

## A Study Of Bacteria Associated With Cuticle And Appendages Of American Cockroach(*Periplanetaamericana*).

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### Abstract

A study of bacteria associated with American cockroaches (*Periplanetaamericana*) was conducted with cockroaches caught within the Prince AbubakarAudu University, Anyigba, Kogi State, Nigeria. The cockroaches were trapped from seven different environments (Male hostel, Female hostel, Staff quarters, Cafeteria, School clinic, Laboratories and PublicToilets). The cockroaches were examined for bacteria attached to their cuticles and appendages. The wash diluent method of isolation was employed and inoculums were plated on Nutrient agar and MacConkey agar. Microscopic examination and standard biochemical tests were conducted on the isolates for presumptive identification. Eleven genera of bacteria were identified among the isolates. Gram positive bacteria isolated were *Bacillus* spp., *Staphylococcus* spp., *Micrococcus* spp., and *Lactobacillus* spp., while *Escherichia coli*, *Pseudomonas* spp., *Proteus* spp., *Salmonella* spp. *Enterobacter* spp., *Serratia* spp. and *Klebsiella* spp. were Gram negative bacteria isolated. Roaches collected directly from the public toilets had mean viable count of  $1.2 \times 10^9$  colony forming units, with the highest occurrence of *Escherichia coli* and *Salmonella* spp. (100% and 60% respectively). Although cockroaches trapped from the School clinic had lowest mean viable count of  $3.6 \times 10^7$  colony forming units, they were found to harbour bacteria of public health significance like *Pseudomonas* spp., *Salmonella* spp., *Klebsiella* spp. and *Staphylococcus* spp., on their external body. In conclusion, cockroaches play significant role in bacterial transmission from one environment to the other. They are risk factors in the transmission of pathogenic bacteria among human and animal populations. Strict hygienic practices must be constantly practiced as a means of reducing transmission of harmful bacteria by these vectors and also reducing the population of cockroaches in our habitations and immediate environment.

**Key Words:** Cockroaches, vector, Bacteria, Public, Health.

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### I. Introduction

Cockroaches are dorsoventrally flattened body insects that eat preferentially decayed vegetable substances. Among the best known pest species are the American cockroach, (*Periplanetaamericana*) which is about 30mm long, is the largest species of common cockroaches, and often considered as pest. They are cosmopolitan and among the most abundant notorious and important insect pests of premises found in toilets, prisons, food manufacturing sites and kitchens, stores, student hostels, households or residential areas, hospitals, restaurants, offices, laboratories etc. (Babaket *al.*, 2013). They have been found to harbour a number of pathogenic and potentially pathogenic bacteria which were carried either on the cuticle or in the gut (Cloarecand Rivault, 1992). As cockroaches engage in their nocturnal forages they shed off part of their wings, appendages and faecal pellets (Wooster and Ross, 1989). Numerous pathogens have been isolated from cockroaches these include fungi like; *Aspergillus* spp., *Candida* spp., *Penicillium* spp., and *Rhizopus* spp. etc. *Cyclosporacayentensis* oocyst, *Entamoebahistolytica* cysts, *Hammerschnidfiallas* spp. are also examples of protozoan parasites recovered from cockroaches. Bacteria like *Salmonella* spp., *Shigella* spp., *Campylobacter* spp., *Pseudomonas* spp., and *Klebsiella pneumoniae* have equally been isolated from roaches (Thyssen *et al.*, 2004).

The success of cockroaches as vectors of bacteria (both pathogenic and non- pathogenic ) is mainly due to their morphologic characteristics, such as locomotion appendices (legs and wings), exoskeleton, small body size and metamorphosis. as vectors , they contribute to dispersion of pathogens by carrying and transmitting them (Purcell and Almeida, 2005) and are known as intermediate or definitive hosts. Transmission between hosts

can occur indirectly by pathogen spread in the environment, normally through faeces and or secretions, and also due to physical contact, when pathogen is adhered to the surface of the vector body.

Sewage and landfills are major sources of pathogenic microorganisms and lack of investments in basic sanitation is a serious public health problem (Nagham et al., 2011). Most of this pathogenic transmission however, comes up as a result of cockroaches been basically omnivorous; they usually feed indiscriminately on garbage and sewage thereby having copious opportunity as vectors of diseases (Graczyk et al., 2005; Palet al., 2005). Their presence generally reduces the perception of human well-being and sanitary standards. Insects are motile and probably the most successful of all animals due to their morphology. They are able to travel quickly, often darting out of site when someone enters into a room, and can fit into small cracks and under doors despite their fairly large size. Insects are cosmopolitan found in different habitations of the world, and as such depend on wide varieties of plant and animals for their food (Beccaloni, 2014). Roaches are known to harbour parasites both externally on their appendages and internally within their gut (Park, 2002). Their presence generally reduces the perception of human well-being and sanitary standards. Insects are motile and probably the most successful of all animals due to their morphology. Studies have shown that exposure to cockroach antigens play an important role in asthma related health conditions (Koura and Kamel, 1990) aside the enteric pathogens of man transmitted by them. The controls of diseases are effectively carried out with the proper management and study of vectors (Salehzadeh et al., 2007). The land use and economy of Anyigbawas predominantly agrarian initially but is fast changing due to the transformation brought about by the economic landscape because of the State University. The level of sanitation in Anyigba is still poor coupled with indiscriminate refuse and sewage disposal in surrounding bushy environments and drains which serve as breeding sites for pests. This study was aimed at evaluating and determining the bacteria genera associated with the external body of *Periplaneta Americana* from different environments within the Prince Abubakar Audu University Anyigba, Kogi State, Nigeria.

## II. Materials and Methods

### Study Area

Anyigba is located in Dekina Local Government Area. It is situated between latitudes 7°15' N- 7°29' N and longitude 7°11' E-7°32' E, with an average altitude of 385m above sea level and total land mass area of 420 Sq.km<sup>2</sup> with an estimated population of 189,976 persons (Ifatimehin et al., 2009). It falls within the tropical wet and dry climatic region and the derived savannah. The annual mean rainfall and temperature are 1250mm and 25°C respectively. Prince Abubakar Audu University was established in 1999 and has a campus which comprises the administrative buildings, faculties (Lecture halls, laboratories and offices), students residential areas, cafeterias, school clinic, staff quarters, etc.).

### Collection of cockroaches

*P. americana* cockroaches were hunted in the night from seven different environments within the campus (Male Hostel, Female Hostel, Staff quarters, Cafeteria, School clinic, Laboratories and Toilets) and transported in sterile specimen bottles to the Laboratory. Ten cockroaches were collected from each of these environments. Cockroaches were identified as *Periplaneta americana* in the Biological Sciences department of Kogi State University, Anyigba (Fig.1).

### Isolation of Bacteria from cockroaches

Wash diluent method of isolation was employed with insect saline (Nagham et al., 2011).

Insect saline solution was prepared by the addition of 0.65g of Sodium Chloride (NaCl) into 100ml of distilled water. Insect saline (10ml) was added to each of the specimen bottle containing one cockroach each and thoroughly shaken for 2min to dislodge micro-organisms from the external surface of the cockroach (Fig. 2).

Sterile test-tubes were placed in test-tube racks for serial dilution. One ml of the solution containing the insect saline and the cockroach was transferred to 9ml of distilled water to make 10<sup>-1</sup> dilution. The process was repeated until a 10<sup>-10</sup> dilution factor was reached for each specimen (Fig. 3).



Figure1:*Periplaneta americana* caught in a kitchen on campus



*Periplaneta americana* collected in specimen bottles. Ten millilitres of insect saline added in the laboratory.

Figure 2:



Figure 3:Test-tubes containing 9ml distilled water and cockroach sample for ten fold dilution.

One ml of each dilution was inoculated into the medium using the pour plate method. The plates were incubated at 35°C for 24 hours. Number of bacteria associated with the external body of *Periplaneta americana* was expressed as colony forming units of bacteria per millilitre of diluent (CFU/ml = no of colonies x reciprocal of dilution factor).

### Presumptive Identification of bacteria

Observation of colonial characteristics was made according to the following criteria (Form, elevation, pigmentation, and size of the colonies). This was followed with Gram staining and Microscopic examination using the oil immersion objective of the binocular microscope. Colonies were subcultured on selective and differential media (Bouamama et al., 2007).

Standard Biochemical tests were done according to the methods of Hammad and Mahdy, 2012.

### III. Results

Results obtained in this study shows that *P. americana* collected from toilets had the highest mean count (1.2 x 10<sup>9</sup>) on the body. Cockroaches from the staff quarters and the male hostel followed with 7.8 x 10<sup>8</sup> and 6.9 x 10<sup>8</sup> CFU of bacteria respectively (Table 1). There was no significant difference in the colony forming units of bacteria based on the location (P > 0.05).

Mean viable count of bacteria on MacConkey agar was observed to be lower than that on Nutrient agar (a non-selective medium) in all the locations tested. The exception was in the colony forming units of bacteria from the school clinic in which case the colony forming unit of bacteria was more on MacConkey agar than on Nutrient agar (Table 1).

**Table 1: Mean colony forming units (CFU) of bacteria isolated from American cockroach trapped within the campus on Nutrient agar and MacConkey agar.**

Environment	Nutrient Agar	CFU/ml MacConkey agar
Male Hostel	4.7x10 <sup>8</sup>	3.5x10 <sup>8</sup>
Female Hostel	6.9x10 <sup>8</sup>	5.8x10 <sup>8</sup>
Staff Quarters	7.8x10 <sup>8</sup>	6.2x10 <sup>8</sup>
Cafeteria	5.8x 10 <sup>8</sup>	2.3x 10 <sup>7</sup>
School clinic	3.6x 10 <sup>7</sup>	4.4x 10 <sup>7</sup>
Laboratory	2.9x 10 <sup>7</sup>	2.1x 10 <sup>7</sup>
Public toilets	1.2 x10 <sup>9</sup>	9.8 x 10 <sup>8</sup>

Presumptive identification of bacteria isolated revealed the presence of seven genera of Gram negative bacteria (Table 3) and four genera of Gram positive bacteria (Table 4).

*Enterobacter* spp. (58.6%), *Bacillus* spp. (55.7%) and *E. coli* (47.1%) were the most frequently isolated bacteria. Though there was no significant difference between the three genera of bacteria most frequently isolated (p < 0.05), there was a significant difference between them and the other genera of bacteria isolated. The frequency of isolation of Gram negative bacteria from the toilets (Table 2) is significantly higher than that of Gram positive Bacteria (Table 3).

**Table 2: Frequency of isolation of Gram negative bacteria from American cockroach trapped within the campus.**

Bacteria	Environments								n=70	%
	MH	FH	SQ	CAF	SC	LAB	PT	total		
<i>E. coli</i>	5	4	4	3	4	3	10	33	47.1	
<i>Enterobacter</i> spp.	6	7	5	6	3	6	8	41	58.6	
<i>Salmonella</i> spp.*	1	2	3	2	4	0	6	18	25.7	

<i>Klebsiellaspp.</i>	3	4	2	3	3	3	2	20	28.6
<i>Pseudomonas spp.</i>	4	3	4	6	6	2	0	25	35.7
<i>Proteus spp.*</i>	3	1	2	2	2	0	5	15	21.4
<i>Serratia spp.</i>	1	3	4	3	0	1	0	12	17.1

**Key:**

MH- Male Hostel, CAF- Cafeteria PT – Public Toilets  
 FH- Female Hostel, SC- School Clinic  
 SQ- Staff Quarters, LAB- Laboratories  
 \*- some isolates biochemically different.

**Table 3: Frequency of isolation of Gram positive bacteria from American cockroach trapped within the campus**

Bacteria	Environments							n=70	%
	MH	FH	SQ	CAF	SC	LAB	PT		
<i>Bacillus spp. **</i>	5	4	3	9	6	10	2	39	55.7
<i>Lactobacillus spp.</i>	2	2	4	6	0	0	3	17	24.3
<i>Staphylococcus spp.</i>	4	3	3	1	2	4	1	18	25.7
<i>Micrococcuspp</i>	2	2	4	4	1	4	0	17	24.3

**Key:**

MH- Male Hostel, CAF- Cafeteria PT – Public Toilets  
 FH- Female Hostel, SC- School Clinic  
 SQ- Staff Quarters, LAB- Laboratories  
 \*\*- some isolates morphologically different

**IV. Discussion**

The number and types of bacteria isolated in this study is consistent with the reports of other studies (Paulet *al.*, 1992; Mpuchaneet *al.*, 2006; Hammad and Mahdy, 2012) *P. americanamove* freely from areas, within and around our environments and they harbour bacteria.

In houses and places with poor hygiene, heavy infestations of pests such as *P. americana* can occur. The presence of cockroaches has severe health implications in the transmission of nosocomial infections. In a study carried out at the medical centres of Khorramshahr county in Iran by Hamid and Shahnaz (2012), pathogenic bacteria were isolated from the external surfaces of all the American cockroaches; the following bacteria pathogens were recovered; *Klebsiellaspp.* (47.90%), *Pseudomonas spp.* (37.00%), *Escherichia coli* (30.10%), *Staphylococcus spp.* (24.60%), *Enterobacter spp.* (19.20%), *Streptococcus spp.* (15.10%), *Serriata spp.* (8.2%), *Bacillus spp.* (4.1%) and *Proteus spp.* (2.7%). The result of this study showed that bacteria isolated from roaches in Prince AbubakarAudu University Anyigba environs were all present in Khorramshahr. The types of bacteria isolated from the cafeteria are consistent with those isolated by Mpuchaneet *al.*, 2006 in kitchens in Botswana.

Some bacteria isolated from cockroaches in food vending environments as recorded in this study may cause food borne illnesses (*Bacillus spp.*, *Staphylococcuspp.*, *Escherichia coli*, *Salmonella spp.*) while others may cause food spoilage (*Pseudomonas spp.*, *Bacillus spp.* and *Enterobacterspp.*)

Bacteria on cockroaches in the laboratory are very serious risk factors in the control of laboratory contaminants. The bacterial load of cockroaches from the laboratory may be an indication of improper handling of bacterial cultures or may have been carried on the cockroaches from another site. Poor sanitation provides ideal conditions for the breeding and multiplication of these pests that have been proven to disseminate bacteria.

Environmental sanitation should be enforced and continuous awareness created on benefits of good waste disposal. Restaurants, kitchens and food vending environments where food items are kept and served should always be kept clean and tidy. Doors, windows and screens must be properly fitted to avoid the entry of insects that are regarded as biological vectors in the transmission of bacteria.

## References

- [1]. Babak, V., Rouhollah, D., Manijeh, M., Mona, S., Nersi, N. (2014). First report of drug resistant bacteria isolated from the Brown-banded cockroach, *Supellalongipalpa* in Ahvaz, south-western Iran. *J. Arthropod-Borne Dis.*, 8(1):53-59
- [2]. Bouamama, L., Lebbadi, M., and Aarab, A. (2007) Bacteriological analysis of *Periplaneta americana* L. (Diptera; Blattellidae) and *Muscadomestica* L. (Diptera: Muscidae) in ten districts of Tangier, Morocco. *African Journal of Biotechnology* 6(17):2038-2042
- [3]. Cloarec, A. and Rivault, C. F. (1992). Cockroaches as carriers of bacteria in multi-family dwellings. *Epidemiol. Infect.*, 109:483
- [4]. Graczyk, T. K., Knight, R., Tamang, L. (2005). Mechanical transmission of human protozoan parasites by insects. *Clin. Microbiol. Rev.* 128-132
- [5]. Hamid, K., Shahnaz, K. (2012). Cockroaches *Periplaneta americana* as carriers of bacteria pathogens, Khorramshahr county, Iran. *Jundishapur. Microbiol.*; 5(1):320-322.
- [6]. Ifatimehin, O. O., Musa, S. D. & Adeyemi, J. O. (2009). An Analysis of the Changing Land use and its Impact on the Environment of Anyigba. *Journal of Sustainable Development in Africa*. 10 (4): 357-364.
- [7]. Mpuchane, S., Matsheka, Gashe, B.A., Allotey, J., M Murindamombe, and Mrema, N. (2006). Microbiological studies of cockroaches from three locations in Gaborone, Botswana. *African Journal of Food Agriculture Nutrition and Development* 6(2) 1-17
- [8]. Nagham, Y.A., Anfal, S.A., Israa, K.A. (2011). Risks associated with cockroaches *Periplaneta americana* as transmitter of pathogenic agents. *Epidemiol infect.* 111:264-293.
- [9]. Pai, H. H., Chen, W. C., Peng, C. F. (2005). Isolation of bacteria with antibiotic resistance from household cockroaches (*Periplaneta americana* and *Blattella germanica*). *Acta Trop.* 93(3):259-265
- [10]. Paul, S. Khan, A.M., Baqui, M.A and Muhibullah, M. (1992) Evaluation of the common cockroach *Periplaneta americana* (L) as carriers of medically important bacteria. *Journal of communicable diseases* .24:206-210
- [11]. Park, S.F. (2002). The physiology of campylobacter species and its relevance to their role as foodborne pathogens. *Int. J. Food microbial.* 74: 177-188.
- [12]. Salehzadeh, A., Tavacol, P., Mahjub, H. 2007. Bacterial, Fungal and parasitic contamination of cockroaches in public hospitals of Hamadan, Iran. *J. vect. Borne. Dis.* 44:105-110.
- [13]. Thyseen, P. J., Moretti, T., Ribeiro, O. B. (2004). The role of insects (Blattodea, Diptera and Hymenoptera) as possible mechanical vectors of helminths in the domiciliary and peridomestic environment. *Cad. Saude. Publica.*, 20: 1096-1102
- [14]. Wooster, Y. and, Ross (1989). Studies of distribution and habitat of cockroaches in southwest Georgia. *Am. J. Trop. Med. Hyg.* 4:1131-1134

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