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Occurrence of Antimicrobial Resistant Enterobacteriaceae and Fungi in effluents from selected Abattoirs in Akoko Local Government, Ondo State

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Abstract

Aim: The aim of the study was to isolate and identify antimicrobial resistant bacteria and fungi from abattoir effluents in Akoko Local Government, Ondo State (AKLGO).

Methodology: Wastewater samples from selected abattoirs in AKLGO were collected and analysed using standard microbiological techniques. Bacteria and fungi from the samples were characterised using morphological attributes, microscopic features, and various biochemical tests. Antimicrobial susceptibility patterns of the isolates were profiled using the Kirby-Bauer's disc diffusion method and interpreted following CLSI guidelines.

Results: Notable bacteria isolated from the samples included *Klebsiella pneumoniae*, *Escherichia coli*, *Serratia marcescens*, *Proteus mirabilis* and *Klebsiella aerogenes*, while fungal species included: *Candida parapsilosis*, *Saccharomyces cerevisiae*, *Candida tropicalis*, *Penicillium echinulatum fassatova*, and *Aspergillus vitis novobranova*. The microbial isolates demonstrated varying susceptibility patterns to the conventional antimicrobial agents employed in this study. Notably, all the bacterial isolates were resistant to Ampicillin, Nalidixic-acid and Ceporex, while Griseofulvin and Nystatin were the least efficacious agents against fungal isolates. The multiple antibiotics resistance index of the isolates was greater than 0.2, which represents a high-risk source of contamination.

Conclusion: The presence of pathogenic antimicrobial resistant organisms in these environments poses a significant health-threat to the populace, thus, these findings underscore the need for proper wastewater treatment and disposal measures at the abattoirs studied.

Keywords: abattoir, effluents, bacteria, fungi antimicrobial resistance, public-health.

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Introduction

Abattoirs are licensed facilities where animals are slaughtered and processed to produce safe meat for human consumption. Key abattoir activities include butchering, hiding removal, managing intestines, rendering, trimming, processing, and thorough cleaning. The slaughtering and meat production process generates significant amounts of wastewater, and when this is not properly treated, it can contribute to environmental contamination, altering local

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microbial ecosystems. This disruption may lead to negative environmental impacts, such as increased methane production by anaerobic bacteria, accelerating global warming [1, 2]. In many developing countries, the sanitary conditions in abattoirs are inadequate, providing an environment for the growth of harmful pathogens [3]. In Nigeria, the demand for beef is rising, but hygiene standards along the meat production chain, especially in abattoirs, are often suboptimal. Many of these facilities have been found to harbour coliform bacteria and pathogens, which makes them unsuitable for safe meat production [4, 5]. Properly equipped, modern abattoirs are essential to ensure the hygienic processing of meat. Without these standards, abattoirs may become sources of contamination [6]. Abattoir wastewater is typically laden with harmful microorganisms and is often discharged directly into nearby water bodies such as rivers and ponds. These waters, frequently used for agricultural irrigation, may become sources of infection, posing risks to farmers and consumers of the crops irrigated with the contaminated water [7]. The United Nations' Sustainable Development Goals (SDGs) include objectives directly related to the meat industry, such as Zero Hunger and Clean Water and Sanitation. As the global population grows and as urbanization and technological advancements increase, there is an escalating demand for water in slaughterhouses and meat processing facilities, intensifying wastewater management challenges [8]. The abattoir industry plays a critical role in Nigeria's livestock sector by providing meat, a vital protein source, while offering employment opportunities [9]. In Ondo State, with a population of over 5 million, the demand for meat is high, particularly in regions like Akoko South West and Akoko North East, where abattoirs operate daily to meet this need. This study examines the pathogens found in wastewater from selected abattoirs in Akoko Local Government Area, Ondo State, and evaluates their antibiotic resistance.

Research Problem

Effluent from abattoirs could cause significant health problems due to the presence of pathogenic microorganisms and antibiotic-resistant bacteria. Therefore, the spectrum and type of pathogens associated with these effluents, as well as their antibiotic susceptibility profile, need to be investigated.

Research Focus

This study focuses on isolating and determining the antibiotic resistance index of potential pathogens Enterobacteriaceae and Fungi in abattoir effluent.

Research Aim and Research Questions

The study aims to isolate and identify potentially pathogenic bacteria and fungi from different abattoir wastewater in selected towns in Akoko LGA, Ondo State and to determine the antibiotic susceptibility patterns of the isolated microorganisms

1. Could pathogenic microorganisms be found in Akoko Local Government Area abattoirs?
2. What is the antimicrobial susceptibility profile of the isolated organisms?

Research Methodology

General Background

Wastewater samples were collected in Bijou bottles from different abattoirs in Akoko Local Government area of Ondo State (ALGAO). The abattoirs are located in Ikare, Ugbe, Akungba and Ayepe all in (ALGAO). Sterile syringes were used to aseptically draw part of the wastewater running of the drainage system into sterile sample bottles and placed immediately inside an ice-pack for transportation to the laboratory.

Instrument and Procedures

All media utilized during the experiment were prepared following the manufacturer's guidelines. This included the use of Sabouraud dextrose agar (SDA), Nutrient Agar, and Eosin methylene blue (EMB) agar. A 0.5 ml aliquot from each sample dilution was introduced into sterile Petri dishes. For bacterial cultures, Nutrient Agar and Eosin Methylene Blue agar were poured, while Sabouraud dextrose agar was used for fungal cultures. Plates were prepared using the pour plate technique and gently swirled for even distribution. The inoculated plates were incubated at 37°C for 24 hours for bacterial growth and 72 hours for fungal cultures. Distinct colonies were isolated, and subcultures were placed on fresh plates to obtain pure cultures. For bacterial identification, Gram staining and biochemical tests (such as citrate utilization, oxidase, catalase, glucose fermentation, indole production, and urease testing) were performed. For fungi, both macroscopic and microscopic analyses were done [10-11]. Antibiotics susceptibility tests: Antimicrobial susceptibility screening was performed based on the Kirby-Bauer disc diffusion method [12]. The isolates were grown on Mueller Hinton Agar for 24 hours; three to five of each colony were suspended in 0.85% saline solution to achieve turbidity equivalent to 0.5 McFarland standard. Using sterile cotton swab sticks, the isolates were swabbed on the entire surface of freshly prepared Mueller Hinton Agar and then ten antibiotics discs, which included Ciprofloxacin, Ceftriaxone, Streptomycin, Ceporex, Ofloxacin, Nalidixic acid, Pefloxacin, Gentamicin, Augmentin, Ampicillin were aseptically placed on the inoculated Mueller Hinton Agar plates using a sterile forceps. The plates were incubated for 18 hours at 37c. Zones of inhibition were measured (mm) and

classified as resistant, intermediate and sensitive in accordance with the standard set by clinical laboratory standard institute [10] guidelines. Antimicrobial Assays: Inoculum standardization used McFarland standard, and susceptibility testing employed disc-diffusion and micro broth dilution methods. Antifungal susceptibility testing utilized the broth microdilution method with Ketoconazole, Fluconazole, Terbinafine, and Griseofulvin [10].

Research Results

The results of this study provide a detailed analysis of the quantitative and qualitative characteristics of isolated organisms, their resistance to antimicrobial agents, and the associated risks to human health and the environment. As shown in Table 1, the spore forming units (SFU/ml) of fungal isolates from effluent samples collected from the abattoirs, ranged from $3.0 \pm 0.07 \times 10^2$ to $4.3 \pm 0.06 \times 10^2$ SFU/ml. notably, samples collected from Akungba had the highest SFU ($4.3 \pm 0.06 \times 10^2$), while sample from Ikare had the lowest SFU ($3.0 \pm 0.07 \times 10^2$).

Table 1. Colony Count of Fungi Isolated from Abattoir Effluent

Source	Fungal Count (sfu)
Akungba	$4.3 \pm 0.06 \times 10^2$
Ayepe	$3.5 \pm 0.06 \times 10^2$
Ikare	$3.0 \pm 0.07 \times 10^2$

As seen in Table 2, varying CFU (colony forming unit) counts were observed in samples from each abattoir, with samples from Ugbe having the highest CFU count of $9.8 \pm 0.15 \times 10^2$ samples from Ayepe with the lowest CFU count of $1.2 \pm 0.07 \times 10^2$. Presented in table 3 are the macroscopic and microscopic features of the fungi species isolated from abattoir effluents, indicating various details including pigmentation and conidiophore morphology.

Table 2. Colony Count of Bacteria Isolated from Abattoir Effluent

Source	Fungal Count (sfu)
Akungba	$6.8 \pm 0.15 \times 10^2$
Ikare	$2.2 \pm 0.12 \times 10^2$
Ugbe	$9.8 \pm 0.15 \times 10^2$
Ayepe	$1.2 \pm 0.07 \times 10^2$

As shown in tables 3 and 4, respectively, the bacterial species identified in this study included: *Proteus mirabilis*, *Klebsiella oxytoca*, *Klebsiella aerogenes*, *Serratia marcescens*, *Serratia liquefaciens*, *Klebsiella pneumoniae*, *Escherichia coli*, *Photobacterium luminescence*, *Enterobacter gregoviae* and *Erwinia ananas*; while the fungal species are as follows: *Penicillium echinulatum fassatiova*, *Aspergillus vitis novobranova*, *Aspergillus tamarii kita*, *Candida parapsilosis*, *Saccharomyces cerevisiae*, *Protium herbariorum*, *Candida tropicalis*, *Eurotium rubrum*, and *Pichia fermentans*.

Table 3. Macroscopic and Microscopic Characteristics of Fungi Species Isolated from Abattoir Effluents

Isolate	Shape	Pigmentation	Elevation	Edge	Opacity	Microscopic Features
<i>Penicillium echinulatum fassatiova</i>	Filamentous	White	Convex	Rough	Translucent	Conidiophores are typically smooth-walled with branches

						bearing flask-shape phialides.
<i>Aspergillus vitis novobranova</i>	Circular	Pink	Convex	Entire	Opaque	Conidiophores are uniseriate, with phialides that are flask-shaped. Conidia are globose to subglobose, borne in chains.
<i>Aspergillus tamaritii</i>	Circular	Creamy	Convex	Entire	Opaque	Conidiophores are often long and unbranched with swollen tips, bearing spherical to ellipsoidal conidia in chains.
<i>Candida parapsilosis</i>	Circular	Creamy	Convex	Entire	Opaque	Oval to elongated yeast cells often forming pseudohyphae.
<i>Saccharomyces cerevisiae</i>	Circular	Creamy	Convex	Entire	Opaque	Oval yeast cells that reproduce by budding, often forming pseudohyphae under certain condition.
<i>Protium herbariorum</i>	Circular	Black	Convex	Entire	Opaque	Conidiophores are typically smooth-walled with branches bearing flask-shaped phialides. Conidia are spherical to ellipsoidal.
<i>Candida tropicalis</i>	Circular	Creamy	Convex	Entire	Opaque	Oval yeast cells with budding reproduction, forming true hyphae under certain conditions.

<i>Eurotium rubrum</i>	Circular	Pinkish Yellow	Convex	Entire	Opaque	Conidiosphere are long, septate, and bear chains of elliptical conidia.
<i>Pichia fermentans</i>	Circular	Creamy	Convex	Entire	Translucent	Oval yeast cells that reproduce by budding, often forming pseudohyphae.
<i>Saccharomyces cerevisiae</i>	Circular	Creamy	Convex	Entire	Opaque	Oval yeast cells that reproduce by budding, often forming pseudohyphae.
<i>Saccharomyces cerevisiae</i>	Circular	Creamy	Convex	Entire	Translucent	Oval yeast cell that produces by budding, often pseudohyphae

Table 4. Biochemical Characteristic of Bacterial Isolates from Abattoir Wastewater

Isolate	Citrate	Oxidase	Indole	Urease	Motility	Glucose	Dextrose	Methyl red	Voges Proskauer	Catalase	Sorbitol	Gram staining	TSI	Maltose	Nitrate reduction	Probable Organisms
A 1	-	-	+	-	+	+	-	+	+	+	- rod	+	A/A	+	+	<i>Klebsiella oxytoca</i>
A2	-	+	+	+	+	+	-	-	+	+	- rod	+	A/A	+	+	<i>Escherichia coli</i>
A3	-	-	+	+	+	+	-	+	+	+	- rod	-	A/A	+	+	<i>Serratia marcescens</i>
A4	-	+	+	+	+	+	-	+	+	+	- rod	+	A/A	+	+	<i>Photobacterium luminescens</i>
A5	-	-	+	-	+	+	-	+	+	+	- rod	-	A/A	+	+	<i>Klebsiella pneumoniae</i>
A 6	-	-	+	+	+	+	-	+	+	+	- rod	-	A/A	+	+	<i>Enterobacter gregoviae</i>
AKG 2	-	+	+	+	+	+	-	+	+	+	- rod	+	A/A	+	+	<i>Erwinia ananas</i>

I1	-	-	+	-	+	+	-	-	+	+	-	-	A/A	+	+	<i>Serratia liquefaciens</i>
I2	-	+	+	+	+	+	-	-	+	+	-	+	A/A	+	+	<i>Escherichia coli</i>
AKG 1	-	-	+	+	+	+	-	+	+	+	-	+	A/A	-	+	<i>Proteus mirabilis</i>
U1	-	+	+	+	+	+	-	-	+	+	-	-	A/A	+	+	<i>Klebsiella aerogenes</i>

Table 5 shows the antibiotics susceptibility pattern of the bacterial isolates in this study. All Isolates were resistant to Ceftriaxone, Ampicilin, Ceporex and Nalidixic-acid. All Isolates were completely susceptible to Ofloxacin, except *Proteus mirabilis* and *Serratia liquefaciens*.

Table 5. Antibiotic Susceptibility Profile of Bacteria Isolates from Abattoir Effluents

Organisms	CPX	TRX	S	CEP	OFX	NA	PEF	CN	AU	PN
<i>Klebsiella oxytoca</i>	R	R	I	R	S	R	R	R	R	R
<i>Escherichia coli</i>	R	R	R	R	S	R	S	S	R	R
<i>Serratia marcescens</i>	I	R	R	R	S	R	R	S	R	R
<i>Photobacterium luminescens</i>	R	R	S	R	S	R	R	R	R	R
<i>Klebsiella pneumonia</i>	I	R	R	R	S	R	S	R	R	R
<i>Enterobacter gregoviae</i>	R	R	R	R	S	R	S	S	R	R
<i>Proteus mirabilis</i>	R	R	S	R	I	R	S	S	R	R
<i>Erwinia ananas</i>	R	R	I	R	S	R	S	R	R	R
<i>Serratia liquefaciens</i>	R	R	R	R	R	R	S	S	R	R
<i>Escherichia coli</i>	R	R	R	R	S	R	R	R	R	R
<i>Klebsiella aerogenes</i>	R	R	R	R	S	R	S	S	R	R

Table 6. Antimicrobial Susceptibility Profile of Fungal Isolates from Abattoir Effluents

Isolates	Antifungal agent				
	KCZ	NYS	FCZ	G	TRB
<i>Penicillium echinulatum fassatiova</i>	0mm	0mm	21mm	0mm	25mm
<i>Aspergillus vitis novobranova</i>	24mm	0mm	18.5mm	0mm	22.5mm
<i>Aspergillus tamarii kita</i>	21mm	0mm	25mm	0mm	0mm
<i>Candida parapsilosis</i>	0mm	0mm	18.5mm	19.5mm	23mm
<i>Saccharomyces Cerevisiae</i>	24mm	20.5mm	32.5mm	0mm	22.5mm
<i>Protium herbariorum link</i>	0mm	22mm	0mm	0mm	21mm
<i>Candida tropicalis</i>	30mm	0mm	30mm	0mm	20mm
<i>Eurotium rubrum</i>	20.5mm	0mm	26mm	0mm	24mm
<i>Pichia fermentans</i>	18.5mm	24.5mm	17mm	0mm	22.5mm

Legend: KCZ- Ketoconazole, NYS- Nystatin, FCZ- Fluconazole, G- Griseofulvin, TRB- Terbinafine

Table 6 displays the results of the antimicrobial susceptibility test conducted on various fungal isolates using different antifungal drugs (Ketoconazole, Nystatin, Fluconazole, Griseofulvin, Terbinafine), indicating the diameter of the zone of inhibition observed around the antifungal drug disc for each fungal isolate. Notably, *Aspergillus vitis novobranova* showed susceptibility to Ketoconazole (24mm) and Terbinafine (22.5mm), while *Saccharomyces cerevisiae* exhibits significant susceptibility to Fluconazole, with a large zone of inhibition (32.5mm)

Table 7. Multiple Antifungal Resistance Index of the Fungal Isolates from Abattoir Wastewater

Organisms	MARI	Source
<i>Penicillium echnulatum fassatiova</i>	0.6	High risk
<i>Aspergillus vitis novobranov</i>	0.4	High risk
<i>Aspergillus tamarii kita</i>	0.6	High risk
<i>Candida parapsilosis</i>	0.4	High risk
<i>Saccharomyces cerevisiae</i>	0.2	High risk

<i>Protium herboriorum link</i>	0.6	High risk
<i>Candida tropicalis</i>	0.4	High risk
<i>Eurotium rubrum</i>	0.4	High risk
<i>Pichia fermentans</i>	0.2	High risk

Table 8. Multiple Antibiotics Resistance Index of the Bacterial Isolates from Abattoir Wastewater

Organisms	MARI	Source
<i>Klebsiella oxytoca</i>	0.8	High risk
<i>Escherichia coli</i>	0.4	High risk
<i>Serratia marcescens</i>	0.6	High risk
<i>Photorhadbus luminescens</i>	0.6	High risk
<i>Klesiella pneumonia</i>	0.7	High risk
<i>Enterobacter gregoviae</i>	0.6	High risk
<i>Proteus mirabilis</i>	0.4	High risk
<i>Erwinia ananas</i>	0.6	High risk
<i>Serratia liquefaciens</i>	0.7	High risk
<i>Escherichia coli</i>	0.6	High risk
<i>Klebsiella aerogenes</i>	0.5	High risk

The results show that the bacteria and fungi in the samples are resistant to antibiotics. This shows the need for better hygiene in slaughterhouses. Further research should be done to find the sources of resistance and ways to stop it.

Discussion

The environment is increasingly acknowledged as a vital factor in spreading and transmitting antimicrobial resistance. This study tackles pathogenic microbial contamination in abattoir wastewater, especially in Akoko Local Government Area, Ondo State. The variety of microorganisms in these effluents highlights the need for strict monitoring and control measures to mitigate health risks related to microbial contamination in wastewater environments. Abattoir wastewater usually contains various contaminants, including organic matter, pathogenic microorganisms, and antimicrobial agents, significantly contributing to environmental pollution.

The bacteria identified from several abattoirs during this study were primarily members of the *Enterobacteriaceae* family, including species such as *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Serratia marcescens*, *Escherichia coli*, and *Proteus mirabilis*. These organisms, many pathogenic, pose notable public health risks. Similar bacterial species have been found in abattoirs in other Nigerian cities such as Enugu, Ilorin, Port-Harcourt, Abuja, and Katsina [11-15]. In tandem with a previous study in Ogbete abattoir in Enugu, all the bacteria isolated belonged to the family *Enterobacteriaceae* [14, 16]. The recovery of bacterial strains from abattoir wastewater in this study emphasizes the considerable contamination and pollution found in the effluent. The presence of microorganisms, especially enteric bacteria, suggests faecal contamination, likely due to the improper disposal of animal waste during the slaughtering process. [17, 15]. These pathogens present a significant public health threat, as exposure to untreated effluent could result in severe gastrointestinal illnesses. Of particular concern are pathogenic strains of *Escherichia coli*, which

are known to cause conditions such as hemolytic uremic syndrome, a major cause of acute kidney failure in children, along with thrombotic thrombocytopenic purpura and bloody diarrhoea [18, 19].

This study's wide range of fungal counts, ranging from 3.0×10^3 to 4.3×10^3 SFU, reflects the heterogeneous nature of fungal contamination in the samples. This study's variability in fungal counts highlights the need for targeted interventions to reduce fungal contamination levels in abattoir effluents. This result aligns with earlier studies showing varying fungal counts in wastewater samples from different sources. The effluent samples gathered from the selected centres in our research revealed a diverse array of fungal isolates, including *Candida parapsilosis*, *Penicillium echinulatum fassatiova*, *Aspergillus vitis novobranova*, *Eurotium rubrum*, and *Pichia fermentans*, among others. This diversity highlights the potential risk of fungal contamination in abattoir effluents, emphasizing the need for targeted control measures. This finding is consistent with previous research identifying fungal species in similar wastewater environments [21, 22].

Microscopic examination showed clear differences in the cellular and structural features of the fungal isolates, which helped identify the species. Differences in shape, colour, height, and edges were noted among the isolates, suggesting their ability to adapt to various environmental conditions. These results align with earlier studies that have recorded the various morphological characteristics of pathogenic fungi in wastewater environments [23, 24]. A significant finding from this study is the isolation of *Candida parapsilosis*, which has been recognized as a major human pathogenic fungus associated with several infections, including urinary tract infections and sepsis. It was reported to be an opportunistic pathogen in cancer patients [10]. The analysis of occurrence frequency showed different levels of abundance among the fungal species, with *Saccharomyces cerevisiae* having the highest frequency. However, this high occurrence highlights the necessity for targeted measures to manage its growth and reduce potential health risks linked to fungal contamination in abattoir effluents. This is in accordance with the findings of Ob-Iren and Ekwueme [25], which also reported *Saccharomyces cerevisiae* as a prevalent fungal species in wastewater samples. The isolation of *S. cerevisiae* from abattoir wastewater emphasizes how well this yeast species can thrive in environments with high organic loads. *S. cerevisiae* is recognized for its capability to ferment sugars, transforming them into ethanol and carbon dioxide, which can be advantageous in wastewater treatment. Under anaerobic conditions, yeasts such as *S. cerevisiae* can help decrease organic pollutants by converting them into less harmful byproducts [26]. However, the uncontrolled discharge of *S. cerevisiae* and other microorganisms into the environment via untreated wastewater can have detrimental effects. The overgrowth of microbial populations in natural water bodies can result in eutrophication, which reduces oxygen levels and negatively impacts aquatic life. Therefore, although *S. cerevisiae* has potential uses in wastewater treatment, managing abattoir effluents is essential to prevent environmental pollution [27].

Antimicrobial resistance is a critical global issue. In this research, *Escherichia coli* obtained from two locations (Ayepe and Ikare) exhibited resistance to most of the antibiotics that were tested. A high resistance rate in *Escherichia coli* was noted by Igwaran et al. [20], and similar results were found by Elemile et al. [28]. In contrast, a study involving *Escherichia coli* from meat chickens in Amsterdam revealed a susceptibility rate of 63.1% to the tested antimicrobials [21]. The resistance of *Escherichia coli* raises serious public health concerns, as it is among the most frequent causes of Gram-negative bacterial infections in humans. Its resistance to extended-spectrum beta-lactamases (ESBLs) has been continually increasing. Moreover, waterborne *Escherichia coli* has been identified as a significant reservoir for antimicrobial resistance, including ESBLs and resistance mechanisms linked to *Klebsiella pneumoniae* carbapenemase [23].

Klebsiella species are frequently present in the gastrointestinal tracts of humans and animals and are typically found in the faeces of healthy individuals. Due to activities in abattoirs and the high concentrations of manure and manure residues in wastewater, *Klebsiella* species can be detected in the effluent from slaughterhouses. Most *Klebsiella* species identified in this study, such as *Klebsiella aerogenes*, are not harmful and act as indicators of the presence of potentially pathogenic microorganisms. However, *Klebsiella pneumoniae*, also detected in this study, can lead to serious infections, including severe pneumonia [29]. Martin and Bachman [30] offer valuable insights into how *Klebsiella pneumoniae* shifts from a harmless colonizer in the human gut to a harmful pathogen. This is particularly pertinent to the discussion of *Klebsiella pneumoniae* in abattoir effluents, where the bacteria can flourish due to the availability of organic matter, such as blood, tissue residues, and manure, typically found in slaughterhouse waste.

In abattoirs, wastewater systems frequently hold large quantities of biological waste, creating an ideal environment for bacteria such as *K. pneumoniae* to thrive. Martin and Bachman [30] emphasize that *K. pneumoniae* is skilled at colonizing the gastrointestinal tract and can spread through contaminated environments. This is directly related to the risk that abattoir effluents may serve as a reservoir for *K. pneumoniae*, facilitating its transmission through water or soil contamination downstream. The effluents could contain strains with accessory genomes that confer virulence factors and antibiotic resistance—characteristics render the bacteria especially hazardous when released into the environment or reintroduced into the food chain.

All bacterial isolates in this study demonstrated resistance to Ceftriaxone, Ceporex, Ampicillin, and Nalidixic acid. Several isolates were resistant to six or more of the antibiotics tested. There were varying levels of resistance

observed in *Escherichia coli* from different communities. These discrepancies could be related to the patterns of antibiotic usage in these communities and the environmental transfer of resistance genes [29]. High resistance to Augmentin was also noted in previous studies by [31]. The elevated resistance of the isolates to Augmentin is concerning, especially since many physicians tend to prescribe it for bacterial infections. This resistance could be attributed to mechanisms like the synthesis of low-affinity β -lactam binding proteins and penicillinase production. [14]. additionally, plasmid-mediated resistance is a likely mechanism utilized by environmental pathogens. A single plasmid can harbour genes that confer resistance to several antibiotics. Infections caused by antibiotic-resistant pathogen strains lead to a more significant strain on healthcare resources [32].

In this study, all bacterial isolates had a Multiple Antibiotic Resistance Index (MARI) exceeding 0.2, which points to contamination from high-risk sources. MARI values above 0.2 often reflect significant environmental exposure to antibiotics. The prevalent use of antibiotics in livestock feed, which promotes growth and prevents infections, plays a role in this situation [33]. Contributes to the emergence of antibiotic-resistant bacteria. Previous studies consistently show that fungi such as *Candida spp* exhibit significant resistance patterns. Kaur *et al.* [34] reported that *Candida parapsilosis* demonstrated a high resistance to commonly used antifungals, aligning with the high-risk classification observed in this study, where it has a MAR index of 0.4. Similarly, *Candida tropicalis* has been noted for its resistance in various studies. Pappas *et al.* [35] highlighted increasing resistance trends among *Candida spp*.

On the other hand, the MAR index for *Penicillium echnulatum fassatiova* and *Aspergillus tamari kita*, both at 0.6, suggests a notable risk of resistance that may not have been extensively documented in previous literature. Although Hedayati *et al.* [36] have discussed the general resistance of *Aspergillus spp*, specific MAR index values and their implications for clinical risks are less frequently addressed. This indicates a potential gap in the literature regarding quantifying resistance levels among these fungi. The high Multi-Drug Antimicrobial Resistance Index (MARI) values in the table indicate a concerning level of antibiotic resistance among the listed organisms. Supporting this, Awolope *et al.* [37] observed that over 95% of *Enterobacteriaceae* isolates in their study demonstrated resistance to multiple antibiotic classes, with MARI values ranging from 0.25 to 0.75. They concluded that such widespread resistance presents considerable environmental and public health risks. The MARI values range from 0.4 to 0.8, categorizing these bacteria as high-risk. This resistance can be attributed to several factors that significantly affect public health and policy. One primary reason for the elevated MARI values is the overuse and misuse of antibiotics in human medicine and agriculture. According to Ventola [38], the inappropriate prescribing of antibiotics contributes significantly to developing resistant strains. When antibiotics are used excessively, bacteria can adapt and evolve, leading to resistance.

Additionally, environmental factors play a role in antibiotic resistance. Antibiotics in the environment, particularly through agricultural runoff and wastewater discharge, can create selective pressure for resistant bacteria [39]. The implications of these high MARIs for public health are profound. Infections caused by these resistant organisms can lead to treatment failures, increased morbidity, and higher healthcare costs. The World Health Organization (WHO) emphasizes that antibiotic resistance is a global public health threat that requires urgent action [40]. The emergence of multi-drug resistant organisms can result in longer hospital stays and the need for more expensive treatments, ultimately straining healthcare systems. In terms of policy, these findings underscore the need for comprehensive antibiotic stewardship programs that promote the responsible use of antibiotics. Strategies should include improving prescribing practices, enhancing infection prevention measures, and increasing surveillance of antibiotic resistance patterns [41]. Public awareness campaigns are also essential to educate healthcare providers and the general public about the risks of antibiotic misuse and the importance of proper hygiene practices.

Nafarnda *et al.* [42] and Armand-Lefevre *et al.* [43] noted that pathogens present in animal carcasses or shed in animal wastes may include rotaviruses, hepatitis E virus, *Salmonella spp.*, *E. coli* O157:H7, *Yersinia enterocolitica*, *Campylobacter spp.*, *Cryptosporidium parvum*, and *Giardia lamblia*. The primary reservoir for *E. coli* O157:H7 has been reported to be healthy cattle in a study in Canada, although this bacterium is also endemic to swine and sheep [44]. These zoonotic pathogens can number in the millions to billions per gram of faeces and have the potential to infect humans. The pathogenic fungi present in animals are also a concern. However, the microbial characteristics of abattoir wastewater and its potential impact on receiving water bodies, which could lead to pollution and increased environmental and public health risks, have not been documented in various regions of Nigeria [45]. The detection of *E. coli* in slaughterhouse wastewater indicates faecal contamination, which means that this water requires special treatment to prevent the spread of this pathogenic organism in the environment. Diseases caused by *E. coli* from animal sources are particularly challenging to treat, especially resistant strains, due to the frequent use of antibiotics in animal feed. The detection of *E. coli* in slaughterhouse wastewater indicates faecal contamination, which means that this water requires special treatment to prevent the spread of this pathogenic organism in the environment. Diseases caused by *E. coli* from animal sources are particularly challenging to treat, especially the resistant strains, due to the frequent use of antibiotics in animal feed [45]. This is in line with the finding of Jega *et al.* [46], who reported that *E. coli* was the most abundant bacteria isolated from slaughterhouse effluent-impacted Tagangu River, Aliero, Kebbi State, North-Western Nigeria. Similarly, Egesi *et al.* [47]. reported that *E. coli* ranks as the second most abundant coliform bacteria isolated from slaughterhouse effluents released into water bodies in Owerri, Nigeria. However, Tesfaye *et al.* [48] reported that *E. coli* is the most prevalent coliform bacteria found in wastewater samples

collected from health facilities, slaughterhouses, downstream rivers, and a wastewater treatment plant in Addis Ababa, Ethiopia. The fungal isolates examined in this study showed differing levels of susceptibility to various antifungal medications. Notably, *Aspergillus tamari novobranova* demonstrated susceptibility to Ketoconazole and Terbinafine, whereas *Saccharomyces cerevisiae* showed significant susceptibility to Fluconazole in a study conducted by de Oliveira et al. [49]. It was discovered that some strains of *Saccharomyces cerevisiae* exhibited different levels of resistance to fluconazole. The study emphasized the need to monitor antifungal susceptibility since certain strains might not respond well to standard treatments [50]. The study of Awari et al. [51] Isolated *Aspergillus spp*, *Fusarium spp*, *Penicillium spp*, *Mucor spp*, and *Candida spp* from wastewater-contaminated soil. These findings underscore the importance of continuous monitoring and careful use of antimicrobials to prevent the emergence and dissemination of resistant strains in the environment. This is consistent with the findings of Saurov et al. [52], who also observed differing levels of susceptibility in fungal isolates when exposed to various antifungal agents. Anele et al. [53] examined the occurrence of fungal contaminants in meat sourced from abattoirs and emphasized the risk of antifungal resistance developing in these settings. The authors discovered a range of fungal species in the meat samples, including some that could be resistant to antifungal agents, which raises concerns regarding food safety and its implications for public health. In conclusion, the high MARI values for the organisms listed reflect a critical public health challenge driven by antibiotic overuse and environmental factors. Addressing this issue through effective policies and practices is crucial to mitigate the threat of antibiotic resistance and protect public health.

Conclusions and Implications

The abattoirs observed in this study release their effluents directly into water sources utilized for domestic, agricultural, and industrial activities. Implementing an efficient waste management system is essential to ensure that abattoir wastewater undergoes treatment before being released into the environment. Additionally, collaboration between regulatory authorities, waste management experts, and other stakeholders is vital to developing and implementing tailored treatment strategies for abattoir effluents.

Suggestions for Future Research

Several strategies for abattoir wastewater management are essential to address antimicrobial resistance and improve public health. First, routine surveillance of abattoirs is crucial for tracking antimicrobial use, which includes regular monitoring and optimizing wastewater treatment processes to remove harmful microorganisms while ensuring compliance with environmental regulations. Second, advanced wastewater treatment technologies should be employed to effectively eliminate pathogenic microorganisms from abattoir effluent before they are discharged into nearby water bodies. Additionally, incorporating filtration systems can help remove fungal spores and other contaminants from the wastewater. Implementing robust disinfection processes, such as UV treatment or chlorination, is vital to reduce pathogens in the effluent further. Lastly, while antibiotics play a critical role in disease prevention and control in animal farming, it is important to advocate for their prudent use among farmers. Encouraging the adoption of alternatives to antibiotics in animal production can help mitigate the risks associated with antimicrobial resistance. By implementing these measures, we can enhance the safety of wastewater management in abattoirs and protect public health.

Declarations

Author Contributions

Conceptualization, K. A. Osei, O. Oluyele and F. O. Adeboye; methodology, K. A. Osei and F. O. Adeboye; validation, O. Oluyele; formal analysis, O. Oluyele; investigation, K. A. Osei and F. O. Adeboye; resources, O. Oluyele, K. A. Osei and F.O. Adeboye; data curation, O. Oluyele, K. A. Osei and F.O. Adeboye; writing– K. A. Osei and F. O. Adeboye; Manuscript draft– O. Oluyele and K. A. Osei; supervision, O. Oluyele; project administration, O. Oluyele. All authors have read and agreed to the published version of the manuscript.

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The authors declare that there are no conflicts of interest regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies, have been completely observed by the authors.

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