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Analysis of bacterial composition in slaughterhouse effluent from a major livestock market in Nigeria

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ABSTRACT

Effluent discharges are point sources of pollution in aquatic ecosystems. Effluents from slaughterhouses which are often discharged untreated into the receiving ecosystem with potential adverse impacts on the ecosystem. The objective of this study was to evaluate the bacterial community profile of slaughterhouse effluent from a major livestock market in Ogun state, South-West Nigeria. The community DNA was extracted and subsequently sequenced using the illumina platform. The top (5) bacterial phyla accounting for over 94.6% of the sequences in the effluent was dominated by Firmicutes (67%) and the least was Euryarchaeota (3.2%). The top five (5) classes were Clostridia (62.11%), Bacteroidia (15.93%), Bacilli (3.97%), Actinobacteria (3.05%) and Methanobacteria (2.95%). The most abundant orders were Clostridiales (62.10%) Bacteroidales (15.90%), Lactobacillales (3.00%), Actinomyctes (2.70%) and Burkholderiales (1.50%). 52 genera were identified (29.60%) while unclassified genera were 65.90%. The results reveal the bacterial community profile of the effluent constituting genera of pathogenic, biotechnological, environmental, veterinary, and public health importance such as *Butyrivibrio, Clostridium, Staphylococcus, Streptococcus, Prevotella, Desulfovibrio, Rhodobacter*, among others. The results are of importance for holistic ecological and human health risk assessments as well as targeted interventions and proper treatment of the effluent before discharge. This will support good health and wellbeing, promote clean water and sanitation, as well as sustain life below water; relevant to the United Nations Sustainable Development Goals 3, 6 and 14 respectively.

Keywords: Abattoir Wastewater; Illumina Platform; Pathogen; Organic Pollution; Aquatic ecosystem

1. Introduction

Environmental pollution caused by direct and indirect human activities are of great concern because of its adverse impact on the ecosystem including the microbial community [1]. Water pollution has become a consistent problem in the society due to poor wastewater management from substantial industrialization, escalating population density and highly urbanized societies [2]. The influx of untreated industrial wastes into water bodies pose more environmental hazards because of the toxic constituents of the waste [3]. These toxic constituents may be bioaccumulated in organisms at various trophic levels and biomagnified through the food chain or web leading to possible deleterious human health risk [4]. Furthermore, anthropogenic activities often render the polluted water difficult to treat and hence domestically useless [5].

Slaughterhouses are integral components of livestock production providing edible meat supply for the teeming populace. In developing countries such as Nigeria, slaughterhouses are poorly developed, often without efficient wastewater treatment facilities encouraging a discharge of effluents into the receiving water body [6, 7]. An example is the Kara Cattle Market, which is situated beside the Ogun River waterway, an important river in the South-West of Nigeria. The river rises in Oyo state and discharges into the Ikorodu axis of the Lagos Lagoon [8]. The impact of activities at the Kara Cattle Market does not only pollute the receiving waterbody, but such activitiesit may also result into environmental changes that create imbalance in the ecosystem, alters the physicochemical properties of the water [9] and possibly lead to loss of microbial community diversity structure also known as biotic homogenization [8].

Biotic homogenization is the process of an increase in the genetic, taxonomic, or functional corollation of biotic groups over time because of anthropogenic modification [10]. Since only a certain proportion of microbial community are active at any point in time while others are latent, understanding the mode of action of biotic homogenization is difficult [11]. A microbial community is made up of both active and dormant members which show different biogeographic patterns with varying responses to environmental factors in the aquatic environment [12]. The dormant communities which represent the area taxa are usually susceptible to the environmental change and ecological drift with a weaker response [13]. Imbalance in the ecosystem would generate unfavorable environmental conditions for active microbes of which adaptation may be difficult to achieve. Consequently, this would have adverse impact on the beneficial roles of the microbial communities such as reversed eutrophication, aeration of the environment, prevention of turbidity to promote light for photosynthesis, among others [14]. The proliferation of pathogenic bacteria, increase in antibiotic resistant species and exchange of resistance genes via horizontal gene transfer have been previously reported as some of the direct effects of slaughterhouse activities on water bodies [15, 8, 6].

Spatial and temporary distribution of bacterial community structure and biodiversity have been well studied using conventional molecular techniques such as 16S rRNA gene ratios, polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE), and another cloning method [16]. These methods may have been used to establish extensive information about the microbial community but are still limited in throughput due to their inability to estimate the overall broad diversity and identification of rare species in complicated environmental systems and can be influenced by extracellular environmental DNA. Compared to previous conventional methods, the advantage of using high-throughput sequencing methods is the capacity to generate thousands of Operational Taxonomic Units (OTU) and multi-million sequences in environmental samples, majority of which are without any laboratory protocols for isolation and identification [17].

There is paucity of data on the community composition of Kara Cattle Market slaughterhouse effluent using high-throughput sequencing methods for this current study site, hence the need for this study. We assessed the bacterial community of the Ogun River receiving end of the Kara Cattle Market effluents using a high-throughput sequencing method (Illumina MiSeq) with the aim to assess the bacterial community and its direct impact on human and/or animal health. The results are expected to feed into measures to ensure good health and wellbeing (United Nations Sustainable Development (UN SDG) 3), clean water and sanitation (UN SDG 6) and sustaining life below water (UN SDG 14).

2. Methods

2.2 Study area

The Kara Cattle Market is situated beside the Ogun River just before the Ojodu-Berger axis, along the Lagos-Ibadan Expressway at coordinates 6.6472 ° N, 3.3829° E in Ogun State, Nigeria. The market is characterized by substantial livestock presence, predominantly cows, sheep, and goats ready for purchase or slaughter in make-shift ranches with traces of old and fresh livestock dung littered all over the surfaces. Non-specific numbers of cattle are slaughtered daily and gutted while the gut contents and the blood are often washed off the site and runs-off via different channels into the Ogun River which is less than 100 m to the slaughterhouse [18, 7].

2.3. Slaughterhouse Effluent Samples Collection

The slaughterhouse effluent samples were collected in January 2016. Five (5) separate samples each were grabbed from the slaughterhouse effluent. Samples were homogenized to generate one composite sample and placed in a sterile 1 L plastic container containing ice packs and conveyed to the Microbiology laboratory at the University of Lagos within 2 h for immediate analysis.

2.4. DNA Extraction of Slaughterhouse Effluent

The total genomic DNA (gDNA) of each sample was extracted employing Presto Soil DNA Extraction kit (Geneaid, Taiwan) in consonant with the producer's instruction.

2.5. Illumina 16S rRNA Gene Sequencing of Slaughterhouse Effluent Samples

The calibre of gDNA was scrutinized on 0.8% agarose gel (loaded 5 µL) for the presence of intact band. The gel was run at 110V for 30 mins. The sample, 1 µL each was filled in Nanodrop 8000 to ascertain A260/280 ratio. The DNA was quantified using Qubit dsDNA HS Assay kit (Life Technologies). One (1) µL of each sample was employed for establishing the concentration using Qubit® 2.0 Fluorometer. The amplicon library was developed using Nextera XT Index Kit (Illumina Inc.) according to the 16S metagenomic sequencing library development protocol. Primers for the amplification of the V3-V4 hyper-variable region V3-F CCTACGGGNBGCASCAG and V4-R GACTACNVGGGTATCTAATCC (~ 460 bps) of 16S rDNA gene of bacteria and archaea were designed and synthesized in-house by Xcelris PrimeX Facility Labs Limited Gujarat, India. The i5 and i7 primers were used to amplify the illumina adaptors, this added multiplexing index sequences and common adapters required for cluster generation (P5 and P7) as stipulated by standard illumina protocol. Purification of amplicon libraries were done by 1X AMpure XP beads, examined on Agilent DNA 1000 chip on Bioanalyzer 2100 and quantified by Qubit Fluorometer 2.0 using Qubit dsDNA HS Assay kit (Life Technologies) [19].

2.6. Cluster Generation and Sequencing

Thereafter, Qubit concentration for the library and the mean peak size from Bio analyzer profile were obtained, then library was loaded onto Illumina platform at suitable concentration (10-20pM) to ensure cluster generation and sequencing. Consequently, Paired-End sequencing permitted the template fragments to be sequenced in both the forward and reverse directions on Illumina platform. The binding of samples to complementary adapter oligos on paired- end flow cell was facilitated by the kit reagents used. The adapters were designed to facilitate selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The opposite end of the fragment was sequenced using the copied reverse strand.

3. Results

A total of 159,982,258 high quality sequence (633 MB mean library size) was obtained by Illumina 2 x 250 bp PE sequencing chemistry to create ~150 MB of data per library. A 97% similarity cutoff was implemented through UCLUST algorithm, these sequences yielded a bacterial operational taxonomic unit (OTU) number that ranged from 151569 to 33130 (Table 1).

3.1. Slaughterhouse Effluent Bacterial Operational Taxonomic Unit (OTU)

Summary shows that a total of 309014 flash/stich reads, 151569 non-chimeric sequences, 33130 OTU, and 8178 OTU with no singletons were recorded in this present study.

A total of 19 phyla (**Table 1**), 63 classes (**Fig. 1**), 122 orders, 234 families, 449 genera, and 523 species were identified in the study. Amongst the major bacterial phyla, Firmicutes was the most dominant with 67.02 % in the sample followed by Bacteroidetes (16.28%), Actinobacteria (4.29%), Proteobacteria (3.81%) and Euryarchaeota (3.23%) (**Table 1**). The top five (5) most abundant phyla accounted for 94.6 % of the total sequences of the effluent sample while the unclassified phyla bacteria have a relative abundance of 5.4% (**Table 1**)

Table 1: Relative abundance of the bacterial phyla in the slaughterhouse effluent

Phylum	Relative Abundance (%)
Firmicutes	67.00
Bacteroidetes	16.30
Actinobacteria	4.30
Proteobacteria	3.80
Euryarchaeota	3.20
Chloroflexi	1.80
Spirochaetes	1.10
Unassigned	0.80
p__TM7	0.50
Tenericutes	0.30
Synergistetes	0.10
Verrucomicrobia	0.10
Planctomycetes	0.10

At the class level, of the top five (5) abundant sequences, Clostridia dominated with 62.11% of the total sample followed by Bacteroidia (15.93%), Bacilli (3.97%), Actinobacteria (3.05%) and Methanobacteria (2.95%) (**Fig. 1)**.

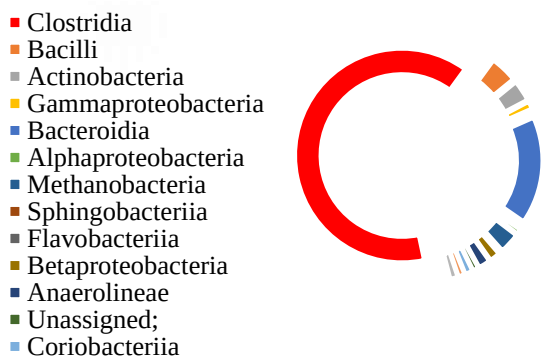

Fig .1. Relative abundance of each class within each microbial community inferring clostridia and Bacteroidia as the most abundant in the slaughterhouse effluent sample

Table 2: Relative Abundance of Bacteria at the order level

Order	Abundance (%)
Clostridiales	62.10
Bacteroidales	15.90
Lactobacillales	3.60
Methanobacteriales	3.00
Actinomycetales	2.70
Burkholderales	1.50
Erysipelotrichales	0.90
Unassigned	0.80
Enterobacteriales	0.70
Rhodobacterales	0.50
Bacillales	0.30
Pseudomonadales	0.30
Methanosarcinales	0.20

At the genus level, a total of 52 genera were identified with a relative abundance of 29.60% while the unclassified genera have a relative abundance of 65.90% (**Tables 1-5**). *Proteiniclasticum* and *Butyrivibrio* dominated the identified genera sequences with a relative abundance of 3.70% each. This was followed by *Streptococcus* (2.90%), *Methanobrevibacter* (2.80%) and *Clostridium* (1.80%). At genus level, the population of enteric bacteria were detected at a very low percentage from 0.10% as well as other pathogenic bacteria and environmental bacteria such as *Staphylococcus*, *Acinetobacter*, and *Enterococcus*.

Table 3: Relative abundance and genus distribution of the bacteria in the slaughterhouse effluent

Taxonomic genus level	Relative abundance ≥ 4.00 – 1.00%
Proteiniclasticum	3.70
Butyrivibrio	3.70
Streptococcus	2.90
Methanobrevibacter	2.80
Clostridium	1.80
Succiniclasticum	1.70
Prevotella	1.60
Ruminococcus	1.60
Corynebacterium	1.30
Treponema	1.00

Table 4: Relative abundance and genus distribution of the bacteria in the slaughterhouse effluent

Taxonomic genus level	Relative abundance ≥1.00 – 0.20%
Peptostreptococcus	0.70
Coprococcus	0.60
Dehalobacterium	0.50
Peptoniphilus	0.30
Bifidobacterium	0.30
Bulleidia	0.30
Rhodobacter	0.30
Desulfovibrio	0.30
Gallicola	0.20
Paludibacter	0.20
Porphyromonas	0.20
Facklamia	0.20
Trichococcus	0.20
Blautia	0.20
Moryella	0.20
Oscillospira	0.20

Table 5: Relative abundance and genus distribution of the bacteria in the slaughterhouse effluent

Taxonomic genus level	Relative abundance ≥0.10%
Staphylococcus	0.10
Micrococcus	0.10
Acinetobacter	0.10
Paracoccus	0.10
Bacillus	0.10
Enterococcus	0.10
Bacteroides	0.10
Chryseobacterium	0.10
Vagococcus	0.10
Anaerovibrio	0.10
Guggenheimella	0.10
Comamonas	0.10
Methanosphaera	0.10
Methanosaeta	0.10
vadinCA11	0.10
Dietzia	0.10
Macrococcus	0.10
Turicibacter	0.10
Pseudobutyrivibrio	0.10
Shuttleworthia	0.10
Schwartzia	0.10
Mogibacterium	0.10
Bosea	0.10
Pyramidobacter	0.10
Anaeroplasma	0.10
Deinococcus	0.10

Most of the species detected fall into the unclassified category, hence, are unable to be identified with their respective genera.

4. Discussion

In this study, a bacterial community comprising of commensals and potential pathogens as well as other categories of bacteria such as the fermenters were detected. Contrary to previous bacterial community studies in Ogun River where the Kara Market slaughterhouse is situated (20, 2, 8, 6), the present methodology has shown its efficiency and reliability for the assessment and quantification of the genetic diversity within ecosystems such as the Ogun River. [17] also reported that Illumina sequencing technology is a prospective and rich way to probe the whole microbial structure alighning with individual taxonomic groups that transpire in the wastewater treatment strategy.

The observed dominant bacteria phylum in this study, Firmicutes is contrary to recent studies on account of the dominant phylum in similar microbial communities. For instance, Kiskova et al. [21] reported the phylum Proteobacteria with the highest abundance (80.39%) followed by Firmicutes (13.05%) and Bacteroidetes (5.64%). In another study by Godoy et al. [22], Proteobacteria was dominant at approximately 53% followed by Firmicutes as the most abundant at 21%. The dominance of these three phyla (Proteobacteria, Bacteroidetes, and firmicutes) in bacteria communities has been frequently reported from some freshwater environments, lake sediments, and microbial fuel cells that produce electricity via the oxidation of organic matter anaerobically. Evidence from various studies indicated that the gut microbiota is dominated by Firmicutes and Bacteroidetes which represent more than 90% of the total community leaving other phyla such as Proteobacteria, Actinobacteria, and Verrucomicrobia as subdominant phyla [16]. Based on the foregoing, the dominance of Firmicutes and

Bacteriodetes in this study may be associated with the evisceration of animal gut microbiota from the Kara market slaughterhouse into the Ogun River through the effluent suggesting its influence on the bacterial community.

The observed presence of bacterial genera such as *Clostridium* and *Staphylococcus* in this study is in consonance with reports from recent microbial evaluations of effluent from the Kara market slaughterhouse [6]. Among the genera identified in this study, *Proteiniclasticum* and *Butyrivibrio* were the most abundant (3.70%). *Proteiniclasticum* is a strict anaerobe while *Butyrivibrio* is a Gram-positive butyrate-forming bacteria commonly found in the gut of ruminant animals where they are involved in the degradation of fiber, protein breakdown, and other essential metabolic activities [23]. *Streptococci* are pathogenic bacteria, commensals of both man and animals, and often present in high densities in human and animal feces [24]. They are known to elicit adverse effects in fish species with implications for extensive fish kills [25]. Their dominance in this study may also suggest the prevalence of Streptococcal infections among most cattle for slaughter at the abattoir. *Streptococcus agalactiae* belonging to group B *Streptococcus* is mostly associated with various livestock infections [26].

Interestingly, the phylum Euryarchaeota is the only representing archaea out of the 5 existing phyla suggesting the dominance of Euryarchaeota among other Archaea in the present study which also agrees with the work of Giwa et al. [17] that identified Archaea in a recent microbial community study. *Methanobrevibacter* (2.80%) dominance in this study is of interest as they are methanogens belonging to the kingdom Euryarchaeota which are commonly isolated from the gastrointestinal ecosystem strictly methane-producing anaerobic archaea. Their dominant presence confirms the high influence of the abattoir wastewater on the bacteria community. Further, *Rhodobacter* and *Desulfovibrio* observed (0.30%) in this study are of environmental and economic importance. For example, *Rhodobacter sphaeroides* is considered a valuable model organism due to its adaptation to heavy metal stress and thus useful as an indicator species and for use in the bioremediation of polluted sites [27].

Among the pathogenic bacteria encountered in the sequence, *Clostridium* and *Treponema* were in abundance of 1.80% and 1% respectively. Some members of *Clostridium* are hazardous pathogens responsible for various foodborne infections and associated toxins such as botulin, a toxin that is of great health concern to both humans and animals [28]. *Treptonema* belonging to the phylum Spirochaete which was in abundance of 1% of the total microbial population in this study is rarely found in slaughterhouse effluent discharging into waterbodies, only a few similar studies reported the presence of this non-native bacteria in their study [29]. Although, these bacteria have pathogenic and non-pathogenic species, the common pathogenic species (*T. denticola* and *T. pallidium*) are not known to cause infection in animals except *T. brennaborense* associated with bovine infection [29]. The presence of bacteria with pathogenic potential among the flora in the effluent is worrisome given the adverse implications of their presence to humans who utilize resources from the effluent impacted river for food production, domestic use, and farming purposes. Pathogenic bacteria of the genus *Staphylococcus, Micrococcus, Acinetobacter, Enterococcus* and *Bacillus* as well as other medically important genus were all found in similar abundance (0.10%) in this study. Further, the relative dominance of *Prevotella* (1.60%) in this study is of tremendous public health interest particularly due to its implication in promoting viral infections such as the coronavirus disease-2019 (COVID-19) [30]. This has been reported to occur through the involvement of *Prevotella* proteins in multiple interactions with the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) which is involve in increasing clinical severity of COVID-19 [30]. This further establishes the impact of the anthropogenic sources on the microbial community in the present study.

5. Conclusion

In conclusion, bacterial abundance based on tracking a single marker gene (for 16S rRNA) of slaughterhouse effluent from the Kara Cattle Market in Ogun state, Nigeria for the first time revealed a bacterial community profile composed of genera of pathogenic, biotechnological, environmental, veterinary, and public health importance. The influence of anthropogenic activities within and around the slaughterhouse was evidenced in the genera of bacteria identified in this study. The important microbial genera include *Clostridium, Staphylococcus, Streptococcus, Prevotella, Desulfovibrio, and Rhodobacter* among

others. The results provide evidence of biotic homogenization because of anthropogenic modification majorly from the slaughterhouse. We recommend holistic ecological and human health risk assessments of the receiving ecosystem as well as targeted and informed management strategies/interventions such as pretreatment of the slaughterhouse wastewater before discharge. This will promote healthiness, clean water, and sanitaion as well as sustenance of life below water (United Nations Sustainable Development Goals 3, 6, and 14 independently).

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7. Authors' contribution

- Olanike Maria Buraimoh (Ph.D.) - Conceptualized the project, designed the experiment, carried out part of laboratory work, and wrote part of the manuscript.
- Odumosu, Bamidele Tolulope. (Ph.D.) - Wrote part of the manuscript and analyzed part of the bioinformatics aspect of the work.
- Sogbanmu, Temitope Olawunmi (Ph.D.) - Wrote part of the manuscript and proof-reading.
- Ojo-Omoniyi Olusola Abayomi (Ph.D.) - Wrote part of the manuscript and proof-reading.

- Afolabi Olumide (MSc.) – Extraction of DNA and bioinformatics
- Akerele, Odunayo Samuel (MSc.) - Collection of samples, laboratory work, and formatting of the manuscript.

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