

Isolation and identification of microorganisms in individuals associated with refuse disposal sites and collection centres in Awka metropolis, Nigeria

Aislamiento e identificación de microorganismos en individuos asociados a los 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. To isolate, characterize and identify pathogens associated with waste dump sites that may be of public health importance.

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: A total of 6 bacterial genera which included *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Staphylococcus epidermidis* and 4 fungal genera namely *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor spp*s and *Candida albicans*. The incidence of microbial isolates from different sampled groups prior to their work differed significantly ($P < 0.05$) when compared with the isolates during their work. It was also observed that the prevalence of different microorganisms isolated after daily activities was higher than that of those isolated before daily activities.

Key words: Refuse dump sites, waste collectors, scavengers, microorganisms, public health.

Resumen

Introducción: A pesar de los riesgos sanitarios inherentes a los vertederos, algunos individuos se ganan la vida rebuscando en los residuos y empaquetándolos para sobrevivir. Aislar, caracterizar e identificar los agentes patógenos asociados a los vertederos que puedan tener importancia para la salud pública.

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 de residuos, centros de recogida y vertederos de residuos en la metrópoli de Awka. Los aislados bacterianos y fúngicos fueron sometidos a pruebas de patogenicidad con ratas Wistar.

Resultados: Un total de 6 géneros bacterianos que incluían *Staphylococcus aureus*, *Streptococcus pneumonia*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Staphylococcus epidermidis* y 4 géneros fúngicos, a saber, *Aspergillus niger*, *Aspergillus fumigatus*, *Mucor spp*s y *Candida albicans*. La incidencia de los aislados microbianos de los distintos grupos muestreados antes de su trabajo difería significativamente ($P < 0,05$) cuando se comparaba con los aislados durante su trabajo. También se observó que la prevalencia de los diferentes microorganismos aislados después de las actividades diarias era mayor que la de los aislados antes de las actividades diarias.

Palabras clave: Vertederos, 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 Ikhuoria¹, 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. Nwobu² 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.

Kimberly³ 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. 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 colonize the waste and begin to degrade them⁴.

The improper disposal of these waste constitute serious health problems, such as transmission of infectious diseases to humans and animals living within the vicinity⁵, as they pollute the air, soil and freshwater bodies.

During the activities of scavengers and waste collectors they are exposed to various infectious agents⁶ as well as to various toxic substances which may cause illness/sickness. They are also exposed to potentially pathogenic bio-aerosols that may lead to the spread of various diseases. Research conducted by Douwes *et al.*⁷ revealed that exposures to bioaerosols in both the occupational and residential indoor environment could have adverse effects with major public health impact, including contagious infectious diseases, acute toxic effects, allergies and cancer. Ajadike⁸, 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. Adesoji⁹ 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.

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 Policy¹⁰. The non-chalant attitude of the people on issues concerning waste management and environmental best practices has become a major source of worry. 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 lands¹¹.

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 were selected based on convenience, accessibility, proximity to the researcher and not necessarily a representative of the entire population.

Study area

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. The culture plates were incubated aerobically at 37°C for 24hrs.

Collection, culture and identification of fungal isolates in waste dump site, scavenger, waste collectors

Waste samples

One milliliter [10ml of sterilised distilled water was added to 1g of waste] of each prepared waste sample was added into 9 ml of 0.1% bacteriological peptone [10^{-1} dilution]. An aliquot [1.0ml] was transferred into the next test tubes and diluted serially in one-tenth stepwise to 10^{-3} dilution using sterile pipettes. From the dilution of 10^{-1} , 10^{-2} and 10^{-3} of each waste sample, 0.1 ml aliquot was transferred aseptically onto freshly prepared Potato Dextrose Agar [PDA] plates of which 0.2 ml of 0.5% Ampicillin was added to inhibit the growth of bacteria and allowing the growth of fungi¹². The inoculums were spread with a sterile bent glass rod. The inoculated plates were inverted and incubated at room temperature for 5 to 7 days.

Antibiotic susceptibility testing

The antibiotic susceptibility of the isolates was determined by the disc diffusion method on nutrient agar. Bacterial isolates were tested against Ciprofloxacin [CFX 5 µg], Streptomycin [S-10 µg], 30µg], Gentamycin [GEN 10µG], Augmentin [AUG 30µg], Chloramphenicol [C 30 µg], Erythromycin [E 15 µg], Ceftazidime [CTX 30µg], Tetracycline [T 30 µg], Ofloxacin [OFL 5µg], Vancomycin [V 30 µg], Rifampicin [R 5µg] and Amoxicillin [AMX 30µg]. Colonies from the slants were picked and inoculated on nutrient broth and incubated for 18hr. Fresh media were prepared. Picking of colonies from the broth cultures was done using sterile applicator stick and proper swabbing onto the surface of the prepared plates was done, after which antimicrobial discs were applied using a sterile forceps. The discs were firmly pressed down to prevent falling off of the discs from the plates during incubation. The plates were incubated at 37°C for 4hours. After incubation, the zones of inhibition formed were measured in two perpendicular planes with the averages determined. After this, the results were interpreted using standard tables to determine if the bacteria are Sensitive [S], Intermediate [I] or Resistant [R] to the antimicrobial drugs. The diameter of the zone of clearance [including the diameter of the disk] was measured to the nearest whole millimeter and interpreted on the basis of CLSI guideline¹³.

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

The results obtained from the waste collectors, scavengers and people living and trading around the waste bin, after daily activities were compared with the results obtained from them prior to work.

Frequency of bacterial and fungal isolates from waste collectors

From the waste collectors 2(11.8%) *Staphylococcus aureus* were isolated prior to waste collection while 15(88.2%) were isolated after waste collection, 2(13.3%) *Klebsiella pneumoniae* were isolated prior to waste collection, while 13(86.7%) were isolated after waste collection from Nasal swab and Hand swab. The remaining isolates were only isolated after work, they are *Streptococcus pneumoniae* 4(5.26%), *Escherichia coli* 6(7.9%), *Pseudomonas aeruginosa* 8(10.5), *Bacillus subtilis* 4(5.26%), *Staphylococcus epidermidis* 2(2.63%), *Aspergillus niger* 7(9.21%), *Aspergillus fumigatus* 2(2.63%), *Mucorspp* 4(5.26%), and *Candida albicans* 7(9.21%) (Table I)

Frequency of bacterial and fungal isolates from scavengers

From the Scavengers, 2(12.5%) *Staphylococcus aureus* were isolated before Scavenging while 14(87.5%) were isolated after Scavenging. The remaining isolates were only isolated after scavenging, they are *Streptococcus pneumoniae* 1(1.92%), *Escherichia coli* 4(7.69%), *Pseudomonas aeruginosa* 6 (11.5), *Klebsiella pneumoniae* 8(15.4%), *Bacillus subtilis* 2(3.84%), *Staphylococcus epidermidis* 1(1.92%), *Aspergillus niger* 4(7.69%), *Aspergillus fumigatus* 3(5.8%), *Mucorspp* 1(1.92%) and *Candida albicans* 6(11.53%) (Table II).

Frequency of bacterial and fungal isolates from people living/trading around refuse dump sites

From people trading around the bins as nobody resides around the main dump sites, no microorganism was isolated before business, but after business, *Staphylococcus aureus* 9(81.8%), *Klebsiella pneumoniae* 1(9.09%) and *Candida albicans* 1(9.09%) were isolated. Few microorganisms were isolated probably because they don't associate directly with the refuse unlike the scavengers and waste collectors (Table III).

Frequency of bacterial and fungal isolates from different inanimate sources.

The most frequently encountered microbial isolates from the waste bins were *Pseudomonas aeruginosa* 21(30%), *Staphylococcus aureus* 14(20%), *Klebsiella pneumoniae* 13(18.57%) and *Bacillus subtilis* 10(14.29%) while the least encountered were *Aspergillus niger* 3(4.29%), *Candida albicans* 3(4.29%), *Aspergillus fumigatus* 2(2.86%) and *Mucormucedo*.1(1.43%).

The most frequently encountered microbial isolates from the waste vehicle were *Klebsiella pneumoniae* 8(30.77%), *Staphylococcus aureus* and *Pseudomonas aeruginosa* 4(15.39%), *Aspergillus niger* and *Mucormucedo* 3(11.54%) while the least encountered were *Escherichia coli*, *Aspergillus fumigatus* and *Bacillus subtilis* 1(3.85%).

The most frequently encountered microbial isolates from the Refuse dump were *Klebsiella pneumoniae* 5(21.74%), *Escherichia coli* 4(17.39), *Aspergillus niger*

3(13.04), while the least encountered were *Mucor* spp. and *Pseudomonas aeruginosa* 2(8.7), *Candida albicans*, *Aspergillus fumigatus*, *Streptococcus pneumoniae* and *Staphylococcus aureus* 1(4.35%).

The most frequently encountered microbial isolates from air around the main refuse dump were *Klebsiella pneumoniae* 7(33.33), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus* 3(14.29), while the least encountered were *Aspergillus niger* 2(9.52) (Table IV).

Table I: Frequency of bacterial and fungal isolates from waste collectors.

WASTE COLLECTORS

Bacterial and fungal isolates	Urine N=20 [%]	Nasal swab N=20 [%]	Hand swab N=20 [%]	Control N=10 [%]	Total N [%]
<i>Staphylococcus aureus</i>	6 (22.2)	5 (22.72)	4 (17.39)	2 (50.00)	17 (22.4)
<i>Streptococcus pneumoniae</i>	3 (11.11)	1 (4.54)	0 (0.00)	0 (0.00)	4 (5.26)
<i>Escherichia coli</i>	3 (11.1)	1 (4.54)	2 (8.70)	0 (0.00)	6 (3.95)
<i>Pseudomonas aeruginosa</i>	3 (11.1)	3 (13.60)	2 (8.70)	0 (0.00)	8 (10.52)
<i>Klebsiella pneumoniae</i>	5 (18.5)	5 (22.72)	3 (13.00)	2 (50.00)	15 (19.73)
<i>Bacillus subtilis</i>	1 (3.70)	2 (9.09)	1 (4.35)	0 (0.00)	4 (5.26)
<i>Staphylococcus epidermidis</i>	1 (3.70)	0 (0.00)	1 (4.35)	0 (0.00)	2 (2.63)
<i>Aspergillus niger</i>	0 (0.00)	2 (9.09)	5 (21.74)	0 (0.00)	7 (9.21)
<i>Aspergillus fumigatus</i>	0 (0.00)	1 (4.54)	1 (4.54)	0 (0.00)	2 (2.63)
<i>Mucor Mucedo</i>	0 (0.00)	2 (9.09)	2 (8.70)	0 (0.00)	4 (5.26)
<i>Candida albicans</i>	5 (18.52)	0 (0.00)	2 (8.70)	0 (0.00)	7 (9.21)
TOTAL	27 (100)	22 (100)	23 (100)	4(100)	76 (100)

Table II: Frequency of bacterial and fungal isolates from the scavengers.

SCAVENGERS

Bacterial and fungal isolates	Urine N=20 [%]	Nasal swab N=20 [%]	Hand swab N=20 [%]	Control N=10 [%]	Total N [%]
<i>Staphylococcus aureus</i>	5 (21.7)	6 (42.9)	3 (23.1)	2 (100)	16 (30.8)
<i>Streptococcus pneumoniae</i>	1 (4.35)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.92)
<i>Escherichia coli</i>	4 (17.39)	0 (0.00)	0 (0.00)	0 (0.00)	4 (7.69)
<i>Pseudomonas aeruginosa</i>	3 (13.00)	2 (14.2)	1 (7.69)	0 (0.00)	6 (11.5)
<i>Klebsiella pneumoniae</i>	6 (26.1)	0 (0.00)	2 (15.39)	0 (0.00)	8 (15.4)
<i>Bacillus subtilis</i>	0 (0.00)	2 (14.28)	0 (0.00)	0 (0.00)	2 (3.84)
<i>Staphylococcus epidermidis</i>	0 (0.00)	0 (0.00)	1 (7.69)	0 (0.00)	1 (1.92)
<i>Aspergillus niger</i>	0 (0.00)	2 (14.28)	2(15.39)	0 (0.00)	4 (7.69)
<i>Aspergillus fumigatus</i>	0 (0.00)	2 (14.28)	1 (7.69)	0 (0.00)	3 (5.76)
<i>Mucor Mucedo</i>	0 (0.00)	0 (0.00)	1 (7.69)	0 (0.00)	1 (1.92)
<i>Candida albicans</i>	4 (17.39)	0 (0.00)	2 (15.39)	0 (0.00)	6 (11.53)
TOTAL	23 (100)	14 (100)	13 (100)	2 (100)	52 (100)

Table III: Frequency of bacterial and fungal isolates from People living/trading around the waste bin.

People living/trading around the bin

Bacterial and fungal isolates	Urine N=10 [%]	Nasal swab N=10 [%]	Hand swab N=10 [%]	Control N=10 [%]	Total N [%]
<i>Staphylococcus aureus</i>	2 (66.67)	3 (75.00)	4 (100.00)	0 (0.00)	9 (81.81)
<i>Klebsiella pneumoniae</i>	0 (0.00)	1 (25.00)	0 (0.00)	0 (0.00)	1 (9.09)
<i>Candida albicans</i>	1 (33.33)	0 (0.00)	0 (0.00)	0 (0.00)	1 (9.09)
TOTAL	3 (100)	4 (100)	4 (100)	0 (0.00)	11 (100)

Table IV: Frequency of bacterial and fungal isolates from different inanimate sources.

Bacterial /Fungalisolates	Waste receptacles N=40[%]	Refuse Vehicles N=10[%]	Refuse [%]	Air around the Dumpsites	Control (N=10)	TOTAL
<i>Staphylococcus aureus</i>	14 (20)	4 (15.39)	1 (4.35)	3 (14.29)	2 (50.00)	24 (16.6)
<i>Streptococcus pneumoniae</i>	0 (0.00)	0 (0.00)	1 (4.35)	1 (4.76)	0 (0.00)	2 (1.38)
<i>Escherichia Coli</i>	3 (4.29)	1 (3.85)	4 (17.39)	0 (0.00)	0 (0.00)	8 (5.55)
<i>Pseudomonas aeruginosa</i>	21 (30.00)	4 (15.39)	2 (8.70)	3 (14.29)	1 (25.00)	31 (21.52)
<i>Klebsiella pneumoniae</i>	13 (18.57)	8 (30.77)	5 (21.74)	7 (33.33)	1 (25.00)	34 (23.61)
<i>Bacillus subtilis</i>	10 (14.29)	1 (3.85)	2 (8.70)	1 (4.76)	0 (0.00)	14 (9.72)
<i>Staphylococcus epidermidis</i>	0 (0.00)	0 (0.00)	1 (4.35)	0 (0.00)	0 (0.00)	1 (0.69)
<i>Aspergillus niger</i>	3 (4.29)	3 (11.54)	3 (13.04)	2 (9.52)	0 (0.00)	11 (7.63)
<i>Aspergillus fumigatus</i>	2 (2.86)	1 (3.85)	1 (4.35)	3 (14.29)	0 (0.00)	7 (4.86)
<i>Mucor mucedo</i>	1 (1.43)	3 (11.54)	2 (8.70)	1 (4.76)	0 (0.00)	7 (4.86)
<i>Candida albicans</i>	3 (4.29)	1 (3.85)	1 (4.35)	0 (0.00)	0 (0.00)	5 (3.47)
Total	70 (100)	26 (100)	23 (100)	21 (100)	4 (100)	144 (100)

Table V: Incidence of different microbial isolates from various sampled groups.

	COLLECTORS			SCAVENGERS			PLAWB			CONTROL			P-value
	PreA	PosA	Mean	PreA	PosA	Mean	PreA	PosA	Mean	PreA	PosA	Mean	
Urine		27	1.35		23	1.15		6	0.30		3	0.15	0.001
Nasal swab	4	23	1.15	2	15	0.75	1	8	0.40	1	2	0.1	0.008
Hand swab	0	23	1.15	0	13	0.65	0	8	0.40	0	1	0.05	0.002

KEY: PreA= Pre Activity, PosA= Post Activity, PLAWB= People living/trading around refuse bins. P is significant at P<0.05.

Table VI: Susceptibility profile of commonly used antibiotics against Gram positive bacterial isolates.

Isolates	Susceptibility	CPX	S	GN	AUG	C	E	CT	V	R
<i>Staphylococcus aureus</i> N=42	NS (%)	17 (40.5)	22 (52.4)	19 (45.2)	25 (59.5)	14 (33.3)	16 (38)	23 (54.8)	20 (47.6)	26 (62)
	NR (%)	25 (59.5)	20 (47.6)	23 (54.8)	17 (40.5)	28 (66.7)	26 (62)	19 (45.2)	22 (52.4)	16 (38)
<i>Streptococcus pneumoniae</i> N=5	NS (%)	4 (80)	4 (80)	5 (100)	4 (80)	3 (60)	4 (80)	4 (80)	2 (40)	3 (60)
	NR (%)	1 (20)	1 (20)	0 (00)	1 (20)	2 (40)	1 (20)	1 (20)	3 (60)	2 (40)
<i>Bacillus subtilis</i> N=6	NS (%)	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	4 (66.7)	2 (33.3)	3 (50)	3 (50)	2 (33.3)
	NR (%)	4 (66.7)	4 (66.7)	5 (83.3)	5 (83.3)	2 (33.3)	4 (66.7)	3 (50)	3 (50)	4 (66.7)

KEY: CPX-Ciprofloxacin,S-Streptomycin,GN-Gentamycin,AUG-Augumentin,C- Chloramphenicol, E-Erythromycin, CT- Ceftriaxone, V- Vancomycin, R- Rifampicin. NS- Number sensitive, NR-Number resistant.

Table VII: Susceptibility profile of commonly used antibiotics against Gram negative bacterial isolates.

Isolates	Susceptibility	CPX	S	GN	AUG	C	CT	AMX	OFX
<i>Escherichia coli</i> N=10	NS(%)	4(40)	3(30)	4(40)	8(80)	2(20)	7(70)	7(70)	4(40)
	NR(%)	6(60)	7(70)	6(60)	2(20)	8(80)	3(30)	3(30)	6(60)
<i>Klebsiella pneumoniae</i> N=24	NS(%)	9(37.5)	4(16.7)	8(33.3)	2(8.3)	12(50)	10(41.7)	10(41.7)	6(25)
	NR(%)	15(62.5)	20(83.3)	16(66.7)	22(91.7)	12(50)	14(58.3)	14(58.3)	18(75)
<i>Pseudomonas aeruginosa</i> N=14	NS(%)	4(28.6)	6(42.9)	6(42.9)	3(21.4)	8(57.1)	3(21.4)	12(50)	3(21.4)
	NR(%)	10(71.4)	8(57.1)	8(57.1)	11(78.6)	6(78.6)	11(78.6)	12(50)	11(78.6)

KEY: CPX-Ciprofloxacin,S-Streptomycin,GN-Gentamycin,AUG-Augumentin,C-Chloramphenicol, CT- Ceftriaxone, AMX-Amoxicillin, OFX- Ofloxacin. NS- Number sensitive, NR-Number resistant.

Discussion

This study revealed that the predominant bacteria in waste dump sites were *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Aspergillus niger*, *Aspergillus fumigatus*, and *Candida albicans*. The bacterial species identified in this study are similar to those reported by Aboagye-Larbi *et al.*¹⁴. Most of the bacteria, which

commonly occur in the air and soil, are opportunistic pathogens which may cause infection. For instance, *Staphylococcus aureus* can cause food poisoning, wound infection and acute osteomyelitis in children and young adults. *Pseudomonas aeruginosa* can also cause wound and burn infections and are difficult to treat with some antibiotics. The microorganisms present in the air may cause infectious diseases in susceptible individuals.

Most of the microbial isolates that were not isolated from the subjects before their daily activities were isolated after their daily activities and the number of microbial isolates from the test subjects were higher than the ones from control subjects. This agrees with the work done by Aboagye-Larbi *et al.*¹⁴. Most of these microorganisms had earlier been reported by Wachukwu *et al.*¹⁰ as being associated with waste decay. The isolates obtained from the dump sites and workers in this study were essentially similar. As earlier reported by Markanday *et al.*¹⁵, most of these isolates are known to be involved in the biodegradation of organic matters. The most frequently isolated fungi in the present study belonged to the genus *Aspergillus*. This conforms with Wienrich *et al.*¹⁶ which suggested that *Aspergillus niger* and *Aspergillus fumigatus* as the leading species of fungi in the biowaste due to their frequency of detection. These two species were also encountered in this study. *Aspergillus* is known to produce aflatoxin, a mycotoxin that is toxic and carcinogenic. High amount of aflatoxins present in contaminated food exerts their toxicological effect in animals and man. *Aspergillus fumigatus* is known to be associated with dust and its endotoxins are found in landfills and compost plants. *Mucormucedo* were identified in and around the environment of the main dump sites and in the collection centers.

Another group of microbial isolates from the waste dump and human are the endospores forming bacteria such as *Bacillus subtilis* and *Escherichia coli*, which are important organisms that cause urinary tract infection and gastroenteritis in children. *Bacillus subtilis* produce spores and are commonly found in the soil, therefore they can easily get through to the scavengers. If waste collectors are not protected, there is tendency of these pathogens gaining entry into the body. The resultant effect will be infection, general body malaise and in some cases death. Wachukwu *et al.*¹⁰ and Aboagye-Larbi *et al.*¹⁴ shared similar views. *Staphylococcus aureus* showed significant increase in the case of scavengers and waste collectors. *Staphylococcus* observed in this group of people may indicate the presence of bacterial infections especially with the *Staphylococcus aureus* which may result in skin injuries or disorders¹⁷. Staphylococcal disease of the skin if left untreated usually results in a localized collection of pus known as an abscess, boil or furuncle. When *Staphylococcus* is in the blood, it can cause high fever, chills and low blood pressure. The direct health risk of concern is mainly for the worker on the field who handles refuse or who live near the disposal sites, if not properly protected.

Of the 4 groups sampled, the number of bacterial and fungal isolates significantly differed between the 4 groups at 5% significance level ($P=0.008$). Further post ANOVA analysis showed that this difference was only between collectors and control and not between any other groups. This may be due to collectors and scavengers handle the

wastes with their bare hands while the PLAWB and control did not. Comparison between the number of organisms isolated from nasal and hand swabs before and after were statistically significant at the 5% significance level ($P=0.001$) and ($P=0.001$) respectively. This meant that the carriage of these microorganisms by waste collectors, scavengers at the waste dump sites might be as a result of their activities, while the carriage by the PLAWB were because of their presence around the bin, as these organisms are airborne and people around the waste bin inhaled them. In addition, carriage of these microbes by some of the control subjects may depend on where they reside as most of the microorganisms were isolated before the day activities. These might explain why most of the Scavengers and workers experienced recurrent respiratory and urinary tract infections, with some presenting with skin rashes.

From the result obtained in this study, antibiotic resistant bacteria were widespread as most of the isolated organisms were resistant to most of the antibiotics for which they were tested. This might be due to either the intrinsic resistance of many microorganisms to antibiotics or acquired resistance of the organisms enabled by the transfer of drug resistance plasmids among members of the isolates. A high level of resistance has been found with members of the family Enterobacteriaceae which were believed to have incidence of pathogenic strains of bacteria with acquired antibiotics resistance. The origin of this resistance can be traced to the faecal constituent of the waste produced by people or animals that have been treated indiscriminately with various types of antibiotics and to natural antibiotic production by soil microorganisms¹⁸.

Conclusion

The study showed that microbial loads at waste dump sites and collection centres pose a great threat not only to scavengers, waste collectors, and people living or trading around collection centers, but to the entire society. The study showed that microorganisms isolated from scavengers and waste collectors in Awka, Nigeria, induced lung and kidney dysfunctions in

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Conflict of Interest

The authors declare that no competing interests exist.

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