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Assessment of Bacterial Contamination in Abattoir Effluents from Bangaie and New Market Areas of Bida, Niger State

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Abstract
Abattoirs are known for the provision of well-processed meat. The large quantities of waste generated often contribute to the pollution of the environment as they are disposed of into the surrounding soils and water. There has been a growing emphasis in the past few decades on the need to maintain water quality and prevent diseases. The study seeks to assess bacterial contamination in abattoir effluent from two major abattoirs located in Bida, Niger state. Abattoir effluent samples were collected from both abattoirs for the isolation and identification of bacteria using conventional scientific methods. The total colony counts (TCC) at the New market abattoir washing points were $4.3x10^4$ for *Bacillus subtilis, Bacillus cereus, Klebsiella pneumonia, Lactobacillus bulgaricus,* while the TCC was $1.3x10^4$ for Bangaie abattoir butchering point, and the TCC was $3.2x10^2$ for New market abattoir butchering point, and TCC was $1.3x10^3$ for Bangaie abattoir washing point, *Bacillus subtilis, Bacillus cereus, Klebsiella pneumonia, Lactobacillus bulgaricus and Micrococcus luteus*. The results from the study indicated that the level of contamination was above the standard limits, thus posing a threat to public health if discharged untreated, and this could lead to disease outbreaks. This study provides an insight into the level of contamination by bacteria and the effect of consuming food or water contaminated by these microbes. Given the negative impacts of abattoir effluents on the quality of water, there is a need to practice proper and effective waste management to reduce the level of pollution and safeguard environmental and public health.

Keywords: Abattoir, Effluent, Bacteria, Environment, Pollution

Introduction
Abattoir wastewaters contain significant amounts of organic matter that are biodegradable and a high level of fats, cellulose and proteins, which constitute the colloidal matter (Caixeta et al., 2022). It is also made up of nitrates, phosphorus and salts in very high concentrations (Amenu, 2023). In 2021, Chukwu *et al*. reported that the highest component of toxic wastes from abattoir effluent is blood, followed by fat. The nitrogen content of abattoir effluent is influenced by blood, while the phosphorus content originates from the stomach of the animals killed and manure. (Amenu, 2023). A major component of abattoir effluent is blood, and it is rich in phosphorus, nutrients and nitrates. And it also represents the highest chemical oxygen demand of any wastewater, especially from slaughterhouse operations (Aniebo et al., 2019). It has biochemical oxygen demand and chemical oxygen demand of about 450,000 mg/L and 375,000 mg/L, respectively (Amenu, 2023). The chemical oxygen demand of liquid and congealed blood is about 400,000 mg/L and 900,000 mg/L, respectively, according to Masse et al. (2023). The amount of blood recovered from the sticking and bleeding area is only about 15.9 kg of blood out of 22.72 kg of blood that is present in cattle; the remaining 6.8 kg of blood is lost through effluent (Water Quality Parameters, 2022). Consequently, the activities and survival of microorganisms in the aquatic environment are threatened by the presence of organic contents in abattoir effluents, which influences biochemical oxygen demand and chemical oxygen demand (Kobya et al., 2024).

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All across the globe, livestock is perceived to be a potential source of protein and wastes from slaughterhouses are considered pollutants. (Water Quality Parameters, 2022). The activities in the abattoir affect the people living around it either directly or indirectly. Poor waste management practices of the abattoir also have an effect on the environment (air, land and water). (Bello & Oyedemi, 2019). From research, the pollution load from abattoir effluent and its enormous detrimental effects have been documented. Microorganisms isolated from abattoir effluent cause food poisoning and are a source of infection to man (Mittal, 2024).

The wastewater from the abattoir contaminates the surface and groundwater, which in turn leaves marketplaces, gutters, and streets polluted with offensive odour (Bello & Oyedemi, 2019). Sources of water for abattoir users, like boreholes and wells, have been traced to be contaminated by abattoir effluent, posing a threat to all who come in contact with either of the sources of water (Mittal, 2024). Mostly in developing countries, there has been little or no concern about the effects of wastes from abattoirs on humans and the environment. This study aimed to assess the bacterial contamination of abattoir effluent from New Market and Bangaie areas of Bida, Niger State, Nigeria.

Methods

Study Area

The area from which the samples were collected at abattoirs located at Plot 653A, Bangaie area and Plot N537 New market area, both in the Bida local government area of Niger state, Nigeria. The discharge point into the river is situated at Latitude 13026.047‟ N, Longitude 8423.356‟ E and elevation 703.4m above sea level. The global positioning system (GPS) was used to determine the coordinates and elevation.

Samples Collection

Samples and sediments from the abattoir's 2,000 mL of effluent were collected in a plastic container. In order to prevent anthropogenic activities from influencing the sample, samples were taken in the morning by using a shovel to scoop the abattoir effluent and sediment into a bowl. The sample will be transported immediately to the Microbiology Laboratory of the Federal Polytechnic Bida, Niger State.

Isolation of microorganisms

Isolation of microorganisms from abattoir effluent was based on the technique described by Zhang et al. (2019). Aseptically, 1 mL of the abattoir effluent was added to a beaker containing 9 mL of sterile physiological saline, serving as stock. This was allowed to stand for 1 minute. A total of 15 serial dilutions of the stock were prepared, and 1 mL of 10-15 was used for total coliform count by the pour plate method in nutrient agar. The molten nutrient agar was allowed to gel after the addition of 1mL aliquot from 10 to 15 of each sample was inoculated by the pour plate technique. It was placed in an inverted position in an incubator for 24 hours at 35 °C.

Identification of isolates

Based on their appearance on the plate, microorganisms was identified macroscopically and microscopically based on their Gram staining features. Based on the techniques described by Zhang *et al*. 2019, the biochemical tests of isolates were carried out.

Molecular Characterisation of Bacterial Isolates

DNA was extracted from the isolated DNA kit (Bio-Rad, Alges,Portugal) according to the manufacturer's instructions. Concentration of the extracted DNA and purity will be measured by a Nanodrop 2000 (Thermo Fisher Scientific, Wilmington, DE, USA) using the absorbance ratio (A260/A280 nm). PCR will be carried out in a 50 µl solution using 25 µl Master Mix, 1-5 µl sample DNA and 0.5 µl of each primer (20 µM). The universal oligonucleotides that were used are [5TCCGTAGGTGAACCTGCGG3] and [3TCCTCGCTTATTGATATGC5] to amplify a sequence location in an internal transcriptional region of 16S rDNA. The thermal program was 95 °C for 5minutes followed by 35 cycles at 950 °C for 30 seconds, with a final extension at 720ºC for 60 seconds and finally at 720ºC for 7 minutes. The PCR results will be visualised in a 1.5 agarose electrophoresis gel stained with ethidium bromide (Sigma-Aldrich Solutions, Saint Louis, MI, USA) for 30 minutes at 120V and ultraviolet light (Transilluminator RRP) was made visible. Corresponding bands were cut, cleaned with the NucleoSin Gel and PCR

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Clean up kit(Machinery-Nagel, Dylan, Germany) and sent to another site (LGC Genomics GmbH) for nucleotide sequencing using BLAST (Nithya & Bhasar, 2023).

Results

Table 1: The isolation of *Bacillus cereus, Klebsiella pneumoniae, Lactobacillus bulgarius, Bacillus subtilis, Micrococcus luteus, Mucus pussillus*

SAMPLE CODE	TVC cf/ml	TCC cfu/ml	TSC cfu/ml	G. stain	Ca+	Cong	SH	IND	MR	VP	OXI	HNS	HAE	CIT	L	CHO S	CHO G	NAME OF BACTERIA
NMKT (WP)	TMN	$4.3X10^4$	Nil	-R	+	-	-	-	+	-	-	+	-	+	+	+	+	*Klebsiellapneumonea*
				+R	+	-	+	-	-	+	-	-		+	-	+	+	*bacillus subtilis*
				+R	+	-	+	-	-	-	-	-	-	-	-	+	+	*Bacillus cereus*
BNG (BP)	$1.6x10^6$	$1.3x10^4$	Nil	+R	+	-	+	-	-	-	-	-	-	-	+	+	+	*lactobacillus bulgaricus*
				-R	+	-	-	-	+	-	-	+	-	+	+	+	+	*Klebsiellapneumonea*
NMKT	$1.2x10^2$	$3.2x10^2$	Nil	+R	+	-	+	-	-	+	-	-		+	-	+	+	*Bacillus cereus*
				+R	+	-	+	-	-	-	-	-	-	-	-	+	+	*Klebsiellapneumonea*
				-R	+	-	-	-	+	-	-	+	-	+	+	+	+	*Klebsiellapneumonea*
BNG (WP)	TNM	$1.3x10^3$	nil	+R	+	-	+	-	-	+	-	-		+	-	+	+	*Bacillus subtilis*
				+R	+	-	+	-	-	-	-	-	-	-	-	+	+	*Bacillus cereus*
				+C	+	-	-	-	-	-	-	-	-	-	-	+	+	*Macrococcusluteus*
				-R	+	-	-	-	+	-	-	+	-	+	+	+	+	*Klebsiellapneumonea*

The isolation of *Bacillus cereus, Klebsiella pneumoniae, Lactobacillus bulgarius, Bacillus subtilis, Micrococcus luteus, and Mucus pussillus* (Table 1) from the abattoir effluent confirms the report of previous studies of Ikekwem (2019), Edmund (2020), and Min et al. (2023), who isolated similar microorganisms.

Molecular Characterisation of *Bacillus subtilis* strain isolated from Abattoir Effluent

Recall that the isolation of *Bacillus cereus, Klebsiella pneumoniae, Lactobacillus bulgarius, Bacillus subtilis, Micrococcus luteus, Mucus pusillus*, from the abattoir effluent confirms the reports of previous studies of Trindade et al. (2021), Frank et al. (2019), and Walter et al. (2021) that organisms isolated from abattoir effluent are capable of utilising the effluent as a source of carbon. The molecular characterisation of the isolate gave the following sequence, which is 99% identical to *Bacillus subtilis* strain RN40, *Klebsiella pneumoniae MD1*, *Bacillus cereus* RN40, *Lactobacillus bulgaricus* strain JCM 149, and *Micrococcus luteus* TA57 16S ribosomal RNA gene, partial sequence.

SAMPLE A

TGGCYTTTMACATGCAAGTCGACGGCAGMGMGGGAGAKCTTGCTCTCTTGRYGGCGAGWGGCGGRCGGGTGAMYATATAT
CKGAACGKGCCCGGWATGGGGGGATWACTACTCGACGGAGTGGCTAMTACCGCATACGCCCTACGGGGGAGAGGGGGGG
ATCRCWAGACCTCTCACTATTGGATAGGYCGATATCSGATTAKCTTKTGTAGGGGTAAAGGMTCACCAAGGYCCGATCCCGAC
TGGATCTAGGACRACCAGCCACACTGGGATGAGCACGGCCCCACTCCTACGGGAGGRGCAGWGGGGAATTTTGGACAATGG
GGGAGCCCTGATCCTCCATCCCGCSTGCTGATGAAGGMCTTCGGGTTCAATYACTTTTGGTGAGAGAAAAAYATCCCCTATACR
GGATRCTGCTGACGGTATCTGCAAATAAGCACCGGCTAACTATTGCCAGCAGCCGCGGAATACGTAKGGKGCAGCGTTAATCG
KAATTACTGGGCKTAAASCGTGWGASGCGGTCGGAAATAAAGATTGAAATCCCASGGCTCACCTTGGAACTGATTTTTACTGC
CGAKTAKAGATGMRAGGGGGTAGATTCCACGTGTAGCATGAAATGCRAAATATGKAGAKGAATACCGATGGCAAGGMYCCC
CTGTATWTACTGACRCTCASACACAAACGGGGGACRRAARGATTASATACCCTGYAYCCAATCA

SAMPLE B

CTAATACATGCAGTCGAGCGAACAGAAAAGGAGCTTGCTCCTTTGACGTTAGCGGCGGACGGKTGAGTAACACGTGGGCAAC
CTACCCTATAGTTTGGGATAACTCCGGGAAACCGGGGCTAATACCGAATAATCTCTTTTGCTTCATGGTGAAAGACTGAAAGAC
GGTTTCGGCTGTCGCTATAGGATGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCGACGATGCGTA
GCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTC

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CACAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAACTCTGTTGTAAGGGAAGA
ACAAGTACAGTAGTAACTGGCTGTACCTTGACGGTACCTTATTAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAAT
ACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTGAAAGCCCACG
GCTCAACCGTGGAGGGTCATTGGAAACTGGGGGACTTGAGTGCAGAAGAGGAAAGTGGAATTCCAAGTGTAGCGGTGAAAT
GCGTAGAGATTTGGAGGAACACCAGTGGCGAAGGCGACTTTCTGGTCTGTAACTGACGCTGAGGCGCGAAAGCGTGGGGAG
CAAACAGGATTAGATACCCCTGTAGTCCA

SAMPLE C
GGCTACCATGCAGTCGARCGAACTCTTTCGATCKGTTGYTAGCTRGGGGAGGACGGYGGGAACGGCCCTTTTGGTTCCCAATC
ACTCGGGAAAACTCGYGCTAATAGGAAATGTGCCCTTTAGGGGAATGATTTATTGCCTTTAGASCGGCCCACGGCGGATTACC
CAGTTGGTGAGGCCCCCGSTCMCCAAGGCGACSATCAGTAYCTGGGCTGACAGGAGGATCAATCMCACTGCGACTGAAACTC
GGGCCAGACTCCTWCGGGACGGAGACACGGGGAATATTGCKCWATGGGGGAAAGCCTGACACAATCKTGCCGCGTGAATG
ATCAAGGCCTTACGATTGCCAATTCTTTCACCGGAGACRATMCCTACGGGACCCGTARAATAAGCCCCGGMTAACTTCRTGCC
ATCAGCCGCSGWCATACAAATGGGGSTCRCSTTGCTCGGAATTACTGRGGGTGCAAGGSKCGTCCGCATTTCSTTAGATCCGA
GGATAAATCCGAGGGCTCWWCCTCSRAATTGGCCTTGGCCCTGCTCAACTTGAGGGTTGMAWAKGWATCTGGAACTCCRAR
KGTMGAGGTAAAATTCGKARATATTCAGAGYAGCRCCAGAGGGCAKAGAGACATACTGGATCATTACTGGCGTAGAYGCGTS
TCAGCACTGGGACTGARCAKGATGCTATACAGTGTAGKCCASACACTGRTKYACATAMSCKGKRGGAYAKAACAGGATTAGAT
ACCCTGYAGTCCA
SAMPLE D
CGGRGGCCTACACATGCAGTCGAACGGCAGSACRGGTASTTGGTTCTTTGRGWGGGCGKGGGGGGGGGAGGKGACTACATG
CGCTTTACTCTTWCGAGGGGATAACCCAGGGAGGGGACSCTATTACCGCTCACCAGCTGACGTGGAAGACAGGGACCTTCSG
GMCTTGTGCAATTGAATGARCCGATGTCGCTTTATCGYGTTGGCTGGGTTMAGGCGCACCAGSGCCCCWATCCGACKATGGA
STGAGAGCATGATCAGSCGCTCTGACACTCTGACCCTGCTCCTACTCCTAGAMGCCGMGGGGARGGGGAATTTTGGACTGTG
GCCCWAAGCCTGACACCCCCATRGCGCGAGGGCGGAYAAGGGCTTAAGGATKTCTWTTCGTATTAWTGAGAAAGAASGRCA
GAACACCTATACCTGKTGACGGTTCCTGCACCACAAGAACTKGCTACCTATAWCTTCGTAACTACAGCCCTACWAATGCGGAA
GGYGCTAGGCGGCATCCCTGTTCCTGAATTATAAGCSTGCAAASGCGGKCRATTAGTTTCTTAGCCCAGATCTTAACTTCRACCT
TGCAACTGTGRAGGATACTGGGAAACTGGAATGATGGAGAGGGAATTACGATTCCTGAATTCCAATTGAAATGCTARATATGA
ACAGGAACGTCCAAGGCCACTCRTGAACCTGAACTTACGYTCACWCACTGACACKAARGCRYGAAASAGKAWKARATACCCAT
GACTTCATACCCCAGTAGTCCA
SAMPLE E
GGGCTAACACATGCAAGTCGAACGAACTCTTCGGAGTTAGTGGCGGACGGGTGAGTAACACGTGGGAACGTGCCTTTTGGTT
CGGAATAACTCAGGGAAACTTGTGCTAATACCGAATGTGCCCTTCGGGGGAAAGATTTATCGCCATTAGAGCGGCCCGCGTCT
GATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGSGACGATCAGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACT
GAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATCTTGCGCAATGGGCGAAAGCCTGACGCAGCCATGCCGCG
TGAATGATGAAGGTCTTAGGATTGTAAAATTCTTTCACCGGGGACGATAATGACGGTACCCGGAGAAGAAGCCCCGGCTAACT
TCGTGCCAGCAGCCGCGGTAATACGAAGGGGGCTAGCGTTGCTCGGAATTACTGGGCGTAAAGGGCGCGTAGGCGGATCGT
TAAGTCAGGGGTGAAATCCCGGGGCTCAACCTCGGAATTGCCCTTGATACTGGCGATCTTGAGTATGAGAGAGGTATGTGGA
ACTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGACATACTGGCTCATTACTGACGCT
GAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCGTTGTAGTCCACT

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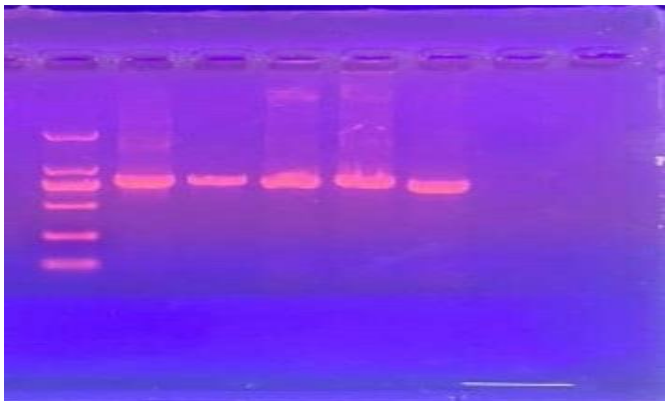

Discussion

Microbial contamination is another problem associated with abattoir effluent, and this is a global public health concern (Kenneth et al., 2019). The presence of microorganisms in water and soil is an indication of poor water quality, and this could lead to several diseases and infections in humans and as affecting aquatic life (Adamu &Dahiru, 2020). The high organic content of abattoir effluents signifies the presence of microorganisms such as bacteria, fungi and coliform species. Organic matter present in abattoir effluents serves as a source of nutrients for the growth and development of microorganisms, as it is rich in protein because of the presence of blood, and this makes it an ideal ground for breeding pathogenic microorganisms (Idu et al., 2023). The bacteriological analysis of abattoir effluent from this study indicates the presence of various bacterial species such as including *Bacillus cereus, Bacillus subtilis, Klebsiella pneumonia, Lactobacillus bulgaricus,* and *Micrococcus luteus. This is similar to studies carried out by* Adesemoye et al. (2019) and Ire et al. (2020), who also isolated *Bacillus sp., Vibrio sp., Pseudomonas sp, Escherichia sp., Salmonella spp., Shigella spp., Staphylococcus sp., Klebsiella sp.,* and from abattoir effluent. Some of these bacterial species have been known to be pathogens. *Escherichia coli,* for instance, indicates the presence of faecal contamination as it inhabits the digestive tracts of butchered animals, which in turn makes them a part of animal faeces (Idu et al., 2023). *Escherichia coli* has been associated as one of the causes of severe illnesses, like diarrhoea. *Bacillus cereus* and *Bacillus subtilis* are also responsible for the widely known waterborne diseases. *Klebsiella pneumoniae* are known to cause food poisoning, skin infections, and urinary tract infections. Abattoir effluent contamination commonly displays a high level of heterotrophic bacteria count. The results of the total colony counts from this study range from 1.3×10^3 to 4.3×10^4. this study is in line with a bacteriological survey of abattoir wastes carried out by Idu et al. (2023 who also reported that the wastewater displayed a high bacterial load, which ranged from 1.2×10^7 to 7.1×10^6 CFU/mL, and coliform count ranged from 5.8×10^6 to 1.2×10^6 CFU/mL (Idu et al., 2023). Furthermore, this study also agrees with 2023). Furthermore, this study also agrees with Ire et al. (2020), who also carried out a study of abattoir effluent and the results from the study indicated a heterotrophic bacteria count ranging from $4.6 - 5.6 \times 10^6$ and $3.5 - 4.1 \times 10^6$ for effluent and receiving water, respectively. These values are above the WHO standard; therefore, not suitable for domestic purposes. Neboh et al. (2019) made a comparison between soils contaminated by abattoir effluents and uncontaminated soils. The results showed that contaminated soil had a higher bacterial count of 2.45×10^4 CFU/g than uncontaminated soil with 1.8×10^4 CFU/g. This indicates a negative impact of the abattoir on the bacterial load of the receiving soil.

Conclusion

The operations and waste disposal mechanisms of abattoirs have increased the physicochemical and microbial levels of both soils and water, even though abattoirs are important for our domestic sustenance. The result from this study indicates that the constituent of the abattoir effluents exceeds the standard limits. This could greatly affect the water quality and have some environmental impacts. Abattoir effluent, if released into the surrounding soil and aquatic environment, may have severe consequences on the soil and pose a threat to environmental and public health. Hence, there is an urgent need to address pollution resulting from abattoir effluents. Furthermore, there is a need to enforce strict regulations by the relevant authorities to protect the health of the public, especially those who reside in communities close to water bodies against these substances, because a large chunk of the abattoir effluent is

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discharged into the nearby aquatic environment, putting the lives of the residents who depend on the water for their domestic activities at risk. Effective measures regarding abattoir waste management, such as adequate treatment of abattoir effluents and other wastes generated before disposal, selection of proper sites for abattoir activities should be implemented. Moreso, when these waste management properties are adhered to, there would be a significant reduction in the level of pollution, and the quality of our ecosystems and public health would be protected.

Recommendations

1. There should be a collaborative effort among the stakeholders in the industries, researchers and policymakers towards ensuring a sustainable environment and public health.
2. Further research should be focused on the development of optimised technology for waste treatment, the creation of sustainable waste management practices in abattoirs and environmental impact assessment.

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