



Spatial Distribution of Bacterial Isolates from Different Abattoirs in Port Harcourt, Nigeria

Azuonwu Testimonies Chikanka¹ and David N. Ogbonna^{2*}

¹*Department of Animal and Environmental Biology, Rivers State University, Nkpolu-Oroworukwo, P.M.B. 5080, Port Harcourt, Nigeria.*

²*Department of Microbiology, Rivers State University, Nkpolu-Oroworukwo, P.M.B. 5080, Port Harcourt, Nigeria.*

Authors' contributions

This work was carried out in collaboration between both authors. Author ATC designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript, managed the analyses of the study and literature searches under the strict supervision of author DNO. Both authors read and approved the final manuscript.

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ABSTRACT

The constituents of wastes generated from abattoir activities create conducive environment for microbial proliferation, most of which are pathogenic. Infections caused by these microorganisms could result to zoonoses. This study was to determine the distribution of bacterial isolates and their biomass from different abattoirs in Port Harcourt. Samples like waste blood, table swab, service water, faecal matter, soil and wastewater from abattoirs in Iwofe, Rumuodomaya and Trans-Amadi were collected from October 2017 to November, 2018 and analysed using standard microbiological procedures. Results obtained revealed that the total heterotrophic bacterial count of blood samples ranged from 8.33×10^1 to 3.33×10^2 cfu/ml for Trans-Amadi and Iwofe abattoirs, table swabs ranged from 6.74×10^4 to 4.88×10^6 cfu/ml, water samples ranged from 1.56×10^4 to 2.07×10^4 cfu/ml; faecal matter had THB counts ranging from 9.97×10^7 to 1.06×10^8 cfu/g; and soil samples ranged from 1.11×10^{10} to 1.17×10^{10} g, while wastewater counts ranged from 1.03×10^8 to 1.08×10^8 cfu/ml. The predominant Bacterial isolates were of the genera *Micrococcus*, *Staphylococcus*, *Serratia*,

*Corresponding author: E-mail: ogbonna.david@ust.edu.ng;

Pseudomonas, *Proteus*, *Klebsiella*, *Escherichia* and *Chromobacterium*, *Serratia* sp. only was isolated from Iwofe and Rumuodomaya abattoirs within April to October while *Chromobacterium* sp. was isolated in Trans-Amadi and Rumuodomaya abattoirs within the months of May to October. Among the isolates, *Escherichia coli* and *Klebsiella* species occurred more compared to others in all the three locations. A higher percentage of microorganisms were recorded in the month of May compared to other months. It is presumed that abattoir wastes harbour many microorganisms of public health importance. The occurrence of these microbes, most of which are enteric pathogens, poses a public health challenge as infections by them could result in illnesses such as gastroenteritis, septicaemia and pneumonia especially in the absence of good hygiene around abattoirs. Proper sanitation in abattoirs as well as management of abattoir wastes is important in reducing the spread of these microorganisms.

Keywords: Abattoir; microorganisms; infections; environmental sanitation.

1. INTRODUCTION

Pollution of the environment has become a worldwide public health issue with the potential to affect human health [1]. Although abattoir operations provide meat for human consumption, a huge amount of wastes are generated as a result of the operations [2]. The untreated wastes including consumable parts of the slaughtered animals and wastewater used in washing the carcass from several abattoirs are disposed of into nearby rivers and streams without treatment [3]. This is as a result of lack of supervision and inappropriate management in these abattoirs, thus resulting in public health risks to the public [4]. This poor management further results in the degradation and contamination of soil, water and therefore result in pollution, global warming, epidemics and loss of biotic life [5]. Indiscriminate disposal of animal dung and other waste products can produce leachates and greenhouse gases like methane, CO₂, etc from decomposing organic wastes which may cause pollution of soils and the atmosphere [6].

In Nigeria, about 80% of abattoirs have been sources of zoonotic diseases which are yet to be controlled [7]. Abattoir wastes are known reservoirs of parasitic organisms such as *Bacillus* spp., *Staphylococcus* spp., *Pseudomonas* spp., *Proteus* spp., *Klebsiella* spp. and *Escherichia coli* [8]. Outbreak of waterborne diseases as a result of abattoir-waste pollution of nearby streams has resulted in the spread of infectious diseases such as typhoid fever, diarrhoea and pneumonia [9]. The risks associated with this practice also includes the presence of antibiotic resistant microorganisms as well as resistance genes which makes treatment of infections resulting from these microorganisms difficult and in most cases, more expensive [10,11]. The situation is of public health importance because the

occurrence of these microorganisms can be of nuisance value. This study was thus aimed at determining the distribution of bacterial isolates in waste samples associated with abattoirs activities in Port Harcourt, Nigeria within the wet and dry seasons.

2. METHODOLOGY

2.1 Sampling Locations

Samples for this study were obtained from the Iwofe, Rumuodomaya and Trans-Amadi abattoirs situated in Port Harcourt, Nigeria. The choice of these abattoirs is based on the large number of persons visiting the abattoirs on daily basis, thus making these locations a beehive of activities by the public. A map showing the sampling locations is presented in Fig. 1.

2.2 Samples Collection

Cow blood samples were collected using sterile 1 ml syringes; faecal matter samples were collected using a sterile spatula to scoop faecal matter recovered from stomach of the cow; wastewater samples were collected in sample bottles as the carcasses were washed; the tap was allowed to run for 1 minute and then the service water samples were collected in sterile sample bottles; table swab samples were collected by swabbing the tables using a sterile swab sticks. All samples were kept in ice-packed coolers and immediately transported to the Microbiology laboratory for analyses. Samples were collected from October 2017 to November, 2018.

2.3 Microbiological Analyses

The samples were serially diluted by adding 1 ml or 1 g of each sample to 9 ml of normal saline

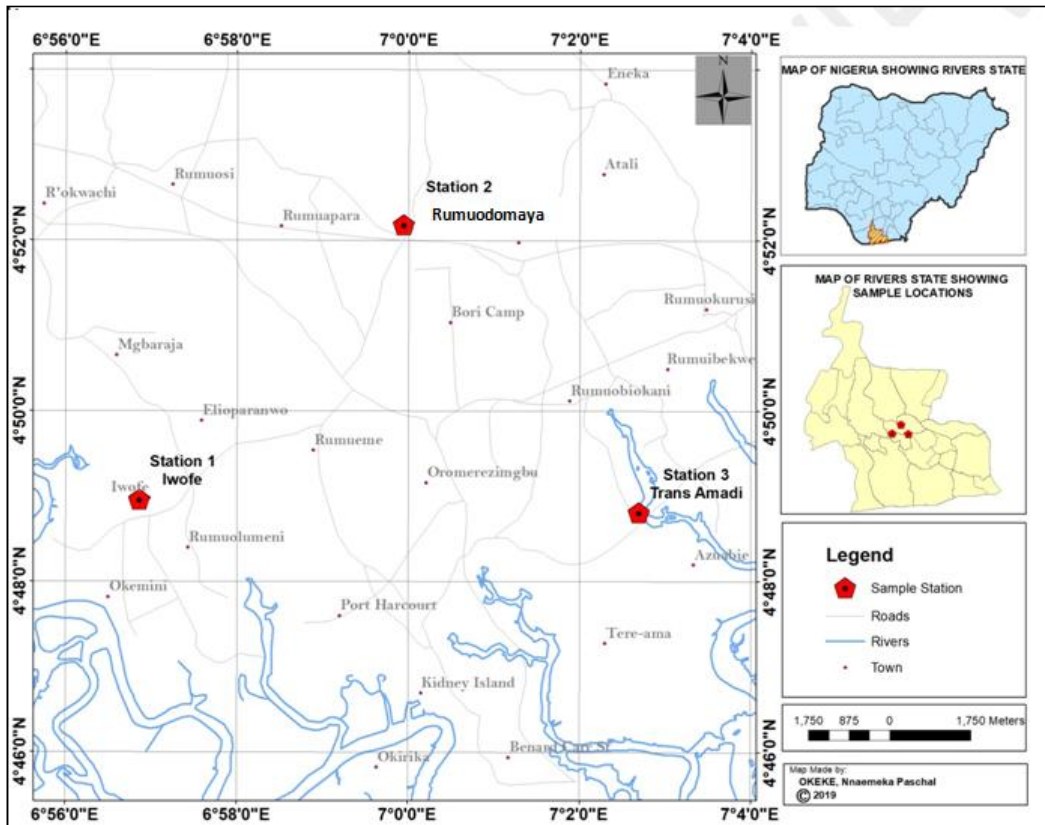


Fig. 1. Map of Port Harcourt metropolis showing the sampling stations

solution and then a ten-fold serial dilution was carried out. Blood samples were diluted to 10^{-2} , swabs to 10^{-4} , service water to 10^{-2} , faecal matter to 10^{-5} , soil to 10^{-7} and wastewater to 10^{-5} . Subsequently, aliquots of the diluted samples were inoculated on prepared, solidified and surface-dried Nutrient agar plates. Plates that had been inoculated on, where incubated at 37°C for 24-48 hours. After 24-48 hours, discreet colonies seen were counted and expressed in colony forming units and thereafter sub cultured on fresh Nutrient agar. Biochemical tests including Gram's stain reaction, sugar fermentation, indole, Methyl Red & Vogues-Proskauer (MRVP), oxidase, catalase, coagulase, motility and citrate were carried out to identify the isolates [12]. Data obtained were analysed using SPSS 22 and presented in tables.

3. RESULTS

Table 1 shows the mean and standard deviation of blood, table swab, service water, faecal matter, soil and wastewater counts (cfu/ml) in the three locations within the period of study. The

blood samples from Iwofe abattoir had a mean total heterotrophic bacteria (THB) count of 3.33×10^2 cfu/ml, while the least counts were from Trans-Amadi abattoir with 8.33×10^1 cfu/ml. There was no significant difference between the waste blood counts in the different locations at a 95% confidence interval. The mean microbial counts for table swab samples from Iwofe abattoir had high THB counts with 6.74×10^6 cfu/ml and Trans-Amadi abattoir having the least with 4.88×10^6 cfu/ml, statistically, there was no significant difference between the table swab counts in the three locations. Rumuodomaya abattoir recorded a high THB count with 2.07×10^4 cfu/ml while Trans-Amadi recorded the least with 1.56×10^4 cfu/ml among the service water samples. There was a significant difference between the THB counts across the locations. Rumuodomaya abattoir recorded high THB counts at 1.06×10^8 cfu/g and Trans-Amadi had the least count of 9.97×10^7 cfu/g from the faecal matter samples. There was a significant statistical difference in the counts between the three locations. A high mean bacterial count was recorded in soil samples for Iwofe abattoir with 1.17×10^{10} cfu/g while Trans-Amadi had the least

Table 1. Microbial counts samples for Abattoir in the three locations

Location	Blood (cfu/ml)	Table swab (cfu/g)	Water (cfu/ml)	Faecal matter (cfu/g)	Soil (cfu/g)	Wastewater (cfu/ml)
Iwofe	$3.33 \times 10^2 \pm 1.42$	$6.74 \times 10^6 \pm 0.12$	$1.85 \times 10^4 \pm 0.26$	$1.04 \times 10^8 \pm 0.05$	$1.17 \times 10^{10} \pm 0.19$	$1.08 \times 10^8 \pm 0.42$
Rumuodomaya	$2.50 \times 10^2 \pm 1.11$	$5.48 \times 10^6 \pm 0.06$	$2.07 \times 10^4 \pm 0.21$	$1.06 \times 10^8 \pm 1.17$	$1.14 \times 10^{10} \pm 0.27$	$1.03 \times 10^8 \pm 0.27$
Trans-Amadi	$8.33 \times 10^1 \pm 0.78$	$4.88 \times 10^6 \pm 0.06$	$1.56 \times 10^4 \pm 0.21$	$9.97 \times 10^7 \pm 0.04$	$1.11 \times 10^{10} \pm 0.21$	$1.07 \times 10^8 \pm 0.31$
P value	0.13	0.22	0.003	0.00	0.69	0.00

P values >0.05 are not significantly different

Table 2. Percentage occurrence of bacterial isolates per month in Iwofe Abattoir

Isolates	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	August	Sept	Oct
<i>Escherichia coli</i>	20.2	16.4	18.1	16.8	16.1	18.2	21.1	16.5	17.8	14.5	15.1	14.3
<i>Klebsiella</i> sp.	17.0	22.4	17.2	18.8	11.3	17.4	16.5	11.3	14.0	17.6	18.1	19.0
<i>Proteus</i> sp.	18.1	15.5	16.4	9.9	13.7	17.4	7.3	4.3	14.7	7.6	6.6	7.7
<i>Salmonella</i> sp.	12.8	13.8	16.4	20.8	14.5	18.2	14.7	14.8	16.3	3.8	17.5	14.9
<i>Bacillus</i> sp.	9.6	12.1	11.2	14.9	16.1	6.6	11.0	17.4	13.2	9.2	6.0	8.9
<i>Serratia</i> sp.	0.0	0.0	0.0	0.0	0.0	4.1	10.1	13.0	9.3	9.2	8.4	6.5
<i>Pseudomonas</i> sp.	9.6	6.9	10.3	11.9	16.9	8.3	8.3	12.2	9.3	12.2	1.8	6.5
<i>Chromobacterium</i> sp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.1	17.5	15.5
<i>Micrococcus</i> sp.	3.2	1.7	5.2	3.0	4.8	5.8	2.8	4.3	1.6	5.3	3.6	2.4
<i>Staphylococcus</i> sp.	9.6	11.2	5.2	4.0	6.5	4.1	8.3	6.1	3.9	1.5	5.4	4.2
Total	100	100	100	100	100	100	100	100	100	100	100	100

Table 3. Percentage occurrence of bacterial isolates per month in Rumuodomaya Abattoir

Isolates	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	August	Sept	Oct
<i>Escherichia coli</i>	20.7	20.6	17.2	19.8	18.5	16.4	17.5	19.0	15.6	15.0	17.0	13.3
<i>Klebsiella sp.</i>	19.0	16.0	16.4	18.3	16.9	18.9	16.1	18.1	13.6	14.2	13.3	15.0
<i>Proteus sp.</i>	19.0	18.3	21.3	19.0	19.4	17.2	16.8	14.3	12.9	12.6	12.1	11.0
<i>Salmonella sp.</i>	9.9	7.6	10.7	12.7	6.5	7.4	7.3	4.8	10.9	11.0	7.3	9.8
<i>Bacillus sp.</i>	7.4	11.5	8.2	0.0	5.6	4.9	3.6	1.9	6.1	4.7	5.5	6.9
<i>Serratia sp.</i>	0.0	0.0	0.0	0.0	0.0	0.0	16.8	14.3	12.9	12.6	12.1	11.0
<i>Pseudomonas sp.</i>	17.4	14.5	13.1	15.9	18.5	18.0	11.7	18.1	11.6	15.0	13.9	15.0
<i>Chromobacterium sp.</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.7	1.9	6.1	10.2	10.3	13.3
<i>Micrococcus sp.</i>	2.5	7.6	6.6	9.5	8.1	10.7	6.6	2.9	4.8	3.9	6.1	3.5
<i>Staphylococcus sp.</i>	4.1	3.8	6.6	4.8	6.5	6.6	2.9	4.8	5.4	0.8	2.4	1.2
Total	100	100	100	100	100	100	100	100	100	100	100	100

Table 4. Percentage occurrence of the isolates per month in Trans-Amadi Abattoir

Isolates	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	August	Sept	Oct
<i>Escherichia coli</i>	18.5	18.9	18.4	18.2	17.1	16.8	17.6	15.5	17.5	16.3	17.0	16.3
<i>Klebsiella sp.</i>	16.7	17.6	13.8	15.5	14.2	14.2	18.6	14.2	13.8	14.4	17.4	13.5
<i>Proteus sp.</i>	17.1	14.9	17.1	14.1	14.2	15.1	17.1	17.8	16.6	18.7	14.7	14.7
<i>Salmonella sp.</i>	12.0	13.5	18.0	17.3	17.5	15.1	8.6	7.3	11.5	11.5	11.6	12.7
<i>Bacillus sp.</i>	10.6	9.5	9.2	10.0	11.4	12.5	13.8	15.5	12.9	12.4	9.4	7.6
<i>Serratia sp.</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6
<i>Pseudomonas sp.</i>	15.3	14.0	14.7	16.8	14.2	16.4	10.5	11.9	11.1	14.4	13.8	14.3
<i>Chromobacterium sp.</i>	0.0	0.0	0.0	0.0	0.0	0.0	3.3	4.6	8.3	8.1	9.4	9.6
<i>Micrococcus sp.</i>	0.9	1.4	0.5	3.2	1.9	3.4	1.4	3.2	2.8	1.9	0.9	3.2
<i>Staphylococcus sp.</i>	8.8	10.4	8.3	5.0	9.5	6.5	9.0	10.0	5.5	2.4	5.8	6.4
Total	100	100	100	100	100	100	100	100	100	100	100	100

Table 5. Total percentage occurrence of isolates within the months

Location/Month	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Total
Iwofe	6.3	7.8	7.8	6.8	8.3	8.1	7.3	7.7	8.7	8.8	11.1	11.3	100
Rumuodomaya	7.6	8.2	7.6	7.9	7.8	7.6	8.6	6.6	9.2	7.9	10.3	10.8	100
Trans-Amadi	8.2	8.4	8.2	8.3	8.0	8.8	7.9	8.3	8.2	7.9	8.5	9.5	100

count of 1.11×10^{10} cfu/g. There was no significant difference statistically between the mean counts obtained. Mean microbial counts for wastewater samples obtained showed that Rumuodomaya abattoir recorded the least THB count of 1.03×10^8 cfu/ml while Iwofe abattoir had the highest count with 1.08×10^8 cfu/ml. There was a significant difference statistically in the counts across the locations.

Tables 2-4 show the percentage occurrence of the bacterial isolates within the sampling period (November 2017 – October, 2018) in the three abattoirs. The tables showed a higher occurrence of *E. coli* in the different locations. *Chromobacterium* sp was isolated between May to October in Trans-Amadi and Rumuodomaya abattoirs, whereas, it was not isolated from Iwofe abattoir. In Rumuodomaya and Iwofe abattoirs, *Serratia* sp was isolated in the months of May and October. Table 5 shows the total percentage occurrence of isolates in the months studied across the locations. Higher microbial counts were recorded in the month of October, 2017 with the highest count from Iwofe with 11.3% and the least from Trans-Amadi with 9.5%.

4. DISCUSSION

The predominant bacteria isolated in this study were of the genera *Micrococcus*, *Staphylococcus*, *Serratia*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Escherichia* and *Chromobacterium*. Similar studies have recorded the presence of these microorganisms from abattoirs in Nigeria and other countries [13-17]. The presence of *Escherichia coli*, *Salmonella* sp., *Klebsiella* sp., *Proteus* sp. and *Serratia* sp. in the soil and wastewater could be as result of faecal contamination and the run-off of wastes into the reservoirs [13,18]. The presence of *Pseudomonas* sp. in these ecologies may be the result of hydrocarbon production during burning of firewood, as this organism has been linked with hydrocarbon oxidation [19]. These bacteria from abattoir wastes may be discharged into water columns and can subsequently be absorbed to sediments, and when the bottom stream is disturbed, the sediment releases the bacteria back into the water columns presenting long term health hazards [20,21]. The accumulation of the faecal materials act as a collection basin for pathogenic microorganisms which may spread between animals and man leading to zoonoses [22]. These zoonotic pathogens can exceed millions to billions per gram of faeces, and may infect humans through

various routes such as contaminated air, contact with livestock animals or their waste products, swimming in water impacted by animal faeces, exposure to potential vectors (such as flies, mosquitoes, water fowl, and rodents), or consumption of food or water contaminated by animal wastes [23,24].

In consonance with this study, isolation of microorganisms from cow waste blood was also reported by Adesoji *et al.*, who isolated bacteria from abattoir wastes in Katsina State, Nigeria [25]. In their study, *E. coli*, *Salmonella* sp. and *Pseudomonas* sp. were isolated from cow waste blood. Isolation of enteric bacteria from the water samples may be linked with the poor construction of the water sources which makes it prone to faecal contamination as well as irregular washing of the water tanks and the non-use of water purifiers. The consequences of anthropogenic pollution during abattoir operations can lead to the transmission of diseases by water borne pathogens, eutrophication of water bodies, accumulation of toxic or recalcitrant chemicals in the soil, destabilization of ecological balance and negative effects on human health [20,26].

The isolation of *Serratia* sp. and *Chromobacterium* sp during the months of May–October (wet season), may be linked with the weather conditions that may have favoured their proliferation in these environments. *Chromobacterium* sp. is often found in aquatic environments and very sensitive to temperature. Its purple colour owes to the antioxidant pigment it produces, called violacein [27]. Infections caused by these microorganisms in humans are rare; however, some species have been reported to cause urinary tract infection, hemophagocytic syndrome, respiratory distress syndrome, fulminant sepsis, pneumonia, gastrointestinal infection, localized cutaneous lesions, localized or metastatic abscesses, osteomyelitis, meningitis, peritonitis, brain abscess and endocarditis [28]. *Serratia* sp. are opportunistic pathogens that have been implicated as causative agents of nosocomial infections including empyema, septicaemia, osteomyelitis and meningitis [29]. Its characteristic red to pink colour (depending on colony's age) stems from its production of a pigment called prodigiosin [30].

5. CONCLUSION

Abattoir wastes have adverse impacts on aquatic and soil environment and this may trigger algal

blooms (eutrophication), depletion of dissolved oxygen, destruction of habitat and fish kills, thereby reducing the population of fishes and other aquatic organisms. This sometimes, may result in the transmission of diseases by water borne pathogens, accumulation of toxic or recalcitrant chemicals in the soil, which may have negative effects on human health. The occurrence of microorganisms of the genera *Micrococcus*, *Staphylococcus*, *Serratia*, *Pseudomonas*, *Proteus*, *Klebsiella*, *Escherichia* and *Chromobacterium* could pose severe threat to consumers of beef if appropriate sanitation is not employed in abattoirs. Occasional consumption of the service water by the slaughter men predisposes them to illnesses caused by these bacteria and in cases where the organisms are multidrug resistant strains, treatment resulting from their infection becomes difficult. Therefore, adequate sanitation and management of abattoir wastes are valid means of preventing or reducing the spread of these microorganisms.

CONSENT

As per international standard or university standard written cow owner consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

Approval for this research was obtained from the Department of Microbiology of the Rivers State University as well as consent from the abattoir authorities.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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