Dedicated to:

My Beloved Parents

and

Siblings

Declaration

I, Papiya Sinha, bearing registration no. Ph.D./1136/2010 dated 22.09.2010 hereby declare

that the subject matter of the thesis entitled "A comparative study on Biofilm production and

Antibiotic resistance profile of coagulase positive Staphylococci from environmental and

clinical isolates of south Assam" is the record of work done by me and the content of the

thesis did not form the basis for award of any degree to me or anybody else to the best of my

knowledge. This thesis has not been submitted in any other university/ institute.

This thesis is being submitted to Assam University, Silchar for the degree of Doctor of

Philosophy in Microbiology.

Date: 5th Sept' 2015

Place: Silchar

(PAPIYA SINHA)

Acknowledgement

At the onset, my deep sense of gratitude to the supreme God, the creator above us, for endowing me knowledge, courage, health and strength to achieve the goal.

I have great pleasure in expressing my sincere gratitude to my teacher and supervisor Prof. Piyush Pandey, Department of Microbiology, Assam University Silchar. It has been really an honour to be his first Ph.D. student. He has always been more than a teacher with his splendid supervision, constant guidance, valuable advice and encouragement. His motivations, patience, ideas, comments, constructive criticisms and enthusiasm led to a successful completion of this study.

My sincere gratitude and thanks are due to Prof. Devashish Kar, Department of Life Science and Bioinformatics, Assam University, Silchar for his support and guidance as Co-Supervisor.

I express my sincere gratitude to Dr. Amitabha Bhattacharjee and Dr. Indu Sharma, Department of Microbiology of Assam University for their continuous support, encouragement and valuable advice.

I owe a debt of gratitude to Prof. G. D. Sharma, Department of Life Science and Bioinformatics, Assam University, Silchar for helping me all through official formalities.

I sincerely thank Prof. Niranjan Nayak, Division of ophthalmology, All India Institute of Medical Sciences (AIIMS) for his useful suggestion about my research.

I can't forget the contribution of Mr. Partha Das sir, ex in-charge, Research cell, Assam university Silchar.

Special thanks goes to Dr. Dipankar Biswas, Senior Scientist, and Dr. Kaushal Yadav, senior research fellow (SRF) of Regional Medical Research Council (RMRC), Dibrugarh for teaching me the molecular techniques.

Credit also goes to Mr. Iyappan, Ph.D. scholar of Annamalai university, Chennai and

Monjur Laskar, Ph.D scholar of dept of life sciences and bioinformatics, assam University,

Silchar for thier contribution in my research work.

I also take this opportunity to thank the Technical, Laboratory and Office staffs

Swetosmita Nath, Joya Das and Mousumi Di, of the Department of Microbiology for

their help and assistance whenever needed.

I am thankful to the scientists and staffs of SAIF, North Eastern Hill University,

Shillong, and Indian Institute of Technology, Guwahati for providing the scanning

electron microscopy (SEM) and confocal laser scanning microscopy (CLSM) facility.

I convey a big 'thank you' to my fellow-mates and the scholars of Soil and

Environmental Microbiology laboratory (SEML, AUS): Th. Nevita, Romita Angom, Aruna

Likhari, Olivia Khunjan, Lakshmi Kshetri and L. Paikhomba Singha for their continuous

support and help whenever needed.

I am really proud to have friends like Ruchira Das, L. Minakshi singha and

Moushumi Shyam Choudhury who were with me like shadow with their constant

encouragement and upkeep in my every ups and downs during this research.

Lastly, my regards and heartiest gratitude for my father, Mr. Prodyut Kumar sinha

and my mother, Mrs. Rita Sinha who are my strength and source of inspiration in all my

tough situation. A very big thanks also goes to my siblings, Probal Sinha, Prashun sinha

and Pushpa Sinha for always being in my side with continuous support and help to carry

out my research.

Thank you all.

Date: 05.09.2015

PAPIYA SINHA

Place: Silchar.

Contents

TITLE	PAGE NO.
DEDICATION	
DECLARATION	
CERTIFICATE	
ACKNOWLEDGEMENT	
LIST OF FIGURES	i-iii
LIST OF TABLES	iv
CHAPTER 1	
INTRODUCTION	1-7
CHAPTER 2	
REVIEW OF LITERATURE	8-36
CHAPTER 3	
MATERIALS AND METHODS	37-51
CHAPTER 4	
RESULTS	57-75
CHAPTER 5	
DISCUSSION	76-86
CHAPTER 6	
CONCLUSION	
CHAPTER 7	87-88
BIBLIOGRAPHY	89-124
ANNEXURE	v-xiv
LIST OF PAPERS PUBLISHED	xv-xvi

LIST OF TABLES

Table No.	Title
2.1	S. aureus virulence mechanisms.
3.1	Lists of primers used.
4.1	Prevalence of <i>S. aureus</i> from clinical sources.
4.2	Prevalence of <i>S. aureus</i> from CA/ environmental sources.
4.3	Preliminary screening of <i>S. aureus</i> isolates.
4.4	Morphological and biochemical characterization of the isolates.
4.5	Distance matrix showing relation between <i>S. aureus</i> clinical isolates (SaC53) and CA/environmental isolates (SaE31).
4.6	Percentage of clinical isolates sensitive/ resistant to different antibiotics.
4.7	Percentage of CA/environmental isolates sensitive/ resistant to different antibiotics.
4.8	Prevalence of <i>S. aureus</i> types in clinical and CA/environmental isolates.
4.9	Comparative biofilm formation pattern of clinical and CA/environmental isolates by CRA method and tube method.
4.10	Prevalence of biofilm formation by tube method in all <i>S. aureus</i> isolates.
4.11	The mean optical densities (ODs) and standard deviations (SDs) of different biofilm phenotypes.
4.12	Sensitivity and specificity of phenotypic and genotypic methods of biofilm evaluation.
4.13	The effect of surface and glucose on biofilm formation by <i>S. aureus</i> isolates.
4.14	Prevalence of <i>icaABCD</i> genes and their biofilm formation pattern.
4.15	Prevalence of <i>hla</i> and <i>hlb</i> genes and their haemolytic pattern.
4.16	Prevalence of <i>sea</i> and <i>sed</i> genes among clinical and CA/ environmental isolates.

LIST OF FIGURES

Figure No.	Title
2.1	Global prevalence of MRSA.
2.2	Schematic circular diagram of the SCC-like MSSA463 chromosome.
2.3	Three main stages of <i>S. aureus</i> biofilm development.
2.4	Schematic diagram of organisation of ica locus in S. aureus.
2.5	Model of PBP2a mediated modulation of biofilm expression and virulence.
3.1	Map showing global location of south Assam.
3.2	Map of study area showing location of three districts of south Assam.
3.3	Diagrammatic representation of biofilm development in PVC material.
4.1	S. aureus grown on Mannitol Salt Agar
4.2	S. aureus small colony variants (SCV) grown on Mannitol Salt Agar
4.3	Gram reaction of S. aureus.
4.4	Catalase reaction of <i>S. aureus</i> .
4.5	Clot formation for coagulase positive test of <i>S. aureus</i> .
4.6	Biochemical Characterization.
4.7	Phylogenetic tree showing relation between S.aureus clinical isolates (Sac53) and CA/environmental isolates (SaE31).
4.8	Antibiotic susceptibility test of <i>S. aureus</i> clinical isolate C1647 by disc diffusion method.
4.9	Comparative MAR index of both clinical and CA/ environmental <i>S. aureus</i> isolates against a range of antibiotics.
4.10	In-vitro biofilm formation by Congo Red Agar (CRA) method.
4.11	In-vitro biofilm formation of <i>S. aureus</i> isolates by Tube Method (TM).
4.12	Comparative adherence values of selected clinical and CA/ environmental isolates of <i>S. aureus</i> by quantitative biofilm estimation method at 620 nm OD.

LIST OF FIGURES

4.13	Correlation between adherence values of selected clinical and CA/environmental isoaltes of <i>S. aureus</i> by quantitative biofilm estimation method at 620 nm OD.
4.14	Comparative biofilm formation on different surfaces by clinical and CA/environmental isolates of <i>S. aureus</i> by TM at 620 nm OD.
4.15	Comparative biofilm formation on different surfaces by WT and SCV isolates of <i>S. aureus</i> by TM at 620 nm OD.
4.16	SEM micrograph of in-vitro biofilm formation on different surfaces
4.17	Effect of different glucose conc. on biofilm formation by selected <i>S. aureus</i> isolates of both clinical and environmental origin.
4.18	Effect of glucose stress on biofilm formation by selected SCV isolates of <i>S. aureus</i> of both clinical and CA/environmental origin.
4.19	Effect of glucose conc. on biofilm formation by selected WT isolates of <i>S. aureus</i> of both clinical and CA/environmental origin.
4.20	Effect of different salt concentration on biofilm formation by all <i>S. aureus</i> isolates of both clinical and environmental origin.
4.21	Effect of different salt stress on biofilm formation by selected SCV <i>S. aureus</i> isolates of both clinical and environmental origin.
4.22	Effect of different salt stress on biofilm formation by selected WT <i>S. aureus</i> isolates of both clinical and environmental origin.
4.23	Effect of different incubation period on biofilm formation by all <i>S. aureus</i> isolates of both clinical and environmental origin.
4.24	Effect of different incubation period on biofilm formation by selected <i>S. aureus</i> isolates of both clinical and environmental origin.
4.25	SEM micrograph of <i>in-vitro</i> biofilm formation at different incubation period
4.26	Effect of pH stress on biofilm formation by all <i>S. aureus</i> isolates of both clinical and environmental origin.
4.27	Effect of pH on biofilm formation by both WT and SCV <i>S. aureus</i> isolates of both clinical and environmental origin.
4.28a	Effect of antibiotic (OX= oxacillin) on biofilm formation by clinical and environmental <i>S. aureus</i> isolates by quantitative biofilm estimation method.

LIST OF FIGURES

4.28b	Effect of antibiotic (OX= oxacillin) on biofilm formation by MSSA and MRSA isolates by quantitative biofilm estimation method at 620 nm OD.
4.29	<i>In-vitro</i> biofilm formation by <i>S.aureus</i> isolates (SaC4075) in tubes.
4.30	SEM micrograph of clinical <i>S. aureus</i> isolate (C4075)
4.31	SEM micrograph of <i>S. aureus</i> SCV isolate (SaC64).
4.32	Molecular detection of icaA gene
4.33	Molecular detection of icaD gene
4.34	Comparative haemolysis pattern exhibited by MRSA and MSSA biotypes of clinical and CA/environmental isolates of <i>S. aureus</i> .
4.35	Comparative haemolytic zone sizes displayed by both clinical and CA/environmental isolates of <i>S. aureus</i> .
4.36	Molecular detection of <i>hla</i> gene
4.37	Molecular detection of hlb gene
4.38 4.39	Frequency of <i>icaA</i> and <i>icaD</i> gene in all <i>S. aureus</i> isolates Frequency of <i>hla</i> and <i>hlb</i> gene in all <i>S. aureus</i> isolates.
4.40	Molecular detection of sea gene
4.41	Molecular detection of sed gene
4.42	Frequency of extracellular enzyme production in clinical and CA/environmental isolates of <i>S. aureus</i> .
4.43	Graph showing zone diameter of extracellular enzyme production in all isolates of <i>S. aureus</i> .
4.44	Comparison of region-wise prevalence rate of <i>S. aureus</i> isolates of south Assam alongwith their methicillin resistance profile.
4.45	Region-wise prevalence rate of different virulence genes of <i>S. aureus</i> isolates of south Assam.