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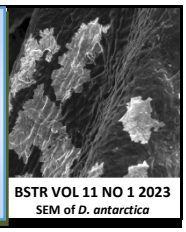

Bacteriological Survey of Abattoir Wastewater in Aba, Abia State, Nigeria

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ABSTRACT

The bacteriological survey of abattoir wastewater in Aba was investigated. Samples of wastewater contaminated with abattoir waste were collected at ten different spots in the abattoir. Nutrient agar was utilised as the media for the total aerobic plate count, McConkey agar was used for the coliform count, and cellulolytic media was used for the cellulolytic count. It was done using the pour plate method. The bacteria were identified using colonial morphology, gram staining, and biochemical assays. All of the aerobic plates, coliform count and cellulolytic count for the abattoir wastewater ranged from 1.03 x10^7 to 7.1 x 10^6 CFU/mL, 5.8 x 10^6 to 1.2 x 10^6 CFU/mL and 3.7 x10^6 to1.3x 10^6 CFU/mL, respectively. *Streptococcus* spp., *Pseudomonas* spp., *Staphylococcus aureus*, *Bacillus* spp., *Micrococcus* spp., *Vibrio cholerae*, *Klebsiella* spp., and *Escherichia coli* were among the bacteria that were isolated. This survey further confirmed the presence of varying bacteria genera in abattoir wastewater and the expedient nature of treating wastewater rather than releasing it to the physical environment as it poses a threat to public health.

INTRODUCTION

The word "abattoir" originates from the French word, "*abattre*", which connotes literarily "bring down". An abattoir is a slaughterhouse or an approved location that is marked, legally authorised and registered by a standard regulatory body for the inspection of animals, appropriate and hygienic slaughtering, processing and efficient preservation and storage of meat products for human consumption [1]. Various activities are known to be carried out in abattoirs and these activities are aimed towards processing the useful and edible portions of slaughtered animals that will be of good use for humans [2,3].

In Nigeria, over a thousand animals ranging from cattle, goats, rams, and chickens are butchered daily in numerous abattoirs scattered around the different parts of the country as a result of rising demand for the consumption of protein-based food substances by the ever-growing population of the country. Wastes in general can be grouped into two groups depending on their origin thus wastes can be organic or inorganic. Wastes emanating from organic sources such as plants and animals are known as organic wastes conversely, those arising from inorganic sources are referred to as inorganic wastes [4,5].

Frank Whittle and Insam [6], in their study, defined abattoir waste as the parts of an animal which are not useful for the manufacturing of food products and can include internal organs, blood, bones, ligaments and tendons. Urine, faeces and carcasses are also included [7]. Abattoir wastewater comprises mainly intestinal content, blood and water. Abattoirs are largely known to cause pollution in the environment through the improper discharge of waste materials arising from the various processes carried out in the abattoir. Microbes like bacteria arising from abattoir wastes in their ubiquitous nature possess the ability to

find their way into several water columns culminating in them getting to the sediment region, through the process of agitation, these sediments stimulate the return of the bacteria into water columns consequently becoming lethal to the sustenance of stability in the health status of the public [8,9]. Various researchers in their independent studies have posited that the bodies of animals can serve as the home of several pathogens [10-12]. In a similar way, these pathogens, such as rotaviruses, hepatitis E virus, *E. coli* O157:H7, *Campylobacter* spp., *Cryptosporidium parvum, Salmonella* spp., *Giardia lamblia*, and *Yersinia enterocolitica*, could be left behind in the waste products of these animals.

In different regions of Nigeria particularly in the Southern part, where Aba metropolis falls on the map, several pathogenic bacteria species have been identified in different abattoir effluents, more predominantly are *Staphylococcus* spp., and *Streptococcus* spp., which lucidly portray the lethal nature of untreated abattoir effluent that is usually released into the environment inhabited by humans thus serving as a serious public health concern [13].

A high percentage of abattoirs in the continent of Africa are usually situated proximally to water bodies, where water utilized in the processing of slaughtered animals is readily accessible [14]. The seeming unending quest to increase the quantity of available meat products has been linked to some pollution problems [15]. Like other forms of discharged sewage, abattoir wastewater eventually finds its way into natural bodies of water such as; groundwater, streams, rivers, lakes, and oceans as a result of natural drainage patterns [16,17]. Also, the disposal of animal blood, rumen content and hooves into water bodies untreated often leads to the rendering of such water bodies unfit and lethal for human consumption.

The untreated dumping of animal waste into the receiving environment has been demonstrated to increase the build-up of toxins in biological systems, deplete the oxygen in the environment, and increase the availability of nutrients [18]. Recent research has shown that in more than 80% of Nigeria's public abattoirs, zoonoses caused by slaughterhouse waste have not yet been entirely eliminated [19]. Activities in the abattoir have been linked with some prevalent diseases such as typhoid fever, pneumonia, diarrhoea, cholera, asthma, respiratory and chest diseases no thanks to the intake of and utilization of water contaminated by sewage from animals involved in the abattoir processes [1,20,21]. This study aims to survey the total bacteria genera domicile in the abattoir water waste and their percentage occurrence.

MATERIALS AND METHOD

Area of Study

Aba, which lies in South-East Nigeria, is the commercial centre of Abia State. The abattoir is situated in the Ogbor Hill area of Aba (**Fig. 1**). The site was selected for the study because it serves as the only site in the area where animal slaughtering activities is at their highest point and is responsible for over 70% of beef produced in Aba. They are all designed and constructed in a distinct form so as to house the slaughtering of cattle within the range of eighty to ninety daily. The animals are slaughtered daily between the hours of eight to ten in the morning.

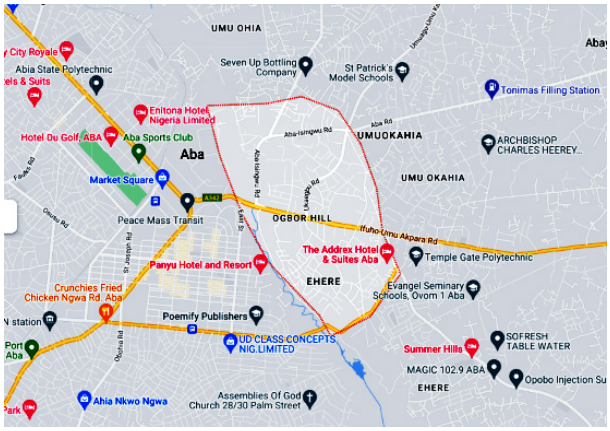

Fig. 1. Map of Aba, Abia State [22].

Sample Collection

The abattoir wastewater samples were collected using sterile 5 mL syringes from ten different locations around the abattoir between April and May 2016. They were then properly labelled, transported in an ice-parked cooler to the Microbiology Laboratory at the Michael Okpara University of Agriculture in Umudike, Abia State, Nigeria, and immediately analysed once they arrived at the lab.

Chemical Reagents

Chemical reagents from BDH Chemicals, Pooles England, and Sigma Chemical Company, St. Louis, Missouri, USA, were used in the investigation and were of analytical grade. The microbiological media used included nutrient agar, which was used to estimate the total heterotrophic aerobic bacteria, purify isolates, and establish stock cultures; thiosulphate bile salt agar (TCBS), which was used to isolate *Vibrio cholerae*; and McConkey agar, which was used to isolate coliforms, both of which were made by Oxoid and Difco Laboratories in England. To isolate cellulolytic bacteria, a cellulolytic medium was created and used.

Enumeration of Total Heterotrophic Bacteria

The wastewater samples were serially diluted ten times with the abattoir wastes. The total viable heterotrophic aerobic count was calculated using the pour plate method. To isolate the total heterotrophic bacteria, coliforms, and *Vibrio cholerae*, 45 °C molten nutrient agar, McConkey agar, and TCBS were then aseptically poured into petri plates containing 1 mL of the appropriate dilution. Colony counts were performed after the plates were incubated at room temperature for 48 hours. They were then conserved by subculturing onto nutrient agar slants, which were used for biochemical testing. The following formulas were used to determine the bacteria load in each situation.

$$\text{TVC(CFU/mL)} = \frac{1}{v} \times \frac{1}{N} \times \frac{1}{D} \qquad [23]$$

Where,

N=Numbers of colonies counted
V=Volume of inoculum
D=Diluting factor

Enumeration of Cellulolytic bacteria

The cellulolytic media mentioned in [24] was utilised for the cellulolytic bacteria count. $CaCO_3$, 2 g, $MgSO_4.7H_20$, 1 g, K_2HPO_4, 1 g, $(NH_4)_2SO_4$, 1 g, cellulose powder, 5 g, and agar, 15 g were included in 1 L of distilled water. Following the plating of the samples in duplicate using the pour plate technique and 1 ml of the proper dilution on Petri dishes, the cellulolytic were then counted. The appropriate Petri dishes were filled with molten media in order to isolate these bacteria. They were mixed thoroughly and then given time to set. These bacteria were counted after 48 hours of incubation at room temperature. On agar plates, colonies of cellulolytic bacteria were counted, extracted, purified by streaking on the cellulolytic media, and then maintained on the medium used for biochemical analysis. The following formulas were used to determine the bacteria load in each situation.

$$\text{TVC(CFU/mL)} = \frac{1}{v} \times \frac{1}{N} \times \frac{1}{D} \qquad [23]$$

Where,

N=Number of colonies counted
V=Volume of inoculum
D=Diluting factor

Characterization and Identification of Bacterial Isolates

The Gram reaction and cell morphology were examined after which the bacterial isolates were characterised and identified. Other tests included those for spore formation, motility, oxidase and catalase production, citrate utilisation, glucose oxidative/fermentation (O/F) utilisation, indole and coagulase production, starch hydrolysis, sugar fermentation, methyl red-Voges Proskauer reaction, and urease production. The tests were conducted in accordance with the methodologies of Collins et al. [25]; Cheesbrough [26]; Adeoye [27]; Agwung-Fobellah and Kemajou [28]; and Ochei and Kolhatkar [29]. The Bergeys Manual of Determinative Bacteriology's keys were used for bacterial identification [30].

RESULTS

The bacteria that were isolated and their frequency of occurrence are displayed in **Table 1**. The bacteria and their percentage occurrence were *Pseudomonas* spp 9.5%, *Staphylococcus aureus* 26.2%, *Bacillus* spp 11.9%, *Micrococcus* spp 4.8%, *Vibrio cholerae* 4.8%, *Klebsiella* spp 21.4% and *Escherichia coli* 7.1%. *Staphylococcus aureus* had the highest of 26.2%, seconded by *Klebsiella* spp with 21.4% occurrence while *Vibrio cholerae* and *Micrococcus* spp showed the lowest percentage occurrence of 4.8%. **Table 2** provides a concise total anaerobic plate count from the wastewater samples from the abattoirs collected from the 10 various places, each ranging from the highest bacterial load to the lowest. 1.03 x 10^7 to 7.1 x 10^6 CFU/mL, coliform count range from 5.8 x 10^6 to 1.2 x10^6 CFU/mL and the cellulolytic count ranged from 3.7x10^6 to 1.3 x 10^6 CFU/mL.

Table 1. Isolated bacteria from abattoir wastewater and their percentage occurrence.

Bacteria	No. of isolates	% occurrence
Pseudomonas spp	4	9.5
Staphylococcus aureus	11	26.2
Klebsiella spp	9	21.4
Streptococcus spp	6	14.3
Bacillus spp	5	11.9
Micrococcus spp	2	4.8
Escherichia coli	3	7.1
Vibrio cholera	2	4.8

Table 2. Bacterial count of abattoir wastewater samples.

Sample location	TAPC	CC	CLC
AWS 1	7.1×10^6	5.8×10^6	3.7×10^6
AWS 2	6.8×10^6	3.6×10^6	2.1×10^6
AWS 3	5.3×10^6	3.3×10^6	2.7×10^6
AWS 4	5.6×10^6	20×10^6	3.2×10^6
AWS 5	4.2×10^6	2.5×10^6	2.0×10^6
AWS 6	1.03×10^7	4.7×10^6	1.3×10^6
AWS 7	1.01×10^7	3.1×10^6	2.2×10^6
AWS 8	3.4×10^6	1.6×10^6	2.5×10^6
AWS 9	3.1×10^7	1.2×10^6	3.4×10^6
AWS 10	1.0×10^7	2.2×10^6	3.0×10^6

Legend:
AWS = Abattoir wastewater site
TAPC = Total aerobic plate count
CC = Coliform count
CLC = Cellulolytic count

DISCUSSION

The study provides results of the bacteriological analysis of abattoir water waste. The bacteria that were isolated from the wastewater from the abattoir included *Staphylococcus aureus*, *Streptococcus* spp., *Pseudomonas* spp., *Micrococcus* spp., *Bacillus* spp., *Vibrio cholerae*, *Klebsiella* spp., and *Escherichia coli*. The investigation found that the wastewater from the abattoir has a significant bacterial load. This outcome is consistent with the works of Adesomoye et al. [31), Eze et al. [32), Idu et al. [5), Ezeronye and Ubalua [12], Edward et al. [10], Bobor et al. [11].

The high bacterial load in the Aba abattoir wastewater depicts a high level of contamination as proved by this study and shows the lethal nature of discharging untreated wastewater to water bodies, hence the need for proper treatment to ensure decontamination [14]. Additionally, this study's findings demonstrated that the total bacteria and total coliform counts are higher than those recommended by the Federal Environmental Protection Agency (FEPA 1991) [33] and the World Health Organisation (W.H.O 2004) [34]. This high bacterial count in the wastewater can be said to be due to the discharge of the whole blood which has a high protein content hence serving as a medium for the growth of bacteria [35).

The occurrence of some bacterial species *Escherichia coli*, *Klebsiella* spp depicts the high tendency of contamination of the wastewater by the untreated faeces of the slaughtered animals disposed into such waters [32,36,37]. The presence of *Vibrio cholerae* in the abattoir wastewater may lead to the spread of cholera, a well-known water-borne disease when this wastewater is disposed untreated into water bodies that serve as drinking water to the populace. Cellulolytic bacteria prevalent in water waste are known to be responsible for the decomposition of cellulose materials present in the wastewater [32].

CONCLUSION

The high bacterial load of the abattoir wastewater clearly suggests contamination as shown by this report and has validated why it is unhealthy to discharge wastewater untreated to the surrounding environment. Hence, more stringent measures should be taken in the enforcement of laws of environmental protection so as to ameliorate the degree of prevalence of environmental pollution and related diseases. Therefore, there is a need to follow strictly the laws guiding waste treatment and disposal. Although beyond the scope of this study, the use of antibiotics in the rearing of animals slaughtered at the abattoir is becoming a growing concern globally since these antibiotics more often than not are not metabolized completely in the system of the animals and thus are released into the environment as

metabolites contained in their urine and faeces. Consequently, this facilitates the widespread of antibiotic resistance genes (ARGs) in aquatic habitats receiving these wastes. Thus, further studies should be done in attempting to profile the distribution and occurrence of different ARGs in waterbodies where these waste materials are disposed into mostly in an untreated form and how they pose a threat to the survival and thriving of humans in the environment. Finally, the conversion of solid waste emanating from abattoir processes through a fermenter into compost and biogas can be a good option, considering the dwindling oil sector of our nation and its associated problems of the release of carbon monoxide, which competes with the Oxygen in the atmosphere.

CONFLICTS OF INTEREST

The authors declare no conflicting interest.

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