International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 31-07-2025, Accepted: 31-08-2025, Published: 15-09-2025
Volume 10, Issue 9, 2025, Page No. 55-58

Isolation and identification of bacterial colonies from the gut of cockroach (*Periplaneta americana*), slender ant (*Tetraponera* spp.*)* and mosquito (*Culicidae* spp.)

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Abstract

Insects such as cockroaches (Periplaneta americana), slender ants (Tetraponera spp.), and mosquitoes (Culicidae spp.) serve as vectors for a variety of pathogenic microorganisms that can affect human health. This study investigated the morphological identification and quantitative enumeration of bacterial colonies isolated from the guts of these insects. Gut samples were collected from various locations and processed according to ISO standard protocols, which included surface sterilization, gut dissection, and homogenization. Culturing was performed using Nutrient agar to support general bacterial growth and MacConkey agar to isolate Gram-negative enteric bacteria selectively. The isolated colonies were further characterized by Gram staining and detailed examination of colony morphology. Results indicated that while all three insect types harbored diverse bacterial communities, the mosquito gut exhibited a particularly high bacterial load. These findings highlight the potential of targeting gut bacteria as a biocontrol strategy to mitigate the insects' role in disease transmission.

Keywords: Bacterial colonies, gut of insects, cockroach, slender ant, mosquito, morphological identification, pathogenic microorganisms

Introduction

Insects comprise over 80% of known animal species and occupy nearly every ecosystem, playing significant roles in processes like pollination, decomposition, and nutrient cycling. However, many insect species also act as reservoirs for various pathogenic bacteria that pose health risks to humans and animals. The gut microbiota of insects not only contributes to digestive and metabolic processes but can also influence the transmission of diseases. In this context, understanding the diversity and dynamics of gut bacteria is crucial. Many insects, including cockroaches, slender ants, and mosquitoes, host a range of bacterial genera such as *Escherichia coli, Klebsiella spp., Enterobacter spp.*, and others that can be either commensal or pathogenic. This study aimed to isolate, culture, and identify bacterial species from the insect gut, compare the bacterial diversity among these species, and determine the potential implications for insect physiology and pathogen transmission.

Materials and Methods

Specimens for this study were collected from three distinct locations: cockroaches from St. Xavier's Hostel in Palayamkottai, slender ants from Keelapattam in Tirunelveli, and mosquitoes from Sathankulam in Tuticorin. Insects were collected in sterile containers and immediately transported under refrigerated conditions to avoid alterations in microbial populations. Upon arrival, insects underwent surface sterilization with 70% ethanol, followed by washes in sterile saline. Using aseptic techniques under a dissecting microscope, the gut was excised and homogenized in sterile saline solution. The homogenized samples were then serially diluted (ranging from 10^{-1} to 10^{-6}) to facilitate the isolation and enumeration of bacteria. Three different media were employed: Nutrient agar for general microbial growth, Nutrient broth for suspension cultures, and MacConkey agar for the selective isolation of Gram-negative bacteria. Standard microbiological techniques, including the spread plate and streak plate methods, were used to obtain discrete colonies, which were then incubated at 37°C for 24–48 hours. Colony morphology was evaluated based on characteristics such as size, pigmentation, shape, margin, elevation, and texture. Finally, Gram staining was performed on purified colonies to differentiate between Gram-positive and Gram-negative bacteria, with particular attention paid to the motility features of the isolates.

Results

Colony-forming units (CFU) were counted after 24 hours of incubation. For cockroaches, dilution series revealed colony counts up to 7.0×10^7 CFU/mL, whereas slender ants showed lower counts—with some dilutions being too low to count—and mosquitoes exhibited high bacterial loads, with counts reaching up to 5.3×10^7 CFU/mL. Detailed cultural characterization showed that bacterial colonies from cockroach guts were diverse in appearance, exhibiting circular and irregular shapes with colors ranging from white and creamy to red and yellow, and textures that were mucoid or sticky. In slender ants, colonies were either circular or irregular with a wide color spectrum including pink, red, and bluish green, while those from mosquitoes were primarily smooth, moist, and slimy. Using MacConkey agar, four distinct Gram-negative bacterial species were isolated and identified: *Escherichia coli* (characterized by pink/red, circular colonies with entire margins and a raised, smooth texture), *Proteus spp.* (displaying colourless, irregular colonies), *Klebsiella spp.* (noted for pink, mucoid, convex colonies with smooth edges), and *Shigella spp.* (with pale, smooth circular colonies). Gram staining confirmed that all these isolates were Gram-negative rods, with *E. coli* and *Proteus spp.* being motile, and *Shigella spp.* and *Klebsiella spp.* non-motile.

Results

The number of bacterial colonies obtained from the gut of sample insects, cultured in nutrient agar medium are tabulated below:

Table 1: Colony count data of cultured bacterial colonies from gut of insects

S. No.	Incubation time (in hours)	Gut of insect	Concentration	No. of bacterial colonies (CFUs/ml)
1.	24	Cockroach	10^{-1}	TNTC
2.	24		10^{-2}	TNTC
3.	24		10^{-3}	1.65×10^{6}
4.	24		10^{-4}	1.21×10^{7}
5.	24		10^{-5}	7.0×10^{7}
6.	24	Slender ant	10^{-1}	TNTC
7.	24		10^{-2}	7.6×10^{3}
8.	24		10^{-3}	4.7×10^{4}
9.	24		10^{-4}	TLTC
10.	24		10^{-5}	TLTC
11.	24	Mosquito	10^{-1}	TNTC
12.	24		10^{-2}	TNTC
13.	24		10^{-3}	TNTC
14.	24		10^{-4}	1.03×10^{7}
15.	24		10^{-5}	5.3×10^{7}

Fig – 11

Fig – 12

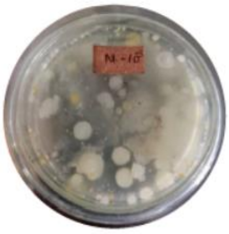


Fig – 13

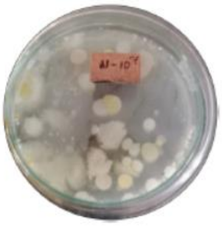


Fig – 14

Fig – 15

Fig 11 to 15: Growth culture of Cockroach's gut bacteria in Nutrient agar medium

Fig – 16

Fig – 17

Fig 16 & 17: Growth culture of slender ants' gut bacteria in Nutrient agar medium

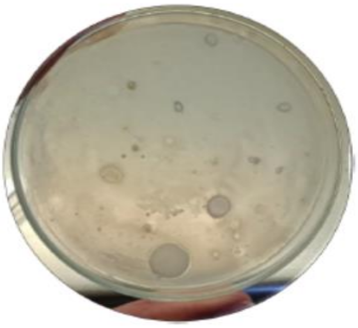


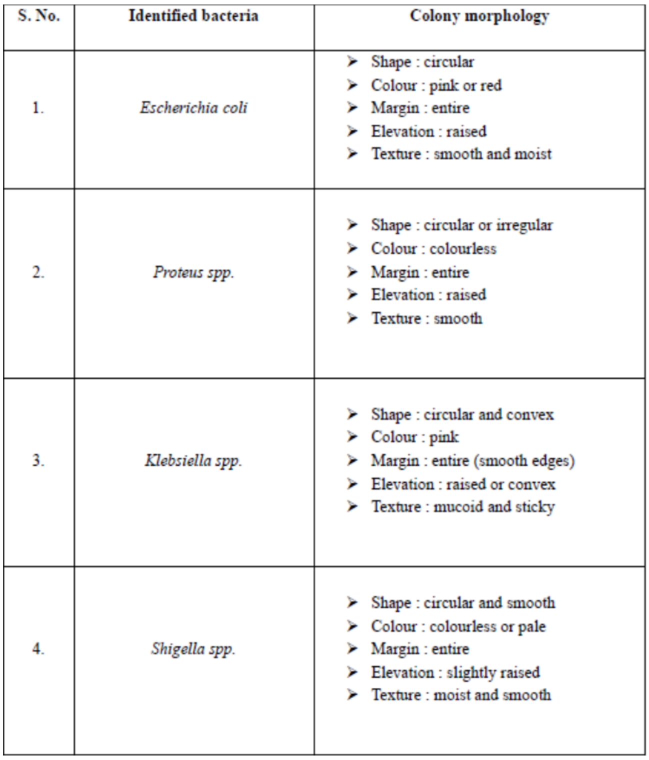
Fig – 18

Fig – 19

Fig 18 & 19: Growth culture of Mosquitoes' gut bacteria in Nutrient agar medium

Isolated and identified bacterial colonies in MacConkey agar medium

Table 3: Cultural characterization of isolated and identified bacterial colonies in MacConkey agar medium



S. No.	Identified bacteria	Colony morphology
1.	*Escherichia coli*	➢ Shape : circular ➢ Colour : pink or red ➢ Margin : entire ➢ Elevation : raised ➢ Texture : smooth and moist
2.	*Proteus spp.*	➢ Shape : circular or irregular ➢ Colour : colourless ➢ Margin : entire ➢ Elevation : raised ➢ Texture : smooth
3.	*Klebsiella spp.*	➢ Shape : circular and convex ➢ Colour : pink ➢ Margin : entire (smooth edges) ➢ Elevation : raised or convex ➢ Texture : mucoid and sticky
4.	*Shigella spp.*	➢ Shape : circular and smooth ➢ Colour : colourless or pale ➢ Margin : entire ➢ Elevation : slightly raised ➢ Texture : moist and smooth

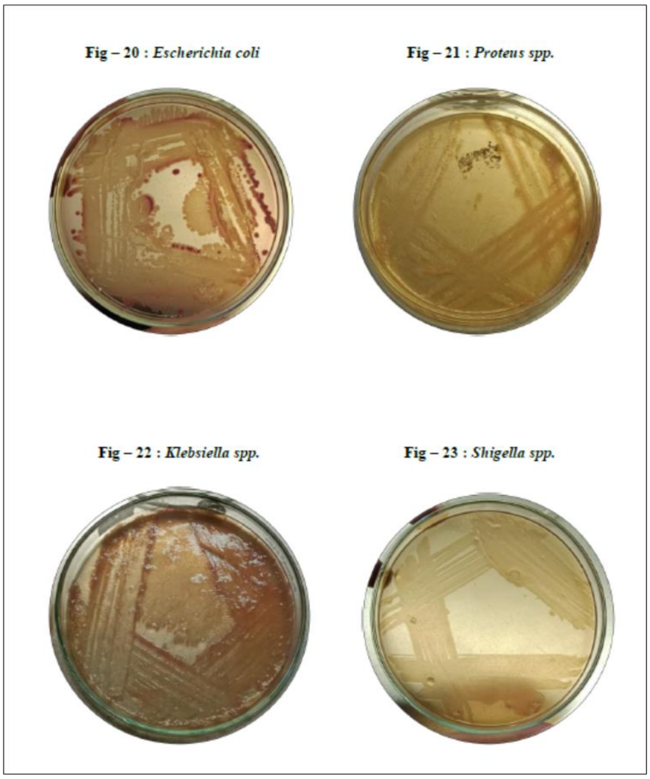


Fig – 20 : *Escherichia coli*

Fig – 21 : *Proteus spp.*

Fig – 22 : *Klebsiella spp.*

Fig – 23 : *Shigella spp.*

Fig 20 to 23: Pure bacterial colonies cultured in MacConkey agar medium

Discussion

The study demonstrates that the insect gut microbiota is highly diverse and variable among different insect species. The presence of a wide range of bacterial colonies in cockroaches, slender ants, and mosquitoes underscores the ecological adaptability and complexity of these microbial communities. In particular, the elevated bacterial counts in mosquitoes suggest that they might serve as a significant reservoir for pathogenic bacteria, thereby enhancing their role as disease vectors. The isolated bacteria, which included several members of the Enterobacteriaceae family, may play roles in digestion, nutrient acquisition, or immune modulation within the insect host. However, they can also pose a public health risk by potentially transferring pathogens to human environments. The differential use of selective media such as MacConkey agar and the confirmatory Gram staining allowed for the accurate identification of Gram-negative enteric bacteria. These insights lay the groundwork for future studies aimed at exploring gut microbiome modulation as a novel strategy for insect biocontrol and disease prevention.

Conclusion

The overall implications of the study provide insight into insect-microbe interactions and their ecological roles. The presence of potentially pathogenic bacteria underscores the need for public health awareness regarding insect-borne diseases. Further research could explore the antibiotic resistance patterns and functional roles of gut microbiota in these insects.

This experiment contributes to understanding the microbial ecology of insects and them impact on both environmental and human health. Biocontrol strategies that target the gut microbiome of insects tends to reduce their survival, reproduction, and ability to spread diseases. Disrupting the gut bacteria can lead to insect mortality, weakened immune systems, or reduced vector competence. *Bacillus thuringiensis israelensis (Bti)* produces endotoxins that destroy mosquito larvae' gut cells, leading to death. Engineered bacteria expressing antipathogen molecules can reduce vector competence. By disrupting the insect gut microbiome through gut – gut-disrupting probiotics, genetically modified bacteria expressing toxins, and engineered microbes, researchers can reduce pest survival, reproduction, and disease transmission.

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