ISSN: 0976-3031

Available Online at http://www.recentscientific.com

International Journal of Recent Scientific Research
Vol. 7, Issue, 11, pp. 14279-14282, November, 2016

International Journal of Recent Scientific Research

Research Article

INCIDENCE OF COMMUNITY ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (CA- MRSA) IN SEAFOOD AND ITS ENVIRONMENT, GUJARAT, INDIA

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ARTICLE INFO

Article History:

Received 16th August, 2016
Received in revised form 25th September, 2016
Accepted 23rd October, 2016
Published online 28th November, 2016

Key Words:

CA-MRSA, Seafood, Panton-Valentine Leukocidine and *lukS*

ABSTRACT

Incidence of CA-MRSA in seafood and its environment was monitored, between 2011 to 2015 and for this purpose a total of 1552 seafood samples were drawn from both retail outlets and fish processing industry that include 379 chilled whole cleaned fish, 308 fresh fish, 614 frozen whole cleaned fish, 124 numbers of processed fish and prawn, 76 water and 51 ice samples. All the confirmed Staphylococci isolates (n= 88) were screened for methicillin resistance on MRSA selective plates and were subjected to Polymerase Chain Reaction (PCR) to screen for Panton-Valentine Leukocidine genes which are associated with CA-MRSA. A total of 14 MRSA (15.91%) were positive for *lukS* gene. This study is significant as it is the first study to detect the presence of CA-MRSA in seafood in India.

INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is a highly versatile, multi-drug resistant, gram positive organism which has the ability to cause a multitude of infections such as soft tissue infections, osteomyelitis, septicemia and bacterial endocarditis (Shambat *et al*., 2012). Reports indicate a major change in epidemiology of MRSA after emergence of Community-Acquired MRSA (CA-MRSA) (Walf *et al*., 2008). CA-MRSA emerged into communities transmitted by healthy individuals without exposure to health care settings and evolved independently from Hospital Acquired (HA) MRSA (Monecke *et al*., 2013; Salgado *et al*., 2003). The emergences of CA-MRSA have been reported as a serious public health issue (Stephens *et al*., 2006). Earlier CA-MRSA were distinguished from HA-MRSA by characteristics such as HA-MRSA strains contained SCC*mec* types I, II, and III, whereas CA-MRSA strains contained type IV and V of SCC*mec*. (Moroney *et al*., 2007). Another striking feature is detection of Panton-Valentine Leukocidine (PVL) genes (*lukS/F)* amongst CA-MRSA which enables them to produce a cytotoxin that cause tissue necrosis and leukocyte destruction (Motoshima *et al*., 2010). These genes are also responsible for severe infections such as skin abscesses and necrotizing pneumonia (Bonnstetter *et al*., 2007). CA-MRSA isolates are resistant to fewer antimicrobial agents as compared with HA-MRSA isolates (Aires de Sousa and Lencastre, 2003).

It is evident that MRSA has become a global infection control problem in humans and animal medications. Although incidences of MRSA in humans and animals (companion and livestock animals) have been widely documented, but the data on the presence of MRSA in seafood is scant. Occurrence of CA-MRSA on fish and it's environment indicate post-harvest contamination plausibly the result of poor personal hygiene amongst fish processing workers or a disease event in fish. Due to limited evidence of MRSA on fish and fish handlers, continuous monitoring and surveillance of resistant microorganisms such as MRSA in aquatic animals is

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recommended by The World Organization of Animal Health (OIE) (Smith and Pearson, 2011).

Gujarat state ranks first in fish production, export and supports livelihood for more than 6 million people associated with various fishing activities. In view of this, monitoring and surveillance of seafood for contamination of CA-MRSA is necessary at regular intervals. The aim of this study is therefore rapid detection and identification of CA-MRSA directly by nucleic acid extraction and detection of PVL toxin producing gene by PCR.

MATERIALS AND METHODS

Sample collection

During the period from 2011 to 2015, a total of 1552 seafood samples was collected from both retail outlets and fish processing industries which included 379 chilled whole cleaned fish samples, 308 fresh fish samples, 614 frozen whole cleaned fish samples, 124 numbers of processed fish and prawn samples, 76 water samples and 51 ice samples. These samples were transported to ICAR-CIFT, Microbiology and Biotechnology Lab, Veraval in sterile collection bag for identification and isolation of *S. aureus.*

Isolation and Identification

The enumeration of coagulase-positive staphylococci was carried out as per the ISO 6888 Part 1 (use of Baird-Parker (BP) agar) and Part 2 (use of Rabbit Plasma Fibrinogen Agar (RPFA): 1999. The isolation of *S aureus* was carried out using standard ISO 6888-1, 2, & 3; 2003 procedures. A typical *Staphylococci* on BP agar with colony morphology of circular, smooth, convex, gray to jet black colony with light coloured margin and the opaque zone was selected. Further confirmation was done by Gram staining, motility, oxidase, catalase and mannitol utilization. The confirmed Staphylococus aureus isolates were screened for MRSA using MRSA II selective plates (Difco, USA) and further Polymerase Chain Reaction (PCR) was carried out for the detection of lukS gene primers. A total of 88 samples (5.68%) were found to be MRSA on MRSA II selective plates. These isolates were selected for DNA extraction and screened for PVL indicator gene *lukS*.

DNA extraction and Polymerase Chain Reaction assay

DNA was extracted from confirmed MRSA isolates on MRSA II selective plates by GenElute Bacterial Genomic DNA Kit (Sigma-Aldrich, Spain) as per manufacturer's guidelines. A monoplex PCR was carried out according to the procedure and primer described in Al Talib *et al*., (2009) with *lukS* of *S. aureus* (151bp) and 16S rRNA of *S. aureus* (597 bp). The amplified product of 151bp was commercially sequenced at SciGenom Labs at Cochin, Kerala. On the basis of maximum identity score, few sequences were selected and aligned with Clustal W (multiple sequence alignment software) and a phylogenetic tree was constructed using the DNASTAR Lasergene 13 software (Wisconsin University, Madison, USA).

RESULTS AND DISCUSSION

In this study, we detected 14 CA-MRSA samples out of a total of 88 MRSA (5.67%) samples screened, that shows the rate of incidence of CA-MRSA is 15.91% in seafood. All these 14 isolates harbored PVL toxin gene *lukS* which enable CA-MRSA to produce a necrotizing cytotoxin. This is confirmed by a clear band at 151bp in the PCR reaction. 7 CA-MRSA were detected from prawn samples, 5 CA-MRSA samples detected from water, and 2 from Ice. None of the whole cleaned processed fish or fresh fish collected from retail market were contaminated with CA-MRSA and the same is depicted in Figure 1.

The amplified PCR product (151bp) was sequenced commercially and a phylogenetic tree was constructed based on the difference in the nucleotide sequences as shown in Figure 2. Three distinct clades were formed among the four isolates viz., lukS #14 with lukS #17 (A); A with lukS #23 (B) and AB with lukS#19 and a distant out group (lukS#22), which is clearly indicating that the possibility of different sources of contamination of CA- MRSA in seafood and its environment.

This is first report of its kind on community associated CA-MRSA in seafood. *lukS* gene from PVL-encoding genes was used in the PCR assay to categorize and discriminate CA-MRSA from HA-MRSA. Reports indicate that Panton-Valentine Leucocidin (PVL) encoding genes are responsible for production of necrotizing cytotoxins in *S. aureus* leading to increased invasiveness and virulence (Al-Talib *et al*., 2009). The study reveals that CA-MRSA may spread from cross-contamination during fish handling. Fish processing workers, water, ice etc may be the potential sources of contamination.

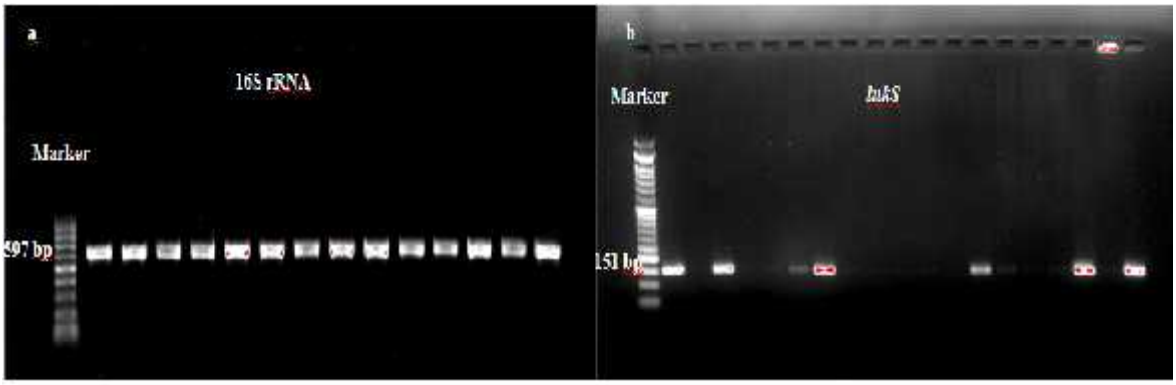

Figure 1 (a) PCR amplification of *Staphylococcus aureus* specific 16S rRNA (597bp)
(b) PCR amplification of PVL producing *lukS* gene (151bp) (M= 100bp ladder)

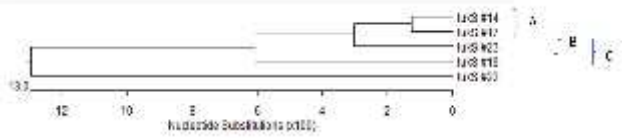

Figure 2 Phylogenetic relationship of *lukS* gene positive CA-MRSA

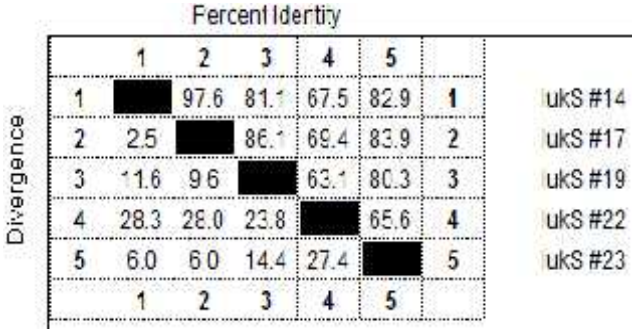

Figure 3 Percentage identity between *lukS* gene positive CA- MRSA

There have been many reports on CA-MRSA in companion animals like dogs, cat, pigs and horses etc, (Yihan *et al*., 2011; Duijkeren *et al*., 2005; Morgan, 2008). Currently there are scant reports on contamination of CA-MRSA in seafood and its associated environments such as water and ice used during processing of seafood. However, in a study by Pokhrel *et al*., (2016) detected PVL gene in 78% of MRSA isolated from clinical samples and it is significantly higher than our study. A slightly lower incidence rate of 12.5% was detected by Wulf *et al*., (2008) in veterinarians and farmers having close contact with pigs in Denmark, whereas, it was 15.91% in our study. The results of the present study are significant in that CA-MRSA capable of producing PVL toxins have shown to cause necrotizing dermatitis, pneumonia and other secondary infections in humans (Rankin *et al*., 2005). Although seafood may only act as carrier of CA-MRSA, they can be a potential reservoir for infections in humans associated with seafood such as fish processing workers, fish handlers and lead to cross-contamination of fish processing environments such as water and ice.

CONCLUSION

In conclusion, CA-MRSA is an emerging pathogen in seafood and its environment. It also indicates that cross-contamination of seafood with CA-MRSA can act as a reservoir for further proliferation of these strains in its environments and can cause infections in healthy persons working with seafood. It is therefore suggested that further research into its current prevalence and epidemiology of CA-MRSA infections in human should be emphasized.

Acknowledgement

Authors are thankful to the Director, ICAR- CIFT, Cochin, Kerala for providing the necessary facilities and fund to carry out this research work. We duly acknowledge the lab work assisted by the technical and supporting staff of the Centre.

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How to cite this article:

Sivaraman, GK *et al*.2016, Incidence of Community Acquired Methicillin-Resistant Staphylococcus Aureus (Ca- Mrsa) In Seafood and Its Environment, Gujarat, India. *Int J Recent Sci Res.* 7(11), pp. 14279-14282.