PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ISOLATES

A THESIS SUBMITTED TO THE

CENTRAL DEPARTMENT OF MICROBIOLOGY

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TRIBHUVAN UNIVERSITY

NEPAL

FOR THE AWARD OF

DOCTOR OF PHILOSOPHY

IN MICROBIOLOGY

BY

DHARM RAJ BHATTA

May 2018

DECLARATION

Thesis entitled **"Phenotypic and molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates"** which is being submitted to the Central Department of Microbiology, Institute of Science and Technology (IOST), Tribhuvan University, Nepal for the award of degree of Doctor of Philosophy (Ph D) is a research work carried out by me under direct supervision of Prof. Dr. Dwij Raj Bhatta, Central Department of Microbiology, Tribhuvan University and co supervised by Dr. Lina M Cavaco.

This research is original and has not been submitted earlier in part or full in this or any other form to any university or institute, here or elsewhere, for the award of any degree.

Dharm Raj Bhatta

May 2018

RECOMMENDATION

This is to recommend that **Mr. Dharm Raj Bhatta** has carried out research entitled **"Phenotypic and molecular characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates"** for the award of Doctor of Philosophy (Ph D) in **Microbiology** under our supervision. To our knowledge, this work has not been submitted for any other degree.

He has fulfilled all the requirements laid down by Institute of Science and Technology (IOST), Tribhuvan University, Kirtipur, for the submission of the thesis for the award of Ph D degree.

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LETTER OF APPROVAL

On the recommendation of Prof. Dr. **Dwij Raj Bhatta**, this Ph D thesis submitted by **Dharm Raj Bhatta**, entitled **"Phenotypic and molecular characterization of methicillin resistant *Staphylococcus aureus* (MRSA) isolates"** is forwarded by Central Department Research Committee (CDRC) to the Dean, IOST, Tribhuvan University.

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major causes of hospital and community acquired infections. Drug resistance among Staphylococci is global burden which is associated with significant morbidity and mortality around the world. This study was conducted to determine the prevalence of MRSA, antibiotic resistance pattern of the isolates and prevalence of Panton Valentine leukocidin (*PVL*) genes among MRSA isolates. This study was conducted for a period of three years (2012-2015) at the Manipal Teaching Hospital, Pokhara, Nepal.

A total of 400 isolates were collected from various clinical specimens including hospital units (Operation theaters and Intensive Care Units). Antibiotic susceptibility testing was performed by Kirby-Bauer disc diffusion method. Primary screening for MRSA was performed by disc diffusion test with cefoxitin (30 μg) and oxacillin (1 μg) discs, further confirmed by cefoxitin MIC test and detection of *mecA* gene by Polymerase Chain Reaction (PCR). Inducible clindamycin resistance was detected by D test. Multiplex PCR was used for the detection of *PVL* genes. Biofilm was detected by microtitre plate method.

Out of 400 *S. aureus* isolates, 139 (34.7%) were MRSA. Among the MRSA isolates, 74 (53.2%) were from inpatients, 58 (41.7%) isolates were from outpatients and 7 (5%) isolates were from hospital units (OT and ICUs). More than 65% of the MRSA isolates were resistant to ciprofloxacin, erythromycin and cotrimoxazole while less than 15% were resistant to amikacin, clindamycin and tetracycline. None of the isolate was resistant to vancomycin. Inducible clindamycin resistance was found in 54 (25.4%) isolates. A total of 148 isolates of *S. aureus* were tested for biofilm assay, 94 (63.5%) were MRSA and 54 (36.5%) were MSSA. Biofilm was detected in 32.4% (48/148) of the isolates. Out of the total of 94 MRSA isolates tested, 39 (41.5%) were biofilm producers. Panton Valentine leukocidin (*PVL*) genes were detected in 79 (56.8 %) of the 139 MRSA isolates. Majority (75.5%) of *PVL* positive strains were isolated from pus samples. High prevalence (90.4%) of *PVL* among community acquired MRSA was found and only 7.1% hospital acquired MRSA were positive for *PVL* genes. No *PVL* genes were detected

among the hospital environmental isolates. Thus, *PVL* can be used as marker for community acquired MRSA. Antibiotic resistance in *PVL* negative MRSA isolates was higher as compared to *PVL* positive MRSA. Out of 112 hospital staff tested, only 8 (7.1%) were found positive for MRSA nasal carrier.

This study showed a high prevalence of MRSA in our hospital. There is need for regular surveillance of antibiotic resistance, standardization of laboratory methods for detecting methicillin resistance and performing antibiotic susceptibility testing in developing countries like Nepal. Screening of erythromycin resistant isolates would minimize clinical failures associated with clindamycin therapy. Association of *PVL* genes among community acquired MRSA may increase their virulence and is a matter of concern. Biofilm formation by MRSA isolates is challenging for clinicians as majority of biofilm producing MRSA isolates were found multidrug resistant.

Key words: Antibiotic resistance, Methicillin-resistant *Staphylococcus aureus* (MRSA), *mecA*, *PVL*, Biofilm, Clinical specimens.

LIST OF ACRONYMS AND ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
AST	Antibiotic susceptibility testing
ATCC	American type culture collection
CDC	Centers for disease control and prevention
CA-MRSA	Community acquired methicillin resistant *Staphylococcus aureus*
CLSI	Clinical and laboratory standards institute
CNS	Coagulase negative Staphylococci
HA-MRSA	Hospital acquired methicillin resistant *Staphylococcus aureus*
HCW	Health care worker
ICU	Intensive care unit
MDR	Multidrug resistant
MIC	Minimal inhibitory concentration
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
NCCLS	National committee for clinical laboratory standards
NNIS	National nosocomial infections surveillance
OPD	Outpatient department
OT	Operation theater
PBP	Penicillin binding protein

PCR	Polymerase Chain Reaction
PVL	Panton Valentine leukocidin
RRS	Regional resistance surveillance
SCC	Staphylococcal cassette chromosome
SSSS	Staphylococcal scalded skin syndrome
SSTI	Skin and soft tissue infections
TSS	Toxic shock syndrome
UTI	Urinary tract infection
VAP	Ventilator associated pneumonia
VISA	Vancomycin intermediate *Staphylococcus aureus*
VRSA	Vancomycin resistant *Staphylococcus aureus*

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