

**EPIDEMIOLOGY OF ORAL DISEASES AND ORAL BACTERIAL
PROFILING USING METAGENOMICS AMONG
TRANSGENDERS & MEN HAVING SEX WITH MEN OF
TAMILNADU**

A Thesis

Submitted by

B. KRISHNAPRASANTH

[D18MS003]

in partial fulfilment for the award of the degree

of

DOCTOR OF PHILOSOPHY



FACULTY OF MEDICINE

(Community Medicine)

BHARATH INSTITUTE OF HIGHER EDUCATION

AND RESEARCH

Chennai - 73

December 2023



Certificate

This is to certify that the corrections as recommended by the Foreign and Indian Examiners for the Thesis entitled, “**EPIDEMIOLOGY OF ORAL DISEASES AND ORAL BACTERIAL PROFILING USING METAGENOMICS AMONG TRANSGENDERS AND MEN HAVING SEX WITH MEN OF TAMILNADU**” submitted by the Candidate **B. KRISHNA PRASANTH**, Reg No. **D18MS003** (Part Time-Internal) in the Department of **COMMUNITY MEDICINE**, Faculty of **MEDICINE** have been made as per the list enclosed.

Signature of the Supervisor

Dr.K. MAHALAKSHMI, Ph.D.,
Professor & Head
Department of Microbiology
Sree Balaji Dental College & Hospital
Velachery Main Road, Chennai - 600 100

BONAFIDE CERTIFICATE

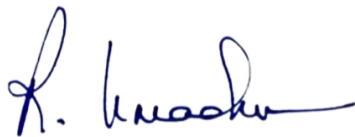
Certified that this thesis titled “**EPIDEMIOLOGY OF ORAL DISEASES AND ORAL BACTERIAL PROFILING USING METAGENOMICS AMONG TRANSGENDERS & MEN HAVING SEX WITH MEN OF TAMILNADU**” is the bonafide work of **Dr. B. KRISHNA PRASANTH [Reg no D18MS003]** who carried out the research under our supervision.

Certified further, that to the best of our knowledge, the work reported herein and in the publications by the scholar does not form part of any other thesis or dissertation, on the basis of which a degree or award was conferred on an earlier occasion of thesis of any other candidate.



Dr.K. MAHALAKSHMI, Ph.D.,
Professor & Head
Department of Microbiology
Sree Balaji Dental College & Hospital
Velachery Main Road, Chennai - 600 100

SUPERVISOR- Dr. K. Mahalakshmi, PhD



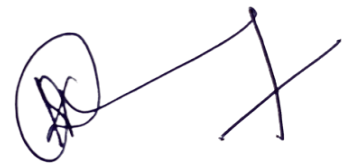
PROFESSOR & H.O.D.
Dept. of Community Medicine
Sree Balaji Medical College
Chennai-600 044.

JOINT-SUPERVISOR- Dr. R. Umadevi, MD

DECLARATION

I declare that this thesis, entitled “**EPIDEMIOLOGY OF ORAL DISEASES AND ORAL BACTERIAL PROFILING USING METAGENOMICS AMONG TRANSGENDERS & MEN HAVING SEX WITH MEN OF TAMILNADU**” is an original and bonafide work done of my own in the partial fulfillment of the requirement for the award of the degree of **Doctor of Philosophy (PhD) in Community Medicine** and submitted to BIHER, carried out under the supervision of **Dr. K. Mahalakshmi**, Professor & Head, Department of Microbiology, Sree Balaji Dental College & Hospital, Bharath Institute of Higher Education & Research, Chennai, Tamilnadu, India and Co-supervision of **Dr. R. Umadevi**, Professor & Head, Department of Community Medicine, Sree Balaji Medical College & Hospital, Bharath Institute of Higher Education & Research, Chennai, Tamilnadu, India.

I further declare that this thesis does not form part of any other synopsis or dissertation on the basis of which a degree was awarded or conferred on earlier occasion on me or any other candidate



PLACE: Chennai

Signature of the candidate

DATE:

Dr. B. Krishna Prasanth

D18MS003

ACKNOWLEDGEMENT

I express my sincere gratitude to Honorable Founder, **Dr. S. JAGATHRATCHAGAN**, Managing trustee, **Dr. J. SRINISHA**, Chancellor, **Dr. MOHAMMED RELA** Vice-Chancellor, **Prof. K. VIJAYA BHASKAR RAJU** for extending their kind support during my research. I express my gratefulness to Pro-Vice Chancellors, **Dr. R.M. SURESH**, **Dr. R. VENKATESH BABU**, **Dr. M. SUNDARARAJAN**, **Dr. SURESH KUMAR SUBBIAH**, Registrar, **Dr. S. BHUMINATHAN** and additional Registrar, **Dr. R. HARIPRAKASH** for giving me an opportunity to pursue this research program. I extend my wholehearted gratitude to our Dean (in-charge) and Medical Superintendent, **Dr. SASI KUMAR** of Sree Balaji Medical College and Hospital for his great support and encouragement.

I would like to thank **Dr. P. NAVEEN CHANDER**, Dean R & D for his constant support and also facilitated to submit the thesis of my work successfully.

I feel indebted to my guide **Dr. K. MAHALAKSHMI**, Professor & Head, Department of Medical Microbiology, Sree Balaji Dental College and Hospital for her constant support, innovative ideas, timely suggestions and erudite guidance in my study and for being a source of inspiration in my endeavors. Her enthusiasm, keen interest, dedicated attitude and continuous help with congruent encouragement is an inexhaustible inspiration which always gives me strength to face the challenges of research. It is my privilege to have worked under the supervision of such an eminent teacher and academic expert. The profound knowledge I gained from her will be benefit me in my future life and career.

I express my deepest gratitude to my co-guide **Dr. R. UMADEVI**, Professor & Head, Department of Community Medicine, Sree Balaji Medical College and Hospital, for her constant support during my entire course of Ph.D. I am very grateful to her as she has introduced me to various national and international faculty with whom I could collaborate for further research work. She has given me valuable suggestions and did fruitful discussions regarding my work and helped me in each step during my Ph.D. period. My madam motivated me in every step during my PhD period. Her expert's opinions meant a lot to me, as I was able to improve my research. I would like to thank her immensely for adjusting the department works and gave time for my research work.

I express my sincere gratitude and thanks to my Doctoral committee members, **Dr. C.P. GIRISH KUMAR**, Scientist-D, National Institute of Epidemiology Chennai and **Dr. S. KALPANA**, Research Officer, Department of Epidemiology, The Tamilnadu Dr. M.G.R. Medical University, Chennai for serving as my DC members whose suggestions and comments were very much valuable for me. I would also like express my gratitude to them for supporting my research protocol and allowing me to further continue with my Ph.D. research work.

I convey my special thanks to **Dr. NAVEEN KUMAR**, IMMUGENIX BIOSCIENCES for his constant support in my laboratory works and helping me throughout my metagenomics research work.

I would like to convey special thanks to colleague and sister **Ms. B. ASHNI**, Assistant Professor of Statistics, Department of Community Medicine, Sree Balaji Medical College for her immense help and guidance in statistics and data analysis during my research.

I extend my heartfelt thanks to my dear friend and colleague **Dr. V.M. ANANTHAEASHWAR**, Assistant Professor, Department of Community Medicine, Sree Balaji Medical College for his untiring support and constant help without which my publications would not have been possible,

I would like to convey my special thanks to **Dr. FARHANA RAHMAN**, Professor, Department of Pharmacology, Sree Balaji Medical College and Hospital, for being there always and helping me during my difficult times. I also like to convey my thanks to **Dr. PRABHU**, Associate Professor, Department of Anatomy, Sree Balaji Medical College for helping me in the official matters related to Ph.D.

I am thankful to all Professors in my department including **Dr. RASHMI GOUR PATEL, Dr. TIMSI JAIN, Dr. T.P. JAYANTHI**, who always support me and make the working place a pleasant environment.

I offer my heartfelt thanks to my friends **Dr. J. DANTI, Dr. S. HARIHARAN, Dr. V. ALJIN, Dr. P.M. AISHWARYA, Dr. M. PONMALAR , Dr. AMIRTHA LEKHA, Dr. T. RAM KUMAR** and other all other staff members of the department of Community Medicine, well-wishers and people who directly or indirectly gave me encouragement and support throughout my career.

Words would not be enough to thank my dear post graduate students **Dr. P. PRAGADEESH, Dr. B.N. SURYA, & Dr. NISHANTH** for being a source of support throughout my PhD work and my undergraduate students **Dr. VIKAS HRUDHAY SHUNKARA** and **Dr. HARSHAN** for helping me during the data collection.

I am indebted to my wife **Dr. LEKHA KRISHNAN** and my dear daughter **DHWANI USHA KRISHNA** for their loving support and co-operation. I am very grateful to my **parents** and **in-laws** who have been the solid pillars in my life and career.

At last, but not the least, I am very grateful and would like to thank **GOD, The Almighty** who has granted me countless blessings, knowledge and opportunities, so that I have been able to accomplish the thesis.

LIST OF TABLES

S.No.	TITLE OF THE TABLE	PAGE No.
1.	DNA concentration and purity of samples estimated using Nanodrop Spectrophotometer and Qubit Fluorimeter	71
2.	Frequency distribution of social domain	85
3.	Frequency distribution of sexual domain	87
4.	Frequency distribution of mental domain	88
5.	Frequency distribution of oral health domain	90
6.	Multivariate analysis of various factors with median scores of social domains	92
7.	Multivariate analysis of various factors with median scores of sexual domains	94
8.	Multivariate analysis of various factors with median scores of mental health domain	96
9.	Multivariate analysis of various factors with median scores of oral health domain	98
10.	Highlights from multivariate regression-variables that are significant risk factors	99
11.	Prevalence of various oral diseases noted in this study	100
12.	Association of various variables with gingival status [Loe & Silness index]	101
13.	Association of various variables with dental treatment needs [DMFT index]	102
14.	Association of various variables with oral hygiene status [OHI-S index]	103
15.	Distribution of reads in each sample of transgender and men having sex with men (MSM)	113
16.	Total number of raw reads and processed reads among transgender and MSM individuals	114
17.	Distribution of alpha diversity among the samples	114
18.	Distribution of beta diversity among the samples	115

19.	Distribution of microbiome domains among the samples	116
20.	Abundance of phylum in subgingival plaque samples of transgender and MSM individuals	118
21.	Abundance of class in subgingival plaque samples of transgender and MSM individuals	120
22.	Abundance of order in subgingival plaque samples of transgender and MSM individuals	121
23.	Abundance of family in subgingival plaque samples of transgender and MSM individuals	124
24.	Abundance of genus in subgingival plaque samples of transgender and MSM individuals	127
25.	Abundance of species in subgingival plaque samples of transgender and MSM individuals	131
26.	The most abundant genus and species in transgender and MSM	139

LIST OF FIGURES

S.No.	TITLE OF THE FIGURE	PAGE No.
1.	Oral health status framework	19
2.	Agarose gel electrophoresis of the subgingival plaque samples	72
3.	Universal sequences for tailing PCR primers	74
4.	Full length 16s PCR amplification	75
5.	Overview of library preparation	75
6.	Data analysis workflow	77
7.	Distribution of age among transgender and MSM individuals	105
8.	Distribution of marital status among transgender and MSM individuals	106
9.	Distribution of educational status among transgender and MSM individuals	107
10.	Distribution of occupation among transgender and MSM individuals	108
11.	Distribution of current living status among transgender and MSM individuals	109
12.	Distribution of income among transgender and MSM individuals	110
13.	Distribution of number of people living with transgender and MSM individuals	111
14.	Distribution of health insurance coverage among transgender and MSM	112
15.	Cluster bar diagram showing species richness of the samples among the two groups.	116
16.	Rarefaction curve showing the species richness of the samples among the two groups.	117
17.	Bubble plot showing abundance of phylum in subgingival plaque samples of transgender and MSM individuals	119
18.	Bubble plot showing abundance of class in subgingival plaque samples of transgender and MSM individuals	121
19.	Bubble plot showing abundance of order in subgingival plaque samples of transgender and MSM individuals	123

20.	Bubble plot showing abundance of family in subgingival plaque samples of transgender and MSM individuals	126
21.	Bubble plot showing abundance of genus in subgingival plaque samples of transgender and MSM individuals	130
22.	Bubble plot showing abundance of species in subgingival plaque samples of transgender and MSM individuals	136