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

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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 these 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.

Key Words: *Pseudomonas* spp; Sediments; Abbatoir; Augumentin; Septrin; Perfloxacin.

INTRODUCTION

The main health concern with human and animal wastes is the high concentrations of pathogenic organisms associated with this type of waste, and the potential it has to spread disease. Animal wastes represent a risk to the quality of local groundwater because of the proliferation of microorganisms that occurs during the process of corpses degradation (Padilla-Gasca *et al.,* 2011).Wing and Wolf

(2000) noted decrease health and quality of life of residents around intensive livestock operations and hinted that respiratory and mucous membrane effects were common with neighbors of intensive swine operation. Abattoir activities have also been found to be associated with some diseases such as pneumonia, diarrhea, typhoid fever, asthma, wool sorter diseases, respiratory and chest diseases (Bello and Oyedemi, 2009). It is generally observed that, waste management legislation in Nigeria, where available, is scattered, scantly, obsolete and non-effective. It is grossly inefficient and non-enforceable, and does not serve as a deterrent to the indiscriminate release of waste into the environment.

Pseudomonas spp, are Gram-negative aerobic bacilli widely distributed in the natural environment and particularly abundant in soils and water, they are opportunistic and ubiquitous pathogens, probably due to their limited nutritional requirements and tolerance of adverse physical and chemical conditions including stream temperatures and sanitizers. *Pseudomonas aeruginosa* shows a wide spectrum of resistance to different classes of antibiotic agents, including third and fourth-generation cephalosporins (cefepime) and carbapenems (imipenem and meropenem) (Black *et al.,* 2002). The emergence of *Pseudomonas spp*, strains with variable and growing levels of antibiotic resistance has generated considerable concern, and various studies have sought to characterize this resistance and establish risk parameters. This phenomenon is complex and has multiple causes, some of which have already been determined whereas others still need to be elucidated. The presence of *Pseudomonas spp,* in aquatic environments facilitates their dissemination, and their association with favourable conditions to antibiotic multi-resistance may cause serious public health problems. Antibiotics have been used to promote animal growth as well as for more traditional therapeutic treatments (Kelly *et al.,* 2013). One of the consequences of this wide use of antibiotic agents in animals is the development of resistance in pathogenic microorganisms and their posterior transmission to humans through food (AHPA, 1998).

The environment is a very important and necessary component for the existence of both man and other biotic organisms. The past two decades have witnessed a heightened concern over environmental degradation from pollution and depletion of natural resources. Organic and inorganic substances have been released into the environment as a result of domestic, agricultural and industrial activities (Lim *et al.,* 2010).

An abattoir is defined as a area which is approved by a regulatory agency or authority for hygienic slaughtering and inspection of animals, possessing and effective preservation and storage of meat products for human consumption (Odo *et al.,* 2022).The release of wastewater especially from abattoirs into the environment has increased in recent time due to the continuous drive to increase meat production to meet the protein needs of the population. The meat processing industry produces large volumes of abattoirs wastewater due to the slaughtering of animals and cleaning of the abattoirs facilities and Meat Processing Plants (MPPs) (Bastillo-Lecompte and Mehrvar, 2015).

Abattoirs are generally known all over the world to pollute the environment either directly or indirectly from their various processes (Adelegan, 2002). Wastewaters are usually released from the abattoirs directly into the ecosystems without adequate treatment process (Mittal, 2006; Arvanitoyannis and Ladas 2008). There by posing serious threats to surface water quality, general environmental safety and health. In Nigeria, the abattoir industry is an important component of the livestock industry providing domestic meat supply to over 150 million people and employment opportunities for teaming population (Nafaranda *et al.,* 2011). They are usually situated near aquatic environment were different untreated waste streams are discharged (Adelegan 2002). And constitute public health concern to authorities. The impact of wastewater effluents on the quality of receiving water bodies are manifold and depend on volume of the discharge, chemical and microbiological

concentration/composition of the effluents (Akpan, 2004). Abattoirs wastewaters contain high amounts of biodegradable organic matter, suspended and colloidal matter such as fats, proteins and cellulose (Caixeta *et al.,*2002). Biodegradable organic matter in receiving waters create high competition for oxygen within the ecosystem leading to high levels of biochemical oxygen demand (BOD) and a reduction in dissolved oxygen, which is detrimental to aquatic life. Nutrients (nitrogen and phosphorus) enrichment in receiving sensitive bodies of water can cause eutrophication by stimulating the growth of algae (called an algal bloom). Blooming and finally collapse of algae may lead to hypoxia/anoxia and hence mass mortality of fish over large areas due to aquatic dissolved oxygen depletion (Foroughi *et al.,* 2010). These effects entail a negative impact on biodiversity, sensitive species may be eliminated, major changes in ecosystem and a number of serious human health hazards may occur. The meat processing industries are under ever increasing scrutiny from environmental authorities to reduce its environment impact (Padilla-Gasca *et al.,* 2011). Adequate operation and efficient processes to treat abattoir effluents are an important stage of meat production chain which requires special attention (Carlos-Hernandez *et al.,* 2010). Large quantities of liquid effluents are generated during slaughtering and meat processing services related to cleaning equipment and associated facilities and the high levels of organic material in these effluents can severely pollute aquatic environments. Liquid residue processing regimes utilize biological treatment technologies by physical-chemical treatments. Some of these pretreatments use traditional chemical formulation (based on sodium alkyl benzene sulphonate) and common sanitizers (such as sodium hypo chlorate), while others utilize biotechnological products (such as enzymes) (Pacheco, 2006). The continuous use of these sanitizing substances can contribute to the selection of incremental resistant microorganisms similarly to antibiotic resistance, which can help reduce membrane permeability and enzymatic inactivation of structures (Lourero *et al.,* 2002).

MATERIALS AND METHOD

Area of Study

This study was conducted in Makurdi, Benue state capital which is located between latitude 7º41'N and latitude 8º 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.

Preparation of Media:

Nutrient Agar (NA)

Twenty-eight (28g) of powdered nutrient agar was weighed using laboratory digital scale and dispensed into 500ml of sterile distilled water in a 1-liter capacity conical flask. The flask was corked with cotton wool wrapped with aluminum foil and autoclaved 121ºC for 15minutes. the media was allowed to cool to 42-45ºC before being used.

Cetrimide Agar (CA)

Nine (9g) of cetrimide agar powder was weighed using laboratory digital scale and dispensed into 500ml of distilled water in a 1-liter capacity conical flask. The flask was corked with cotton wool wrapped with aluminum foil and autoclaved at 121ºC for 15minutes the media was allowed to cool to 42-45ºC before being used.

Isolation of Organisms in waste water and sediments:

Nine (9g) of the cetrimide agar was weighed and dissolved in 250ml of distilled water, brought to boil on the hot plate in order to dissolve completely. It was then autoclaved at 121ºc for 15minutes and was allowed to cool.

The waste water and sediment samples were serially diluted using 1ml into 9ml of sterile diluted water and 1ml of dilution 10^{-2} and10^{-4} were introduced into Petri-dishes and cooled media prepared above was poured into the Petri-dishes, swirled and allowed to solidify, inverted and incubated at 37ºc for 18-24hours, after which plates were observed and all the colonies with characteristics green colour were picked, purified and saved in slants for further work.

Nutrient agar (NA) powder weighing seven (7g) was dispensed into 250ml of distilled water in a conical flask. After mixing the solution, it was heated gently to dissolve and then autoclaved. The agar was allowed to cool to about 45ºC then poured into the Petri-dish containing the samples using pour plate method. The plates were incubated at 37ºC for 18-24 hours. After 24 hours, the colonies observed on the plates were counted.

Identification of Bacterial Isolates

All the *pseudomonasspp* 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 carried out to differentiate gram positive from gram negative organism. The bacteria colonies was picked with a wire loop and smeared on grease free glass slide and allowed to air dry and fixed on the smear by heat. The smear was then covered with Lugol's iodine for 30-60 seconds and then rapidly washed off with distilled water. The water was tipped off and the smear was covered with drops of crystal violet stains for 30-60seconds and then rapidly washed off with distilled water. The water was tipped off and the smear was covered with Lugol's iodine for 30 – 60 seconds and washed off with distilled water. The smear was decolorized with acetone or alcohol for 10second and immediately washed off with distilled water. The smear was counter stained with safranin for 10-60seconds and washed with distilled water. The back of the side was wiped clean allowed to dry and examined microscopically with oil immersion at 100x objective.

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 30^0C for 16-18hours, 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

This test was used to detect organisms that utilize the amino acid tryptophan. A sterile wire loop was used to inculcate organism in a test tube containing 5ml of reptide water (medium) and incubated for 48hours at 57^0c. After incubation, 5.0ml of kovoc's reagent was added into the test tube and allowed to stand for 15mins. A rose spank color to indicate positive reaction.

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 15ib for 15minutes 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, 3mls 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 differentiate those bacteria that produce the enzyme catalase such as *staphylococci* from non-catalase producing bacteria such as *streptococci*. This test was carried out according to the method of CheeseBrough. (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 soaks 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).

RESULT AND DISCUSSION

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 *Pseudomonasaeruginosa,*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 7.2×10^3Cfu/ml was recorded from waste water sample. While the sediment has the highest colony count of 3.0×10^8Cfu/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 15.10×10^5Cfu/ml was recorded from waste water sample. While thesediment has the highest colony count of 2.0×10^8Cfu/ml and the lowest colony count of 35.2×10^3cfu/ml. Table 4 shows the total colony count of waste water and sediments sample from Modern Market Abattoir.The highest colony count of 1.6×10^9Cfu/ml and the lowest colony count of 22.0×10^3Cfu/ml was recorded from waste water sample. While the sediment has the highest colony count of 1.0×10^9Cfu/ml and the lowest colony count of 36.8×10^3Cfu/ml. The total colony count of waste water and sediments sample from North Bank Abattoir. The highest colony count of 3.2×10^7Cfu/ml and the lowest colony count of 8.0×10^3Cfu/ml was recorded from waste water sample. While the sediment has the highest colony count of 2.0×10^9Cfu/ml and the lowest colony count of 17.6×10^5Cfu/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 (11mm), Gentamycin (13mm) Amoxilin (15mm) Streptomycin (16mm) Perfloxacin (17mm), Ciprofloxacin (18mm) and Amoxalin being the most susceptible with the zone of 20mm.

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 x 10^5
	2	10^6	40	4.0 x 10^7
	3	10^8	16	1.6 x 10^9
Sediments	1	10^2	456	35.6 x 10^3
	2	10^4	252	825.2 x 10^5
	3	10^6	144	14.4 x 10^7
	4	10^8	2	2.0 x 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 x 10^3
	2	10^4	140	14.0 x 10^5
	3	10^6	72	7.2 x 10^7
	4	10^8	16	1.6 x 10^9
Sediments	1	10^2	368	36.8 x 10^3
	2	10^4	252	25.2 x 10^5
	3	10^6	7	7.0 x 10^6
	4	10^8	10	1.0 x 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	***Pseudomonasaeruginosa***
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. Wastes from abattoir are deposited on the land or channelled into water resource leading to pollution (Adeyemo *et al.,* 2002). Abattoir effluent waste water has a complex composition and can be harmful to the environment. For example, the discharge of animal waste water and blood into streams would deplete the dissolved oxygen (DO) of the aquatic environment thus reducing the survival of aquatic

life (Nwachukwu *et al.,* 2011). The waste from the slaughtering and dressing grounds in the abattoir are washed into open drainages untreated and the decomposition processes of these wastes can introduce enteric pathogens and excess nutrients into the surrounding surface waters which also percolate into the underlying aquifers to contaminate the hand dug wells which serve dual purpose of drinking water for the butchers and others working in the abattoir and the people in the neighbourhood thus, causing diseases such as diarrhoea, typhoid fever and pneumonia. 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 include 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 was 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 genus 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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