

Search for Coagulase-Positive *Staphylococcus*, *Salmonella* spp. and *Listeria monocytogenes* from Cockroaches in Residential Kitchens

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Cockroaches can carry and disseminate microorganisms. The objective of this study was to investigate the presence of coagulase-positive *Staphylococcus*, *Salmonella* spp. and *Listeria monocytogenes* from cockroaches in residential kitchens. The study was conducted from March to December 2010 and involved 120 inspections. In each kitchen, three traps were placed and left for 24 hours. The samples were subjected to microbiological analysis for *Salmonella* sp., *L. monocytogenes* and coagulase-positive *Staphylococcus*. The insects were also quantified, sexed and classified. Twenty-nine cockroaches were collected, none of which was contaminated with *Salmonella* sp., coagulase-positive *Staphylococcus* or *L. monocytogenes*. However, microorganisms of the genus *Citrobacter* sp. (23.52%), *Enterobacter* sp. (23.52%), *Proteus* sp. (23.52%), *Serratia* sp. (5.88%), *Flavimonas* sp. (2.94%), *Morganella* sp. (2.94%), *Providencia* sp. (2.94%), *Alcaligenes* sp. (2.94%), *Staphylococcus* sp. (8.82%) and *Acinetobacter* (2.94%) were found. Thus, we observed that cockroaches can carry microorganisms which reinforces the importance of the control of these insects.

Key Words: Microorganism, Bugs, Health public, Food-borne disease, Food safety.

Cockroaches (Blattodea: Blattidae) are adaptable because of their nutritional requirements, reproductive potential and ability to hide, which protects them from detection and predators¹. These bugs have been around for 400 million years, and during this period, they have undergone little change in appearance. They can be feed on almost anything that has some nutritional value².

In urban areas, cockroaches take advantage of favourable conditions: they are omnivores and exhibit necrophagy, coprophagy and high reproductive potential. They also have the ability to adapt to different environments easily hide in small gaps³.

Approximately 4000 species of cockroaches exist, of which 1200 live in the neotropical region, representing 460 genera (in the neotropical region). They have spread across all zoogeographical regions. Some species have adapted to urban conditions and have spread throughout the world; these species include *Blatta*

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orientalis, *Blatella germanica*, *Periplaneta americana*, *Periplaneta australiase* and *Supella longipalpa* ⁴.

The cockroaches are vectors for many microorganisms and distribute them on surfaces, utensils and food. Several bacteria can cause FBD, including *Staphylococcus aureus*, *Salmonella* sp. and *Listeria monocytogenes* ⁵.

S. aureus (a species of coagulase-positive *Staphylococcus*) is one of the most common pathogens responsible for bouts of food-borne intoxication. Food poisoning provoked by this microorganism is due to ingestion of an enterotoxin produced and released by bacteria during their growth in food, which represents a public health risk ⁶.

Another organism that gets attention is *Salmonella* sp., which diffuses widely in nature and may be present in soil, air, water, waste water, animals, humans, food and faeces^{7,8}. However, the natural habitat of *Salmonella* sp. is the intestinal tract of animals such as cattle, pigs and poultry ⁹. Moreover, it is important to emphasise that *Salmonella* spp. is considered the leading cause of food-borne disease the State of Rio Grande do Sul, southern Brazil ¹⁰.

In recent years, *L. monocytogenes* has acquired extreme importance both in industrial environments and in residential kitchens; it is a bacterium that adapts to different environmental conditions and grows rapidly over wide ranges of temperature and pH ¹¹. *L. monocytogenes* is unquestionably pathogenic to humans, and unlike other food-borne pathogens that cause gastrointestinal symptoms, the main clinical symptoms are initially similar to a cold with fever and malaise. However, *L. monocytogenes* infection may progress to meningitis, meningoenphalitis, septicaemia, abortion or premature birth ¹².

According to the Secretariat of Health Surveillance⁵, residences are places where the greatest proportion of outbreaks of food-borne disease have been reported (45.3%), followed by food services in restaurants (19.6%) and schools (10.1%). Therefore, it is important to verify whether cockroaches present in residential kitchens carry microorganisms that cause food-borne disease, as they are frequently encountered in these locations. Thus, this study aimed to search for the presence of coagulase-positive *Staphylococcus*,

Salmonella sp. and *L. monocytogenes* carried by cockroaches in residential kitchens. In addition, we completed a quantitative survey of residential kitchens and took samples of cockroaches present in those locations, as well as made sure that they can carry microorganisms that cause food-borne disease.

MATERIALS AND METHODS

The study was conducted from May to December 2010 in 120 kitchens in a randomly selected residential city in the far-west region of Santa Catarina, Brazil. Three traps were placed (disinfected with 70% alcohol) in each residence. The traps were baited with attractive food (sugar, powdered chocolate and onion), installed during the day, and left in place for 24 h. The traps were identified in accordance with the collection point number. The cockroaches were living and healthy because they had just been used for microbiological study. When captured, they were transferred to the microbiology laboratory at the University of West of Santa Catarina (UNOESC), Brazil.

In the microbiology laboratory, the insects were subjected to a temperature of less than -10°C for a period of 10–15 min. to the immobilization of these. After, the cockroaches were immersed in tubes containing peptonated saline 0.1% and then agitated manually for 10 seconds.

Subsequently, this mixture was microbiologically analysed for *Salmonella* sp., *Listeria monocytogenes* and coagulase-positive *Staphylococcus*.

All the microbiological tests were conducted according to regulations No. 62 of 2003, the Ministry of Agriculture, Livestock and Supply (MAPA)¹³, which formalises the official analytical methods for microbiological analysis for the control animal products and water ¹³.

Coagulase-positive *Staphylococcus* was plated in 100µl dilutions of concentrations of 0.1, 0.01 and 0.001mL on plates containing Baird-Parker agar (DIFCO, France). These plates were incubated at 37°C for 48 h. Characteristic colonies (black with halos) were subjected to gram staining and biochemical tests to confirm the presence of catalase and coagulase. The results were expressed in CFU/cockroach.

In the survey of *Salmonella* sp., 0.01 ml was used for the initial sample added to 0.09 ml of 1% buffered peptone water (DIFCO, France) and incubated for 16–20 h at 36±1°C. Subsequently, 1 mL was inoculated simultaneously in this sample test tube containing tetrathionate broth (MERCK, Germany). These were incubated at 41±0.5°C for 24 h. Then, these samples were separately streaked onto brilliant green phenol red lactose sucrose agar (OXOID, England) and xylose lysine deoxycholate (MERCK, Germany) and incubated at 36±1°C for 18–24 h.

Characteristic colonies were confirmed by biochemical and serological tests, including citrate utilization, urea hydrolysis, nitrate reduction, lysine de-carboxylation, oxidase production, methyl red, Voges-Proskauer, triple-sugar iron, motility, indole production, H₂S production and decarboxylation of ornithine tests¹⁴.

The results were choice in the presence or absence of *Salmonella* sp. For *L. monocytogenes*, we used 0.1 ml of pre-diluted sample and pre-enriched Listeria enrichment broth (broth LEB- DIFCO, France) and inoculated tubes containing 10 ml of Fraser broth (AES, Bruz Sedex).

We then incubated the tubes at 30±1°C for 24–48 h.

After this period, these samples were streaked on Oxford agar (AES, Bruz Sedex) and incubated at 30±1°C for 24–48 h. Characteristic colonies (black colonies surrounded by a dark halo) underwent gram staining and biochemical tests (methyl red, Voges-Proskauer, nitrate reduction, motility, indole production, H₂S production and hemolysis on blood agar 5%).

After microbiological testing, the cockroaches were transferred to flasks containing 70% alcohol and transported to the laboratory of zoology and botany at the UNOESC, where quantification, sex determination and classification at the species level were conducted with the help of a stereoscopic microscope and a dichotomous key of Buzzi⁴.

RESULTS AND DISCUSSION

Of the 120 residential kitchens evaluated in fifteen (12.5% of houses) cockroaches were captured. Among the 15 residences where insects were found, 60% were constructed of wood, 20% masonry and 20% mixed wood-masonry.

Table 1. Cockroaches number collected in residential kitchens, 2010.

Species	Number of individuals	Dimorphism	
		Male	Female
<i>Blatella germanica</i>	6	2	5
<i>Periplaneta americana</i>	7	4	2
<i>Periplaneta australasiae</i>	7	3	4
<i>Supella longipalpa</i> *	9	8	0
Total	29	17	11

*One individual of this species was of unidentified sex.

We found 29 specimens of the cockroaches; 17 were males, 11 were females and one was unidentified. Of the cockroaches encountered, 6 individuals (20.68%) were *P. americana*, 7 individuals (24.13%) were *P. australasiae*, 7 were individuals (24.13%) *B. germanica*, and 9 individuals were (31.03%) *S. longipalpa* (Table 1). All of these species are synanthropic and common in Brazil and other tropical countries¹⁵.

None of the 29 cockroaches analysed

were contaminated with *Salmonella* sp., coagulase-positive *Staphylococcus* or *L. monocytogenes*, the microorganisms of interest in this study.

However, 80% of the other microorganisms that were found were from the family Enterobacteriaceae (Table 2), with the highest number of occurrences from the genera *Citrobacter* spp. (23.52%), *Enterobacter* spp. (23.52%), *Proteus* spp. (23.52%), *Serratia* spp. (5.88%), *Morganella* sp. (2.94%) and *Providencia* sp. (2.94%).

Table 2. Genus and species of microorganisms isolated from the cockroaches, 2010.

Genus	Species	Occurrence	Frequency (%)
<i>Alcaligenes</i> sp.	<i>Alcaligenes faecalis</i>	01	2.94
<i>Acinetobacter</i> sp.		01	2.94
<i>Citrobacter</i> spp.	<i>Citrobacter amalonaticus</i>	03	8.82
	<i>Citrobacter freundii</i>	04	11.76
	<i>Citrobacter kosen</i>	01	2.94
<i>Enterobacter</i> spp.	<i>Enterobacter aerogenes</i>	04	11.76
<i>Enterobacter gergoviae</i>		01	2.94
<i>Enterobacter</i> sp.		03	8.82
<i>Flavimonas</i> sp.		01	2.94
<i>Morganella</i> sp.	<i>Morganella morganii</i>	01	2.94
<i>Proteus</i> spp.	<i>Proteus freundii</i>	01	2.94
	<i>Proteus mirabilis</i>	02	5.88
	<i>Proteus myxofaciens</i>	01	2.94
	<i>Proteus vulgaris</i>	04	11.76
<i>Providencia</i> sp.	<i>Providencia rustigianii</i>	01	2.94
<i>Serratia</i> spp.	<i>Serratia fonticola</i>	01	2.94
	<i>Serratia marcescens</i>	01	2.94
<i>Staphylococcus</i> sp.		03	8.82
Total		34	100.00

Although we did not encounter coagulase-positive *Staphylococcus*, the genus *Staphylococcus* sp. was present, making up 8.82% of the total.

The number of cockroaches found in this study may have been influenced by many different factors because, according to Soares¹ the adaptation of many species of cockroaches to homes may be due to domestic heating (in temperate countries), the lack of natural enemies in buildings and the wide availability of small shelters such as cracks and crevices in the walls of houses, especially wooden homes.

In this study, 15 insects in 120 inspections were found. The climatic variation can be explained by climatic variation during the period of collection. According to meteorological data provided by Epagri/Ciram¹⁶, the mean temperature registered in the months from June to December 2010 (the period when the study was conducted) was 17.8°C. Of the 29 cockroaches collected, 12 were collected in December, which had the highest average temperature (21.15°C) during the collection period, corroborating the conclusions made by Soares¹.

According to Lopes¹⁵, low temperatures and the lack of food are crucial in stimulating the

search for new areas of colonization for the species *B. germanica*. In addition, Oliveira² found that this insect prefers humidity and heat. Buzzi⁴ found that the optimum temperature for development and proliferation of *P. americana* is 29°C. Dutra *et al.*¹⁷ found that cockroaches prefer places with high temperatures of between 30 and 33°C.

Of the total number of cockroaches analysed in this study, none were contaminated with *Salmonella* sp., coagulase-positive *Staphylococcus* or *L. monocytogenes*. Cloarec *et al.*¹⁸ corroborated these results by analysing low-income housing and found cockroaches in 52 different homes; however, none of the samples were contaminated by coagulase-positive *Staphylococcus*.

Many of the microorganisms found in this study are deteriorating, capable of breaking down food, but some can be pathogenic to humans, or indicate the presence of pathogens, as in the case of microorganisms of the Enterobacteriaceae family. Cockroaches are no longer just a socio-economic problem; they are becoming a greater risk for public health. Some genera that are considered non-pathogenic may eventually act as opportunistic pathogens, e.g. *Citrobacter* spp., *Enterobacter*

spp., *Proteus* spp., and *Serratia* spp., among others. Many of these microorganisms, pathogenic or not, cause food spoilage¹⁹.

The genus *Citrobacter* spp. was present in 23.52% of the samples in this study; according to Felipe¹⁹, these microorganisms are frequently isolated from clinical specimens and are opportunistic pathogens that are also found in soil, water, sewage and foods.

Chaichanawongsaroj²⁰ found that in public catering establishments and residences, the species *Citrobacter* spp. were isolated from cockroaches, in addition to other genera, such as *Serratia* spp., *Enterobacter* spp., *Proteus* spp. and *Morganella* spp. The results of this study are similar to the findings of Prado²¹ who conducted a study in Ribeirão Preto/SP in which the microorganisms of the genera *Enterobacter* (55%), *Serratia* (26%), *Citrobacter* (14.5%) and *Providencia* were isolated from the cockroach species *P. americana*.

In this study, we found many species of the genus *Enterobacter*, which represented 23.52% of the samples. These naturally occurring microorganisms are found in sweet water, soil, sewage, plants, vegetables and the faeces of humans and animals²². Many species, most notably *E. cloacae*, *E. sakazakii*, *E. aerogenes*, *E. agglomerans* and *E. gergoviae*, are opportunistic pathogens at the sites of burns and wounds; they also cause urinary tract infections and occasionally septicaemia and meningitis¹⁹.

Another genus of the family Enterobacteriaceae, which represented 23.52% of the samples, was *Proteus*, which can be found in the intestines of humans and many animals, soil and polluted water²³. *Proteus* is considered a human pathogen that causes urinary tract infections²⁴ and invades secondary septicaemic lesions frequently found in burns patients¹⁹.

The presence of the genus *Staphylococcus* sp. (coagulase-negative), found in our study (8.82% of the samples), is of concern based on the results obtained in several studies^{25, 26, 27}, which demonstrated that coagulase-negative *Staphylococcus* may have genes responsible for production of toxins involved in food poisoning outbreaks.

In relation to gender, *Serratia* spp. (5.88% of isolates) does not present the risk for transmission of food-borne disease, but it has

clinical importance, since in the hospital it is an opportunistic pathogen, causing septicaemia and urinary tract diseases¹⁹. This genus occurs in water, soil, plants, insects and animals²⁸.

The genus *Flavimonas* was also isolated from the analysed samples from these cockroaches (2.94%). These microorganisms are pathogens that can infect immuno-depressed patients, especially those who undergo surgery or have indwelling venous catheters.

Other clinically important microorganisms found were bacteria from the genus *Providencia* (2.94%), which can be isolated from diarrhoea in humans, as well as from wounds, burns and bacteraemia¹⁹. *Providencia* frequently cause urinary tract infections and contribute to stone formation in the kidney; they are also related to skin lesions and diarrhoea in children²⁹.

Alcaligenes spp., which occurred in 2.94% of the samples, can be culled from the cockroaches, as well as from contact with contaminated soil and water, since these microorganisms are normally found in these environments. *Alcaligenes* is associated with both local and systemic infections, such as meningitis, pneumonia, urinary tract infections and osteomyelitis²⁹.

Acinetobacter spp., which occurred in 2.94% of the analysis performed, persist in hospital environments for long periods due the expression of virulence factors. *Acinetobacter* spp. are important causative agents of infections in ICU patients and are usually associated with lower respiratory tract infections³⁰.

The presence of other bacteria from the family Enterobacteriaceae (*Morganella* spp. and *Providencia* spp., which made up 2.94% of samples) also presents risks to public health, since these are important human pathogens that cause a variety of diseases including nosocomial infections, urinary tract infections, septicaemia and wound infections³¹.

According to Rafael³² problems with synanthropic animals can be combated by cleaning the insides and outsides of homes or businesses disposing of scraps of food and rubbish, And avoiding moist, dark, unventilated environments with many cracks where cockroaches can hide. Thus, cockroaches can have the ability to transmit diseases to humans and food spoilage-causing bacteria to food; they carry various microorganisms

of concern to the medical and food industries.

Microorganisms that belong to the family Enterobacteriaceae have been isolated from cockroaches can cause various infections. Although we did not find our microorganisms of interest in this study, it appears that these insects can be vectors of opportunistic bacteria and potential pathogens.

In this sense, the need to educate the general public about the control of these insects in residences, as well as in other places such as hospitals, schools and food services, becomes apparent.

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