DOI: 10.31357/fmesmph.2012.00142

Role of *Staphylococcus aureus* in Atopic Dermatitis

by

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Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Master of Philosophy in Medical Sciences on 2011

I certify that the candidate has incorporated all corrections, amendments and additions recommended by the examiners.

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The work described in this thesis was carried out by me under the supervision of Prof. Neluka Fernando and Dr. Neelika Malavige and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma

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ABST	- antibiotic sensitivity testing
AD	- Atopic dermatitis
ATCC	- American type culture collection
CA	- Community acquired
CD	- Cluster of differentiation
CFU	- Colony forming unit
CLA	- Cutaneous lymphocyte associated antigen
EDTA	- Ethylenediamietetraacetic acid
egc	- Enterotoxin gene cluster
FLG	- Filament aggregating protein/Filaggrin
HA	- Hospital acquired
i NOS	- Inducible nitric oxide Synthase
ICAM	- Intercellular adhesion molecule
IDEC	- Inflammatory dendritic epidermal cell
IFN-γ	- Interferon gamma
IgE	- Immunoglobulin type E
IL	- Interleukin
ISAAC	- International study of asthma and allergies in childhood
LC	- Langerhans cell
MHC	- Major histocompatibility complex
MRSA	- Methicillin resistant *Staphylococcus aureus*

NCTC	- National collection of type cultures
PAMP	- Pathogen associated molecular patterns
PMN	- Polymorphonuclear neutrophils
PRR	- Pattern recognition receptor
PVL	- Panton valentine leukocidin
RANTES	- Regulated on activation, normal T cell expressed and secreted
SA	- *Staphylococcus aureus*
SAg	- Staphylococcal superantigen
SC	- Stratum corneum
SE	- Staphylococcal enterotoxin
SE*l*	- Staphylococcal enterotoxin like toxin
TAE	- Tris-acetate ethylenediamietetraacetic acid
TCR	- T cell receptor
Th	- T helper
TLR	- Toll-like receptor
Tm	- Melting temperature
TNF	- Tumor necrosis factor
Treg	- Regulatory T cell
TSLP	- Thymic stromal lymphoprotein
TSST-1	- Toxic shock syndrome toxin type 1

Acknowledgements

I am indebted to my supervisors Prof. Neluka Fernando, Dr. Neelika Malawige and Dr. Graham Ogg for their advice, comments, encouragement and guidance throughout my research which helped me complete this work very successfully. I also gratefully acknowledge my supervisors for creating this postgraduate research opportunity for me.

My sincere gratitude goes to Dr. S.D. Kamaladasa at Department of Medicine, Faculty of Medical Sciences, University of Sri Jayawardanapura, Prof. J.K.K Seneviratne and all the dermatologists at Dermatology Clinic, Lady Ridgeway Hospital for Children for helping me in diagnosis and sample collection from patients. I am also grateful to Dr. D.H. Karunatilaka at Sri Jayawardanapura General Hospital for helping me in sample collection from controls.

I appreciate the technical assistance given by Mr. Rohan Mahendra at Department of Microbiology, Faculty of Medical Sciences, University of Sri Jayawardanapura and I also take this opportunity to thank all the members of academic and non-academic staff in the Department of Microbiology for their help in various other ways.

I would also like to extend my heartfelt gratitude to my parents, my sisters and my brother for their numerous supports. Last but not least I am grateful to the Research committee, University of Sri Jayawardanapura and the Medical Research Council, UK for providing financial assistance for this research.

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ABSTRACT

Introduction: *Staphylococcus aureus* (SA) colonize most patients with atopic dermatitis (AD). SA is able to secrete staphylococcal enterotoxins (SEs), staphylococcal enterotoxin-like toxins (SE*l*s) and Toxic shock syndrome toxin – 1 (TSST-1), which have superantigenic properties. SAgs activate various cells important in the pathogenesis of AD.

Objectives: To determine the association of SA colonization patterns and densities in lesional and nonlesional skin in patients with varying severity of AD, to determine the antibiotic sensitivity patterns of SA isolates from Sri Lanka and to determine the role of different SAgs in the pathogenesis and clinical disease severity of AD.

Methodology: Skin and nasal swabs collected from 100 patients with AD and 120 controls were cultured. Colony counts were obtained for skin samples, and antibiotic sensitivity testing was performed in cases positive for SA. Severity of AD was graded using the Nottingham Eczema Severity Score. Bacterial DNA was extracted. SAg genes were detected by separate PCRs.

Results: SA colonization was seen in 59 patients (59%) and 16 controls (13%). Skin colonization was seen in 57 patients (57%) compared with 10 controls (8%), and nasal colonization of SA was seen in 45 patients (45%) and 9 controls (8%). Lesional skin of most patients (52/57; 91%) had SA densities of > 300 CFUs/cm^2. Colonization rates with

SA significantly increased with increasing age (Spearman correlation coefficient $R = 0.9$, $P < 0.05$) and increasing duration of lesions in patients with AD (Spearman $R = 0.87$, $P < 0.05$). Isolates from eight patients (13.5%) were found to be methicillin-resistant *S. aureus* (MRSA). Only 6 isolates (10%) were susceptible to penicillin and 22 (37%) to erythromycin, while 28 (47%) isolates had erythromycin-induced resistance to clindamycin. 52/59 (88%) of SA isolates from patients and 5/16 (31%) from healthy individuals possessed at least 1 type of SAg. Except SE*I*L and SEH, each SAg type was expressed significantly higher (P<0.05, fisher exact test) in patients when compared to controls. SE*I*M, (46%), SEI (44%), SEG (39%), SE*I*N (36%) and SE*I*O (34%) were the most abundant SAg genes in isolates from patients. SE*I*M (P=0.0380) and SE*I*O (P=0.0158) were appeared to be significantly associated with milder disease. But these were not significant after correcting for multiple comparisons. Possession of SEB gene was significantly higher (0.0186) from SA isolated of patients who were aged 5 years or older compared to SA isolated of younger patients. Genes for classical superantigens such as SEA, SEB, SEC, SEE and TSST were significantly associated with patients with moderate to severe disease when the colonizing strain possessed not more than 2 SAg genes in total.

Conclusions: SA colonization appears to be associated with increased severity of AD, consistent with its role in disease pathogenesis. Patients with AD are more likely to be colonized with SA strains resistant to conventional antibiotics. Emergence of community acquired MRSA appears to be a significant problem in Colombo and could worsen with the indiscriminate use of antimicrobials. Patients with AD are also a reservoir of SAg producing SA strains. SAgs associate with AD in a cohort of patients from Sri Lanka consistent with a role in disease pathogenesis.