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Isolation of Antibiotic Resistant Bacteria from Abattoir Waste Water: A Case Study of Makurdi Metropolis

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Abstract

The prevalence of antibiotic-resistant bacteria in the livestock population is strongly correlated with antibiotic usage (non-specific usage), as the selection and dissemination of resistant bacteria are heavily augmented under selective pressure caused by antibiotics. This study, therefore, is aimed at isolating antibiotic-resistant bacteria from an abattoir wastewater in the Makurdi metropolis. The samples were collected and diluted into four folds. One mile (1ml) of the samples each pour plated on Nutrient agar (NA), MacConkey agar (MCA), Mannitol salt agar (MSA), and Eosin methylene blue agar (EMBA) for isolation and identification; and their antibiotic susceptibility patterns were determined using disc diffusion method. The results obtained showed that the mean value of bacteria count ranged from 0.57×10 - 5.94×10. The organisms isolated were species of Escherichia coli, Staphylococcus, Bacillus, Klebsiella, Streptococcus, Proteus Shigella, and Salmonella. Staphylococcus spp showed the highest prevalence of 7(22.58%), Klebsiella and Bacillus 6(19.35%), Escherichia coli 5(16.13%), Streptococcus, Proteus and Shigella 2(6.45%) while Salmonella spp have the least prevalence of 1(3.23%). Among the isolates, Klebsiella spp was resistant to 60% of the drugs used, while Salmonella spp was susceptible to all the drugs used. This research result reveals that all isolates from abattoir wastewater are susceptible to gentamycin and septrin. While other bacteria Staphylococcus, Shigella, Proteus, Streptococcus, Bacillus, and Escherichia coli also showed varying degrees of resistance and susceptibility. Therefore, abattoir wastewater should be treated before being discharged into water bodies.

1. Introduction

Environmental problems have increased over the last four decades with improper management practices being largely responsible for the gross pollution of the aquatic environment with a concomitant increase in water-borne diseases especially typhoid fever, cholera, diarrhea, and dysentery. Effluent is an out-flowing of water from a natural body of water or a human-made structure. Effluent is defined by the United States Environmental Protection Agency as "wastewater-treated or untreated-that flows out of a treatment plant, sewer, or industrial outfall. Generally refers to wastes discharged into surface waters" (USEPA, 2016). An abattoir is a special facility designed and licensed for receiving, holding, slaughtering, and inspecting meat animals and meat products before releasing to the public. Nevertheless, the slaughtering of livestock continues to increase as a result of the increase in demand for meat and its products. An abattoir has been defined as a premise approved and registered by the controlling authority for hygienic slaughtering and inspection of animals, possessing and effective preservation and storage of meat products for human consumption (Alonge, 2011). In Nigeria, the location and operation of several private and government abattoirs, with Benue State not excluded, are generally unregulated. Abattoir operation could be very beneficial to man; in that, it provides meat for human consumption and other useful by-products, still, it can be very hazardous to public health concerning the waste it generates (Adeyemi *et al.*, 2017). Abattoirs generate large amounts of solid waste and

effluents such as rumen contents, blood, and wastewater (Ayodele *et al.*, 2015). Abattoirs often have difficulties in disposing of the solid wastes and wastewater in an environmentally acceptable fashion and in many instances untreated rumen contents, blood, and/or other abattoir effluents and wastewater are released into the environment (Bellani *et al..*, 2010). The resulting pollution not only causes problems related to odor, flies, and hygiene, but surface and groundwater can be polluted with pathogens (Shuval *et al..*, 2012). Abattoir wastewater may be defined as water that has been used in cleaning up slaughtered cattle, sheep, pig, and goats carcasses, the floor for slaughter halls, personnel, and slaughter equipment. Abattoir wastewater is characterized by the presence of a high concentration of whole blood of the slaughtered food animals and suspended particles of semi-digested and undigested feed within the stomach and intestine of slaughtered and dressed food animals (Coker *et al..*, 2019).

Over the recent past, the public has become increasingly alarmed by the new scientific data that have made their way into the media about the connection between the overuse of antibiotics in both medicine and agriculture, and the spread of antibiotic-resistant bacteria mostly through wastewater. The prevalence of antibiotic resistance in a population is strongly correlated with antibiotic usage (nonspecific usage), as the selection and dissemination of resistant bacteria are heavily augmented under selective pressure caused by antibiotics. Antibiotic resistant bacteria arising from abattoir wastewater enter human environments and more about with people and goods thus, creating trans-border resistance. Knowledge of the transfer of resistance via plasmids, phages, transposons, and free DNA has increased considerably since the 1970s and 1980s (Levy, 2006). When the transfer was considered uncommon, it was thought that only Zoonitic bacteria from animals could infect humans, and the transfer of resistance genes from animals to humans was thought to be rare (Lacy 2008). The fear now is that antibiotic resistance bacteria, pathogens, or non-pathogens to humans are selected in the intestinal flora of animals, contaminate foods of animal origin, and transfer to their gut. The recommended levels for antibiotics were just 5-10ppm in the 1950s, but have increased 10-20 folds since then (Tenover *et al.*, 2019, Dewey *et al..*, 2018). Humans and animals live in close association with large numbers of bacteria with the majority in the large intestines, where they are exposed to antimicrobial agents, exchange genetic materials with other bacteria, on excretion, contaminate the environment or colonize other materials and humans. Contamination of carcass with feacal flora inevitably occurs. Food of animal origin may serve as a vehicle to transport resistant bacteria and resistance genes between animals and humans. The prevalence and degree of antibiotic resistance are found in the indicator of selective pressure of antibiotic usage (Wiggins, 2013). They correlate with the amounts and types of antimicrobial agents consumed by this population (Lester *et al.,* 2000, Van den Bogaard, 2011). This study aims to isolate antibiotic-resistant bacteria from abattoir wastewater.

2. Materials and Methods

2.1 Study Area

The study was carried out in Makurdi metropolis, the capital city of Benue state. North bank abattoir, Wadata market abattoir and Modern market abattoir. These abattoir sites were chosen because they are very popular among other abattoirs in Makurdi (Figure 1).

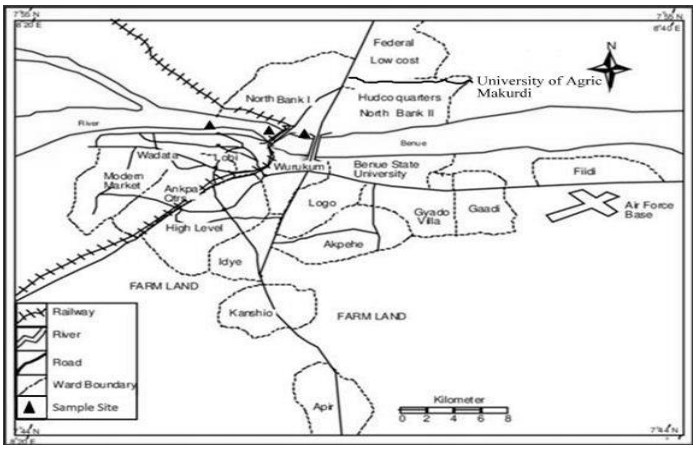

Figure 1: Map of Makurdi showing the study area.

2.2 Collection of Waste Water Samples

The wastewater samples were collected from different abattoirs within the Makurdi metropolis, the abattoirs include; the North bank market abattoir, Wadata market abattoir, and Modern market abattoir. Clean sterile containers were used and an ice-box container was also used to transfer the wastewater to maintain its flora. The samples were collected 3 hours before they were processed. Serial dilution of wastewater samples was carried out using test tubes. After serial dilution, the samples were transferred from the test tubes (1m1) and were plated on nutrient agar (NA), MacConkey agar (MCA), Mannitol salt agar (MSA) and Eosin methylene blue Agar (EMBA), and the plates were then incubated at 37C for 24 hours. The plating method used was the spread plate method. At the end of the incubation period, colonies that developed on the plates were picked and subcultured on Nutrient Agar to obtain pure cultures. The pure cultures were maintained on agar slants for further characterization and identification.

2.3 Characterization and identification of isolates

The isolates were characterized using the following biochemical test. The isolates were identified by comparing their characteristics with those of known taxa using the schemes of Cowan *et al.*, (2018).

2.3.1 Gram Stain

A thin smear of the inoculum was made on a glass slide with the use of a wire loop and a drop of distilled water. The smear was heat fixed, and the slide was placed on the staining rack, the fixed smear was then covered with crystal violet for 60 seconds, and was rapidly washed with clean water. The slide was then flooded with Lugol's iodine for 60 seconds after which it was washed off with clean water. The smear was decolorized with acetone alcohol and washed immediately with clean water. Counter-staining was done using saframine for 30 seconds and was washed off with clean water, the stained smear was air-dried. And the stained smear was viewed under the microscope using an oil immersion objective (X100). A deep or dark purple color indicated gram-positive bacteria, while a pale or dark red color indicated gram-negative strains of bacteria.

2.3.2 Catalase test

A drop of hydrogen peroxide was placed on a clean grease-free slide. Using a sterile wooden stick test colony was placed on the hydrogen peroxide on the slide, immediate bubbling indicated a positive result while no bubbles indicated a negative result.

2.3.3 Citrate test

This test is used to determine the ability of a microorganism to utilize carbon as the sole carbon and energy source of nitrogen. Simon citrate agar medium was dispensed into test tubes and sterilized at 121°C for 15 minutes. The bottles containing Simon citrate medium and test organism were then incubated at 37°C for 24 - 48 hours. A color change of the medium from green to blue indicates a positive reaction while retention of the original green color indicates a negative result as seen in the table.

2.3.4 Urease test

The isolate was inoculated in a bijou bottle containing three milliliters of christenseris modified urea agar and incubated for 3 - 12 hours, pink color indicates a positive urease test.

2.3.5 Indole test

Bacteria isolate was inoculated in a test tube containing distilled water inoculated for 48 hours, 0.5m/s of Kovac indole reagent was added and shacked gently. The formation of a red surface layer within 10 minutes indicates a positive indole test.

2.3.6 Oxidase test

The Oxidase reagent strip was moistened with sterile distilled water, a wire loop was used to pick a colony of bacteria inoculums and rubbed on the moistened strip, a red-purple color within 20 seconds indicates a positive oxidase test.

2.3.7 Hydrogen sulphide agar (H_2S)

Hydrogen sulphide agar slants were prepared and allowed to gel. The agar slants were then stabbed with the test organism and incubated at 37°C for 24 hours. The formation of a black precipitate indicates a positive result, otherwise negative.

2.3.8 Antibiotic sensitivity test

All the bacterial isolates were tested for their sensitivity to antibiotics using a disc diffusion method by Bauer *et al.* (2017). A multi-disc containing the following septrin 30ug, ciprofloxacin (CPX) 10ug, Amoxicilln (AM) 30ug. It was investigated using commercial antibiotic discs placed on nutrient agar plates previously seeded

within 18 hours, both cultures of the test organism. The plates were incubated at 37°C for 48 hours, after which zones of inhibition were examined and interpreted accordingly (Chortky *et al.*, 2018).

3. Results

The total bacteria count was obtained by counting discrete colonies on Nutrient agar respectively. The number of the colonies counted was multiplied by the reciprocal of the dilution factor plated, and divided by the volume of inoculums used, to obtain the colony forming unit per milliliter (CFU/M) of each of the samples as seen in table 1. Results from the biochemical identification and characterization are shown in Table 2. Table 3 show the percentage prevalence of bacteria isolates across location, *Staphylococcus* had the highest percentage of 7(22.58%) and *Salmonella* had the lowest percentage of 1(3.23%). Table 4 shows the antibiotic susceptibility of bacteria isolates (Gram Positive), *Bacillus*, *Streptococcus,* and *Staphylococcus*, all are resistant bacteria using multi-drug disc in the diffusion method. Table 5 show the antibiotic susceptibility of bacteria isolates (Gram Negative), results show that cumulative resistance of bacteria to antibiotic was also present, it was observed that *Klebsiella spp*, had the highest resistance pattern, while *Salmonella spp* had the lowest or no resistance pattern, using multi-drug disc in diffusion method. Antibiotic resistance patterns among the bacteria isolates were presented and the results were revealed. Spices like *Shigella spp*, *Klebsiella spp*, *Escherichia* coli, *Streptococcus spp*, *Bacillus spp*, *Proteus spp*, *Salmonella spp,* and *Staphylococcus spp* were found to be resistant to more than one antibiotic. Thus they showed multi-drug resistance, this resistance is believed to have been acquired as a result of animals being fed with antibiotics and this resistance can be transferred to individuals within the communities who drink from untreated water sources. The bacteria isolated in this study are known to be potential pathogens of man capable of causing a variety of diseases- *Staphylococcus* causes Infections of the skin, and deeper tissues, and *Staphylococcus* enteritis and food poisoning. *Escherichia coli* causes diarrhea, urinary tract, and kidney infections, and septicemia. *Shigella* causes bacillary dysentery and *Klebsiella* causes continuous watery diarrhea characterized by watery stools.

Table 1: Mean Total Viable Count of Samples

Sample	Mean viable count± S.D	Total viable count
North bank	$3.95 \times 10^6 \pm 0.99 \times 10^5$	$2.50 \times 10^6 \pm 5.94 \times 10^5$
Modern market	$2.56 \times 10^6 \pm 5.66 \times 10^5$	$2.47 \times 10^6 \pm 4.10 \times 10^5$
Wadata	$4.07 \times 10^6 \pm 1.27 \times 10^5$	$2.02 \times 10^6 \pm 0.57 \times 10^5$
P-value	0.036	0.517

Df=1 P< 0.05

Table 2: Cultural, Morphological, and Biochemical Characteristics of Isolates

Colony color	Colony shape	Morphology	Gram reaction	Catalase	Citrease	Urease	Indole	Oxidase	Hydrogen sulphide	Bacteria isolate
Cream	Circular	Cocci	+	+	+	_	_	_	_	*Staphylococcus spp*
Cream	Circular	Cocci	+	_	_	_	_	_	_	*Streptococcus spp*
White	Irregular	Rod	+	+	+	+	_	_	_	*Bacillus spp*
Mucoid pink	Circular	Rod	_	+	+	+	_	_	_	*Klebsiella spp*
Pale	Circular	Rod	_	+	+	+	_	_	+	*Proteus spp*
Pale	Circular	Rod	_	+	+	_	_	_	+	*Salmonella spp*
Pale	Circular	Rod	_	+	_	_	_	_	_	*Shigella spp*
Pale	Circular	Rod	_	+	_	_	+	_	_	*Escherichia coli*

Table 3: Percentage Prevalence of Bacteria Isolates Across Location

Bacteria isolates	North bank	Modern market	Wadata market	Total
Staphylococcus spp	3(9.68)	2(6.45)	2(6.45)	7(22.58)
Streptococcus spp	1(3.23)	1(3.23)	0(0.00)	2(6.45)
Bacillus spp	2(6.45)	3(9.68)	1(3.23)	6(19.35)

Klebsiella spp	2(6.45)	1(3.23)	3(9.68)	6(19.35)
Proteus spp	0(0.00)	1(3.23)	1(3.23)	2(6.45)
Salmonella spp	0(0.00)	0(0.00)	1(3.23)	1(3.23)
Shigella spp	1(3.23)	0(0.00)	1(3.23)	2(6.45)
Escherichia coli	2(6.45)	1(3.23)	2(6.45)	5(16.13)
Total	11(35.48)	9(29.03	11(35.48)	31(100.00)

Table 4: Antibiotic Susceptibility of Bacteria Isolates (Gram Positive)

Bacteria species	CPX (mm)	E (mm)	LEV (mm)	CN (mm)	APX (mm)	RD (mm)	AMX (mm)	S (mm)	NB (mm)	CH (mm)
Staphylococcus spp	20	Nil	20	25	20	Nil	Nil	24	25	26
Streptococcus spp	Nil	Nil	25	25	25	25	25	25	Nil	Nil
Bacillus spp	24	17	Nil	19	22	Nil	Nil	Nil	23	27

*Keys:*CPX = Ciprofloxacin, E = Erithromycin, LEV = Levofloxacin, CN = Gentamycin, APX = Ampiclox, AMX = Amoxicillin, S = Septrin, NB = Norfloxacin, RD = Rifampicin, CH = Chloramphenicol, mm = millimeter.

Table 5: Antibiotic Susceptibility of Bacteria Isolates (Gram Negative)

Bacteria species	CPX (mm)	SXT (mm)	S (mm)	PN (mm)	CEP (mm)	OFX (mm)	NA (mm)	PEF (mm)	CN (mm)	UA (mm)
E.coli	Nil	Nil	20	21	20	24	25	25	25	26
Shigella spp	25	26	25	25	26	25	25	25	20	Nil
Klebsiella spp	24	Nil	25	Nil	Nil	14	Nil	Nil	22	Nil
Salmonella spp	25	25	25	20	19	25	25	25	18	20
Proteus spp	23	26	25	20	19	Nil	18	Nil	24	24

Keys: CPX = Ciprofloxacin, SXT = Septrin, S = Streptomycin, PN = Amplicin, CEP = Ceporex, OFX = Tarivid, NA = Nalidixic acid, PEF = Reflacine, CN = Gentamycin, UA = Augmentin, mm = millimeter.

4. Discussion

This study investigated the bacteria associated with abattoir wastewater. From the result of this study, the bacteria associated with abattoir wastewater include species of *Escherichia*, *Salmonella*, *Shigella*, *Klebsiella*, *Bacillus*, *Proteus*, *Streptococcus,* and *Staphylococcus*. *Escherichia* coli were more consistently isolated from the wastewater followed by *Bacillus* and *Staphylococcus* species. This study agrees with other investigations that had isolated bacteria from wastewater (Coker *et al.* 2001),(Lateef, 2004). From the statistical analysis using ANOVA, there was a significant difference between the colony count in the different locations. North bank had the highest bacteria count ($2.50 \times 10^6 \pm 5.94 \times 10^5$), followed by the modern market ($2.47 \times 10^6 \pm 4.10 \times 10^5$), and Wadata had the lowest bacteria count ($2.02 \times 10^6 \pm 0.57 \times 10^5$).In this study, Staphylococcus has the highest prevalence counting at 7(22.58%), while Salmonella has the lowest prevalence counting at 1(3.23%). The presence of *Escherichia* coli in the water indicates contamination with feacal matter. *Bacillus* and *Staphylococcus* species are widely distributed in the environment, especially on animal farms (Prescott *et al.* 2008). The level of resistance to antimicrobial agents is a reflection of misuse or abuse of these agents in the environment especially on animal farms (Umoh *et al.*, 1990; Malik *et al.*, 1994).In this study, *Staphylococcus, Klebsiella, Bacillus,* and *Escherichia Coli* out of others are antibiotic-resistant bacteria due to B lactamase which is present in the species. This study agrees with the report of Person (2015|) and (2011) who found out that the production of B lactamase by bacteria makes them highly resistant to B lactamase antibiotics. Only gentamicin was effective against all the species of *Escherichia* tested. It was also observed that amoxicillin was effective against *Staphylococcus* species. This could be because these antibiotics are commonly used and are likely to be abused. Similarly, the resistance to cotrimoxazole, pefloxacin, ciprofloxacin, and amoxicillin reflects the widespread use of these antibiotics in Makurdi and Nigeria at large.

5. Conclusion

This study has shown that abattoir wastewater contains pathogenic bacteria including E. coli. The bacteria are commonly resistant to the frequently used antibiotics (Amoxycillin, Pefloxacine, and Cotromoxazone). Among the bacteria, most of them showed a multi-drug resistance. The abattoir wastewater should be carefully

managed or treated; it could be a source of disease outbreaks in Makurdi. The isolation of these pathogens is worrisome because the wastewater from all these abattoirs drains into River Benue which is the major river in the city and during scarcity of water people used the water from this river for domestic purposes.

6. Recommendations

Based on the results of the study, the recommendation is that;

- A study should be carried out on the river Benue or water bodies that receive wastewater from neighboring abattoirs within Makurdi.
- The abattoir wastewater should be treated before being discharged into water bodies.
- The public should be enlightened on the abuse of antibiotics, especially animal farmers.
- Administration of antibiotics to the animal should follow the regulations strictly.
- Regular monitoring of abattoir wastewater should be undertaken.

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Appendix