Superinfection with an endosymbiotic bacterium *Parvibaculum lavamentivorans* leads to manifest *Leishmania donovani* as Post kala azar dermal leishmaniasis

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Background

- Post-kala-azar dermal leishmaniasis (PKDL) is a well-known dermal sequel of visceral leishmaniasis (VL) or kala-azar.
- This is not known whether this is due to changes in the parasite genome or due to some host factors. Our group has been of the opinion that PKDL is a result of *in vivo* generation of quasi-species of *Leishmania donovani* either as *in vivo* hybridization of various endemically circulating species within the host cells or due to superinfection with other organisms (1).
- Therefore, this study was undertaken to discover the presence of organism inside the parasite by TEM. For confirmation weather it is bacteria or other species, Whole Genome Sequencing was performed using Illumina technology (2)

Objective

To investigate the possibility of superinfection in Leishmania parasites isolated from PKDL patients, with other organism, .

Methodology

Isolation
- Isolation of Leishmania parasites from skin lesions of PKDL patient.
- Identification and characterization of the isolate .

TEM
- Visualization of internal structure of PKDL strain by transmission Electron Microscopy (TEM)

WGS
- Whole Genome Sequencing (WGS) of the PKDL strain using 2x100bp pair ends on an illumina HiSeq2500 platform.
- De-novo assembly, annotations and Other bioinformatics analysis

Results

- In TEM, Surprisingly we observed some suspicious and unsure structure inside the parasite, we found those structure resembling to bacteria. This indicates the possibility of endosymbiotic infection of the Leishmania (Figure 1).

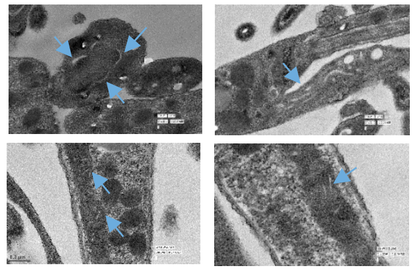

Figure 1 : TEM images showing the internal structure .

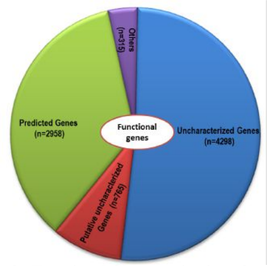

Figure 2 : Functional Characterization of Genes present in *Leishmania spp.* (PKDL)

- Whole genome sequencing produced 36.3 million reads, corresponding to more than 110-fold sequencing depth. Low-quality and adopter sequences were trimmed from the reads and filtered out depending upon phred quality score (*Q* score) of individual base (Table1). At least 138 tRNAs were identified in the genome. This number is significantly higher than other *Leishmania* isolates sequenced so far. It showed clear difference between the VL and PKDL strains (Figure 2). The BLAST analysis suggested that the 11,281 sequence reads had homology with *Leptomonas seymouri* (possibly coinfection) as reported earlier als. However we found 893 contigs of a heterotrophic bacterium *Parvibaculum lavamentivorans* DS-1 in the sequencing data, which covers 53.8% of the *Parvibaculum lavamentivorans* DS-1 genome (Figure 3). This indicates the confirmation of endosymbiotic infection of the Leishmania by this bacterium and could be cause of PKDL manifestations (2).

Nucleotide sequence accession number.

The whole-genome sequence described here has been deposited at GenBank under the accession no. LBGS00000000.

S. No	Genome Descriptions	Size/Number
1	Filtered Genome Size	27.8mb
2	Total number of Contigs	1100
3	G+C %	55.80
4	Total SNPs & INDELs	6712
	Synonymous SNPs	744
	Non-synonymous SNPs	1068
	Frame-shift INDELs	20
5	Tandem Repeats (TRs)	28,800
6	Coding Genes	8,336
7	Orthologous genes	7876
8	tRNAs	138

Table 1: Brief summary of *Leishmania spp.* (PKDL) genome

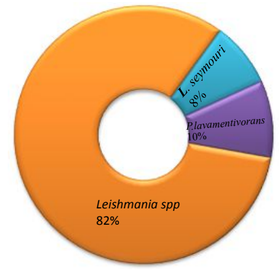

Figure 3 : Systematic representation of presence of *L. seymouri* and *P.lavamentivorans* in *Leishmania spp.* (PKDL)

Discussion and Conclusion

In Sudan and India, about 5-20% VL patients manifest as PKDL in the form of maculopapular skin lesions. These lesions may appear several months or years after the cure of VL, concomittently or even without prior history of VL. The known risk factors associated with the development of PKDL are previous treatment for VL with antimonials. The bacterium *P. lavamentivorans* is reported to degrade anti-leishmnaia drug antimony (Sb). We hypothesize that *P.lavamentivorans* after caming in contact with leishmanial parasite made a symbiotic relation and produced a different leishmania quasi-species symbiodinium which eventually results in the skin manifestations.

Acknowledgement

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References

1. *Singh S, Sharma U, Mishra J 2011. Post-kala-azar dermal leishmaniasis: recent developments. Int J Dermatol 50:1099–1108. doi:.10.1111/j.1365-4632.2011.04925.x [PubMed] [Cross Ref]*
2. *Anil Kumar Gupta, Saumya Srivastava, Amit Singh, and Sarman Singh De Novo Whole-Genome Sequence and Annotation of a Leishmania Strain Isolated from a Case of Post-Kala-Azar Dermal Leishmaniasis Genome Announc. 2015 Jul-Aug; 3(4): e00809-15.*