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Isolation of Bacteria and Fungi from Effluent from Gusau Modern Abattoir

Abdullah Manir¹, Mehraj Gul², Safiullah Khan Achakzai³, Safia Gul⁴, Allah Nawaz Khan⁵, Mehmoona Safeer⁶, Safiullah Khan⁷, Uzma Ayaz⁸, Hamid Shah⁹, Muhammad Junaid Alam¹⁰, Habibullah¹⁰, Matiullah¹¹, Asif Ali¹², Sana Ashiq¹³, Kanwal Ashiq¹⁴, Unsah Tabassim¹⁵, Nadia Hameed¹⁶.

¹BSC Microbiology (UDUS)

²National Institute of Health, Islamabad.

³Center for Advance Studies in Vaccinology & Biotechnology (CASVAB) University of Baluchistan, Quetta.

⁴Assistant Professor Department of Botany Sardar Bahadur Khan Women University Barewary Road Quetta.

⁵Department of Botany, University of Agriculture Faisalabad.

⁶P.hD Scholar Department of Chemistry, Hazara University, Mansehra, Pakistan.

⁷Lecturer in Government College NO.1 D.I. Khan.

⁸Department of Plant Breeding & Molecular Genetics, University of Poonch Rawalkot Azad Jammu & Kashmir.

⁹College of Pharmacy, Pharmaceutics Department University of Sargodha, Sargodha Pakistan.

¹⁰Department of Microbiology, Quaid-i-Azam University Islamabad.

¹¹Department of Animal Sciences, Quaid-i-Azam University Islamabad.

¹²Department of Zoology and Biology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi, Pakistan.

¹³Sharif Medical Research Centre and Dental College Lahore.

¹⁴Department of Pharmacy Superior University Lahore.

¹⁵M.Phil Scholar Chemistry, Women University Bagh Azad Kashmir.

¹⁶Assistant Professor Chemistry, Government Post Graduate College Bagh Azad Kashmir.

Corresponding Author's Email: Manirabdullahi010@gmail.com

ABSTRACT

The present research on the Isolation of Bacteria and Fungi from Wastewater Emanating from Gusau Modern Abattoir revealed a very high contamination of the wastewater by different bacterial and fungal isolates. The bacterial species isolated includes: *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, and *Shigella dysenteriae*. An average total count of 4.6×10 was observed for the bacterial isolates. Fungal species isolated were: *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Oidiodendron griseum*. Similarly, an average fungal count of 5.3×10 cfu/ml was recorded. Total coliform count of the wastewater ranged between 400-1100MPN/100mml. All there counts exceeded the World Health Organization's standard limit for wastewater. Thus, the wastewater is not supposed to be discharged into the environment without adequate treatment. The bacterial species isolated from the wastewater have also shown some pattern of resistance to some commonly used antibiotics. Thereby, endangering the lives of community members through the spread of untreatable infectious diseases.

Key words. Isolation, Bacteria, Fungi, Effluent, Guasue Modern abattoir.

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INTRODUCTION

The abattoir is a specialized facility approved and registered by regulatory authority for inspection of animals, hygienic slaughtering, processing and effective preservation and storage of meat products for human consumption [1]. Abattoir effluents are waste water derived from animal slaughtering activities in abattoirs, consisting mainly of intestinal contents, blood and water. Abattoir effluent

like other types of discharged sewage, eventually enter natural bodies of water like ground water, streams, rivers, lakes and oceans as a result of natural drainage pattern and sequence [2-3]. These water bodies are used by human beings for drinking, household, industrial, agricultural (irrigation), swimming and other recreational purposes. Drinking water and recreational water have been implicated in the transmission of pathogens, and it was opined that the source of contamination could be either sewage or infected animals[4-5].

A number of bacteria species, including coliforms and Listeria can be present in the Intestines of some humans and animals, including birds without causing infection [6]. Hence, listeria is could be transmitted through water bodies contaminated by abattoir effluent.

The disposal of abattoir effluent which feeds natural bodies of water and the monitoring of the bacterial status of such effluent are of public health significance [7-8], especially in developing countries like Nigeria, where abattoir effluent are discharged untreated. Abattoir effluent, like other types of industrial sewage are supposed to undergo various stages of treatment to eliminate or remove bacterial content before being discharged into drainage to enter the natural bodies of water [8-9].

Furthermore, the presence of various types of bacteria species in abattoir effluent makes it a conducive environment for the transmission of antimicrobial resistance amongst them [10]. Antimicrobial resistance has generally undergone near exponential increase in the past decades [11]. Prophylactic use of common broad spectrum antibiotics as well as empirical preemptive therapy in high risk settings, or indiscriminate usage, particularly in developing nations, has further accentuated this trend, especially in patients with underlying malignancy[11-12].

Fungi are widely distributed in nature and can occur as unicellular yeast or filamentous and, multicellular molds. Despite their wide occurrence, little attention has been given to their presence and significance in aquatic environments [13]. In 1980s and 1990s, more cases of health problems caused by fungal contaminated drinking water were reported from Finland and Sweden [14-15]. Fungi have been reported from all types of water [16-17].

The blood is collected by the authorities, but, the intestinal content are discharged into the drainage within the abattoir. This drainage channels into many areas of the abattoir were meat is washed and meat can get contaminated from this point due to splashes of water from the drainage. It also passes near the slaughter slab were it can contaminate if mishandled. Water bodies also get contaminated from discharge of untreated abattoir effluent.

Because of the possible health consequences of the disposal of untreated abattoir effluent in the environment, as they are a major source of contaminants to our meat and to our sources of drinking water, there is the need to carry out a microbiological assessment of the wastewater and draw the attention of the government and managers of abattoir environment to the implications.

The aim of this research is to study the microbiological quality of wastewater emanating from Gusau Modern Abattoir.

The objectives of this research are:

- To isolate bacteria and fungi from abattoir wastewater.
- To determine the bacterial and fungal load of the abattoir wastewater.
- To determine the sensitivity of the bacterial isolates to some common antibiotics.

MATERIAL AND METHODS

Study Area

Gusau modern abattoir is the main abattoir in the State. Cattle, sheep, goats and camels are the animals that are slaughtered for human consumption in the abattoir with an average daily slaughter figure of about 40 heads of cattle, 6 camels, 5 goats and 25 sheep.

Sample Collection and Analysis

A total number of six (6) samples of wastewater were examined. Samples were collected from the main drainage of Gusau modern abattoir which carries animal's intestinal, stomach and urinary discharges and water. The samples were collected for a period of three (3) days, two (2) samples per day. Sample were collected at 9:00am and after 11:00am daily in sterile plastic container (500ml volume), aseptically transported to UDUS microbiology laboratory and kept at low temperature and then, analyzed.

Serial Dilution

Serial decimal dilution of each sample was prepared by using sterile water as diluents. 1g of a well-mixed sample was transferred into a test tube of 9ml sterile water labelled 10^{-1} . It was then shaken thoroughly, and then, 1ml was transferred from this test tube to another test tube of 9ml sterile water labelled 10^{-2} . It

was shaken thoroughly, and the procedure was repeated to obtain 10^{-3} , and 10^{-4} test tubes. A control will be prepared with purely sterile water with no sample [18].

Culture Media and their Preparation

All microbiological media used were prepared according to the directions of the manufacturers and they include:

Nutrients Agar

A total of 4.8g was weighed properly and poured into a sterile conical flask -200ml of water was added and swirled to dissolve, and then beated . The medium was autoclaved of 121°C for 15minutes The medium was allowed to solidify on the media on the laboratory bench before use [18].

MacConkey Broth

Mac Conkey broth was used for coliform count. Both single strength and double strength broth was prepared. A total of 8.0grams of commercially available Mac Conkey broth powder was suspended in 100ml and 200ml of sterile distilled water for single strength and double strength broth respectively. Both were heated for 15minutes, and then transferred into sterile test tubes, 10ml each, and then, Durham tubes were inverted in all the test tubes and they were autoclaved at 121°C for 15minutes [19].

Potato Dextrose Agar

Potato Dextrose Agar was used for the isolation, was prepared according to manufacturer's instruction. The molten medium was poured into conical flasks, plugged with aluminum foil. The medium was sterilized by autoclaving at 121°C at a pressure of 15 pound for 15 minutes. After sterilization, 15 ml of medium was aseptically dispensed into sterile petri dishes and allowed to solidify. The petri dishes were labeled accordingly [19].

Preparation of Nutrient Agar slant

A total of 1.3gm nutrient agar powder was dissolved in 100ml distilled water. This was boiled to dissolved and sterilized by autoclaving at 121°C for 15minutes after dispensing in bijou bottles. The medium was allowed to solidify in a slanting position and later packed and stored at 4°C in the refrigerator [18].

Preparation of Mueller-Hinton Agar

A total of 7.6gm Mueller-Hinton (MH) agar powder was weighed and suspended in 200ml of distilled water and boiled for 10 minutes to dissolve. The agar was sterilized byautoclaving at 1210C for 15 minutes. The agar was allowed to cool in a water bath and thereafter [18].

Urease Agar

This was prepared by suspending 2.4gm of urea base in 95ml of distilled water. It was dissolved by shaking and the suspension was sterilized by autoclaving at 121oC for 10minutes. Then 2gm of urea was dissolved in 5ml of distilled water and boiled for about 30minutes. The two solutions were allowed to cool in a water bath set at 45oC. The urea was added to the urea base and the mixture was then dispensed, in 5ml amounts into half ounce bottles. The media bottles were then kept in a slant position to solidify [18].

Simmon's Citrate Agar

A total of 4.6gm SCA powder was suspended in 200ml of distilled water. This was boiledto dissolve completely. It was then dispensed into half ounce bottles (5ml per bottle) and sterilized by autoclaving at 121oC for 10 minutes. They were then placed in a slanting position to solidify [18].

MR/VP Broth

A total of 3grams was suspended in 200ml of deionised water, it was swirled and mixed, then, hot plated for 15minutes, It was then dispensed into tubes and sterilized by autoclaving for 15minutes at 121°C [19].

Triple Sugar Iron Agar

A total of 6.5gm TSI powder was suspended in 100ml deionised water, soaked for 10minutes, swirled and mixed then boiled. It was then dispensed into tubes and sterilized by autoclaving for 15minutes at 121oC. They were later paced in a slanting position to solidify, ensuring that the slant is over a butt at about 3cm deep [18].

Analysis of Microbial Load

Total Viable Count

For this purpose, dilutions were made upto 10-5 for each samples. 0.1ml of dilution of 10-5 for each sample was inoculated on Nutrient agar medium using pour plate method for water samples.

Most Probable Number of Coliforms

In the procedure, a series of MacConkey broth tubes are inoculated with measured amounts of the water sample to be tested. The series of tubes may consist of three or four groups of three, five or more tubes. The more tubes utilized, the more sensitive the test. Gas production in any one of the

tubes is presumptive evidence of the presence of coliforms. The most probable number (MPN) of coliforms in 100 ml of the water sample can be estimated by the number of positive tubes. The Most probable number (MPN) technique for coliform and total coliform was used for the water analysis. The procedure, which involved the use of three dilutions (10, 1, and 0.1 ml) of each sample, was adapted from [20].

Total Count of Fungi

Fungal colonies were counted based on their color on the surface and reverse side of each plate and reported as frequency of occurrence of isolated fungi per sample.

Identification of Isolates

Gram Staining

In the staining process, a thin smear was prepared by adding a drop of sterile water on a glass slide and then a heat sterile wire loop was used to pick a part of a single significant colony and it was emulsified with the drop of sterile water on the glass slide to make a thin smear. It was then heat fixed by passing it over a Bunsen flame twice. It was then stained with crystal violet for 60 seconds, then washed with water and then treated with iodine for another 60 seconds. It was then washed with water and then counter stained with safranin for another 60 seconds. It was then be washed with water and allowed to air dry. The stained smears were examined microscopically using 100X oil immersion objective and 10X eye piece [21].

Biochemical Test

Urease Test

A portion of each test colony was streaked on urea agar slant and incubated for 24hrs at 37°C. Urease positive organisms turned the medium red. *Salmonella* is urease negative [22].

Citrate Test

A colony of the test organisms was inoculated on the surface of simmon's citrate agar slant and incubated overnight at 37°C for 24hrs. The color changed to blue indicating that the organism utilized the citrate as a sole source of carbon [18].

Indole Test

Tryptone water was inoculated with a colony of the test organisms and incubated at 37°C for 48hrs. 1ml of Kovac's reagent was run down the side of the tube into the medium. This test was for the ability of the organism to convert tryptophan (amino acid) to indole. Indole production was indicated by a deep-red coloration at the top of the broth, production of a yellow ring indicated indole negative [19].

Methyl Red Test

This test determines the ability of the test organism to ferment glucose and produce a pH of 4.5. Peptone water culture of suspected *Salmonella* organism was inoculated into glucose phosphate peptone water and incubated at 37°C for 48hrs. Thereafter 5 drops of methyl red indicator were run down the side of the tube. A pink ring on the surface of the medium indicated a methyl red positive reaction [23].

Voges Proskaur Test

This test was used to detect which of the isolates were able to produce a neutral red end point acetyl methyl carbinol (acetone) from glucose fermentation or its reductive product butylenes glycerol. The test is usually used to differentiate between Gram negative organisms especially members of the Enterobacteriaceae. Monica cheese [24] Inoculate the suspected organism into a test tube containing buffered glucose peptone water and incubate at 37°C for 24 hours. Into the incubated medium, add 0.6% w/v solution of A and 0.2ml of solution B Shake the mixture and live to stand. A red color is a positive result. While the development of a yellow color indicates a negative reaction. Solution A Contains 5g of - naphlho100ml absolute ethyl alcohol Solution B contains100ml Distilled water 40g potassium hydroxide.

The alkalis oxidize the acetyl methyl carbonyl (acetone) to diacetyl which gives the pink color.

Catalase Test

This test was done according to monica cheese [24]. The test was performed by dropping a loopful of the isolate mix with the hydrogen peroxide on the slide. The production of gas bubbles (O₂) from the mixture which will occur almost immediately is a positive reaction.

Reaction on Triple Sugar Iron Agar

This test determines the ability of the test organism to produce hydrogen sulfide, gas and ferment glucose. A colony of the test organism was stabbed and the slant surface streaked and incubated overnight at 37°C for 24hrs. Hydrogen sulphide production gas production and sugars fermentation indicated TSI positive [18].

Identification of Fungi

Isolated fungi were identified by examining both microscopic and macroscopic characters. The Identification was aided by using identification kof Barnett [25-28].

Antibiotic Susceptibility Test

Antibiogram of the test isolates was determined using disc diffusion technique [29]. Antimicrobial disc were obtained from Oxoid Limited, Basingstoke Hampshire, England. Each colony of the test isolate was picked with a wire loop and inoculated into nutrient broth and incubated for 3hours. The turbidity of each broth culture was adjusted to correspond to 0.5 McFarland turbidity standards (corresponding to approximately 108cfu/ml). Each standardized broth culture was used to inoculate the surface of the Mueller-Hinton (MH) agar plates and the excess fluid drained into disinfectant jar. The surface of each inoculated plate was allowed to dry. Using a disc dispenser, the antibiotic discs were aseptically placed on the surface of the inoculated agar plates and then incubated for 24hrs at 37°C. After incubation, the plates were examined for zones of inhibition round each disc. The diameters of the zones were measured with a meter rule and recorded. Each test was conducted three times and the mean inhibition zone diameter (IZD) recorded to the nearest millimeter. Each test isolates was classified as either resistance, intermediate or sensitive to the test antibiotics in accordance with the criteria given [30].

RESULTS

The results of this research are presented in Tables 1 to 10. Table 1 shows the Bacterial colony count of Isolates. Table 2 shows the coliform count of the abattoir wastewater. Table 3 shows the morphological characteristics of bacterial colonies from the wastewater samples. Table 4 shows the biochemical characteristics of bacterial isolates from the wastewater samples. Table 5 shows the frequency of occurrence of bacterial isolates from the wastewater samples. Table 6 shows sensitivity test of bacterial isolates to some common antibiotics. Table 7 shows the sensitivity of profile of bacterial isolates to some common antibiotics. Table 8 shows the macroscopic and microscopic characteristics of fungal species isolated from the wastewater samples. Table 9 shows the frequency of occurrence of fungal species isolated from the wastewater samples. Table 10 shows the total count of fungal species isolated from the wastewater samples.

Table 1: Bacterial Load of Wastewater from the Abattoir Drainage

Sample Code	Number of Colonies	Total Count(cfu/ml)
A1	4 30	4.3×10^{-7}
A2	443	4.43×10^{-7}
B1	476	4.76×10^{-7}
B2	482	4.82×10^{-7}
C1	470	4.7×10^{-7}
C2	457	4.57×10^{-7}

Table 2: Coliform Count of Wastewater from the Abattoir Drainage

Sample Code	No of 10ml positive	No of 1ml positive	No of 0.1ml positive	MPN/100mml
A1	1	3	3	400
A2	3	3	3	1100
B1	1	3	3	400
B2	1	3	3	400
C1	3	3	3	1100
C2	1	3	3	400

MPN: Most Probable Number

Table 3: Morphological Characteristics of Bacterial Colonies

Sample Code	Colony Characteristics
A1	Small circular white colonies with smooth edges
	Small milky colonies with rough edges
	Small circular white colonies, raised with smooth edges
A2	Small circular white colonies with smooth edges
	Small circular white colonies, raised with smooth edges
	Large white colonies with rough edges and raised surface
B1	Small circular white colonies with smooth edges
	Large milky flat colonies with rough edges
	Large milky flat colonies with rough edges
B2	Small milky colonies with rough edges
	Small circular white colonies, raised with smooth edges
	Large white colonies with rough edges and raised surface
C1	Small circular, raised white colonies with rough edges
	Small circular white colonies, raised with smooth edges
C2	Small circular, raised white colonies with rough edges
	Small milky colonies with rough edges
	Large milky flat colonies with rough edges

Table 4: Biochemical Characteristics of Bacterial Isolates

Sample Code	Gram Reaction	Catalase Test	Citrate Test	Indole Test	Urease Test	MR Test	VP Test	Glucose Test	Lactose Test	Sucrose Test	Motility Test	H2S Test	Gas Test	Identified Organisms
A1i	-rods	+	+	-	-	+	-	+	+	+	+	-	-	<i>Pseudomonas aeruginosa</i>
A1ii	-rods	+	+	-	-	-	+	+	+	+	+	-	+	<i>Enterobacter aerogenes</i>
A1iii	-rods	+	-	+	-	+	-	+	+	-	+	-	+	<i>Escherichia coli</i>
A2i	-rods	+	+	-	-	+	-	+	+	+	+	-	-	<i>Pseudomonas aeruginosa</i>
A2ii	-rods	+	-	+	-	+	-	+	+	-	+	-	+	<i>Escherichia coli</i>
B1i	-rods	+	+	-	-	+	-	+	+	+	+	-	-	<i>Pseudomonas aeruginosa</i>
B1ii	-rods	+	-	+	+	+	-	+	+	-	+	+	-	<i>Proteus vulgaris</i>
B1iii	-rods	+	+	+	-	+	-	+	+	+	-	-	+	<i>Klebsiella pneumoniae</i>
B2i	-rods	+	+	-	-	-	+	+	+	+	+	-	+	<i>Enterobacter aerogenes</i>
B2ii	-rods	+	-	+	-	+	-	+	+	-	+	-	+	<i>Escherichia coli</i>
B2iii	-rods	+	-	+	+	+	-	+	+	-	+	+	-	<i>Proteus vulgaris</i>
C1i	-rods	+	+	-	-	+	-	+	-	-	-	-	+	<i>Shigella dysentriiae</i>
C1ii	-rods	+	-	+	-	+	-	+	+	-	+	-	+	<i>Escherichia coli</i>
C2i	-rods	+	+	-	-	+	-	+	-	-	-	-	+	<i>Shigella dysentriiae</i>
C2ii	-rods	+	+	-	-	-	+	+	+	+	+	-	+	<i>Enterobacter aerogenes</i>
C2iii	-rods	+	+	+	-	+	-	+	+	+	-	-	+	<i>Klebsiella pneumoniae</i>

Table 5: Frequency of Occurrence of Bacterial Isolates

Names of Isolates	Frequency of Occurrence (%)
<i>Escherichia coli</i>	25%
<i>Pseudomonas aeruginosa</i>	18.75%
<i>Klebsiella pneumoniae</i>	12.5%
<i>Enterobacter aerogenes</i>	12.5%
<i>Shigella dysentriiae</i>	18.75%
<i>Proteus vulgaris</i>	12.5%
Total	100%

Table 6:Sensitivity Test of Bacterial Isolates to Some Common Antibiotics

Names of Isolates	Zone STR	of CEF	Inhibition NIT	(mm) CXC	CIP	GEN	AUG	OFL
<i>Eschericia coli</i>	13	0	0	10	28	23	0	33
<i>Enterobacter aerogenes</i>	12	0	16	11	25	25	0	34
<i>Pseudomonas aeruginosa</i>	13	0	14	10	29	22	0	31
<i>Klebsiella pneumoniae</i>	13	11	0	28	14	25	0	30
<i>Proteus vulgaris</i>	17	12	0	27	25	30	0	31
<i>Shigella dysentriiae</i>	15	0	11	27	16	28	0	32

KEY: STR: Streptomycin, CEF: Ceftriaxone, NIT: Nitrofurantoin, CXC: Cloxacillin, CIP: Ciprofloxacin, GEN: Gentamycin, AUG: Augmentin, OFL: Ofloxacin.

Table 7: Sensitivity Profile of Bacterial Isolates to Some Common Antibiotics

Names of Isolates	STR	CEF	NIT	CXC	CIP	GEN	AUG	OFL
<i>Eschericia coli</i>	I	R	R	R	S	S	R	S
<i>Enterobacter aerogenes</i>	I	R	I	R	S	S	R	S
<i>Pseudomonas aeruginosa</i>	I	R	I	R	S	S	R	S
<i>Klebsiellapneumoniae</i>	I	R	R	S	I	S	R	S
<i>Proteus vulgaris</i>	I	R	R	S	S	S	R	S
<i>Shigella dysentriiae</i>	I	R	R	S	I	S	R	S

KEY: STR: Streptomycin, CEF: Ceftriaxone, NIT: Nitrofurantoin, CXC: Cloxacillin, CIP: Ciprofloxacin, GEN: Gentamycin, AUG: Augmentin, OFL: Ofloxacin, S: Susceptible, I: Intermediate Susceptible, R: Resistant.

Table 8: Macroscopic and Microscopic Characteristics of Fungi Isolated from Abattoir Effluent

Macroscopy	Microscopy	Identified Isolates
The upper surface of colonies was olive green with white edge, granular surface and green coloration on the reverse side	The conidiophore was thick walled, hyaline and slightly roughened, erect, long, aseptate with a vesicle at the top with phialides and with short conidial chains.	<i>Aspergilus flavus</i>
The colonies were widely spread, black, with smooth white edges and spongy surface densely packed and brown on the reverse side	The conidiophore was long, erected from the base to the vesicle, smooth walled, hyaline with globes conidial head.	<i>Aspergilus niger</i>
The colony was widely spread, dark green with smooth white edges and spongy surface and brown on the reverse side	The conidiophore was long, narrow at the base and broad near the vesicle, smooth walled hyaline.	<i>Aspergilus fumigatus</i>
The colony is white with smooth edge and tuft surface and on the reverse side is brown in colour	The macroconidia are canoe shaped, multiseptate which contain 3-6 septations and slightly pointed at the end.	<i>Fusarium oxysporum</i>
The colony was white with cottony surface and on the reverse side is brown in coloration.	Dark mycelium hyaline, long mycelium cell and branched at the upper part, no septation of branches set off from the main hyphae.	<i>Rhizoctonia solani</i>
The colony was white with irregular edge and spongy surface and brown in colour on the reverse.	The conidiophore was irregularly branched at the upper portion and the branches segmented into rod-shaped conidia which were in chains.	<i>Oidiodendron griseum</i>

Table 9:Frequency of Occurrence of Fungal Species Isolated

Names of Isolates	Number of Colonies of Isolates	Frequency of Occurrence
<i>Aspergilus niger</i>	12	38.7%
<i>Aspergilus fumigatus</i>	7	22.6%
<i>Aspergilus flavus</i>	4	12.9%
<i>Rhizoctonia solani</i>	2	6.4%
<i>Fusarium oxysporum</i>	3	9.7%
<i>Oidiodendron griseum</i>	3	9.7%
Total	31	100%

Table 10: Total Count of Fungal Species Isolated (cfu/ml)

Sample Code	Number of Colonies	Total Count
A1	6	6.0×10^3
A2	5	5.0×10^3
B2	5	5.0×10^3
B2	4	4.0×10^3
C1	7	7.0×10^3
C2	4	4.0×10^3

DISCUSSION

The total bacterial count of wastewater samples from Gusau Modern Abattoir revealed an average count of 4.6×10 cfu/ml and an average Fungal count of 5.2×10 cfu/ml. This is higher for the wastewater according to World Health Organization's standard limit (1×10^2 cfu/ml). Any water Contaminated to this extent is neither good for domestic use nor it is supposed to be discharged into the environment directly without treatment. The abundance of microorganisms may be due to the abundance of nutrients as revealed by [31]. The average bacterial and fungal count in this research are similar to the findings of [32] which was 4.9×10 to 7.3×10 cfu/ml of bacteria and average fungal count of 1.17×10 cfu/ml from wastewater in Sokoto abattoir.

Total coliform count Most Probable Number MPN/100mml of water from this research ranged from 400 to 1100MPN/100mml, which is also above the World Health Organization's limit for drinking water. This concurs with the findings of [33] that reported a total coliform count of 400 to 1100MPN/100mml from Agege abattoir soil in Lagos state. High coliform count is an indication of the likelihood of the presence of pathogenic organisms, most of which causes waterborne diseases.

From the present study, bacteria and fungi isolated from the wastewater samples include *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Shigella dysentriae*, *Aspergilus niger*, *Aspergilus flavus*, *Aspergilus fumigatus*, *Rhizoctonia solani*, *Fusarium oxysporum*, and *Oidiodendron griseum*. This is also similar to the findings of [33].

The presence of *Escherichia coli* and its high occurrence of 25% in the wastewater samples from Gusau Modern Abattoir is an indication of fecal contamination. *Escherichia coli* are part of the normal flora of the human intestines. Some strains of *Escherichia coli* have been linked to diarrhea, gastroenteritis and urinary tract infections [33]. *Pseudomonas aeruginosa* and *Shigella dysentriae* are second to *Escherichia coli* from this research with a percentage occurrence of 18.75% each, followed by *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *Proteus vulgaris* which all have a percentage occurrence of 12.5% each.

The bacterial isolates from this study were resistant to ceftriaxone and augmentin, intermediate or partially sensitive to streptomycin, and sensitive to ofloxacin and gentamycin. *Escherichia coli*, *Enterobacter aerogenes* and *Pseudomonas aeruginosa* were resistant to cloxacillin but sensitive to ciprofloxacin. *Klebsiella pneumoniae* and *Shigella dysentriae* were sensitive to cloxacillin but intermediately sensitive to ciprofloxacin while *Proteus vulgaris* was susceptible to both cloxacillin and ciprofloxacin. *Enterobacter aerogenes* and *Pseudomonas aeruginosa* were intermediately sensitive to nitrofurantoin while *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris* and *Shigella dysentriae* were resistant to it. This findings are similar to those of [18] who reported antibiotic resistant strains of *Escherichia coli* and *Salmonella* spp from Nsukka abattoir and meat seller's tables at Nsukka market.

The fungal isolate with the highest occurrence in this study was *Aspergilus niger* with a percentage occurrence of 38.7%, followed by *Aspergilus fumigatus* and *Aspergilus flavus* with occurrence of 22.6% and 12.9% respectively. *Fusarium oxysporum* and *Oidiodendron griseum* both have a percentage occurrence of 9.7% while *Rhizoctonia solani* have the least occurrence of only 6.4%. The Isolated fungal species also colonies concurs with the findings of [31-34] who reported *Aspergilus* spp from Agege abattoir in Lagos state, Nigeria.

CONCLUSION

It can be concluded from the results of this research that wastewater emanating from Gusau Modern Abattoir have a high bacterial count of 4.6×10 cfu/ml and a high fungal count of 5.2×10 cfu/ml and high coliform count of 1,100 which exceeded the World Health Organization's standard limit the implication is that, these wastewater can be a source of contamination to our meat at the point of processing within the slaughter house, and also to our water bodies when discharged untreated. The findings from this study have also shown that the zoonotic pathogens from the abattoir wastewater are multiple antibiotic resistance to antibacterial agents used. This development is of serious public health significance

because the resistant isolates may be transferred to the consumers of such meat who will subsequently develop resistance to the therapeutic agents.

RECOMMENDATIONS

It can be recommended that;

- An adequate waste management system should be design for abattoir wastewater to ensure that wastewater are treated before discharge.
- Government agencies and relevant stakeholders should enlighten the general population on the possible impact of pollution from abattoir wastewater.
- Were possible, abattoir wastewater should be directed to irrigation lands as they are rich in nutrients and manure.
- Livestock farmers, animal health workers and veterinarians should avoid indiscriminate use and abuse of antibiotics in the management of livestock diseases and health Problem.
- Consumers of meat should cook their meat properly before consumption.

REFERENCES

1. Alonge D.O. (2001). Textbook of Meat hygiene in the tropics. 5th edition, *Farmcoo press Ibadan, Nigeria* Pp 61.
2. Madigan MT, Martinko JM, Parker J (1997). *Biology of Micro organism*, 8th Ed. New York. Prentice Hall
3. Pelcar MJ, Chan ECS, Kreig NR (2002). *Microbiology* 5th Ed. New Delhi. Tata McGraw – Hill.
4. Muniesa M, Jofre JG, Aljaro C, Blanch AR (2006). Occurrence of Escherichia coli O157: H7 and other enterohaemoragic E. coli in the environment. *Environ. Sci. Technol.* 40:7141-7149.
5. Sehgal R, Kumar Y, Kumar S (2008). Prevalence and geographical distribution of *Escherichia coli* O157 in India; a 10-year survey. *Royal Society of Tropical Medicine and Hygiene* 102:380-383.
6. Ramaswany V, Crescence VM, Rejitha JS, Lekshmi MU, Dharsana KS, Prasad SP, Vijila HM (2007). Listeria: review of epidemiology and pathogenesis. *J. Microbiol. Immunol. Infect.* 40:4-13. .
7. Black RE, Levine M, Clement ML, Hughes TP, Blaser MJ (1988). "Experimental Campylobacter jejuni infection in Humans" *J. Infect. Dis.* 157:472 – 479.
8. Hug A, Sack RB, Nizam A, Longini IM, Nair GB, Ali A, Morris J.G, Khan MNH, Siddique AK, Yunus M, Albert MJ, Sack DA and Colwell RR (2005). Critical Factors Influencing the occurrence of Vibrio cholerae in the environment of Bangladesh *Appl. Environ. Microbial.* 71:4645-4654
9. Nester EW, Roberts CE, Pearsall NW Anderson GO, Nester MT (1998). *Microbiology: A human Perspective* 2nd Ed. Boston. McGraw-Hill
10. Mach PA, Grimes DJ (1982). R-plasmid Transfer in a Wastewater Treatment Plant. *Applied and Environmental Microbiology.* 44: 1395-1403. th Ed. New York. Prentice Hall
11. Safdar A, Armstrong D (2003). Antimicrobial activities against 84 Listeria monocytogenes isolates from patients with systemic listeriosis at a comprehensive cancer center (1995-1997). *J. Clin. Microbiol.* 41:483-485.
12. Bondarianzadeh D (2007). Food Risk to Babies Listeriosis. *Nutrition Today.* 42:236-239.
13. [13]. Kirk, P.M., Cannon, P.F., David, J.C. and Stalpers, J.A. (2001). Ainsworth and Bisby's Microbiology Review Dictionary of the Fungi, 9th Edition. CAB International, Wallingford. Pp. 23-45.
14. Muittari, A., Kuusisto, P., Virtanen, P., Sovijarvi, A., Gronroos, P., Harmoinen, A., Antila, P. and Kellomaki, L. (1980). *An Epidemic of Extrinsic Allergic Alveolitis Caused by Tap Water*. Clinical Allergy, 10:77–90.
15. Aslund, P. (1984). Skin irritations cause by moulds (in Swedish). *Var Foda*, 36:327-336.
16. Mirian, U.Y., Rita, D.P.R., Sueli, F.Y.O., Celso, V.N., Tânia, U.N. and Benedito, P.D.F. (2007). Yeasts and Filamentous Fungi in Bottled Mineral Water and Tap Water from Municipal Supplies. *Brazilian Archives of Biology and Technology an International Journal*, 50:1-9.
17. Okpako, E.C., Osuagwu, A.N., Duke, A.E. and Ntui, V.O. (2009). Prevalence and Significance of Fungi in Sachet and Borehole Drinking Water in Calabar, Nigeria. *African Journal of Microbiology Research*, 3:56-61.
18. Onwumere, O. S. (2010). Isolation and Phenotypic Characterization of *E. coli* O157:H7 and *Salmonella* spp in a Slaughter House and Meat Tables at Nsukka Market, Enugu State, Nigeria. (Thesis). University of Nigeria Nsukka.
19. Oyeleke, S. B. and Manga, S. B. (2008). *Essentials of Laboratory Practicals in Microbiology*. First Edition, Tobest Publishers, Minna, Nigeria.
20. Shittu, O. B., Olaitan, J. O., and Amusa, T. S. (2008). Physico-chemical and Bacteriological Analysis of Water Used for Drinking and Swimming Purpose in Abeokuta, Nigeria. *Afr. J. of Biomedical Res.*, vol.11: 285-290.
21. Barnett, H.L. and B.B. Hunter, (1972). Illustrated Genera of Imperfect Fungi. *Burgers Publishing Minneapolis*, U.S.A., pp. 90 – 169.
22. Bauer, A.W; Kirby, M.M; Shries, J.C and Turk, M. (1966): Antibiotic susceptibility testing by a standardized single disk diffusion mtd. *American J. of Clin. Pathology*, Vol. 36 pp 493-496.
23. Cadmus, S. I., Olugasa, B. O. and Ogundipe, G. A. (1999). The Prevalence and Importance of Bovine Tuberculosis in Ibadan, in *Proceedings of the 37th Annual Congress of the Nigerian Veterinary Medical Association*, pp. 65-700.
24. E

25. Adwars P.R. and Ewing, W.H. (1972): Identification of enterobacteriacea. 3rd Edition; *Burgess Publishing Company*, Minneapolis Minnesota.
26. Gracey, J.F. (1986): Meat Hygiene, England Bailliere Tindal ELBS Publishers.
27. Klich A. M. (2002). *Identification of Common Aspergillus species*. Published by the Central Bureau Voor Schimmel cultures, Utrecht. Pp 1-115.
28. Larone, D. H. (2004). *Medically Important Fungi: A Guide to Identification*. ASM press, Washington D.C. 23-234.
29. Loverdo, J., Martel, H and Mallet, J. (1906): *Les Abattoirs Public H*. Dunod et Pinat, Editeurs, Paris.
30. Monica cheese brough (2005). *District Laboratory Practice in Tropical Countries*. Cambridge University Press.
31. Ogbonna, D. N. and Igbenijie, M. (2006). Characteristics of Microorganisms Associated with Waste Collection sites in Port Harcourt City, Nigeria. *Nigerian Journal of Microbiology*, 20(3): 1427-1434.
32. Ogofure, A. G., Ologbosere, O. A., Aluyi, H. S. A., Beshiru, A. and Omeje, F. I. (2016). Physicochemical and Microbiological Profile of Bacterial and Fungal Isolates of Ikpoba River in Benin City: Public Health Implications. *African Journal of Environmental Science and Technology*. 10(3): 67-76.
33. Olugasa B. O., Cadmus, S. I. B. and Atsanda, N. (2000). Actualization of Standards for Beef Quality and Control in South western Nigeria, *Proceedings of the 10th International Congress on Animal Hygiene in Maastricht*, Netherlands. 1: 67-71.
34. Samson A. R., Hoekstra S. E. and Prisvad C. J. (2004). *Introduction to Food and Airborne Fungi*, Seventh Edition. Published by the Centraal Bureau Voor Schimmel cultures, Utrecht. Pp. 12-124
35. Tortora, G. J., Funke, B. R. and Case, C. L. L (2007). *Microbiology: An Introduction*. 9th Edition. Benjamin/Cummings Publishing Company Inc, California.

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