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Isolation and Molecular Characterization of Microflora from the Midgut of *Armigeres Subalbatus* Species Collected from Buldhana District, India.

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Abstract

Mosquitoes are of great importance to human health. A number of studies have shown that midgut and salivary gland microflora have an impact on malaria parasite burden through colonization mechanisms, involving either direct Plasmodium microbiota interaction or bacterial-mediated induction of mosquito immune response. The objective of this study was to isolate and identify the microflora from the midgut of *Armigeres subalbatus* species. A total of 10 field-collected adult Armigeres mosquitoes were anesthetized by chloroform and dissected. 70% of ethanol was used for surface sterilization of mosquitoes and laboratory equipment, followed by rinsing Armigeres mosquitoes two times with 1X PBS. Individually dissected midguts were transferred to 1.5ml Eppendorf tube containing 100µl of phosphate buffered saline (PBS) and homogenized. Gut homogenate was serially diluted (10 folds) in PBS and was directly pour plated on sterile nutrient agar media for 24 h at 35 ± 2 °C. From all field collected adult *Armigeres subalbatus* mosquitoes, total 26 bacterial isolates were obtained. From a total of 26 identified microflora, *Bacillus cereus* was the most abundant microbiota comprised 34.61% of the total species isolated. *Pseudomonas aeruginosa* was the second most abundant microbiota comprised 23.07% of the total species isolated. *Staphyllococcus epidermidis* was found to comprise 19.23% of total isolates. *Pseudomonas geniculate* & *Serratia marcescens* were found to constitute 11.53% each of the total identified microflora. The results of present investigation are quite encouraging & it can be used as a baseline for studying the relationship between microbiota and mosquitoes, and for the development of a new biological control.

Keywords: Mosquitoes, Armigeres subalbatus, Microbiota, Identification.

INTRODUCTION

Mosquitoes transmit a wide range of pathogens that cause diseases in humans and other animals. All mosquito species are aquatic during the immature stages [1]. Studies conducted in the early 1900s noted that larval and adult stages of mosquitoes harbor extracellular microbes in their digestive tract [2,3]. During the last 10 years, their roles for mosquito biology have been broadly studied. Some findings emphasize that they play important roles in immunity, food digestion, fertility, and fecundity, which ultimately affect larval growth, adult fitness, vector populations, and disease prevalence [4]. Besides, these studies evidenced positive and negative effects of these gut microbial communities on vector competency through host-parasite interaction [5,6], thereby significantly influencing disease transmission potential [7,8]. Moreover, mosquito-midgut bacteria are used as vehicles to express molecules in the gut and suppress parasite colonization [9,10]. This

technique is known as paratransgenesis, which emerged as a novel vector controlling strategy. Here, the symbiotic bacteria associated with vectors may genetically modify for the expression of an effector molecule and reintroduced through food to the mosquito midgut.

Studies conducted to isolate and identify bacterial species in field-collected Anopheles and Aedes mosquitoes using microbe culturing techniques reported the presence of a wide range of bacterial taxonomic groups in the midgut [11,12]. Similarly, a wide range of bacterial microflora including *Pseudomonas cepacia*, *Entrobacter agglomerans* and Flavobacterium species were identified in the midgut of three laboratory-reared Anopheles mosquito species [13]. Furthermore, the gut microflora varied depending on the sugar and blood feeding status of mosquitoes with reduced susceptibility of these mosquitoes to parasite development [13]. Moreover, midgut microfloral diversity depends on the ecological niche and geographical locations of vector mosquitoes. Straif and his colleagues [14] identified *Enterobacter agglomerans* and *Escherichia coli* as the most frequently isolated bacteria from midgut of field collected *An. gambiae* and *An. funestus* mosquitoes in Kenya and Mali.

The identification of suitable midgut bacteria is the fundamental requirement in the paratransgenic techniques. These identified organisms should be able to colonize in the midgut of an interested disease vector and should be transferred to the next generation through transstadial passage [15]. The number of recent studies has used culture dependent and culture-independent approaches to characterize the microbial communities in the midgut of different mosquito species including Aedes, Culex, Anopheles, Coquillettidia, and Mansonia [16–21]. However, little is known about the midgut microflora of Culex mosquitoes, and very few studies have been conducted on midgut microbiota [15,22,23]. In addition, no studies have been carried out to screen the midgut bacteria in *Mansonia annulifera* mosquitoes.

The midgut microflora of mosquitoes influence mosquito physiology, and also significantly alter vector competence [24]. As parts of the digestive system, the salivary glands harbor fewer bacterial microflora than the midgut of the mosquitoes [25]. However, Sharma et al. [26] showed that the salivary glands harbor more diverse microbial communities than the midgut in *An. culicifacies*. Along with ecological factors such as sugar feeding, blood meals drastically alter mosquito gut microbial composition; these blood-fed midguts are enriched with Pseudomonas species [27].

These microbes in the gut of blood-fed mosquitoes may provide the additional genetic capacity to cope with oxidative stress due to catalase, manganese superoxidase dismutase, superoxide dismutase (Fe), heme oxygenase, alkyl hydroperoxide reductase (AhpC), and glutathione peroxidase enzymes in blood fed mosquitoes; such kind of genetic tolerance and fitness in mosquitoes is conferred by bacterial microflora in the stressful gut environment induced by a blood meal [27].

Thus, the resident microbiota in malaria vectors can enhance or suppress the development of the parasites that are to be transmitted to the mammalian host. Isolation and identification of microflora in the midgut and salivary glands of Armigeres mosquito species will provide data for further analysis of the tritrophic interaction of microbiota with the development of Plasmodium parasite in the mosquito vector for integrated malaria control in endemic areas. Therefore, the aim of this study was to assess, isolate and identify bacterial microflora from the midgut and salivary glands of field collected Armigeres mosquitoes in some malaria endemic areas of Ethiopia.

The abundance of disease-transmitting vectors due to limited vector control activities and higher receptivity in these areas have been identified as major challenges to maintain the disease-

free status in the country, for JE and filariasis. Therefore, alternative vector control methods are needed as additional tools to be incorporated into the integrated vector management [28]. Paratransgenesis could be a better control tool for vector management. Hence, the present study was conducted to determine the microflora in the midgut of wild-caught *Cx. tritaeniorhynchus*, *Cx. gelidus*, and *Ma. annulifera* which has been limitedly assessed world-wide.

MATERIALS AND METHODS

Selection of Study Area and Sampling Sites:

The study was conducted in the district of Buldhana, Maharashtra state of India. The Buldhana district is one of the most diversified regions in Maharashtra State of India. The study was conducted both in rural and urban areas and samples were obtained from the two selected villages of rural and two sites of urban areas.

The climatic condition of this district is characterized by a hot summer, well-distributed rainfall during the south-west monsoon season, and generally dry weather during the rest of the year. The cold season is from December to February. The average annual rainfall in the district is 796.6 mm (31.37 inches). During summer, the mean daily maximum temperature was 42.3^{O} C and the minimum was 27.4^{O} C, and it decreased toward winter with a mean daily maximum temperature of 27.6^{O} C and a minimum of 15.1^{O} C (Buldhana Gazetteer 2015).

Field Collection of Adult Mosquitoes:

Entomological surveys were conducted at each selected location from June 2018 to May 2022 for four year at Buldhana district by hand collection method by using suction tube and insect net according to the standard guidelines described by the World Health Organization [35]. A large number of specimen were collected by visiting various places among thirteen talukas of Buldhana district at 15 days interval, by one man one hour search method. The collection of the adult specimens were collected from houses, cattle sheds, store rooms, Buses stand, railway stations, water bodies and hostels with the help of an improvised mouth aspirator and a torch suggested by WHO (1975). Collected adult mosquitoes were transferred safely to the presterilized adult rearing cages. All collected adult mosquito samples were transported and housed at the insectary facility at the Department of Zoology, Shri Vyankatesh College, Deulgaon Raja, Maharashtra, India, until taken for further experiments.

Processing and Identification of Field-Caught Adults:

Unfed (non-blood-fed) live mosquitoes captured from the field were sorted within 6-8 hours after collection.The mosquitoes were sacrificed using a cold shock. Mosquito species, namely *A. subalbatus* was segregated based on key morphological characteristics [29–31]. The adult mosquitoes were anesthetized using chloroform. For isolation of midgut bacterial population, a total of 10 adult female *A. subalbatus* mosquitoes were selected for dissection. The mosquito samples were surface sterilized with 75% ethanol for 5min followed by washing twice with phosphate buffered saline (PBS) before the dissection and separation of midgut for isolation of bacteria. Individually dissected midguts were transferred to 1.5 ml Eppendorf tube containing 100µl of phosphate buffered saline (PBS) and homogenized with a sterilized micro-pestle [32].

Culturing and Isolation of Bacteria:

Gut homogenate was serially diluted (10 folds) in PBS and was directly pour plated on sterile nutrient agar media (Himedia, India). The media was allowed to solidify in the laminar hood and the plates were later transferred to an incubator maintained at 37°C for 24-48 hrs. All the steps from dissection to midgut bacteria isolation were done in a sterile environment to avoid

contamination. A negative control was also performed to confirm the sterility of the procedure, in which the phosphate buffered saline (PBS) from mosquito second wash, was inoculated with media and incubated at 37°C for 24-48 hrs. Bacterial colonies obtained on the plate were differentiated according to their colony morphology like shape, size, colour, margin, opacity, elevation etc. Morphologically distinct colonies were selected for subculture on a nutrient agar plate until a presumably pure single colony was obtained.

Morphological and Biochemical characterization of isolates:

The isolates were characterized by morphological and biochemical characteristics. Biochemical characterization of isolates was done by performing various tests susch as indole, methyl red, voges prauskar, citrate, lactose, xylose, maltose, fructose, dextrose, galactose, raffinose, trehalose, melibiose, sucrose, L-arabinose, mannose, inulin, sodium gluconate, glycerol, salicin, glucosamine, dulcitol, inositol, sorbitol, rhamnose, cellobiose, melizitiose, α-methyl mannose, xylitol, arabinose, citrate, malonate, sorbase, nitrate reduction, urease and starch hydrolysis. Finally the isolates were identified on the basis of 16S rRNA gene sequencing.

Genomic DNA Extractions and Sequencing:

Bacterial Genomic DNA was isolated using the InstaGene™ Matrix Genomic DNA isolation kit. The procedure was followed according to the instructions given in kit. The PCR amplifications were performed using universal primers 27F (5′ AGAGTTTGATCMTGGCTCAG 3′) and 1492R (5′ TACGGYTACCTTGTTACGACTT 3′) [33] targeting 16S rRNA gene sequences. For Polymerase Chain Reaction (PCR) 1µl of template DNA was added in 20µl of PCR reaction solution. The primers 27F/1492R was used for bacteria and then PCR reaction performed with initial denaturation 94°C for 2 minutes and then 35 amplification cycles at 94°C for 45 seconds, 55°C for 60 seconds and 72°C for 60 seconds. Final extension was done at 72°C for 10 minutes. DNA fragments were amplified (1,400bp) by using universal primer including a positive (*E. coli* genomic DNA) and negative control. The amplified product was visualized on a 1% agarose gel containing ethidium bromide using a UV transilluminator. The PCR amplicons were then purified using the Montage PCR Clean up kit (Millipore). The purified products were sequenced using the 518F/800R primers. Sequencing reactions were performed using a ABI PRISM® BigDye™ Terminator Cycle Sequencing Kits with AmpliTaq® DNA polymerase (FS enzyme) (Applied Biosystems).

Phylogenetic Analysis of Midgut Bacteria Isolated from Mosquito species:

Homologous sequences were searched in the GenBank database using BLAST and also using the EzTaxon server (http://www.ezbiocloud.net/eztaxon/identify) [34]. Homologous sequences retrieved from the databases were aligned using the CLUSTALW program, and Phylogenetic relatedness was performed through neighbor joining algorithm, using Kimura-2 parameter method for distance calculations, incorporated into MEGA 6.0 package. Robustness of the trees was examined through 1000 bootstrap replicates, and the consensus tree was used for analysis.

RESULTS:

Bacterial Isolates from the Midgut of *A. subalbatus*:

A total of 26 bacterial strains were identified from the midgut of ten selected vector mosquitoes. The majority of the midgut isolates (53.84%; 14/26) were categorized under the phylum Firmicutes. The other nine species were from the Proteobacteria (34.61%; 9/26) and

Psedomonadota (11.53%; 3/26) phyla. The list of bacteria species identified from the midgut lumens of each mosquito species is illustrated in Table 1.

A total of 5 bacterial species of 4 genera were identified which belonged to three major phyla: Firmicutes, Proteobacteria and Psedomonadota. Phyla Firmicutes and Proteobacteria were the major species representing phyla and covers about 53.84% and 34.61% respectively of total identified species; rest 11.53% comes in phylum Psedomonadota. In phylum Firmicutes, the isolated bacteria belonged to one broad class ie. Bacilli. In phylum Proteobacteria, the isolated bacteria belonged to one broad class ie. Gamma-proteobacteria Psedomonadota was the least bacterial species representing phylum. The isolates belonging to phylum fermicutes were identified as *Bacillus cereus* & *Staphylococcus epidermidis*. The isolates belonging to phylum Proteobacteria were identified as *Pseudomonas aeruginosa* & *Pseudomonas geniculate*. The isolates belonging to phylum Psedomonadota identified as *Serratia marcescens*.

All the bacterial species have been summarized in Table 01 according to their abundance in their respective regions. From a total of 26 identified microflora, *Bacillus cereus* was the most abundant microbiota comprised 34.61% of the total species isolated. *Pseudomonas aeruginosa* was the second most abundant microbiota comprised 23.07% of the total species isolated. *Staphyllococcus epidermidis* was found to comprise 19.23% of total isolates. *Pseudomonas geniculate* & *Serratia marcescens* were found to constitute 18.33% each of the total identified microflora.

The isolate DMS-17 was subjected to the molecular 16S rRNA gene sequencing method. The sequence of the DMS-17 isolate was obtained as follows. In the phylogenetic tree, the isolate sequence showed similarity with *Bacillus cereus.*

Isolate DMS-17 Merged Sequence:

TGGGCCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGCGT
AGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTAC
GGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGC
GTGAGCGATGAAGGCCTTCGGGTCGTAAAGCTCTGTTGTTAGGGAAGAACAAGTACCG
GAGTAACTGCCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCG
CGCAGGTGGTTCCTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCATTG
GAAACTGGGGAACTTGAGTGCAGAAGAGGAAAGTGGAATTCCACGTGTAGCGGTGAAA
TGCGTAGAGATGTGGAGGAACACCAGTGGCGAAGGCGACTTTTCTGGTCTGTAACTGA
C

The isolate DMS-18 was subjected to the molecular 16S rRNA gene sequencing method. The sequence of the DMS-12 isolate was obtained as follows. In the phylogenetic tree, the isolate sequence showed similarity with *Staphylococcus epidermidis.*

Isolate DMS-18 Merged Sequence:

GCGATTACTAGCGATTCCGGCTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAG
AACGGTTTTATCGGATTAGCTCCCCCTCGCGGGTTGGCAACCGTTTGTACCGTCCATTGT
AGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCT
CCGGTTTATCACCGGCAGTCTCCTTAGAGTGCCCAACTGAATGATGGCAACTAAGAATA
AGGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAAC
CATGCACCACCTGTCACCGTTGCCCCCGAAGGGGAAACTATGTCTCCATAGTGGTCACC
GGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCAC
CGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCAGG
CGGAGTGCTTAATGCGTTAGCTGCAGCACTGAGGGGCGGAAACCCCCCAACACTTAGC
ACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTC

GCGCCTCAGTGTCAGTTACAGACCAGACAGTCGCCTTCGCCACTGGTGTTCCTCCAAAT
CTCTACGCATTTCACCGCTACACTTGGAATTCCACTATCCTCTTCTGCACTCAAGTTCCCC
AGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATCAGACTTAAAGAACCA
CCTGCGCGCGCTTTACGCCCAATAAATTCC

The isolate DMS-4 was subjected to the molecular 16S rRNA gene sequencing method. The sequence of the DMS-4 isolate was obtained as follows. In the phylogenetic tree, the isolate sequence showed similarity with *Serratia marcescens.*

Isolate DMS-4 Merged Sequence:

AACTCCGGGAAACCGGGGCTAATACCGGATGGTTCCTTCCTCCGCATGGAGGAAGGCGG
AAAGACGGTTTCGGCTGTCACTTACAGATGGGCCCGCGGCGCATTAGCTAGTTGGTGGG
GTAATGGCCTACCAAGGCAACGATGCGTAGCCAACCTGACAGGGTGATCGGCCACACTG
GGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATG
GACGAAAGTCTGACGGAGCAACGCCCCGTGAGCGAAGAAGGCCTTCGGGTCGTAAAG
CTCTGTTGTAAGGGAAGAACAAGCATCGGTTAACTGCCGGTGCCCTGACGGTACCTTAC
CAGAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACATAGGTGGCAAGCGTT
GTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCCTTTAAGTCTGATGTG

The isolate DMS-12 was subjected to the molecular 16S rRNA gene sequencing method. The sequence of the DMS-4 isolate was obtained as follows. In the phylogenetic tree, the isolate sequence showed similarity with *Pseudomonas geniculata.*

Isolate DMS-12 Merged Sequence:

ATTTTAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTACCTTATAGATTGGGATAACT
CCGGGAAACCGGGGCTAATACCGAATAATACTTTTTAACACATGTTTTGAAGTTGAAAGA
CGGTTTCGGCTGTCACTATTAAATGGACCCGCGGCGCATTAACTAGTTGGTGAGGTAACG
GCTCACCAAGGCAACGATGCGTAACCAACCTGAAAGGGTGATCGGCCACACTGGGACT
GAAACACGGCCCAAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCACAATGGACGA
AAGTCTGATGGAACAACGCCCCGTGAGTGAAAAAGGATTTCGGTTCGTAAAACTCTTTT
GCAAGGGAAAAACAAGTAGCGTAATAACTGGCGCTACCTTGACGGTACCTTGTTAAAAA
GCCACGGCTAATTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGG
AATTATTGGGCGTAAAGCGCGCGCAGGTGGTTCCTTAAGTCTGATGTGAAAGCCCCCGG
CTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAAAAAAGGATAGTGGA
ATTCCAAGTGTAGCGGTGAAATGCGTAAAGATTTGGAGGAACACCAGTGGCGAAGGCG
ACTGTCTGGTCTGTAACTGACACTGAGGCGCGAAAGCGTGGGGAGCAAACAGGATTAG
ATACCCTGGTAGTC
CACGCCG

The isolate DMS-7 was subjected to the molecular 16S rRNA gene sequencing method. The sequence of the DMS-7 isolate was obtained as follows. In the phylogenetic tree, the isolate sequence showed similarity with *Pseudomonas aeruginosa.*

Isolate DMS-7 Merged Sequence:

GCTTCATGCAGGCGAGTTGCAGCCTGCAATCCGAACTGAGAATAGATTTATGGGATTTGC
TTGACCTCGCGGTTTTGCTGCCCTTTGTTCTATCCATTGTAGCACGTGTGTAGCCCAGGT
CATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGGTTTGTCACCGGCAGTC
ACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTGCGCTCGTTGCGGG
ACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACTCTG
TCCCCCGAAGGGGAACGCTCTGTCTCCAGAGTTGTCAGAGGATGTCAAGACCTGGTAA
GGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCA
ATTCCTTTGAGTTTCAGCCTTGCGGCCGTACTCCCCAGGCGGAGTGCTTAATGCGTTTGC
TGCAGCACTAAAGGGCGGAAACCCTCTAACACTTAGCACTCATCGTTTACGGCGTGGAC

TACCAGGGTATCTAATCCTGTTCGCTCCCCACGCTTTCGCGCCTCAATGTCAGTTACAGA
CCAG

DISCUSSION:

Microbial symbionts in the insects exist at different organs such as the gut, ovaries, Malpighian tubules, and hemocoel [35]. The microbiota in the gut is of precise interest because it is the first contact point between the parasites and epithelial surfaces [36]. The prevalence of insect gut microflora especially in *A. subalbatus* mosquitoes has been investigated through classical culture-based methods or metagenomic based on 16S rRNA gene sequencing [37– 39]. The bacterial composition of mosquitoes sampled from natural habitats is highly variable but often contain a core microbiome that is dominated by a small number of taxa. However, it may vary subject to the insect species, geographical origin, ecological niche, source of food, and gender [40]. This microbiota can influence the capacity of insects to transmit disease-causing pathogens through various mechanisms. Therefore, understanding the interaction between vectors with their microbiota and transmitting pathogens may be useful to limit disease transmissions by exploring the potential role of microbiota in modulating infections that could lead to alternative disease control strategies [40]. Paratransgenesis is one such approach that controls the transmission of pathogens by arthropod vectors using gut microflora. This approach consists of the use of genetically altered symbiotic bacteria that secrete effector molecules that kill the infectious agent, disturb the reproduction, make the vector less competent, or reduce the life span of the host. However, this needs screening of microbiota among locally available disease-transmitting vectors using culture-dependent methods since the availability of gut microflora may vary with the geographical location [32].

The studies have carried out to screen the midgut microflora in mosquito species under different genera including Aedes, Anopheles, Culex, and Mansonia. However, only a few studies have been conducted to evaluate the prevalence of gut microflora in A. Subalbatus. A study conducted by Kang et al. [41] has reported the presence of bacterial members from *Rickettsiaceae* and *Enterobacteriaceae* families. This is the only available information regarding the midgut microbes of this species. Thus, the present study contributes to filling the knowledge gap regarding the prevalence of gut bacterial flora in the above mosquito species. Examination of 16S ribosomal RNA amplicons from culturable microflora isolated from the midgut of adult female *A. subalbatus* from Buldhana district of India revealed the presence of 26 bacterial species under 4 bacterial genera. All species identified were fit into three Phyla: Proteobacteria (02 species), Firmicutes (02 species), and Pseudomonata (1 species). These three bacterial phyla have commonly been recorded in the gut of many insect species including mosquitoes [16, 17, 18].

There are some evidence on the usage of gut microbacteria available in the insects for control approaches in the means of vector suppression, inhibition of parasite/pathogenic development in the invertebrate hosts, oviposition attractants in the development of lethal ovitraps, and transformation carriers in expressing molecules. Apart from mosquitoes, achievements were made from kissing bug gut inhabiting microbe, *Rhodococcus rhodnii*, for the prevention of the Chagas disease and sand fly gut inhabiting microbe, *Enterobacter cloacae*, for reducing Leishmania parasite transmission [42].

Table No. 1: Midgut microflora identified in A.Subalbatus

Phylum	Class	Isolate	Bacterial Species identified	Total No. of Isolates	Percent Distribution
Proteobacteria	Gamma-proteobacteria	DMS 7	*Pseudomonas aeruginosa*	6	23.07
		DMS 12	*Pseudomonas geniculate*	3	11.53
Fermicutes	Bacilli	DMS 17	*Bacillus cereus*	9	34.61
		DMS 18	*Staphylococcus epidermidis*	5	19.23
Psedomonadota	Gamma-proteobacteria	DMS 4	*Serratia marcescens*	3	11.53

CONCLUSION

This study describes isolation and identification of microbiota in the midgut of field collected *Armigeres subalbatus* species.To our knowledge, it is the first study providing an in depth description of the microbiota diversity in midgut of *Armigeres subalbatus* mosquitoes from india. Our findings indicated that, *Bacillus cereus* species had the dominant microbiota identified from *Armigeres subalbatus*. Moreover, *Armigeres subalbatus* has found to comprise more diversified microbiota. Since there is only a few published information on the midgut microflora of *Armigeres subalbatus*, the present study provides fundamental information to the literature. For paratransgenic applications, the feasibility of the gut microbes could be evaluated. Therefore, the present data strongly encourage further investigations to explore the potential usage of these microbes through the paratransgenic approach which is a novel eco-friendly vector control strategy.

Conflicts of Interest

The authors declare that they have no conflict of interests

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