respectively in Nigeria.

A total of 25 bacterial isolates were obtained from the abattoir effluent samples collected from the two abattoirs. Isolates obtained after characterization revealed the presence of *Escherichia coli*, *Pseudomonas aeruginosa,* and *Streptococcus* spp. The presence of these microorganisms is worrisome mainly because these microorganisms are opportunistic human pathogens and even though may not infect healthy humans but may infect immune-compromised individuals [13]. Similar findings were reported by Ezeronye and Ubalua [15], who reported the isolation of *Streptococcus faecalis*, *E. coli*, *Staphylococcus* spp., *Clostridium* spp., and *Salmonella* spp. among other organisms from Aba River as a result of contamination from abattoir effluents. Also, Bala [16] reported the isolation of similar organisms from water contaminated with fecal material in Jimeta-Yola, Nigeria.

The presence of *Escherichia coli*, *Streptococcus* spp., and *P. aeruginosa* in this study give credence to human fecal contamination of these sites. The isolation of *E. coli* and other coliforms is an indication of recent human contamination of the sampling points, and is of great public health concern [17]. While, the presence of *Pseudomonas* spp. within the abattoir environment is probably due to the presence of hydrocarbons within the abattoir. This observation supports the report by Faria and Bharathi [18] that *Pseudomonas* spp. is widespread in the environment and concluded that they could contribute to the oxidation of hydrocarbons in the environment.

The emergence of antimicrobial resistant bacteria increases in environment where antimicrobials are indiscriminately used in the public. In Nigeria and other developing countries, acquired bacterial resistance to antimicrobial agents is common and the complex socio-economic and behavioral factors associated with this phenomenon include abuse of antibiotics among other complex factor. In this study, majority of the isolates were susceptible to the antibiotics used against expectations with ciprofloxacin, STR, and others having the highest sensitivity on *P. aeruginosa*, and *E*. coli. However, resistance was observed in E. coli against NAL,

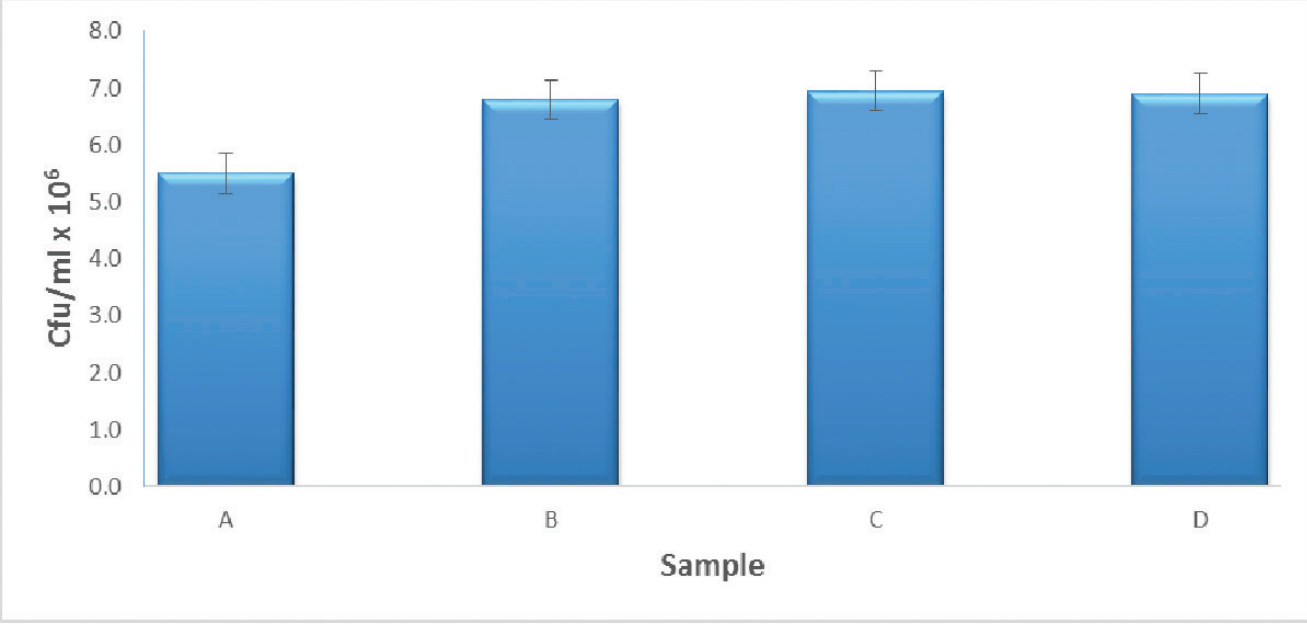

Figure 2. Total heterotrophic bacterial load from Abakaliki abattoir. A = sampling point A (Slab), B = sampling point B (Channel), C = sampling point C (Butchering), and D = sampling point D (River).

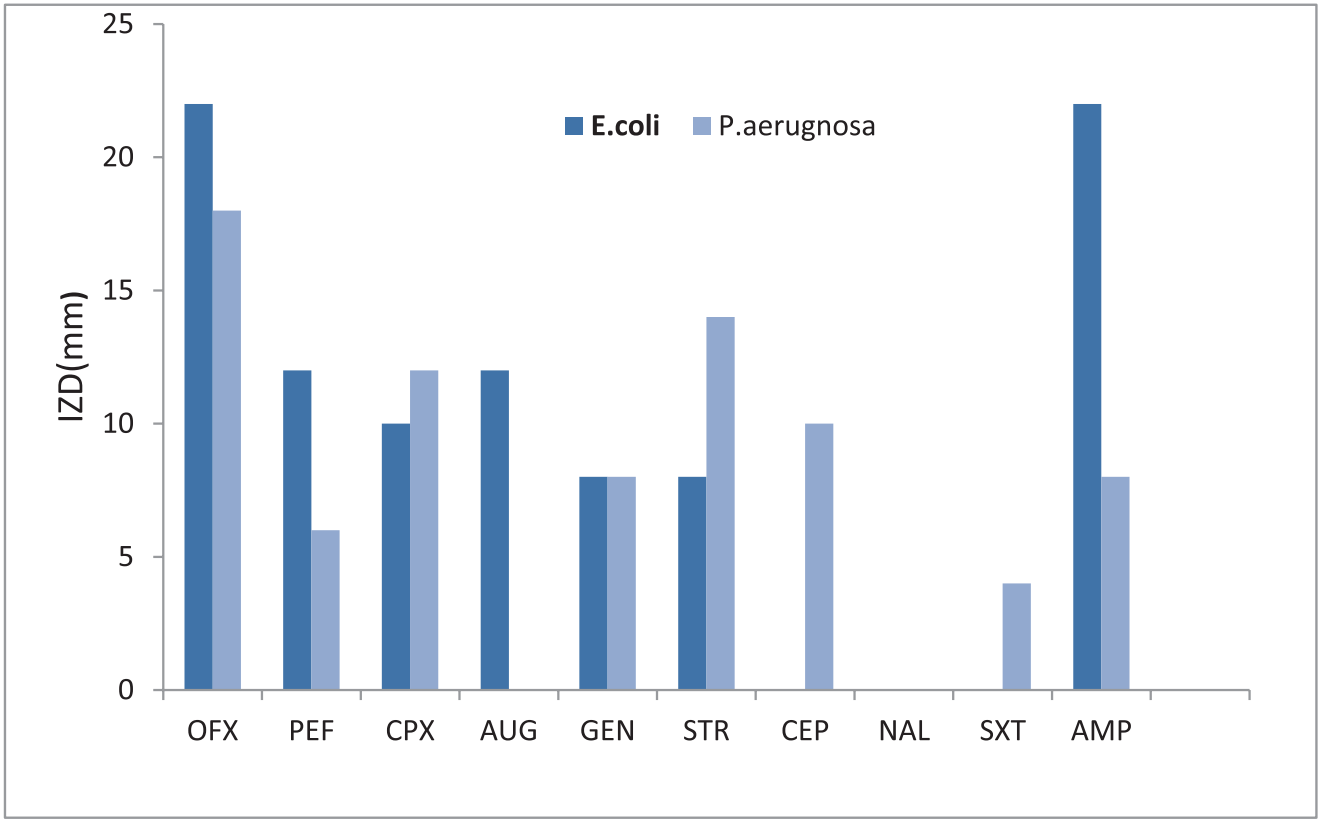

Figure 3. Antibiotic susceptibility pattern of *P. aeruginosa* and *E. coli* from Abakaliki abattoir. PEF = pefloxacin; CIP= ciprofloxacin; IZD = inhibition zone diameter.

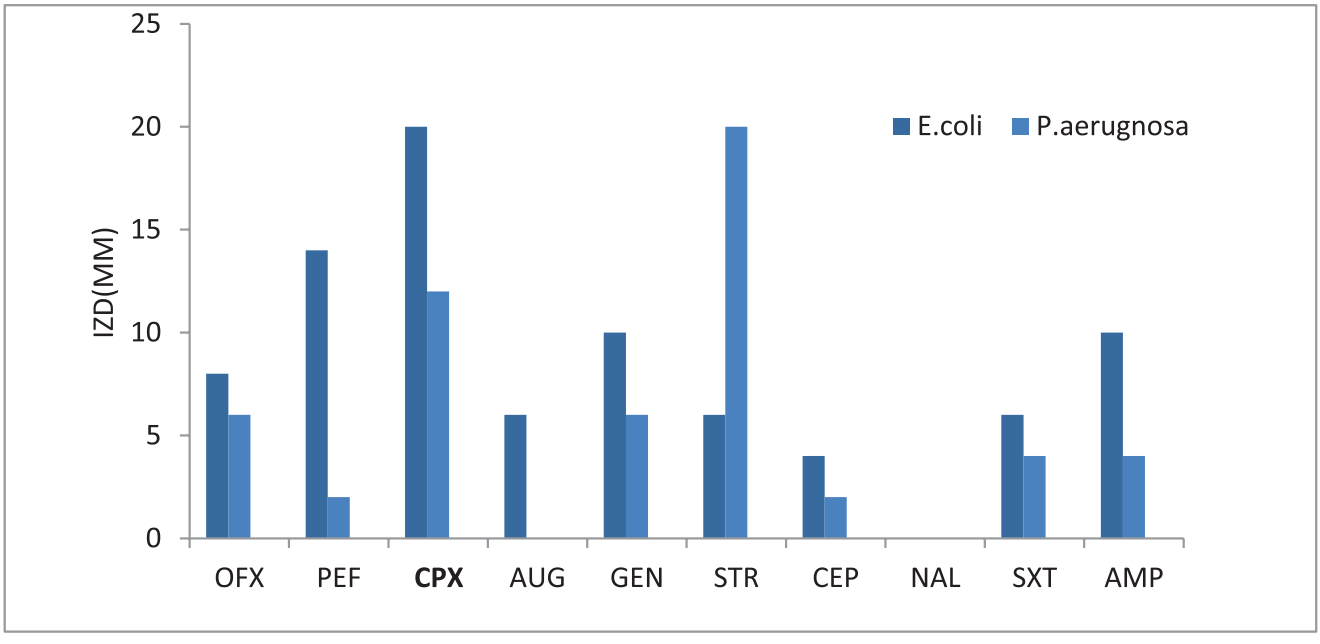

Figure 4. Antibiotic susceptibility pattern of *P. aeruginosa* and *E. coli* from Nkwo–Ezzangbo abattoir. PEF = pefloxacin; CIP= ciprofloxacin; IZD = inhibition zone diameter.

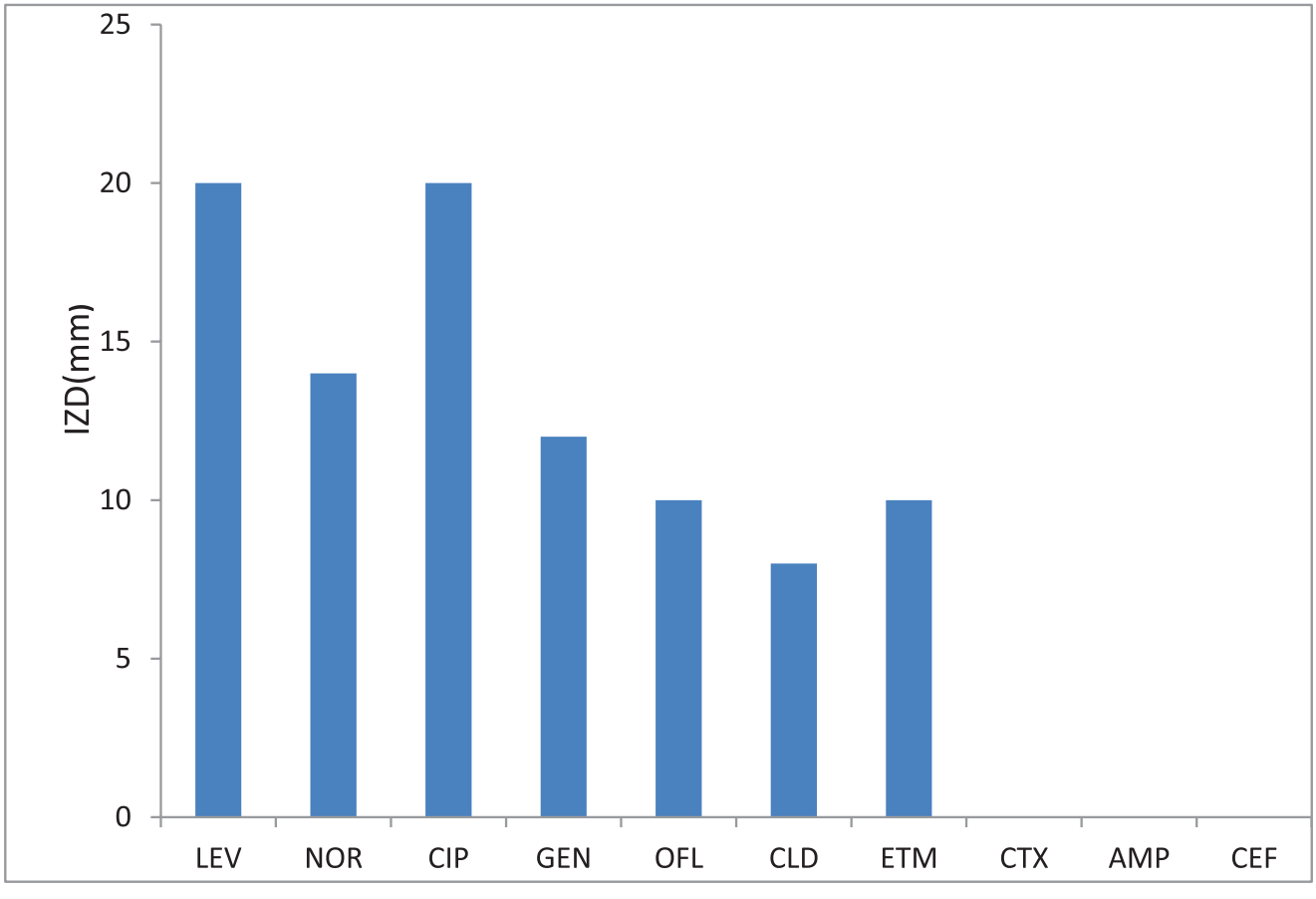

Figure 5. Antibiotic resistance pattern of *Streptococcus* spp. from Ezzangbo abbatoir. CIP = ciprofloxacin; CLD = clindamicin; CTX = cetriaxone; CEF = cifixime; IZD = inhibition zone diameter.

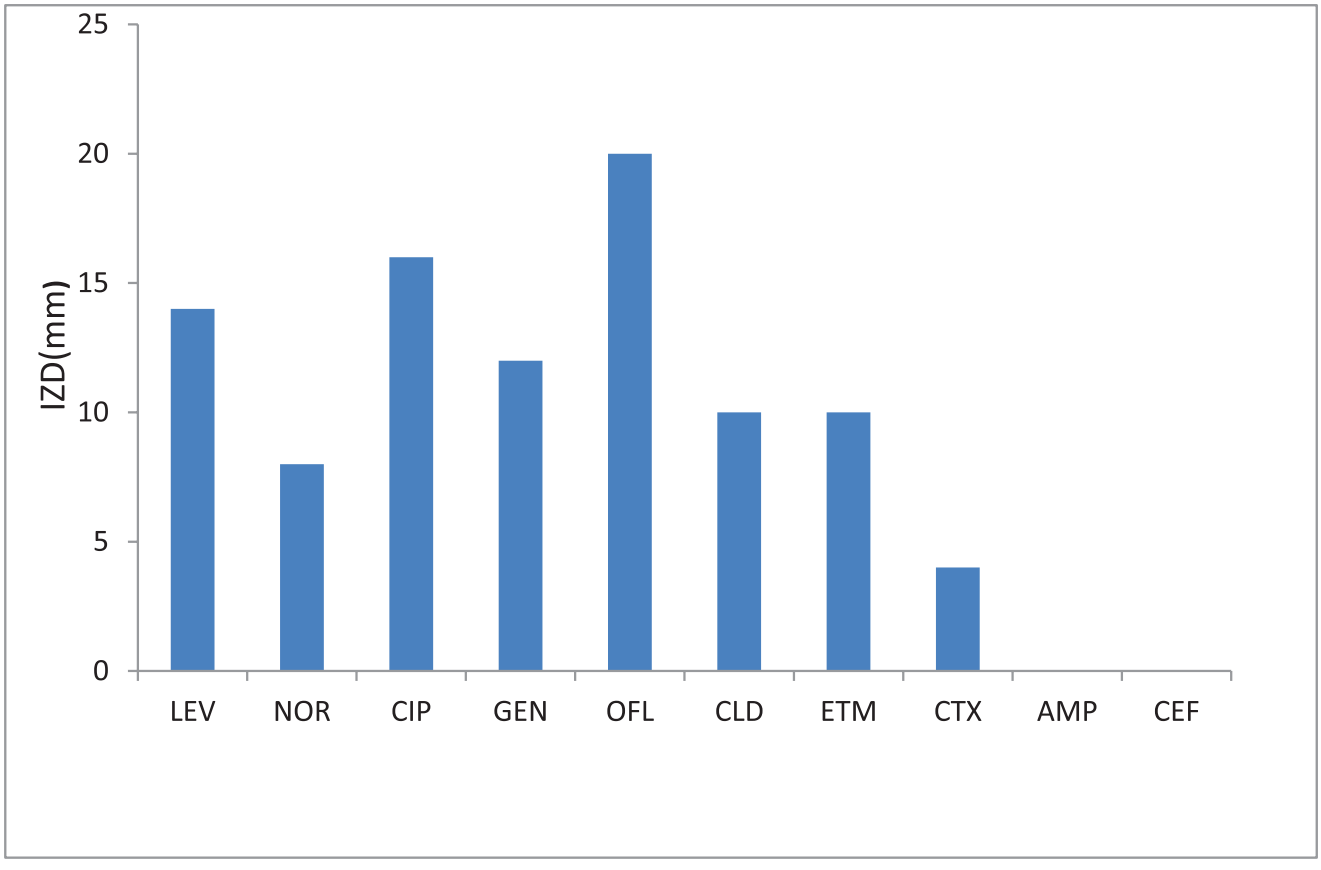

Figure 6. Antibiotic resistance pattern of *Streptococcus* spp. from Abakaliki abbatoir. CIP = ciprofloxacin; CLD = clindamicin; CTX = cetriaxone; CEF = cifixime; IZD = inhibition zone diameter.

SXT, and CEP, while P. aeruginosa was resistant to AUG and NAL (Figs. 3 and 4). Also, environmental *Streptococcus* spp. isolated from both abattoir effluents has considerable levels of antibiotic susceptibility. Isolates showed sensitivity to a wide range of clinically relevant antibiotic agents against fluoroquinolones such as LEV, NOR, ciprofloxacin, OFX, and other classes of antibiotics such as GEN, clindamycin, and ETM, while resistance was observed in cetriaxone and AMP, respectively (Figs. 5 and 6). Our result is contrary to the study by Lister et al. [19] who recorded that isolates demonstrated resistance to a wide range of antimicrobial agents. The possible reason may be due to difference in time, variation of environment, and the type of contaminated effluents.

Multiple bacterial resistances to drugs had earlier been reported in aquaculture environments by other workers across the globe [20]. Puah et al. [21] had reported up to six different resistance pattern and resistance to (two or more drugs) in 93% of tested isolates. Resistance to multiple antibiotics can lead to occurrence of newly emerging resistant bacteria which may be transmitted to consumers causing infection that are difficult to treat.

The fact that antimicrobial resistant genes are common in environment and play an important role for bacterial survival, the prevalence of multi-drug resistance bacteria in abattoir effluent is probably due to a multitude of biological as well as ecological factors. Because they are multi-drug resistant implies that there is possibility of these bacteria to harbor plasmids with several genes conferring resistance to a broad array of antibiotics. These suggest that there is high chance of spreading these pathogens and the associated resistant genes to humans and animals.In conclusion, the research has described the bacterial profile and antimicrobial resistance pattern of *P.aeruginosa*, *E. coli*, and *Streptococcus* species from abattoir effluents in Abakaliki area. On-site observation of the abattoirs especially at Ezzangbo shows that the sanitary and hygienic conditions of the abattoir are far from ideal. Also, this study observed that untreated abattoir wastewater discharged into water bodies within the study area, contains antibiotic resistant bacteria that could impact on public health. The

importance of adopting appropriate abattoir wastewater treatment measures to prevent the chances of contaminating water bodies and ground water is therefore recommended.

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