Antibiotic Resistance Pattern of Gram-Negative Bacteria Isolated from Abattoir Wastewater, Amosun, Ibadan, Nigeria

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Abstract
Abattoirs generate enormous volume of wastewater, containing high level of pathogenic bacteria, antibiotics and organic matter. Discharge of untreated abattoir wastewater can cause surface and groundwater pollution and negatively affect aquatic ecosystem, thereby, posing risks to public health. This study is aimed at determining antibiotic resistance pattern of the Gram-negative bacteria isolated from abattoir wastewater in Amosun, Ibadan, Nigeria. Wastewater samples were aseptically collected from the abattoir in sterile bottles using standard method and was analyzed for their physicochemical properties. Standard pour plate method was used for bacterial enumeration and isolation using Nutrient agar and MacConkey Agar, respectively. Identification of the isolates was done using conventional methods. Antibiotic susceptibility test was performed against 5 selected antibiotics using the Kirby-Bauer's disk diffusion technique according to the Clinical and Laboratory Standards Institute guidelines.

The mean physicochemical parameters of the wastewater showed a pH of 6.33, turbidity of 250 NTU and a strong odour. The mean total heterotrophic bacteria count was 2.42 x 10^2 CFU/mL. A total of 30 Gram-negative bacteria including: *Pseudomonas* spp. (11), *Enterobacter* spp. (7), *Klebsiella* spp. (4), *Aeromonas* spp. (2), *Providencia* spp. (2), *Escherichia* coli (1), *Proteus* sp. (1), *Serratia* sp. (1), and *Alcaligenes* sp. (1) were isolated. The antibiotic susceptibility profile showed a total resistance to amoxicillin- clavulanic and high resistance rates: 96.6% to both ceftriaxone and ceftazidime, 73% to imipenem. The antibiotic resistance of diverse potential pathogens in abattoir wastewater suggests a critical need for sustainable wastewater treatment and regulate the use of antibiotics in animal husbandry.

Keywords: Gram-negative bacteria, wastewater, abattoir, antibiotic resistance.

Introduction

Abattoirs are known as slaughterhouses which play a significant role in the meat processing industry by contributing to the global food supply chain and processing millions of animals daily (Bello and Oyedemi, 2009). The high demand for meat leads to the generation of large amount of wastewater, airborne pollutants, organic and inorganic materials are generated during different operations in the abattoir. Wastewater from abattoir can pose public health risks to the people within the abattoir environment, cause harm to aquatic life and affects soil and vegetation if not properly managed and treated (Gufe et *al*., 2025).

Abattoir wastewater consist of pathogens that are of major concern as they can cause cross contamination due to the spread of microbes to food and non-food materials (Rodriguez- Lopez *et al*., 2020). Pathogenic bacteria such as *E. coli*, *Salmonella*, *Campylobacter*, *Pseudomonas*, *Klebsiella*, *Enterobacter* and *Serratia* spp. have been reported in abattoir wastewater (Homeier-Bachmann *et al*., 2021). Human diseases caused by Gram-negative bacteria include: sepsis, endotoxic shock, pneumonia, diarrhea, meningitis and urinary tract infections. Among the genera that commonly infect humans are *Yersinia* spp., *Shigella* spp., *Salmonella* spp., *Escherichia* spp., *Proteus* spp., *Enterobacter* spp., *Klebsiella* spp., and *Citrobacter* spp. (Oliveira and Reygaert, 2023).

Commercial agriculture, especially animal husbandry sector majorly relies on the use of antimicrobials for improving animal health and production. Antibiotics are used in livestock either as therapeutics (treating individual animals) or metaphylactic which involves treating the whole herd due to the presence of diseases in some animals (Homeier-Bachmann *et al*., 2021). The problem of antibiotic misuse in animals and humans in Nigeria, is majorly due to wrong and sub-standard prescriptions by untrained medical professionals (Shaibu *et al*., 2021). Antibiotics are also misused in animal husbandry for promoting growth, increasing feed efficiency and weight gain, preventing diseases in healthy animals, rather than treating only diseased animals. The use of antibiotics at sub-therapeutic doses can result into the development of antibiotic-resistant bacteria and spread of antibiotic residues to the food chain (Sawa *et al*., 2020).

Antibiotic resistance is the mechanism by which an organism adapts to survive in the presence of antibiotics by pumping out, altering the antibiotic-binding site, or making antibiotics compatible, thereby shielding itself from the effects of antibiotics (Samrot *et al*., 2023). Consumption of antibiotics can change the human microbiota and favor bacteria that are resistant to antibiotics. Through sewage and effluents, antibiotic resistant bacteria and partially metabolized antibiotics can find their way into farmland and surface waterways after being expelled into the environment (Verburg *et al*., 2021).

There is an alarming increase in antibiotic resistance among Gram- negative bacteria thereby, making them one of the most important public health issues in the world. Due to the high risk of diseases, death rate and the frequent need for patients to be in the intensive care unit (ICU), these bacteria are of great clinical significance in hospitals (Oliveira and Reygaert, 2023). Bacteria can spread through their ability to form biofilms that can persist over time in the environment (Rodriguez- Lopez *et al*., 2020). Moreover, biofilms can aid the horizontal transfer of antibiotic resistance genes thereby raising concerns regarding the dissemination of antibiotic resistant bacteria (Ghidini *et al*., 2022).

Antibiotic resistant Gram-negative bacteria from abattoir wastewater sources include: some strains of *E. coli* strains which have developed resistance to fluoroquinolones and carbapenems (Sawa *et al*., 2020). Report have shown that *Acinetobacter* spp. are often resistant to multiple antibiotics, including carbapenems and colistin. *Pseudomonas* spp. are often resistant to antibiotics like beta-lactams and fluoroquinolones. *Klebsiella* spp. are resistant to the penicillins (Denissen *et al*., 2024).

Materials and Methods

Study Area: The study area was Ibadan Central Abattoir, Amosun, Akinyele Local Government Area, kilometer 16, Ibadan- Oyo express way Ibadan, Nigeria and located on latitude *7°22'39.22"N and longitude 3°54'21.28"E*. It is the largest abattoir in Nigeria (Kola-Aderoju and Ityonzughul *et al*., 2024) with large number of slaughtered cows daily.

Sample Collection

The abattoir wastewater samples were collected into sterile 200mL bottles at four different locations and the samples were pooled together. Samples were collected bi-monthly for the period of two months (May and June 2024). The samples were collected during the day when the activity was at its peak and were transported at 4°C in ice packs to the to the Microbiology Laboratory, KolaDaisi University for immediate microbiological analysis.

Determination of the Physicochemical Parameters of the Abattoir Wastewater

The physicochemical properties including: Temperature, pH, odor, colour, Total Dissolved Solids (TDS), dissolved Oxygen (DO), conductivity and turbidity were analysed. The physicochemical parameters were analysed based on the American Public Health Association series of Standard Methods of Examination of Water and Effluent (APHA, 2012).

Determination of the Mean total Heterotrophic Bacteria Count

The total heterotrophic bacterial count was done using standard pour plate and serial techniques to estimate the number of heterotrophic bacteria present in the abattoir wastewater. Exactly 1mL of the fourth ten-fold serial dilutions was aseptically put in sterile petri plates. On cooling to 45°C, 20 mL of sterilized nutrient agar were added to each plate and gently mixed. The plates were allowed to solidify and incubated at 37°C for 24 to 72 hours. The mean total heterotrophic bacteria count was derived using the standard mean formula and was expressed as CFU/mL.

Isolation and Identification of Gram-negative Bacteria

Gram-negative bacteria were isolated using standard pour plate method, 1 ml of the wastewater sample were added to petri-dishes and mixed with 20mL of cooled, sterilized molten MacConkey agar aseptically. After solidifying, the petri-dishes were inverted and incubated at 37°C for 24 hours. Pure cultures of Gram-negative bacteria were obtained and stored in cryovial tubes at 4°C.The isolates were identified using Gram reaction, biochemical test such as catalase, urease, methyl red, indole, oxidase, motility, Voges Proskauer, citrate and sugar fermentation tests.

Antibiotic Susceptibility Testing

The antibiotic susceptibility test was performed on Mueller-Hinton agar using the Kirby-Bauer disk diffusion technique. The test was performed against 5 selected antibiotics including: Ceftriaxone 30µg, Ceftazidime 10µg, Imipenem 10µg, Amoxicillin-clavulanic acid 30µg, Sulfamethoxazole- trimethoprim 25µg. The diameters of the inhibitory zones surrounding the disks were measured, recorded and interpreted using the Clinical and Laboratory Standards Institute guidelines, CLSI guidelines 2024.

Data Analysis

The data obtained from the abattoir wastewater analysis were collated and analyzed Descriptive statistics was done using MS Excel (Microsoft Corp., USA).

Results

Physicochemical Analysis of Abattoir Wastewater

The mean physicochemical parameters of the abattoir wastewater revealed that the temperature was 33°C, pH was 6.33, dark brown in colour, odour was strong, turbidity was 250 NTU, conductivity was 2000 µS/cm, total dissolved solid was 1400 mg/L, and dissolved oxygen was 4.5 mg/L (Table 1).

Table 1: The Mean Values of the Physicochemical Parameters of Abattoir Wastewater

Physicochemical analysis	Results	WHO Standard of abattoir wastewater
Temperature (°C)	33	< 40
pH	6.33	6.5-9.5
Color	Dark brown	Platinum
Turbidity (NTU)	250	100
Conductivity (µS/cm)	2000	1400
Total Dissolved Solid (mg/L)	1400	1000
Dissolved Oxygen (mg/L)	4.5	7.5
Odor	Strong	Weak

The Mean Total Heterotrophic Bacterial Count (THBC)

The mean value for the total heterotrophic bacteria count obtained from the abattoir wastewater is 2.42 x 10^2 cfu/mL (Table 2).

Table 2: The Mean Total Heterotrophic Bacteria Count

Abattoir wastewater	Total Heterotrophic Bacteria Count (10^4 CFU/mL)
Pooled Sample 1	218
Pooled Sample 2	220
Pooled Sample 3	290
Pooled Sample 4	240
Mean	242

Isolation and Identification of Gram-negative Bacteria

A total of 30 Gram-negative bacteria belonging to nine different genera were isolated and identified as follows: *Pseudomonas* spp. (11), *Entero*bacter spp. (7), *Klebsiella* spp. (4), *Aeromonas* spp. (2), *Providencia* spp. (2), *Proteus* sp. (1), *Escherichia* sp. (1), *Serratia* sp. (1) and *Alcaligenes* sp. (1) (Table 3).

Table 3: Percentage Distribution of Gram- negative Bacteria Isolated from Abattoir Wastewater

S/N	Gram-negative bacteria	No of isolates	Frequency of occurrence (%)
1	*Enterobacter* spp.	7	23.3
2	*Pseudomonas* spp.	11	36.7
3	*Klebsiella* spp.	4	13.3
4	*Providencia* spp.	2	6.7
5	*Aeromonas* spp.	2	6.7
6	*Escherichia coli*	1	3.3
7	*Proteus* sp.	1	3.3
8	*Serratia* sp.	1	3.3
9	*Alcaligenes* sp.	1	3.3

Antibiotics Susceptibility Profile of the Gram-negative Bacteria

The antibiotic susceptibility profile showed total resistance to amoxicillin-clavunanic acid. The isolates showed high resistance rates: 96.6% to both ceftazidime and ceftriaxone, 73% to both imipenem and sulfamethoxazole-trimethoprim (Figure 1). It was also revealed that all the *Aeromonas* spp. (2), *Proteus* sp. (1), *Escherichia coli*. (1), *Serratia* sp. (1) and *Alcaligenes* sp. were resistant to the all the tested antibiotics. *Pseudomonas* spp., *Enterobacter* spp. and *Providencia* spp. showed total resistance to three antibiotics including: amoxicillin-clavunanic, ceftazidime and ceftriaxone (Table 4). All the isolates exhibited multidrug resistance.

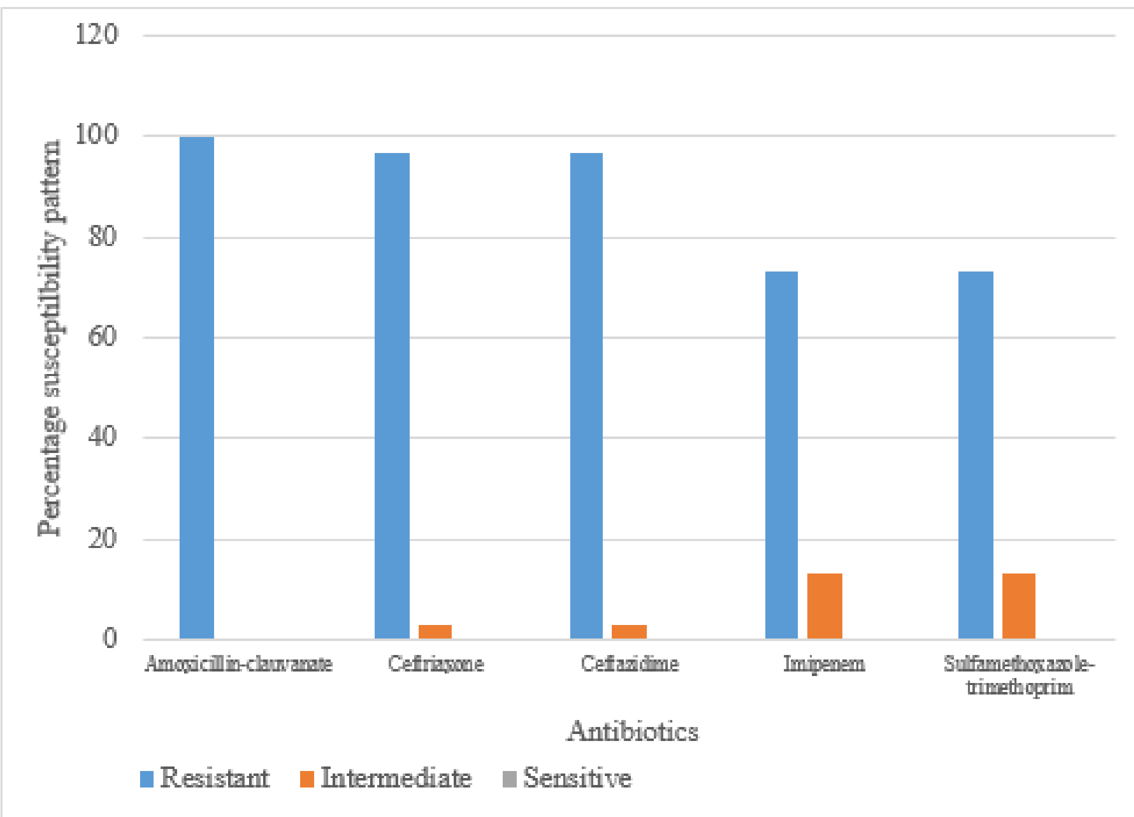

Fig. 1: The percentage antibiotics susceptibility profile of all the Gram-negative bacteria.

Table 4: The Antibiotics Susceptibility Profile of the Gram-negative Bacteria

	AUG			CAZ			CTX (N)			IMP			SXT		
Isolates	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
Pseudomonas spp.	11	0	0	11	0	0	11	0	0	6	2	3	7	0	4
Enterobacter spp.	7	0	0	7	0	0	7	0	0	6	0	1	5	0	2
Klebsiella spp.	4	0	0	3	1	0	3	1	0	3	0	1	3	0	1
Providencia spp.	2	0	0	2	0	0	2	0	0	1	1	0	1	1	0
Aeromonas spp.	2	0	0	2	0	0	2	0	0	2	0	0	2	0	0
Proteus spp.	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
Serratia spp.	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
Escherichia coli	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
Alcaligenes spp.	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0
Total	30	0	0	29	1	0	29	1	0	22	3	5	22	1	7

Keys: AUG: Amoxicillin-clavulanic acid   CAZ: Ceftazidime   CTX: Ceftriaxone
IMP: Imipenem   SXT: Sulfamethoxazole-trimethoprim   N: Numbers
R- resistant   I- intermediate   S- sensitive

Discussion

Abattoir wastewater is a significant source of environmental pollution and the presence of pathogenic bacteria poses a major challenge due to their ability to cause gastrointestinal diseases, urinary tract infections and other severe health issues. The discharge of abattoir wastewater directly into the environment without prior treatment is a great threat to water bodies as it affects the aquatic life and the food chain (Gufe *et al*., 2025).

The physicochemical analysis of the abattoir wastewater which revealed a pH of 6.33, is lower than the recommended standard (WHO, 2011) and thus indicate that the wastewater is slightly acidic. The acidic nature could enhance the survival and growth of pathogens, potentially leading to the spread of disease through improper disposal. The temperature which was 33°C is within the permissible limit of <40°C (WHO, 2011). The turbidity which showed 250 NTU is higher while, the dissolved oxygen of 4.5 mg/l is lower compared to the finding of another study carried out on abattoir wastewater from Northern part of Nigeria (Bate *et al*., 2023). The low dissolved oxygen observed in this study suggests low levels of oxygen available for microbial degradation. The total dissolved solids (TDS) of 1400 mg/l is in agreement with a study conducted on abattoir wastewater in Imo state Nigeria (Chinakwe *et al*., 2022). The dark brown colour of the wastewater is not within the WHO recommended standard. The odor which was very unpleasant is suggesting the presence of organic matter and this is similar to the findings of another study in Lagos state (Ademola *et al.,* 2017). The conductivity of 2000 µS/cm is higher than the recommended standard (WHO, 2011).

The mean total heterotrophic bacteria count which was 2.42×10^2 CFU/mL is not in agreement with another study on abattoir who reported a higher microbial load (Awari *et al*., 2024). This microbial load exceeded the WHO recommended level of 100 CFU/mL reported by Bate *et al.* (2023). The high microbial load indicates that the abattoir releases effluents that are of major public health concern to the environment.

Gram-negative bacteria, including *Escherichia coli*, *Klebsiella*, and *Pseudomonas* have been implicated in human infections (Ali and Gujiba, 2024). The presence of Gram-negative bacteria in the abattoir wastewater revealed a significant presence of potentially pathogenic bacteria, raising concerns about water quality and public health threat. The recovery of nine different genera such as *Pseudomonas* spp., *Enterobacter* spp., *Klebsiella* spp., *Aeromonas* spp., *Escherichia coli*, *Proteus* sp., *Serratia* sp., *Alcaligenes* sp. and *Providencia* spp., is in contrast with the findings of another study carried out in Eastern part of Nigeria who reported five genera of Gram- negative bacteria from abattoir wastewater (Awari *et al.,* 2024). The presence of diverse range of potentially pathogenic bacteria in this study suggest a public health threat to humans, animals and plants with direct or indirect contact with the wastewater or the receiving water bodies. This highlights the need for effective treatment strategies to remove or reduce microbial contaminants before discharge into the environment.

The antibiotic susceptibility testing of the Gram-negative bacterial isolates from abattoir wastewater which revealed a concerning level of resistance to different antibiotics is an indication of the spread of antibiotic resistance menace to the environment. The total resistance to amoxicillin-clavulanate and high resistance rates to; ceftazidime, ceftriaxone, imipenem and sulfamethoxazole- trimethoprim observed in this present study suggests the misuse of antibiotics in animal husbandry. The observation that the isolated Gram-negative bacteria were totally resistant to amoxicillin-clavulanic acid and showed high resistance to ceftazidime is in agreement with the report of Olawale *et al*. (2020) on abattoir wastewater Kwara state Nigeria. Also, the high resistance rate to ceftazidime in this study is similar to the high resistance to the same antibiotic reported in Enterobactericeae from abattoir wastewater in Germany (Homeier-Bachmann *et al*., 2021). The high resistance showed to imipenem by the recovered Gram–negative bacterial isolates is in contrast to another study conducted on wastewater from poultry slaughterhouse which reported no resistance to the same antibiotic (Savin *et al*., 2021). High resistance to imipenem, one of the last line antibiotics used in treating multidrug resistant Gram-negative infection observed in this study can lead the spread of infections which require longer hospital stays, higher health cost and mortality due to difficult to treat infections.

The total resistance of *E. coli* to all the 5 tested antibiotics is not in agreement with resistance to only one antibiotic reported in *E. coli* carried out on abattoir wastewater (Onuoha *et al*., 2018). However, the observation that *Pseudomonas* spp. showed total resistance to amoxicillin-clavulanic acid, is similar to the findings of another study carried out on abattoir wastewater from Abakaliki, Nigeria (Onuoha *et al*., 2018). This study which revealed high resistance rates in *Pseudomonas* spp. to both ceftazidime is in contrast to the low resistance reported in *Pseudomonas* spp. from abattoir effluents in Ogun state, Nigeria (Akpan *et al*., 2020). The multidrug resistance pattern observed in *E. coli*, *Enterobacter* spp. and *Klebsiella* spp. is also similar to another study conducted on Enterobacteriaceae from different sources including abattoir effluents in Ethiopia (Tesfaye *et al*., 2019). This study that revealed multidrug resistance in all the nine identified genera indicates widespread of multiple antibiotic resistant bacteria in abattoir wastewater. The widespread of these multidrug resistant bacteria in abattoir wastewater that are channeled directly to the environment indicate a major public health threat to humans as it can lead to treatment failure or delay if not properly controlled by effective wastewater treatment and antibiotic stewardship practices.

Conclusion

The physicochemical parameters not conforming to the recommended standard, high microbial load, significant presence of potentially pathogenic bacteria coupled with high antibiotic resistance rates in the abattoir wastewater indicates the alarming levels of pollution, contamination and spread of antibiotic resistant bacteria which pose a significant threat to the soil health, water quality, public health and sustainability of the environment.

Recommendation

The abattoir industry should implement effective waste treatment technologies to reduce pollution and bacterial contamination and regular monitoring and testing of wastewater should be conducted to ensure compliance with environmental regulations.

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