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Microbiological Investigation of Waste Water Discharge from Temple and Nkwelle Abattoir

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Abstract

The increasing volume of waste generation and inadequate disposal systems in Nigeria, particularly due to anthropogenic activities such as the indiscriminate location of slaughterhouses near residential areas, poses significant environmental and public health risks. This study investigated the microbiological quality of wastewater discharged from two slaughterhouses (Temple and Nkwelle) in Umunya, Oyi Local Government Area, Anambra State. Wastewater samples were collected using sterile containers and immediately transported to the microbiology laboratory for analysis. Bacterial isolation was conducted using Nutrient agar, Salmonella/Shigella agar, and MacConkey agar, while fungal isolation was performed using Potato Dextrose agar. The Total Heterotrophic Bacterial Counts (THBC) for Temple and Nkwelle slaughterhouses were 6.8×10^5 CFU/mL and 5.2×10^5 CFU/mL, respectively, while the Fungal Counts were 1.4×10^4 CFU/mL and 1.2×10^4 CFU/mL, respectively. Bacterial isolates included *Escherichia coli, Bacillus spp., Citrobacter spp., Streptococcus spp., Proteus spp., Enterobacter faecalis, Klebsiella spp., Lactobacillus spp., and Staphylococcus aureus*. Fungal isolates comprised *Penicillium spp., Aspergillus spp., and Mucor spp*. The findings indicate that slaughterhouse wastewater discharges contain high microbial loads of pathogenic bacteria and fungi, posing serious environmental contamination and public health risks, including potential waterborne diseases and ecological disruption. This study underscores the urgent need for sustainable and environmentally friendly waste management practices in slaughterhouse operations. Recommendations include implementing proper wastewater treatment systems, enforcing regulatory measures, and promoting public awareness to mitigate the adverse impacts on the environment and human health.

Keywords: Slaughterhouse wastewater, Microbial contamination, public health, Environmental pollution, Pathogenic microorganisms, Waste management.

Introduction

The slaughterhouse is known to provide domestic meat to the people, as well as generate employment opportunities. Ogbonnaya (2018) reported that the slaughterhouse industries in Nigeria are less developed and therefore do not have adequate facilities for the treatment of slaughterhouse waste waters before disposal. Hence, slaughterhouse waste water is contaminated with microorganisms of diverse species (Nafarnda et al., 2012; Agu et al., 2023) and can constitute potential health risks from waterborne pathogens (Nafarnda et al., 2012; Awari et al., 2023; Ezeokoli et al., 2023). Due to the high population density and inadequate sanitation, the rivers located around the slaughterhouse are usually turned into dumping sites for refuse and wastes. The people living along a particular segment of the river simply demarcate a portion of the river that is relatively upstream with respect to their location and draw water from there for their domestic uses.

In Nigeria, the slaughterhouse industry is an important component of the livestock industry, providing domestic meat supply to over 150 million people and employment opportunities for a teeming population. However, the slaughterhouse industries are less developed in developing countries like Nigeria. Facilities for the treatment of slaughterhouse waste waters are lacking, unlike in developed countries where these facilities are adequately provided (Adams et al., 2009). Furthermore, Awari et al. (2023) described the ubiquity, importance, and harmful effects of microorganisms, which demonstrates the different areas in which they are deployed to meet human needs, including agriculture (Agu et al., 2013; Agu et al., 2022; Agu et al., 2015; Aniekwu et al., 2024; Egurefa et al., 2024; Ifediegwu et al., 2015; Okafor et al., 2016; Okonkwo et al., 2023; Orji et al., 2022), biotechnology, engineering, environmental sciences (Agu et al., 2014; Mbachu et al., 2014; Orji et al., 2014; Umeoduagu et al., 2024; Uwanta et al., 2023), medicine, and pharmaceutical sciences. Potential health risks from waterborne pathogens can exist in water contaminated by slaughterhouse waste waters, runoff from feedlots, dairy farms, grazed pastures, fallow and sod amended with poultry litter, grassland treated with dairy manure, and sewage sludge treated land. Such contamination of water bodies from slaughterhouse wastes could constitute significant environmental and public health hazards (Aquaplus, 2003).

Contamination of the environment by slaughterhouse waste water, which is the main focus of this study, could constitute significant environmental and health hazards (Coker et al., 2001; Awari et al., 2023). Adelegan (2002) documented that animal blood is released untreated into the stream while the consumable parts of the slaughtered animal are washed directly into the flowing water. In addition, wastes from slaughterhouses also contain undigested feed, flesh bits, fats, and bones. These slaughterhouse wastes are characterized by a high level of organic matter (Coker et al., 2001; Nafarnda et al., 2006), which supports the growth of microorganisms. Some of the microbes isolated from surface water polluted by discharge of slaughterhouse waste water have been shown to cause diseases such as acute gastroenteritis, salmonellosis, and typhoid fever caused by enteropathogenic *Escherichia coli*, *Salmonella paratyphi* strains, and *Salmonella typhi* respectively (Denis et al., 2005; Muoghalu and Omoch, 2000). Studies carried out in Canada and Nigeria by several researchers, including (Denis et al., 2005; Muoghalu and Omoch, 2000) reveal high microbial load in slaughterhouse waste

water. The wastewater from slaughterhouse processes can be regarded as point source or nonpoint source of water pollution owing to the fact that waste from these processes can either be directly dumped or indirectly through runoff actions into the same river. Point sources occur when pollutants are emitted directly into the water body. Surface waters being a normal habitat for aquatic animals could have consequential impact on man either directly or indirectly when polluted since less than 1% of the world's fresh water is readily accessible for human use (UNESCO, 2006). According to reports by (WHO, 2005) and (UNESCO, 2006), large numbers of surface water bodies in developing countries are polluted. The people that make use of water from such water bodies are definitely adversely affected by the effects of the discharges from slaughterhouses waste water and other pollution sources, thereby putting a large proportion of the users at environmental and public health risks (Nafarnda et al., 2012; Osibanjo and Adie, 2007; Adepeju et al., 2023). In addition, other pollution sources that discharge their wastes into the receiving water bodies and have considerable pollution effect. These sources may include cassava processing industries, paint industries, rubber factories, sawmills, oil mills, motor servicing workshops, car wash services, sand dredging operations, as well as the activities of cattle-rearing.

This research paper strategically aimed at the microbiological quality of wastewater from two slaughterhouses situated at Oyi L.G.A, Umunya, Anambra State.

Research Method

3.1 Sample Collection

Sterile containers were used to collect waste water discharge from slaughter house from Umunya slaughter house, Anambra State and was transported to Microbiology Laboratory.

3.2 Sterilization of Materials

The laboratory apparatus required for analysis were sterilized with autoclave at temperature 121^{o}C for 15 minutes. This was done to remove and kill any microorganism that might have contaminated the laboratory apparatus. The entire working surface including the work bench was equally disinfected with 70% ethanol.

3.3 Procedures for Microbiological Analyses

3.3.1 Media Preparation

The different media which includes Nutrient agar, Salmonella agar, Shigella agar and MacConkey agar was used in culturing bacteria. With the guide of the manufacturing instructions, and was autoclave at 121^{o}C for 15 minutes and allow cooling.

3.3.2 Serial Dilution

Each sample was serially diluted using Normal Saline. 9mls of distilled water was measured out into each test tube labeled 10^{-1}, 10^{-2}, to 10^{-6} Using separate sterile pipettes; 1ml of wastewater from abattoir sample was measured out into the first test tubes and properly mixed. Using a different pipette, 1ml from the first test tube was pipette into the second test tube

already containing 9ml of distilled water. Using a different pipette, 1ml from the second test tube was pipette into the third test tube and lastly 1ml from the third test tube was pipette and then discards.

This was followed by pour plate technique.

3.3.3 Culture and Isolation (Pour Plate) of Bacteria

Ali routs (0.1ml) of the inoculum (10^{-5}) was poured into Nutrient Agar and spread evenly on it with the aid of a sterile glass spreader. The plate was allowed to dry for 15minutes. The plates were incubated at 35^{o}C for 24 hours. The same were done for MacConkey, and Eosine Methylene Blue. An un-inoculated agar plate of Nutrient, Eosine Methylene Blue and MacConkey agar served as control.

After the incubation period, the plates were brought out of the incubators and the colonies were counted and each count was expressed in colony forming unit per ml (CFU/ml).

Pure culture were obtained by sub-culturing the different colonies into nutrient agar plate by streaking method. The plates were again incubated at 35^{o}C for 24 hours.

3.3.4 Sub-Culturing and Maintenance of Pure Bacterial and Fungal Isolates

After incubation, colonies that developed on plates were randomly picked and sub-cultured on fresh nutrient agar to obtain pure culture of the isolates. Using wire loop, it was inoculated on Petri dishes containing Nutrient agar for bacterial counts at 37^{0}C for 24hours and Potato Dextrose Agar for fungal counts at 25^{o}C for 72hours. After purification the isolates were maintained using freshly prepared nutrient agar and PDA slants for bacteria and fungi respectively slants and were kept at 4^{o}C for identification of the different Isolates.

3.4 Identification of Various Bacterial Isolates

Identification of isolates was based on colonial features including macroscopic examination (such as motility, Gram-staining reaction, cell arrangement and shape) and biochemical features. The results obtained were then compared with standard references for proper identification of the isolates; Bergey's manual was used for bacteria identification.

The following biochemical tests were carried out for the characterization and identification of the organisms according to the method of (Cheesbrough, 2016).

I. Catalase test
II. Oxidase test
III. Indole test
IV. Sugar fermentation
V. Coagulase test
VI. Gram's staining

3.4.1 Gram Staining:

Is a useful tool for identification of bacteria in specimen and cultures by their gram reaction and morphology. This technique divides bacteria into two groups- Gram positive and Gram negative.

Gram Positive and Gram Negative: the classification is based on the differences in the composition and permeability of bacteria cell walls. The Gram positive bacteria retain the stain, crystal violet while the Gram negative bacteria are discolored and are able to take up the counter stain according to (Cheesbrough, 2006).

Procedure:

1. A thin smear of the culture was made on a clean grease free slide and the film was allowed to air dry and fixed by gently passing the slide over the flame three times.
2. The smear was covered with crystal violet solution and allowed to stain for 60 seconds.
3. Excess stain was dipped off and washed with clean water.
4. The smear was stained with lugol's iodine and allow for 1minutes and washed with clean water
5. The smear was decolorized with few drops of alcohol solution and was immediately washed off using clean water.
6. The smear was then counter stained with safranine solution for about 2 minutes and wash off with a running tap.
7. The stained smear was allowed to air dry, after which a drop of oil immersion was placed on it and examined with x100 objective lens.

After gram staining of the culture on the plates, further biochemical test were carried out on the organisms isolated for further identification.

3.4.2 Sugar Fermentation Test

This was done to investigate the ability of isolates to utilize sugar. The following sugars were used: glucose, manitol, sucrose and lactose. One gram (1g) of each sugar was dissolved in 10ml of water in different test tubes. One point five gram (1.5g) of bacteriological peptone and 1ml of bromo-thimoblue tubes. The mixture was dispensed into test tubes, sterilized by autoclaving with inverted Durham tubes at 115^{o} C for 10 minutes and incubated for 24 hours at 37^{o}C. Change of color from blue to yellow indicated positive test result, which also signifies the production of acid. Presence of air bubbles at the top of the Durham tube indicated gas production and absence of air bubbles indicated no gas production.

3.4.3 Catalase Test:

This test was useful in differentiating bacteria that produce the enzymes catalases such as *staphylococcus* species from non-catalases producing bacteria such as ***streptococcus*** species. Catalase acts as a catalyst in the breakdown of hydrogen peroxide and water according to (Cheesbrough, 2006).

Procedure:

1. Two millimeters of hydrogen peroxide solution was poured in a test tube.
2. With the aid of a sterile glass rod/wire loop, several colonies of the test organisms was collected from the nutrient agar plate and immersed in the hydrogen peroxide
3. Bubbling was checked
4. Active bubbling of the mixture indicates positive results.

3.4.4 Oxidase Test

This test was always used in the identification of bacteria that produce the enzyme cytochrome oxidase. To perform this test, a piece of filter paper was soaked with a few drops of oxidase reagents (1% tetramethyl p-phenylenediamine dihydrochloride) according to (Cheesbrough, 2006).

A colony of the test organism was then smeared on the filter paper, when the organism is oxidize producing the phenylenediamine, the reagents will be oxidized to deep purple colour.

Procedure:

1. A piece of filter paper was placed in a clean petri dish and two drops of freshly prepared oxidize reagent was placed on it.
2. Development of blue purple colour was checked for within few seconds.
3. The blue purple colour produced indicates a positive result.

3.4.5 Indole Test:

This test is important in the identification of entero bacteria especially, Escherichia coli, that are able to breakdown the amino acid, tryptophan according to (Cheesbrough, 2006).

In this test, the test organisms cultured in a medium, which contains tryptophan, indole production is detected by Kovac's or Erhlich's reagent containing 4(p) dimethryl aminobenzaidehyde, this reacts with the indole to produce a red coloured compound.

Procedure:

1. The test organism was inoculated into a bijou bottle containing 3ml of sterile Trytone water.
2. This was incubated at 37^{o}C for 48hours

3.4.6 Citrate Test:

Simmon's culture medium, a modification of koser's medium with agar was used, agar was added to solidify the medium that was dispersed in test tubes, autoclaved at 121^{o} C for 15 minutes and allowed to set as slopes. The Simon's medium constituted loser's medium (11g), agar (30g) and bromothymol blue (40ml) as an indicator according (2006).

The solidified medium was inoculated with the test organism and incubated at 37^{0} C for 96 hours. Blue colour and streak of growth indicated Positive result.

3.5 Fungal Sub-Culturing

The discrete colonies on the pour plate were picked with a sterile loop and streaked on Petri dishes containing solidified medium. The organisms were sub-cultured onto new plate several times until pure culture was obtained.

3.6 Identification of Fungal Isolates

This was carried out by wet mount and slide culture method as described by larone(1995).

3.6.1 Wet Mount

A drop of 70% alcohol was added onto a clean microscope slide and with a sterile platinum wire loop, a colony from the isolate was transferred onto the drop of the 70% alcohol on the slide. Before the alcohol dried out one drop of the lactophenol cotton blue was added and the slide was covered using a cover slip. The slide were then taken for microscope examination using an objective (40x) of the light microscope.

3.6.2 Slide Culture

This was done to observe the morphological characteristics of the isolates without disturbing the arrangement of spore hyphae. A 100mm square agar block (PDA) each were placed on sterile sides. The slides were incubated with speck of fungal colony and aseptically Petri dish containing small amounts of water helped to prevent the agar from drying out .The Petri dishes were covered and incubated at 25-30ºC. Viable growth was examined at intervals when the viable growth was observed, the cover slip were removed and the lactophenol cotton blue mount was performed and examined microscopically for sporulation.

4.0 Results

The total heterotrophic bacteria, coliform and fungal counts of the swimming pool samples were recorded as follows, the total heterotroic bacteria count ranged between 6.8×10^5 to 2.6×10^5CFU/ml, the total coliform count ranged between 5.2×10^5 to 2.0×10^5CFU/ml in MacConkey Agar, the total fungal count ranged between 1.4×10^4 to 1.2×10^4CFU/ml in PDA. The bacterial isolates includes *Bacillus*spp., *Staphylococcus* spp., *Proteus*spp., *Klebsiella*spp., and *Escherichia* spp, while the fungal isolates were *Aspergillus* spp and *Penicillum*spp. The bacteria counts of waste water samples from slaughter houses were shown in table 4.1, the total fungal counts of waste water samples from slaughter houses were shown in table 4.2, the presence of microorganisms in the both slaughter waste waters were shown in in table 4.3, the biochemical identification of bacteria isolated from the recreational water samples were shown in table 4.4 while the characteristics of fungal isolates were shown in in table 4.5

Table 4.1: Bacterial Counts of Waste Water Samples from the Slaughter Houses

PARAMETER ANALYSED	TSWW	NSWW
THBC (CFU/ml)	$6.8x10^5$	$5.2x10^5$
TCC (CFU/ml)	$3.2 x10^5$	$2.2 x10^5$
FCC (CFU/ml)	$2.6 x10^5$	$2.0 x10^5$

Key: TSWW = Temple Slaughter Waste Water

NSWW = Nkwele Slaughter Waste Water

THBC = Total Heterotropic Bacteria Count

TCC = Total Coliform Count

TFC = Total Fecal Count

Table 4.2: Fungal Counts of waste water Samples from the Slaughter Houses

Waste Water Samples From SWW	Total viable fungi count (CFU/ml) on PDA plates
TSWW	$1.4 x 10^4$
NSWW	$1.2 x 10^4$

KEY: PDA = Potato Dextrose Agar

TSWW = Temple Slaughter Waste Water

NSWW = Nkwele Slaughter Waste Water

Table 4.3: Presence or Microorganisms in the Slaughter Waste Water

Suspected Microorganism	TSWW	NSWW
Bacillus spp	√	√
Escherichia spp	√	√
Klebsiella spp	√	√
Proteus spp	√	√
Staphylococcus spp	√	√
Penicillum spp	√	0
Aspergillus spp	√	√

KEY:

TSWW = Temple Slaughter Waste Water Umunya Oyi LGA

NSWW = Nkwele Slaughter Waste Water

Table 4.4: BIOCHEMICAL IDENTIFICATION OF BACTERIA ISOLATED FROM THE SLAUGHTER HOUSE

S/N	Code	Colour	Shape	Elevation	Opacity	Margin	Size	Texture	Gram RXN	Shape	Catalase	Oxidase	Motility	Citrate	Indole	MR	VP	Glucose	Lactose	Fructose	Manitol	Sucrose	Galactose	Probable organism
1	TSWW	Pale	Circular	Flat	Opaque	Entire	Large	Moist	-ve	Rod	-	+	-	+	+	-	-	+	+	+	+	+	-	*Escherichia* spp
2	NSWW	Creamy	Circular	Raised	Translucent	Wavy	Small	Mucoi	-ve	Rod	-	+	+	-	+	-	+	-	+	+	-	-	-	*Proteus* spp
3	TSWW	Milky	Round	Elevated	Translucent	Entire	Small	Mucoi	+ve	Cocci	+	-	-	+	-	-	-	+	-	+	+	-	-	*Staphylococcus* spp
4	NSWW	Creamy	Circular	Flat	Opaque	Entire	Small	Moist	-ve	Rod	-	-	-	+	-	+	+	+	+	+	+	-	-	*Klebsiella* spp
5	TSWW	Green	Circular	Flat	Opaque	Entire	Small	Moist	-ve	Rod	-	-	-	+	-	+	+	+	+	+	+	-	-	*Bacillus* spp

Key: + = positive; - = negative; MR = methyl red; VP = Vogues Proskauer; TSWW = Temple Slaughter Waste Water, NSWW = Nkwele Slaughter Waste Water

Table 4.5: Macroscopic and Microscopic Characteristics of Fungi Isolated From the Slaughter Houses Samples

SAMPLE	Colony Morphology	Microscopic Morphology	Probable Organisms
TSWW	White to cream yellow colonies	Brush like structures, Septate Hyphae, Condiosphores simple or Branched Philalides and Condia Produced in dry Chains from the tips of the Phialides	*Penicillum* sp.
NSWW	Slightly brown colonies	Septate and Hyaline Hyphae, Smooth and Colourless Conidiosphores and Spores and a Swollen Vesicle giving rise to Phialides where Conidia arise	*Aspergillus* sp.

KEY:

TSWW = Temple Slaughter Waste Water

NSWW = Nkwele Slaughter Waste Water

4.1 Discussion

The result of this study shows that the bacteria isolated from waste water slaughter house samples include: *Escherichia* spp, *Bacillus* spp, *Proteus* sp., *Klebsiella* spp, and *Staphylococcus* spp while the fungi isolated includes *Penicillum* spp., and *Aspergillus* spp.. The total viable counts of the samples ranged between 2.6×10^5 to 6.8×10^5 CFU/ml in Temple slaughter waste water samples while in Nkwelle slaughter waste water sample the viable count ranged between 2.0×10^5 to 5.2×10^5CFU/ml. The total fungal count of samples from Temple slaughter waste water recoded 1.4×10^4 CFU/ml, while the fungal count from the sample from Nkwelle slaughter waste water recorded 1.2×10^4 CFU/ml.

This study therefore revealed the potential health risk associated with use of Nkwelle slaughter waste water and Temple slaughter waste water facilities without adequate treatment. The presence of pathogenic organisms in these slaughter houses poses a lot of harm to humans who get in contact with them.

The result of this study is was line with many other works including Atuanya, (2022) in "Waster Water qualities of government and private slaughter houses and their effects on Ikpoba River, Benin City, Edo State, Nigeria". The microbiological quality of slaughterhouse waste water is a significant environmental and public health concern. Slaughterhouse wastewater contains a high load of organic matter, suspended solids, nutrients, and pathogenic microorganisms, including bacteria, viruses, and parasites.

Some of the microbes isolated from surface water polluted by discharge of slaughter house waste water have been shown to cause diseases such as acute gastroenteritis, salmonellosis and typhoid fever caused by enteropathogenic *Escherichia coli*, *Salmonella paratyphi* strains and *Salmonella typhi* respectively (Denis *et al*., 2005; Muoghalu and Omoch,

2000). Studies carrid out in Canada and Nigeria by several researchers including (Denis *et al*., 2005; Muoghalu and Omoch, 2000) reveal high microbial of slaughter house waste water.

The waste water from slaughter house processes can be regarded as point source or nonpoint source of water pollution owing to the fact that waste from these processes can either be directly dumped or indirectly through runoff actions into the same river.

The microbiological quality of slaughterhouse waste water is a critical concern that requires attention from slaughterhouse operators, regulators, and the scientific community. Effective treatment and management practices can reduce the microbiological contaminants in slaughterhouse waste water and prevent environmental and public health impacts.

5.0 Conclusion

In conclusion, the microbiological quality of waste water from slaughterhouses is a significant concern that requires attention and effective management. The presence of harmful microorganisms in slaughterhouse wastewater (SWW) can pose serious environmental and public health risks. To mitigate these risks, it is essential to:

1. Implement proper slaughterhouse practices and hygiene measures.
2. Ensure effective treatment of slaughterhouse wastewater using appropriate technologies.
3. Regularly monitor the microbiological quality of slaughterhouse wastewater.
4. Comply with regulatory standards and guidelines.
5. Adopt sustainable and environmentally friendly management practices.

By taking these steps, the microbiological quality of slaughterhouse wastewater can be improved, reducing the risk of waterborne diseases and environmental pollution. This is crucial for protecting public health, environmental sustainability, and the overall well-being of our ecosystem.

Recommendations

a) Implement anaerobic digestion and advanced treatment technologies.
b) Conduct regular monitoring and testing for microbiological contaminants.
c) Develop and implement effective management plans for slaughterhouse wastewater.
d) Encourage sustainable and environmentally friendly practices in slaughterhouses.
e) Support research and development of new and innovative treatment technologies.

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