Bacterial Flora Found in Common Indoor Insects

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Abstract: The most common indoor arthropods that are considered pests include cockroaches, flies, and ants. They are often associated with unsanitary conditions because they thrive in environments with high organic content such as sewage, garbage, and other decomposing material. Although these insects are very capable of hosting and transmitting bacteria, entomologists and medical professionals rarely consider them to be of medical significance. As a result, an experiment was conducted in which sticky traps were utilized to collect specimen within various buildings on Texas A&M University's campus. The collected specimens included cockroaches, house flies, fire ants, and a cricket. The specimens were then swabbed on agar plates and the plates were left for 72 hours to allow for microbial growth. In addition, different segments of the captured specimens were swabbed to determine which area of the exoskeleton contained the highest concentration of bacteria. The results indicated that all the specimens had microbial growth on their plates. However, the cockroach, *Periplaneta fuliginosa*, and cricket, *Acheta domesticus*, had the most bacterial growth out of all the specimen. In both *Periplaneta fuliginosa* and *Acheta domesticus*, the wings were the areas that showed the most microbial growth. The presence of microbes indicates that these specimens do have the potential to have medical importance because they are found in indoor environments where human activity is prevalent. The fact that most of the growth developed from their wings indicates that they could easily spread pathogens during flight by releasing it into the air.

The presence of nuisance arthropods found indoors has rarely been considered by entomologists and medical professionals to be of any medical significance. The most common indoor insects, specifically cockroach, fly, and ant species are often considered to be linked with unsanitary conditions (Cranshaw 2009). The American cockroach (*Periplaneta americana*) (Blattodea: Blattidae) is one of the most common arthropods associated with unsanitary conditions. This idea is largely due to the fact that these insects are often found in areas with high organic content, such as sewage, rotting food, and decomposing material (Atiokeng et. al 2017). In a survey of the bacterial growth of different species of cockroaches, it was found that the American cockroach is the most competent vector in terms of mechanical transmission (Bena et. Al 2018). The idea that these cockroaches can transmit bacterial and fungal infections as a mechanical vector has been proven in many studies, including in a case study in the intensive care unit of a hospital in Goiania, Brazil (Lemos et. Al 2006). In this study, it was found that the presence of cockroaches increased the rate of nosocomial *Aspergillus*

sp. And *Penicillium sp.* infections in patients. In terms of bacterial infections, a study of cockroaches in urban environments found a total of 56 bacterial species on samples collected, 14 of which are known human pathogens (Rivault, Guyader 1993). Another cause for the cockroach's medical importance is its relationship to an increase in asthma in those that are frequently exposed (Pomés, Arruda 2014). One of the greatest concerns of their transmission of bacteria is caused by their ability to aggravate asthma in those that are allergic or frequently exposed to them, which in some cases is due to the presence of bacteria such as *Streptococcus pneumoniae, Mycoplasma pneumoniae,* and others (Eggleston 2017). It has also been recorded that cockroaches are capable of carrying some species of parasites, but their ability to transmit these parasites mechanically is unknown (Atiokeng et. al 2017). Another common pest insect is the common house fly (*Musca domestica*), which has been found to transmit bacteria such as *Eschericha coli* and *Salmonella* species and parasitic species such as *Giardia lambilla* and *Dipylidium* species (Heidelberg 2019). The household ant species most commonly found indoors have also been found to transmit a plethora of bacteria, with indoor samples on average producing 75% more bacterial colonies than outdoor samples (Alharbi et al 2019).

While it has been reported in studies that 92.3% of bacterial growth on these cockroaches was found on the exoskeleton, it is not apparent of which body part has the highest growth (Austin et. Al 2007). In this experiment, the body sections were tested from various arthropods found indoors, collected from different locations across the Texas A&M University campus (College Station, TX), all of which have high levels of human activity at many times during the day. These collected samples were analyzed for their bacterial flora, along with an analysis of the congregation of bacterial growth on different body sections of the arthropod, including the legs, abdomen, antennae, and mouthparts.

Materials and Methods

Over a five-week time course, collections of indoor arthropods (consisting of *Periplaneta fuliginosa*, *Musca domestica*, *Acheta domesticus,* and *Solenopsis xyloni)* were collected using a collection of trapping methods. The first method was tape trapping, using clear sticky tape to trap the arthropod without harming the integrity of the sample. Another trapping method used was the jar trap, in which a mason jar lined with sticky tape is used to keep the samples from being damaged (Wang et. al 2019) For both trapping methods, a bait of white bread (H-E-B brand) and onion will be added, as it has been proven to increase the number of arthropods collected (Bennett 2006). These traps will be placed in dark, moist areas, as these are where the highest number of arthropods are found indoors (Kattes 2009). The traps were placed at dusk and retrieved after 8-12 hours to prevent from being tampered with by students and faculty at the university. A total of 10 traps were used (5 tape traps and 5 jar traps) and placed on varying indoor areas of campus, including the first floor of the Heep center, Medical Sciences Library, West Campus Library, Veterinary Medical Sciences Building, Wehner Building, Heldensfeld Hall, Academic Plaza, Janice and John G. Thomas Honey Bee Facility, Rosenthal Meat Science and Technology Center, and Kleberg Center. This baiting and trapping will be repeated a total of three times, each of which will be in the same locations with the same baiting conditions. The insect sample collections will then be analyzed for their bacterial flora. To do this, individual cotton swabs were used to swab the legs, mouthparts,

antennae, and abdomen of each sample that was large enough to ensure isolation. The swabs were used to streak a Hardy Diagnostics Nutrient Agar plate to determine the level of bacterial and fungal growth. These plates will be stored in an incubator at approximately 37 °C. The plates were incubated for 24 hours, then initially checked for bacterial and fungal growth. The plates were allowed to grow for a total of 3 days, with analysis of growth every 24 hours. The plates were then compared to determine which body segment has the highest microbial growth, on which species and in which building on campus.

Results

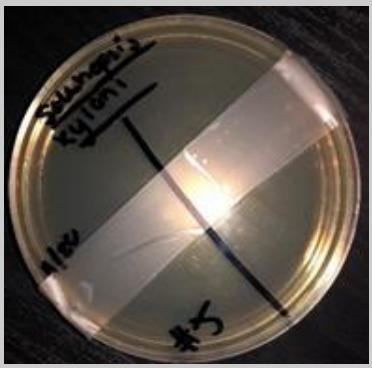
There was a total of eight plates, each streaked according to their respective species. Streaked specimen included *Acheta domesticus*, *Solenopsis xyloni*, *Musca domestica*, and *Periplaneta fuliginosa*. The two larger specimen, *Periplaneta fuliginosa* and *Acheta domesticus*, were swabbed on wings, mouthparts, legs, and head.

The plates were observed every 24 hours and pictures were taken to track the growth of each specimen (Tables 1-3).

Table 1. A comparison of growth and colony number on agar plates 24 hours after being swabbed.

Name of Species	**Pictures**	**Growth**	**Colonies**
Periplaneta fuliginosa **Smokybrown cockroach**		No	No colonies observed

Periplaneta fuliginosa **Smokybrown cockroach**		Yes	Wings: 31 circular colonies. Back legs: Two circular and one punctiform colony. Front legs: No colonies observed. Mouthparts: Two circular, one irregular, and one punctiform colony.
Musca domestica **House fly**		Specimen #1: No Specimen #2: No	Specimen #1: No colonies observed. Specimen #2: No colonies observed.
Musca domestica **House fly**		Specimen #3: No Specimen #4: No	Specimen #3: No colonies observed. Specimen #4: No colonies observed.

Solenopsis xyloni **Southern fire ant**		Specimen #1: No Specimen #2: Yes	Specimen #1: No colonies observed. Specimen #2: 9 punctiform colonies observed.
Solenopsis xyloni **Southern fire ant**		Specimen #3: Yes Specimen #4: Yes	Specimen #3: 5 punctiform colonies. Specimen #4: 9 punctiform colonies.
Solenopsis xyloni **Southern fire ant**		No	No colonies observed

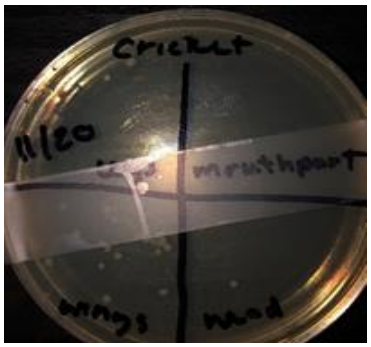
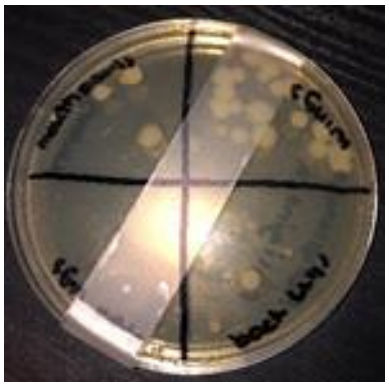
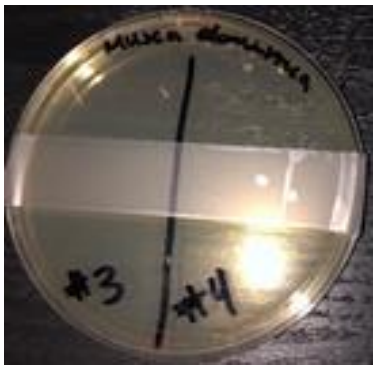
Acheta domesticus **House cricket**		Yes	Legs: Three punctiform colonies. Mouthparts: No colonies observed. Head: One punctiform colony. Wings: 23 punctiform colonies.

Table 2. A comparison of growth and colony number on agar plates 48 hours after being swabbed.

Name of Species	**Pictures**	**Growth Y/N**	**Colonies**
Periplaneta fuliginosa **Smokybrown cockroach**		No	No colonies observed.
Periplaneta fuliginosa **Smokybrown cockroach**		Yes	**Front leg**s: no colonies observed. **Back legs**: 3 flat,circular colonies. **Wings**: 15 circular colonies with 1 dark growth. **Mouthparts**: 3 irregular, flat colonies and 4 punctiform colonies.

Musca domestica **House fly**		Specimen #1: No Specimen #2: No	Specimen #1: No colonies observed. Specimen #2: No colonies observed.
Musca domestica **House fly**		Specimen #3: No Specimen #4: Yes	Specimen #3: No Specimen #4: Two punctiform colonies.
Solenopsis xyloni **Southern fire ant**		Specimen #1: No. Specimen #2: Yes.	Specimen #1: No colonies observed. Specimen #2: 10 circular colonies and 14 punctiform colonies.

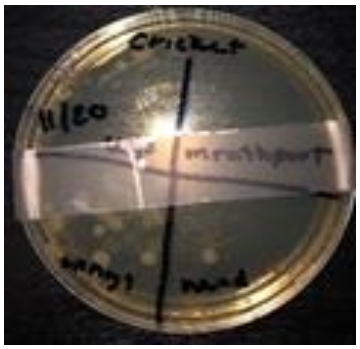
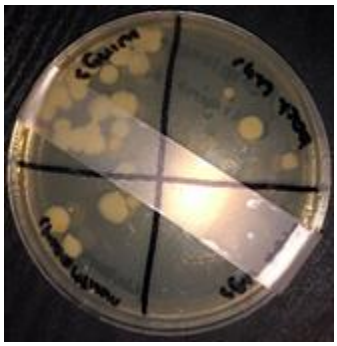
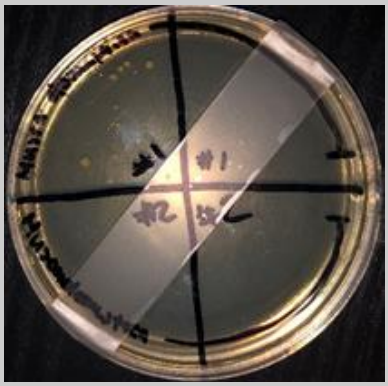
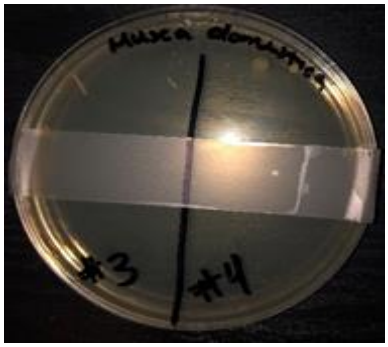
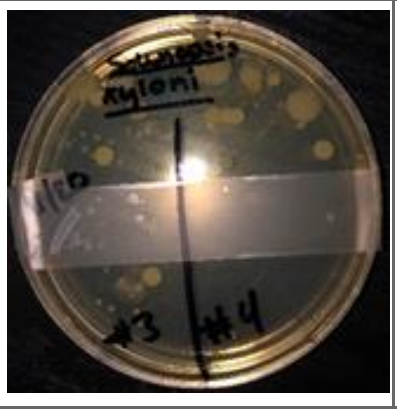
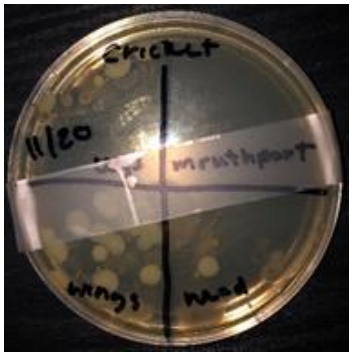
Solenopsis xyloni **Southern fire ant**		Specimen #3: Yes Specimen #4: Yes	Specimen #3: 5 circular colonies and 14 punctiform colonies. Specimen #4: 12 circular colonies and 11 punctiform colonies.
Solenopsis xyloni **Southern fire ant**		No	No colonies observed.
Acheta domesticus **House cricket**		Yes	Mouthparts: No colonies observed. Wings: 17 flat, circular colonies. Head: One circular colony and two punctiform colonies. Legs: Three circular colonies and one irregular colony.

Table 3. A comparison of growth and colony number on agar plates 72 hours after being swabbed.

Name of Species	**Picture**	**Growth Y/N**	**Colonies**
Periplaneta fuliginosa **Smokybrown cockroach**		No	No colonies observed.
Periplaneta fuliginosa **Smokybrown cockroach**		Yes, but no growth on the front legs	Wings: 24 circular and one punctiform colony.Back legs: 4 circular and two punctiform colonies. Front legs: One punctiform colony. Mouthparts: 4 circular and 4 punctiform colonies.

Musca domestica **House fly**		Specimen #1: Yes Specimen #2: No	Specimen #1: 9 punctiform colonies. Specimen #2: No colonies observed.
Musca domestica **House fly**		Specimen #3: No Specimen #4: Yes	Specimen #3: No colonies observed. Specimen #4: One circular and one irregular colony.
Solenopsis xyloni **Southern fire ant**		Specimen #1: Yes Specimen #2: Yes	Specimen #1: Two circular colonies. Specimen #2: 32 circular colonies.
Solenopsis xyloni **Southern fire ant**		Specimen #3: Yes Specimen #4: Yes	Specimen #3: Six circular and 22 punctiform colonies. Specimen #4: 12 circular colonies and 16 punctiform colonies.

Solenopsis xyloni **Southern fire ant**		No	No colonies observed.
Acheta domesticus **House cricket**		Yes	**Wings**: 15 circular colonies observed. **Legs**: 8 circular colonies with dark growth found in 4 of them. **Body**: 1 circular colony with 2 punctiform colonies and 3 fuzzy colonies. **Mouthparts**: No colonies observed.

After 24 hours, five plates had grown bacterial colonies out of twelve specimens plated. This number rose to six after 48 hours and to eight after 72 hours.

Every plate had growth within 72 hours with the exception of one *Solenopsis xyloni* (#5), one *Periplaneta fuliginosa*, and two *Musca domestica* (#3) plates. This represented 4 out of 12 specimens swabbed. Plates with little growth (characterized as less than 5 colonies after 72 hours) included *Solenopsis xyloni* (#1) and *Musca domestica* (#4). The plate with moderate growth (characterized as 5-15 colonies after 72 hours) was *Musca domestica* (#1) with nine colonies. The remaining plates all had over 25 colonies on the plate after 72 hours. *Solenopsis xyloni* (#2, 3, and 4), *Acheta domesticus*, and *Periplaneta fuliginosa*. The latter two specimen were large enough to have different body segments swabbed and individually plated. For these two plates, the number of colony growths on the wings outnumbered colonies on every other segment, followed by legs, then mouthparts. Between both species, *Periplaneta fuliginosa* had more growth overall, with 40 colonies after 72 hours compared to *Acheta domesticus* having 29 colonies.

Between specimens of the same species, the largest difference was between both *Periplaneta fuliginosa*. One plate of this species had the most growth of all specimens while the other had no growth. Between *Solenopsis xyloni*, specimens #1 and #5 had little and no growth, respectively, while specimens #2, #3, and #4 had over twenty-five colonies on each plate. Between *Musca domestica*, specimens #2 and #3 had no growth, #4 had two colonies, and #1 had nine colonies, although all nine were small, punctiform colonies.

Discussion

After reviewing the results of the agar plates streaked with microbes from *Periplaneta fuliginosa* specimens, it is immediately apparent that the "Smokybrown cockroach" species commonly carries various different bacteria species, as the agar plates clearly cultivated multiple bacterial colonies. This initial observation opposes the aforementioned notion that cockroaches do not vector clinically significant pathogens, and therefore are not of medical importance. Aside from the question whether or not the specimens transmit various medically significant pathogens, this study was posed at discerning which body parts of said specimens had the largest amount of microbial growth. After observing the cultures taken from the 6 cockroach specimens, it is evident that the wings contain far more bacterial populations than any other region of the specimen, such as the mouthparts or the legs. The significance of this observation revolves around the correlation between high levels of exposure to cockroaches and the exacerbation of pre-existing asthma conditions. One could speculate that the increased concentration of bacteria and other microorganisms on the wings of the cockroach specimens is to blame for the aggravation of asthma in some individuals. This could potentially be due to the fact that some species of cockroaches, such as the *Periplaneta fuliginosa* that was observed in the experiment, are capable fliers, and therefore could spread these microorganisms around a house or building as they flap their wings, although it must be noted that most species of cockroaches do not fly. A second species that was swabbed, streaked and cultivated was the house fly, otherwise known as *Musca domestica*. Out of the 12 *Musca domestica* specimens observed, 9 of the specimens produced no microbial growth after cultivation. For the 3 specimens that produced microbial growth, such growth was predominantly limited to several small punctiform colonies. Although these housefly specimens were observed to vector a smaller number of bacteria than the cockroach specimens, the specimens cultivated bacterial growth nonetheless, and therefore should be considered medically significant. As for the concentration of bacteria on the specimens' various body regions, the specimens were deemed too small to be able to accurately swab specific body regions, and the results do not reflect any specific body region of *Musca domestica*.

The *Solenopsis xyloni* (southern fire ant) had intriguing discoveries. In the first 24 hours, only three of the five specimens collected had bacterial growth of more than five punctiform colonies. The first and fifth specimens had no bacterial growth. At the 48 hour interval, specimens 2, 3, and 4 showed growth in the previous punctiform colonies turned to circular colonies as well as developing new punctiform colonies However, at the end of the 72 hour interval, specimen 1 had grown circular colonies with all the rest of the specimens with the exception of specimen 5. It was expected for all specimens to experience some growth throughout the 72-hour period; Except, we experienced no growth from specimen 5 which was interesting and could have to do with the location that the specimens were collected at. Varying locations are exposed to different types of bacteria so it could have had an effect on the different rates of growth of bacteria.

Although *Solenopsis xyloni* is too tiny to swab exact body parts for the most bacterial growth, this intriguing discovery of the delayed and continuous bacterial growth can have significant medical significance. SInce *Solenopsis xyloni* is a microscopic species that is attracted to any potential food

source, it would be simple for them to navigate through any crack and crevice into households or buildings. Therefore, they can easily spread bacterial species- whether benign or dangerous- within three days.

The *Acheta domesticus* (house cricket) is an interesting discovery of the amount of bacterial growth on its different body parts in a relatively progressive time span. Within 24 hours, it had the most punctiform colonies on the wings (twenty-three punctiform colonies) while the legs and head altogether had only four punctiform colonies. At the 48 hour interval mark,the legs had no significant bacterial growth. In contrast, the head developed one circular colony and 2 punctiform colonies and the wings had seventeen flat circular colonies. However, by the 72-hour mark, the legs had gained eight circular colonies with dark growth within. Most interestingly, the body of the cricket had fuzzy colonies, which indicates fungal growth. This indicated that crickets were capable to be hosts of both bacterial and fungal growth- both that could be potentially dangerous pathogens.

Acheta domesticus mouthparts intriguingly did not have any bacterial growth indicated from the sample swabs. It was hypothesized that the mouthparts would have the most bacterial growth, yet it was actually the wings that had the most bacterial colonies and growth. This may be explained that the wingspans of crickets have more surface area than its mouthparts to pick up any bacterial species. Although *Acheta domesticus* isn't known for biting, it still could make any contact into any residential housing or human occupancy area.

A flaw that should be mentioned is that only one cricket specimen was examined in this experiment. Therefore, it would not be concise to utilize this result as an accurate representation for the cricket population within College Station boundaries. However, the fact that bacterial and fungal growth was found does indicate enough medical significance to prove that it is possible that both microorganisms have the capability to thrive on crickets.

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