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ORIGINAL

Pathogenicity Testing of Microbial Isolates from Refuse Dumps sites and collection centres in Awka metropolis, Nigeria

Pruebas de patogenicidad de aislados microbianos procedentes de vertederos y centros de recogida de basuras en la metrópoli de Awka, Nigeria

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Abstract

Introduction: Despite the attendant health risks inherent in waste dump sites, certain individuals make their living by foraging and packing the waste for survival.
Methods: A total of 280 samples were collected from the waste collectors, scavengers, and people living and trading around refuse dump sites, collection centers, and refuse dump sites in Awka Metropolis. The bacterial and fungal isolates were further subjected to pathogenicity testing using Wistar rats.
Results: The histology of the kidneys and lungs was altered except for the control group, which was injected with normal saline. There were moderate and profuse macrophage infiltrations, with Bowman's capsule distorted in the majority of the kidneys. It was observed that none of the microorganisms altered the histology of the liver. The histology of the kidney and the lungs of the albino Wistar rats was altered, which is indicative of pathoogenicity.
Conclusión: the pathogenic nature of the organisms isolated in this study indicates that the activities of scavengers and waste collectors pose a serious health risk to the public. This study therefore calls for a proper regulatory system for waste disposal.

Key words: Refuse dump sites, pathogenicity, Waste collectors, Scavengers, Microorganisms, Public health.

Resumen

Introducción: A pesar de los riesgos para la salud inherentes a los vertederos, algunas personas se ganan la vida rebuscando y empaquetando los residuos para sobrevivir.
Material y métodos: Se recogieron un total de 280 muestras de recolectores de residuos, carroñeros y personas que viven y comercian en los alrededores de los vertederos, centros de recogida y vertederos de residuos de la metrópolis de Awka. Los aislados bacterianos y fúngicos se sometieron además a pruebas de patogenicidad utilizando ratas Wistar.
Resultados: La histología de los riñones y los pulmones estaba alterada, excepto en el grupo de control, al que se le inyectó solución salina normal. Había infiltraciones moderadas y profusas de macrófagos, con la cápsula de Bowman distorsionada en la mayoría de los riñones. Se observó que ninguno de los microorganismos alteraba la histología del hígado. La histología del riñón y de los pulmones de las ratas Wistar albinas estaba alterada, lo que es indicativo de patogenicidad.
Conclusión: La naturaleza patógena de los organismos aislados en este estudio indica que las actividades de los carroñeros y recolectores de residuos suponen un grave riesgo para la salud de la población. Por lo tanto, este estudio reclama un sistema de regulación adecuado para la eliminación de residuos.

Palabras clave: Vertederos, patogenicidad, Recolectores de residuos, Carroñeros, Microorganismos, Salud pública.

Introduction

Domestic solid waste is any unwanted or discarded solid materials from residential activities that cause environmental, social and health problems. The World Health Organization refers to waste as something which the owner no longer wants at a given time and space which has no current or perceived market value. In the words of Ikhuoria[1], waste is refuse, garbage, ashes and rubbish that are derived from places of human and animal habitation. He further grouped solid waste elements into two – decomposable refuse and non-decomposable refuse. Nwobu[2] in his study of solid waste disposal and management in Awka, Anambra state, defined solid waste as anything discarded or unwanted whose physical state is solid or semisolid.

Kimberly[3] carried out a study on composition of solid waste in Florida State, United States of America. In his study, he made a classification of solid wastes based on the material composition. These include garbage, or food waste, paper, glass, cars and other household wastes. The daily activities of humans give rise to a large variety of wastes and when these waste materials are disposed off, microorganisms of different types such as bacteria, fungi and worms (helminthes) colonize the waste and begin to degrade them[4]. As a result, they break down the unprocessed or organic components of waste into inorganic forms, which can readily serve as sources of nutrients for a variety of other organisms.

Ajadike[5], states that urban waste crisis arises in Nigeria because of three fundamental factors namely, rapid increase in urban population, heavy consumption pattern of urban dwellers and the inefficiency of the authorities whose statutory responsibilities includes efficient waste disposal in cities. Adesoji[6] took a study of solid waste disposal in Ibadan, he discovered that various landfill sites and open dump sites in the town are mismanaged and these sites harbor disease carrying pathogens such as rat, cockroaches, mosquitoes, houseflies, fleas etc.

It is evident that most environmental, economic and health related problems in human and the environment can be attributed to the incidence of solid wastes. This work goes further to ascertain the situation in the study area.

Though there are available methods of waste disposal, such as composting, landfill and incineration, open dumping continues to be the only method available in Nigeria particularly in major cities like Port Harcourt, Awka, Nnewi and Onitsha even though these are strongly discouraged in the National Sanitation Policy[7]. Wastes are left on the streets for days or weeks, without proper sorting before they are disposed to the final dumpsites or relocated to open lands[8].

Materials and methods

Study design

This prospective study was performed to determine some microorganisms in individuals associated with refuse dumpsites and collection centers in Awka metropolis.The sampling method used was a Convenience Sampling Technique, a non-probability sampling technique where the subjects [Waste collectors, scavengers and people living/ trading around the refuse dumpsites] were selected based on convenience, accessibility, proximity to the researcher and not necessarily a representative of the entire population.

Study area

The samples were collected in Awka metropolis consisting Awka, Nibo, Nise, Amawbia, Okpuno and Umuokpu, where refuse collecting centers and the main dump site are located.

Ethical consideration

Ethical clearance was obtained from the Faculty of Health Sciences and Technology and Authorization from the Anambra State Waste Management Authority. Informed Consent was also sought from various waste scavengers and waste collectors who willingly volunteered to be part of this study. It entailed the purpose of the study, benefits, privacy/confidentiality and conflict of interest. Participation was absolutely voluntary and each subject had the opportunity to participate or opt out at any point in the course of the survey.

Sampling period and sample population

The study was carried out between June 2016 and August 2016, using scavengers and waste collectors within the age bracket of 18-45 years and Control subjects of same age bracket.A total of 350 samples were collected, 30 samples from individuals living and trading around refuse dump sites, 60 samples from waste collectors, 60 samples from waste scavengers, 10 samples from waste vehicles, 40 samples from waste collection centers and 10 samples from main refuse dump sites, and 140 samples from the Control group.

Microbial analysis

Waste Sample

Waste Samples [20g] were collected from different portion of the main dump sites and collection centers for even distribution, to ensure that no organisms were missed. The samples were collected in sterile containers, using a special spatula. Thereafter, 1g of each prepared waste sample was added into 9ml of 0.1% bacteriological peptone [10-1dilution] shaken vigorously for at least 1 minute. The diluents were left to sediment for a short period. Further ten-fold serial dilutions were made up to 10-4, using sterile pipettes. Cultures from the last 2 dilutions [10-3 and 10-4] were made by transferring an aliquot [0.1ml] into surface dried Nutrient agar, and

MacConkey Agar plates and spread evenly with a spreader [i.e. bent glass rod]. The culture plates were incubated aerobically at 370C for 24hrs.

Haematoxylin& Eosin staining (H&E)

Sections were freed from wax with xylene for 2mins and brought to distilled water through descending grades of ethyl alcohol [95, 90, and 70] and rinsed in running water; Stained with Erhlich's haematoxylin for 30 minutes and rinsed in running tap water. They were differentiated with 1% acid alcohol until only nuclei were stained, rinsed in running tap water and 'blued' in Scott's tap water substitute for 3 minutes. Furthermore, the sections were rinsed in tap water, counter stained with Eosin for 2 minutes, dehydrated in ascending grades of alcohol [70, 90, 95], taken back to hot air oven for 5mins where all traces of water was removed, cleared in xylene and mounted in DPX[Di butyl phthalate polystyrene xylene]. These procedure were successfully carried out with the help of a histopathologist.Cell nuclei would be indicated by bluecolour, cytoplasm by pinkcolour, collagen and muscle fibres by various shades of pinkcolour.

Statistical analysis

Data collected were subjected to statistical analysis using percentages, Student's t-test and analysis of variance (ANOVA).Values will be deemed significant at $P<0.05$.

Results

Histology result

Plate 1

A. Control Kidney tissue shows normal histology. Glomeruli and tubules are intact. **B.** Kidney tissue infected with *Candidaalbicans* from subjects show cellular hyperplasia of the Bowman's capsule epithelial lining with mild infiltration of inflammatory cells within the interstitial tissue. **C.** Kidney tissue infected with *Escherichia coli* from subjects show distorted Bowman's capsule epithelial lining with infiltration of inflammatory cells within the interstitial tissue. **D.** Kidney tissue infected with *Klebsiella pneumoniae* from subjects show mildly distorted Bowman's capsule epithelial lining (H&E x 100).

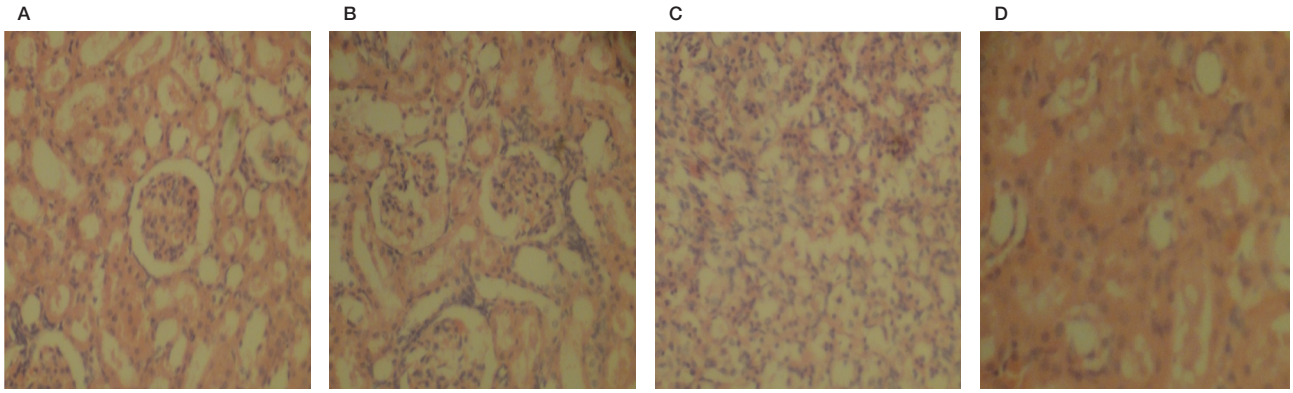


Plate 2

A. Kidney infected with *Aspergillus niger* from subjects show distorted Bowman's capsule epithelial lining with infiltration of inflammatory cells within the interstitial tissue. **B.** Kidney tissue infected with *Staphylococcus aureus* from subjects show distorted Bowman's capsule epithelial lining and edema with infiltration of inflammatory cells within the interstitial tissue. **C.** Kidney tissue infected with *Bacillus subtilis* from subjects show mildly distorted Bowman's capsule epithelial lining. **D.** Kidney tissue infected with *Pseudomonas aeruginosa* from subjects show mildly distorted Bowman's capsule epithelial lining (H&E x 100).

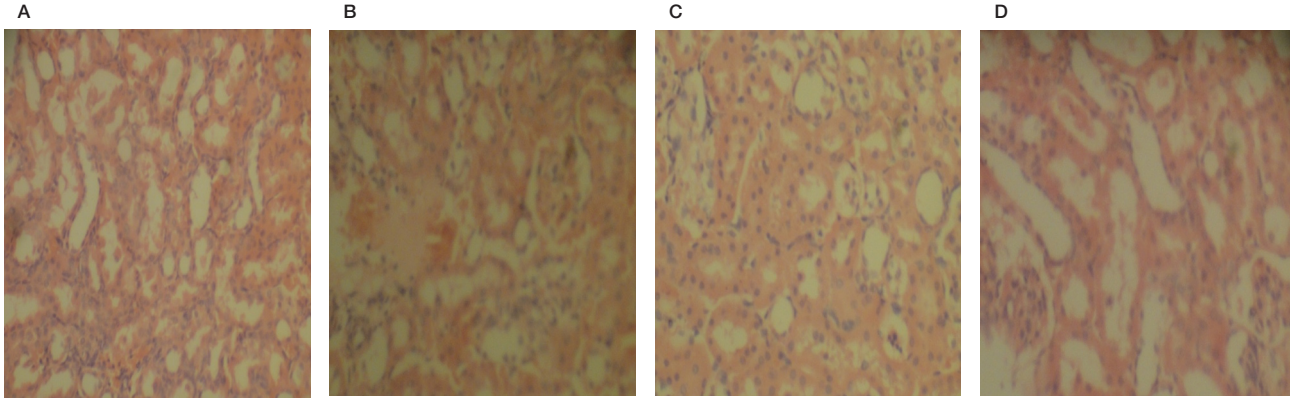


Plate 3

A. Control lung tissue shows normal histology (H&E x 100). **B.** Lung tissue infected with *Aspergillus niger* from subjects show moderate infiltration of inflammatory cells. **C.** Lung tissue infected with *Bacillus subtilis* from subjectsshow moderate infiltration of inflammatory cells.**D.** Lung tissue infected with *Candida albicans* from subjects shows moderate infiltration of inflammatory cells (H&E x 100).

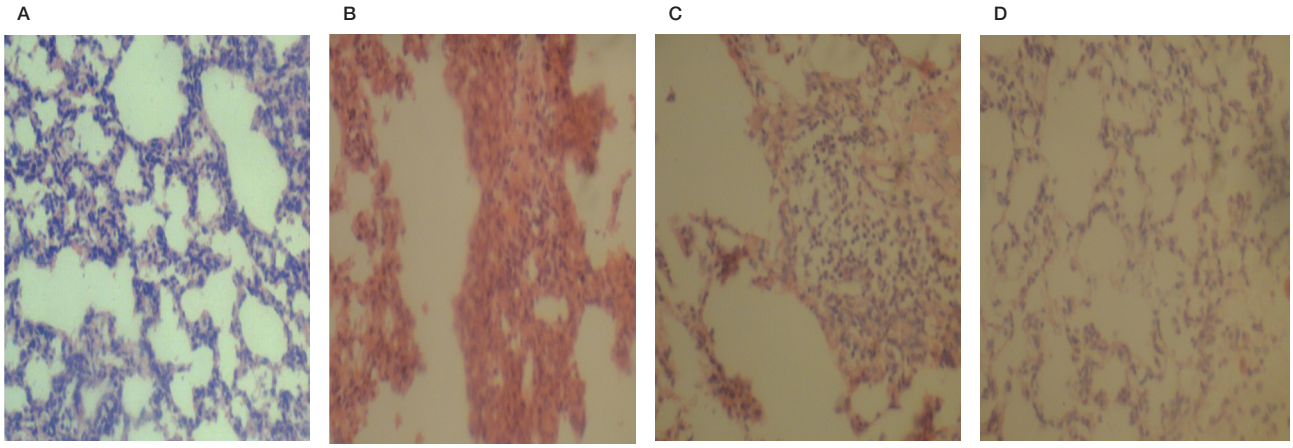
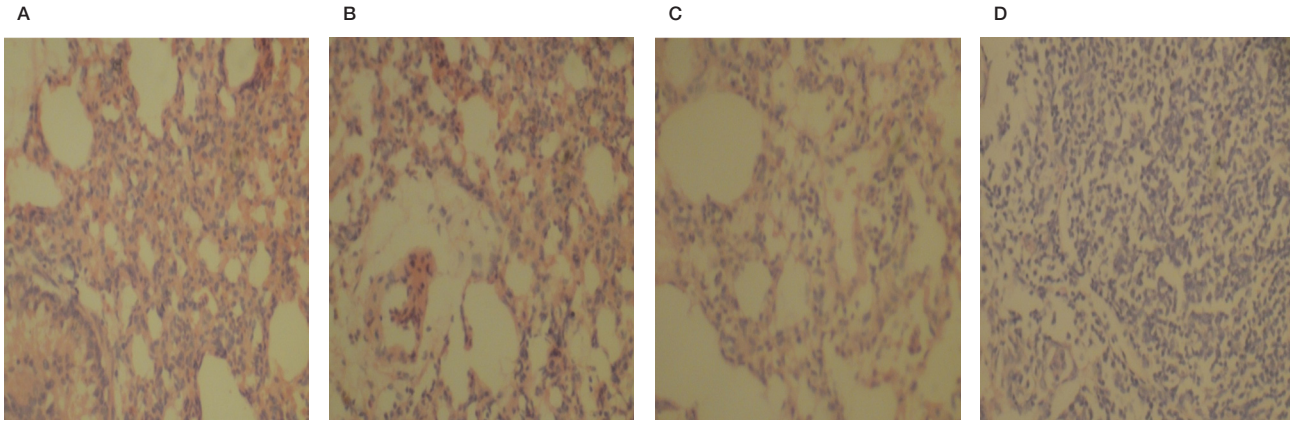


Plate 4

A. Lung tissue infected with *Escherichia coli* from subjects show moderate infiltration of inflammatory cells. **B.** Lung infected with *Klebsiella pneumoniae* from subjects show moderate infiltration of inflammatory cells.**C.** Lung infeted with *Pseudomonas aeruginosa* shows moderate infiltration of inflammatory cells. **D.** Lungs tissue infected with *Staphylococcus aureus* shows profuse infiltration of inflammatory cells.



Discussion

Wistar rat studies revealed histological alteration of some of the vital organs which included the kidneys and lungs. Observed alteration in these organs may be due to damage to these organs by the isolates, as the same microbial isolates injected into the Wistar rats were later isolated from the lesions found in the organs.

According to the histology results, the kidneys showed cellular hyperplasia of the epithelial lining of the Bowman's capsule and oedema with mild infiltration of inflammatory cells within the interstitial tissues as had been previously reported by Cheong *et al*.[9] in workers exposed to industrial waste. The lungs showed moderate and profuse infiltration of the inflammatory cells which normally occurs when there is an injury, giving an insight into the pathogenic nature of the isolates. All the microbial isolates in this study apparently did not have the ability to invade the liver of the rats as these appeared not to be infected. No pathological lesions were observed in any of the control animals. This was an indication that the effects observed with the infected animals might be due to the activity of the microbial isolates with which they were infected indicating that some of the organisms isolated were pathogenic and may cause serious damage to the host, if left untreated. Although none of the experimental rats died when they were exposed to the isolates, these isolates might be opportunistic pathogens and could be hazardous to those body defenses that are compromised. This view was expressed by Chhabra *et al*.[10] where *Aspergillus spp* killed immunosuppressed rat

and altered the histology of the normal rats without killing them. Continous exposure to these microorganisms may cause harm. Hence, the need of public awareness in respect of the dangers associated with improper waste disposal and management.

Conclusion

All the microbial isolates in this study apparently did not have the ability to invade the liver of the rats as these appeared not to be infected. No pathological lesions were observed in any of the control animals. This was an indication that the effects observed with the infected animals might be due to the activity of the microbial isolates with which they were infected indicating that some of the organisms isolated were pathogenic and may cause serious damage to the host, if left untreated.

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References

Aboagye-Larbi, H., Acheampong, M.A., Kyei, S.K., Carboo, D., (2014). The Potential Health Hazards Associated With Waste Scavenging in Ghana: A Case Study of Three Selected Dumpsites in Tema Metropolis. Int. J. Environ. Sci. Toxic. Res. Vol. 2(10):199-209

1. Ikhuoria IA. Analysis of the special Attributes and chemical methods of solid waste management in Nigeria. Environment Review. 2002; 49 (1).

2. Nwobu E. Solid Waste Disposal and Management in Awka.An unpublished PGD Project, Department of Geography, Meteorology and Environmental Management.NnamdiAzikiwe University, Awka; 2022.
3. Kimberly. Study on composition of solid waste in Florida State, United States of America; 2005.

4. Wachukwu CK, Mbata CA, Nyenke CU. The Health Profile and Impact Assessment of Waste Scavengers (Rag Pickers) in Port Harcourt, Nigeria.Journal of Applied Sciences. 2010; 10: 1968-72.

5. Ajadike JC. Urban Solid Wastes; "Problems and Management in Nigeria" in G.E.K. Oformata and P.O.Phil-Eze (eds.) Geographical perspective on Environmental Problems and Management in Nigeria, Nsukka Department of Geography, University of Nigeria, Nsukka.2001.

6. Adesoji AO. Solid Waste Management Problems in Ibadan Wiser publication Ltd, Ibadan. 2004.

7. Anthony M. Well Fact Sheet. Solid Waste Disposal in Ghana. Director of Waste Management, Kumasi.Ballows, H., Herman, I.S., (1990). Manual of Clinical Microbiology. 5th; 2005.

8. Mbata CA. Studies on the burden of solid waste biodegrading microbes and health risks on waste scavengers in Port Harcourt.Ph.D. Thesis, Nigeria. 2008.

9. Cheong H, Kim EA, Choi J, Choi S, Shi J, Choi DS, et al. Ground rounds: an outbreak of toxic hepatitis among industrial waste disposal workers. Environmental Health perspective. 2007; 115: 107-12.

10. Chhabra D, Dhakad NK. Study on pathogenicity of the Aspergillus species in environmentally immunosuppressed rats. Veterinary world. 2008;1 (3):69-70.