ECRONICON OPEN ACCESS
EC MICROBIOLOGY
Research Article

Molecular Typing of *Mycobacterium leprae* by Variable Number Tandem Repeats in Multi-Case Families of Leprosy

Santosh Chokkakula[1]*, Idress Hamad Attitalla[2], Madan Mohan Male[3] and Aldrussi A.S. Ibrahim[3]

[1]*School of Life and Health Sciences, Adikavi Nannaya University, India*

[2]*Omar Al-Mukhtar University, Faculty of Science, Microbiology Department, Libya*

[3]*University of Benghazi, Faculty of Science, Botany Department, Benghazi, Libya*

***Corresponding Author**: Santosh Chokkakula, School of Life and Health Sciences, Adikavi Nannaya University, Rajahmundry-535105, India.

Received: April 12, 2016; **Published:** September 08, 2016

Abstract

During the study period 2011 - 2013, 14 multicase families have been identified from unique screening of 1084 houses from Andhra Pradesh and Odisha states. In all multicase families, all first identified individuals were noted as MB. A total of 34 cases were successfully screened from multicases families, SSS samples were obtained, DNA was isolated and subjected to PCR-VNTR analysis. Families- 1, 7, 11, 13 have copy number 4 and families -4, 14 have copy number 5 for locus (GGT) 5. Most of the families have copy number 2 for locus 21 - 3. The copy number 9 of (GTA) 9 have linked with households of family- 1, 13, 14 and copy number 13 has seen in family 4. Copy number 11of (AT) 17 locus has coupled with households of family- 1, 4 and copy number 12, 13 have associated with families 13 and 14. Copy number 8 of (AC) 9 locus has associate with households of family- 1; 9 copy number in 4 and 14 families; 10 copy number in family 13. For 6 - 7 locus, households of family- 1, 3, 6 have 7 copy number; households of families -2, 5, 12, 13 and 14 have copy number 6; 5 copy number has seen in family- 4. Households of family- 1, 6, 8, 13 and 14 have 5 copy number; households of family -4 has copy number 4; 6 copy number has seen in family- 5 and 12 for locus 27 - 5. 23 - 3 locus has copy number 2 in multicase families. 12 copy number of (TTC) 21 locus has connected with households of families- 1, 13 and 14; families- 2 and 4 have copy number 10. Multicase families those have same copy number of VNTR loci have been infected with leprosy patient in the households. The multicase families whose have different copy number have infected with leprosy bacilli from the environment.

Keywords: *Mycobacterium leprae; Multi Case Family; Leprosy; DNA; Diseases*

Introduction

Leprosy is chronic granulomatous disease caused by *Mycobacterium leprae* and associated with the disability, stigma and discrimination to the affected individuals [1]. Though often considered a disease of antiquity, it is found most commonly today in tropical and sub-tropical regions [2]. Global efforts to eradicate leprosy have been largely successful in controlling its spread. Despite these efforts, the disease remains endemic countries, with 232,857 cases reported cases globally in 2012 [3]. India accounted for 1.27 of cases reported in 2011-2012, emphasizing the need for greater scrutiny of its epidemiology. Andhra Pradesh and Odisha are states which have high number of registered cases annually. During the year 2011 - 2012, about 7820 leprosy cases were registered in the state of Andhra Pradesh and their prevalence rate of leprosy is 0·58/10,000 where as in Odisha the prevalence rate is 0.99/10,000 and 8312 new cases were detected in 2011 - 2012 [4]. The detection rate of new cases in each year and the national prevalence reflects the continuing spread of leprosy. In such areas the strain typing and strain differentiation are very helpful to identify the source of infection, transmission of infection and spreading of disease [5,6].

In recent years molecular typing methodologies have complemented conventional infectious disease. With the publication in 2001 of the complete genome sequence of an isolate from Tamil Naidu, India called TN strain; selection of potential polymorphic genomic markers for strain typing was feasible. This contained short tandem repeats (STR) regions that had potential for genetic polymorphism by expansion or contraction of repeats and therefore for strain typing of M. leprae. Lists of loci were targeted for strain typing [7] and multi locus

variable number tandem repeat analysis (MLVA) as means for molecular differentiation of M. leprae, within and amongst leprosy patients emerged. For VNTR analysis, PCR and sequencing was most sophisticated.

Leprosy patients can carry out abundant number of bacilli and they casually spill out their bacilli to outside through skin, nasal ulcers and saliva [8,9]. People who are close contact in their family have received droplet nuclei mainly by coughing and sneezing. Such leprosy patients can transfer the bacilli in a house or in a community who are in contact with households and neighbors and have other social relationships. The peoples who are close contact with leprosy have highest risk of contracting leprosy themselves. Risk with leprosy was estimated that leprosy was about nine times higher in household patients and about four times higher in neighboring houses of patients compared with households that had no such contact with patients [10,11].

Several studies reported that *M. Leprae* can see in environment and this environment bacterial can also involve in transmission leprosy disease [12]. A report from North India noted that soil samples have been associated with *M. Leprae* bacilli [13]. Study from Indonesia reported that *M. Leprae* DNA has also been reported in water samples [14]. So the present study involves strain typing of *M. lepra*e in 14 multicase families and explains whether first affected person in multicase families have involved in leprosy transmission or environmental bacteria have associated with leprosy bacilli transmission.

Materials and Methods

Screening and enrolment of cases

Since 1989, LEPRA- India (non-profit and nongovernmental organization) takes care for patients in Andhra Pradesh, Assam, Bihar, Jharkhand, Madhya Pradesh, Odisha and Sikkim states of India with mission to restore health, hope and dignity to people affected by leprosy. In the field of leprosy, it works closely with government, national and international organisations. As referral centre, it connected with the patients in free diagnosis, free treatment of leprosy, TB, HIV, Filariasis and eye care. The Blue Peter Public Health and Research Centre (BPHRC) is research wing of LEPRA-India which attain samples from the entire referral centres of India for the research purpose. In the present study we selected, Andhra Pradesh and Odisha (Aliabad, Hyderabad districts from Andhra Pradesh and Somper, Koraput from Odisha) where high case detection rate was identified. From all the 4 districts, 9 Periphery Health Centres (2 from Andhra Pradesh and 7 from Odisha) were located and samples were collected from those PHC using the prospective study design. During the study period 2011 to 2013, 14 families were identified as multicases families. Among 14 families, 2 families have identified as all households has leprosy. In all families, the first identified person was identified as MB. The history of all families' household contacts and diagnosis date was summarised in Table 1. From all the patients, the SSS (Slit Skin Smear) was collected and were stored in a separate aliquot containing 70% ethanol and then stored at -20 until DNA extraction was done.

PHC Code	No. of families	First Person	Diagnosis date	Classification	Total no of house holds	Total leprosy patients in family	Contacts Diagnosis date
AD	Family - 1	Jade Satyavanu	22/02/13	MB	4	4	22/02/2013
AD	Family - 2	Mandokar rakesh	21/01/13	MB	2	2	10/01/2011
AD	Family - 3	Jade Anchna	22/02/13	MB	4	4	22/02/2013
S3	Family - 4	Surubali Sha	30/05/12	MB	8	2	22/5/2012
S3	Family - 5	Sarbe Sha	30/05/12	MB	4	2	28/05/2012
S3	Family - 6	Nirupama Sha	30/05/12	MB	4	2	22/5/2012
S3	Family - 7	Santhosini Sha	30/05/12	MB	8	2	22/5/2012
S3	Family - 8	Bhagya Rana	30/05/12	MB	5	2	22/5/2012
S3	Family - 9	Bipin Rana	30/05/12	MB	5	2	22/5/2012
K4	Family - 10	Bhagaban Saura	15/04/13	MB	5	2	15/04/2013

S1	Family - 11	Bhagsban	06/08/12	MB	6	3	20/08/2012
S2	Family - 12	Prashla Guru	13/08/12	MB	4	2	13/08/2012
S2	Family - 13	Padamabati padhan	20/08/12	MB	5	2	20/08/2012
S2	Family - 14	Sridhara Badi	06/05/13	MB	8	3	06/05/2013

Table 1: *Personal and clinical data of multicase families, MB- Multi Bacillary.*

DNA Extraction

According the manufactures instructions, the total genomic DNA from SSS (Slit Skin Smear) samples were extracted by DNeasy Blood & Tissue Kit, Qiagen, Germany (cat No.69504), briefly ethanol soaked samples were centrifuged and then neutralized with phosphate buffer followed by ATL and Proteinase-K treatment. Break down of cell wall and cell membrane was carried out by overnight incubation, then the sample was mixed with AL buffer and ethanol and heat at 700C for 20 min. Transfer the sample in to spin columns, washed the sample with AW1 and AW2 buffer. Finally, the DNA was eluted in 50-100µL with AE buffer with centrifugation. The DNA was stored in -40C until PCR was performed.

Amplification of VNTR loci repeats

Amplification of 9 STR loci have been done by using selected primers which were described previously for genotyping of *M. leprae* in clinical specimens [15]. These designed primers were obtained from Eurofins Genomics India Pvt. Ltd, Bangalore and list of primer was noted in (Table 2). The PCR was performed with 96 well Thermal Cycler, BIO RAD, and Ver. 1.065 and 25 µL of reaction mixture contains 12.5 µL of red dye master mix, 8.5 µL of nuclease free water, 1 µL forward primer, 1 µL reverse primer and 2 µL of genomic DNA. Cycling conditions as follows: initial denaturation for 10 min at 95°C, followed by 40 cycles, each comprised of 90 s of denaturation at 94°C, 90 s of annealing at 60°C, and 90s of extension at 72°C. A final extension step of 10 min at 72°C was performed prior to storage at 4°C. For evaluation of PCR yield, gel electrophoresis was performed by 2% using 5 µL PCR product to perceive the positive amplification STR locus.

Loci	Forward Primer	Reverse Primer	PCR Product length	Predominant Copy Number
(GTA) 9	AGCCTTAGTCGCGCAGATG	TCCGCTGTCCGTCCGCTGA	307	9
(GGT) 5	GCAGCGGTGTAACAGCATAGC	TGTCTGCCTTGCGAAACGGTC	242	5
(AT) 17	TCTCCAACATGCTGCGACA	GTACAGCGGCCTGATCGAA	181	17
21 - 3	GAATCTGACCTTTCGGAAATG	CGATGCAGCTTCCTACGG	312	3
(AC) 9	AGCGCCCGTTGTCGATAG	GACTGGATGTCGGCACCCC	236	9
27 - 5	ATTGAGCAGATGGCCGGTC	AGCAGTCGGCACGCCCTT	327	5
6 - 7	GCCATCGTTGTCGGTTCATC	CGGAGGAGGTGGGTACGGT	270	7
23 - 3	CCGAAGCCCTGGACGAAG	GCCGTAAATCCGCTCCC	243	3
(TTC) 21	GGACCTAAACCATCCCGTTT	CTACAGGGGGCACTTAGCTC	200	21

Table 2: *Primers used for amplification of 9 Short Tandem Repeat (STR) loci.*

Sequencing and VNTR Analysis

Amplify the specific regions of STR locus have been done by designed primers and analysis identification of number of repeat units was done by these primers. The PCR amplicons obtained by PCR amplification were first subjected to purification by DNA purification kit according to the manufactures instructions. Thus the PCR products with positive amplification signals have sent to Vimta Labs Pvt. Ltd, Hyderabad, India for sequencing analysis. ABI genetic Analyser 3730 (Applied Biosystems) has associated with sequences generation

and a nucleotide BLAST of the sequence of samples was performed with *M. leprae* genome at LEPROMA for detection copy number. The results obtained by nucleotide blast were compared with blasted regions of positive strain TN. So that copy number of each locus has clearly analysed.

Ethical Approval

All human slit skin smear (SSS) samples were obtained in accordance with a defined protocol which had been approved by institutional ethical review board, LEPRA India-BPHRC, Hyderabad. Consent has been obtained from patients.

Results

Patients description and diagnosis

During the study period from 2011 - 2013, over all 14 multicase families have been identified by unique house hold screening of 1084 houses from Andhra Pradesh and Odisha. Through careful findings of lesions, nerve involvements, reactions, tenderness etc., the affected individuals have been identified (as a first person) then all households have also screened. So 14 multicase families in which all first person as MB and 2 families as all leprosy affected individuals have been traced. Successful identification of 34 cases from all multicases families has done and details were summarized in (Table 1). SSS samples were obtained from all 34 cases and their DNA was subjected to PCR-VNTR analysis.

VNTR-STR analysis for identification of copy number in multicase families

The VNTR profiles of 14 Multicase families containing 34 *M. leprae* isolates were considered for our analysis. The VNTR profiles obtained in this study were compared to VNTR profiles from a published *M. leprae* VNTR database. (GGT) 5 locus has two types of copy number 5 and 4 with 4 being the most predominant. The 4 copy number of (GGT) 5 locus has associated in family- 1, 7, 11, and 13. The 5 copy number of (GGT) 5 locus has associated in family- 4, 14 and rest of the families have mixed copy number 4 and 5. 21 - 3 locus was less allelic variation and most of the families have associated with copy number 2. The VNTR locus (GTA) 9 was polymorphic in the studied population with copy number ranging from 9 to 18 and 9 being the most predominant. The identical copy number 9 of (GTA) 9 have linked with households of family- 1, 13 and 14. The copy number 13 has seen in family 4 and rest of the families have mixed copy number ranging from 9 to18.

Families	All affected persons	(GGT)5	21 - 3	(GTA)9	(AT)17	(AC)9	6 - 7	27 - 5	23 - 3	(TTC)21
TN	-	5	2	9	17	9	7	5	3	21
Family - 1	P.1	4	2	9	11	8	7	5	2	12
	P.2	4	2	9	11	8	7	5	2	12
	P.3	4	2	9	11	8	7	5	2	12
	P.4	4	2	9	11	8	7	5	2	12
Family - 2	P.1	4	2	10	9	6	6	4	3	10
	P.2	5	2	13	10	7	6	6	3	10
Family -3	P.1	5	3	10	9	9	7	4	2	9
	P.2	5	3	11	10	8	7	6	2	13
	P.3	4	2	14	11	10	7	5	2	14
	P.4	4	2	15	12	16	7	4	2	16
Family - 4	P.1	5	3	13	11	9	5	4	3	10
	P.2	5	3	13	11	9	5	4	3	10

Family - 5	P.1	4	3	18	11	9	6	6	2	15
	P.2	5	2	16	12	8	6	6	2	18
Family - 6	P.1	4	2	11	13	7	7	5	3	21
	P.2	5	2	9	14	8	7	5	3	19
Family - 7	P.1	4	2	14	9	9	5	4	2	20
	P.2	4	2	13	16	8	6	5	2	12
Family - 8	P.1	4	3	11	21	10	7	5	3	21
	P.2	5	2	10	24	9	8	5	3	20
Family - 9	P.1	4	2	13	14	8	10	4	2	19
	P.2	5	2	14	16	7	6	5	2	16
Family - 10	P.1	4	2	17	11	6	6	6	2	18
	P.2	5	2	16	12	16	10	4	2	13
Family - 11	P.1	4	2	10	13	7	8	4	3	14
	P.2	4	2	10	14	8	7	5	3	15
	P.3	4	2	10	16	9	6	5	2	18
Family - 12	P.1	4	3	15	14	8	6	6	2	19
	P.2	5	2	14	13	8	6	6	2	8
Family - 13	P.1	4	2	9	12	10	6	5	3	12
	P.2	4	2	9	12	10	6	5	2	12
Family - 14	P.1	5	2	9	13	9	6	5	2	12
	P.2	5	2	9	13	9	6	5	2	12
	P.3	5	2	9	13	9	6	5	2	12

Table 3: *Allelic distribution of 9 STR loci of M. leprae strains from multicase families.*

(AT)17 locus is obviously highly polymorphic and found 11 copy number was predominant. The identical copy number 11of (AT) 17 have linked with households of family-1and 4. Copy number 12 and 13 have associated with families 13 and 14 respectively and rest of the families have different copy number ranging from 9 to 24. (AC)9 locus was also observed as polymorphic but it lesser than the (AT) 17 locus and 8 copy number and 7 copy numbers were identified as abundant. The single copy number 8 have associate with households of family-1; 9 copy number in 4 and 14 families; 10 copy number in family 13. Remaining families have allied with different copy number. For 6 - 7 locus, households of family- 1, 3, 6, have 7 copy number; households of families - 2, 5, 12, 13 and 14 have copy number 6; 5 copy number have seen in family- 4.

27 - 5 locus has been coupled with copy number 4, 5 and 6 in which 5 copy number is the most predominant. Households of family- 1, 6, 8, 13 and 14 have 5 copy number; households of family- 4 have copy number 4; 6 copy number have seen in family- 5 and 12. And rest of the families have different copy numbers. Only two types of copy numbers 2 and copy 3 have been identified in 23 - 3 locus with 2 being predominant. Most of the households of family have copy number 2. (TTC) 21 locus have linked with highest allelic variation than all the present study locus and have number ranging from 9 to 21. 12 copy number have connected with households of families- 1, 13 and 14; families- 2 and 4 has copy number 10 and rest of the families have different copy number ranging from 9 to 21.

Discussion

Strain tying and strain differentiation is a method which useful to distinguishing members of the same microbial species from one another on the basis of genotype. Strain typing explains that one isolate is same or it's different. Several molecular typing methods have been employed to distinguish the *M. leprae* strains over the years. Identification leprosy transmission in households of multicase families

still unknown concern. The first affected person in households is the direct agent to transmission of disease in multicase families. There is possibility to transmission of bacteria from environment to multicase families may occur [16]. The present study involves strain typing of *M. leprae* in 14 multicase families containing 34 patients. This method of study can notably explain that persons in the multicase families either affected with leprosy patient in the same household or with leprosy affected patient from outside thereby to easy understanding of leprosy bacilli transmission.

The STR profile of present study population was compared with reference sequenced strain TN, of South Indian origin to identify shared genetic markers. Our results for locus (GGT)5 exhibiting 4 copy allele as higher, is similar to reported from Colombia [17], Brazil [18], South India [19], Mumbai [20] however interestingly, Philippines leprosy patients have shown 6 copy number of (GGT)5 locus [21]. The 4 copy number of (GGT) 5 locus has associated in family- 1, 7, 11, and 13. The 5 copy number of (GGT) 5 locus has associated in family- 4, 14 and rest of the families have mixed copy number 4 and 5. All households of multicase families have same copy number of indicates, households gets affected with first person. In the present study 2 copy number of 21 - 3 locus was predominant as that of South India [19], North India [20] and Thailand [22] however Philippines leprosy patients having 3 copy number as a predominant [21]. In China [23] and Brazil [18] leprosy patients have associated with 3 copy number of 21 - 3 loci which results were differ from the present results where 2 types of copy numbers were identified and most of the multicase families have associated with copy number 2. Predominance of allele with 9 copies for loci (GTA). 9 was observed in our population which is similar to that reported earlier from studies conducted in South India and Mumbai [19,20]. Miyako Kimura [24] and colleagues reported variation in the (GTA) 9 locus from Cebu and identified as the most abundant repeat units were 9 and then 10 which support our present result. In Philippians populations 9 repeat was the most abundant followed by 11 and 12 [21] which were also slightly resembles to the present results. Families-1, 4, 13 and 14 have identical copy number indicates those families infected with first affected person in the family.

Isolates from South India [19], North India [25] leprosy patient's predominance of (AT) 17 locus with copy number 11 alike our present study however Thailand [22], Philippines [21], Colombia [17], China [23] and Brazilian [18] leprosy patient showed predominance of allele with copy number 13, 13, 12, 12 and 14 respectively. Families 1, 4, 13 and 14 have single copy number and those households get infected with leprosy patient from the family. Microsatellite (AC) 9 predominantly exhibit allele with copy number 8. This finding is similar to those reported from Thailand [22], Brazil [18], China [23] and South India [19] but Philippians [21], Colombia [17] leprosy patients have associated with 9 and 7 copy number of (AC) 9 respectively. 1, 4, 13 and 14 families have been coupled with the single copy number which describes that these multicase families get infected with one of the leprosy affected patient in the total households. 6 - 7 locus was less polymorphic with 6 copy number 54 (54%) and 7 copy number 32 (32%) as a predominant which support Colombian [17], Thailand [22] and South Indian [19] leprosy patients. Most of the families have allied with same copy number for 6 - 7 locus.

27 - 5 clearly described as 27 - 5 repeat was conserved with 5 times. In Mumbai leprosy population 5 copy number of 27 - 5 locus was predominant which is resembles to our study [20]. In North Indian population 5 and 6 copy number was high [25] and in South Indian patients 5 and 4 copy number was high [19], in these studies South Indian leprosy patients as like of our results but not alike of North Indian report. The 27 - 5 locus has predominant copy number with 5 and from the patients of Thailand and Philippines those are resembles with our study [22, 21] however Colombian leprosy patients have 4 and 5 copy number as high [17]. Brazilian leprosy patients have been associated with two types of repeat units 4 and 5 but in our study the copy number of 27 - 5 locus have ranging from 4 to 6 and 5 copy number was high however China leprosy population have only one type of copy number 5 [23].

Households of family- 1, 6, 8, 13 and 14 have 5 copy number; households of family -4 have copy number 4; 6 copy number have seen in family- 5 and 12. 23 - 3 locus was less polymorphic with 2 types of copy number 2 and 3. Thailand [22], Mumbai [20] have been associated with two types of copy number as like of our report but Philippians [21], Colombian [17], China [23] and Brazilian [18] shown one type of copy number 2. Most of the households of multicase family have copy number 2. The 21 bp locus (TTC) 21 has highest polymorphic over all the present study VNTR locus and families- 1, 2, 4, 13 and 14 have single copy number for locus (TTC)21. Several studies have been conducted and stated that *M. leprae* bacteria can survive few days in environmental sources like water, soil and some human excretory.

The DNA of M. leprae have seen soil sample from Indonesia [14]. Studies from North India reported that M. leprae have identified in soil samples of some endemic areas [12,13].

Conclusion

Our present study concluded that multicase families which have same copy number of VNTR loci have been infected with leprosy patient in the households. The multicase families whose have different copy number have infected with leprosy bacilli from the environment.

Acknowledgement

We heart fully thank Dr. Aparna, Group leader, Microbiology Division, LEPRA India- Blue Peter public Health and Research centre, Hyderabad, India for providing leprosy slit skin smear samples and for providing good laboratory facility to carry out this work. All the field and administrative staff, Leprosy programme officers of Hyderabad, Adilabad, Koraput and Sonepur districts are kindly acknowledged for their support in data collection.

Bibliography

1. Scollard DM., *et al*. "The continuing challenges of leprosy". *Clinical Microbiology Reviews* 19.2 (2006): 338-812.
2. Britton WJ and Lockwood DN "*Leprosy Lancet*". 363.9416 (2004):1209-1219.
3. World Health Organization report 2012. Leprosy today (2014).
4. National Leprosy Eradication Programme (NLEP) Progress Report, India for the year 2011-12.
5. Linder K., *et al*. "Relapses vs. reactions in multibacillary leprosy: proposal of new relapse criteria". *Tropical Medicine and International Health* 13.3 (2008): 295-309.
6. Shetty VP., *et al*. "Clinical, histopathological and bacteriological study of 52 referral MB cases relapsing after MDT". *Leprosy Review* 76.3 (2005): 241-252.
7. Shin YC., *et al*. "Variable numbers of TTC repeats in Mycobacterium leprae DNA from leprosy patients and use in strain differentiation". *Journal of Clinical Microbiology* 38.12 (2000): 4535-4538.
8. Davey TF and Rees RJ., "The nasal discharge in leprosy: clinical and bacteriological aspects". *Leprosy Review* 45.2 (1974): 121-134.
9. Kotteswaran G., *et al*. "Skin adnexa in leprosy and their role in the dissemination of M. Leprae". *Leprosy Review* 52.4 (1980): 475-481.
10. Fine PE., *et al*. "Ponnighaus Household and dwelling contact as risk factors for leprosy in northern Malawi". *American Journal Epidemiolology* 146.1 (1997): 91-102.
11. Van Beers SM., *et al*. "Patient contact is the major determinant in incident leprosy: implications for future control". *International Journal of Leprosy and Other Mycobacterium Diseases* 67.2 (1999): 119-128.
12. Turankar RP., *et al*. "Dynamics of Mycobacterium leprae transmission in environmental context: deciphering the role of environment as a potential reservoir". *Infection Genetics Evolution* 12.1 (2012): 121-126.
13. Lavania M., *et al*. "Detection of viable Mycobacterium leprae from environmental soil samples: insights into possible sources for transmission of leprosy". *Infection Genetics Evolution* 8.5 (2008): 627-631.
14. Matsuoka M., *et al*. "Mycobacterium leprae DNA in daily using water as a possible source of leprosy infection". *Indian Journal of Leprosy* 71.1 (1999): 61-67.

15. Kimura M., *et al*. "Rapid variable-number tandem-repeat genotyping for *Mycobacterium leprae* clinical specimens". *Journal of Clinical Microbiology* 47.6 (2009): 1757-1766.

16. Desikan KV and Sreevatsa. "Extended studies on the viability of Mycobacterium leprae outside the human body". *Leprosy Review* 66.4 (1995): 287-295.

17. Nora CC., *et al*. "Identification and comparison of Mycobacterium leprae genotypes in two geographical regions of Colombia". *Leprosy Review* 80 (2009): 316-321.

18. Fontes AN., *et al*. "Genetic diversity of *Mycobacterium leprae* isolates from Brazilian leprosy patients". *Leprosy Review* 80.3 (2009): 302-315.

19. Shinde V., *et al*. "VNTR typing of Mycobacterium leprae in South Indian leprosy patients". *Leprosy Review* 80.3 (2009): 290-301.

20. Sanjana K., *et al*. "Distribution of *Mycobacterium leprae* Strains among Cases in a Rural and Urban Population of Maharashtra, India". *Journal of Clinical Microbiology* 50.4 (2012): 1406-1411.

21. Sakamuri RM., *et al*. "A continuation: study and characterisation of *M. leprae* short tandem repeat genotypes and transmission of leprosy in Cebu, Philippines". *Leprosy Review* 80.3 (2009): 272-279.

22. Sopa S., *et al*. "Molecular epidemiology of leprosy based on VNTR typing in Thailand". *Leprosy Review* 80.3 (2009): 280-289.

23. Yan X., *et al*. "VNTR typing studies of Mycobacterium leprae in China: Assessment of methods and stability of markers during treatment". *Leprosy Review* 80.3 (2009): 261-271.

24. Miyako K., *et al*. "Rapid Variable-Number Tandem-Repeat Genotyping for Mycobacterium leprae Clinical Specimens". *Journal of Clinical Microbiology* 47.6 (2009): 1757-1766.

25. Lavania M., *et al*. "Molecular typing of *Mycobacterium leprae* strains from northern India using short tandem repeats". *Indian Journal of Medical Research* 133 (2011): 618-626.

Volume 3 Issue 4 September 2016
© All rights reserved by Santosh Chokkakula., *et al.*