Microbiota Diversity Associated with Midgut and Salivary Gland of *Aedes aegypti* and *Aedes albopictus*

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ABSTRACT

Objective: The gut and salivary gland contain diverse micro biota and play an important role in vector competence and disease transmission. In light of this, this study aimed to screen the salivary gland and midgut microbiota associated with *Aedes aegypti* and *Aedes albopictus* from Kathmandu and Lalitpur districts of Nepal.

Methods: An analytical cross-sectional study was conducted from April 2017 to October 2017 in Kathmandu and Lalitpur districts of Nepal. The field collected mosquitoes larvae were reared in the laboratory until the adult emergence and identified morphologically using standard key. The dissected salivary gland and gut samples were homogenized, suspended in phosphate buffered saline and inoculated in the culture media for bacterial growth which were further identified.

Results: *Pseudomonas aeruginosa* was predominant bacteria in the gut and salivary gland of *Ae. aegypti*. Similarly, in *Ae. albopictus, Serratia marcescens* was predominant in gut while, *Acinetobacter* spp. was predominant in salivary gland. Simpsons diversity index (D), Shannon weaver diversity index (H) and Evenness (E) were found to be the highest viz, 0.81, 1.83 and 0.88 in the gut of *Ae. aegypti*.

Conclusion: This study had provided a comprehensive overview of the bacterial population in the gut and salivary gland of *Aedes aegypti* and *Aedes albopictus*. It was found that the most bacterial genera were common to both vectors, although some variation was found in gut and salivary gland. This distribution suggests that there are no host-specific bacterial genera.

Key words: Dengue, *Ae. aegypti*, *Ae. albopictus*, microbiota, vector competence

INTRODUCTION

Mosquitoes poses a continuous public health threat due to their ability to transmit various diseases like Dengue, Zika fever, Chikungunya, Yellow fever, Malaria, Japanese encephalitis, Lymphatic filariasis etc (Yadav et al. 2016). Dengue, considered as the most important acute systemic arthropod-borne viral infection in humans, is becoming a global health concern, expanding its territory from tropical region to most subtropical regions of the world with over 2.5 billion people living in high-risk areas and 390 million infections per year (Guzman et al. 2010; Simmons et al 2012; Roth et al. 2014; Musso et al. 2015).The major vectors responsible for the transmission of dengue virus include *Aedes aegypti* and *Aedes albopictus* which are also considered as the important disease vectors for many arboviruses including, Zika virus, Chikungunya virus and Yellow fever virus (Kraemer et al. 2015). After the first record of dengue outbreak in 2004 (Pandey et al. 2004), Nepal had faced numerous outbreaks subsequently in the year 2006 to 2019 providing the evidences that DENV is one of the major emerging infectious diseases in

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Nepal (Pandey et al. 2008; Rijal et al. 2020). During the 2010 outbreaks, the first isolated and genetically characterized dengue virus isolation was reported from Nepal and entomological survey identified *Ae. aegypti* from all epidemic areas (Pandey et al. 2013). An entomological survey carried out in 2009 showed the presence of *Ae. aegypti* larvae in Kathmandu and Lalitpur districts of Nepal (Gautam et al. 2009).

From the several previous studies, it is evident that mosquitoes harbor bacterial flora that directly or indirectly play a role in physiological, metabolic, immunological functions and various other developmental activities in mosquito as well as alter the vector competency to transmit pathogens(Dillon and Dillon 2004; Minard et al. 2013; Coon et al. 2014). As an efficient method for vector control, a number of bacterial flora isolated from the gut of medically important mosquitoes species have been utilized for manipulating their midgut bacteria to modulate the vector competency, a process known as paratransgenesis (Hurwitz et al. 2011; Wang et al. 2012; Yadav et al. 2015). It has been hypothesized that mosquitoes acquired bacterial flora from the surrounding environment where they breed(Buck et al. 2016). Despite the fact that microbiota residing in the vector contribute to mosquitoes functions, most studies were focused on bacterial communities in the midgut compartment (Lindh et al. 2005; Cirimotich et al. 2011; Dinparast et al. 2011; Wang et al. 2011; Boissière et al. 2012; Osei-Poku et al. 2012; Terenius et al. 2012; Tchioffo et al. 2013) and have not been fully assessed in salivary gland, a key organ for virus and parasite replication (Minard et al. 2013).

In case of Nepal, literatures elucidating the microbial diversity in those vector species are rare. However, little is known about the microbial diversity in the gut of *Ae. aegypti* which revealed the presence of bacterial groups including known genera such as *Staphylococcus*, *Acinetobacter*, *Pseudomonas* and *Bacillus* (Thapa et al. 2017). In this study, we for the first time, attempted to explore the microbial diversity from gut and salivary gland of both the dengue vectors in Nepal.

MATERIALS AND METHODS

Study site and description

Altogether 17 sites in Kathmandu and Lalitpur districts were selected based on the prevalence of *Ae. aegypti* and *Ae. albopictus* as mentioned in the previous study (Gautam et al. 2009).

Kathmandu district is located at 27°42′N 85°20′E and covers an area of 395 km^2. Similarly, Lalitpur district is situated in the southeastern part of the Kathmandu valley between 27°32′13″N and 27°49′10″N and 85°11′31″E and 85°31′38″E and covers an area of 119 km^2.The Kathmandu valley has a temperate climate with three seasons. The summer season lasts from May through June, with a mean daily temperature of 32°C. The monsoon season (July through September) brings can be associated with periodic flooding. Most areas of the Kathmandu Valley receive 176.4ml of annual rainfall. The winter season (October through April) has a mean daily minimum temperature of -2° C. The annual humidity is 75% in average. Daily temperature fluctuations of 11 to 17°C occur in most of Nepal's interior regions.

Sample collection and dissection

The potential habitats of *Aedes* mosquitoes in water holding artificial containers were searched in and around the houses. Larvae and pupae samples were collected - using dropper and dipper technique. Live specimens were transferred to the laboratory of Natural History Museum, Tribhuvan University, Swayambhu, Kathmandu, Nepal. They were reared in the presterilized plastic cups until adult emergence, fed with 10% sucrose solution and identified to species level using standard morphological keys (Darsie and Pradhan 1990). Altogether, 56 *Aedes* sample were processed (29 *Ae. aegypti* and 27 *Ae. albopictus*). Midguts and salivary glands were then dissected from individual mosquito over a sterile glass slide containing a drop of 1x PBS, then transferred to a microcentrifuge tube containing 150µl of sterile PBS. The dissected contents were separately homogenized using a sterilized micro pestle (Chandel et al. 2013) and obtained content was considered a sample for the enumeration and isolation of bacteria.

Enumeration, isolation and identification of bacteria

Homogenates from gut and salivary gland were serially diluted (10 folds) in PBS up to 10^{-6} and spread on nutrient agar (NA) for enumeration and streaked on different media: NA, blood agar (BA) MacConkey agar (MA) and chocolate agar (CA) (Himedia, India) and were incubated aerobically at 37°C for 24-48 hours. The sterility of all reagents were checked and controls for the efficiency of sterilization were treated like the

other samples. Distinct bacterial colonies obtained on the plates were counted and the concentration of bacterial load was calculated in terms of CFU/ml and were differentiated based on their colony morphology. Morphologically distinct colonies were subcultured on nutrient agar plates for obtaining a pure culture. Colonies from pure culture were identified by Gram staining and biochemical investigation following Bergey's Manual of Systematic Bacteriology (Holt et al. 1994).

Statistical analysis

SPSS (21.0 version) was used to calculate frequencies and percentage of the data which later were computed using the Pearson's Chi-Square test. Student's t test was applied to compare the mean. A p-value of < 0.05 was considered statistically significant. Diversity indices were analyzed by calculating Simpson's Diversity Index, Shannon Diversity Index and Evenness by using the formula in Excel spreadsheet 2010.

RESULTS

Average bacterial load in vectors

An average bacterial load in gut and salivary gland of *Ae. albopictus* was 2.3× 10^9 CFU/ml and 1.6× 10^9 CFU/ml respectively. The Student's t-test showed significant difference between the mean count of bacterial load (p= 0.002). The average bacterial load in gut and salivary gland of *Ae. aegypti* was 1.28×10^9CFU/ml and 1.16×10^9CFU/ml respectively and the Student's t-test showed no significant difference between the mean count (p=0.697). Likewise, there was significant difference between the mean counts in gut of the two vector species *Ae. aegypti* and *Ae. albopictus* (p=0.00) and no significant difference in the salivary gland of both vector species (p=0.177) was observed.

Table 1: Average bacterial load in gut and salivary gland of *Aedes aegypti* and *Aedes albopictus*

	Gut	Salivary gland	p-value (Student's t- test)
Ae. albopictus	2.3×10^9	1.6×10^9	0.002
Ae. Aegypti	1.28×10^9	1.16×10^9	0.697
p- value (Student's t- test)	0.00	0.177	

Bacterial isolates from the gut of *Aedes aegypti*

A total of 8 different culturable bacterial species were identified from the gut of *Ae. aegypti*. Among all bacterial species, *Pseudomonas aeruginosa* (51.70%) and *Citrobacter freundii* (51.70%) were the dominant species whereas, CONS (3.4%) and *Proteus mirabilis* (3.4%) were the least represented species (Figure 1). Out of 29 samples, 24 showed polymicrobial growth whereas 5 showed monomicrobial growth. Of the total isolates, 89% were Gram negative and 11% were Gram positive.

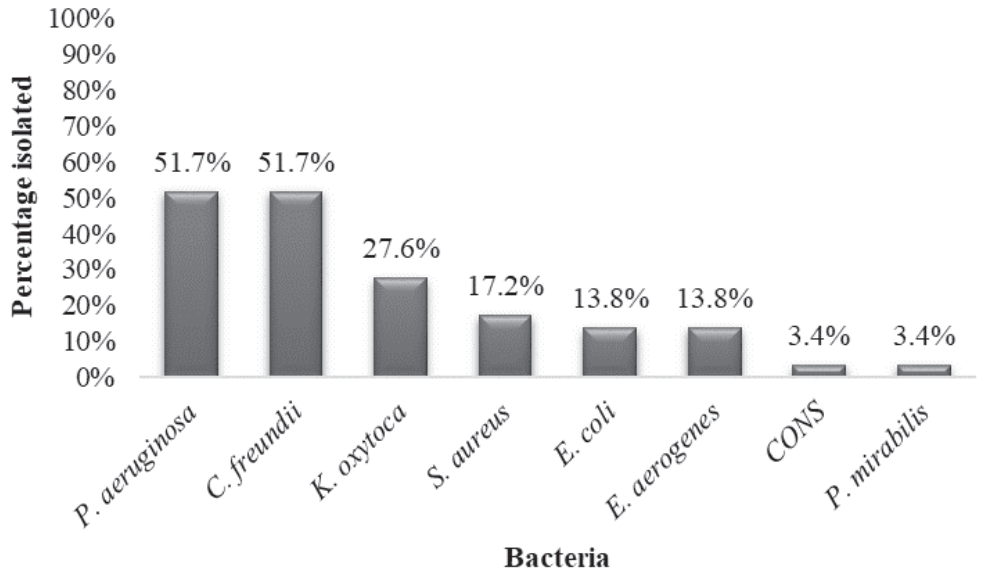

Figure 1: Bacterial isolates from the gut of *Ae. aegypti*

Bacterial isolates from the salivary gland of *Ae. aegypti*

A total of 4 different bacterial species were identified from the salivary gland of *Ae. aegypti*. Among all the identified bacterial isolates, *Pseudomonas aeruginosa* (96.6%) was the dominant species followed by Cogulase negative *Staphylococcus* (CONS) (24%), *Acinetobacter* spp. (17.2%) and *Staphylococcus aureus* (6.9%) (Figure 2). Out of 29 samples, 13 showed polymicrobial growth whereas 16 showed monomicrobial growth and 79% belong to Gram negative and 21% to Gram positive.

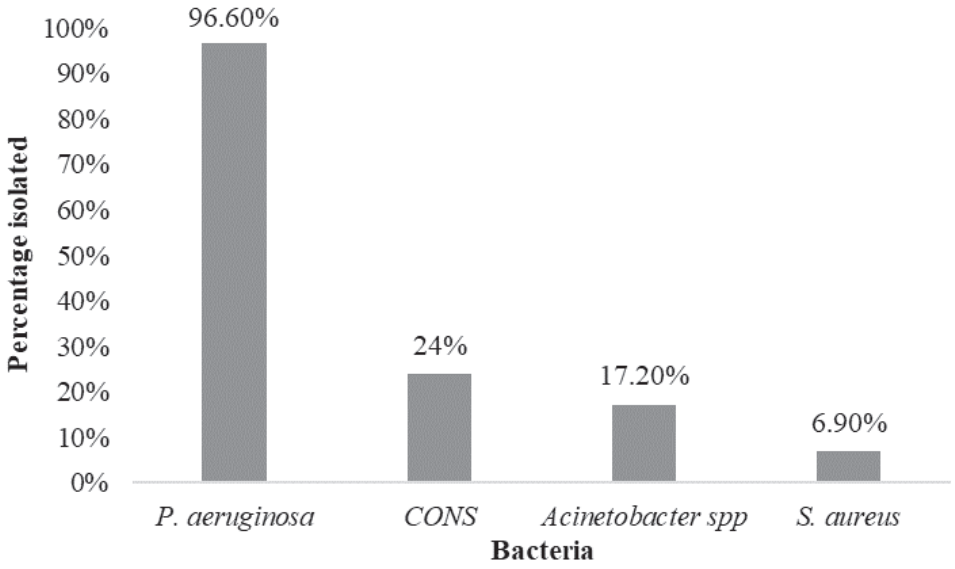

Figure 2: Bacterial isolates from the salivary gland of *Ae. aegypti*

Association between bacterial isolates in gut and salivary gland of *Ae. aegypti*

P. aeruginosa, *S. aureus* and CONS were common in both gut and salivary gland of *Ae. aegypti*. *P. aeruginosa* and CONS were significantly higher in salivary gland (p <0.05) than in gut whereas, there was no significant difference in the abundance of *S. aureus* in the gut and salivary gland (p > 0.05).

Table 2: Association between bacterial isolates in *Aedes aegypti*

Bacterial isolates	*Ae. aegypti*		P value (Chi square test)
	Gut N (%)	Salivary gland N (%)	
P. aeruginosa	15 (51.7)	28 (96.6)	0.00
S. aureus	5 (17.2)	2 (6.9)	0.2
CONS	1(3.4)	7(24.1)	0.02
C. freundii	15 (51.7)	-	-
K. oxytoca	8(27.6)	-	-
E. coli	4 (13.8)	-	-
E. aerogenes	4 (13.8)	-	-
P. mirabilis	1 (3.4)	-	-
Acinetobacter spp	-	5 (17.2)	-

Bacterial isolates from the gut of *Ae. albopictus*

Altogether 9 different bacterial species were identified from the gut of *Ae. albopictus*. Among all, *Serratia marcescens* (70.4%) was the predominant bacterial species (Figure 3). Out of 27 sample processed, 15 showed polymicrobial growth whereas 12 showed monomicrobial growth. Gram negative were dominating accounting for 98% of the bacterial isolates whereas only 2% were Gram positive.

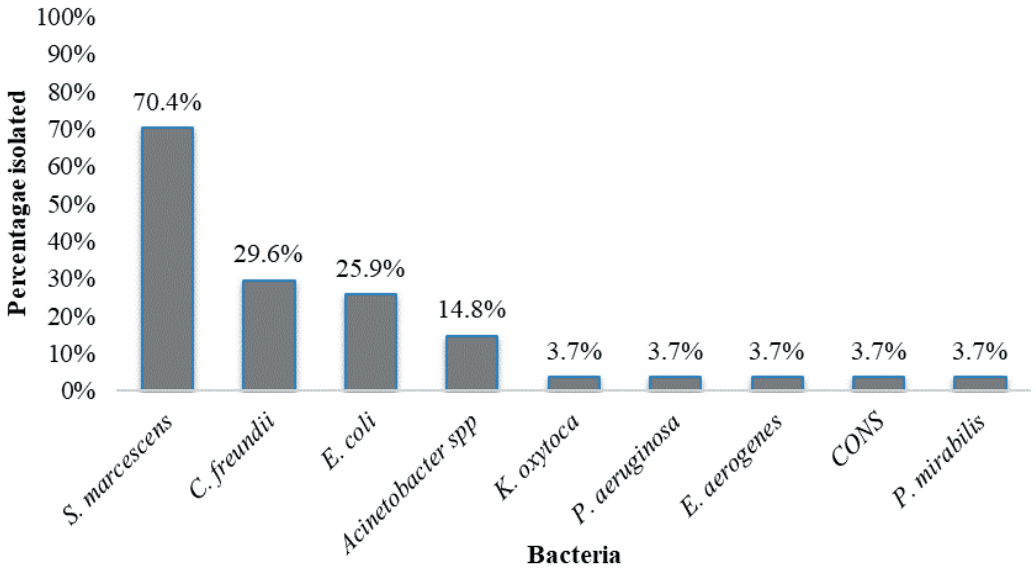

Figure 3: Bacterial isolates from the gut of *Ae. albopictus*

Bacterial Isolates from salivary gland of *Ae. albopictus*

Four bacterial species were identified from the salivary gland of *Ae. albopictus*. Among all, *Acinetobacter* spp. (100%) was the dominant one followed by *P. aeruginosa* (22.2%), *B. subtilis* (18.5%) and *S. aureus* (3.7%) (Figure 4). Out of 27 samples, 12 showed polymicrobial growth whereas, 15 showed monomicrobial growth. Gram negative bacteria were found in large proportion (85%), followed by Gram positive bacteria (15%).

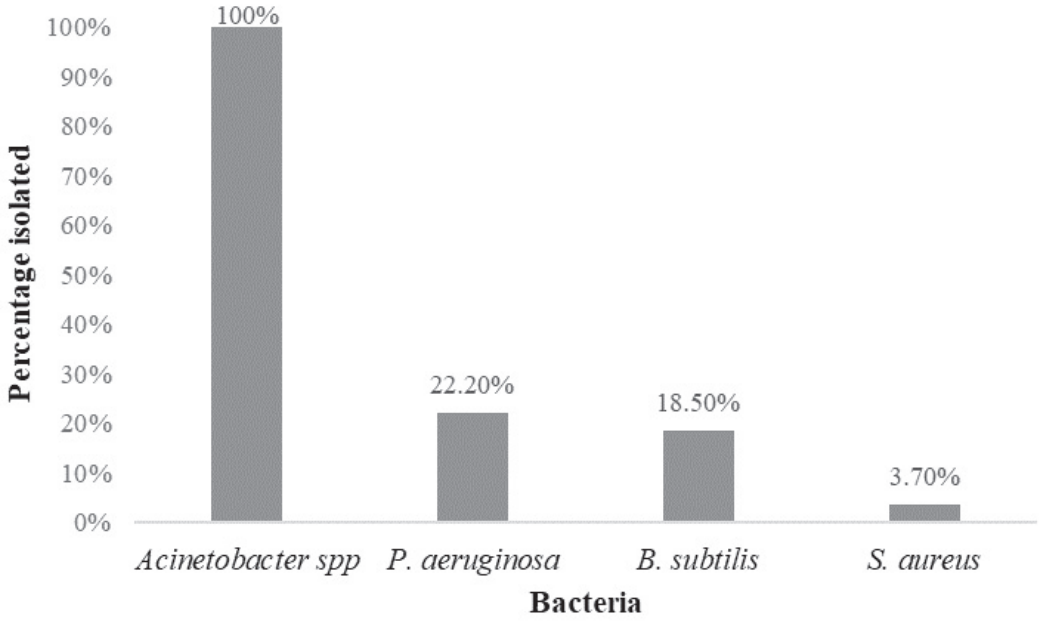

Figure 4: Bacterial isolates from the salivary gland of *Ae. albopictus*

Association between bacterial isolates in gut and salivary gland of *Ae. albopictus*

Pseudomonas aeruginosa and *Acinetobacter* spp. were common in both gut and salivary gland of *Ae. albopictus*. *P. aeruginosa* was significantly higher in salivary gland ($p < 0.05$) than in gut.

Table 3: Association between bacterial isolates in *Aedes albopictus*

Bacterial isolates	*Ae. albopictus*		P value
	Gut N (%)	Salivary gland N (%)	
Pseudomonas aeruginosa	1 (3.7)	6 (22.2)	0.043
Acinetobacter spp.	4 (14.8)	27 (100)	-
Staphylococcus aureus	-	1 (3.7)	-
Bacillus subtilis	-	5 (18.5)	-
Klebsiella oxytoca	1(3.7)	-	-
Escherichia coli	7 (25.9)	-	-
Enterobacter aerogenes	1 (3.7)	-	-
Proteus mirabilis	1 (3.7)	-	-
Citrobacter freundii	8 (29.6)	-	-
Serratia marcescens	19(70.4)	-	-

Statistical association between bacterial flora in *Ae. aegypti* and *Ae. albopictus*

As presented in table 4, 11 different bacterial species were isolated from *Ae. albopictus*, whereas in case of *Ae. aegypti* a total of 9 bacterial species were isolated. *S. marcescens* and *B. subtilis* were found to be present only on *Ae. albopictus*. *Klebsiella oxytoca, P. aeruginosa* and CONS were found to be significantly higher in *Ae. aegypti* than in *Ae. albopictus* ($p < 0.05$). However there was no significant difference in the abundance of *Escherichia coli, Proteus mirabilis, Citrobacter freundii, Enterobacter aerogenes* and *S. aureus* in vectors ($p > 0.05$).

Table 4: Association between bacterial isolates from *Aedes aegypti* and *Aedes albopictus*

Identified Bacterial flora	*Ae. aegypti* N (%)	*Ae. albopictus* N (%)	P value (Chi square test)
S. marcescens	-	19 (70.4)	-
K. oxytoca	8 (27.6.)	1 (3.7)	0.015
E. coli	4 (13.8)	7 (25.9)	0.253
C. freundii	15 (51.7)	8 (29.6)	0.093
E. aerogenes	5 (17.2)	1 (3.7)	0.102
P. mirabilis	1 (3.4)	1 (3.7)	0.959
S. aureus	5 (17.2)	1 (3.7)	0.102
P. aeruginosa	28 (96.6)	7 (25.9)	0.00
Acinetobacter spp.	5 (17.2)	27 (100)	-
CONS	8 (27.6)	1 (3.7)	0.015
B. subtilis	-	5 (18.9)	-

Distribution of bacterial flora in dengue vectors collected from Kathmandu and Lalitpur districts

Altogether 11 bacterial species were identified from Kathmandu district whereas only 8 bacterial species were identified from Lalitpur. *S. aureus, P. mirabilis* and *B. subtilis* were found to be absent in Lalitpur district (Table 5 and 6).

Table 5: Isolated bacterial flora in dengue vectors from Kathmandu district

Isolated organisms	*Ae. albopictus*		*Ae. aegypti*	
	Gut No. (%)	Salivary gland No. (%)	Gut No. (%)	Salivary gland No. (%)
S. marcescens	14 (82.35)	-	-	-
C. freundii	3 (17.64)	-	4 (23.5)	-
K. oxytoca	1 (5.88)	-	8 (47)	-
E. coli	2 (11.76)	-	3 (17.6)	-
Acinetobacter spp.	2 (11.76)	17 (100)	-	3 (17.6)
P. aeruginosa	1 (5.88)	2 (11.76)	4 (23.5)	17 (100)
E. aerogenes	1 (5.88)	-	3 (17.6)	-
CONS	1 (5.88)	-	1 (5.88)	3 (17.6)
P. mirabilis	1 (5.88)	-	1 (5.88)	-
S. aureus	-	1 (5.88)	5 (29.4)	2 (11.76)
B. subtilis	-	3 (17.64)	-	-

Table 6: Isolated bacterial flora in dengue vectors from Lalitpur district

Isolated organism	*Ae. albopictus*		*Ae. aegypti*	
	Gut No. (%)	Salivary gland No. (%)	Gut No. (%)	Salivary gland No. (%)
S. marcescens	5 (50)	-	-	-
C. freundii	5 (50)	-	11 (91.6)	-
E. coli	5 (50)	-	1 (8.33)	-
Acinetobacter spp	1 (10%)	10 (100)	-	2 (16.6)
B. subtilis	-	2 (20)	-	-
E. aerogenes	-	-	1 (8.33)	-
P. aeruginosa	-	4 (40)	11 (91.6)	11 (91.6)
CONS	-	-	-	2 (16.6)

Diversity indices of midgut and salivary gland bacterial isolates of *Aedes aegypti* and *Aedes albopictus*

In case of *Ae. aegypti*, Simpsons diversity index (D), Shannon weaver diversity index (H') and Evenness (E) of gut were found to be 0.81, 1.83 and 0.88 respectively whereas the salivary gland had 0.52, 0.97 and 0.7 respectively. Gut of *Ae. albopictus* had Simpsons diversity index of 0.74, Shannon weaver diversity index of 1.63 and Evenness of 0.74. Likewise, salivary gland had Simpson's diversity index of 0.48, Shannon weaver diversity index of 0.89 and Evenness of 0.64 (Table 7).

Table 7: Diversity indices of midgut and salivary glands bacterial isolates of *Aedes aegypti* and *Aedes albopictus*

Indices	*Ae. aegypti*		*Ae. Albopictus*	
	Gut	Salivary gland	Gut	Salivary gland
Simpsons Diversity Index(D)	0.81	0.52	0.74	0.48
Shannon weaver diversity Index (H')	1.83	0.97	1.63	0.89
Evenness (E)	0.88	0.7	0.74	0.64

DISCUSSION

Ae. aegypti and *Ae. albopictus* are the most important vectors of arboviruses globally and have expanded their territory worldwide (Reiner et al. 2009). This study aimed to explore the diversity of midgut and salivary gland microbiota of two vector species *Ae. aegypti* and *Ae. albopictus* collected from two hilly districts of Nepal, to delineate the bacterial communities which potentially will provide an insight on the ecology and probable role in host survival, community interactions and protection against natural enemies (Zouache et al. 2010)

From the total 56 *Aedes* sample processed (29 *Ae. aegypti* and 27 *Ae. albopictus*) altogether eleven different bacterial species viz; *P. aeruginosa, Acinetobacter* spp., *C. freundii, S. marcescens, K. oxytoca, E. coli, S. aureus, E. aerogenes,* CONS, *B. subtilis* and *P. mirabilis* were identified. This finding was in accordance with the result reported by other researchers and most of the bacterial genera had already been reported from the mid gut of *Aedes* and other mosquitoes species as well (Yadav et al. 2015; Thapa et al. 2017). All of these bacterial species were identified from *Ae. albopictus* whereas only 9 bacterial species were found to be associated with *Ae. aegypti*. *S. marcescens* and *B. subtilis* were found to be associated with *Ae. albopictus* only. Chi square test showed that the bacteria namely *K. oxytoca*, *P. aeruginosa* and CONS were significantly higher in *Ae. aegypti* than in *Ae. albopictus* ($p<0.05$). *P. aeruginosa* was found to be highly abundant in both *Ae. aegypti* (51.7% in gut and 96.6% in salivary gland) and *Ae. Albopictus* (3.7% in gut and 22.2% in salivary gland) and was found to be significantly higher in salivary gland than in gut of both the vectors ($p<0.05$). Earlier studies also reported this bacterium to be commonly present in mosquito's guts. Peck and Walton (Peck and Walton 2006) demonstrated that a high level of *P. aeruginosa* improved larval growth of *Culex quinquefasciatus* in a phosphorus-rich medium. In this study, *Acinetoba*cter species was found to be highly abundant in *Ae. albopictus* (100% in salivary gland and 14.8% in gut) compared to *Ae. aegypti* (17.2% in salivary gland only). Previous study had shown the prevalence of *Acinetobacter* to be 70% in *Ae. Albopictus* (Minard et al. 2013). *Acinetobacter* spp.is commonly present in rhizosphere, water as well as skin of some vertebrates, used as the food sources, for laying eggs, hatching larvae by the mosquitoes species and their association may favour the survival of mosquitoes (Doughari et al. 2011). Similarly, *Acinetobacter baumannii* and *Acinetobacter johnsonii* potentially involved in both blood digestion and nectar assimilation in *Ae. albopictus* (Minard et al. 2013). In this study, *Serratia marcescens* was the predominant bacterial

isolates in the gut of *Ae. albopictus* (70.4%) but absent in *Ae. aegypti*. However, conversely, a study carried out by Gusmao et al (Gusmão et al. 2007) showed the high prevalence of *S. marcescens* in *Ae. aegypti*. The genus *Serratia* has a wide host range and isolated from various insects. Furthermore, *Serratia marcescens* was frequently isolated as a pathogen of insectary-reared insects, identified in intestinal tube of *Lutzomyia longipalpis* and in blood and sugar fed *Lutzomyia longipalpis* (Krieg 1987; Grimont and Grimont 1992). Likewise, the same organism from the midgut of *An. albimanus* provided an evidence that *Serratia marcescens* has a wide host range and is an important species contributing to paratransgenesis (Gonzalez et al. 2003). Similarly, *Enterobacter* species isolated from the gut of *Ae. aegypti* and *Ae. albopictus* was previously identified in *Ae. aegypti* eggs which suggested the transovarial transmission of this bacteria (Gusmão et al. 2010). Here, majority of the isolates from gut but not the salivary gland belongs to Enterobacteriaceae family and various researches also showed the dominance of this family in *Ae. aegypti* and *Ae. albopictus* (Terenius et al. 2012; Moro et al. 2013)

In this study, the bacterial load was higher in *Ae. albopictus* compared to *Ae. aegypti* and the gut of both the vectors harbored more bacterial flora than salivary gland. As evidences from the earlier studies, bacteria were identified from salivary glands and other organs to a lesser extent than gut, the latter being a key organ for nutrition and an interface with the external environment providing favorable space for the multiplication of microorganisms (Dillon and Dillon 2004, Pidiyar et al. 2004; Rani et al. 2009).This study showed the dominance of Gram negative bacteria in the gut and salivary gland of both the vectors. It was in consistent with previous studies where the dominance of Gram negative bacteria were reported from different mosquitoes species (Lindh et al. 2005; Dong and Manfredini 2009; Cirimotich et al. 2011). Similarly, in this study, 89% of the isolated bacteria from the gut and 79% from the salivary gland of *Ae. aegypti* were Gram negative bacilli which is in accordance with the study carried elsewhere which was 85% (Ramirez et al. 2012) and 76% (Gusmão et al. 2010). In an attempt to study the microbial diversity of whole body of *Ae. aegypti* from Nepal, Gram negative bacteria were identified in larger proportion (63%) followed by Gram positive bacteria (37%) (Thapa et al. 2017). In the same study, *Staphylococcus* spp., *Pseudomonas* spp. and *Acinetobacter* spp. were the most common bacterial flora. Gram negative bacteria tend to offer more protection against *Plasmodium* infection than Gram positive bacteria (Cirimotich et al. 2011), suggesting the important role of these gut bacteria in reducing disease transmission.

It was hypothesized that mosquito vectors acquired bacterial flora from the surrounding environment (Buck et al. 2016). A large number of bacterial genera identified in this study, such as *Acinetobacter, Klebsiella, Pseudomonas, Enterobacter, Bacillus* had already been reported to be common in breeding habitats of those vector species (Smith and Walker 1998; Osei-Poku et al. 2012). This study however has a limitation since it has focused on the aerobic and facultative anerobic bacteria and the anerobic bacteria were excluded.

The diversity index is used to quantify the diversity of individuals in a certain community as well as describes its numerical structure. Simpson's diversity index is used to measure the probability of any two randomly drawn individuals from an infinitely large community belonging to different species (Chandel et al. 2013). The value ranges from 0 to 1, with close to 1 indicates high diversity and close to 0 indicates low diversity. In this study the value of Simpson's diversity index ranged from 0.48 to 0.81 (maximum in gut of *Ae. aegypti* and minimum in salivary gland of *Ae. albopictus*). Another widely used index to compare the diversity between various habitats is Shannon index (H). The Shannon index ranges between 1.5 to 3., where values greater than 3 indicates rich and stable diversity and below 1.0 indicates that the diversity is not stable due to pollution and habitat degradation (Clarke 2001). In this study the Shannon diversity index ranged between 0.89 to 1.83 (minimum in salivary gland of *Ae. albopictus* and maximum in gut of *Ae. aegypti*). Evenness index is commonly used for the determination of closeness of the species as well as shows how well they are evenly distributed among the individuals. The highest evenness was recorded for the gut of *Ae. aegypti* indicating that bacterial species in the gut of *Ae. aegypti* were evenly distributed as compared to other categories of individuals. The lowest evenness was recorded from the salivary gland of *Ae. albopictus* indicating that species were less evenly distributed and some species more dominant than the other. In this study, the Simpson's diversity index (D), Shannon weaver diversity index (H) and evenness (E) in the gut

of *Ae. abopictus* were 0.74, 1.63 and 0.74 respectively which was in accordance with the study carried out by Zouche et al. 2010 where H was 1.16 to 2.45, D was 0.63 to 0.89 and E was 0.80 and 0.86. Likewise, the value of H and E for *Ae. aegypti* was in accordance with the previous study carried out in Nepal which were 1.34 and 0.97 (Thapa et al. 2017).

CONCLUSIONS

To the best of our knowledge, there is no published literature elucidating the bacterial diversity in midgut and salivary gland of *Ae. aegypti* and *Ae. albopictus* in Nepal. Most of the bacterial genera identified in this study have been demonstrated to play a key role in insect's function. Thus, identification of microbiota in *Aedes* with potential role on vector competency and survival may open a new horizon for potentially novel approach to contain the vector targeting the bacteria associated with the host mosquito.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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