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Microbiological Assessment and Antibiotic Susceptibility of Orogodo River Exposed to Abattoir Effluent in Delta State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The content of abattoir effluent and its receiving water body, Orogodo River in Agbor, Delta State. Total heterotrophic and coliform counts were assessed using standard microbiological techniques. The viable heterotrophic bacteria count for the rainy season ranges from 7.7x105-4.6x104cfu/ml. Total coliform counts 2.6x105 -3.4x104 MPN/ml respectively. A total of 11 bacteria were isolated, antibiotic susceptibility pattern of bacteria was determined using the disk diffusion method. Five out of the bacteria isolates were resistant to at least four antibiotics. Out of all the antibiotics used 90 isolates were susceptible to erythromycin, 5% were resistant, also, 85 were susceptible to ofloxacin with 5% resistance and 10% were intermediate. Ampicillin had the highest resistance of 40%. The Molecular identification of multiple antibiotic-resistant bacteria was carried out using a 16S rRNA gene sequence. The identified bacteria include Shigella flexneri, Enterobacter hormaechei, Proteus

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vulgarus, Morganella morganii, and Pseudomonas oryzae. Data were statistically analyzed to determine the level of significance. The microbial population of bacteria isolated was found to be higher in the abattoir wastewater sample 7.7x105 and the point of entry sites 4.0x105 when compared to the downstream and upstream sample stations. The high microbial load and presence of multiple antibiotic-resistant bacteria in the river water is alarming and can lead to public health risks when such water is consumed or used for cleaning meat during processing. There is a need for proper management of abattoir waste, An on-site pretreatment strategy (collection of waste, treatment, processing, anaerobic digestion, and safe disposal) is highly recommended and should be enforced by relevant authority to safeguard the environment and the populace that depend on the Orogodo river water.

Keywords: Abattoir effluent; Shigella flexneri; Proteus vulgaris; Morganella morganii; antibiotic susceptibility; 16S rRNA sequencing.

1. INTRODUCTION

Safe and clean water is a basic requirement for every human being for a healthy life. Globally fresh water is becoming a limited resource due to population expansion, anthropogenic contaminations and climate change [1]. The lack of quality health care systems and insufficiency in the supply of potable water, has resulted in developing countries being on alarming list of water borne disease outbreaks (WHO, 2018).

Abattoir is a large scale facility designed to slaughter and process animals for meat production and meat products for the populace and also provides job opportunity for several people. Due to increase in population, the high demand for protein products has undoubtedly increased the number of animals slaughtered on daily basis, and subsequently the quantity of abattoir effluent (waste water) generated, owing to the fact that large quantities of water are used in processing the animals. These waste waters are subsequently discharged into nearby receiving surface waters. The indiscriminate discharge of untreated waste water pollutes the environment thereby posing serious threat to human health and veterinary safety [2].

The disposal of untreated abattoir waste water into open drainages or neighboring streams, paves way for enteric pathogens and excess nutrients which favors the proliferation of these pathogens in surface water [3]. Animals are known reservoir for members of the Enterobacteriaceae family. *Salmonella* spp.*, Klebsiella* spp.*, E. coli, Proteus* spp.*, Shigella, Enterobacter* spp.*, Citrobacter spp* and *Serratia spp*. have been isolated from waste water and sludge from abattoir. These pathogens cause enteric diseases and gastroenteritis. Various route of transfer include, consuming inadequately cooked contaminated food and animal products, and contaminated water. Shigellosis or bacilliary dysentery caused by *Shigella* spp., and Salmonellosis caused by *Salmonella* spp. are examples of diseases associated with vegetables, food substances and products processed or in contact with polluted water [4].). *Enterobacter* spp. which has been implicated in septicemia, urinary tract infections, cerebral abscess and intestinal infections, has been known to mainly inhabit the intestinal guts of animals and can be shed in feces and waste water when these animals are slaughtered. The effluent generated during slaughtering and processing of animals often contain a mixture of organic waste (blood, paunch manure, urine and feces), chemicals and microorganisms, some of which may exhibit resistance to antibiotics.

The emergence and spread of antibiotics resistance bacteria represent a critical global challenge. While much attention has been given to antibiotics resistant within clinical settings, the role of environmental reservoirs such as water bodies in amplifying and disseminating resistant genes is increasingly recognized Aminov, 2010; Baquero *et al* [5].

This study aims to examine the bacteriological quality of river receiving abattoir effluent, determine the antibiogram of the recovered species, and identify resistant bacterial species using 16s rRNA gene sequencing. This will help to provide vivid information and treatment options to these pathogenic bacteria. This research is motivated by the need to evaluate the environmental implications of improper waste disposal practices and to provide insights that can inform policy and management strategies to mitigate the spread of antibiotics resistance (Nafaranda, *et al,* 2011)

Antibiotic resistance is becoming an emergent threat to human, animal health and environmental safety, and it requires immediate response. Antimicrobial resistance in livestock-associated bacteria has increased as a result of inappropriate and frequent use of antimicrobials or therapeutics, as well as growth promoters, in animal husbandry [6]. These animals may be unable to metabolize the antibiotics, which they then pass out through feces or release into the environment during the slaughtering process [7]. Furthermore, antimicrobial drugs used in animal husbandry can result in the development of cross-resistance to antimicrobials often utilized in human medicine (Marshall and Levy, 2011). This might result in antibiotic treatment failure, higher cost of treatment due to trial treatments and consequently death (Santajit and Indrawattana, [8] Resistance, 2017). Livestock harboring MAR bacteria can disseminate to humans by direct contact and cross contamination of food products (De Krekar *et al*., 2016; Ye *et al.*, 2018). Process water that accumulates at various stages of the slaughtering process (e.g. scalding and eviscerating water) contains numerous multi-drug resistant (MDR) bacteria, could be one possible cross-contamination route [9].

Species of the *Enterobacteriaceae* family have been reported to harbor several antibiotic resistance genes [10]. The wastewater from abattoir, are reservoir for spread of antimicrobial-resistant bacteria with clinical importance into the environment [9]. These bacteria can thrive in aquatic ecosystems, potentially leading to the spread of resistance genes through horizontal gene transfer mechanisms [11]. Antibiotic resistant organisms are responsible for thousands of death annually and this is projected to increase dramatically as a global health hazard [12].

Although the slaughtering of cows in Agbor abattoir are done on well built slabs situated away from the river, the waste are thereafter released into the river without any form of treatment . (Rousham *et al.*, [13]. Residents in Agbor, Delta State, use the river water for domestic purposes, and irrigation by small scale farmers. Most of the butchers wash slaughtered meat directly in the river and also use the water to wash themselves after the day's activity. Since these animals are a potential source of antibiotic-resistant bacteria, using untreated water from such contaminated source increases the risk of public health threats (Stanje *et al*., 2016).

So far, insufficient studies have been carried on the impact of abattoir effluent on this important source of water to the Agbor, Delta State. Thus, it is important to examine the bacteriological quality of the river water and the antibiotic susceptibility profile of the recovered microbial species. This is to be able to proffer solution to the increasing cases of resistant intestinal gastroenteritis in Agbor, Delta State.

2. MATERIALS AND METHODS

2.1 Sample Collection

Surface water samples were collected from receiving river water, following the method of Gallardo *et al*. [14]. Sterile pyrex glass bottles were rinsed and used to collect the water samples from four (4) different sites (Point A, B, C and D) along the river's flow. Point A is the discharge point where the abattoir is situated, about 50m from Point B. Point B is 50m from C. Point C is midstream which is 50m away from point D. Point D is the downstream, about 100m from point A. The water samples from points A, B, C and D served as test samples while, samples collected upstream (where there is less or no human activities) served as control. Water samples were collected at the early hours of the morning (7am-8am) which coincided with the period of animal slaughtering and utilization of the river by butchers.

Sampling was done during the wet or rainy season (June to July) using a wide mouthed 500 ml sterilized Pyrex glass bottles with tight screw dust proof stoppers. Bottles were filled, leaving a head space of 2cm, and subsequently covered, labeled, stored on ice, and transported immediately to the laboratory for analysis.

2.2 Isolation and Enumeration of Bacteria

Isolation and enumeration of bacteria from the water samples were done according to the method of Ajuzieogu *et al*. [15]. From each sample, one (1) ml was homogenized in 9 ml of 0.85% normal saline using Heindolph vortexing machine. Serial dilutions (10-fold) of the samples were prepared and dilutions (10^{-3}- 10^{-5}) of samples were plated out on freshly prepared Nutrient Agar (NA), Salmonella-Shigella Agar (SSA) and Eosin Methylene Blue (EMB) Agar using the spread plate method, and plates incubated at 37°C for 24 h. Colonies were observed, and the number of colonies on each agar plate were counted and recorded. Distinct

colonies were purified by subculture on NA plates and later transferred into agar slants for preservation at 4°C, for further analysis. Total coliform counts were enumerated on Lactose broth using the multiple-tube most probable number (MPN) fermentation technique. The presumptive, confirmed and completed tests were carried out as described in the Standard Methods for the Examination of Water and Wastewater, American Public Health Association (Eaton *et al*., 2005). The numbers of positive findings were enumerated and statistical tables (MPN tables) were used to determine bacteria counts.

2.3 Antibiotic Susceptibility Methods

Antibiotic sensitivity of the isolates was done using the Kirby Bauer disc diffusion technique described by Ajuzieogu *et al*. [16] and interpreted by adopting the breakpoints of Clinical and Laboratory Standard Institute (CLSI document M100-S24, 2014 and CLSI guideline M45, 2015). Mueller-Hinton agar (MHA) was prepared according to the manufacturer's instructions. The medium was cooled to 45–50°C and poured into plates. Plates were allowed to set on a level surface to a depth of approximately 4 mm. When the agar had gelled, plates were allowed to dry before use. An 18–24 h-old broth culture of the isolates were standardized by diluting to 0.5 Mcfarland's standard. A sterile cotton swab stick was inserted into the standardized inoculums (1×10^8 CFU/ml) each, drained to remove excess inoculum load and inoculated by spreading on the surface of prepared Mueller Hinton Agar (MHA) plates. The inoculated MHA plates were subsequently allowed to dry for a few minutes at room temperature with the lid closed; thereafter the antibiotic impregnated discs of known concentrations were aseptically placed on the inoculated MHA plates, 15 mm away from the edge of the plates with the aid of sterile forceps. The plates were then incubated at 37°C for 18–24 h. After which, the diameters of the zones of inhibition were measured with a metre rule and recorded in millimetres. In this study, Maxi disc antibiotic sensitivity disc by Rapid Labs™ was used. Antibiotic impregnated discs of known concentrations included; Gram-negative: Trimethoprim/sulfamethoxazole (SXT) (30 µg), Chloramphenicol (CHL) (30 µg), Ciprofloxacin (CPX) (30 µg), Ampicillin (AMP) (10 µg), Gentamycin (GEN) (30 µg), Ofloxacin (OFX) (10 µg), Streptomycin (STP) (30 µg), Nalidixic Acid (NAX) (30 µg). Gram-positive: Norfloxacin (NOR) (10 µg), Gentamycin (GEN) (10 µg), Ampicillin (AMP) (10 µg), Ampicillin (AMP) (10 µg), Rifampin (RIF) (5 µg), Ciprofloxacin (CPX) (10 µg), Streptomycin (STP) (30 µg), Trimethoprim/sulfamethoxazole (SXT) (30 µg), Erythromycin (E) (10 µg). Characterization of the resistance (R), intermediate (I) or sensitive (S) profile of the isolates was determined by measuring the diameters of the zones of inhibition, then compared with the interpretative chart to determine the resistant, intermediate or sensitive nature of the isolates to the antibiotics used, with the aid of the interpretative chart by CLSI document M100-S24, 2014.

2.4 Molecular Studies

2.4.1 Extraction of genomic (Chromosomal) DNA

The bacteria were grown in Luria Bertani (LB) broth medium for 24 hours at 37°C and the genomic DNA of the bacteria was extracted using "Illustra bacteria genomic Prep Mini Spin Kit (GE Healthcare, USA) according to the manufacturer's instructions. The quality of the extracted DNA was checked by gel electrophoresis on 1% agarose gel stained with ethidium bromide and viewed under a UV trans illuminator [17].

2.4.2 Amplification of 16S rRNA gene sequence

The 16S rRNA gene fragment of the extracted DNA was amplified using an automated PCR thermocycler. The isolated genomic DNA was subjected to 16S rRNA gene amplification using universal bacterial primers (27F: 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R: 5′-GGTTACCTTGTTACGACTT-3′). These primers were synthesized at Inqaba Biotech Ltd (Pretoria, South Africa). The amplification reaction was carried out by preparing a 25µl reaction mix consisting of: 12.5 µl of 2X ready master mix(containing the dNTPs, Taq DNA polymerase, $MgCl_2$, and the reaction buffer); 2 µl of template genomic DNA; 1 µl each of forward and reverse primers (5µm), and 8.5 µl of nuclease free water. The PCR thermal cycling programme used was as follows: initial denaturation at95°C for 5min; 30cycles of denaturation, annealing and extension at 94°C, 52°C and 72°C for 30s,30s and 1min 25s respectively, followed by a final extension at 72°C for 10min and kept at a hold temperature of 4°C [17]. The size of the amplified fragments was verified by electrophoresis of the products on 2% Agarose gel stained with

ethidium bromide and viewed under a UV transilluminator.

2.4.3 Sequence determination of PCR amplified products

The 16S rRNA gene sequences of the PCR amplified products was determined with a Dye terminator sequencing kit and the product were analyzed with an ABI Prism DNA sequencer. The sequencing service was provided by Inqaba Biotech Ltd (Pretoria, South Africa).

2.5 Data Analysis

Data obtained were statistically analyzed using a One-Way Analysis of variance (ANOVA). to compare significant differences between the mean values of total heterotrophic counts and total coliform counts, at $p \leq 0.05$. The statistical package used is Statistical Package for the Social Sciences (SPSS) version 25.

3. RESULTS

3.1 Bacterial Counts for Water Samples from Different Sampled Points

Bacterial counts recorded for water samples from different sampled points are presented in Table 1. Point A recorded the highest mean total heterotrophic bacteria counts (7.7×10^5 cfu/ml) and mean total coliform counts (2.6×10^5 cfu/ml), while Point D recorded the least mean total heterotrophic bacteria counts (3.7×10^3 cfu/ml)and mean total coliform counts (1.9×10^2 cfu/ml) downstream. Upstream samples which served as control recorded appreciable population of total heterotrophic bacteria counts (1.2×10^4 cfu/ml), but low total coliform counts (0.18×10^2 cfu/ml).

3.2 Morphological and Biochemical Characteristics of Bacterial Isolates

The morphological characteristics, Gram reaction and biochemical characteristics of the bacterial isolates are displayed in Table 2. Majority of the bacterial isolates were Gram negative rods, except *Micrococcus* species that was Gram positive cocci. The tentative identities of the isolates were; *Shigella* spp., *Enterobacter* spp., *Proteus* spp., *Morganella* spp. and others.

3.3 Distribution of Bacteria Across Sample Points

The distribution of the bacterial isolates across the sampled points are presented in Table 3. The control (upstream) had the least distribution of bacterial isolates (*Klebsiella* spp., *Salmonella* spp., *Acinetobacter* spp.), while point C and A had the highest distribution of bacterial isolates (*Klebsiella* spp., *Salmonella* spp., *Acinetobacter* spp., *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp., *Morganella* spp., *Escherichia coli, Proteus* spp.).

3.4 Antibiotic Susceptibility Profile of Bacterial Species

The antibiotic susceptibility profile of the bacterial species is shown in Table 4. The bacterial species displayed multi-antibiotic resistance to some of the antibiotics they were exposed to, while other bacteria were susceptible to the antibiotics used. Five out of the 11 bacterial isolates were resistant to at least four antibiotics. The antibiogram profile of bacterial isolates are as shown in Fig 1. The vertical axis is the y axis which shows the percentage susceptibility, resistance and intermediate values while, the horizontal axis is the x axis which indicates the antibiotics used for this study. Out of all the antibiotics used 90% of isolates were susceptible to erythromycin, 5% were resistant, while 85% were susceptible to ofloxacin with 5% resistance and 10% were intermediate. Ampicillin had highest resistance of 40%, as presented in Fig. 1.

3.5 Molecular Identities of Bacterial Isolates

The molecular characterization and identification of the bacterial isolates revealed their identities to be *Shigella flexneri* strain ISEM01, *Proteus vulgaris* strain ISEM03, and *Morganella morganii* strain ISEM04, as displayed in Table 5. The agarose gel electrophorectic amplifcation of the16S rRNA genes of the bacteria isolates is displayed in Plate 1.

4. DISCUSSION

This study examined the bacteriological quality of river receiving abattoir effluent, determined the antibiogram of the recovered bacterial species, and identified resistant bacterial species using 16s rRNA gene sequencing.

Point A recorded the highest mean total heterotrophic bacteria counts (7.7×10^5 cfu/ml) and mean total coliform counts (2.6×10^5 cfu/ml), while Point D recorded the least mean total

heterotrophic bacteria counts (3.7 x 10^3 cfu/ml)and mean total coliform counts (1.9 x 10^2 cfu/ml) downstream (Table 1). This confirms the input and bacterial contamination of the receiving river from abattoir effluent discharges. Akpan *et al*. [18] reported similar findings in their study, although, they reported higher total coliform counts from their downstream point, than that reported in this study. Total heterotrophic counts and Total coliform counts from midstream (at the point of abattoir effluent release into river) samples were 13.6 x 10^6 cfu/ml and 24.0 x 10^6 cfu/ml, respectively, while downstream (75m after the point of effluent in flow) values of same were 5.2 x 10^6 cfu/ml and 15.0 x 10^6 cfu/ml, respectively, Akpan *et al*. [18]. Upstream samples in this study which served as control recorded appreciable population of total heterotrophic bacteria counts (1.2 x 10^4 cfu/ml), but low total coliform counts (0.18 x 10^2 cfu/ml). This observation could be attributed to absence of abattoir discharges at that point (Upstream), compared to the other sampled points in the river. The low bacterial counts observed upstream implied that the abattoir effluent is a major reason for increased bacterial load observed at the other sampled points (A to D), and is the major contributor of pathogenic bacteria to the Orogodo River. Akpan *et al*. [18] also reported relatively decreased Total heterotrophic counts (0.5 x 10^6 cfu/ml) and Total coliform counts (0.11 x 10^6 cfu/ml) from the upstream point in their study. From the results, it was observed the bacterial counts decreased as the distance increased further away from the discharge point (Point A), downstream. This could be as a result of several factors; dilution, diffusion, adsorption and adhesion of the organic matter in the abattoir effluent, on aquatic plant surfaces and debris, as the effluent flowed along the river from point A to D. Statistical analysis revealed there was no significant difference ($P \leq 0.05$) between mean total heterotrophic counts at Point B and C,100m away from Point A. However, there was significant difference between mean total heterotrophic and total coliform counts at Point A (discharge point) and other sampled points (Table 1). This is in contrast to the reports of Akpan *et al*. [18] and Joseph *et al*. [19] who reported there was no significant difference ($P \leq 0.05$) between the mean bacterial counts and total faecal coliform count of abattoir wastewater discharged into the receiving water body at the beginning and the receiving water bodies at 75m and 350m respectively, downstream. A river normally describes a natural flow of water running in a channel. However, there are especially in arid regions, rivers that only flow during the wet season and totally dry in the dry season. The Orogodo river is a short shallow municipal river located in Delta State, that flows through Agbor, and ends up in River Ethiope, Southern Nigeria [20]. It is a flowing river, also sampling was during the rainy season, hence, the reason for decreased bacterial load at Point B, C and D, and the significant difference in bacterial population between Point A and Points 100 m and 150 m away from the point of discharge (Point A). The mean total coliform counts at Point A exceeded the FEPA and EPA's effluent limit for discharge of industrial effluent into surface waters in Nigeria (400MPN/100 ml). These are indicative of the contributory role of the abattoir effluents in the contamination of the river with potential bacterial pathogens Nafarnda et al., [21] Olaiya et al., [22] Akpan et al., [18].

Bacteria isolated and identified in this study included; *Salmonella* spp*., Morganella morganii, Escherichia coli, Pseudomonas* spp*., Citrobacter* spp*., Enterobacter* spp*., Klebsiella* spp*., Proteus* spp*., Bacillus* spp*., Shigella* spp.,and *Acinetobacter* spp. Joseph *et al*. [19] isolated and identified similar species; *Escherichia coli,Enterobacter* spp*., Salmonella* spp, *Shigella* spp., and *Klebsiella* spp. Findings from Kwadzah and Iorhemen (2015), Ojekunle and Lateef [23], Shukri *et al*. [24] and Njoku *et al*. [25] also underpin these findings.

Table 1. Total heterotrophic bacteria and total coliform counts enumerated from water samples

Sampled sites	Mean heterotrophic count (cfu/ml)	Standard deviation (SD)	Mean coliform Count (CFU/ml)	Standard deviation (SD)	P-value
Upstream	$1.2x10^4$	$1.20±0.14^{AB}$	$0.18x10^2$	$0.02± 0.03^{A}$	0.05
A	$7.7x10^5$	$77.00±0.42^{J}$	$2.6x10^5$	$5.60±0.42^{I}$	0.05
B	$2.0x10^5$	$20.00±0.64^{H}$	$3.4x10^2$	$3.40±0.42^{H}$	0.05
C	$1.9x10^4$	$19.00±1.13^{H}$	$1.6x10^3$	$1.60±0.28^{FG}$	0.05
D	$3.7x10^3$	$0.37±0.03^{A}$	$1.9x10^2$	$1.90±0.14^{E}$	0.05

Columns that appear with the same superscript are not significantly different

Table 2. Morphological and biochemical characterization of bacterial isolates

	Morphological			Biochemical								
Isolates	**Cell shape**	**Cell arrangement**	**Gram stain**	**Mo**	**Ca**	**Ox**	**Co**	**In**	**Ur**	**Ci**	**Glu**	**La**	**Possible isolate**
1	cocci	Single	+	+	-	-	-	-	+	+	+	-	*Micrococcus* spp.
2	rod	Chain	-	-	+	-	-	-	-	-	+	-	*Shigella* spp.
3	rod	Single	-	-	+	-	-	-	-	+	+	-	*Enterobacter* spp.
4	rod	Chain	-	+	+	-	-	+	+	+	+	-	*Proteus* spp.
5	rod	Chain	-	+	+	-	-	+	+	-	-	+	*Morganella* spp.
6	rod	Single	-	+	+	+	-	-	+	-	+	-	*Pseudomonas* spp.
7	rod	Single	-	-	+	-	-	-	+	-	+	+	*Citrobacter* spp.
8	rod	Single	-	+	+	-	-	+	-	-	+	+	*Escherichia coli*
9	rod	Single	-	+	+	+	-	-	-	+	+	-	*Salmonella* spp.
10	rod	Single	-	-	+	-	-	-	+	+	+	+	*Klebsiella* spp.
11	rod	Chain	+	-	+	-	-	-	+	-	+	-	*Bacillus* spp.

Mo-Motility; Ca- Catalase; Co- Coagulase; Ox- Oxidase; In- Indole; Ur-Urease; Ci- Citrate; Glu- Glucose; La- Lactose; +- positive and – is negative

Table 3. Distribution of bacteria across sample points bacterial isolates sample sites

	Upstream (Control)	**Point A**	**Point B**	**Point C**	**Point D**
Bacillus spp.	-	+	-	+	-
Pseudomonas spp.	-	-	+	+	-
Klebsiella spp.	+	-	+	+	-
Enterobacter spp.	-	+	+	+	+
Salmonella spp.	+	+	+	+	+
Morganella spp.	-	+	+	+	-
Shigella spp.	-	+	-	+	+
Escherichia coli	-	+	+	+	-
Proteus spp.	-	+	+	-	+
Citrobacter spp.	-	+	-	-	+
Acinetobacter spp.	+	-	-	+	-

A= Abattoir effluent sample from butchering section, B=Point of entry of abattoir effluent and municipal drains sample, C = Mid-stream sample, D =Downstream sample, + = Detected, —= Not detected

Table 4. Antimicrobial susceptibility profile of bacterial isolates

Isolates	OFX (mm)	CPX (mm)	GEN (mm)	STP (mm)	ERY (mm)	NA (mm)	SXT (mm)	AMP (mm)	NOR (mm)	CHL (mm)	RIF (mm)
Shigella spp.	22(S)	7(R)	17(S)	15(S)	11(R)	5(R)	20(S)	17(S)	6(R)	12(R)	6(R)
Enterobacter spp.	17(S)	17(S)	19(S)	14(I)	3(R)	9(R)	5(R)	7(R)	2(R)	8(R)	8(R)
Bacillus spp.	11(R)	16(I)	9(R)	19(S)	15(R)	12(R)	18(S)	6(R)	16(I)	17(I)	15(S)
Klebsiella spp.	17(S)	22(S)	21(S)	16(S)	17(I)	11(R)	19(S)	17(R)	16(I)	11(R)	22(R)
Proteus spp.	17(S)	5(R)	16(S)	14(I)	19(I)	9(R)	17(S)	7(R)	14(I)	4(R)	13(I)
Morganella spp.	16(S)	3(R)	7(R)	9(R)	15(R)	15(I)	12(I)	4(R)	13(I)	18(S)	20(S)
Citrobacter spp.	21(S)	18(I)	16(S)	15(S)	21(I)	15(I)	18(S)	16(I)	15(I)	19(S)	18(S)
Acinetobacter spp	18(S)	18(I)	13(I)	12(I)	22(I)	11(R)	18(S)	6(R)	13(I)	18(S)	23(S)
Escherichia coli	22(S)	20(I)	5(R)	17(S)	15(R)	16(I)	21(S)	6(R)	9(R)	17(I)	5(R)
Salmonella spp.	15(I)	17(S)	19(S)	13(I)	16(I)	15(I)	3(R)	15(I)	16(I)	18(S)	12(I)
Pseudomonas spp.	19(S)	3(R)	6(R)	15(S)	17(I)	3(R)	11(I)	12(R)	6(R)	16(I)	11(I)

Zone of inhibitions in (mm). AMP- Ampicillin, SXT-Trimethoprim/sulfamethoxazole, OFX-Ofloxacin, CPX- ciprofloxacin, NOR-norfloxacin, GEN-Gentamicin, ERY-Erythromycin, CHL-Chloramphenicol, NAX-Nalidixic Acid, STP-Streptomycin

Table 5. Molecular identification based on 16S rRNA sequencing data

Isolate Code	Identified Bacteria	% Similarity	Assigned GenBank Accession Number
A	*Shigella flexneri* strain ISEM01	98%	MW290510
G	*Proteus vulgaris* strain ISEM03	98%	MW290512
K	*Morganella morganii* strain ISEM04	98%	MW290513
B	*Enterobacter hormaechei* strain ISEM02	98%	MW290511
V	*Pseudomonas oryzae* strain ISEM05	98%	MW290514

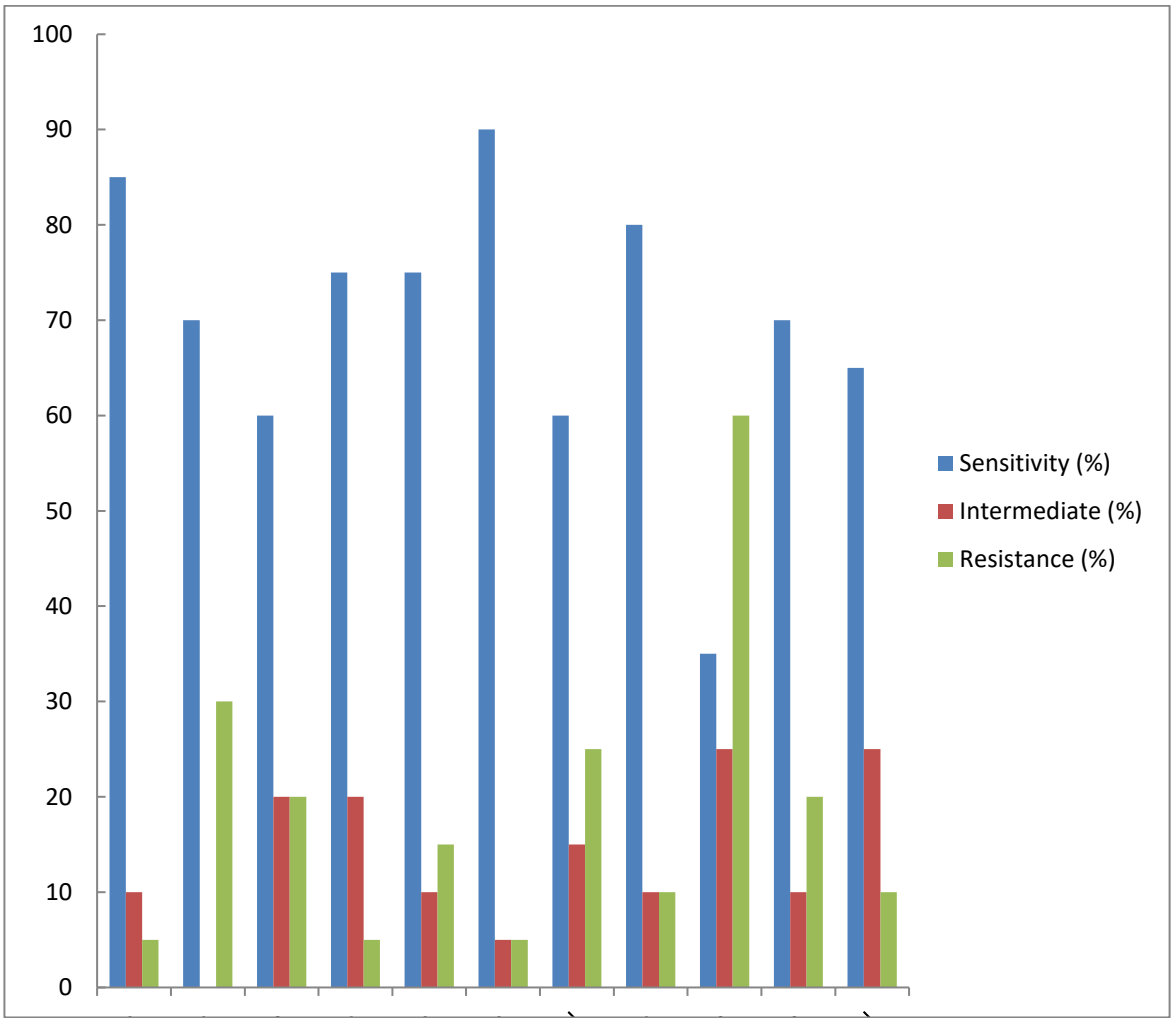

Fig. 1. Percentage antibiogram profile of bacterial isolates

The control (upstream) had the least distribution of bacterial isolates (*Klebsiella* spp., *Salmonella* spp., *Acinetobacter* spp.), while point C and A had the highest distribution of bacterial isolates (*Klebsiella* spp., *Salmonella* spp., *Acinetobacter* spp., *Bacillus* spp., *Pseudomonas* spp., *Salmonella* spp., *Morganella* spp., *Escherichia coli, Proteus* spp.). This is due to the direct discharge of untreated abattoir effluent to the Orogodo river. This aligns with the findings of Nafarnda *et al*. [21], Adebowale *et al*. [3], Akpan *et al*. [18] and Joseph *et al*. (2021), who recovered similar bacterial species from midstream points (point of abattoir effluent discharge into recipient river).

The bacterial species displayed multi-antibiotic resistance to some of the antibiotics they were exposed to, while other bacteria were susceptible to the antibiotics used. Five out of the 11 bacterial isolates were resistant to at least four antibiotics. Out of all the antibiotics used 90% of isolates were susceptible to erythromycin, 5% were resistant, while 85% were susceptible to Ofloxacin with 5% resistance and 10% were intermediate. Ampicillin had highest resistant of 40%, as presented in Fig. 1.

Shigella spp. was resistant to Ciprofloxacin, Rimfapin, Nalidixic acid, Chloramphenicol, and Norfloxacin (Alizadeh-Hesar *et al*., 2015). *Morganella* spp. was resistant to Ciprofloxacin, Gentamycin, Streptomycin and Ampicillin. *Proteus* spp. resistant to Ciprofloxacin, Nalidixic acid, Ampicillin and Chloramphenicol [27] *Enterobacter* spp. was resistant to Erythromycin, Nalidixic acid, Sulfamethoxazole, Ampicilin, Norfloxacin and Chloramphenicol. *Pseudomonas* spp. was resistant Ciprofloxacin, Gentamycin, Nalidixic acid and Norfloxacin. Antibiotics susceptibility profile revealed that abattoir effluent harbor antibiotic resistant strains which is

introduced into the river water upon disposal. This thus creates a potential gene pool from which antibiotic resistance may be transferred, resulting in the emergence and spread of antibiotic resistance bacteria. This can also spread across to other aquatic animals which could accumulate them in their tissues and then transfer to humans on consumption. Jega *et al*. [28]reported *Enterobacter* sp., *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Citrobacter* sp. recovered in their study, expressed multiple antibiotic (MAR) against at least Septrin (sulfamethoxazole),Chloramphenicol, Amoxicillin, Augmentin, Gentamycin, Tarivid (ofloxacin) and Streptomycin. These findings were also underpinned by the report of Akpan *et al*. [18], that multiple antibiotic resistance was expressed by 77% of Gram negative bacteria recovered in their study [29,30].

Among the 11 bacteria isolated in the study 5 isolates were resistant to at least four categories of antibiotics. Multiple antibiotic resistant bacteria were further identified using molecular characterization. The molecular phylogenetic identification supported by physiological properties assigned MW290510, MW290511, MW290512, MW290513 and MW290514 as a close relative of *Shigella flexneri* strain ISEM01, *Enterobacter hormaechei* strain ISEM02, *Proteus vulgaris* strain ISEM03, *Morganella morganii* strain ISEM04 and *Pseudomonas oryzae* strain ISEM05 respectively (Table 5). All strains were observed to be unique to abattoir wastewater [31].

Enterobacter homorchae strain ISME01 is a Gram-negative, rod shaped, facultative anaerobe, non-spore forming bacteria belonging to the family *Enterobacteriaceae*. They are associated with bacteriemia, septic arthritis, endocarditis, urinary tract infection, sepsis, intra-abdominal infection and soft tissue infection, and infections in neonates. The risk factors in neonates include premature birth and low birth weight (Kanishka *et al*., 2020).

Morganella morganii strain ISME04 is a specie belonging to the family *Enterobacteriaceae.* It is normally found in the environment and intestinal tracts of mammals as part of normal flora. *Morganella morganii* is an opportunistic pathogen which causes sepsis, abscess, bacteremia and urinary tract infection (UTI).

Shigella flexneri strain ISME01 is a Gram-negative, rod shaped, entero-invasive bacteria. It invades the colonic and rectal epithelium of humans causing acute mucosal inflammation characteristic of shigellosis, which is responsible for significant mortality and morbidity in young children and immunocompromised adults. It is the most endemic form of shigellosis which causes shigellosis related deaths. It is possible to transmit *Shigella* from meat washing into the river. This process can pick up and spread antibiotic resistant *Shigella* to cause disease outbreaks among humans [29].

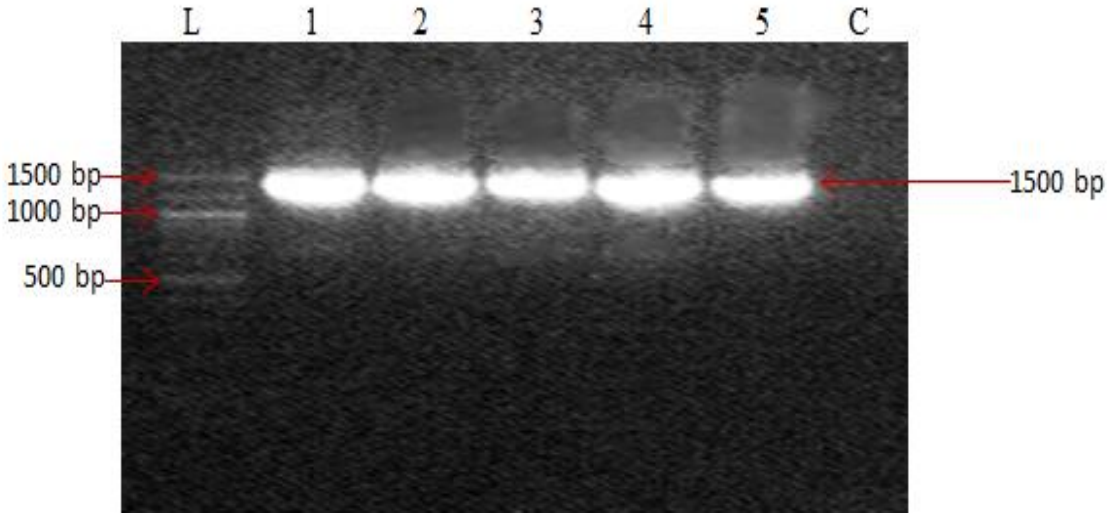

Plate 1. 16s rRNA genes amplified from genomic DNA of the bacterial isolates

Key:Lane L = ladder; Lane 1 = Isolate A;Lane 2 = Isolate B;Lane 3 = Isolate G;Lane 4 = Isolate K;Lane 5 = Isolate V;Lane C = Control

Proteus vulgaris is a nosocomial pathogen which produces infections in human. It causes urinary tract infections and produce bacteriaemia, pneumonia and focal lesions in debilitated patients or those receiving intravenous infusion. *Pseudomonas* spp. Recovered from abattoir effluents have been implicated for expressing multi-antibiotic resistance [30]. However,*Pseudomonas oryzae* has not been reported before now in abattoir effluents, nor its recipient river water, and for the expression of multi-antibiotic resistance. Findings from this study has thus established the presence of multi-antibiotic resistant *Pseudomonas oryzae* in river receiving abattoir effluent [32].

5. CONCLUSION

The Orogodo river water in Agbor community, Delta State, is known to be used for different purposes such as fishing, bathing, irrigation, domestic use, drinking, and once upon a time, for recreational purpose. This study established the fact that abattoir effluent is the major pollutant of Orogodo River owing to findings from microbiological and antibiotic susceptibility tests conducted. The recipient river surpassed the WHO standard for drinking water and permissible limit for the discharge of industrial effluent. Also, the bacterial pathogens introduced from the abattoir effluent into the recipient river possessed multi-antibiotic resistance, thereby creating a pool for possible transfer of antibiotic resistance genes to indigenous microbial population, butchers and other abattoir operators who use the water for bathing and to consumers of vegetables planted by the river bank, irrigated with water from the river. However, the antibiotic susceptibility profile of bacterial pathogens in this study will assist in providing treatment options for patients who are infected with these bacteria.

Emergence of new antibiotics resistance in pathogenic bacteria is becoming a major threat to health facilities and to treatment of common infections. They are the leading cause of life threatening infections in clinical facilities culminating into prolonged treatment period, failure in treating mild infections and in extreme cases, death. This increases healthcare cost and economic burden on families (WHO, 2021). Therefore, it is recommended that Orogodo River water should be treated before use, abattoir workers should be sensitized on the impact of antibiotic resistance and the need to treat abattoir effluent before discharge into recipient river.

6. LIMITATION

We acknowleged limitation in the seasonal nature of sampling, and therefore, suggest further research on comparison of seasonal variations and its impacts on microbial load.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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