Microbes, mosquitoes and malaria

Avinash Sharma, Kamlesh Jangid and Yogesh Shouche

Every living organism harbours microbes that are intimately associated with its body surfaces and mosquitoes are no exception to this. Microbes in the mid gut of mosquitoes have special importance because of their proximity to mosquito-transmitted pathogens in this tract and potential to influence disease transmission. Although microbial community structure in the mid gut of different mosquito species has been analysed using culture based and culture independent methods for almost a decade[1], recent developments in high throughput sequencing technologies have allowed studies at much greater depths[2,3]. This additional information has given a new dimension to better understand microbial community dynamics in the mosquito mid gut and its role in disease transmission. Here, we summarize three recent publications that have made some important observations probably raising more questions than answered.

Wang *et al.*[2] used Roche® 454-pyrosequencing to study the microbial community structure during the life-cycle of wild and lab-reared Kenyan mosquito strain. Specific regions of the eubacterial 16S rRNA gene amplified from mixed-community DNA during different developmental stages of the mosquito were used to study community dynamics in the mosquitoes. Although the mid gut was found to harbour distinctly different bacteria in different developmental stages, the phyla Cyanobacteria, Proteobacteria, Bacteroidetes, Actinobacteria and Firmicutes together represented 90.7–99.9% of the sequences in all the stages. In general, the microbial community diversity decreased progressively from larvae to adults and further upon blood meal in adults. Whereas the larvae and pupae microbial communities were dominated by Cyanobacteria, those in newly emerged adults showed predominantly Enterobacteriaceae and Propinobacteriaceae. Restructuring of the larval intestine during metamorphosis and change in the gut environment may be responsible for this shift. At the species level, as many as 69.4% of the sequences were closely affiliated to *Thorsellia anopheles*, originally isolated from the mid gut of *Anopheles arabiensis* and subsequently detected in *Anopheles gambiae*. Adult development was associated with an increase in Flavobacteriaceae from 13.2% to 61.7% and decrease in Enterobacteriaceae from 37.4% to as low as 5.5%. Family SAR11 which contains oligotrophic bacteria was relatively constant around 4–5%. Upon blood meal, the community structure showed a significant change within 2 days with predominance of Proteobacteria. However, after 4 and 7 days post-blood meal, Flavobacteria especially the genus *Elizabethkingia* which was also reported in lab and field mosquitoes, predominated the communities. Regardless of this dynamically changing community, both wild and lab-reared mosquitoes seemed to harbour comparable microbial communities.

In contrast, Boissière *et al.*[3] using a similar strategy reported significant differences between the microbial communities in lab-reared and natural populations of *Anopheles gambiae* from two different locations in Cameroon. Larvae were reared in laboratory and experimentally infected with *Plasmodium falciparum* from a single donor. Blood-fed females were dissected after 8 days and the total DNA from individual gut samples was used for microbial community analysis and *Plasmodium* infection status. Although sample-specific differences were noted, microbial communities in general were dominated by Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes and Fusobacteria. Between lab-reared and field collected mosquitoes, microbial communities in lab-reared mosquitoes were dominated by Flavobacteria, specifically the genus *Elizabethkingia* as reported by Wang *et al.*[2]. In contrast, mosquitoes from natural habitats showed predominance of Proteobacteria. Further, Gram-positive bacteria were detected only in natural populations and were absent in lab-reared mosquitoes. The relative proportion of alpha-, beta- and gamma-proteobacteia in the microbial communities differed between the mosquito larvae collected from the two sites. Possibly, the differences resulted from either the genetic variation between different mosquito strains or due to difference in the experimental design. Whereas Wang *et al.*[2] used rain water for propagation of mosquitoes in microcosm, Boissière *et al.*[3] used habitat water for their study. In addition, Wang *et al.* used larvae from *Anopheles gambiae* colony maintained in the insectary, whereas Boissière *et al.* used larvae collected from their natural habitats.

Although microbial community dynamics is an important component of transgenic mosquito research, it cannot directly implicate mid gut microbiota in the transmission of diseases, which has been an actively studied area in this research[4]. Cirimotich *et al.*[5] studied the role of naturally occurring microbes in

454 Pyrosequencing

Pyrosequencing technology as developed by 454 Life Sciences is one of the modern massively parallel sequencing procedures that relies upon the release of inorganic pyrophosphate (hence the name) with every nucleotide incorporation during strand synthesis. In the first step, target DNA is fragmented and clonally amplified on beads in an emulsion PCR (emPCR) to generate multiple copies. The beads are then overlaid onto a PicoTitrePlate™ with millions of wells in such a way that each well has a single bead. Subsequent sequencing reactions that occur on each bead make use of polymerase, luciferase, ATP sulphurylase and microfluidics cycles of the four nucleotides over the PicoTitrePlate™. The incorporation of a nucleotide during strand synthesis on each bead releases a pyrophosphate, which acts as the substrate for a luminescence reaction that is measured by the CCD camera. The signal intensity is directly proportional to the number of nucleotides incorporated. Thus, several million reactions are carried out simultaneously and with present-day technology ~ 500 bp sequence read can be generated on each bead leading to massive output.

the transmission of malaria. Using a series of experiments and pure cultures of bacteria isolated from natural populations of *A. arabiensis* from Zaire, they demonstrated that Gram-negative bacteria inhibit *Plasmodium* development in mosquitoes whereas Gram-positive bacteria have negligible effect. Further experiments with one isolate, *Enterobacter* sp. (Esp_Z) suggested that inhibition was possibly mediated by a soluble, short-lived factor rather than a direct contact with *Plasmodium*. Further, reversal of inhibition by free radical scavengers like vitamin C and glutathione suggested possible involvement of reactive oxygen species. It is however intriguing that given the extensive coverage of pyrosequencing, *Enterobacter* sp. was not detected by Boissière *et al.*[3]. On the contrary, a strong correlation between presence of Enterobacteriaceae and *Plasmodium* positivity was in fact noted. The genetic differences between the mosquito populations studied in these two reports[3,5] along with other factors including experimental strategy might explain these contradictory observations.

These studies generate key information toward the development of mosquito species that are refractory to transmission of parasites. Regardless of the stark differences, they establish that mosquitoes, like humans, harbour a 'core gut microbiota' but individual mosquitoes may carry their own specific flora. This core community comprises genera, such as *Asaia*, *Burkholderia*, *Serratia*, *Ralstonia*, *Acinetobacter*, *Pseudomonas*, *Sphingomonas*, *Staphylococcus*, *Streptococcus*, *Escherichia*/*Shigella*, *Elizabethkingia* and *Thorsellia*. In addition, the inhibitory effect of specific members of natural microbiota is also established. However, the contrasting observations suggest that there is much more to be learnt about the microbial community dynamics in mosquito mid gut warranting a detailed study of different mosquito populations from diverse environments.

1. Pidiyar, V. J., Jangid, K., Patole, M. S. and Shouche, Y. S., *Am. J. Trop. Med. Hyg*., 2004, **70**, 597–603.
2. Wang, Y., Gilbreath 3rd, T. M., Kukutla, P., Yan, G. and Xu, J., *PLoS One*, 2011, **6**, e24767. Epub 21 Sep. 2011.
3. Boissière, A. *et al.*, *PLoS Pathog*., 2012, **8**, e1002742. Epub 31 May 2012.
4. Mourya, D. T., Gokhale, M. D., Pidiyar, V., Barde, P. V., Patole, M., Mishra, A. C. and Shouche, Y., *Acta Virol*., 2002, **46**, 257–260.
5. Cirimotich, C. M., Dong, Y., Clayton, A. M., Sandiford, S. L., Souza-Neto, J. A., Mulenga, M. and Dimopoulos, G., *Science*, 2011, **332**, 855–858.

Avinash Sharma, Kamlesh Jangid and Yogesh Shouche are in the Microbial Culture Collection, National Centre for Cell Science, Pune University Campus, Ganeshkhind, Pune 411 007, India.*
**e-mail: yogesh@nccs.res.in*

CURRENT SCIENCE

Display Advertisement Rates

India	Tariff (Rupees)*					
Size	No. of insertions	Inside pages		Inside cover pages		Back cover pages	
		B&W	Colour	B&W	Colour	B&W	Colour
Full page	1	12,000	20,000	18,000	30,000	25,000	35,000
	2	21,600	36,000	32,000	54,000	45,000	63,000
	4	42,000	70,000	63,000	1,05,000	87,000	1,20,000
	6	60,000	1,00,000	90,000	1,50,000	1,25,000	1,75,000
	8	75,000	1,25,000	1,15,000	1,90,000	1,60,000	2,20,000
	10	90,000	1,50,000	1,35,000	2,25,000	1,85,000	2,60,000
	12	1,00,000	1,65,000	1,50,000	2,50,000	2,10,000	2,90,000
Half page	1	7,000	12,000	We also have provision for quarter page display advertisement: **Quarter page**: 4,000 per insertion (in Rupees) **Note:** For payments towards the advertisement charges, Cheque (local/multicity) or Demand Drafts may be drawn in favour of '**Current Science Association, Bangalore'.** ***25% rebate for Institutional members***			
	2	12,500	22,000				
	4	23,750	42,000				
	6	33,500	60,000				
	8	42,000	75,000				
	10	50,000	90,000				
	12	55,000	1,00,000				

Contact us: Current Science Association, C.V. Raman Avenue, P.B. No. 8001, Bangalore 560 080 or E-mail: csc@ias.ernet.in

Last date for receiving advertising material: Ten days before the scheduled date of publication.