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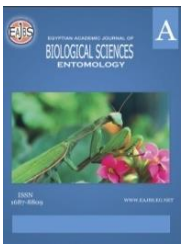

Cockroaches (*Periplaneta americana* Linnaeus [Dictyoptera: Blattidae]) as Potential Vectors of Bacteria in Federal University Wukari, Nigeria

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ABSTRACT

The cockroaches have become the most common domestic insect pest of public health and epidemiological importance. Their presence raises safety concerns, especially as carriers of food-borne pathogens and food-spoilage organisms. Hence, this study aimed to document the bacteria isolated from the body surface and gut of cockroaches at Federal University Wukari, Nigeria. Using a swab bacteriological technique, investigations were carried out on the body surface and gut of cockroaches picked from sewers, kitchens, and bathrooms from student's hostels and opened fields of the institution. Nutrient and MacConkey agar were used as media. Students t-test was used to statistically compare the bacterial load recorded on samples retrieved from open field vis-à-vis the hostels. From the two sampled sites, the gut had higher colony forming unit (Cfu/ml) of ($1.5 \times 10^6 \pm 33435.80$) vis-à-vis the body surface ($6.9 \times 10^4 \pm 13388.88$). Marginally higher bacterial isolates were observed on cockroaches retrieved from the open field (25 bacterial isolates) than from students' hostels (23 bacterial isolates). In this study, *Staphylococcus aureus* was the most predominantly isolated bacterial species from both locations followed by *Escherichia coli*. Species with lower frequencies were *Proteus mirabilis*, *Bacillus* species, and *Pseudomonas aeruginosa*. Control and preventive strategies for cockroaches are paramount in suppressing the contamination of food by microbes associated with cockroaches. Additionally, optimum hygiene practices should be instituted in the hostels, food canteens, and clinics/hospitals of the University; and indeed, the host community to avoid the build-up of breeding sites of cockroaches which are known to carry and spread pathogenic bacteria.

INTRODUCTION

Cockroaches (*Periplaneta americana* Linnaeus [Dictyoptera: Blattidae]) are found in all over the world. They are important disease-causing organisms and are therefore of public health concern. There are over 4,500 known species of cockroaches. However, only 30 species are considered as pest, out of which, *Blattella germanica* (German cockroach), *Blatta orientalis* (Oriental cockroach), *Periplaneta americana* (American cockroach), *P.*

australasiae (Australian cockroach), *and Supella longipalpa* (brown-banded cockroach) are the most predominant and of public health importance (Shahraki *et al*., 2013; Nuwer, 2013; Sarwar, 2015). Among them, *P. americana* and *Blattela germanica* (Figure 1) are the most predominant within the southern guinea savanna zone of Nigeria (Omudu and Eyumah, 2008).

The feeding mechanism and dirty breeding habits of cockroaches make them the mechanical carriers for various human intestinal pathogens (fungi, bacteria, viruses, and parasites) (Graczyk *et al*., 2005; Kinfu and Erko, 2008). Various pathogenic microorganisms have been isolated from cockroaches, including bacteria (Mehainaoui *et al*., 2021; Memona *et al*., 2017), viruses (Tetteh-Quarcoo *et al*., 2013; Du *et al*., 2018), various fungi (Salehzadeh *et al*., 2007; Kassiri *et al*., 2018), and parasites (Tetteh-Quarcoo *et al*., 2013; Dokmaikaw and Suntaravitun, 2019; Donkor, 2020). Most of them are intestinal pathogens, with bacteria as the main pathogens. At present, local and foreign researchers tend to carry out studies on cockroach pathogens in hospitals (Memona *et al*., 2017; Chehelgerdi and Ranjbar, 2021; Mehainaoui *et al*., 2021) and residential areas (Hamu *et al*., 2014; Farah Haziqah *et al*., 2017). Such studies have hardly been conducted in Wukari, Taraba State of Nigeria.

Cockroaches prefer to live in a variety of habitats, especially in warm, dark, humid, and food-abundant places (Adenusi *et al*., 2018; Dokmaikaw and Suntaravitun, 2019). More so, they are omnivores and eat any organic food but prefer food sources such as candy, meat products, starch, and grease. They also feed on plants, vegetables, and fruits (Chamavit *et al*., 2011), and catering places are ideal breeding places. The body parts (appendages, mouthparts, antennae) and secretions of cockroaches are the means through which they disseminate pathogens (Memona *et al*., 2017).

Foodborne diseases are widespread and escalating public health problems worldwide. Cockroaches harbour a variety of pathogenic microorganisms, a quarter of which are foodborne pathogens (Chehelgerdi and Ranjbar, 2021). Hence, they are important reservoirs and mechanical vectors for foodborne pathogens. In general, the role of cockroaches in human infection is poorly understood and has been a controversial issue for many years.

In this part of the world, open defecation (defecation in the open field and refuse dumps) is widespread as a result of inadequate toilet facilities and water supply,. The University is not completely immune to this. More so, food canteens within the campus process goat and cow intestines around their premises. These amidst others, make the environment favourable for cockroach proliferation. The risk to human health arising from cockroach infestations have been reported (Tetteh-Quarcoo *et al*., 2013) as there are reports of the isolation of various pathogenic bacteria, human helminth, and protozoan parasites from them. The behaviour, feeding habits, body structure, and mobility of cockroaches make them well adapted for the mechanical transmission of oocysts, cysts and larvae of parasites and also microbes that cause diseases, some of whose epidemiology is still poorly understood in developing countries (Tatfeng *et al*., 2005; Ghosh and Gayen, 2006; Bouamamaa *et al*., 2010).

Despite various work done on bacteria associated with cockroaches, there is a dearth of such comprehensive study in Wukari, Nigeria. This study is aimed at filling this research gap by providing some information about the bacterial communities associated with cockroaches; *P. Americana*; the a predominant species in Wukari, Nigeria.

MATERIALS AND METHODS

Description of the Study Area:

This study was conducted at two purposively selected sites at Federal University Wukari. Wukari is within the Southern Guinea savanna zone of Taraba State, Nigeria. It is one of the largest local Government areas in the State, with coordinates; latitude 7.850°N and longitude 9.78°E. It has an annual precipitation of 1205 mm and an average temperature of 26.8°C, with a population of over 10,000 (Okrikata and Yusuf, 2016; Samuel *et al*., 2022)

Collection and Identification of Cockroach Samples:

Since *P. americana* is the most predominant species in the study area (Omudu and Eyumah, 2008; Elijah and Lamidi, 2023), it was selected for the study. A total of 40 adult *P. americana* samples were collected manually using hand gloves and sterile entomological forceps. Twenty were collected from the students' hostels, and another twenty from the open field, and both sites are situated within Federal University Wukari, Nigeria. In the hostels, preference was given to sewers, lavatories and areas where students cook, as insects retrieved from these spots are believed to have sanitary significance. Each cockroach sample was collected into separate sterile sample bottles to avoid cross-contamination and was immediately moved to the Biology Laboratory of the same institution where they were killed using chloroform. Representative samples were identified as *P. americana* using distinguishing physical features (see Figure 1) of the dominant species in the study area (Omudu and Eyumah, 2008; Elijah and Lamidi, 2023) along with morphological characteristics and keys in Pratt and Smith (1988).

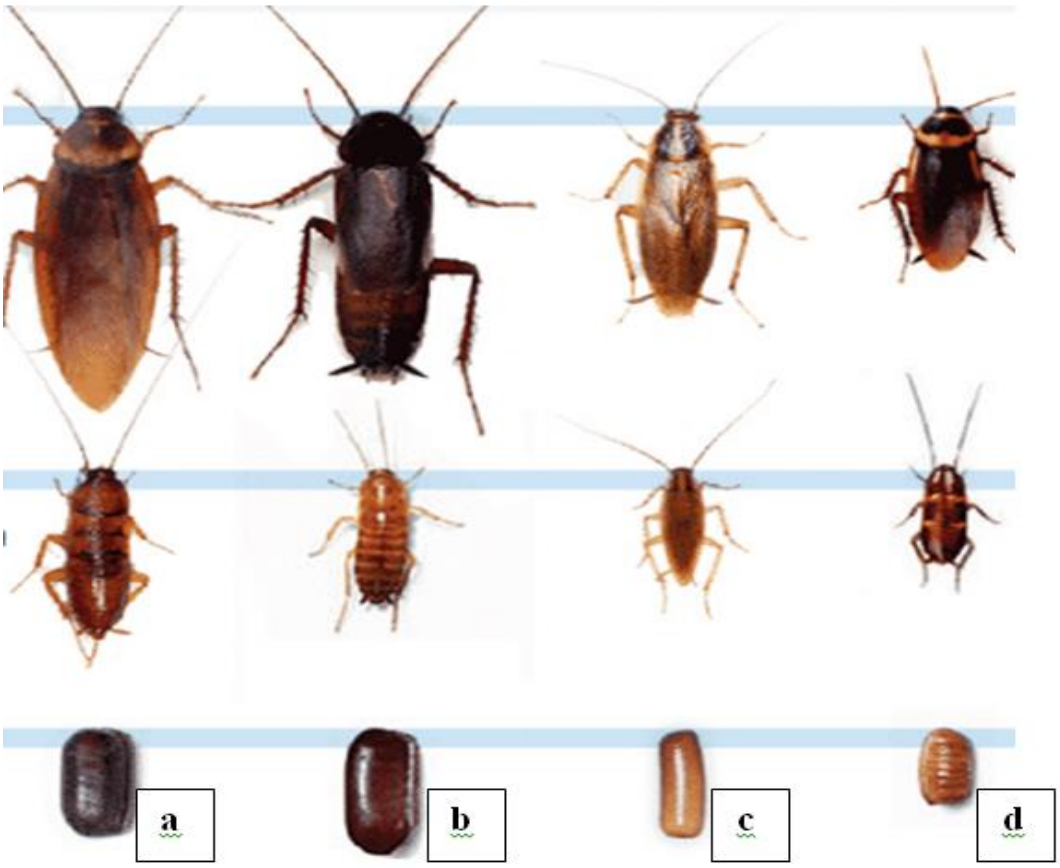

Fig. 1. Eggs, Nymphs and Adults **a.** *Periplaneta americana* **b.** *Blatta orientalis* **c.** *Blattela germanica* **d.** *Supella longipalpa* (**Source:** Makumana, 2018)

Bacteriological Analysis of the Cockroaches:

The body surface of each cockroach sample was washed with vortexing (Vortex 2 IKA; Germany) in 5ml sterile physiological saline (8.5 g Nacl in a litre of distilled water) for 2 minutes and the wash was taken as body surface homogenate. After body surface washing, the cockroaches were soaked in 90% ethanol for 5 minutes to decontaminate their body surface and dried. This was followed by washing with sterile saline to remove traces of ethanol. The samples were then aseptically dissected using autoclaved-sterilized dissecting needles under a dissecting microscope (Model STM-82; India). The entomological dissecting kits (fisher scientific) used for dissecting the samples for examination, were inserted into a beaker containing ethanol, and flamed between dissections. The excised gut was homogenized in 5ml of sterilized normal saline water (Tachbele *et al*., 2006).

One millilitre of each homogenate was added to 9ml of physiological saline. Serial dilution (tenfold) was done on each of the suspensions from the cockroaches. These were used as inoculum. They were streaked and plated on Nutrient agar and MacConkey agar in triplicates and cultured. The inoculum from the body surfaces and guts of the samples were respectively cultured in plates which were designated as A – T for body surfaces, and a – t for guts for each culture medium. Isolated bacterial colonies were characterized using macroscopic morphology, gram staining, biochemical reactions, and sugar fermentation tests to identify the bacterial species (Chessbrough, 2006; Cappucino and Sherman, 2014). The tests were done following standard protocol (Garrity, 2001).

Enumeration of Isolates:

Pure isolates were observed, counted, and expressed in colony forming unit/milliliter (cfu/ml). The total microbial count (cfu/ml) for bacteria was recorded using the model outlined by Ukoroije and Bobmanuel (2019):

$$cfu/ml = N\,x\,D/A$$

Where:

N = Number of colonies counted.

D = Dilution factor (10^{-3}).

A = Aliquot (volume of sample used for inoculation).

Statistical Analysis:

Two-tailed paired student's t-test was used to compare the microbial count in the gut and body surface of cockroaches collected from the students' hostel and open field for each of Nutrient agar and MacConkey agar. The level of significance was pegged at 5% level of probability.

RESULTS

Of the 40 cockroaches sampled for this investigation, 32(80%) were found to harbour one or more species of bacteria on the body surface or in the gut. For the open field, 17 out of 20 carried bacterial species, and the corresponding figure for the students' hostel is 15 of 20 samples. While biochemical and sugar fermentation tests revealed the presence of 6 isolates which are *Proteus mirabilis, Bacillus* spp., *Enterobacter aerogenes, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli* on the body surfaces of samples retrieved from the open field, only 4: *S. aureus, P. aeruginosa, Bacillus* spp. and *E. coli* were isolated from their guts (Table 1). Corresponding isolates from the hostels were *P. aeruginosa, S. aureus, Bacillus* spp., *E. coli,* and *S. aureus, Bacillus* spp. and *E. coli*, respectively (Table **2**).

Table 1. Biochemical characteristics and identification of isolates retrieved from the open field in Federal University Wukari using Nutrient and MacConkey agar.

Plates[a,b]	Cell Shape	Biochemical test					Sugar fermentation test					Suspected organisms
		Gram stain	Catalase	Oxidase	Indole	Citrate	Glucose	Lactose	Sucrose	H_2S	Gas	
Body surface												
MCA Plate A	Rods	-	+	-	-	-	+	-	-	+	+	*Proteus mirabilis*
MCA Plate B	Rods	+	+	-	-	+	+	-	+	-	-	*Bacillus* spp.
MCA Plates C, D, F	Rods	-	+	-	-	+	+	+	+	+	+	*Enterobacter aerogenes*
MCA Plate E	Rods	-	+	+	-	+	-	-	-	-	+	*Pseudomonas aeruginosa*
MCA Plates G, L, M, N, O, P	Cocci	+	+	-	-	+	+	+	+	-	-	*Staphylococcus aureus*
NA Plate Q	Cocci	+	+	-	-	+	+	+	+	-	-	*Staphylococcus aureus*
Plates H, J	Rods	+	+	-	-	+	+	+	+	-	-	*Bacillus* spp.
Plates I, K, T	Rods	-	+	-	+	-	+	+	+	-	+	*Escherichia coli*
Gut												
NA Plates a, b, c	Cocci	+	+	-	-	+	+	+	+	-	-	*Staphylococcus aureus*
NA Plate d	Rods	-	+	+	-	+	-	-	-	-	+	*Pseudomonas aeruginosa*
NA Plates h, j	Rods	+	+	-	-	+	+	+	+	-	-	*Bacillus* spp.
MCA Plate k	Rods	-	+	-	+	-	+	+	+	-	+	*Escherichia coli*

MCA = MacConkey agar

NA = Nutrient agar

[a] = a cockroach sample may simultaneously contain bacterial species on the body surface.

[b] = a cockroach sample may simultaneously contain > 1 bacterial species.

Alphabets (A – T and a - t) = Codes used on the plate(s) for easy comprehension for isolates from body surfaces and guts, respectively.

Plates within alphabets A – T and a – t not listed did not have bacterial growth.

+ = Positive

- = Negative

Table 2. Biochemical characteristics and identification of isolates retrieved from hostels in Federal University Wukari using Nutrient and MacConkey agar.

Plates[a,b]	Cell shape	Biochemical test					Sugar fermentation test					Suspected organisms
		Gram stain	Catalase	Oxidase	Indole	Citrate	Glucose	Lactose	Sucrose	H_2S	Gas	
Body surface												
MCA Plates A, B, C, D	Rods	-	+	+	-	+	-	-	-	-	+	*Pseudomonas aeruginosa*
NA Plates E, M, N	Cocci	+	+	-	-	+	+	+	+	-	-	*Staphylococcus aureus*
NA Plates H, J	Rods	+	+	-	-	+	+	+	+	-	-	*Bacillus* spp.
MCA Plates K, P, Q	Rods	-	+	-	+	-	+	+	+	-	+	*Escherichia coli*
NA Plate O	Rods	-	+	-	+	-	+	+	+	-	+	*Escherichia coli*
Gut												
MCA Plates b, c, d	Cocci	+	+	-	-	+	+	+	+	-	-	*Staphylococcus aureus*
NA Plate l	Cocci	+	+	-	-	+	+	+	+	-	-	*Staphylococcus aureus*
MCA Plates h, j	Rods	+	+	-	-	+	+	+	+	-	-	*Bacillus* spp.
NA Plate k	Rods	+	+	-	-	+	+	+	+	-	-	*Bacillus* spp.
MCA Plates i, k	Rods	-	+	-	+	-	+	+	+	-	+	*Escherichia coli*
NA Plate o	Rods	-	+	-	+	-	+	+	+	-	+	*Escherichia coli*

MCA = MacConkey agar

NA = Nutrient agar

[a] = a cockroach sample may simultaneously contain bacterial species on the body surface.

[b] = a cockroach sample may simultaneously contain > 1 bacterial species.

Alphabets (A – T and a - t) = Codes used on the plate(s) for easy comprehension for isolates from body surfaces and guts, respectively.

Plates within alphabets A – T and a – t not listed did not have bacterial growth.

+ = Positive

- = Negative

Using Nutrient agar as a culture medium, Table 3**,** shows higher bacterial colonies in the guts of cockroach samples retrieved from the students' hostels in the University vis-à-vis those from the open field. However, the differences as detected by Student's t-test were due to random variation ($p>0.05$). On the other hand, Table 3 also showed higher and significant ($p < 0.05$) bacterial colonies on the body surface of samples retrieved from the open field vis-à-vis those on samples from the hostels. Results of bacterial isolates using MacConkey agar showed higher but insignificant ($p>0.05$) colonies from samples retrieved from students' hostel in comparison to those of open field for both gut and body surface (Table 4).

Table 3. Comparison of bacteria colony counts in the guts and body surfaces of cockroaches retrieved from students' hostel and open field in Federal University Wukari using Nutrient agar.

Variables	Mean colony count from samples from students' hostel	Mean colony count from samples from open field	Mean difference[1]	t-value	P-value
Gut					
Number of Colonies	159.80±32.44	143.35±18.97	16.45±37.58	0.438	0.667^{ns}
Population in cfu/ml	$1.5x10^{6}$±33435.80	$1.4x10^{5}$±20152.21	$1.6x10^{4}$±39039.26	0.418	0.681^{ns}
Body surface					
Number of Colonies	70.65±12.71	120.01±16.71	-49.36±20.99	-2.35	0.030^{*}
Population in cfu/ml	$6.9x10^{4}$±13388.88	$1.2x10^{6}$±16702.04	$-5.0x10^{4}$±21460.08	-2.37	0.029^{*}

[1] - Values indicates means (±) for students' hostel minus means (±) for open field
* = significantly different ($p\leq0.05$)
^{ns} = not significantly different ($P > 0.05$)
cfu – colony forming unit.

Table 4. Comparison of bacteria colony counts in the guts and body surfaces of cockroaches retrieved from students' hostel and open field in Federal University Wukari using MacConkey agar.

Variables	Mean colony count from samples from students' hostel	Mean colony count from samples from open field	Mean difference[1]	t-value	P-value
Gut					
Number of Colonies	75.45±13.12	60.25±7.81	15.20±15.72	0.996	0.333^{ns}
Population in cfu/ml	$9.2x10^{4}$±2312.34	$5.3x10^{4}$±7813.98	$3.8x10^{4}$±24473.31	1.571	0.134^{ns}
Body surface					
Number of Colonies	100.65±17.27	82.15±12.26	18.50±21.18	0.873	0.394^{ns}
Population in cfu/ml	$8.0x10^{4}$±19418.04	$7.4x10^{4}$±13966.14	$1.8x10^{4}$±23918.89	0.873	0.395^{ns}

[1] - Values indicates means (±) for students' hostel minus means (±) for open field
* = significantly different ($p\leq0.05$)
^{ns} = not significantly different ($P > 0.05$)
cfu – colony forming unit.

Staphylococcus aureus which is known to cause Pneumonia, Sepsis, Peritonitis, Impetigo, and other ailments is the most predominant isolate on samples from the hostels (47.83%) and open field (48.00%). This is followed by *E. coli* known to cause Bloody diarrhoea, Colitis, Pulmonary effusion, amidst others. Their corresponding frequencies (%) were 26.09% in the hostels' samples, and 20.00% in the open field samples (Table 5).

Table 5. Frequency of occurrence of Bacterial species isolated from the samples of cockroaches collected from the open field and hostels of Federal University Wukari

Suspected organisms	Frequency[a,b]	Percentage (%)	Some infectious diseases caused	References
Hostel				
Pseudomonas aeruginosa	4	17.39	Pneumonia, Bacteraemia, Endocarditis, Meningitis	Bodey *et al*. (1983); Renolds and Kollef (2021)
Escherichia coli	6	26.09	Bloody diarrhea, Colitis, Pulmonary effusion	Griffin and Tauxe (1991)
Bacillus spp.	2	8.69	Septicemia, Meningitis, Osteomyelitis, Endocarditis	Kotiranta *et al*. (2000)
Staphylococcus aureus	11	47.83	Pneumonia, Sepsis, Peritonitis, Impetigo, Arthritis, Osteomyelitis	Kim *et al.* (2014), Ghalehnoo (2018)
Open field				
Proteus mirabilis	1	4.00	Prostatitis, Urethritis, Cystitis	Ioannou and Vougiouklakis (2020)
Bacillus spp.	3	12.00	Septicemia, Meningitis, Osteomyelitis, Endocarditis	Kotiranta *et al*. (2000)
Escherichia coli	5	20.00	Bloody diarrhea, Colitis, Pulmonary effusion	Griffin and Tauxe (1991)
Enterobacter aerogenes	3	12.00	Urinary tract infection (UTI), Soft tissue infections, Respiratory infections, Osteomyelitis, Endocarditis	Davin-Regli and Pagès (2015)
Pseudomonas aeruginosa	1	4.00	Pneumonia, Bacteraemia, Endocarditis, Meningitis	Bodey *et al*. (1983); Renolds and Kollef (2021)
Staphylococcus aureus	12	48.00	Pneumonia, Sepsis, Peritonitis, Impetigo, Arthritis, Osteomyelitis	Kim *et al.* (2014), Ghalehnoo (2018)

a = a cockroach sample may simultaneously contain bacterial species on the body surface.
b = a cockroach sample may simultaneously contain > 1 bacterial species.

DISCUSSION

Many health problems of humans can be attributed to cockroaches. They are thus important public health pests in both rural and urban areas (Dokmaikaw, and Suntaravitun, 2019). The feeding mechanism and dirty breeding habits of cockroaches make them the most likely mechanical carriers for various human intestinal pathogens (fungi, bacteria, viruses, and parasites) (Graczyk *et al*., 2005; Kinfu and Erko, 2008).Various pathogenic microorganisms have been isolated from cockroaches, including bacteria such as *Enterobacter, Pseudomonas* spp. *Staphylococcus* spp. and *Enterococcus* spp. (Memona *et al*., 2017; Mehainaoui *et al*., 2021), viruses like Rotavirus and Enterovirus (Tetteh-Quarcoo *et al*., 2013; Du *et al.*, 2018), various fungi e.g., *Candida, Aspergillus*, *Rhizopus,* and *Mucor* (Salehzadeh *et al*., 2007; Kassiri *et al*., 2018), and parasites such as hookworm, *Cryptosporidium* spp., *Entamoeba histolytica,* and *Cyclospora* spp. (Tetteh-Quarcoo *et al*., 2013; Dokmaikaw and Suntaravitun, 2019; Donkor, 2020). Many of these are intestinal pathogens.

Seven bacterial species were macroscopically and biochemically identified in this study. Across the body surfaces and guts of the cockroach samples assessed, the predominant bacteria isolated were *S. aureus, E. coli, P. aeruginosa*, and *E. aerogenes*. This disagrees with the findings of Pai *et al*. (2005) whose study in homes in a Taiwan town noted *Enterobacter agglomerans*, *Citrobacter freundii*, *Hafnia alvei,* and *Enterobacter cloacae* as the most predominant bacterial species.

In this study, we further observed that; while *S. aureus* was the most predominantly isolated bacterial species from both locations followed by *E. coli*; the least were *P. mirabilis*, *Bacillus* species, and *P. aeruginosa.* This varies slightly with the findings of Jalil *et al*. (2023) whose study in Babylon, Iraq, found *Bacillus* spp. along with *Staphylococcus* spp. and *E. coli* as the most predominant bacterial species associated with *P. americana*. The slight variation noted may be due to differences in the area of study and the anthropogenic

behaviours therein. *Staphylococcus aureus*, *E. coli*, *E. aerogenes*, and *Bacillus* spp. are pathogenic bacteria which are known to cause serious diseases such as Gastroenteritis, Anthrax and Pneumonia (Ehling-Schulz *et al.*, 2019). *Proteus* spp., *P. aeruginosa*, *Enterobacter* species and some *Bacillus* species are opportunistic bacteria which can also cause serious harm to animal and human health (Berg *et al*., 2005).

Marginally higher bacterial isolates were observed on cockroaches retrieved from the open field (25 bacterial isolates) than from students' hostels (23 bacterial isolates). We also noted that the occurrence of bacterial isolates on the body surface of the cockroach samples was lower than those retrieved from the gut. This is at variance with the report of Makumana (2018) who isolated more bacterial species from the body surfaces of cockroaches than from their guts in Zimbabwe. The high microbial densities of bacterial species harboured in the gut and surfaces of the cockroaches are a public health risk as they increase the likelihood of transmission of infections.

Contrary to the report by Fotedar *et al.* (1991), which showed that the most frequently identified bacterial species from cockroaches were Gram-negative bacilli, in the family *Enterobacteriaceae*; we isolated 11 gram negative and 12 gram positive species. Hence, we observed widespread bacterial contamination of cockroaches collected from the surveyed locations as reported by Pai (2013) and Fakoorziba *et al*. (2014).

Conclusion

Despite increased awareness about the importance of sanitation, cockroaches that are known to be associated with filthy environments are still potential sources of bacterial food poisoning. This is indicated by the isolation of a number of pathogenic bacteria from the *P. americana* samples predominated by *S. aureus*, *E. coli* and *P. aeruginosa*. Not less than 80% of the cockroach samples were found to be contaminated by the pathogenic organisms in the sampling sites. Overall, higher bacterial colonies were documented from the guts than the body surfaces of the cockroach samples. It is thus imperative that the waste management system is strengthened by the agencies charged with the responsibility to so do. More so, optimum hygiene practices should be advocated for in the hostels, food canteens, open gardens, hospitals/clinics, classrooms, and offices in the University, and in the host community at large.

Declarations:

Ethical Approval: Not applicable.

Authors Contributions: Associate Professors Emmanuel Okrikata and Hakan Bozdoğan designed the experiments, recommended the methodologies used in the study and wrote the manuscript. Mr. Bon-Ekhn Luka collected the samples and executed the studies. Associate Professor Hakan Bozdoğan corrected the final manuscript. All authors read and approved the final manuscript.

Competing Interests: The authors declare that they have no competing interests.

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