OPEN ACCESS
Indiana Journal of Agriculture and Life Sciences
Abbriviate Tittle- Ind J Agri Life Sci
ISSN *(Online)*- xxxx-xxxx
Journal Homepage Link-https://indianapublications.com/journal/IJALS

INDIANA PUBLICATIONS
PRODUCTIVE AND QUALITY RESEARCH

Research Article

Volume-01|Issue-01|2021

Characterization of Staphylococcal Exotoxin (PVL) Gene of Methicillin Resistant Staphylococcus Aureus (MRSA) From Foods of Animal Origin, In Southern Assam, India

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Article History
Received:25.06.2021
Accepted:05.07.2021
Published:15.07.2021

Citation
Parbin, N. (2021).Characterization of Staphylococcal Exotoxin (PVL) Gene of Methicillin Resistant Staphylococcus Aureus (MRSA) From Foods of Animal Origin, In Southern Assam, India. *Indiana Journal of Agriculture and Life Sciences, 1*(1),6-9.

Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) carrying the important virulence determinant, Panton-Valentine leukocidin (PVL), is an emerging infectious pathogen. The present study was aimed to isolate and identify the MRSA from food samples of animal origin and to perform the molecular characterization of the PVL exotoxic gene from the MRSA positive isolates. In carrying out the first PVL prevalence study in Southern Assam, India. We screened 73 isolates of *S. aureus*.

Keywords:*Staphylococcus aureus*, Methicillin Resistant *Staphylococcus aureus*(MRSA), PVL..

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SUMMARY

The present study showed that though the occurrence of MRSA from positive *Staphylococcus aureus*isolates obtained from retail meat samples in this region is neither too low nor too high. 100 retail meat and fish samples and obtained a total of 36 Methicillin Resistant *Staphylococcus aureus* (MRSA) isolates. The result showed that though MRSA is present in this region but it might not be that much pathogenic or infectious.

INTRODUCTION

Methicillin-resistant ***Staphylococcus aureus*** (MRSA) is a major cause of nosocomial infections, and the increasing prevalence rates were noticed by the researchers in recent years. Otherwise, the epidemiology of MRSA infection has changed in the past decades because of the emergence of the strains acquired outsides the healthcare environment named community-associated *Staphylococcus aureus* (CA-MRSA) (Hsueh PR, Teng LJ *et al.*, 2004).

Methicillin resistance in *Staphylococcus aureus*is conferred by the mecA gene, which encodes an altered penicillin binding protein (PBP 2). The mecA gene is harbored in a large mobile genetic element (referred to as the staphylococcal chromosomal cassette mec (SCCmec)) that has a unique chromosomal integration locus. Sequence analyses defined three major (SCCmectypes I, II, and III) among nosocomial MRSA strains. SCCmec types are distinguished on the basis of their sizes, which range from 26 and 67 kb, and genetic compositions, in which their genomes include recombinases and antibiotic resistance genes.

The SCCmecC is integrated in the MRSA chromosome at the 3' end of open reading frame X at the specific site attBSCC, which is located near the origin of replication in the staphylococcal chromosome and flanked by direct and repeated sequences. SCCmecC carries the mecC gene, a region encompassing the mecA gene and its regulators mecRI and mecI and the ccr complexes, the recombinase genes region, which is responsible for SCCmec mobility (Katayama *et al*. 2000).

Panton Valentine Leukocidin (PVL)

Panton-Valentine leukocidin is a bi-component beta-barrel toxin that causes leukocyte lysis or apoptosis via pore formation (Kaneko J, Kamio Y. 2004). PVL is encoded by two genes, LukF-PV and LukS-PV, which are carried on a variety of lysogenic bacteriophages.

Identification of MRSA in food animals led to logical concerns about foodborne contamination, and MRSA has been identified in retail meat in Europe, Asia and North America; however, the role of

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foodborne contamination in human MRSA infection or colonization is currently unclear and much further study is required to elucidate this potential problem. One aspect that has to be considered is the amount of MRSA in contaminated meat. During slaughtering processes, MRSA can be contaminated on carcasses, contributing to the high contamination rates of MRSA on retailed meats in fresh markets. The super antigens can be found in those MRSA strains. Therefore, meats and meat products act as a vehicle in transmission of MRSA to the butchers and consumers.

MATERIALS AND METHODS

A total of 100 samples were collected from meat (which included beef, mutton, pork and chicken) and fish consumed by the human, from January to June, 2017. The collected samples were brought to the laboratory aseptically in chilled state and processed.

Approximately 2.5g of the sample was collected from the meat or fish sample, extracted aseptically and homogenized with 9 ml of peptone water incubated at 37 °C for 24 hours.

After incubation, the inoculam from the peptone was streaked on MSA plates. The colonies of *Staphylococcus aureus* species gave golden yellow colonies. Isolated colonies of purified strains grown on solidified agar plates were observed and morphological data was recorded.

The desired isolates and distinct colonies were identified on the basis of their morphological and biochemical character. Coagulase test was done to isolate the coagulase positive *Staphylococcus aureus.* Biochemical test were performed according to the procedure of Cruickshank *et al*. (1975). The biochemical tests involved catalase, oxidase, IMViC test, urease test and TSI test.

Antibiotics susceptibility test was performed by Kirby Bauer's Disk Diffusion Method. Antibiotic disks of Oxacillin and Cefoxitin antibiotic were used and the zones of inhibitions were measured in millimeter with standard chart provided in CLSI guideline (CLSI 2011).

For genotypic characterization of Methicillin resistant *Staphylococcus aureus*simplex PCR was performed by targeting PVL gene. PCR was performed for all isolates which showed resistance against the respective antibiotics i.e. Oxacillin and Cefoxitin. For partial gene PCR amplification, primer (table 1) specific for targeting the PVL gene were used for reaction with bacterial DNA as template.

Table 1. Oligonucleotide used as primer for amplification of PVLgene

Primer pairs	Gene	Sequence(5'-3')	Amplified product size (bp)
PVL F:	PVL	5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3'	433
PVL R:		5'-GCATCAACTGTATTGGATAGCAAAAGC-3'	

RESULTS

The 100 samples collected consisted of 44 samples of fish and 56 samples of meat which included chicken, mutton, beef and pork. Out of the 100 isolates 80 were found to be positive for Staphylococcus aureus. The isolation was identified on the basis of observation of various morphological. Among all the 80 morphologically confirmed *Staphylococci*, a total 73 isolates were confirmed to be *Staphylococcus aureus*on the basis of the biochemical tests performed of all the 73 isolates of colonies isolated from food samples of animal origin, all of the 73 samples were subjected to antibiotic susceptibility test performed by the disc diffusion method. Out of the 73 isolates (42 from meat samples and 31 from fish samples) only 43 isolates were resistant to Oxacillin and only 36 isolates were resistant to Cefoxitin (table 2) *i.e.* their zone of inbition was < 1.6 µg and < 2.2 µg respectively. So from these 73 *Staphylococcus aureus* positive isolates only 36 isolates were taken as MRSA positive isolates.

Table 2. Screening of MRSA

Antibiotic	Sensitivity (%)(n=73)	Intermediate (%)(n=73)	Resistivity (%)(n=73)
Oxacillin	34.5	6.84	58.90
Cefoxitin	42.4	8.21	49.31

Simplex PCR of thgese 36 MRSA iaolates was done out of which only 4 isolates were found to be harbouring PVL exotoxin gene of 433bp. PCR result revealed that only 11.11% of Methicillin resistant *Staphylococcus aureus* isolates encoded PVL gene.

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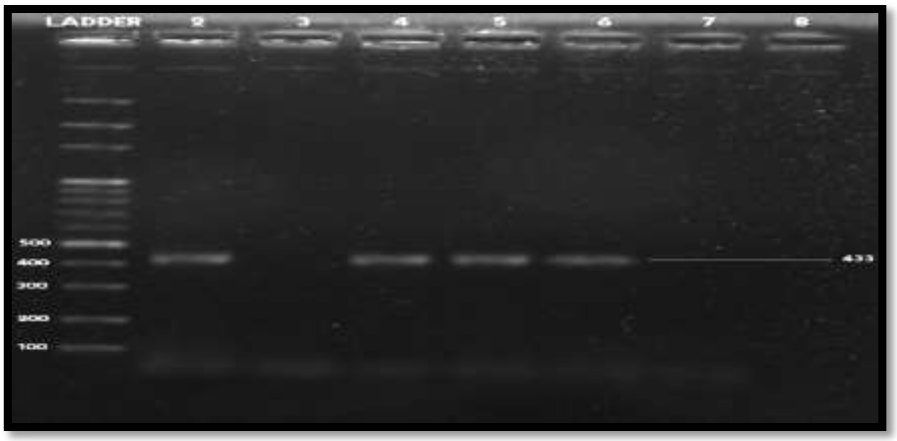

Figure 1: Photo Plate 15: Gel Image showing amplification of PVL gene. Ladderusedis 100 bp, lane 2, 4, 5 and 6 shows amplified PVL gene (433 bp), lane 8 is –ve control

DISCUSSION

In the present study, 80 (80 %) out of 100 (fish and meat which included chicken, mutton, pork and beef) sample were isolated which appeared golden yellow colonies on MSA medium. These isolated samples were then further characterized by a series of biochemical tests (Catalase, oxidase, coagulase, and IMVIC test). The results revealed that isolates were Catalase (+), oxidase (-), Indole (-), MR (+), VP (+), Citrate utilization (+), Urease (+), TSI (A/A).

Of the 100 samples 56 are meat samples which included 21 mutton, 9 pork, 15 beef and 11 chicken samples of which 42 meat samples *i.e.* 17 mutton, 7 pork, 11 beef and 7 chicken (*i.e.* 86% mutton, 77.77% pork, 71.42% beef and 63.63% chicken meat respectively) are found to be positive for *Staphylococcus aureus*and out of the 44 fish sample 31 sample are found to be harbouring the *Staphylococcus aureus* organism i.e. 70.45% fish sample is contaminated with *Staphylococcus aureus*.

Now the antibiotic susceptibility test showed that out of the 73 *Staphylococcus aureus* positive isolates, only 36 isolates are resistant to both Oxacillin and Cefoxitin antibiotic. These 36 samples included 13 fish samples, 9 beef samples, 4 pork samples, 4 chicken samples and 6 mutton samples (*i.e.* 41.93% fish samples, 81.81% beef samples, 57.14% pork samples, 57.14% chicken samples and 35.29% mutton samples) are resistant to both Oxacillin and Cefoxitin. So from this study it is seen that 81.81% beef samples, 57.14% pork samples, 35.29% mutton samples and 57.14% chicken samples are found to be MRSA positive.

In this study simplex PCR was used to detect the PVL which flanked at 433 bp from 36 samples (23 meat samples and 13 fish samples). The PVL gene was detected in 11.11% (n=4) of Methicillin Resistant *S.aureus* from 2 fish samples and 2 meat samples (1 beef and 1 chicken sample), and absence of PVL was detected from maximum of the MRSA positive samples.

CONCLUSION

The present study showed that though the occurrence of MRSA from positive *Staphylococcus aureus* isolates obtained from retail meat samples in this region of the world is neither too low nor too high but the pathogenicity of these MRSA strains i.e. the occurrence of PVL exotoxin among these MRSA strains is relatively low. The result showed that though MRSA is present in this region but it might not be that much pathogenic or infectious. But maintaining preventive measures and more hygiene may help in eradicating the occurrence of MRSA and its toxic gene PVL totally from this area making us free from health risks.

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