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The Prevalence Of Antibiotic-Resistant Bacteria On Mosquitoes Collected From A Recreational Park

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Abstract

It is well known that a mosquito bite results in a puncture wound in the host's skin. This wound represents a portal of entry for various infectious agents, including several viruses known to cause illness in humans. However, the role of mosquitoes as vectors of harmful bacteria is unknown. Furthermore, the increasing prevalence of antibiotic resistant bacteria in the natural environment warrants research investigating the carriage of harmful antibiotic-resistant bacteria by mosquitoes. Pools (n=57) of 10 mosquitoes each were collected from a popular recreational park with a carbon dioxide trap. Each pool was homogenized in peptone water and assayed for the presence of antibiotic-resistant bacteria by enumeration on blood agar containing one of five antibiotics (ampicillin, tetracycline, ciprofloxacin, triclosan, and oxacillin). Approximately 91% (52) of the pools contained at least one antibiotic-resistant bacterium, while 29% (13) contained more than 200 bacteria resistant to at least one antibiotic. Approximately 70% of the pools contained bacteria resistant to multiple antibiotics. Resistance was most prevalent toward triclosan, with 77% (44) of pools harboring resistant bacteria. Our results show that mosquitoes represent potential vectors of large populations of antibiotic-resistant bacteria. Genetic analyses of mosquito homogenates are ongoing to determine the identity of the bacteria and therefore the likelihood that mosquitoes are also vectors of human bacteria pathogens.

Keywords: Antibiotic-Resistant Bacteria, Mosquitoes, Recreational Park

1. Introduction

Antibiotic resistant bacteria have existed in the environment for many years, but have become an increasingly important issue. This is a direct result of two factors, including (i) limited progress in the development of novel antibiotics, and (ii) the continued acquisition of antibiotic resistance by bacteria[1]. As the prevalence of community-associated infections increases, it becomes crucial to identify the reservoirs of bacteria that represent a public health threat. While it is established that a mosquito bite represents a portal of entry for viruses found in mosquito saliva[2], the role of mosquitoes in the transmission of bacteria, especially antibiotic resistant bacteria, is unknown. A female mosquito requires a single bloodmeal in order to lay her eggs. Since the average female mosquito only lives long enough to lay up to three clutches of eggs, it follows that a mosquito serving as a reservoir for pathogenic bacteria could potentially transmit the bacteria to human hosts up to three times[3]. While the rate of carriage of antibiotic resistant bacteria by mosquitoes is unknown, it could represent an important link between environmental reservoirs of harmful bacteria and human health. Furthermore, understanding the relationship between mosquitoes as vectors of potentially harmful bacteria, and the bacteria they harbor can provide information about the ecology of potential pathogens. Therefore, the objectives of this study were to evaluate the carriage rate of antibiotic susceptible and resistant bacteria, including MRSA, on common woodland mosquitoes as well as to determine the community structure of bacteria associated with mosquitoes.

2. Materials and Methods:

2.1 mosquito collection and processing:

On six nights between August 25th and September 29th, 2009 mosquitoes were collected from a wooded area adjacent to a recreational park. Carbon dioxide was used as a mosquito attractant by hanging blocks of dry ice near a battery-powered fan that was used force mosquitoes into a fine mesh collection net. Traps were deployed prior to sunset and allowed to run overnight. The following morning, the net was sealed and transported to the lab where the mosquitoes were immediately separated into pools of ten mosquitoes each. Each pool was homogenized in a 1.5 mL microfuge tube and resuspended in 1.5 mL of peptone water.

2.2 culture conditions:

An aliquot (50 µl) of each homogenate was inoculated onto plain blood agar and blood agar containing an antibiotic to a concentration of 25 µl ml^{-1}. The antibiotics were chosen because of their common usage and included ampicillin, tetracycline, triclosan, and ciprofloxacin. To prevent fungal growth, all blood agar contained the antifungal agent cyclohexamide. ORSAB (oxacillin resistance screen agar base) was also inoculated with the homogenate to screen for the presence of methicillin resistant Staphylococcus aureus (MRSA). All media were incubated at 37˚ C for 48-72 hours following which bacteria colonies were counted.

2.3 DNA isolation, PCR analysis, and denaturing gradient gel electrophoresis (DGGE) analysis:

DNA was isolated from two mosquito pools from each collection date using beat beating and phenol/chloroform extraction[4]. The isolated DNA was then subjected to PCR analysis with primers BAC 341f and BAC 534r targeting the 16S rRNA gene under the following reaction conditions: denaturation- 94ºC for 45", annealing- 55ºC for 45", and elongation- 72º for 45". The reaction concluded with a final extension step of 72º for 6 min. The amplified community DNA was then subjected to analysis by DGGE, during which the PCR-amplified DNA is separated in a gradient of chemical denaturant based on sequence differences among the bacteria in the community. This analysis results in banding patterns that represent the different bacteria present in the communities and can be compared to determine similarities and differences among the mosquito pools. The pools were subjected to DGGE analysis on a 40%-60% Bis-Acrylamide gel, for 16 hours at 60V, and post-stained with SYBR® Green nucleic acid stain.

3. Results:

3.1 enumeration of antibiotic resistant bacteria:

A total of 57 pools of ten mosquitoes each were collected during the study and over 91% of these were found to contain bacteria resistant to at least one antibiotic. In total approximately 14,000 antibiotic resistant bacteria were isolated and on average approximately 749 bacteria were isolated from each mosquito. Moreover, 10 pools contained bacteria resistant to four of the five antibiotics tested, and 1 pool contained bacteria resistant to all five antibiotics tested. Bacteria isolated from the mosquitoes exhibited varying levels of resistance to the antibiotics tested. Resistance to ampicillin and triclosan was most prevalent among the pools, as 5,658 and 4,980 colonies were resistant, respectively. Additionally, approximately 1,962 and 1,365 bacterial colonies were found to be resistant to ciprofloxacin and tetracycline, while resistance to oxacillin was the least prevalent with 246 colonies recorded (Figure 1). The majority of antibiotic resistant bacteria detected were resistant to ampicillin and triclosan (Figure 1), while more mosquito pools were found to contain bacteria resistant to triclosan (44) than to any other antibiotic (Figure 2).

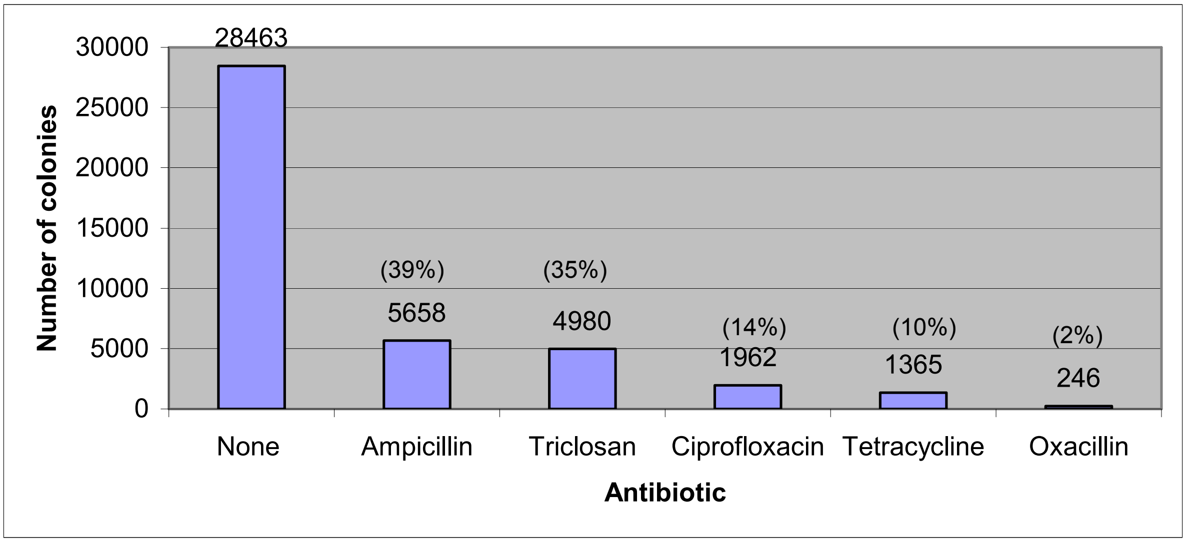

Figure 1: Quantification of antibiotic resistant bacteria isolated from mosquitoes.

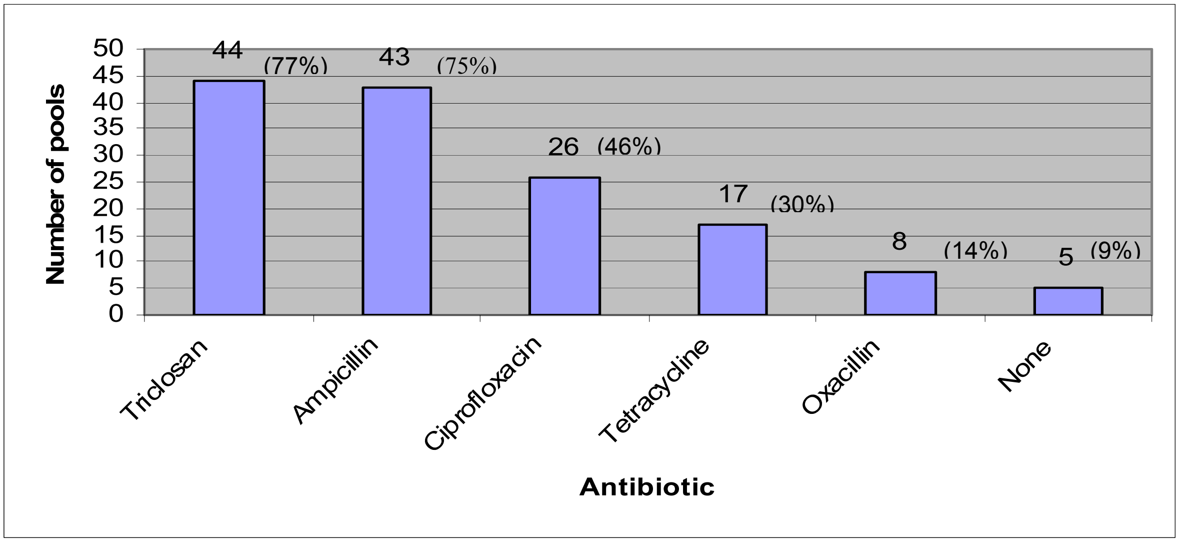

Figure 2: The number of pools containing bacteria resistant to each antibiotic.

3.2 denaturing gradient gel electrophoresis

DGGE analysis of two DNA pools from each mosquito collection (12 samples) revealed that the produced banding patterns were similar among all samples as seen in Figure 3.

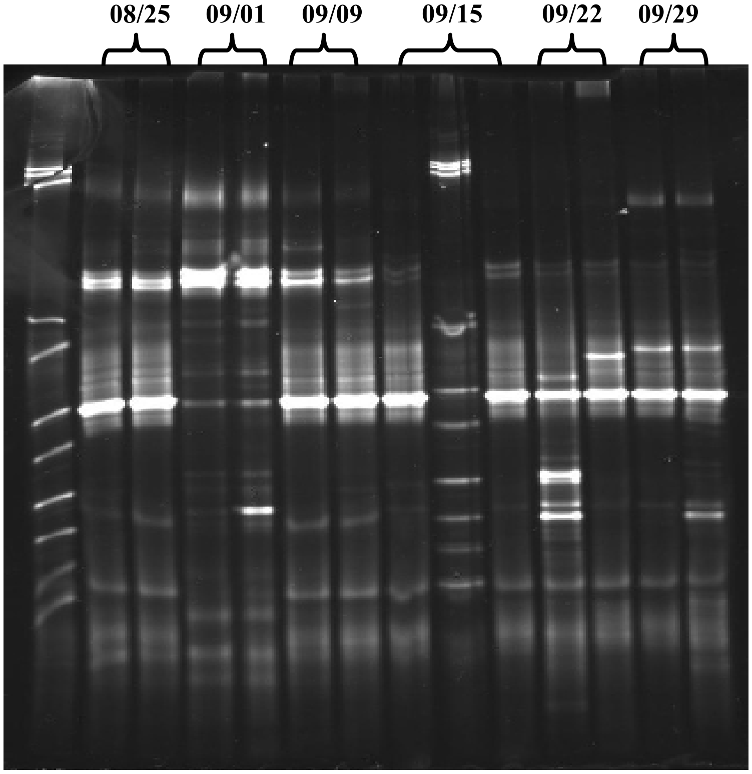

Figure 3: Image of DGGE analysis using two samples for each collection date.

4. Discussion:

As the threat to public health of antibiotic resistant bacteria increases, it becomes increasingly important to identify potential reservoirs of such microbes. This action can provide the public with a better understanding of how antibiotic resistant bacteria are transmitted and also how people can better protect themselves. Since mosquitoes have been shown to harbor and transmit pathogens such as viruses, the objective of this study was to determine (i) if woodland mosquitoes are also carriers of bacteria resistant to five common antibiotics including ampicillin, tetracycline, triclosan, ciprofloxacin, and oxacillin and (ii) the structure of the bacteria community associated with mosquitoes.

The majority of mosquito pools (91%) assessed in this study contained bacteria resistant to at least one antibiotic. With the amount of antibiotic resistant bacteria in the environment on the rise, it makes sense that woodland insects such as common mosquitoes could potentially come into contact with resistant bacteria. The high prevalence of resistant bacteria is not surprising considering that mosquitoes are known to “swarm”, forming dense “clouds” of mosquitoes during mating rituals. This activity could facilitate cross contamination of bacteria between mosquitoes, thereby increasing the numbers of pools exhibiting detectable levels of resistant bacteria.

Our results showed that of the 57 mosquito pools tested, 43 (75%) pools contained mosquitoes resistant to triclosan and ampicillin. This high prevalence can be partially explained by noting that ampicillin resistance is already widespread throughout the natural environment, mostly as a result of its historic use for treatment of bacterial infections. Additionally, triclosan is widely used in many commercial products, including soaps and lotions. In the cases of both ampicillin and triclosan, the principle route of environmental exposure is through the sanitary sewer system. Disposal creates frequent opportunities for environmental bacteria to adapt and acquire antibiotic resistance mechanisms.

DGGE analysis revealed strong similarities among the bacteria communities associated with twelve mosquito pools collected throughout a one-month period. This result is contrary to the expected results, as environmental microbial assemblages are known to be generally heterogeneous and diverse. The nesting and swarming behavior of mosquitoes likely contributed to the homogeneity observed in this experiment. Furthermore, common habitats and hosts shared by mosquitoes can also promote similarity among bacteria assemblages associated with mosquitoes. The results of the DGGE analysis will be supplemented by sequencing DNA extracted from bacterial isolated from each pool, as well as from bands excised from the DGGE gel. Identifying the bacteria detected in this study will facilitate further assessment of the public health risk that mosquitoes pose while revealing the ecology surrounding the relationship between bacteria and mosquitoes.

Our methodology appeared to reveal important and detailed information about the carriage of bacteria, including antibiotic resistant bacteria, by mosquitoes. First, the process of collecting mosquitoes is best facilitated by dry-ice traps, as the trapping capacity of traditional mosquito light powered traps is increased by 400-500% when coupled with baiting through the use of dry ice[5]. The sublimation of dry ice results in the emission of CO_2, which mimics the respiration of CO_2 by humans and represents the mechanism by which mosquitoes use to detect humans. Tryptic soy agar was used as the primary isolation media as it provides nutrients necessary to support the growth of diverse bacteria [4] and can be enriched with whole blood to facilitate the growth of an even wider range of bacteria. Finally, a goal of our study was to establish fundamental knowledge about the relationship between bacteria assemblages and the mosquito. Therefore, DGGE analysis was used to graphically show changes in bacteria communities associated with the mosquito pools throughout the one-month sampling period. DGGE is a useful tool for comparing communities of bacteria[5] and our results suggest that the communities of bacteria associated with mosquitoes are consistent over time.

5. Conclusion:

After culturing 57 different pools of mosquitoes it was found that 52 pools (91%) contained resistant bacteria. It was also shown that of the five antibiotics tested, the greatest number of bacteria were resistant to ampicillin, followed by triclosan, while bacteria were most susceptible to tetracycline and oxacillin. Finally our data revealed, for the first time, the consistency in structure among communities associated with mosquitoes. Taken together, these data provide useful information on which future analyses to assess transmission rates and geographic differences can be based.

6. References

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