

Original research Articles

Isolation and Identification of some Microbial Pathogens Associated with the External Body Surface of *Periplaneta americana* in Umuahia, Abia State.

ABSTRACT

AIM: This study was carried out to isolate and identify the microbial pathogens of medical importance on the external body surfaces of *Periplaneta americana* collected from toilet areas, residential/hostel areas and health facilities in Umuahia metropolis, Abia State, south-eastern Nigeria.

STUDY DESIGN: cross sectional study.

PLACE AND DURATION OF STUDY: The study was carried out in Umuahia metropolis, Abia State, south-eastern Nigeria between October-December, 2014.

METHODOLOGY: A total of 100 cockroaches from toilets (30), residential/hostel areas (46) and health facilities (24) in Umuahia were collected and their external body parts screened for bacterial, fungal and parasitological carriage. Sterile physiological saline used as washing fluid of the external surface of each cockroach was cultured by the streak plate method. The isolated bacteria were identified using standard bacteriological methods. Antibiotics susceptibility tests were done by the disc diffusion method.

RESULTS: Results showed that all the cockroaches were positive for different species of medically important bacteria and fungi. *Escherichia coli* were the most common bacteria in the cockroaches' from toilet (21.14%) and residential/hostel areas (22.37%) while, *Staphylococcus aureus* (21.74%) was the most frequent isolate obtained from cockroaches obtained from health facilities. *Aspergillus* species (33.16%) was the most prevalent fungal group. Quantitative analysis of the total viable count and microbial loads of the bacterial and fungal isolates from the three sites revealed that the isolates obtained from toilet areas were high as compared to those from residential areas.

Antibiotic susceptibility of all the bacteria isolates revealed a common pattern of multiple drug resistance amongst isolates from health facilities when compared to those from toilet and residential areas.

CONCLUSION: The result indicated that *Periplaneta americana* is a possible reservoir of several pathogens via their bodies and thus may spread multiple drug resistant species.

Keywords: Microbial pathogens; external surface; *Periplaneta Americana*.

1. INTRODUCTION

Cockroaches are considered one of the most adaptable insect groups ever in human history [1]. Evidence of the continued co-existence with people throughout history is testimony to how adaptable some cockroaches are to the habits of people [2]. They are born invertebrates and omnivorous scavengers in nature and have been noted by scientists to have survived more than 300 million years. Of the over 4,000 known species of cockroaches, only a dozen can be considered as pests. Syanthropic animal species, including cockroaches, live in most human habitations especially where food is stored, processed, prepared, or served [3]. Apart from human food, they also consume putrefied and decaying organic matter [4], and thus are easily, sited crawling in and/or near broken sewage lines or refuse dump sites in major towns and cities around the world. During the day, these insects hide in isolated locations or in gaps of the walls, but become active at night when they can move about unnoticed [5].

The American cockroach, *Periplaneta americana* is by far the most serious and predominant metropolitan pest in Umuahia, Abia State, Nigeria and her environs judging by how often they are commonly sited in large numbers in many households, kitchens/restaurants, offices, grocery stores, broken sewage and refuse disposal sites in and around Umuahia metropolis. The medical importance of cockroaches is much greater than generally realized as they have been shown to harbor a number of pathogenic and non-pathogenic microorganisms [6]. Cockroaches are known vectors of human entero-pathogens as recent research findings have provided substantial evidence of the isolation of various human pathogens from these insects [7, 8]. [9] suggested that cockroaches are possible vectors of *Klebsiella pneumonia* in the hospital environment. In a related study of bronchial asthma by [10], 40% of patients in China were found to be allergic to German cockroaches.

Furthermore, a comparative analysis of pathogenic organisms in cockroaches from different socio-economic community settings in Edo State, Nigeria showed that organisms such as *Bacillus spp.*, *Escherichia coli*, *Proteus mirabilis*, *proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Citrobacter freundii* and *Salmonella spp* can be harbored on the body surface and alimentary tract of cockroaches [11].

Indeed, numerous published papers recognized the association of cockroaches with several infectious diseases and spread of pathogenic and drug resistant microbes such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella spp.*, *Salmonella typhi* and even some fungi, viruses, protozoa and helminthes [3]. Over 4% of cockroaches collected from hospitals, houses, animal sheds, grocery stores, and restaurants in India harbored multiple drug resistant *Salmonella* [12]. [13] isolated *Shigella boydii*, *Shigella dysenteriae*, and *Salmonella typhimurum*, from cockroaches collected from hospital kitchens in Malaysia. In a study, 70% of cockroaches collected from hospitals in Iran yielded *Salmonella* spp. and some of the isolates were resistant to antimicrobial drugs [14].

2. MATERIAL AND METHODS

This study was carried out in Umuahia; the capital city of Abia State in Southern Nigeria, and has a population of 359, 230 people according to official figures from the 2006 Nigerian Census. A total of 100 adult American cockroaches (*Periplaneta americana*) were collected at random from various locations/settings within the Umuahia capital city. They include those collected from toilet areas, hospitals/clinics/health centres (collectively grouped as health facilities) and student dwellings/residential areas.

Cockroaches were collected by three (3) methods; sterile screw-capped jar traps, sticky traps and by capture with sterile hand-gloves [15, 16]. Only cockroaches caught whole and alive were considered in the study. Identification of cockroaches was performed in accordance with [17]. The collected cockroaches were brought to the Microbiology laboratory of the Department of Microbiology, Michael Okpara University of Agriculture, Umudike and killed in a sterile jar using chloroform soaked cotton. The external body surface was washed by vortexing in 5 ml sterile physiological saline for two minutes, and the washing fluid was taken as external body homogenate sample. 1 ml of each homogenate was inoculated separately into 9 ml of physiological saline. A 10 fold serial dilution was carried out on the wash suspension of the cockroaches and was adequately streak plated on the various media used in triplicates and incubated at 37 °C for 18-24 hr [18].

Microscopy of parasites on different body parts was also done using appropriate techniques. Morphological characteristics were used to identify the parasites after wet sediment from the cockroach washing was mixed with a drop of iodine solution [19].

Quantitative analysis of the viable bacterial and fungal loads from the external surface of the cockroaches was done by a modification of the Mile's method. In brief, 0.1ml of the diluted wash suspension of the cockroaches was cultured by streak plate on media used in triplicates. The

colony forming units (CFU) was calculated as CFU/ml of washing from cockroaches after incubation at 37°C for 24 hrs and the mean count of plates taken [6].

Fungal cultures were made with Sabouraud Dextrose Agar (SDA) impregnated with antibiotics and incubated for 2-5days. The isolates were identified using lacto-phenol cotton blue in which a portion of the fungi were collected using sterile needle and placed on the microscope slide. The fungal isolates were identified on the basis of their colonial morphology on the SDA medium and type of hyphae after subcultures were made.

The isolation and identification of bacteria on the body surface of the study cockroaches were done by standard methods. Aliquots of 0.1ml of the diluted wash suspension were cultured on plates of MacConkey Agar, Mannitol Salt Agar (MSA), Nutrient agar (NA), and Chocolate Agar in triplicates. Isolated bacterial colonies were identified and characterized using macroscopic morphology, Gram stain reaction and biochemical tests [18].

The antibiotics sensitivity of the isolates was tested against the following antibiotics streptomycin (30mcg), tarivid (10mcg), peflacin (10mcg), ciproflox (10mcg), Augmentin (30mcg), Gentamicin (10mcg), septrin (30mcg), ceporex (10mcg) and Ampicillin (30mcg) using Kirby Bauer antibiotics disc method [9]. A colony of the test organisms was picked with sterile wire loop and immersed in peptone water. The turbidity of the suspension was compared against a reference 0.5 Mcfarland tube. The suspension of the organism was streaked on the entire of nutrient agar and the antibiotic disc was placed on the centre of the plate using forcep. The plates were incubated at 37°C for 24 hours. The diameter of the zone of inhibition was measures using CLSI standard guidelines.

3. RESULTS

A total of 100 cockroaches from toilet areas (30), health facilities (24) and residential/hostel areas (46) were captured, processed and analyzed for bacteria, fungi and parasite carriage on their external body parts.

105 **Table 1: Number of Cockroaches Analyzed From the Sample Areas**

Habitat/Sample Area	Nº. of Samples Collected	Percentage
Toilet areas	30	30
Hospital/clinic/health centres	24	24
Student dwelling/residential areas	46	46
Total	100	100

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107 A total of 245 bacterial isolates were isolated and identified, majority of which had a higher frequency of occurrence in toilets and
108 dwelling areas when compared with those obtained from health facilities.

109 **Table 2: Relative Abundance of Bacteria Isolates on the External Body Surfaces of Cockroaches Collected**

Bacteria isolates	Toilet area Nº (%)	Residential/hostel (%)	Nº Health facilities (%)	Nº Cumulative Nº (%)
<i>Bacillus</i> spp.	15 (12.20)	10(13.16)	8 (17.39)	33 (13.47)
<i>Escherichia coli</i>	26 (21.14)	17 (22.37)	6(13.04)	49 (20.00)
<i>Klebsiella</i> spp.	22 (17.89)	12 (15.79)	8(17.39)	42(17.14)
<i>Pseudomonas aeruginosa</i>	9(7.32)	7(9.21)	4(8.70)	20(8.16)
<i>Proteus vulgaris</i>	7(5.69)	5(6.58)	1(2.17)	13(5.30)
<i>Proteus mirabilis</i>	5(4.07)	3(3.95)	1(2.17)	9(3.67)
<i>Shigella</i> spp.	5(4.07)	3(3.95)	-(0)	8(3.27)
<i>Staphylococcus aureus</i>	14(11.38)	9(11.84)	10(21.74)	33(13.47)
<i>Streptococcus</i> spp.	6(4.88)	3(3.95)	2(4.35)	11(4.49)
<i>Staphylococcus epidermidis</i>	8(6.50)	4(5.26)	4(8.70)	16(6.53)
<i>Salmonella</i> spp.	6(4.88)	3(3.95)	2(4.35)	11(4.49)
Total	123	76	46	245

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111 Table 3 compares the bacterial loads of these isolates and their standard deviations with respect to the habitat/sample area they
112 were obtained from.

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115 **Table 3: Microbial Load of Bacteria Isolates on the External Body Surfaces of Cockroaches Collected**

Bacteria isolates	Toilet area Mean load (CFU/ml)	Residential areas load (CFU/ml)	Mean Health facilities (CFU/ml)	Mean load
<i>Bacillus</i> spp	$8.0 \pm 2.9 \times 10^6$	$7.8 \pm 3.1 \times 10^6$	$3.1 \pm 4.2 \times 10^6$	
<i>Escherichia coli</i>	$10 \pm 3.2 \times 10^6$	$9.6 \pm 4.7 \times 10^6$	$5.2 \pm 2.0 \times 10^6$	
<i>Klebsiella</i> spp	$9.0 \pm 3.0 \times 10^6$	$6.0 \pm 2.5 \times 10^6$	$3.8 \pm 3.8 \times 10^6$	
<i>Pseudomonas aeruginosa</i>	$6.0 \pm 1.2 \times 10^6$	$4.5 \pm 1.9 \times 10^6$	$3.4 \pm 0.7 \times 10^6$	

<i>Proteus vulgaris</i>	$11 \pm 2.1 \times 10^6$	$8.0 \pm 0.1 \times 10^6$	$2.1 \pm 0 \times 10^6$
<i>Proteus mirabilis</i>	$9.8 \pm 1.9 \times 10$	$8.7 \pm 0.8 \times 10^6$	$2.4 \pm 0 \times 10^6$
<i>Shigella</i> spp	$6.0 \pm 0.8 \times 10^6$	$3.2 \pm 4.2 \times 10^6$	-
<i>Staphylococcus aureus</i>	$5.2 \pm 4.0.9 \times 10^6$	$2.0 \pm 0.01 \times 10^6$	$2.0 \pm 1.0 \times 10^6$
<i>Streptococcus</i> spp	$3.4 \pm 0.9 \times 10^6$	$4.0 \pm 0.2 \times 10^6$	$2.16 \pm 0.01 \times 10^6$
<i>Staphylococcus epidermidis</i>	$4.0 \pm 0.14 \times 10^6$	$2.1 \pm 5.1 \times 10^6$	$1.0 \pm 2.0 \times 10^6$
<i>Salmonella</i> spp	$3.0 \pm 4.6 \times 10^6$	$2.0 \pm 0.02 \times 10^6$	$2.0 \pm 0.8 \times 10^6$

In all, 196 fungal isolates were obtained from the three areas sampled, out of which *Aspergillus* spp. (33.16%) were the most frequently obtained fungal organism from cockroaches obtained from the three habitat groups analyzed followed by *Mucor* spp (17.86), *Penicillium* spp., (15.31), *Rhizopus* spp (6.63) and *Candida* spp (27.04). They were identified on the basis of their cultural and microscopic characteristics.

Table 4: Relative Abundance of Fungi Isolates on the External Body Surfaces of Cockroaches Collected

Bacteria isolates	Toilet area	Residential/hostel	Health facilities	Cumulative
	N°. (%)	N°. (%)	N°. (%)	N°. (%)
<i>Aspergillus</i> spp.	30 (32.61)	21 (30.43)	14 (40.00)	65 (33.16)
<i>Mucor</i> spp.	17 (18.49)	14 (20.30)	4 (11.43)	35 (17.86)
<i>Penicillium</i> spp.	13 (14.13)	10 (14.49)	7 (20.00)	30 (15.31)
<i>Rhizopus</i> spp.	8 (8.70)	5 (7.25)	-(0)	13 (6.63)
<i>Candida</i> spp.	24 (26.09)	19 (27.53)	10 (28.57)	53 (27.04)
Total	92	69	35	196

Table 5 show the microbe load of the fungal isolates. Cockroaches from toilet areas were found to harbor the highest loads of the fungal organisms.

Table 5: Microbial Load of Fungi Isolates on the External Body Surfaces of Cockroaches Collected

Fungal isolates	Toilet area	Residential areas	Health facilities
	Mean load (CFU/ml)	Mean load (CFU/ml)	Mean load (CFU/ml)
<i>Aspergillus</i> spp.	$5.7 \pm 6 \times 10^6$	$4.3 \pm 3.89 \times 10^6$	$3.3 \pm 5.12 \times 10^6$
<i>Mucor</i> spp.	$5.2 \pm 3.0 \times 10^6$	$3.0 \pm 2.5 \times 10^6$	$2.0 \pm 4.0 \times 10^6$
<i>Penicillium</i> spp.	$3.1 \pm 1.8 \times 10^6$	$2.4 \pm 2.0 \times 10^6$	$1.4 \pm 2.1 \times 10^6$
<i>Rhizopus</i> spp.	$3.9 \pm 2.6 \times 10^6$	$3.0 \pm 1.6 \times 10^6$	-
<i>Candida</i> spp.	$3.0 \pm 4.0 \times 10^6$	$2.1 \pm 1.8 \times 10^6$	$1.8 \pm 0.74 \times 10^6$

129 Parasitological investigations gave a total of 167 parasites .The parasite with the highest frequency of occurrence was *Ascaris*
 130 *lumbricoides* which had an abundance of 30%, 32.79% and 50% respectively from the three habitats analyzed in this study (*i.e* toilet
 131 areas, residential/hostel dwellings and health facilities).
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133 **Table 6: Number of Parasites Identified On External Body Surfaces Cockroaches Collected**

Parasites	Toilet area N°. (%)	Residential/ Hostel N°. (%)	Health facilities N°.(%)	Cumulative (%)	N°.
<i>Endolimax nana</i>	15(16.67)	10(16.40)	3(18.75)	28(16.77)	
<i>Giardia lamblia</i>	26(28.89)	18(29.51)	4(25.00)	48(28.74)	
<i>Ascaris lumbricoides</i>	27(30.00)	20(32.79)	8(50.00)	55(32.93)	
<i>Entamoeba histolytica</i>	22(24.44)	13(21.331)	1(6.25)	36(21.56)	
Total	90	61	16	167	

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137 **Table 7: Antibiotic Susceptibility of the Bacterial Isolates from External Body Surfaces Cockroaches Collected from**
 138 **Toilet and residential/hostel areas.**

Bacterial Isolate	N°. tested	N°. sensitive (%)								
		OFX	PEF	CRO	AU	CN	S	CEP	SXT	PN
<i>Bacillus</i> spp	25	23(92.00)	24(96.00)	25(100)	12(48.00)	16(64.00)	0(0)	5(20.00)	2(8.00)	8(32.00)
<i>Escherichia coli</i>	43	43(100)	42(97.68)	43(100)	21(48.84)	9(20.93)	2(4.65)	8(18.60)	0(0)	5(11.63)
<i>Klebsiella</i> spp.	34	34(100)	34(100)	34(100)	27(79.41)	11(32.35)	8(23.53)	18(52.94)	6(17.65)	9(26.65)

<i>Pseudomonas aeruginosa</i>	16	15(93.75)	13(81.25)	16(100)	10(62.50)	5(31.25)	1(6.25)	2(12.50)	0(0)	0(0)
<i>Proteus vulgaris</i>	12	12(100)	12(100)	12(100)	2(16.67)	1(8.33)	3(25.00)	10(83.33)	0(0)	2(16.67)
<i>Proteus mirabilis</i>	8	8(100)	8(100)	8(100)	4(50.00)	2(25.00)	4(50.00)	6(75.00)	0(0)	3(37.50)
<i>Shigella</i> spp.	8	6(75)	4(50)	5(62.50)	3(37.50)	0(0)	0(0)	5(62.50)	0(0)	1(12.50)
<i>Staphylococcus aureus</i>	23	23(100)	17(73.91)	23(100)	15(65.22)	0(0)	8(34.78)	2(8.70)	0(0)	10(43.49)
<i>Staphylococcus epidermidis</i>	12	9(75.00)	10(83.33)	11(91.67)	7(58.33)	2(16.67)	0(0)	3(33.33)	5(41.67)	8(8.33)
<i>Salmonella</i> spp.	9	8(89.89)	6(66.67)	8(88.89)	4(66.67)	5(55.55)	1(11.11)	3(33.33)	0(0)	0(0)

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140 **KEY: OFX=Ofloxacin, PEF= Peflacine, CRO = Ceftriazone, AU = Augmentin, CN = Gentamicin, S = Streptomycin, CEP =**
141 **Ceporex, SXT = Septrin and PN = Ampicillin.**

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143 Results of the bacterial organisms' susceptibility to the antibiotics used revealed that isolates from cockroaches gotten from health
144 facilities exhibited an increased rate of multiple drug resistance as against those from cockroaches obtained from toilet and residential/hostel
145 areas.

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147 **Table 8: Antibiotic Susceptibility of the Bacterial Isolates from External Body Surfaces Cockroaches Collected**
148 **Obtained From Health Facilities**

Bacterial isolates	N°. tested	N°. sensitive (%)								
		OFX	PEF	CRO	AU	CN	S	CEP	SXT	PN
<i>Bacillus</i> spp.	8	5(62.50)	6(75.00)	7(87.50)	1(12.50)	2(25.00)	0(0)	0(0)	1(12.50)	1(12.50)
<i>Escherichia coli</i>	6	6(100)	5(83.33)	6(100)	2(33.33)	0(0)	0(0)	2(33.33)	0(0)	0(0)
<i>Klebsiella</i> spp.	8	7(87.50)	8(100)	8(100)	5(75.00)	3(37.00)	2(25.00)	4(50.00)	1(12.50)	2(25.00)
<i>Pseudomonas aeruginosa</i>	4	3(75.00)	3(75.00)	4(100)	2(50.00)	1(25.00)	0(0)	0(0)	0(0)	0(0)
<i>Proteus vulgaris</i>	1	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
<i>Proteus mirabilis</i>	1	1(100)	1(100)	1(100)	0(0)	0(0)	0(0)	1(100)	0(0)	0(0)
<i>Staphylococcus aureus</i>	10	8(80.00)	7(70.00)	9(90.00)	4(40.00)	20(20.00)	10(10.00)	0(0)	10(100)	0(0)
<i>Staphylococcus epidermidis</i>	4	3(75.00)	3(75.00)	4(100)	3(75.00)	1(25.00)	0(0)	1(25.00)	1(25.00)	1(25.00)
<i>Salmonella</i> spp.	2	1(50.00)	1(50.00)	2(100)	0(0)	0(0)	0(0)	0(0)	0(0)	1(50.00)

KEY: **OFX**=Ofloxacin, **PEF**= Peflacine, **CRO** = Ceftriazone, **AU** = Augmentin, **CN** = Gentamicin, **S** = Streptomycin, **CEP** = Ceporex, **SXT** = Septrin and **PN** = Ampicillin.

4. DISCUSSION

The study isolated ten (10) bacterial organisms, five (5) fungal species and 4 parasitic organisms from the 100 cockroaches screened. The isolation of several bacterial, fungal and parasitic organisms on the external body parts of cockroaches agrees with previous studies on the subject matter [8, 3]. The high prevalence of *Escherichia coli* (20.00%) and *Klebsiella* spp (17.14%) in cockroaches collected from the three areas agrees with the findings of [1]. However Staphylococcus areas appeared to be higher in those obtained from health facilities (21.74%). The isolation of *S. aureus* was in agreement with the reports of [10] on the comparative analysis of pathogenic organisms in cockroaches from different community settings in Edo State, Nigeria. A total of 196 fungal organisms were isolated from the external body parts of cockroaches from the three areas covered by this study which was in agreement with [1]. The presence of these organisms in very large numbers was at variance with the report of [6]. Non isolation of *Rhizopus* spp in this study from cockroaches obtained from health facilities disagrees with the findings of [20]. High prevalence of *Aspergillus* spp (33.16%) agrees with the work of [4]. More parasites were recovered from Cockroaches obtained from toilet areas than the other habitats sampled and agree with the reports with [8]. The antibiotic susceptibility analysis of the bacterial isolates showed a common pattern of multiple drug resistance to the antibiotics used. [3] in their study also found a similar situation in *Blatella germanica*, which was a possible cause of the spread of multi-drug resistant bacteria. However, the antibiotics resistance pattern observed in this study was found to be a little bit different from the pattern shown by *Salmonella* species obtained from health facilities as against the pattern noticed by [13] the pattern of antibiotic susceptibility of bacterial species isolated from the external body surfaces of cockroaches collected from toilet, residential and hostel areas varied widely with the susceptibility pattern obtained in health facilities detailed in Tables 7 and 8, which further confirms the spread of antibiotics resistant bacteria in health facilities [11]. These cockroaches therefore could act as mobile reservoirs of such bacteria and could start a fresh outbreak of life threatening septicemia infections [6] amongst the inhabitants of Umuahia the metropolis.

5. CONCLUSION

In conclusion, the findings of this study showed that American cockroaches (*Periplaneta americana*) collected from toilet and residential/hostel areas and even those from health facilities, harbored a great diversity of microorganisms (Bacteria, fungi and parasites). Thus, suggests *Periplaneta americana* to be a possible reservoir of several pathogens via their bodies and thus may spread multiple drug resistant species with its attendant public health concerns and in the transmission of community acquired and nosocomial infections.

An effective waste disposal system should be instituted and strengthened by the appropriate agencies saddled with the responsibility of waste disposal. Furthermore, good hygiene practices such as covering of food, heating of left-over food before consumption and washing of kitchen utensils and laboratory equipment before use plus the use of insecticides will go a long way in reducing contact with these dangerous vectors.

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