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Antibiotic Resistance Profile of Bacteria from Abattoir Wastewater and Environmental Media in Ado-Ekiti, Nigeria

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Abstract
The slaughter of livestock continues to rise due to the growing demand for meat and its products. Antimicrobials have played a central role in the fight to reduce infectious diseases for most of the past century. Soil and wastewater samples were collected from Ado Ekiti abattoir in Ekiti State. The antibiotic susceptibility of the bacterial isolates was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar. The antibiotics tested included β-lactams, specifically ampicillin (10 µg) and cefotaxime (30 µg); aminoglycosides, represented by gentamicin (10 µg); fluoroquinolones, with ciprofloxacin (5 µg); tetracyclines, including tetracycline (30 µg); and sulfonamides, tested as trimethoprim-sulfamethoxazole (25 µg). The findings revealed varying levels of susceptibility, with *Bacillus spp., Staphylococcus aureus*, *Streptococcus spp*., and *Listeria spp.* showing the highest susceptibility across all tested antibiotics. Similarly, *Escherichia coli, Salmonella spp., Shigella spp., Vibrio spp*., *Klebsiella pneumoniae, Enterobacter spp*., and Proteus spp. were generally sensitive to most antibiotics, with inhibition zones of 25 mm or more for ciprofloxacin, cefotaxime, gentamicin, and chloramphenicol. However, *Pseudomonas aeruginosa* exhibited notable resistance, especially to ampicillin (10.5 mm), cefotaxime (12.3 mm), tetracycline (9.8 mm), erythromycin (8.9 mm), and trimethoprim-sulfamethoxazole (10.7 mm), highlighting its multidrug-resistance. Notably, resistance to erythromycin was observed in *E. coli, Salmonella spp., Vibrio spp., Proteus spp.,* and *Pseudomonas aeruginosa,* suggesting limited efficacy of this antibiotic against these pathogens. These findings emphasise the importance of continuous surveillance and prudent antibiotic use to reduce the risks of antimicrobial resistance in abattoir environments.

Keywords: Abattoir, Antibiotic Susceptibility, Antimicrobial Resistance, Multi-Drug Resistance, Waste Water

1. Introduction

Antimicrobials have played a leading role in the fight to reduce infectious diseases for much of the past century. They are mainly used to treat infectious diseases in humans and animals, but are also highly valuable in preventing infections. Untreated waste from abattoirs that is released into the environment, particularly water bodies, presents a significant public health risk. These wastes have been found to contain high levels of contaminants, including pathogens of mainly enteric origin, such as *Escherichia coli, Klebsiella, Shigella*, and *Salmonella*, and have also been shown to exhibit resistance to antibiotic treatment (Stanley *et al*., 2016) [28]. Slaughterhouses produce large quantities of wastewater containing antimicrobial-resistant bacteria (AMRB), which are released into the environment (Stefan *et al*., 2021) [29]. Antibiotic-resistant bacteria are commonly detected in livestock, including *Escherichia coli, Pseudomonas aeruginosa, Salmonella spp., Staphylococcus aureus, Shigella spp., Klebsiella spp., Enterococcus spp.*, and *Streptococcus spp*. As pathogens, they cause difficult-to-treat infections; as commensals, they may serve as a reservoir of resistance genes for other bacteria.

Consequently, outbreaks caused by these pathogens have been linked to various types of food. For example, Salmonella outbreaks have been associated with contaminated poultry, eggs, cheese, ice cream, fresh produce, and chocolate, while STEC outbreaks are mainly connected to fresh produce and ground beef (Kapperud *et al.,* 1990 [18]; Voetsch *et al.,* 2004 [33]; Werber *et al.,* 2005; Centres for Disease Control and Prevention [CDC], 2015 [9]; Herman *et al*., 2015 [16]; Colavecchio *et al*., 2017 [11]). This impact is especially significant for vulnerable patients, who may be unable to bear the higher costs or worsened illness, ultimately leading to greater disability and increased mortality (NCDC, 2023). Therefore, it highlights the urgent need for intervention to address these issues, particularly in developing countries such as Nigeria.

However, despite these challenges, reports indicate that most Nigerian abattoirs lack proper facilities for waste treatment. These wastes are either disposed of in open dumps or discharged into nearby streams, thereby posing a threat to the environment and increasing the risk of AMR with serious public health repercussions. The failure of waste management authorities to handle the waste produced, coupled with the indiscriminate disposal practices, has transformed many beautiful cities into expansive ghettos and dumpsites. It is undeniable that a healthy environment is closely linked to good human health (Ajah *et al*, 2015).

Therefore, this study examined the occurrence of antibiotic-resistant bacteria in environmental media at abattoirs in Ado-Ekiti, Nigeria, offering valuable insights into the risks associated with antibiotic resistance related to abattoir activities in the city and broader urban areas.

2. Materials and Methods

2.1 Study area

The study was carried out at the Ado-Ekiti municipal abattoir in Ado-Ekiti, Ekiti State. It is the largest abattoir in the state, slaughtering over 700 cattle each week. Ado-Ekiti is the largest town and the capital of Ekiti State, situated between latitudes 7°34’ and 7°41’ North of the equator and longitudes 5°11’ and 5°16’ East of the Greenwich meridian (Awowusi, 2010) [7]. It covers an area of 29,300 hectares. According to the 2006 census, Ado-Ekiti has a population of 444,057, but current projections estimate the population of Ado-Ekiti in 2025 to be 578,000, representing a 3.77% increase from 2024 (Metrotrends, 2025) [19].

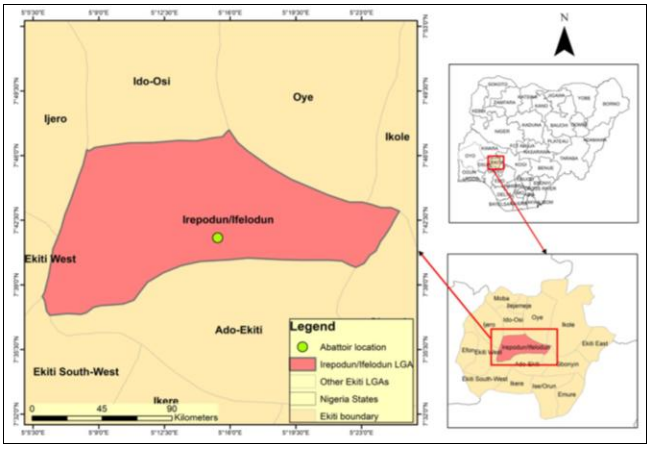


Fig 1: Map of Ado Ekiti

2.2 Collection of Abattoir Wastewater Samples

Abattoir wastewater samples were collected from two selected slaughterhouses (Ibadan and Ado Ekiti, Nigeria), obtained from the wastewater discharge outlet, soil, and contact surface. Wastewater samples were collected in sterile 500 mL glass bottles, contact surface samples were collected using a swab stick, and soil samples were collected in a polythene bag. The wastewater samples and swab sticks were transported on ice (4°C) to the laboratory and stored at 4°C until further analysis (APHA, 2017) [5].

2.3 Isolation and Characterisation of Bacterial Strains

Total Heterotrophic Bacterial Count (THBC) and Total Coliform Count (TCC) were measured using pour plate and membrane filtration methods, respectively. Wastewater samples were serially diluted in sterile physiological saline from 10^1 to 10^6. For THBC, 0.1 mL of each appropriate dilution was spread on Nutrient Agar (Oxford, UK) and incubated at 37°C for 24–48 hours. Following incubation, colonies were counted and reported as CFU/mL (Prescott *et al*., 2021). For TCC, 100 mL of wastewater was filtered through a 0.45 µm membrane filter, then placed on MacConkey agar (Oxoid, UK) and incubated at 37°C for 24 hours. Pink or mucoid colonies typical of coliforms were counted and documented as CFU/mL (Cheesbrough, 2006) [10].

Bacterial strains were further isolated from wastewater using serial dilutions (10^1 to 10^6) and spread plating on agar supplemented with abattoir wastewater and MacConkey agar, followed by incubation at 37°C for 24–48 hours. Distinct colonies were chosen and initially identified through Gram staining and biochemical tests. Further confirmation involved additional biochemical assays, including indole, catalase, oxidase, citrate, and urease tests, as well as the API 20E kit (bioMérieux, France) for identifying enteric bacteria (Ei *et al.,* 2015) [12].

2.4 Screening for Multidrug-Resistant (MDR) Bacteria

The antibiotic susceptibility of the bacterial isolates was determined using the Kirby-Bauer disk diffusion method on Mueller-Hinton Agar. The antibiotics tested included β-lactams, specifically ampicillin (10 µg) and cefotaxime (30 µg); aminoglycosides, represented by gentamicin (10 µg); fluoroquinolones, with ciprofloxacin (5 µg); tetracyclines, including tetracycline (30 µg); and sulfonamides, tested as trimethoprim-sulfamethoxazole (25 µg).

2.5 Statistical analysis

Correlation analysis was conducted to determine relationships between bacterial growth, pollutant degradation, and environmental factors. For antimicrobial susceptibility, inhibition zone diameters were compared using Student's t-test or ANOVA, where applicable.

3. Results and Discussion

The current study evaluates the incidence of antibiotic-resistant bacteria in environmental media in an abattoir in Ado-Ekiti, Nigeria.

3.1 Antimicrobial Sensitivity Patterns of Bacterial Isolates from Abattoir Samples

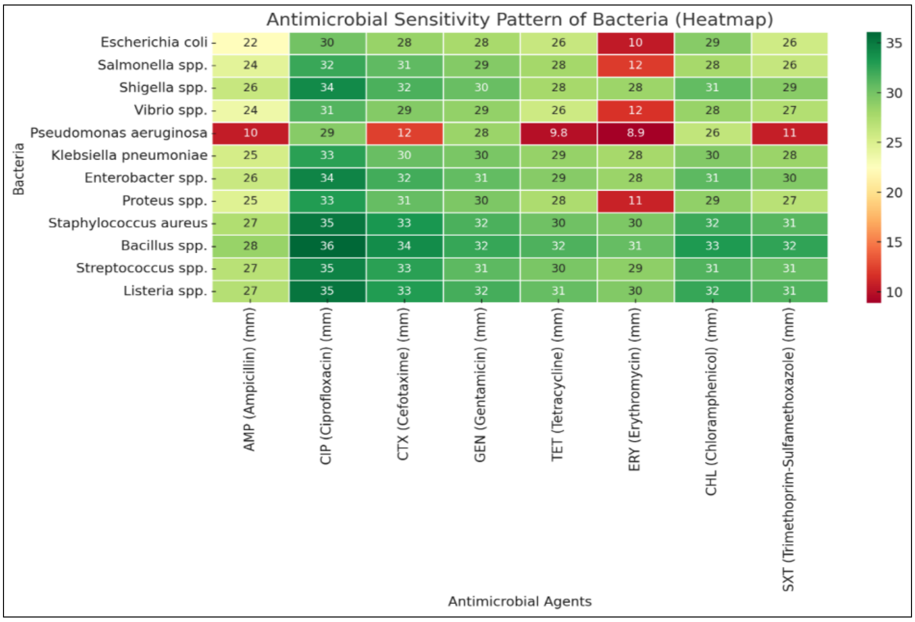
Findings show that the zone of inhibition indicates whether each bacterium is resistant or susceptible to different antibiotics (see Fig 2). For example, *Escherichia coli*, a common pathogen, is sensitive to most antibiotics tested, with a relatively large zone of inhibition for Ampicillin (22.3 mm), Ciprofloxacin (30.1 mm), Cefotaxime (28.5 mm) (Alabi *et al*., 2025) [4], and Gentamicin (27.8 mm). However, it shows resistance to erythromycin, with a smaller inhibition zone of 10.4 mm, marked as resistant (R), meaning it cannot be controlled by this antibiotic. Likewise, other bacteria, such as *Salmonella spp., Shigella spp.,* and *Vibrio spp*., show varying levels of sensitivity, while *Pseudomonas aeruginosa* is resistant to several antibiotics, including Ampicillin and Cefotaxime, as indicated by the (R) label. *Salmonella spp*. also exhibits good sensitivity to many antibiotics, with strong inhibition by Ciprofloxacin (32.2 mm), Cefotaxime (30.8 mm), and Gentamicin (29.4

mm). However, similar to *Escherichia coli*, it is resistant to erythromycin, with a smaller inhibition zone of 12.1 mm (R), implying resistance to this antibiotic.

Additionally, *Shigella spp.* was sensitive to a range of antibiotics, including Ciprofloxacin (34.1 mm), Cefotaxime (31.6 mm), and Gentamicin (30.5 mm). It does not show resistance to any of the tested antibiotics, with good inhibition across the board. This suggests that *Shigella spp.* is relatively sensitive to the antibiotics tested. Vibrio spp. also demonstrates sensitivity to many antibiotics, especially Ciprofloxacin (31.3 mm) and Cefotaxime (29.4 mm) (Kalule *et al.*, 2019) [17]. It shows some resistance to erythromycin with an 11.7 mm (R) inhibition zone, indicating it is less sensitive to this antibiotic compared to others. *Pseudomonas aeruginosa*, however, is particularly concerning because it shows resistance (R) to multiple antibiotics. For example, Ampicillin and Cefotaxime exhibit microscopic zones of inhibition (10.5 mm and 12.3 mm, respectively), and they are also resistant to Tetracycline (9.8 mm) and Erythromycin (8.9 mm). This high level of resistance means that *Pseudomonas aeruginosa* is challenging to treat with common antibiotics (Xiao *et al*., 2023) [35]. *Klebsiella pneumoniae* displays sensitivity to several antibiotics, including Ciprofloxacin (32.7 mm) and Cefotaxime (30.5 mm). It shows relatively minor zones of inhibition for Tetracycline (28.6 mm) and Erythromycin (27.9 mm), but overall, it is more susceptible than *Pseudomonas aeruginosa.*

Furthermore, *Enterobacter spp*. shares a similar sensitivity pattern with *Klebsiella pneumoniae*. It exhibits good sensitivity to Ciprofloxacin (34.5 mm) and Cefotaxime (31.9 mm), but shows moderate resistance to erythromycin (28.4 mm). Overall, it is generally sensitive to the antibiotics tested. *Proteus spp.* shows a similar trend to *Enterobacter spp.,* with high sensitivity to Ciprofloxacin (33.1 mm) and Cefotaxime (30.6 mm). It also exhibits resistance to erythromycin (10.9 mm), similar to other bacteria, such *as Escherichia coli* and *Salmonella spp*. Furthermore, Staphylococcus aureus, a Gram-positive bacterium, exhibits good inhibition by most antibiotics, particularly Ciprofloxacin (35.2 mm), Cefotaxime (33.4 mm), and Gentamicin (31.6 mm). It appears to be more sensitive to antibiotics compared to Pseudomonas aeruginosa, although it still shows moderate resistance to erythromycin, with a zone of inhibition of 29.7 mm (Puvača *et al*., 2022; Talat *et al*., 2023) [24, 32].

Further findings also revealed that Bacillus spp. Exhibits good sensitivity to most antibiotics, especially Ciprofloxacin (36.1 mm) and Cefotaxime (34.0 mm). It is susceptible to Gentamicin (32.3 mm) and shows no significant resistance to the antibiotics tested. *Streptococcus spp.* shows similar sensitivity patterns to Bacillus spp., with potent inhibition by Ciprofloxacin (34.6 mm) and Cefotaxime (32.7 mm). It does not show significant resistance to any antibiotics, indicating it is relatively easy to treat. Finally, *Listeria spp., like Streptococcus spp*., is sensitive to most antibiotics, particularly Ciprofloxacin (35.3 mm) and Cefotaxime (33.1 mm). It also has a good response to Gentamicin (31.8 mm), with no resistance seen to the tested antibiotics. The findings underscore the need for continuous surveillance and prudent antibiotic use to mitigate antimicrobial resistance risks in abattoir environments that have the potential to contaminate environmental media with direct contact with humans, such as water and soil. Some of the intermediate stages require prompt intervention to prevent total resistance due to ecological factors (Burnham, 2021 [8]; Miani *et al.,* 2023 [20]; Gufe *et al.,* 2025), as antibiotics continue to be used in animal treatment and husbandry (Foyle *et al.,* 2023) [13].



	AMP (Ampicillin) (mm)	CIP (Ciprofloxacin) (mm)	CTX (Cefotaxime) (mm)	GEN (Gentamicin) (mm)	TET (Tetracycline) (mm)	ERY (Erythromycin) (mm)	CHL (Chloramphenicol) (mm)	SXT (Trimethoprim-Sulfamethoxazole) (mm)
Escherichia coli	22	30	28	28	26	10	29	26
Salmonella spp.	24	32	31	29	28	12	28	26
Shigella spp.	26	34	32	30	28	28	31	29
Vibrio spp.	24	31	29	29	26	12	28	27
Pseudomonas aeruginosa	10	29	12	28	9.8	8.9	26	11
Klebsiella pneumoniae	25	33	30	30	29	28	30	28
Enterobacter spp.	26	34	32	31	29	28	31	30
Proteus spp.	25	33	31	30	28	11	29	27
Staphylococcus aureus	27	35	33	32	30	30	32	31
Bacillus spp.	28	36	34	32	32	31	33	32
Streptococcus spp.	27	35	33	31	30	29	31	31
Listeria spp.	27	35	33	32	31	30	32	31

Fig 2: Antimicrobial Sensitivity of Bacterial Isolates from water Sample

3.2 Bacterial Load in Abattoir Wastewater

The results indicate varying categories of bacteria (see Fig 3), including heterotrophic bacteria, total coliforms, *Faecal coliforms, Escherichia coli*, pathogenic and opportunistic bacteria, suggesting potential risk to public health. These findings are consistent with previous studies (Adeyemi *et al*., 2018; Savin *et al*., 2020) [3, 26]. Similarly, total coliform counts were elevated, reaching 8.9 × 10^5 CFU/mL, indicating substantial faecal contamination from the content of animal intestine (Adebowale *et al*., 2016; Sule *et al*., 2018) [2, 30]. Additionally, all casing cleaning, including flushing, soaking, scraping, and rinsing, is done at the slaughter and dressing floor, which is also a contributing factor to the total coliform load in the wastewater. Additionally, faecal coliforms were present at a concentration of 6.7 × 10^3 CFU/mL, further confirming the faecal origin of the contamination.

Additionally, pathogenic bacteria, including *Salmonella* spp., *Shigella* spp., and *Vibrio* spp., were detected in both samples, posing significant health risks. In addition, *Staphylococcus* spp. Counts were recorded at 9.2 × 10^3 CFU/mL, while *Pseudomonas* spp. counts reached 6.7 × 10^3 CFU/mL and 7.5 × 10^3 CFU/mL, respectively (Adeyemi *et al*. 2018; Savin *et al*. 2020) [3, 26]. The findings indicate that abattoir wastewater harbours a high bacterial load, including coliforms and potential pathogens, which can pose serious environmental and public health concerns. As such, exhibiting dangerously high bacterial contamination levels, including the presence of harmful and disease-causing organisms (Sule *et al*., 2018 [30]; Sule *et al*., 2021). If discharged without proper treatment, such wastewater could seriously endanger public health and contaminate water bodies used for drinking, agriculture, or recreation.

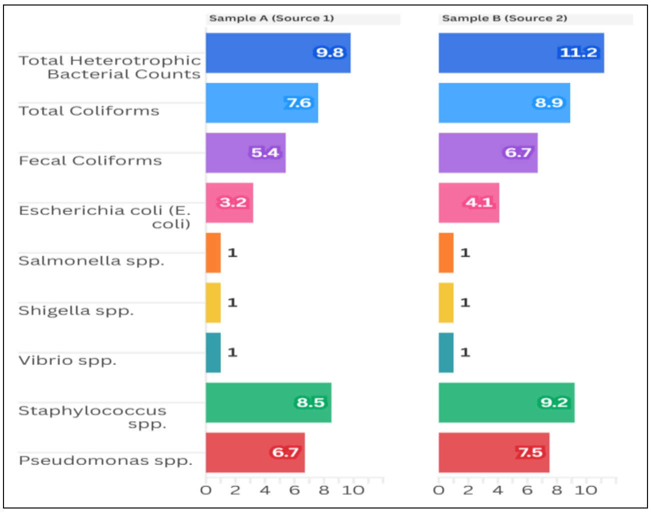


Fig 3: Bacteria Load present in Abattoir Wastewater

3.3 Regarding bacterial load in Abattoir-impacted soil

Findings revealed different types of bacteria with significantly higher heterotrophic bacteria in (4.2 × 10^7 CFU/g), indicating greater microbial activity near the wastewater discharge point (see Fig 4). This may result from the method used for skinning the slaughtered animal and the sanitary facilities at the abattoirs (Astruc & Terlouw, 2023; Nastasijevic *et al*., 2023 [6]; Gufe *et al*., 2025). Similarly, total coliform counts were high (2.7 × 10^6 CFU/g), indicating substantial faecal contamination in the soil closer to the wastewater source. Notably, the wastewater from skinning and burning was channelled to the same ground (soil), and the contents of the burning, such as ashes and hydrocarbons, might influence the bacterial load in the soil. Additionally, faecal coliforms were significantly high (1.9 × 10^5 CFU/g), supporting the impact of wastewater discharge on soil contamination. This poses a risk to human health if such bacteria are present in high levels in water or other environments, potentially causing gastrointestinal illnesses and urinary tract infections. Furthermore, *Staphylococcus spp*. was recorded at 4.1 × 10^3 CFU/g in Sample B, while *Pseudomonas spp.* was present at 2.6 × 10^3 CFU/g in Sample B, indicating the presence of faecal contamination in soil around the abattoirs, resulting from the animal slaughtering and evisceration processes (Moreroa & Basitere, 2022 [21]; Gufe *et al*., 2025).

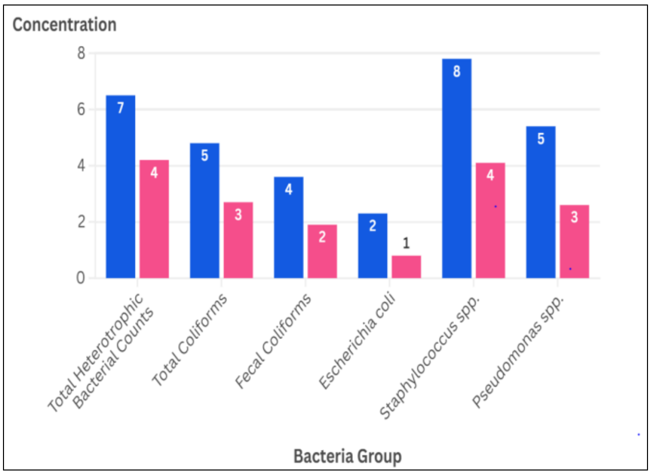


Fig 4: Bacteria Load in soil near wastewater discharge and distance from discharge

3.4 Bacterial Load from Cotton Swab Samples Collected from Abattoir Surfaces

The bacterial contamination levels on abattoir table surfaces (see Fig 5), specifically those used for selling meat, were compared in two sets of samples. Results consistently showed higher bacterial counts, suggesting poor hygiene conditions or more recent contamination. This was evident from the total heterotrophic bacterial counts, which provide a general indication of overall bacterial presence. Similarly, the heterotrophic bacterial counts were significantly high (5.9 × 10^7 CFU/cm²), suggesting extensive microbial colonisation on surfaces. Moreover, total coliform counts were recorded at 3.4 × 10^6 CFU/cm², indicating substantial faecal contamination, which is consistent with studies by Adeyemi *et al.* (2018) [3] and Savin *et al.* (2020) [26]. The detection of faecal coliforms is a serious concern because it directly indicates poor hygiene practices and the risk of foodborne illnesses spreading from contaminated surfaces to meat products.

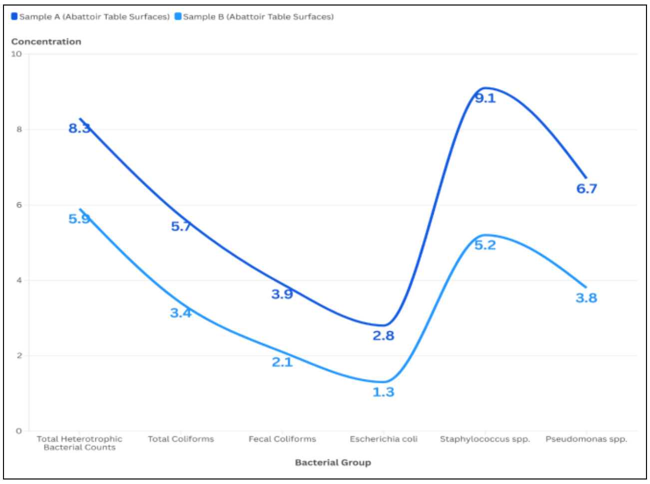


Fig 5: Bacteria Load on table surfaces in the sampled Abattoir

Additionally, further findings revealed that pathogenic isolates such as *Staphylococcus* spp. and *Pseudomonas* spp. were also present in high numbers. For instance, *Staphylococcus* spp. were recorded at 5.2×10^3 CFU/cm^2, indicating a possible risk of foodborne infections, *as well as Pseudomonas* spp. with 3.8×10^3 CFU/cm^2, which are primarily disease-causing bacteria in humans (Adamu & Dahiru, 2020; Rajpal *et al*., 2022; Shende *et al*., 2022) [1, 25, 27]. These findings highlight the poor hygiene conditions of abattoir table surfaces used for selling meat, with microbial contamination posing potential risks for cross-contamination and public health concerns. However, the pathogenic microbial load might be influenced by the nature of water used in the abattoir.

Overall, these findings highlight a worrying state of surface hygiene in the abattoir, especially for the tables. The high bacterial counts across all groups indicate an urgent need for improved cleaning procedures, stricter hygiene standards, and regular monitoring to safeguard public health and ensure food safety.

4. Conclusion

This study investigated the occurrence of antibiotic-resistant bacteria in environmental media in Ado-Ekiti, Nigeria. Results showed that *Pseudomonas aeruginosa* exhibited significant resistance, especially to ampicillin (10.5 mm), cefotaxime (12.3 mm), tetracycline (9.8 mm), erythromycin (8.9 mm), and trimethoprim-sulfamethoxazole (10.7 mm), highlighting its multidrug-resistant characteristics. Notably, erythromycin resistance was detected in *E. coli, Salmonella spp., Vibrio spp., Proteus spp.,* and *Pseudomonas aeruginosa*, indicating limited effectiveness of this antibiotic against these pathogens. Conversely, *Bacillus spp., Staphylococcus aureus, Streptococcus spp.,* and *Listeria spp*. showed the greatest susceptibility across all tested antibiotics. Therefore, these findings provide evidence-based insights into the influence of abattoir operations on the antimicrobial resistance (AMR) challenge, aiding the development of policies to regulate and monitor abattoir activities in the effort to combat AMR.

Competing interest

The authors declare no conflict of interest.

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