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Antibiogram of *Pseudomonas* isolates and potential public health impact of an abattoir effluent in Benin City, Nigeria

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Abstract:

Background: Bacteria from abattoir wastes are often linked to livestock carcasses previously exposed to continuous antimicrobial use and misuse; thereby creating opportunity for community spread of multidrug resistant (MDR) strains such as *Pseudomonas* spp. The aim of this study was to investigate the antibiogram of *Pseudomonas* isolates and bacteriological quality of an abattoir effluent in lieu of its potential public health impact.
Methodology: Water samples were collected weekly for six weeks from discharge point (DP) of the abattoir effluent, effluent receiving canal confluence point (CP), and 500 m upstream (US) and 500 m downstream (DS) from points where CP made contact with the Ikpoba River, Benin City, Nigeria. Bacteria spp. were isolated, enumerated (heterotrophic bacterial plate, coliform, *Escherichia coli* and *Pseudomonas* counts) and identified using standard microbiological techniques. Identity of *Pseudomonas* isolates was confirmed by PCR while antibiogram of selected isolates was evaluated and interpreted according to the disk diffusion method of the Clinical and Laboratory Standards Institute (CLSI).
Results: Heterotrophic bacteria plate counts (HPC) varied from $1.1\times10^3 \pm 0.28$ CFU/ml to $1.95\times10^6 \pm 0.48$ CFU/ml; total coliform counts ranged between 0.0 and $1.2\times10^6 \pm 0.28$ CFU/ml while mean *E. coli* count varied from 0.0 to $4.9\times10^5 \pm 0.49$ CFU/ml, and *Pseudomonas* counts were between 0.0 to 1.4×10^3 CFU/ml. The selected strains of *Pseudomonas* spp (n=50) showed resistance to oxacillin (100%), vancomycin (52%), tetracycline (50%), gentamycin (26%) and ceftriaxone (20%), while they were sensitive to ceftazidime (82%), ofloxacin (80%) and amikacin (74%). MDR phenotype was observed in 9 (18%) of the test isolates.
Conclusion: The study revealed that untreated abattoir effluent was a considerable source of MDR *Pseudomonas* spp. among other bacteriological pollutants (e.g. HPC, coliform and *E. coli*) that could compromise the quality of the receiving river in lieu of public health concerns of riverside communities that depend on this vital water resource for their subsistence.

Keywords: *Pseudomonas*; MDR; antibiogram; abattoir effluent; public health

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Antibiogramme des isolats de *Pseudomonas* et impact potentiel sur la santé publique d'un effluent d'abattoir à Benin City, Nigeria

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Abstrait:

Contexte: Les bactéries provenant des déchets d'abattoir sont souvent liées à des carcasses de bétail précédemment exposées à une utilisation et une mauvaise utilisation antimicrobiennes; créant ainsi une opportunité de propagation communautaire de souches multirésistantes (MDR) telles que *Pseudomonas* spp. Le but de cette étude était d'étudier

l'antibiogramme des isolats de *Pseudomonas* et la qualité bactériologique d'un effluent d'abattoir au lieu de son impact potentiel sur la santé publique.
Méthodologie: Des échantillons d'eau ont été prélevés chaque semaine pendant six semaines à partir du point de rejet (DP) de l'effluent de l'abattoir, du point de confluence du canal de réception des effluents (CP) et à 500 m en amont (US) et 500 m en aval (DS) des points où le CP a établi le contact avec la rivière Ikpoba, Benin City, Nigeria. Bacteria spp. ont été isolés, dénombrés (plaques bactériennes hétérotrophes, coliformes, dénombrements d'*Escherichia coli* et de *Pseudomonas*) et identifiés à l'aide de techniques microbiologiques standard. L'identité des isolats de *Pseudomonas* a été confirmée par PCR tandis que l'antibiogramme des isolats sélectionnés a été évalué et interprété selon la méthode de diffusion sur disque du Clinical and Laboratory Standards Institute (CLSI).
Résultats: le nombre de plaques de bactéries hétérotrophes (HPC) variait de $1,1\times10^3 \pm 0,28$ UFC/ml à $1,95\times10^6 \pm 0,48$ UFC/ml; le nombre total de coliformes variait entre 0,0 et $1,2\times10^6 \pm 0,28$ UFC/ml tandis que le nombre moyen d'*E. coli* variait de 0,0 à $4,9\times10^5 \pm 0,49$ UFC/ml, et le nombre de *Pseudomonas* était compris entre 0,0 et $1,4\times10^3$ UFC/ml. Les souches sélectionnées de *Pseudomonas* spp (n=50) ont montré une résistance à l'oxacilline (100%), à la vancomycine (52%), à la tétracycline (50%), à la gentamycine (26%) et à la ceftriaxone (20%), alors qu'elles étaient sensibles à la ceftazidime (82%), l'ofloxacine (80%) et l'amikacine (74%). Le phénotype MDR a été observé dans 9 (18%) des isolats testés.
Conclusion: L'étude a révélé que les effluents d'abattoir non traités étaient une source considérable de MDR *Pseudomonas* spp. entre autres polluants bactériologiques (par exemple HPC, coliformes et *E. coli*) qui pourraient compromettre la qualité de la rivière réceptrice au lieu des préoccupations de santé publique des communautés riveraines qui dépendent de cette ressource vitale en eau pour leur subsistance.

Mots-clés: *Pseudomonas*; MDR; antibiogramme; effluent d'abattoir; santé publique

Introduction:

The meat processing industry consumes 29% of the total freshwater used by the agricultural sector worldwide (1). This creates an opportunity for the generation of large amounts of wastewater that are ultimately discharged into the environment. Wastes from such facilities have been reported to be of public health concern, especially in developing countries such as Nigeria, where abattoir effluents are directly disposed into streams and rivers with little or no treatment (2). Incidentally, many of these streams and rivers also serve as water resource for domestic, agricultural, recreational, as well as drinking purposes for communities downstream (3).

Contamination of rivers by abattoir wastes could constitute significant environmental and health hazards, promoting growth of disease-causing organisms including *Pseudomonas* spp. among others (4,5). Auwalu et al., (6) reported a significant nexus between abattoir effluents and diseases such as typhoid fever, diarrhoea illnesses, respiratory diseases, cough, foot and mouth diseases and dengue, affecting abattoir neighboring communities in Malaysia while Bello and Oyedemi (7) associated diseases such as pneumonia, diarrhoea, typhoid fever, asthma, wool sorter disease, and respiratory and chest diseases, with abattoir activities in Ogbomoso, Nigeria.

The genus *Pseudomonas* comprises Gram-negative, motile, aerobic Gamma-proteobacteria grouped into the family Pseudomonadaceae (8). There are 191 validly described species in this genus; many of which demonstrate a great deal of metabolic diversity and consequently colonize a wide range of ecological niches including soil, water, humans, plant and animal surfaces, cosmetics, medical products and instruments, and foods of animal and vegetal origins (9,10). *Pseudomonas* spp. are opportunistic pathogens with intrinsic antimicrobial resistance due to low outer membrane permeability as well as extensive efflux pump system (11). The United States Center for Disease Prevention and Control (CDC) reported *Pseudomonas* species as one of the top 18 antibiotic-resistant pathogens that pose serious threats in the US (12). The document further observed that more than 6700 multidrug resistant (MDR) *Pseudomonas* infections with 440 deaths per year were reported in 2019 (12). *Pseudomonas* has been incriminated in infection of the urinary tract, blood steam, pneumonia, pharyngitis, and many other medical conditions with remarkably high morbidity and mortality (13).

Abattoir effluents are particularly pivotal in the spread of MDR pathogens in the community. Many isolates from this environment emanate from livestock carcasses previously exposed to continuous antimicrobial use and misuse for therapeutic and/or prophylactic purposes, as well as growth promoters. This creates ample opportunity for such MDR strains to be transferred via the food chain to humans and spread in the population (5). The spread of MDR pseudomonads from different sources to humans and the environment indicates the frequent dissemination of resistance genes by horizontal gene transfer (HGT), since many of these genes are located in plasmids, integrons,

or transposons (10).

Odjajdare et al., (14) reported a significant correlation between wastewater effluent quality and that of receiving waters, indicating that release of effluents containing MDR *Pseudomonas* spp into the environment could potentially compromise the public health of persons who depend on such resource for sundry uses. Beside the higher rate of fatality among patients with resistant bacteria related infections, the occurrence of MDR infection mandates longer hospitalization and therapies with complex and expensive treatment modalities especially in low-and middle-income countries like Nigeria. According to the Organization for Economic Cooperation and Development (OECD), 2.4 million deaths due to MDR related infections are expected in Europe, North America, and Australia, with an associated cost of up to US$3.5 billion per year in the next 30 years (12). These impact and cost estimates are expected to be even worse in Africa and other developing countries where there is severe shortage of infrastructure and healthcare delivery facilities.

Antibiotic resistant pseudomonads in abattoir have been previously reported in Nigeria (15,16,17), thus highlighting a growing public health concern over possible outbreak of difficult to treat community acquired *Pseudomonas* infections. It is therefore imperative that surveillance of resistance patterns of *Pseudomonas* strains from the environment are regularly and continuously conducted to generate data that would help clinicians choose correct empirical treatment in lieu of public health preservation. Thus, the aim of this study was to investigate the antibiotic resistance profile and public health impact of *Pseudomonas* species isolated from an abattoir effluent that empties into the Ikpoba River, Benin City, Nigeria.

Material and Methods:

Study site and sampling

The study was conducted in an abattoir that introduces its effluent into the Ikpoba River, Benin City, Nigeria. Fig 1 shows the sampling sites as derived from Google map using a Global Positioning System (GPS). Samples were collected weekly for six weeks from four (4) points within the abattoir catchment including abattoir effluent discharge point (point source; DP), effluent receiving canal (confluent point; CP), and 500 m upstream (upstream; US) and 500 m downstream (downstream; DS) from the point where CP makes contact with the Ikpoba River.

The samples were collected in 1-liter capacity sterile plastic containers in duplicates and transported in cooler boxes containing ice packs to the Microbiology Laboratory of Benson Idahosa University for further analysis. All samples were analyzed within 24 hours of sample collection.

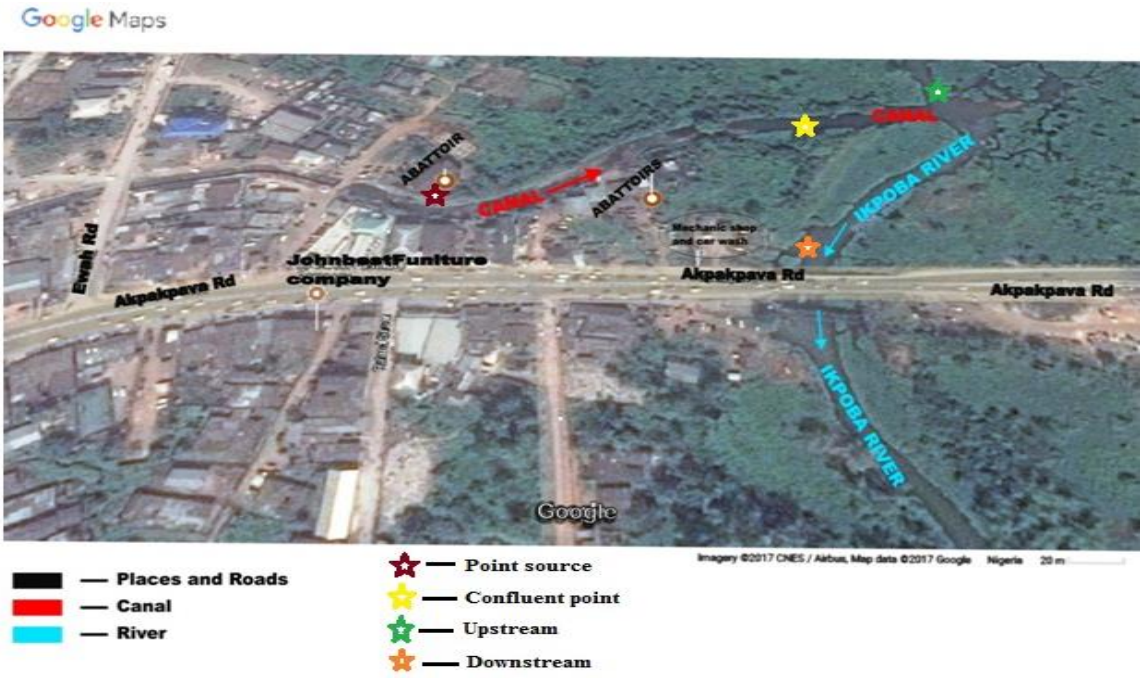

Fig 1: Images of the abattoir catchment as derived from Google map using a Global Positioning System (GPS). www.googlewmaps.com

Microbiological analysis

Each water sample was serially diluted and used to inoculate sterile nutrient agar, MacConkey agar, EMB agar and Cetrimide agar (containing 10% glycerol) plates following standard spread plate technique. All plates were incubated at 37°C for 24-48 hrs. After incubation, total (heterotrophic) bacterial counts (TBC) otherwise known as heterotrophic plate counts (HPC), total coliform counts, *E. coli* and *Pseudomonas* counts were recorded from the respective agar plates. Coliforms on MacConkey agar appeared as pink colonies; *E. coli* on EMB agar appeared as green colonies with metallic-sheen; while *Pseudomonas* spp. colonies present with green pigmentation on Cetrimide agar.

Pseudomonas isolates were phenotypically identified using colonial, cultural, morphological (Gram staining, motility) and biochemical (catalase and oxidase) characteristics (18). Presumptive *Pseudomonas* isolates were then subcultured onto sterile nutrient agar plates and subsequently stored on nutrient agar slants at 4°C in the refrigerator until needed for further analysis.

Antibiotic susceptibility testing of *Pseudomonas* isolates

The antibiotic susceptibility test against 8 selected antibiotics was performed and interpreted based with the disk diffusion method of the Clinical and Laboratory Standards Institute (19) using 24 hours culture of *Pseudomonas* isolates (n=50) on Mueller Hinton agar (HIMEDIA®, India, REF-M173) plates. The paper antibiotic disks (Mast Diagnostics, Merseyside, UK) used were amikacin (30µg), ceftazidime (30µg), ceftriaxone (30µg), gentamicin (10µg), ofloxacin (5µg), oxacillin (1µg), tetracycline (30µg), and vancomycin (30µg).

Molecular confirmation of *Pseudomonas* isolates

DNA extraction

DNA extraction from pure cultures of phenotypically identified *Pseudomonas* isolates (n=50) was carried out as described by Odjadjare and Igbinosa (3) with slight modifications. Single colonies of presumptive *Pseudomonas* isolates grown overnight at 37°C on nutrient agar plates were picked with sterile wire loop, suspended in 200µl of sterile nuclease free water (Life Science Biotechnology, Solon, South Africa) and the cells lysed by heating on a heating-block (Fisher Scientific, Model: 2052-1CEFS, California, U.S.A) for 15 mins at 100°C. The cell debris was removed by centrifugation at 11,000×g for 2 mins using a MiniSpin microcentrifuge (Model: Mini-14K, TOMOS Life Science Group, Shanghai, China). The cell lysates (5µl) were used as DNA template in the PCR assays immediately after extraction.

PCR amplification of target genes

PCR was performed in a GenePro Thermal Cycler (Model: TC-E-96G Hangzhou Bioer Technology Co., China LTD). The PCR master mix (Inqaba Biotech, South Africa) for a typical 20µl reaction included; 2×Master mix (10µl), nuclease free water (3.0µl), Primer F (5pmol/u) (1.0µl), Primer R (5pmol/u) (1.0µl) and DNA template (5.0µl). The primers used were; *Pseudomonas* genus 16S rRNA forward (5'-GACGGGTGAGTAATGCCTA-3') and 16S rRNA reverse (5'-CACTGGTGTTCCTTCCTATA-3') primers (20).

The PCR conditions for amplification were as follows; initial denaturation at 95°C for 5 mins., followed by 10 cycles of denaturation at 94°C for 15 sec, annealing at 53°C for 30 sec and 72°C for 45 second. This was repeated for another 25 cycles with exception of the 72°C elongation step, which was increased by 5 sec at every cycle; and a final extension step of 72°C for 10 min. Amplicon size of the gene was 617 bp (20). The amplified PCR products were held at 4°C on completion of the reaction until it is used for further analysis.

Agarose gel electrophoresis

The PCR products were resolved by electrophoresis in 1.8% agarose gel in 1× TAE buffer (4.85g of Tris, 0.37g of EDTA and 1.64g of sodium acetate in 1000 ml of distilled water) stained with ethidium bromide (5µl/100ml). Five microlitre (5µl) of the PCR products was mixed with 5µl loading dye and loaded onto sample wells. Electrophoresis was run at 100 V for 30 to 45 mins. The resolved bands were visualized under UV transilluminator and photographed (3).

Statistical analysis

Calculation of means and standard deviation was done using Microsoft Excel Office 2013. Comparisons of means were analysed statistically, using the one-way analysis of variance (ANOVA) and Pearson chi-square. Relationships were tested using the Pearson correlation index. All statistical analyses were performed using SPSS 23.0 software. Values were deemed to be statistically significant at 95% confidence interval (i.e. $p<0.05$).

Results:

Bacteria load estimation and identification

The total heterotrophic bacterial counts (TBC) during this study ranged between 1.1 × 10^3±0.28 CFU/ml and 1.95×10^6±0.48 CFU/ml (Table 1). The highest bacterial count was observed in samples collected at CP in week 6, while the lowest was observed in week 4 at US. Bacterial counts at DP and CP were relatively higher than those recorded at US and DS; however, the observed difference was not statistically significant across sampled points. There was significant correlation between TBC and coliform counts (p<0.01) and TBC versus *Pseudomonas* count (p<0.05). However, there was no significant correlation between TBC and *E. coli* count.

Table 2 shows the total coliform counts during the study. The highest coliform count was observed in the sixth week at CP (1.2×10^6±0.28 CFU/ml) while the lowest (0.0) were observed in weeks 2 and 3 at US. There was no significant correlation between coliform count and other bacterial counts (e.g. *E. coli* and *Pseudomonas* counts), except with TBC as earlier indicated. The mean *E. coli* count during the study ranged from 0.0 in US and DS (weeks 2 to 4) to 4.9×10^5±0.49 CFU/ml in CP (week 6) (Table 3). *E. coli* count did not vary significantly with sampling point and time. There was also no significant correlation between *E. coli* and other bacterial counts (including TBC, coliform and *Pseudomonas* counts).

Table 1: Mean total bacterial counts of test samples (CFU/ml) of abattoir effluents in Benin City, Nigeria

Sampled points	Sampling Weeks					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Point source (DP)	1.98 ×10^5±0.14[a]	2.85×10^5±0.7	1.97×10^5±0.19	1.19×10^5±0.2	1.79×10^5±0.15	1.67×10^6±0.64
Confluent point (CP)	1.16 ×10^5±0.24	1.71×10^6±0.14	3.5 ×10^4±0.2	2.3 ×10^5±0.5	2.88×10^5±0.19	1.95×10^6±0.48
Upstream (US)	4.05 ×10^5±0.35	2.75×10^5±0.4	2.6×10^5±0.3	1.1×10^3±0.28	2.68×10^4±0.36	1.63×10^4±0.49
Downstream (DS)	5.2×10^4±0.14	4.9×10^4±0.21	5.6×10^4±0.35	2.7×10^4±0.14	8.4×10^4±0.2	7.1×10^3±0.28

[a]values represent mean ± standard deviation

Table 2: Mean total coliform counts of test samples (CFU/ml) of abattoir effluents in Benin City, Nigeria

Sampled points	Sampling weeks					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Point source (DP)	3.25×10^4±0.19[a]	7.15 × 10^5±0.12	5.15×10^5±0.35	2.8 ×10^5±0.85	6.0×10^4±0.89	1.56×10^4±0.28
Confluent point (CP)	3.5 ×10^4±0.42	9.25 × 10^5±0.7	8.1×10^5±0.28	8.65×0^5±0.35	2.7×10^5±0.49	1.2×10^6±0..28
Upstream (US)	2.9 ×10^4±0.14	99.5×10^2±0.71	3.7 × 10^4±0.1	1.3×10^3±0.1	7.65×10^3±0.35	1.15×10^4±0.31
Downstream (DS)	3.1×10^4±0.14	0±0	0±0	7.5×10^3±0.4	5.7 ×10^4±0.42	1.39× 10^5±0.14

[a]values represent mean ± standard deviation

Table 3: Mean *Escherichia coli* counts of test samples (CFU/ml) of abbatoir effluents in Benin City, Nigeria

Sampled points	Sampling weeks					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Point source (DP)	$2.0\times10^{4}\pm0.7^{a}$	$8.0\times10^{3}\pm0.11$	$6.0X10^{3}\pm0.85$	$1.14\times10^{4}\pm0.22$	$2.9\times10^{4}\pm0.56$	$1.9\times10^{5}\pm0.28$
Confluent point (CP)	$5.5\times10^{3}\pm0.71$	$1.3\times10^{4}\pm0.14$	$1.4\times10^{4}\pm0.14$	$7.4\times10^{3}\pm0.78$	$2.8\times10^{4}\pm0.49$	$4.9\times10^{5}\pm0.49$
Upstream (US)	$1.8\times10^{4}\pm0.42$	0±0	0±0	0±0	0±0	0±0
Downstream (DS)	$3.1\times10^{4}\pm0.28$	0±0	0±0	0±0	$5.1\times10^{4}\pm0.53$	$1.13\times10^{4}\pm0.12$

[a]values represent mean ± standard deviation

Table 4: Mean *Pseudomonas* counts of test samples (CFU/ml) of abbatoir effluents in Benin City, Nigeria

Sampled Points	Sampling Weeks					
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Point source (DP)	$1.0\times10^{2}\pm10^{a}$	$3.3\times10^{2}\pm18$	0±0	$4.1\times10^{2}\pm14$	$1.4\times10^{3}\pm65$	$5.5\times10^{2}\pm25$
Confluent point (CP)	$3.25\times10^{2}\pm85$	$6.0\times10^{2}\pm10$	0±0	$1.8\times10^{2}\pm65$	$7.0\times10^{2}\pm30$	$6.0\times10^{2}\pm0$
Upstream (US)	0±0	0±0	0±0	0±0	0±0	$2.0\times10^{0}\pm0.7$
Downstream (DS)	0±0	0±0	0±0	$6.5\times10^{1}\pm5$	$2.5\times10^{1}\pm0.5$	0±0

[a]values represent mean ± standard deviation

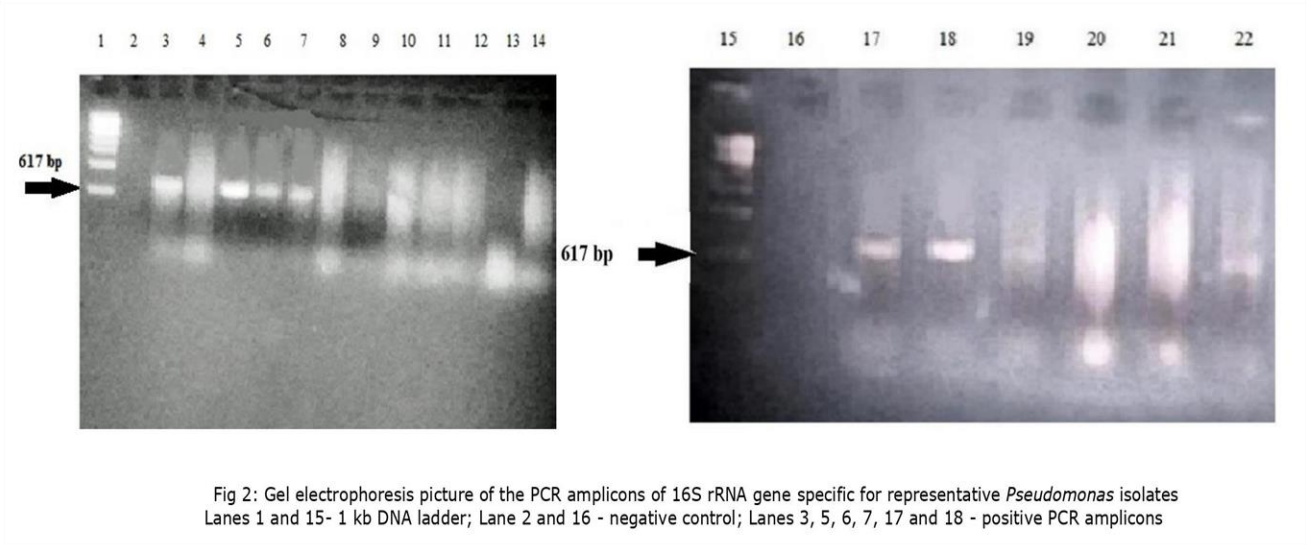

Fig 2: Gel electrophoresis picture of the PCR amplicons of 16S rRNA gene specific for representative *Pseudomonas* isolates
Lanes 1 and 15- 1 kb DNA ladder; Lane 2 and 16 - negative control; Lanes 3, 5, 6, 7, 17 and 18 - positive PCR amplicons

Presumptive *Pseudomonas* counts on cetrimide agar are shown in Table 4. The highest *Pseudomonas* count (1.4×10^{3} CFU/ml) was observed at DP in week 5 while the lowest (0.0) were recorded at US in weeks 1 to 6 and DS in weeks 1,2,3 and 6. *Pseudomonas* count for CP varied significantly ($p<0.05$) with counts from US and DS. However, *Pseudomonas* count did not vary significantly with sampling time. *Pseudomonas* count did not also correlate with other bacterial counts except for TBC as earlier mentioned. Fifty (50) presumptive *Pseudomonas* strains isolated and selected based on cultural, morphological and biochemical characteristics were confirmed by genus specific PCR (Fig 2).

Table 5: Antibiotic susceptibility profile of *Pseudomonas* spp isolated from abattoir effluents in Benin City, Nigeria

Antibiotic class	Antibiotics	Antibiogram (n=50)		
		Resistant (%)	Intermediate (%)	Sensitive (%)
Aminoglycoside	Amikacin	0	23 (26)	37 (74)
	Gentamicin	13 (26)	17 (34)	20 (40)
Cephem	Ceftazidime	1 (2)	8 (16)	41 (82)
	Ceftriaxone	10 (20)	38 (76)	2 (4)
Fluoroquinolone	Ofloxacin	4 (8)	6 (12)	40 (80)
Penicillin	Oxacillin	50 (100)	0	0
Tetracycline	Tetracycline	25 (50)	17 (34)	8 (16)
Glycopeptide	Vancomycin	26 (52)	24 (48)	0

Table 6: Multiple drug (MDR) resistance profile of the *Pseudomonas* isolates[a]

S/N	MDR Phenotype	No of isolates with MDR Phenotype
1.	OFX, GM, T, CRO	1
2.	GM, T, CRO	7
3.	OFX, T, CRO	1

[a]MDR profiles presented in the table are exclusive of the non-pseudomonal antibiotics (oxacillin and vancomycin); OFX = Ofloxacin; GM = Gentamycin; T = Tetracycline; CRO = Ceftriaxone

Antibiogram of *Pseudomonas* isolates

The antibiogram of the *Pseudomonas* strains indicated that the isolates were resistant to oxacillin (100%), vancomycin (52%), tetracycline (50%), gentamycin (26%) and ceftriaxone (20%); whereas, they were sensitive to ceftazidime (82%), ofloxacin (80%) and amikacin (74%). *Pseudomonas* isolates exhibited intermediate susceptibility (76%) to ceftriaxone (Table 5).

The MDR phenotypes involving four antibiotic classes (fluoroquinolone, cephem, aminoglycoside and tetracycline) excluding resistance to the non-anti-pseudomonas antibiotics (oxacillin and vancomycin) was observed in 9 (18%) of the 50 selected isolates (Table 6).

Discussion:

There are conflicting reports in the literature with regards to the public health significance of heterotrophic bacterial load in waters. Hellard et al., (21) observed that there was no clinical correlation with elevated heterotrophic bacterial load in water. However, Payment et al., (22) associated high bacterial load with waterborne diseases, while Allen et al., (23) submitted that there is no epidemiological evidence that higher heterotrophic bacteria density has any public health significance. The bacterial load observed in this study was similar to that reported by Atuanya et al., (24) but relatively higher than those observed by Nafarnda et al., (25) and Onuoha et al., (4). Shukri et al., (26) reported far higher bacterial load in Kalerwe abattoir effluents which empties into the Nsooba channel of Uganda.

The conflicting reports on health significance of heterotrophic plate counts (HPC) in water notwithstanding, several countries across the globe have set different allowable maximum limits for bacterial loads in water samples ranging from 20 to 1,000 CFU/ml (23). This implies that the water sources in our study (across all sampled sites) were of poor bacteriological quality with reference to heterotrophic bacterial load. The HPC index of poor water quality was further corroborated by the significant correlation observed between bacterial load and coliform ($p<0.01$), and between bacterial load versus *Pseudomonas* densities ($p<0.05$). The observation therefore suggests that bacterial load is an important index for measuring the possible presence of coliform and potential pathogens such as *Pseudomonas* in water samples.

Coliforms are a range of bacteria in the family Enterobacteriaceae, used as indicator of faecal pollution from warm blooded animals (27). They are important water quality index for determination of the relative risk of possible presence of microbial pathogens of faecal origin in a water sample (28). The total coliform count in this study was similar to that reported by Ezeigbo et al., (29) but relatively lower than those observed in previous studies (24, 30, 31). The coliform load observed in the abattoir effluent was generally higher than the WHO limit (<1000/100ml) for wastewater effluent indicative of risk of transmission of bacterial infections through the use of untreated wastewater (32, 33). This observation validates the poor bacteriological quality of the effluent and its potential negative public health impact.

However, contrary to the report of WHO (32), Grobrow et al., (34) observed that there is no direct correlation between numbers of any indicator and enteric pathogens, and Havelaar et al., (33) reported that many earlier classic failures of coliforms to identify waterborne pathogens have resulted in waterborne outbreaks associated with water sources prejudged to be free of faecal coliforms. These assertions were corroborated by the lack of significant correlation between coliform and *E. coli,* and coliform versus *Pseudomonas* counts as observed in this study. The observation is consistent with the opinion of Havelaar et al., (33) who stated that epidemiologic studies often fail to show relationship to microbial indicators, due to widely fluctuating ratio of pathogens to faecal indicators and their varying virulence.

The total *Pseudomonas* counts (TPC) observed in the abattoir effluent (DP) was generally higher than the zero pathogen limits envisaged by WHO (32). The presumptive *Pseudomonas* load was significantly ($p<0.05$) higher in the effluent (DP) and CP compared to US and DS, suggesting that the abattoir effluent was a major contributor of potentially pathogenic *Pseudomonas* strains to the Ikpoba River. The *Pseudomonas* loads were similar to those reported by Odjadjare et al., (14) in municipal wastewater effluents in South Africa. However, Havelaar et al., (33) reported *Pseudomonas* density in hospital wastewater relatively higher (10^4 to 10^6 CFU/100ml) than those observed in this study.

Pseudomonas spp. are regarded as emerging waterborne pathogens. They have been incriminated in a number of waterborne outbreaks including those associated with use of recreational waters (35), showers, hot tubs and swimming pools (36). Thus, discharge of the abattoir effluent under study into the Ikpoba river portends serious public health risks to those who depend on the river water for sundry uses. It is therefore imperative for stakeholders in the public health sector to include *Pseudomonas* in the list of potential pathogens screened for in routine surveillance of abattoir waste effluents in the interest of public health.

Notwithstanding the inherent antimicrobial resistance of *Pseudomonas* spp. to many commonly deployed antibiotics, previous reports suggested that the degree of resistance to antipseudomonal agents vary considerably (37). Consistent with the observation in this study, *Pseudomonas* spp. from environmental sources were reported to be sensitive to amikacin (38,39), ceftazidime (40), gentamycin (10,14,17) and ofloxacin (14,41,42). Ceftazidime was the most effective antibiotics recorded in this study with 82% sensitivity, followed by ofloxacin (80%), amikacin (74%) and gentamycin (40%). Conversely, previous studies have reported *Pseudomonas* resistance to amikacin (37), ceftazidime (5,10,15,16), gentamycin (5,15,40) and ofloxacin (17).

In agreement with the findings of this study, pseudomonads resistance was previously reported against oxacillin and vancomycin (11, 14), as well as tetracycline (5,42). Although Igwe et al., (43) reported 20% sensitivity of *Pseudomonas* isolated from women with UTI in a tertiary hospital in Abakaliki, Nigeria, there is a general dearth of information in the literature on pseudomonads susceptibility to oxacillin. In a similar vein, there is scarcity of information on vancomycin susceptible pseudomonads, despite Agwu et al., (44) report of 52% sensitivity to the drug among isolates from delayed foot ulcers in Ekpoma, Nigeria. Lower sensitivity of *Pseudomonas* spp. to vancomycin was also previously reported in the literature (14,45). The observations suggest that these antibiotics were generally ineffective against *Pseudomonas* spp. and therefore should not be administered in the therapeutic management of *Pseudomonas* infections.

Despite the fact that oxacillin and vancomycin are known to be non-traditional anti-pseudomonas antibiotics, they were included in this study because previous reports (14,43,44,45) have documented strains of *Pseudomonas* spp sensitive to these drugs. Hence, it was necessary to test whether these observations marked an evolving trend or were probably one-off discoveries on the antibiogram of this organism. None of the isolates in the current study were sensitive to both antibiotics. However, it will be important to further investigate the biological, molecular and physiological basis of *Pseudomonas* sensitivity to these anti-

biotics if and when they are isolated in the future. Contrary to the observation of this study, Odjadjare et al., (14) reported *Pseudomonas* isolates from municipal wastewater effluents which were generally sensitive to tetracycline.

The MDR phenotypes observed in this study were similar to those reported previously (5,40,42), indicating the MDR coverage of the fluoroquinolones, cephems, aminoglycosides and tetracyclines. However, contrary to the observation of this study, reports elsewhere on MDR phenotypes among *Pseudomonas* spp. did not involve the fluoroquinolones and amino-glycosides (10,14). Our observation suggests that abattoir effluent is a considerable source of MDR *Pseudomonas* strains, and poses risk to public health through the dissemination of community acquired antibiotic resistant bacteria. Chika et al., (15) asserted that MDR *Pseudomonas* in the community portends danger for the healthcare sector because such pathogens could spread to the hospital environment via community-acquired infections when patients report to the hospital for medical attention and become hospitalized.

The multiple antibiotics resistance observed in this study did not come as a surprise, as food animal production often apply antibiotics for therapeutic, prophylactic and growth enhancement purposes. Such practices enhance the chances of increased transfer and spread of antibiotics resistance determinants in the environment through the discharge of abattoir wastes containing unmetabolized drugs or their metabolic intermediates (17). The findings in this study revealed that untreated abattoir effluent was a considerable source of MDR *Pseudomonas* spp. among other bacterio-logical pollutants (e.g. HPC, coliform and *E. coli*) that could compromise the quality of the receiving river as well as the public health of riverside communities that depends on this vital water resource for their subsistence. There is therefore an urgent need to educate stake-holders involved in the slaughter house business on the importance of effluent treatment before discharge into the environment. This will help to prevent the dissemination and spread of antibiotics resistant bacteria in the community and preserve the public health.

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