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Evaluation of German Cockroach, *Blattella germanica* from Different Niches for Bacterial Contamination

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Abstract: Cohabitating nature of cockroach with humans is on the increase in most rural and underdeveloped areas of Cross River State including Calabar South. It is therefore important to screen the bacterial carriage of this pest because of shared ecological niche. Sticky trap method was used to capture the pest and properly identify by an Entomologist Dr Ogban, Cross River University of Technology, Calabar. They were killed using chloroform. Pour plating culturing technique was used for bacterial isolation, isolates were characterized and identified using standard bacteriological methods. The research result reveals eleven (11) bacterial species (*Bacillus, Klebsiella, E. coli, P. aeruginosa, S. aureus, Proteus, S. epidermidis, Streptococcus, Salmonella, Shigella* and *Enterococcus*). *E. coli Klebsiella* and *Bacillus* were the most frequently isolated organisms from the external surface of latrines and living house cockroaches. *Klebsiella, E. coli* and *Enterococcus* species were predominant in the gastrointestinal tract (GIT) of cockroaches from same ecological niche except that in living houses, the presence of *Enterococcus* species were insignificance compare to other isolates. There was a significant difference at $P = .05$ in isolates from cockroaches captured from latrines area to those from living houses. The degree of contamination based on sum total of each isolate from External and GIT parts of cockroaches from both experimental niche was; *E. coli* > *Klebsiella* > *S. aureus* > *Bacillus* > *Shigella* > *P. aeruginosa* = *Samonella* = *Enterococcus* > *Streptococcus* > *S. epidermidis* > *Proteus*. This study encourages the use of some natural and home friendly cockroach eradication techniques that pose no harm to home dwellers and it environ. This include timely removal of bushes around the living environ, frequent sealing of cracks around the kitchens, and bathrooms to reduce potential hidings areas. Getting rids of all foods remains on kitchen tables, floor etc. Application of boil mint leaves at different corners of the house. These will naturally scare the presence of this pest from human habitation and serve as a better strategy to minimize cockroach-associated infections.

Keywords: Cockroach, External Body Surface, Gastrointestinal Tract (GIT), Latrines, Living Houses, *E. coli*, Klebsiella and Bacillus Species

1. Introduction

There are over 4,600 known species of cockroach of which 30 are associated with human habitations. Their apparent ubiquity stems from their special ability to tolerate a wide range of environmental conditions ranging from cold to tropical heat [1]. Some of the most common species include German cockroach (B. germanica), American cockroach (Periplaneta americana), brown-banded cockroaches (Supella longipalpa) and Oriental cockroaches (Blatta orientalis). B. germanica is the most important and most common species of cockroach found everywhere in the world [2]. They have special preference for starchy, sugary and fermented foods. They sometimes feed on dead or wounded cockroaches of their own or other species. Cockroaches are commonly found around hospitals, sick rooms, areas of intensive care, surgical sections, living rooms, toilet areas etc. and are presumed to play a pivotal role in diseases transmission and are, therefore, threat to public health [3]. They are presumed to harbor pathogenic organisms such as Bacillus, *Salmonella*, *Escherichia coli*, *Listeria monocytogenes*, Enterococcus faecalis, Aspergillus niger, although the distributions of these

organisms to disease causation differ depending on location, environmental and sanitary conditions [4]. Hence, this research reports the evaluation of B. germanica samples trapped from different ecological niches in Calabar South Local Government Area of Cross River State for bacterial contamination.

2. Materials and Methods

2.1. Study Area

The study was carried out between March and April, 2019 in Calabar South, Cross River State. The area was chosen because of the low sanitary condition such as unkempt bushes, heaps of refuse etc. serving as a conducive environment for proliferation of different insects such as housefly, blackfly, cockroaches among others. Some houses in this area are still making use of pit latrines, few houses that make use of water closet are unable to keep them in good condition because of non-availability or irregular supply of water. This area has mean annual rain fall of 1830mm and two distinct seasons; the rainy season (April-September) and dry season (October-March).

2.2. Sample Collection

Altogether, 80 adult cockroaches were randomly trapped at night using stick trap, 40 from toilets and 40 from living houses. Each sample was placed in a universal bottle and transported immediately to Microbiology Laboratory, Cross River University of Technology for analysis. Trapped samples (Cockroaches) were killed using chloroform and examined under a dissecting microscope for affirmation of identity using standard taxonomy keys.

2.3. Bacterial Cell Count, Isolation and Characterization of Bacterial Species

Each cockroach was taken with sterile forceps and put into 10 mL of sterile saline in a universal bottle. After closing the bottle with a screw cap, it was vortexed several times to wash free the microbial contaminants on the external surface. Approximately 1.0 mL of the wash-saline was added to 9.0 mL of sterile saline to get a 10^{-1} dilution. This was serially diluted ten-fold down to 10^{-10}. A 1.0 mL inoculum was taken and plated simultaneously in nutrient agar (NA) and MacConkey agar (MA) respectively. After incubation for 24 hours in a humidified incubator, the plates were examined and the bacterial colonies counted. Subsequently, plates with well separated colonies were taken and discrete colonies isolated. Each colony was purified by streaking and re-isolating three successive times in agar. Purified colonies were then characterised using standard bacteriological techniques. This technique was used to determine the contamination of the external surfaces. Thereafter, the cockroach was placed in 70% ethanol to decontaminate the external surface. Then it was rinsed in distilled water and allowed to dry at room temperature. The cockroach was aseptically dissected to remove the gastrointestinal tract (GIT) using sterile forceps, scalpels and scissors. The contamination of the GIT was done by homogenizing the gut and other abdominal organs in a sintered glass tissue grinder. A 1.0g amount of the homogenised tissue was suspended in 9.0 mL of saline and the suspension serially diluted ten-fold. Each dilution was plated simultaneously in NA and MA as earlier described**.**

2.4. Data Analysis

SPSS version 20 was used for descriptive statistics. The student Unpaired T-test compared the means value of isolates from different parts of the sample. ANNOVA compared the significant difference at P = .05 between isolates from different ecological niche.

3. Results

Table 1. *Bacterial Isolates Identified from External and GIT of B. germanica.*

Bacterial Isolate	External Surface		GIT	
	Latrines	L. Room	Latrines	L. Room
Bacillus Sp	5	4	3	1
Klebsiella Sp	7	6	9	5
E. coli Sp	8	7	7	6
P. aeruginosa	4	3	1	0
S. aureus	3	3	4	4
Proteus Sp	1	1	0	0
S. epidermidis	1	2	0	0
Streptococcus Sp	1	2	1	1
Salmonella Sp	2	2	2	2
Shigella Sp	1	2	2	4
Enterococcus Sp	0	0	5	3
Total	33	32	34	26

E. coli = Escherichia coli, P. aeruginosa = Pseudomonas aeruginosa, S. aureus = Staphylococcus aureus, S. epidermidis = Staphylococcus epidermidis, GIT = Gastro-intestinal tract, L. Room = Living Room

Evaluation of external and GIT of cockroaches captured from latrines and living houses reveal 11 genera of bacteria identified as Bacillus, Klebsiella, E. coli, P. aeruginosa, S. aureus, Proteus, S. epidermidis, Streptococcus, Salmonella, Shigella and Enterococcus species respectively.

With reference to cockroaches caught from latrines, E. coli species was the most frequently isolated flora, isolated 8 times out of 33 samples. This was followed by Klebsiella, Bacillus, Pseudomonas, S. aureus, Salmonella, Proteus, S. epidermidis, Streptococcus, Shigella species with the respective rates of isolation shown in Table 1. Of the 32 cockroach samples caught in the living houses, 7 yielded E coli and Klebsiella (6), Bacillus (4), Pseudomonas and Staphylococcus (3 each); S. epidermidis, Streptococcus, Salmonella and Shigella (2, each); and Proteus species (1) (Table 1). There was a significant difference at P = .05 in isolates caught from latrines area to those from living houses.

3.1. Bacterial Contaminants from External Surface of Cockroaches Caught from Latrines and Living Houses

The external surface of cockroaches from latrines yielded *E. coli* as the highest isolated organism found in 8

samples (24%). This was followed by *Klebsiella, Bacillus, P aeruginosa and Staphylococcus aureus* with isolation rate of 21, 15, 12 and 10% respectively. *Salmonella* was isolated in 2 (7%) cockroach sample. *Proteus, S. epidermidis, Streptococcus, and Shigella* occurred just once (3%). *Enterococcus* species was not found among these group of samples. *E. coli* was also the most frequently isolated 7 (22%) among samples obtained from living houses followed by *Klebsiella* 6 (19%), *Bacillus* 4 (13%). *P. aeruginosa* and *S. aureus* occurred in 3 (9%) samples each, *S. epidermidis, Streptococcus, Salmonella and Shigella* species occurred in 2 (6%) samples each, *Enterococcus* was not among the isolates obtained from living houses (Figure 1).

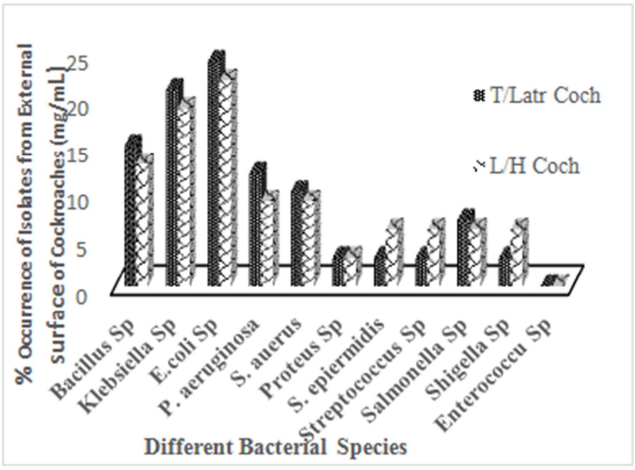

Figure 1. *Showing the Distribution of Different Bacterial species from External surface of Cockroaches captured from Toilet/latrines and Living Houses.*

3.2. Frequency of Isolation of Bacterial Contaminants from the GIT of Cockroaches Obtained from Latrines and Living Houses

Among the bacteria genera isolated from the GIT of cockroaches obtained from latrines, *Klebsiella* species occurred in 9 out of 34 specimens (27%) followed by *E. coli, Enterobacter* and *Staphylococcus* species with 21, 15 and 12% occurrence respectively. *Bacillus* occurred in 3 cockroach samples (9%) while *Shigella* and *Salmonella* species occurred in 2 (6%) each and *Pseudomonas, Streptococcus* and *Salmonella* species in 1 (3%) each. For cockroaches captured from living houses, *E. coli* showed the highest occurrence (23%) followed by *Klebsiella* (19%), and *Staphylococcus* and *Shigella* species (15% each), *Enterobacter* species occurred in 3 (12%) cockroach samples, *Salmonella* species in 2 (8%), while *Bacillus* and *Streptococcus* species occurred in 1 (4%) each. *Pseudomonas* S*p* was not isolated from the GIT of this group of cockroaches (Figure 2).

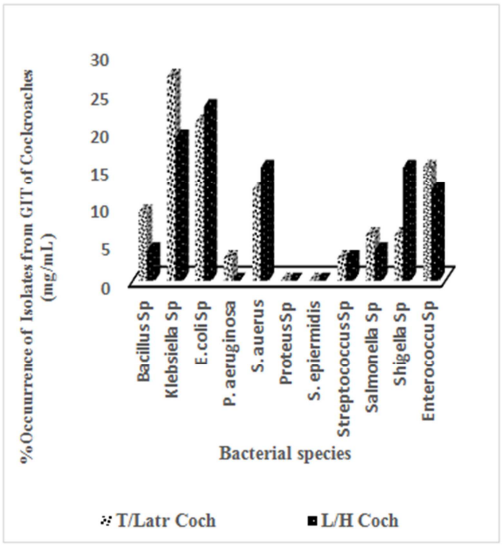

Figure 2. *Showing the Distribution of Different Bacterial species from GIT of Cockroaches captured from Toilet/latrines and Living Houses.*

3.3. Total Number of Bacterial Isolates from Cockroaches According to Body part (External and GIT) and Source (latrines and living houses)

Of 65 cockroach samples captured, 33 from latrines and 32 from living houses, *E. coli* yielded the highest 15 (23%) from external surface of cockroaches, this was followed by *Klebsiella* 13 (20%), *Bacillus* 9 (14%) *P. aeruginosa* 7 (11%), *S. aureus* 6 (9%), *Salmonella* 4 (16%). *S epidermidis, Streptococcus* and *Shigella* species had a total occurrence of 3 (5%) each, *Proteus* was the least with occurrence rate of 2 (3%).

The internal part (GIT) of sixty (60) cockroach samples were analysed, 14 (23%) was *Klebsiella,* 13 (22%) was *E. coli.* 8 (13%) was from *S. aureus* and *Shigella,* 6 (10%) came from *Salmonella*, 2 (3.3%*)* came from *Streptococcus. P. aeruginosa* was the least isolate 1 (1.7%), *Proteus* and *S. epidermidis* were not isolated from this group of sample (Figures 3 and 4).

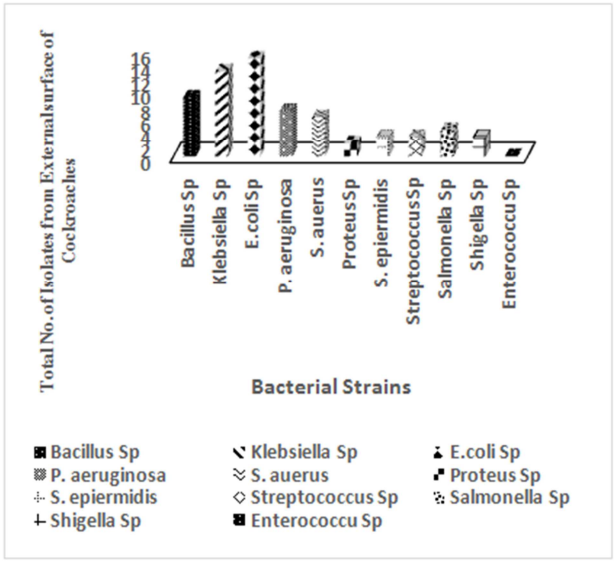

Figure 3. *Total number of Each Isolate from External Surface of Cockroaches Captured from Latrines and Living Houses.*

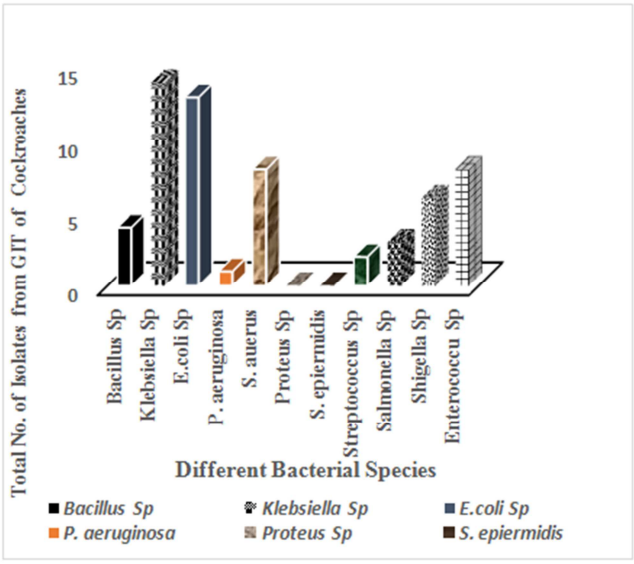

Figure 4. *Total number of Each Isolate from GIT of Cockroaches Captured from Latrines and Living Houses.*

The degree of contamination based on sum total of each isolate from External and Internal (GIT) parts of cockroaches from both experimental sources was; E. coli > Klebsiella > S. aureus > Bacillus > Shigella > P. aeruginosa = Samonella = Enterococcus > Streptococcus > S. epidermidis > Proteus (Figure 5).

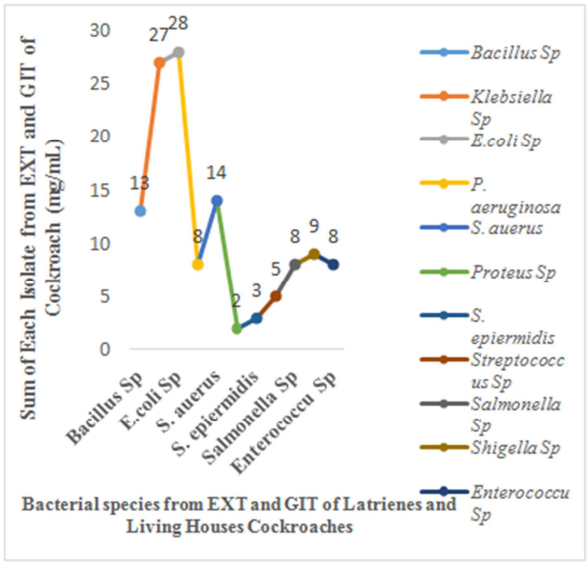

Figure 5. *Showing sum total of Each Bacterial Isolates from External and GIT of cockroaches captured from latrines and Living Houses.*

Key
EXT = External
GIT = Gastrointestinal tract

4. Discussion

Cockroaches are commonly seen in many human habitations, particularly in kitchens where foods are stored, processed, prepared, or served as well as around latrines in contact with human faeces and everywhere in the hospital environment [5]. They are ubiquitous and possess nocturnal and omnivorous attributes that make them mechanical transmitters of pathogenic organisms including bacteria, fungi, parasites and viruses [6, 7].

The evaluation of *B. germanica* from different niches for bacterial contamination was conducted on 80 cockroach samples captured from latrines and living houses in Calabar South, Cross River State, Nigeria. Eleven bacterial genera (*Bacillus, Klebsiella, E. coli, P. aeruginosa, S. aureus, Proteus, S. epidermidis, Streptococcus, Salmonella, Shigella* and *Enterococcus* species) were isolated and identified using standard bacteriological techniques.

Cockroaches caught from latrines showed high occurrence of *E. coli* species, isolated 8 times out of 33 samples, followed by *Klebsiella, Bacillus, Pseudomonas, S. aureus, Salmonella, Proteus, S. epidermidis, Streptococcus* and *Shigella* species with the respective rates of isolation shown in Table 1. Of the 32 cockroach samples caught in the living houses, *E coli* were found in 7 samples and *Klebsiella* (6), *Bacillus* (4), *Pseudomonas* and *Staphylococcus* (3 each); *S. epidermidis, Streptococcus, Salmonella* and *Shigella* (2, each); and *Proteus* species (1).

E. coli, Klebsiella and *Bacillus* species were the highest contaminant of the external surface of cockroaches captured from latrines and living houses.

Klebsiella, E. coli and *Enterococcus* were the most frequent isolated organisms from the GIT of latrines. This was same for living houses except that the presence of *Enterococcus* was insignificant compare to the above mentioned organisms. *E. coli* was the leading isolate among other organisms from external surface, GIT of latrines and living houses, whereas *Klebsiella* was leading other isolates from GIT of latrines cockroaches respectively.

The sum total of each isolate from external and GIT of latrines and living house cockroaches reveal the level of contamination in the order of *E. coli* > *Klebsiella* > *S. aureus* > *Bacillus* > *Shigella* > *P. aeruginosa* = *Samonella* = *Enterococcus* > *Streptococcus* > *S. epidermidis* > *Proteus.*

The Presence of *E coli* outside and inside of the cockroach shows faecal contamination which is not surprising given that latrines and other dirty places are common habitats for them. On the other hand, a good number of these isolates have been associated with food borne infections and human intestinal diseases such as abdominal cramps, bloody diarrheal, vomiting, pneumonia, blood stream infection, wound infection, urinary tract infection, bacteraemia, and liver abscesses [8, 9]. Mpuchane *et al.* (2006) [10] had also observed these pathogens as common isolates from cockroaches trapped from kitchen and living houses in the Central/Broadhurst, Old Naledi and Tlokweng of Gaborone, Botswana. Feleke *et al.* (2016) [11] also reported high occurrence of *Klebsiella* (17.7%), *E. coli* (16%) and *Citrobacter* (15%) species as leading isolates from external and internal surfaces of cockroaches found in the hospital environment; adding that *E. coli* was also the predominant isolate from nonhospital cockroaches.

The presence of *Enterobacter* Sp and absence of *S. epidermidis* from the internal GIT of cockroaches has not been reported by other worker and may reflect differences in cockroach species and locations in which cockroaches were trapped.

The presence of cockroach in human habitation is a serious public health problem. Thus, regular control of this pest by strict implementation of pest control regulations is of primary importance for the safety of public health.

5. Conclusion

Different bacteria species were isolated from cockroaches trapped from different ecological niches. *E. coli, Klebsiella and Bacillus* were the principal isolates from both external and GIT part of cockroaches except that *Enterococcus* species replaces *Bacillus* in GIT. These pathogens are threat to human health as they are major causative agents of human intestinal diseases, blood and organ infection. This research advocates the use of natural/harmless cockroach eradicative measures at homes with high population of children. These include routine removal of bushes around the living environ, frequent sealing of cracks around the kitchens, and bathrooms to reduce potential hidings areas. Getting rids of all foods remains on kitchen tables, floor etc. Application of boil mint leaves at different corners of the house. These will naturally scare the presence of this pest from human habitation and serve as a better strategy to minimize cockroach-associated infections.

Consent

It is not applicable.

Ethical Approval

It is not applicable.

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