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ANTIBIOTIC SUCESPBILITY PATTERN OF *PSUEDOMONAS SPECIES* ISOLATED FROM WASTE WATER AND SEDIMENTS FROM ABATTOIR IN MAKURDI METROPOLIS

Odo Joel Inya[2]*, Aernan Paulyn.Tracy [1] and Okechukwu, G.N.[1]

[1]Department of Microbiology, University of Agriculture P M B 2373, Makurdi, Benue State, Nigeria.

[2]Departments of Fisheries and Aquaculture University of Agriculture P M B 2373, Makurdi, Nigeria

Corresponding author email. E-mail: odojoel@gmail.com

Abstract

To determine the antibiotic susceptibility pattern of *Pseudomonas spp* isolated from waste water and waste water sediments from abattoir in Makurdi metropolis. The samples of waste water and sediment were collected from drainage point immediately after slaughter slab where the solid parts (sludge) of the sewage was separated with the use of wire mesh to enable free settling sediment. Thus, samples were collected from four different abattoirs, located in North Bank, Wurukum, Modern Market and Wadata area of Makurdi metropolis. All the *pseudomonas* spp were examined microscopically. The samples were analyzed morphologically, culturally, and further subjected to biochemical tests using standard microbiological practices. The Kirby Bauer disc diffusion method was used for antibiotic susceptibility testing. From the quantification of isolates, the colony count ranged between 35.2x 10^3Cfu/ml to 2.0x10^9 Cfu/ml in waste water sediments. Colony count in waste water ranged from 7.2×10^3 Cfu/ml to 1.6×10^9 cfu/ml. The isolates showed resistance to Augumentin, Chloramphanicol, Septrin, while Ciprofloxacin, Amoxalin, Streptomycin and perfloxacin were highly susceptible and effective. Adequate treatment of waste water from this abattoir is highly recommended to reduce contamination and spread of infections leading to public health hazards. Furthermore, The butchers, sellers and workers in abattoirs should be educated on the importance of practicing good personal and environmental hygiene so to stop the spread of these organisms.

Keywords: *Pseudomonas* spp; Sediments; Abbatoir; Augumentin; Septrin; Perfloxacin

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Introduction

The high levels of harmful organisms found in human and animal waste, as well as the risk of disease transmission, are the key health concerns. Because of the microbial growth caused by the decomposition of animal remains, animal wastes pose a threat to the quality of the nearby groundwater (Padilla-Gasca et al., 2011). Wing and Wolf (2000) reported a decline in people' health and quality of life near intensive livestock operations and suggested that neighbours of an intensive swine farm frequently experienced respiratory and mucous membrane consequences. Activities related to abattoirs have also been linked to illnesses like pneumonia, diarrhoea, typhoid fever, asthma, diseases of wool sorters, respiratory illnesses, and chest illnesses (Bello and Oyedemi, 2009). It is generally acknowledged that Nigeria's waste management laws, when they exist, are dispersed, sparse, out-of-date, and ineffective. It is utterly ineffective, unenforceable, and ineffective in preventing the careless disposal of garbage into the environment.

Pseudomonas spp. are Gram-negative aerobic bacilli that are extensively distributed in nature and are especially prevalent in soils and water.They are opportunistic pathogens that are everywhere, likely as a result of their low nutritional needs and tolerance for challenging physical and chemical conditions, such as stream temperatures and sanitizers, as well as their limited nutritional requirements. A wide range of antibiotic classes, including as 3rd amd 4th -generations cephalosporins (cefepime) and carbapenems (imipenem and meropenem), are resistant to Pseudomonas aeruginosa (Black et al., 2002). Numerous studies have attempted to describe this resistance and set risk parameters in response to the introduction of *Pseudomonas spp*. strains with varying and increasing levels of antibiotic resistance. This phenomenon is intricate and has a variety of reasons, some of which have already been identified and others of which still require clarification. Pseudomonas species can spread more easily in aquatic environments, and their correlation with conditions that favour antibiotic multi-resistance can have major negative effects on public health. Both conventional medicinal procedures and animal growth promotion have employed antibiotics (Kelly et al., 2013). The resistance of harmful germs and their later transmission to humans through food are two effects of this widespread usage of antibiotics in animals. (AHPA, 1998).

For the survival of both humans and other biotic species, the environment is a crucial and essential element. Concern over environmental degradation brought on by pollution and the depletion of natural resources has grown over the past two decades. The environment has been exposed to both organic and inorganic pollutants as a result of human, agricultural, and industrial activity (Lim et al., 2010).

A location designated as a "abattoir" by a regulatory body or authority is one where animals are slaughtered and inspected in a hygienic manner, and where meat products for human consumption are effectively preserved and stored (Odo et al., 2022). Due to the ongoing push to boost meat production in order to meet the population's protein needs, the leakage of wastewater, particularly from abattoirs, into the environment has grown recently. Due to the slaughter of animals and the cleaning of the slaughterhouse facilities and Meat Processing Plants (MPPs), the meat processing sector generates huge volumes of abattoirs wastewater (Bastillo-Lecompte and Mehrvar, 2015).

The broad consensus across the globe is that abattoirs cause environmental pollution through a variety of activities, either directly or indirectly (Adelegan, 2002). Typically, wastewater is not adequately treated before being released from abattoirs into ecosystems (Mittal, 2006; Arvanitoyannis and Ladas 2008). Consequently, there are grave risks to the safety and health of the environment, surface water quality, and other factors. The slaughterhouse industry in Nigeria is a significant sector of the livestock industry, providing approximately 150 million people with domestic meat supplies and employment possibilities (Nafaranda et al., 2011). They are typically located close to water where various untreated waste streams are released (Adelegan, 2002). and cause authorities to be concerned about public health. There are many factors that affect how wastewater effluents affect recipient water bodies' quality, including the volume of the discharge, the concentration of chemicals and microbes, and the makeup of the effluents (Akpan, 2004). High levels of biodegradable organic materials, suspended and colloidal debris, including lipids, proteins, and cellulose, are present in the wastewater from abattoirs (Caixeta et al., 2002). The high levels of biochemical oxygen demand (BOD) and decreased dissolved oxygen caused by biodegradable organic matter in receiving waters result in intense competition for oxygen throughout the ecosystem, which is harmful to aquatic life. By encouraging the growth of algae, nutrient enrichment in receiving sensitive bodies of water can lead to eutrophication (called an algal bloom). Algae blooming and eventually collapsing might cause hypoxia/anoxia, which would cause fish populations to die off in significant numbers owing to a lack of dissolved oxygen in the water (Foroughi et al., 2010). These consequences include a detrimental influence on biodiversity, the potential extinction of vulnerable species, significant ecosystem changes, and a number of grave risks to human health. Environmental regulators are scrutinising the meat processing sectors more and more in an effort to lessen their impact on the environment (Padilla-Gasca et al., 2011). An essential element of the meat production chain that needs specific attention is the operation and treatment of abattoir effluents (Carlos-Hernandez et al., 2010). Due to the high levels of organic material in these effluents, which are produced in large numbers during slaughtering and meat processing services related to cleaning equipment and related facilities, aquatic ecosystems can be badly contaminated. Processing methods for liquid residues combine physical-chemical and biological treatments. While some of these pretreatment methods make use of conventional chemicals (based on sodium alkyl benzene sulphonate) and everyday sanitizers (like sodium hypo chlorate), others make use of biotechnological goods (like enzymes) (Pacheco, 2006). Similar to antibiotic resistance, the continued use of these cleaning agents may select for increasingly resistant germs, reducing membrane permeability and enzymatic inactivation of structures (Lourero et al., 2002).

Materials and Methods

Area of Study

This study was conducted in Makurdi, Benue state capital which is located between latitude 7^{o}41'N and latitude 8^{o} 28'E. The rainfall is bifocal (April- July and September to October) with a short spell sometimes in August (usually referred to as August break. This annual rainfall is between 1000mm-1500mm. the vegetation of the area is guinea, savannah, river Benue divides the town into north and south banks, the mean annual temperature is about 26°C while the relative humidity is between 60-80%.

Sample Site and Sample Collection

Samples of waste water and sediments were collected from four different abattoirs in Makurdi metropolis of Benue State. The samples of waste water and sediment were collected from drainage point immediately after slaughter slab where the solid parts (sludge) of the sewage was separated with the use of wire mesh to enable free settling sediment. The sediments and waste water were scooped using sterile hand towel and transfer into sterile sample bottles, labeled and transported to the laboratory for analysis.

Materials Used

Wire loop, swab stick, forceps, autoclave, microscope, weighing balance, test tubes, conical flasks, test tube racks, Aluminium foil, hand gloves, Pressure cooker, sterile sample bottle, Petri-dishes, glass shoes, distilled water, foil paper, cotton wool, media (nutrient agar, cetrimide, cled), marker, white tape, pipette, Reagents (Lugol's iodine, crystal violet, normal saline, safranin, peptone water, antibiotic disc.

Identification of Bacterial Isolates

All the *pseudomonas spp* bacteria were examined microscopically. They were later referred to appropriate genus and species following various morphological and biochemical tests (Lescott *et al*., 2008)

Gram Staining

This is done to distinguish between gramme positive and gramme negative organisms. The bacteria colonies were collected using a wire loop, spread on a grease-free glass slide, let air dry, and then fastened with heat. The smear was then quickly wiped off with distilled water after being treated with Lugol's iodine for 30 to 60 seconds. The water was turned on, droplets of crystal violet stain were applied to the smear for 30 to 60 seconds, and then the stain was quickly removed with distilled water. The water was turned off, Lugol's iodine was applied to the smear for 30 to 60 seconds, and then the smear was rinsed off with distilled water. The smear was promptly removed with distilled water after being decolored for 10 seconds with acetone or alcohol. The smear was rinsed with distilled water after being counter stained with safranin for 10 to 60 seconds. The back of the side was thoroughly cleaned, let to dry, and then viewed under a 100x oil immersion objective under a microscope.

Antibiotic Sensitivity of the Bacterial Isolates

Antimicrobial susceptibility Test Disc were used in the agar diffusion test method for in vitro susceptibility testing. Pure cultures of the isolates were inoculated into sterile normal saline and adjustment to march 0.5 McFarland standards. And then inoculated into Mueller Hinton agar using sterile swab sticks and then allowed to dry for five minutes. The filter paper discs impregnated with specified concentration of microbial agents were gently placed on the surface of the culture using sterile forceps. The antimicrobial diffused through the agar to form a gradient. After incubation at 30ºC for 16-18 hours, the zones of inhibition around the discs were measured. The antibiotic discs used were Septrin (30µg), Ciprofloxacin (10µg), Gentamycin (10µg), Perfloxacin (20µg), Amoxalin (20µg), Streptomycin (30µg), *Amoxilin* (15µg), Augmentin (30µg), Chloramphenicol (30µg), seprin (11µg). This was done for *Pseudomonas spp* isolates; clinical Laboratory Standard Institute, (2015) standard was used for the interpretation of the zones of inhibition.

Biochemical Test

The following biochemical tests were carried out on the isolate: citrate, catalase test, urease test, Oxidase test, coagulase test.

Indole Test

The tryptophan-using organisms were sought out using this assay. Using a sterile wire loop, an organism was cultured for 48 hours at 570°C in a test tube containing 5 ml of reptilian water (medium). 5.0 ml of Kovac's reagent was added to the test tube after incubation and left there for 15 minutes. A swat with a rose tint indicates a favourable response.

Urease test

This test was used to detect the ability of the organisms to produce urease enzymes. Urease in the presence of water converted urea to ammonia and carbon dioxide. Urease test using Christensen urea broth 2.1g urea agar base was weighed and dissolved in 100mls of distilled water in a conical flask. The agar was heated over a laboratory hot plate gently and sterilized with autoclave at 121^{o}C 15 ib for 15 minutes after which it was allowed to cool to 50^{o}C.

Urea concentrates was weighed (0.4g) and dissolved in 10mls sterile distilled water. This solution was poured aseptically into the sterile urea agar base and mixed gently after which it was dispensed, 3 mls into bijou bottle. The test organism was heavily inoculated on the medium using sterile wire loop and incubated at 37^{o}C for 3-12 hours. Pink colour indicates a positive urease test while absence of pink colour indicates negative urease test.

Catalase test

This test was used to distinguish between bacteria that produce the catalase enzyme, such as Staphylococci, and those that do not, such as Streptococci. The CheeseBrough approach was followed in the execution of this test. (2005)

Procedure: a drop of distilled water was made on one end of a clean slide and a drop of 3% hydrogen peroxide was made on the other end of the slide. A sterile wire loop was used to obtain a colony of the organism and emulsified on drop of distilled water made on the slide. The loop was then sterilized by passing it through flame. The same colony was emulsified on the hydrogen peroxide on the slide, the slide was then observed for bubble formation. Presence of bubbles indicates the presence of *Stapphlococcus spp*.

Citrate Test

This test was used to study the ability of the organisms to utilize citrate present in Simmon's medium as a sole source of carbon.

Materials used: Simmon's citrate agar, test bacterial culture.

Procedure: A bacterial colony was inoculated directly on simmon's citrate agar. A positive test was indicated by the appearance of growth with blue colour while a negative test showed no growth with original green colour.

Oxidase test

Is used to assist in identification of *Pseudomonas, Neisseria, Vibrio, Brucella* all of which produces the enzyme *cytochrome oxidase.*

A piece of filter paper was placed in a clean Petridish and 2 to 3 drops of 1% aqueous solution to oxidase reagent (tetrameltryl–p-phenyl diamine) was added to it such that it soak completely. A wire loop was used to pick a colony of the test organism and smeared on the filter paper. The development of a blue purple colour in 10 second showed a positive oxidase result and the absence of the blue purple colour indicates a negative result (Cheesbrough, 2016).

Coagulase Test

This test is used to differentiate staphylococcus aureus which produces the enzymes coagulase from *staphyloccus* epidermidis which do not produce the enzyme coagulase. A drop of distilled water was placed on two different point on a clean glass slide. A colony of a test organism was then emulsified in the water. One the suspension, a drop of plasma was added while the suspension serves as a control. Mixed gently and clumping of the sample was observed within ten seconds indicating a positive test, while a negative test shows no camping of the sample (slide coagulase test).

Results

Table 1 shows the frequency of occurrence of bacteria *spp* isolated from waste water and sediments from abattoir. *Escherichiacoli* was the most frequently occurring, 38% followed by *Bacillus spp*, 30.7% and the least was *Pseudomonas aeruginosa*, 7.6%. The total colony count of waste water and sediments sample from Wurukum Abattoir is shown on table 2. The highest colony counts of 1.6×10^{9} Cfu/ml and the lowest colony count of 7.2×10^{3} Cfu/ml was recorded from waste water sample. While the sediment has the highest colony count of 3.0×10^{8} Cfu/ml and the lowest colony count of 35.2×10^{3} Cfu/ml. The total colony count of waste water and sediments sample from Wadata Abattoir is shown in table 3. The highest colony counts of 1.6×10^{9} Cfu/ml and the lowest colony count of 15.10×10^{5} Cfu/ml was recorded from waste water sample. While thesediment has the highest colony count of 2.0×10^{8} Cfu/ml and the lowest colony count of 35.2×10^{3} Cfu/ml. Table 4 shows the total colony count of waste water and sediments sample from Modern Market Abattoir. The highest colony count of 1.6×10^{9} Cfu/ml and the lowest colony count of 22.0×10^{3} Cfu/ml was recorded from waste water sample. While the sediment has the highest colony count of 1.0×10^{9} Cfu/ml and

the lowest colony count of 36.8×10^3 Cfu/ml. The total colony count of waste water and sediments sample from North Bank Abattoir. The highest colony count of 3.2×10^7 Cfu/ml and the lowest colony count of 8.0×10^3 Cfu/ml was recorded from waste water sample. While the sediment has the highest colony count of 2.0×10^9 Cfu/ml and the lowest colony count of 17.6×10^5 Cfu/ml in table 5. Table 6 shows the susceptibility pattern of *Pseudomonas aeurginosa* to antibiotics using Gram negative antibiotics disc. *Pseudomonas aeurginosa* showed resistance to the following antibiotics, Chloramphenicol and Septrin, but susceptible to these antibiotics with their zone of Inhibition ranging from seprin (11 mm), Gentamycin (13 mm) Amoxilin (15 mm) Streptomycin (16 mm) Perfloxacin (17 mm), Ciprofloxacin (18 mm) and Amoxalin being the most susceptible with the zone of 20 mm.

Table 1: Frequency of occurrence of bacteria *spp* isolated from waste water and sediments from abattoir

S/N	Isolates	Frequency of isolation	Frequency (%)
1	*E.coli*	15	38.4
2	*Bacillus spp*	12	30.7
3	*Staphylococcus aureus*	9	23.0
4	*Pseudomonas aeruginosa*	3	7.6

Table 2: Total colony Count of Waste Water/Sediment Sample from Sample from Wurukum Abattoir

	S/N	Dilution factors	No. of colonies	Colony count (cfu/ml).
Waste water	1	10^2	72	7.2×10^3
	2	10^4	80	8.0×10^5
	3	10^6	40	4.0×10^7
	4	10^8	16	1.6×10^9
Sediments	1	10^2	352	35.2×10^3
	2	10^4	156	15.6×10^5
	3	10^6	7	7.0×10^6
	4	10^8	3	3.0×10^8

Table 3: Total colony Count of Waste Water/Sediment Sample from Sample from Wadata Abattoir

	S/N	Dilution factors	No. of colonies	Colony count (cfu/ml).
Waste water	1	10^4	152	15.2×10^5
	2	10^6	40	4.0×10^7
	3	10^8	16	1.6×10^9
Sediments	1	10^2	456	35.6×10^3
	2	10^4	252	825.2×10^5
	3	10^6	144	14.4×10^7
	4	10^8	2	2.0×10^8

Table 4: Total colony Count of Waste Water/Sediment Sample from Sample from Modern Market Abattoir

	S/N	Dilution factors	No. of colonies	Colony count (cfu/ml).
Waste water	1	10^2	220	22.0×10^3
	2	10^4	140	14.0×10^5
	3	10^6	72	7.2×10^7
	4	10^8	16	1.6×10^9
Sediments	1	10^2	368	36.8×10^3
	2	10^4	252	25.2×10^5
	3	10^6	7	7.0×10^6
	4	10^8	10	1.0×10^9

Table 5: Total Colony Count of Waste Water/Sediment Sample from North Bank Abattoir

	S/N	Dilution factors	No. of colonies	Colony count (cfu/ml).
Waste water	1	10^2	80	8.0×10^3
	2	10^6	32	3.2×10^7
Sediments	2	10^4	176	17.6×10^5
	4	10^8	20	2.0×10^9

Table 6 : Susceptibility Pattern of *Pseudomonasaeruginosa* to Antibiotics using Gram Negative Antibiotics Disc.

Antibiotics	*Pseudomonas aeruginosa*
Ciprofloxacin	18
Amoxilin	15
Augumentin	–
Gentamycin	13
Perfloxacin	17
Amoxalin	20
Streptomycin	16
Seprin	11
Chloramphanicol	–
Septrin	–

KEY

- Resistance

Discussion

From all the samples analyzed, *E. coli*, *Bacillus spp*, *Pseudomonas spp*, and *Staphlococcus aureus*, were foundin the sediments and waste water from the different abattoirs Makurdi metropolis. Pollution results when abattoir waste is dumped on the ground or drained into a water supply (Adeyemo et al., 2002). The complicated composition of abattoir effluent waste water makes it potentially hazardous to the environment. For instance, the discharge of blood and animal waste into streams would reduce the amount of dissolved oxygen (DO) in the aquatic environment, limiting the chance that aquatic life would survive (Nwachukwu et al., 2011). The waste from the abattoir's slaughtering and dressing grounds is dumped into open drains untreated, and as it decomposes, it can introduce enteric pathogens and excess nutrients into the nearby surface waters and underlying aquifers, contaminating the hand-dug wells that serve as both a source of drinking water for the butchers and other employees of the abattoir and the local residents, leading to disease. This level of contamination is considered not good for both domestic use and or direct discharge into

water bodies without treatment. *Pseudomonas spp* (7.6%), was less occurring from this work. This does not conform to the findings of (Falodun and Adekanmbi, 2016) who isolated strains of *Pseudomonas* (29.6%) from waste water generated in abattoir effluent in Ibadan. The noticed disparity may be due to the nature of the waste water, nutrient availability, the unhygienic environments of the abattoir provide means for these organisms to grow, multiply thus increasing the rate at which these organisms are spread. Environmental factors which are conducive for these microorganisms aid them to grow in this environment. Such factors includes adequate pH, temperature, nutrients, oxygen all aid these organisms thrive and being able to contaminate larger water when in contact (Federov *et al.,* 1993). The poor growth could also be linked with inadequate environmental factors reported by Ferervo *et al*. (1993). *Pseudomonas spp* obtained from these samples have shown different characteristic sensitivity and resistance pattern when antibiotic disc were introduced into the isolates from this work, the sensitivity was high and the resistance was low this is in line with the findings of (Merlin *et al.*, 2011). The ability of these species to form resistance is due to the presence of some resistant genes. This is not surprising giving the rate at which antibiotic drugs is being abused thus promoting the acquisition of resistant genes by the colony (Akan *et al.,* 2010). The high resistance level among this genera may be partly attributed to possible transfer of resistant trait from indigenous micro flora associated with source of raw materials used in the abattoir (Osinbajo and Addie, 2007). Since water is often used by individuals, contaminations from abattoir effluent are very possible. There is need to control the transfer and spread of infectious disease and antibiotic resistance through abattoir effluent.

Conclusion

It has been observed that *Escherichia coli*, *Bacillus spp*, *Staphylococcus spp* and *Pseudomonas spp* were isolated from both sediment and waste water in abattoir across Makurdi metropolis. *Pseudomonas spp* had a high sensitivity and low resistance to antibiotics.

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