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Potential Risk of Transmission of *Escherichia coli* and *Salmonella* spp. Infections by the *Musca domestica* Fly and the *Periplaneta americana* Cockroach in the City of Cotonou (South Benin)

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Abstract

Background: The occurrence of bacterial infections sometimes involves synanthropic flies and cockroaches, as mechanical vectors of pathogenic microorganisms. The aim of this study was to identify the different species of flies and cockroaches that cohabit with humans in the city of Cotonou and determine the contribution of *Musca domestica* and *Periplaneta americana* in the transmission of bacteria responsible for infections in humans. **Methods:** Capture sessions during the day for flies and at night for cockroaches were carried out in 4 arrondissements of the city of Cotonou. The insects collected were transported to the Center of Research Entomological of Cotonou for identification, then sent to the Section Hygiene of Water and Food for microbiological analysis, enumeration and testing for human pathogenic bacteria. **Results:** A total of 351 flies and 26 cockroaches were collected at the sites, including two synanthropic species belonging to two families each. *Musca domestica* and *Periplaneta americana* were the most abundant species found after identification in the city of Cotonou. Thermo-tolerant coliforms and *Escherichia coli* were found on all specimens at varying concentrations. *Salmonella* spp. bacteria were identified in the fly population from the Dantokpa dump. **Conclusion:** Flies and cockroaches can transmit microorganisms to humans. The presence of *Salmonella* spp. among the specimens revealed that infections fre-

quently caused by contaminated food or water are also transmitted to humans by flies. The presence of these germs on flies and cockroaches represents a potential risk of mechanical transmission to humans. It is, therefore, essential to continue investigations in order to assess the species of bacteria propagated, improve control strategies against these troublesome insects and adopt better hygiene conditions for better living.

Keywords

Musca domestica, *Periplaneta americana*, Mechanical Transmission, *Salmonella* spp. and *Escherichia coli* Infections, Cotonou

1. Introduction

Toxi-infections are a major public health problem worldwide, particularly in Africa [1]. According to the WHO, in 2020, around 1 in 10 people worldwide will fall ill each year after eating contaminated food, resulting in more than 420,000 deaths [2]. Infants, young children, pregnant women, the elderly and the chronically ill are most at risk. Apart from the major parasitic and viral diseases, a number of pathologies are attributed to bacteria. They are generally transmitted through contaminated food and water. The bacteria responsible for these illnesses manifest themselves through infections, including *Salmonella* spp. and *Escherichia coli*, from which a third of children under the age of five suffer, according to WHO data for 2021 [3]. In Benin, as in most underdeveloped countries, they constitute a health problem due to the difficulty of access to drinking water and the poor management of the population's environment [4].

After man, insects are the most studied living beings because of their impact on health, the environment and habitat [5]. Among these insects are *Musca domestica*, a fly, and *Periplaneta americana*, a cockroach commonly found in our environment. They play a key role in the epidemiology of diarrhoeal diseases, with sometimes serious consequences for human health [6]. Because of their close association with humans, they are considered mechanical vectors of microorganisms [7], such as bacteria of the genus *Escherichia coli*, *Salmonella* spp., *Staphylococcus* spp., *Proteus* spp., *Shigella* spp., *Bacillus* spp., *Vibrio cholerea* and others implicated in several infections including urinary tract infections, toxi-infections, typhoid, dysentery, and nosocomial infections [8] [9]. The saprophagous nature of flies and cockroaches means they are not only pathogenic vectors via their exoskeletons and excrement, but also reservoirs for the multiplication of bacteria likely to cause a bacterial epidemic [8] [10].

The poor management of urban areas and the lack of hygiene due to the presence of waste and contaminated water in the vicinity of open-air or itinerant food sales outlets expose populations to increased risks of food-borne bacterial infections [11]. In order to assess the risk of bacterial transmission in the Coto-

nou commune and raise public awareness of the need for behavioral change in terms of food protection and cleanliness at points of sale, we need to know the microbiological profile of flies and cockroaches. With this in mind, the present study has been entitled "Evaluation of the risk of transmission of *Escherichia coli* and *Salmonella* spp. bacterial infections by the *Musca domestica* fly and the *Periplaneta americana* cockroach in the city of Cotonou". Its aim is to identify the different species of flies and cockroaches that cohabit with humans in the city of Cotonou and determine the role of *Musca domestica* and *Periplaneta americana* in the transmission of bacteria responsible for infections in humans.

2. Materials and Methods

2.1. Study Area

The city of Cotonou (6˚21'36"N; 2˚26'24"E) is the economic capital of Benin, concentrating all the country's administrative and political functions. It is located in the Littoral department and is the only commune, with 13 arrondissements and 143 city districts [12]. The climate is equatorial, with two rainy and two dry seasons alternating: a long rainy season from mid-March to mid-July; a short dry season from mid-July to mid-September; a short rainy season from mid-September to mid-November; and a long dry season from mid-November to mid-March. Rainfall occurs mainly between March and July, with a peak in June (300 to 500 mm). Average monthly temperatures range from 27˚C to 31˚C. The population of Cotonou is estimated at 679,012, divided into 4 health zones [12]. In addition, this locality lacks access to drinking water and environmental management, which exposes the population to a high risk of contamination by diseases linked to insalubrity [4].

2.2. Type of Study and Sampling Site Selection Criteria

This was a descriptive, cross-sectional and analytical study that ran from December 2022 to March 2023. Flies were collected at food markets, a slaughterhouse and a number of dumpsites in the city of Cotonou. These were the Dantokpa and Gbégamey markets; the Agla, Gbégamey and Dantokpa dumps; and the Cotonou slaughterhouse. Markets and slaughterhouses are open places located in vulnerable neighborhoods where hygiene and sanitation conditions are inadequate. They offer several varieties of uncooked foodstuffs, including fresh and dried vegetables, fruit, animal products and cooked foods, as well as live animals. As for the dumps, they are very dirty places, as they result from the assembly of household waste from the surrounding concessions. Authorization from the Company of Waste Management and Sanitation was, therefore, required to collect flies from the dumps.

Cockroaches were collected in two households (Agla and Dantokpa) in damp, dark and warm areas.

Figure 1 and **Figure 2** respectively show the sites where the captures were made.

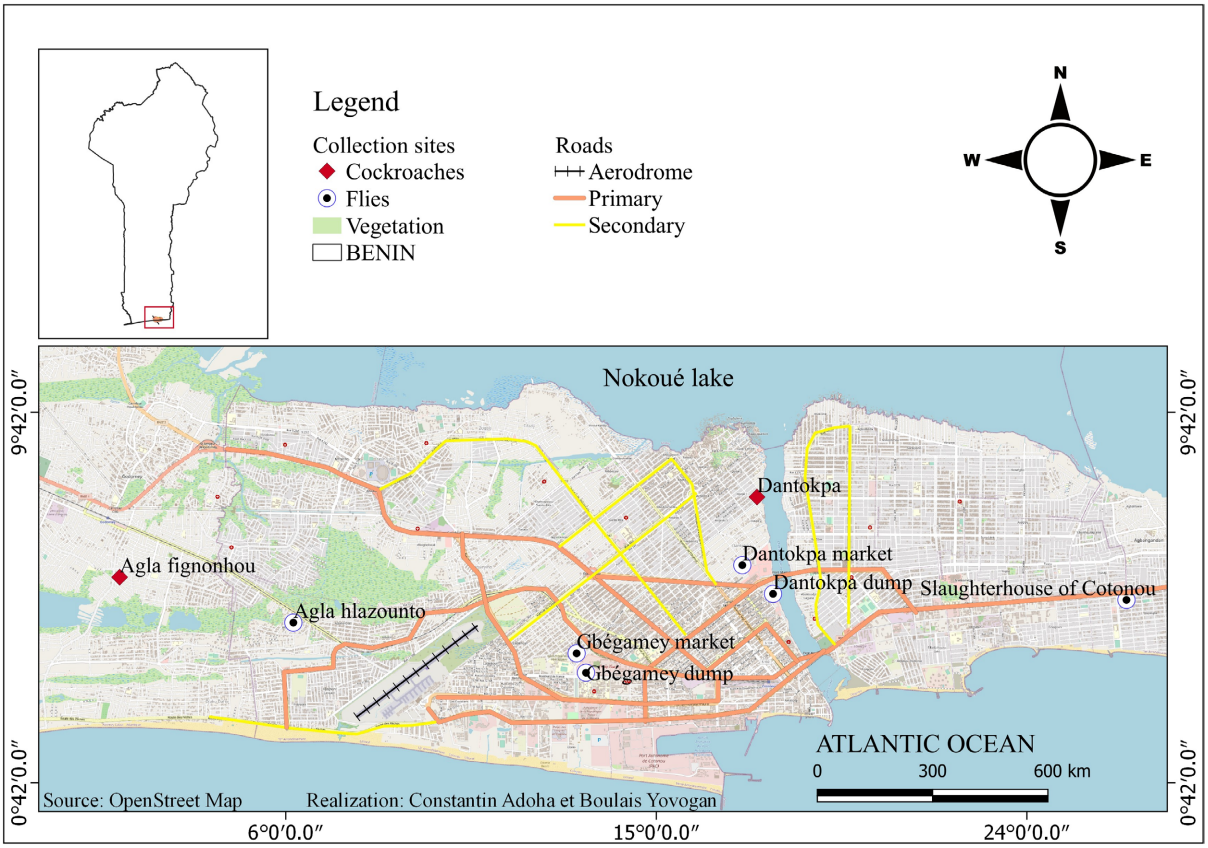

Figure 1. Map of fly and cockroach capture sites in the city of Cotonou.

Figure 2. Images of some collection sites.

2.3. Fly Collection

The investigations took place from december 26, 2022 to february 10, 2023 in 4 neighborhoods, namely Akpakpa, Agla, Dantokpa, and Gbégamey diversified

between the abattoir, the dump and the market. Flies were caught on different days in the 4 localities at two respective times of day, 8:30 a.m.-10:00 a.m. and 2:00 p.m.-4:00 p.m., corresponding to different levels of sunshine. Adult flies of both sexes were collected using Bawa *et al.* traps [10] [13] represented by **Figure 3**. The trap was a plastic bottle with a capacity of 1.5 L, cut 1/3 at the top. The upper part of the bottle, shorter than the lower part, is inverted to form a cone leading into the body of the bottle (A). A bait of beef casings, left to ferment for 36 hours, is placed inside the bottle and then deposited on the floor to attract the flies (B). Bait exposure time was 5 to 10 min. Once the trap containing the bait had been exposed, the cut-off top part of the bottle was placed on the trap and all the flies attracted by the smell of the bait were trapped. The whole device was sealed with adhesive tape (C) and placed in a cooler for transport to the Centre de Recherche Entomologique de Cotonou (CREC).

2.4. Cockroach Collection

The cockroaches were captured during the night of January 2 to 30, 2023 in the Agla and Dantokpa neighborhood between 8:00 pm and midnight, corresponding to their foraging hours. Cockroaches are omnivorous and will eat a bit of everything, so trapping was done in two ways [8]. First, pieces of sugar or bread were placed on A4 paper to attract them. Secondly, the cockroaches were collected directly with the gloved hand and placed in sterile jars for transport to the CREC (**Figure 4**).

(A) (B) (C)

Figure 3. (A) Fly trap without bait; (B) Fly trap without bait; (C) Trapped flies.

Figure 4. Image showing cockroach capture.

2.5. Morphological Identification of Flies and Cockroaches

At the CREC, the flies collected were cold anesthetized in a freezer for 15 minutes, then isolated from the bait and identified using standard morphological keys [9]. Cockroaches were also anaesthetized and identified using standard keys [14]. Only identified *Musca domestica* flies and *Periplaneta americana* cockroaches were transferred to sterile plastic bags according to catch area, under aseptic conditions. Codes (Table 1) were assigned to each plastic bag for sampling, and then sent to the Section Hygiène des Eaux et Aliments (SHEA) laboratory for microbiological analysis.

2.6. Microbiological Analysis of Flies and Cockroaches

Thermo-tolerant coliforms (indicator of other microorganisms) and two enterobacteria including *Escherichia coli* (indicative of faecal contamination) and *Salmonella* spp. (pathogenic bacteria) were sought, counted and identified on the *M. domestica* flies and *P. americana* cockroaches collected and identified.

At the SHEA laboratory, 1 g of each sample was aseptically collected in sterile stomacher bags for preparation of the 1/10 stock suspension using Buffered Peptone Water (EPT) broth. The mixture was ground and homogenized and successive decimal dilutions were made to culture and isolate the bacteria.

2.7. Culture and Isolation of Bacteria

For the enumeration of coliforms and *Escherichia coli*, 1mL of each decimal dilution was taken and introduced into two different sterile petri dishes, 10^{-2} and 10^{-3} respectively. These 1 mL dilutions were then inoculated by pouring 15 mL to 20 mL of Tryptone Bile Glucuronide (TBX) agar into the plates. The agar was then incubated at 44˚C for 24 h ± 2 h. Colonies of *Escherichia coli* and thermo-tolerant coliforms were counted on TBX agar. At the same time, *Salmonella* testing was carried out. The same 1/10 stock suspension was incubated at 37˚C for 18 h ± 2 h for the pre-enrichment step. Then 0.1 ml of the pre-enriched broth (1/10 suspension) was added to Rappaport-Vassiliadis (RV) broth and incubated at 41.5˚C for 21 h ± 3 h. After incubation of this mixture, a small quantity of Rappaport-Vassiliadis (RV) broth was plated onto Hektoen agar by the

Table 1. Presentation of codes by collection site for fly and cockroach specimens.

Flies			Cockroaches		
Collection sites	Codes	Legends	Collection sites	Codes	Legends
Agla	M1	Dump	Agla	C1	Sump
Akpakpa	M2	Slaughterhouse	Dantokpa	C2	Kitchen
Gbégamey	M3	Dump	-	-	-
	M4	Market	-	-	-
Dantokpa	M5	Market	-	-	-
	M6	Dump	-	-	-

dial method and incubated at 37˚C for 21 h ± 3 h. On TBX agar, *Escherichia coli* appeared as blue colonies, whereas thermotolerant coliform colonies were colorless. These characteristics enabled us to count easily. The cultural characteristics of bacterial colonies on Hektoen agar were used to identify suspect colonies. Thus, any flat, yellow bacterial colony with a bulging center and a regular outline was considered suspicious of *Escherichia coli*, and any green colony with or without a black center was considered suspicious of *Salmonella*. Colonies suspect of *Escherichia coli* and *Salmonella* were purified on nutrient agar for confirmation by biochemical tests.

2.8. Biochemical Identification

Isolates on nutrient agar were plated on a conventional enterobacteria identification mini-gallery consisting of Simmons Citrate, Kligler Hajna, Mannitol-mobility, Urea-indole and Lysine decarboxylase media to confirm suspected *Escherichia coli* and *Salmonella* colonies. The mini-gallery was incubated at 37˚C for 18 to 24 hours. All desired characteristics were recorded. Bacteria were identified using the Enterobacteriaceae Identification Chart.

2.9. Data Analysis

The coordinates of the collection points were recorded using the OSM Tracker for Android TM application. The data from this study were recorded in Excel 2016 and then analyzed directly in Excel, in order to take stock of the species captured and bacteria counted. The chi-square test was calculated using SPSS software to see if there was a significant difference between the abundances of thermotolerant coliforms and *E. coli* according to the capture sites. All differences were considered significant at $P < 0.05$.

3. Results

3.1. Attractiveness of Fly and Cockroach's Traps

A total of 351 flies and 26 cockroaches were collected in Cotonou. Two species of flies were identified: *Musca domestica* and *Chrysomya* spp.; and two species of cockroaches: *Periplaneta americana* and *Blattela germanica*. *M. domestica* accounts for 72% and *P. americana* for 88.46% of harvests. However, both species of flies (*M. domestica* and *Chrysomya* spp.) and cockroaches (*P. americana* and *B. germanica*) were regularly caught at the study sites (Table 2 and Table 3).

On the whole, flies seem to be more frequent in dumps than in markets and the Slaughterhouse.

Cockroaches seem to be more frequent in sump than in kitchens.

3.2. Microbiological Profile Fly Populations Caught in Cotonou

Laboratory analyses showed that the flies studied were all contaminated with thermo-tolerant coliforms and *E. coli* (Figure 5). There was a significant difference between the abundances of thermo-tolerant coliforms and *E. coli* depending

Table 2. Distribution of fly species inventoried at sites in Cotonou.

Sites	Agla	Akpakpa	Gbégamey		Dantokpa		Percentage of species
	Dump	Slaughterhouse	Dump	Market	Market	Dump	
Musca domestica	35	85	29	25	45	34	72%
Chrysomya spp.	5	2	20	12	29	30	28%
Total	40	87	49	37	74	64	100%

Table 3. Distribution of cockroach species inventoried at sites in Cotonou (Benin).

Sites	Agla	Dantokpa	Percentage of species
	Sump	Kitchen	
Periplaneta americana	14	9	88.46%
Blattella germanica	3	0	11.54%
Total	17	9	100%

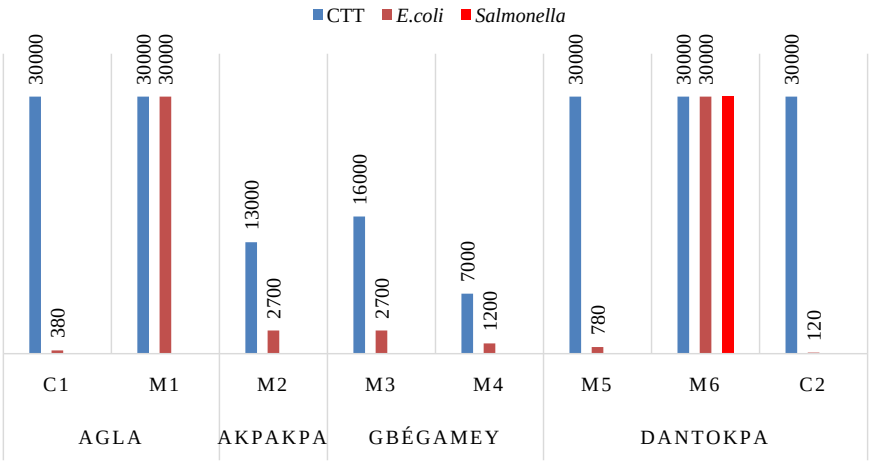

Figure 5. Histogram of bacteria isolated from collected flies and cockroaches.

on the capture sites (P = 0.000). The Dantokpa landfill had the same microbiological profile as the other capture sites, with one particularity for *Salmonella* spp. (Table 4).

3.3. Cockroach Populations Caught in Cotonou

Bacteria isolated from *Periplaneta americana* cockroaches were selected according to two capture sites (Figure 5). Thermo-tolerant coliforms and *E. coli* were the only bacteria found among the three selected. In Table 5, we observed the high significant difference between the abundances of thermo-tolerant coliforms and *E. coli* according to capture site (P = 0.000).

Microbiological analyses carried out on the specimens collected and sampled showed that the flies and cockroaches collected were all contaminated with coliforms (Figure 5). The Akpakpa and Gbégamey fly populations were found to contain fewer bacteria (CTT ranged from 7000 - 16,000 CFU/g and *E. coli* from

Table 4. Results of microbiological analysis of fly populations.

Codes	Thermo-Tolerant Coliforms (CTT)	*Escherichia coli*	*Salmonella* in 1 g	P-value	P-value 1
	CFU/g				
M1	$>3 \times 10^4$	$>3 \times 10^4$	Absent	**0.000**	**0.000**
M2	1.3×10^4	2.7×10^3	Absent		
M3	1.6×10^4	2.7×10^3	Absent	**0.000**	
M4	7×10^3	1.2×10^3	Absent		
M5	$>3 \times 10^4$	7.8×10^2	Absent	**0.000**	
M6	$>3 \times 10^4$	$>3 \times 10^4$	Present		

CFU/g: Colony Forming Unit per gramm.

Table 5. Results of microbiological analysis of cockroach populations.

Codes	Thermo-Tolerant Coliforms (CTT)	*Escherichia coli*	*Salmonella* dans 1 g	P-value
	UFC/g			
C1	$>3 \times 10^4$	3.8×10^2	Absente	**0.000**
C2	$>3 \times 10^4$	1.2×10^2	Absente	

1200 - 2700 CFU/g) than the Agla and Dantokpa populations (CTT was 30,000 CFU/g and *E. coli* from 780 - 30,000 CFU/g), with *Salmonella* present in the Dantokpa dump. However, the lowest concentration of *Escherichia coli* was found in the fly population of the Dantokpa market (780 CFU/g). The presence of *Escherichia coli* was also low, while the presence of CTT was high in cockroaches (CTT is 30,000 CFU/g and *E. coli* varies from 120 - 380 CFU/g).

4. Discussion

Musca domestica and *Periplaneta americana* are two synanthropic vector species of several groups of micro-organisms, as documented by several authors [6] [7] [13] [15], but little is known about the most frequently propagated bacteria likely to cause bacterial infections in the city of Cotonou in particular and in Benin in general.

This study firstly provides information on the diversity of flies and cockroaches encountered, and secondly on the microbiological profile of these insects causing *Escherichia coli* and *Salmonella* spp. bacterial infections in the city of Cotonou. The effectiveness of the attractiveness of the traps used was assessed by the diversity of flies caught in the field. In fact, the traps used showed their effectiveness, as they yielded 253 flies of the species *Musca domestica*, or 72% of the collection, against 98 flies of the genus *Chrysomya* spp., or 28% of the collection. The predominance of the *Musca domestica* species justifies the title of "the most abundant common fly in West Africa", as shown by the work carried out by Dawaye *et al.* in Cameroon [6] and Komono *et al.* in Côte d'Ivoire [13], who

respectively had 55.11% and 62.6% of the same species after collection. The same trap also enabled us to obtain 23 cockroaches of the species *Periplaneta americana*, or 88.46%, compared with 3 cockroaches of the species *Blattella germanica*, or 11, 54%, which contrasts with the results of collections by Memona *et al.* in Lahore, Pakistan [15] and Mehainaoui *et al.* in Algeria [16], who respectively obtained 35% of *Periplaneta americana* species versus 45% of *Blattella germanica* species and 100% of *Blattella germanica* species. These results are satisfactory given that the study was carried out during the dry season, a period when fly and cockroach outbreaks are rare. As for microbiological analyses, all flies and cockroaches carried coliforms (thermotolerant and *E. coli*) with a considerable microbial quantum (>3.10^4 CFU/g) likely to cause bacterial infections, which is in line with the research work of Dawaye *et al.* [6], which also found a microbial quantum >3.10^4 CFU/g in the flies. The housefly (*Musca domestica*) has always been parasitized by pathogens fatal to human and animal health, the vast majority of which are bacterial germs, as noted in the work of Bahrndorff *et al.* [17] in Denmark and the meta-analysis data of Khamesipour *et al.* [18]. The American cockroach (*Periplaneta americana*) can also spread a whole range of bacterial infections, and in itself represents a danger to human health, as shown by the work of Malik [8] in Lahore, Pakistan, and Toufik *et al.* [7] in the commune of Biskra, Algeria. The presence of coliform bacteria at capture sites indicates contamination by organic matter (animal and/or plant) [19]. The presence of coliform by these flies and cockroaches in the Agla and Dantokpa neighborhood can be explained by the existence of major agri-food processing activities and the practice of agricultural activities. These results are in line with those of Craun *et al.* [19] who showed that the urban and peri-urban physical environment is the most contaminated by coliforms. Similarly, the high number of thermo-tolerant coliforms observed suggests the presence of other bacteria pathogenic to humans, in line with the work of Dawaye *et al.* [6] in Cameroon. The Salmonella found on the flies could have originated from animal excrement after slaughter in food markets. This is in line with studies by Abdus *et al.* [20]. As salmonella are enteric pathogenic bacteria, these results indicate a potential risk of bacterial infections through water and food contamination in the Cotonou commune; this is in line with the work of Thomson *et al.* carried out in 2017 [21].

As the type of bait used in this study would not have attracted enough cockroaches, it would be interesting to consider the use of other types of bait for future work. In addition, other bacteria (such as *Staphylococcus* spp.) are also responsible for bacterial foodborne infections, and their carriage by these flies and cockroaches deserves to be explored.

The principal means of combating bacterial infections has always been hygiene-based prevention. However, these infections continue to be a major public health problem, especially in low- and middle-income countries, also because of bacterial resistance to antibiotics, according to Blaak *et al.* and Herindrainy [22] [23]. Thus, in addition to standardized practices for controlling infections caused

by microorganisms, which take into account the five keys to safer food, to know: practice cleanliness, separate raw from cooked food, cook food thoroughly, maintain food at the right temperature and use safe water and products, according to the WHO [24], an approach must be implemented to eliminate insect vectors of bacteria as far as possible through vector control programs.

5. Conclusion

In the course of this study, two species of synanthropic flies and cockroaches were identified in the city of Cotonou. *Musca domestica* and *Periplaneta americana* are two insects that live in an unhealthy environment, thus favorable to contamination and microbial proliferation. The results of the microbiological analysis obtained showed a high level of thermo-tolerant coliforms in all specimens, suspecting the presence of other pathogenic bacteria, and a high level of *Escherichia coli* responsible for several infections. Poor management of household waste, agro-economic transformation activities and poor sanitation are the likely sources of contamination of flies and cockroaches by fecal bacteria. The presence of *Salmonella* spp., responsible for infection, is thought to be involved in the spread of diarrhoeal diseases through food contamination at the Dantokpa site, a market widely visited in the sub-region. Interpretation of the results of the microbiological analyses confirmed that flies and cockroaches, in particular *Musca domestica* and *Periplaneta americana*, play a role as mechanical vectors in the occurrence of *Escherichia coli* and *Salmonella* spp. bacterial infections, generally caused by the absorption of contaminated food and water. The presence of these bacteria on flies and cockroaches shows the high risk of their transmission within the population. It is, therefore, essential to set up entomological surveillance systems that take public hygiene into account, in order to reinforce strategies to combat the vectors of these various bacteria. Similarly, insect control and public health measures need to be stepped up to reduce the risk of bacterial infection.

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Authors' Contributions

TFT, BBEA, RO, and MA designed the research; BBEA, CND, JK, IJGY, SDZ and FH conducted data collection; all authors conducted data analysis. TFT, BBEA, DSK and VD coded the data; TFT and BBEA led the drafting with substantive input from DSK and VD in the results section; all authors revised the manuscript. All authors read and approved the final manuscript.

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Ethics Approval

Not applicable to fly and cockroach collection. The actors involved in the work have given their informed consent. SGDS authorization has been obtained.

Availability of Data and Materials

Data is contained within the article.

Conflicts of Interest

The authors declare that they have no competing interest.

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