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MOLECULAR IDENTIFICATION OF METHICILLIN RESISTANCE AND VIRULENCE MARKER IN *STAPHYLOCOCCUS AUREUS*

A PROJECT REPORT PRESENTED BY

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to the Board of Study in Plant Sciences of the
POSTGRADUATE INSTITUTE OF SCIENCE

in partial fulfillment of the requirement
for the award of the degree of

MASTER OF SCIENCE IN MEDICAL MICROBIOLOGY

Of the

UNIVERSITY OF PERADENIYA
SRI LANKA
2009

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14 OCT 2019
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MOLECULAR IDENTIFICATION OF METHICILLIN RESISTANCE AND VIRULENCE MARKER IN *STAPHYLOCOCCUS AUREUS*

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Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial and community-acquired pathogen of worldwide concern. In Sri Lanka, MRSA was first described in 1989 and have emerged subsequently as a major nosocomial pathogen in hospital environments. Therefore, accurate methods to detect methicillin resistance among *S. aureus* strains are essential to ensure optimal antimicrobial therapy and management of infection control caused by these strains.

Objectives

To detect the *S. aureus* species marker-the *fem*B gene, *mecA* gene encoding the penicillin binding protein2a which is associated with staphylococcal methicillin resistance and a key virulence factor, Panton-Valentine Leukocidin(PVL) gene which causes leukocyte destruction and tissue necrosis and is responsible for severe infection with *S. aureus*.

Design: Prospective laboratory analysis

Setting: GENETECH, Colombo 8; Department of Microbiology, University of Peradeniya.

Methods and results

A clinical MRSA isolate, confirmed by disc diffusion method was obtained from Apollo Lanka Hospital in order to establish the PCR assay for the detection of *femB* , *mecA* and *PVL* genes.

Forty-three clinical isolates of *S. aureus* were identified by the conventional laboratory methods of colony morphology, Gram stain, catalase and slide and tube coagulase. Using the PCR, *femB* gene was detected in 36 of the 43 isolates.

The MICs of oxacillin for all 43 isolates were determined by Mueller-Hinton Agar dilution method. 31 isolates had MIC's of $\leq$2 mg/L (Methicillin sensitive *S. aureus*-MSSA) and 12 isolates were MRSA with values of 4 and $\geq$ 128 mg/L. Using PCR, all 12(28%) MRSA strains gave positive results for *mecA* gene. Furthermore, 10 of the 31 isolates identified as MSSA were *mecA* positive. All 7 *femB* gene negative isolates were *mecA* positive.

Eleven out of the 36 (30%) *S. aureus* isolates were positive for the PVL gene. None of the *femB* negative isolates carried the *PVL* gene. 10 of them were MSSA and 1 was MRSA.

A further 43 clinical specimens from which *S. aureus* was isolated and ABST performed in the routine clinical microbiology laboratory-THP were directly tested in order to compare the time required to detect MRSA using conventional methods vs. PCR assay. Among 43 *S. aureus* isolates from patients, 18 (42%) were MRSA by conventional disc diffusion testing. Test results were obtained in 48-72hrs. All 43 clinical specimens were directly examined for *femB*, *mecA* and *PVL* genes using PCR. 15 specimens were failed to detect in PCR assay. Of the 28 specimens, *femB* gene was detected in 24(86%). Again indicating a discrepancy between conventional and molecular identification of *S aureus.*

The *mecA* gene was detected in all 14 of the 18 (78%) specimens from which MRSA were isolated. In addition, 2 specimens from which MSSA had been reported were positive for *mecA.* Four specimens which expressed the *mecA* gene were negative for *femB.*

Fourteen out of 43 specimens tested directly yielded positive results for *PVL* gene. 7 of them were MSSA and 7 were MRSA. The *PVL* gene was not detected in any of the *femB* negative specimens.

Direct testing by PCR required 4 hours for DNA preparation, PCR performance and detection of PCR products into the gel, contrasting with the 48-72 hours required for conventional testing. A minimum of 25-26 bacterial cells in 1µl of specimens were detectable using this assay.

This study shows that conventional methods for detection of methicillin resistance like disc diffusion and MIC are time consuming. PCR offers a rapid and accurate identification of species, methicillin resistance and virulence detection for clinical use. However, its use may be limited to molecular diagnostic laboratories as technical expertise is required and cost is high (currently ≈ Rs.3600).