Cross Current International Journal of Medical and Biosciences
Abbreviated Key Title: Cross Current Int J Med Biosci
ISSN: 2663-2446 (Print) & Open Access

OPEN ACCESS

Volume-1 | Issue-6 | Nov-Dec 2019 |

Research Article

Bacteria Associated With the American Cockroach Periplanata Americana (Dictyoptera: Blatellidae) In Amassoma, Bayelsa State, Nigeria

Ukoroije, Rosemary Boate[1*] and Bobmanuel Rosetta Bekinwari[2]

[1]Biological Sciences department, Niger Delta University Wilberforce Island, P. M. B. 071, Bayelsa State, Nigeria
[2]Biology department, Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt. P. M. B. 5047, Rivers State, Nigeria

***Corresponding author:**
Ukoroije, Rosemary Boate

Received: 11.11.2019
Accepted: 18.11.2019
Published: 28.12.2019

Abstract: Cockroaches have become the most common peridomestic insect pest of public health and epidemiological importance. The presence raises safety concerns, especially as carriers of food-borne pathogens and food-spoilage organisms. Using a swab bacteriological technique, investigations were carried out on the wings, legs and mouth parts of cockroaches trapped from sewers, kitchens and bathrooms from household in Amassoma, Bayelsa State. The density of microorganisms by standard plate count was used to analyze the samples. Microbial load gotten were 62 x 10^3 – 76 x 10^3 cfu/ml^{-1} for wings, 45 x 10^3 –53 x 10^3 cfu/ml^{-1}for legs and 36 x 10^3- 41 x10^3 cfu/ml^{-1} for mouthparts respectively regarding heterotrophic bacterial count and 59 x 10^3 - 62 x 10^3 cfu/ml^{-1}, 41 x 10^3 - 53 x 10^3 cfu/ml^{-1} and 36 x 10^3 - 43 x 10^3 cfu/ml^{-1} for fecal coliform count respectively. Five bacteria genera of the family Enterobacteriaceae, known as Opportunistic pathogens and responsible for food spoilage were identified such as *Escherichia coli* (33.3%) most frequently isolated, *Citrobacter* specie (16.7%), *Enterobacter* specie (16.7%) while Pseudomonas specie (22.2%) and Klebseilla specie (11.1%) were the least isolated. Highest bacteria count was recorded from the wings followed by the legs and the mouth. Cockroaches can readily move from contaminated zones such as faeces to food preparation areas spreading food spoilage and disease-causing organisms onto the food. Proper care in disposal of food remnants and overall cleanliness at the households prevent cockroaches from foraging in the kitchen, bedroom and toilet.
Keywords: Cockroach, Bacteria, Microorganisms, *Escherichia coli*, Pathogens.

INTRODUCTION

Cockroaches (*Periplaneta americana*) have become a significant domestic pest that are not only repugnant because of their association with dirt, but because of their possible health risks in spreading diseases, causing allergies, tainting food odours and contaminating food and food processing environments. Increased infestation of the American cockroach in buildings has increased with urbanization. Poor management of urban refuse has been linked with the increase in the population of cockroaches in urban areas (Bonnefoy *et al.*, 2008). Cockroaches have even flourished in the streets where foods are vended. Cockroaches have also been isolated from various environments including hospitals, food industries and landfill sites (Jeffery *et al.*, 2012). They are also common pests in bakeries, food processing facilities and kitchens (Adler *et al.*, 2002).

While the causal relationship between cockroaches and disease still needs to be established, they also pose danger in the dairy industry since they carry microorganisms including Salmonella specie (Gashe and Mpuchane, 2000). In another study, 98.5 %

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DOI: 10.36344/ccijmb.2019.v01i06.001

of cockroaches from hospitals and residences were carriers of microorganisms and involved in the aetiology of nosocomial infections (Branscone, 2002). At the household level, a relationship has been established between cockroach infestation and standards of hygiene. Various studies have revealed that cockroaches aggregate in corners in kitchens, especially around the refrigerators and in the bathrooms around chests, around plumbing connections within or between rooms and/or flats (Jeffery *et al*., 2012). As cockroaches (nymphs and adults) are engaged in their nocturnal forages, they drop off shed skins and fecal pellets. Most of the Gram positive bacteria isolated from the cuticle were coagulase negative Staphylococci (Tatfeng *et al*., 2005; Prado *et al*., 2002). It is possible that the antimicrobial agents that are present in the secretions produced by the male accessory glands may have a role in selecting against certain types of bacteria (Gillio, 2003).

Cockroaches are possible vectors of pathogenic bacteria in hospital environments (Gliniewicz *et al*., 2003). Up to 54 % of isolates from hospital environments were found to be human pathogens (Cotton *et al.,* 2003). More than 33.3 % of cockroach isolates were resistant to more than three antimicrobials. Resistance covered a large diversity of microbes including Salmonella, (Gashe and Mpuchane, 2000), enterobacteria and coagulase negative staphylococci (Prado *et al*., 2002). There has been great concern about cockroaches carrying and spreading microorganisms as they forage in the houses, at the same time on to foodstuffs and other working areas in the kitchen, which could result in allergic reactions from consumers. The current study was carried out to determine the microbial load and diversity associated with cockroaches' wings, legs and mouth part.

1.1 Statement of Problem

Cockroaches are omnivorous scavengers and consume any organic food source available to them. Although they prefer sweets, meats and starches, they are also known to consume other items such as books and decaying matter. Cockroaches feed on human excreta as well as human food and as such at least twenty two species of bacteria, viruses, fungi and protozoa have been isolated and experimentally confirmed from cockroaches as well as five species of helminthes worms and intestinal diseases such as diarrhea, dysentery, typhoid fever and cholera, causing public health problems (Tatfeng, *et al*., 2005; Ghosh and Gayen, 2006; Bouamama, *et al*., 2007). Therefore, this study is aimed at finding the bacterial load and diversity associated with cockroaches' wings, legs and mouth part.

2.0 MATERIALS AND METHODS

2.1 Sample Collection

A total of 120 adult cockroaches were manually handpicked using hand gloves and sterile entomological forceps from sewers, kitchen (cabinets and sinks), toilets and bathrooms into a sterile container from the girl's hostel of Niger Delta University (Hostel F and CHS 1 wing C). Ninety (90) Cockroaches were used for the study. Cockroaches were fed on bread and bread crumbs and were taken into Microbiology Laboratory, Niger Delta University for the bioassays.

2.2 Sampling Method

The bacteriology enumeration method used was swab technique in order to determine surface-adhering bacteria. The legs, wings and mouth parts each from the sample cockroach were swabbed and the swab stick and its contents were deep into prepared peptone water for six hours before serially diluted following aseptic techniques. This was then shaken vigorously by hand before appropriate aliquots were transferred into diluents. Further dilutions were made as deemed necessary. Nutrient agar was used for enumerating aerobic mesophilic bacteria; MacConkey agar was used for enumeration of fecal coliforms.

2.2.1 Culture Method

Adopting the procedure of Cheesbrough, (2006) 1-ml serially diluted peptone solution from 10^{-3} dilution factor, each from the swab sample (wings, legs and mouth part), was platted in triplicate onto the prepared agar, after which it was incubated at 37°C for 24hrs. Eighteen (18) different pure isolates, 9 from samples treated with nutrient agar and MacConkey agar were randomly selected based on their morphology and colors, in the ratio of 3:3:3 and subculture into a prepared nutrient Agar (10.08g into 360 ml of distilled water and autoclaved).

2.2.2 Enumeration Of Isolates

Pure isolates were observed, counted and expressed in cfu/ml. Total microbial count (cfu/ml^{-1}) for bacteria was recorded using the formula Cfu/ml = (N/A) x (1/D). Where, N = number of colonies counted, A = aliquot (volume of sample use for inoculation) and D = dilution factor (10^{-3}).

2.2.3 Characterization of Isolates

Isolates were characterized base on morphology identification (where the isolates were carefully identified and compared with the use of hand lens for certain colour consistency and shape), microscopic appearance (Gram staining technique where positive bacteria retain the purple color of the stain, while gram negative bacteria turn pink coloration with rod like shape) and biochemical characteristic such as Kligler iron agar test to indicate lactose and glucose fermentation and hydrogen sulfide gas production; Catalase test used to isolate several colonies from a pure isolate used to pick out several colonies from a pure isolate and Indole test to examine coloration of isolates on the surface layer Cheesbrough, (2006).

2.2.4 Motility Test

The test insect was placed on a cover slip and a drop of water/Vaseline was added on each corner of the cover slip. The slide was inverted such that the central of it depress over the cover slip to enable the cover slip stick on the slide so that when the slide is inverted the drop of bacterial culture would be suspended on the central depression of the slide. The slide was then subjected to the microscope with x100 objective power to examine motility of the organism Cheesbrough, (2006).

2.3 Statistical Analysis

The collected data was analyzed statistically using one way ANOVA (Analysis of Variance). Duncan's Multiple Range Test was used to determine significant difference between treatments at alpha level $P<0.05$.

3. RESULT

The high prevalence of bacteria harbored in the body surface of the cockroaches is of public health risk, increasing the likelihood of transmission of several infections. Indeed, cockroaches captured in homes or other locals, habitually contain large number of micro-organisms in general and bacteria specifically. Household foods are prone to contamination with food borne bacteria associated with cockroaches' resulting to public health hazard especially regarding the synanthropic nature of cockroaches and the habitual visits to toilets, cesspools, soak away, bathrooms drainages and dustbins, thus picking up bacteria and other pathogenic organisms on their legs, wings and in their mouth parts and even guts which they deposit onto the food of humans Craczyk *et al*., (2005). In this study, bacteria isolated from the cockroach samples were *Escherichia coli, Psuedomonas specie, Klebsiella specie, Enterobacter specie and Citrobacter specie.*

Represented on table 1 is the bacteria load from the three swab locations of the cockroach. The highest bacteria count was recorded from the wings, followed by the legs and the mouth part which ranged from 62×10^3 - 76×10^3 cfu/ml^{-1} for wings, 45×10^3 - 53×10^3 cfu/ml for legs and 36×10^3 - 41×10^3 cfu/ml^{-1} for mouthparts respectively for heterotrophic bacterial count. Regarding fecal Coliform count the bacterial load were 59×10^3 - 62×10^3 cfu/ml^{-1} for wings, 41×10^3 - 53×10^3 cfu/ml^{-1} for legs and 36×10^3 - 43×10^3 cfu/ml^{-1} for mouthpart respectively.

Table 1: Bacterial count in cfu/ml for *P. americana* wings, legs and mouth parts inoculated in Nutrient and MacConkey agar for their total heterotrophic and fecal Coliform count.

Sample replicate	Nutrient Agar (cfu/ml)			MacConkey Agar (cfu/ml)		
	Wings	Legs	Mouth part	Wings	Legs	Mouth part
R1	$63x10^3$	$53x10^3$	$36x10^3$	$57x10^3$	$41x10^3$	$43x10^3$
R2	$76x10^3$	$51x10^3$	$41x10^3$	$62x10^3$	$45x10^3$	$43x10^3$
R3	$62x10^3$	$45x10^3$	$39x10^3$	$59x10^3$	$53x10^3$	$39x10^3$
Mn±S.E	$67.0±7.8^c$	$48.0±3.0^a$	$38.7±2.5^a$	$59.3±2.5^b$	$46.3±6.1^b$	$37.7±6.1^b$

Different superscripts letters (a, b and c) indicate significant difference among treatments at alpha level p<0.05.

A total of five bacteria isolates were identified to their species as shown on table 2. *Escherichia coli,* (33.3%) which was the most frequently isolated, followed by *Citrobacter specie* (16.7%) and *Enterobacter specie* (16.7%). While *Pseudomonas specie* (22.2%) and *Klebseilla specie* (11.1%) had the least isolated frequency. There was significant difference ($p>0.5$) in *Escherichia coli, Pseudomonas specie, Enterobacter specie, Klebsiella specie and Citrobacter specie* count across *P. americana* wings, legs and mouthparts.

Table 2: Frequency and percentage frequency of the identified Bacteria from *P. americana* wings, legs and mouth part

Identified bacterial species	wings	legs	Mouth part	Frequency (f)	% Frequency
E. coli	20	20	20	60	33.3%
Citrobacter	10	10	10	30	16.7%
Enterobacter	-	10	20	30	16.7%
Pseudomonas	20	10	10	40	22.2%
Klebseilla	10	10	-	20	11.1%

The bacteria isolate appeared at different frequencies in the samples location. Some of them appeared twice and others appeared only once. All the five bacteria species; *Escherichia coli, Psuedomonas specie, Klebsiella specie, Enterobacter specie, and Citrobacter specie* identified were all associated with the legs. *Escherichia coli,* was present in all the sample parts of the cockroaches as shown on fig. 1 below.

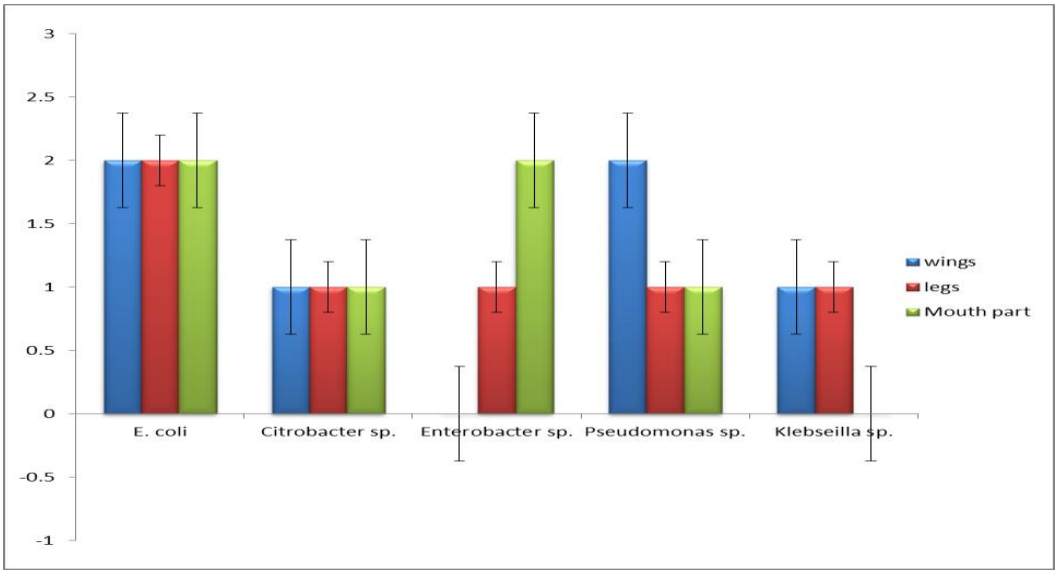

Fig. 1: Bar chart showing the identified bacteria and their frequencies of occurrence

Source: AuthorsThere was significant difference (p>0.5) in the identified bacteria species count and their frequencies of occurrence: *Escherichia coli, Pseudomonas specie, Enterobacter specie, Klebsiella specie and Citrobacter specie* across *P. americana* wings, legs and mouthparts.

4. DISCUSSION

The microbial association of cockroaches is much greater than generally realized as they have been shown to carry diverse pathogenic and non-pathogenic bacterial flora, different protozoa, pathogenic helminthes, fungi, and viruses Bennett 2008; Marriott and Gravani 2006; Tatfeng *et al*., 2005). Cockroaches feed on human excreta as well as human food, thus are potential transmitters of diseases such as dysentery, typhoid, cholera and other food-borne infections which have been experimentally confirmed (Tatfeng *et al*., 2005; Ghosh & Gayen, 2006; Bouamama *et al*., 2007). It was reported that 98% of cockroaches found in medical facilities could carry pathogens on their integuments or digestive tracts (Cloarec *et al*., 1992). Indeed, many potential pathogens such as *Escherichia coli*, *Enterobacter cloacae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Klebsiella* specie, *Salmonella typhi* and *Shigella dysenteriae* were isolated from cockroaches collected in hospitals (Tachebe *et al*., 2006; Salehzadeh *et al*., 2007).

The survey of surface bacterial loads of the cockroaches trapped from sewers, kitchen (cabinets and sinks) and bathrooms revealed mean total counts (Mn±S.E) of 67.0±7.8, 48.0±3.0, 38.7±2.5 for heterotrophic bacterial count, 59.3±2.5, 46.3±6.1, 37.7±6.1 for fecal coliform count, from the three swab locations (wings, legs and mouth part) ranging respectively. Higher bacteria count was recorded from the wing, then the legs, and the least was recorded from their mouth parts. There was a significant difference (P>0.05) among the wings, legs and mouthparts of the cockroaches from which swab was taken.

In this study, 18 bacterial isolates of 5 bacterial species. The predominant bacteria isolated from the captured cockroaches were *Escherichia coli, Pseudomonas specie, Enterobacter specie, Klebseilla specie and Citrobacter specie* across the cockroach wings, legs and mouthparts. In contrast, the most common bacteria species frequently isolated from the three swab regions of the cockroaches was *Escherichia coli,* (33.3%) followed by *Citrobacter specie* (16.7%) and *Enterobacter specie* (16.7%), *Pseudomonas specie* (22.2%) and *Klebseilla specie* (11.1%) was the least frequently isolated. There was significant difference (p>0.5) in *Escherichia coli, Pseudomonas specie, Enterobacter specie, Klebseilla specie and Citrobacter specie* count among sample locations. This report is in conformity with that of Chaichunawongsaroj *et al*., (2004), Tenaillon *et al*., (2010) who stated that the occurrence of *E. coli* bacteria and other gram negative bacteria in cockroaches was very highly present in cockroaches trapped from urban environments.

The high prevalence of bacteria harboured in the body and surfaces of the cockroaches is a public health risk, increasing the likelihood of transmission of infections. The present results showed widespread bacterial contamination of cockroaches collected from the surveyed locations. Indeed, cockroaches captured in homes, offices, hospitals, or other locals habitually contain a large number of micro-organisms (Fu *et al*., 2009; Bouamama *et al*., 2010). In hospital environments, cockroaches could be efficient carriers of nosocomial infections through dispersal and spread of pathogenic agents, especially to patients in intensive care, neonatal units, long-term care facilities, and nursing homes (Fakoorziba *et al*., 2010; Pai 2012).

Several authors reported that cockroaches collected from hospitals have more bacterial counts

than cockroaches found in residential areas due to their permanent contact with infested sites. Hospital environments may be more conducive to accruing bacteria from many different contaminated sources such as water and food causing high rates of bacterial prevalence. Multidrug-resistant bacterial strains of medical importance have also been isolated from cockroaches in different hospitals and urban environments (Oliva *et al*., 2010; Pai *et al*., 2004; Salehzadeh *et al*., 2007).

It is known that sanitation-improved sites carry less pathogens and synanthropic organisms (Marriott and Gravani 2006; Carling and Bartley 2010). The present results showed that there was a significant difference in bacterial abundance in percentage or in species richness between the different outer surfaces swabs obtained.

Cockroaches use their mouthparts and legs for grooming thus increasing the likelihood of direct contact with contaminated surfaces. Cockroaches can readily move from contaminated zones (garbage) and create the opportunity to spread disease-causing organisms on food and food preparation areas. Many studies have highlighted a possible and potential risk of human contamination through bacteria carried by cockroaches in connection with human habitats (Oliva *et al*., 2010; Pai *et al*., 2004). Moreover, association of cockroach with the human environment can cause direct food contamination and several health problems such as allergic responses (skin rashes, watery eyes, and sneezing) particularly in patients who have lung disease such as asthma (Chew *et al.,* 2006; Safari *et al*., 2009).

5. CONCLUSION

Control and preventive measure of cockroaches is the key to eliminating contamination of food by micro-organisms such as bacteria associated with cockroaches. The use of an integrated pest management system that incorporates cultural methods, safe hygiene/good sanitation and the use of biopesticides is a positive measure aimed at reducing infestation of pathogen spread by cockroaches that cannot be overemphasized.

ACKNOWLEDGEMENT

The authors are grateful to Dr Rosemary B. Ukoroije for supervision and compilation, Mr. Richard Otami Abalis for the microbial and statistical analyses and Dr Rosetta B. Bobmanuel for editing and vetting of this work. Your efforts are well appreciated and won't be forgotten.

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