Original Research Article

<https://doi.org/10.20546/ijcmas.2018.709.394>

Incidence and Molecular Characterization of *Staphylococcus aureus* Isolated from Meat Products

B. J. Shathish Sharma¹, S. Rathnapraba^{1*}, S. Meignanalakshmi¹, S. Manoharan²,
S. Saranya¹ and K. Vijayarani¹

¹Department of Animal Biotechnology, Madras Veterinary College, TANUVAS, Chennai - 07,
Tamil Nadu, India

²Vaccine Research Centre – Bacterial Vaccine, Centre for Animal Health Studies, TANUVAS,
Chennai - 51, Tamil Nadu, India

*Corresponding author

ABSTRACT

The presence of microorganisms in food is predictable, which causes significant threat to the public health. *Staphylococcus aureus* is one such foodborne bacterial pathogen that causes various foodborne outbreaks worldwide. The incidence of *S. aureus* in food samples is common and it has been characterized as economically important foodborne bacterial pathogens. In the present study, the incidence of *S. aureus* was investigated in various ready to eat meat products (n=100) purchased from retail market in Chennai. Overall 20% incidence of *S. aureus* was in different meat products. The prevalence of *Staphylococcus aureus* in different meat products were 25.6%, 33.3%, 23.8% and 3.7% for chicken, mutton, pork and beef products respectively. All the presumptive colonies of *S. aureus* was further screened by femA gene specific PCR. The results revealed that out of 20 samples, 11 samples showed positive for femA gene specific PCR. Sequence analysis of *S. aureus* revealed that all the isolates have 100% homology with the other *S. aureus* isolates in the NCBI database. All the isolates were screened for their antibiotic resistant pattern. The results revealed that all the isolates showed 100% resistant towards Penicillin, Vancomycin and Methicillin antibiotics. The present study results concluded that, a relatively higher incidence of *S. aureus* in the meat products marketed in Chennai. Hence, the hygienic quality of the food products needs to be improved to avoid major foodborne outbreaks.

Keywords

Staphylococcus aureus,
Meat products, femA,
Methicillin resistant and
prevalence

Article Info

Accepted:
20 August 2018
Available Online:
10 September 2018

Introduction

Foodborne illness is the major problem in worldwide. Among that, bacterial pathogens are the causative agents of two third of foodborne outbreaks. Meat is an ideal medium for the growth of many organisms because they contain moisture, rich in nitrogenous

compounds (e.g. amino acids, peptides and proteins), minerals and necessary growth factors. Meat being a nutritious material, promotes the growth of the organism easily (Anu *et al.*, 2015). These pathogenic microorganisms may cause an illness from slight skin infection to serious foodborne such as pneumonia and septicaemia (Lowy, 1998).

Staphylococcal intoxication is one of the most common bacterial foodborne outbreaks in world (European commission and health 2003). *Staphylococcus aureus* produce several enterotoxins (SEs) which leads to intoxication in humans when ingested through contaminated food. Enterotoxins are highly stable to heat, so that they will be active after cooking and pasteurization which in turn resulted in food poisoning (Nagarajappa *et al.*, 2012 and Normanno *et al.*, 2005). Methicillin resistant *Staphylococcus aureus* (MRSA) has been recently emerged as a health concern (Hanson *et al.*, 2011). Staphylococcal food poisoning symptoms includes gastroenteritis resulting a sudden onset of nausea, vomiting, abdominal cramps and diarrhea (Balaban and Rasooly, 2000). *Staphylococcus aureus* food poisoning is life threatening in worldwide and in India, the rate of infection is still higher because of warm and humid climate (Bhatia and Zahoor, 2007).

The present study was aimed to investigate the incidence and molecular characterization of methicillin resistant *Staphylococcus aureus* in meat samples in and around Chennai zone.

Materials and Methods

Sample collection

A total of 100 meat products (ready to eat and frozen meat) were collected from different retail outlets in Chennai. These samples includes raw chicken, chicken sausage, cutlet, kheema, pakoda, nuggets, meat balls, raw mutton, mutton balls, pork sausage, kheema, ham, bacon, beef khabab, cubes, kheema and sausage. These samples were further screened for the presence of *S. aureus*.

Enrichment of samples

Each sample (1 g) was homogenized with 9 ml of buffered peptone water using mortar and

pestle. Then the homogenate was transferred in to sterile test tubes and incubated at 37°C for overnight.

Isolation and identification

Staphylococcus aureus (ATCC BAA 976) procured from American Type Culture Collection (ATCC, USA) was used as reference strain. For isolation of *Staphylococcus aureus*, the pre-enriched samples were serially diluted and streaked on the mannitol salt agar (MSA) plates and incubated at 37°C for overnight. The characteristics colonies were then inoculated in mannitol salt broth (MSB). Further the gram staining was performed using HiMedia kit (K001) to identify the presence of single type of colony. Subsequent to gram staining, the biochemical tests were performed using HiStaph identification kit (KB004-HiMedia Pvt. Ltd., Mumbai).

Detection and molecular characterization of *S. aureus*

DNA extraction

DNA was extracted as per Kwasaki *et al.*, (2005) with slight modification. Overnight culture (1ml) was centrifuged at 10000 rpm for 3 minutes, then the pellet was suspended in 1ml of tris – EDTA buffer (TE buffer pH-8) and then centrifuged at 10000 rpm for 3 minutes. Finally the pellet was suspended in 20µl of Nucleuses free water (NFW) and boiled for 10 minutes. After that it was centrifuged at 10000 rpm for 3 minutes and supernatant was collected and quantified to check the concentration and purity of the DNA.

Polymerase chain reaction

The samples confirmed by culture methods were subjected to PCR targeting fem A gene.

To amplify the 296 bp portion of fem A gene the primer sequence FP: 5' TATGAGTTAAAGCTTGCTGAAGGTT 3' and RP: 5'TTACCAGCATTACCTGTAATCTCG3' were used (Kwasaki *et al.*, 2005). PCR was carried out in the thermocycler (Master Cycler, Gradient and Eppendorf, Germany) with the final volume of 20µl reaction containing 10µl of red dye master mix (amplicon- 2X), 1 µl of (10 Pico moles) each primer, 2 µl of DNA template and 6µl of nuclease free water. The reaction was carried with following cycling conditions; initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 2 min, annealing at 58°C for 30 sec, extension at 72°C for 30 sec and final extension at 72°C for 5 min and analyzed in 2% agarose gel electrophoresis.

Sequence analysis

The PCR purified samples (Qiagen gel extraction kit, Germany) was subjected to sequence analysis (Eurofins Genomics India Pvt. Ltd., Bengaluru, India). The sequences were aligned, assembled and analyzed using DNA Star Laser gene V. 7.0 software. The phylogenetic tree was constructed with 1000 bootstrap value using a Maximum Likelihood method with MEGA software version 6.0.

Antibiotic susceptibility test

All the isolates of *Staphylococcus aureus* were tested for their antibiotic susceptibility pattern using disc diffusion method. Turbidity of the overnight culture was adjusted to 0.5 McFarland standard and spread evenly over the Muller Hinton agar (MHA) plates and the disc containing the specific concentrations of 10 antibiotics (HiMedia laboratories Pvt. Ltd., Mumbai) were placed on the surfaces of the agar plates and incubated at 37°C for overnight. The diameter of the inhibition

zones were measured as per the manufacturer's instructions.

Results and Discussion

The processed meat products are public health hazard due to the possible presence of foodborne pathogenic bacteria which cause toxicities and outbreaks (Raji *et al.*, 2007). *Staphylococcus aureus* is one of the most economically important foodborne bacterial pathogen in worldwide. In the present study, 100 meat products (ready to eat and frozen meat) were screened for the presence of *Staphylococcus aureus* by both conventional culture and molecular method. The overall incidence of *Staphylococcus aureus* in meat samples was found to be 20%. The prevalence of *Staphylococcus aureus* in different meat products were 25.6%, 33.3%, 23.8% and 3.7% for chicken, mutton, pork and beef products respectively (Table 1). Many researchers have reported different levels of *S. aureus* incidence in various meat products. EI-Jakee *et al.*, (2013) reported that about 6% of occurrence of *Staphylococcus aureus* in chicken products. Similarly, Lubna *et al.*, (2015) reported about 65.6% and 43.3% prevalence of *S. aureus* in beef and pork products. Likewise, Hanson *et al.*, (2011) reported that the incidence of *S. aureus* as 17.8%, 6.9% and 3.6% in chicken, beef and pork products were contaminated with *S. aureus*. The variation in the incidence may be due to the number of samples screened, environmental parameters and sampling.

Detection of toxin genes by polymerase chain reaction allows the determination of potentially hazards pathogenic bacteria present in the samples irrespective of the strain. For this reason, PCR may be considered more sensitive than immunological methods. Moreover, the PCR have remained rapid, highly specific and direct method for detection and identification of pathogens. The high

specificity of the technique is based on the use of species specific primer sequences that can be used for the identification of the bacterial pathogens. In this study, PCR technique was used to identify the toxin gene such as fem A for species specific amplification of *Staphylococcus aureus* because the identification of pathogenic bacteria at species level is more specific and accurate.

Fem A is the gene which encodes a protein that facilitates the methicillin resistance property in *S. aureus*. All the presumptive colonies were screened for the presence of *Staphylococcus aureus* targeting fem A gene and the results revealed that, out of 20 samples, 11 samples showed positive for *Staphylococcus aureus* (Figure 1). Similar study was also conducted by Wang *et al.*, (1997) to screen the *S. aureus* targeting 296 bp

portion of femA gene. A study conducted by Pelisser *et al.*, (2009) targeted fem A gene for the detection of *Staphylococcus aureus* in meat and meat products.

Sequence analysis of *S. aureus* revealed that all the isolates have 100% homology with the *S. aureus* sequences available in the NCBI database. Phylogenetic tree was constructed based on a 296 bp portion of *femA* gene of *Staphylococcus aureus* along with other coagulase negative *Staphylococci spp.* The tree was generated using the MEGA6 Maximum Likelihood method. The value on each branch represents the percentage of bootstrap replications supporting the branch. A total of 1000 bootstrap replications were calculated. *Macrocooccus caseolyticus* was used as an out group for the phylogenetic tree construction (Figure 2).

Table.1 Incidence of *Staphylococcus aureus* in meat samples by conventional culture method

S. No	Sample details	No. of the samples screened (n)	No of positive samples	Per cent positivity
1	Chicken products	43	11	25.6
2	Mutton products	9	3	33.3
3	Pork products	21	5	23.8
4	Beef products	27	1	3.7
	Total	100	20	20.0

Table.2 Antibiotic susceptibility of *Staphylococcus aureus* isolates

S. No.	Name of the antibiotic disc	Antibiotic susceptibility (%)
1.	Vancomycin (30 mcg)	100(R)
2.	Penicillin (10 units)	100(R)
3.	Gentamicin (10mcg)	62(R)
4.	Ampicillin (10 mcg)	64.7(R)
5.	Streptomycin (10 mcg)	28.5(I)
6.	Chloramphenicol (30mcg)	12.1(S)
7.	Methicillin (5 mcg)	100(R)
8.	Tetracycline (30 mcg)	12.5(R)
9.	Erythromycin (15 mcg)	70(R)
10.	Ciprofloxacin (5 mcg)	68.8(S)

Fig.1 PCR amplified product of 296 bp *Fem A* gene. Lane 1: 100 bp ladder, Lane 2: *Staphylococcus aureus* (ATCC BAA 976), Lane 3-7: *Staphylococcus aureus* isolates and Lane 8: Non template control (NTC)

Fig.2 Phylogenetic analysis of *fem A* gene portion of *Staphylococcus aureus* isolates

The phylogenetic tree revealed four distinct group namely *S. saprophyticus*, *S. simulans*, *S. epidermidis* and *S. hemolyticus*. Frequently the *S. saprophyticus* species group includes *S. xylosus* and *S. saprophyticus* while the *simulans* species groups is comprised of *S. carnosus* and *S. piscifermentans*. The *epidermidis* species group is comprised of *S. carnosus* and *S. piscifermentans* the *epidermiditis* species group is composed of *S. epidermidis*, *S. capitis*, *S. caprae* and *S. saccharolyticus* and the *haemolyticus* species group encompasses *S. haemolyticus*, *S. hominis* and *S. devriesei* (Piette *et al.*, 2009 and Moura *et al.*, 2012).

In the present study, the main cluster grouped several *S. aureus* strains and these strains are homologous to those from meat products. Four of the *S. aureus* isolates form the close evolutionary relationship with the *S. aureus* isolates from Australia and three of the isolates form close evolutionary relationship with the Greece isolates. The *S. saprophyticus*, *S. simulans*, *S. epidermidis* and *S. hemolyticus* form separate cluster from the present isolates and also from other *S. aureus* isolates.

Antimicrobial resistant in Methicillin resistance *Staphylococcus aureus* (MRSA) isolates infections leads to high morbidity and mortality worldwide. The prevalence of MRSA infections can vary from country to country (Gordon and Lowy, 2008). According to the susceptibility test results, all the isolates were identified as sensitive against Ciprofloxacin (68.5%) and Chloramphenicol (12.1%). Resistance rate against Gentamicin, Ampicillin, Tetracycline and Erythromycin were determined as 62%, 64.7%, 12.5% and 70% respectively. Penicillin, Vancomycin and Methicillin antibiotics had the highest resistance (100%). The results were showed in table 2. In a similar study by Kelman *et al.*, (2011), they found that the *S. aureus* isolates

showed highest resistant to methicillin, tetracycline, penicillin, erythromycin, gentamicin and ampicillin. Further the results were also supported by the findings of Fitsuma (2017).

Investigating the presence of pathogenic bacteria in food products is essential to control its occurrence and to establish effective prevention mechanisms. The present study clearly indicated that higher prevalence of multidrug resistant *S. aureus* in ready to eat and frozen meat products marketed in Chennai, India. This is recognized as environmental hazard to the food supply and to human health. The femA PCR assay was employed for rapid detection of *S. aureus* in ready to eat meat products. This resulted in high risk of food poisoning to the consumers. Hence, there is a need for strict hygienic and preventive measures to the manufacturer, distributors and consumers of these products. Further proper and regular surveillance of foods for multidrug resistant pathogens needs to be in place to prevent occurrence of antimicrobial resistance.

Acknowledgement

This work was supported by Department of Biotechnology, Govt. of India, New Delhi.

References

- Anu, S. A., and Gayathri, T. 2015. Isolation and Characterization of Microorganisms from Raw Meat Obtained from Different Market Places in and Around Chennai. Journal of Pharmaceutical, Chemical and Biological Sciences. 3(2): 295-301.
- Balaban, N., and Rasooly, A. 2000. Staphylococcal enterotoxins. International Journal of Food Microbiology. 61:1-10.

- Bhatia, A., and Zahoor, S. 2007. *Staphylococcus aureus* Enterotoxins: 2007. A Review. Journal of Clinical and Diagnostic Research. 3:188-197.
- EI-Jakee, J., S. A. Marouf, S. Nagwa, H. A. Eman, I. A. Sherein, A. A. Samy, and Walaa, E. E. 2013. Rapid Method for Detection of *Staphylococcus aureus* Enterotoxins in Food. Global Veterinaria. 11(3): 335-341.
- European Commission and Health. 2003. Staphylococcal Enterotoxins in Milk Products, Particularly Cheeses. European Commission.
- Gordon, R. J., and Lowy, F. D. 2008. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clinical Infectious Diseases. 46(5): S350–S359.
- Hanson, B. M., A. E. Dressler, R. P. Hraper, E. Wardyn, L. K. Roberts, J. S. Kroeger, and Smith, T. C. 2011. Prevalence of *Staphylococcus aureus* and methicillin-resistance *Staphylococcus aureus* (MRSA) on retail meat in Iowa. Journal of Infection and Public Health. 4(4):169-174.
- Kwasaki, S., N. Horikoshi, Y. Okada, K. Takeshita, T. Sameshima, and Kawamoto, S. 2005. Multiplex PCR for simultaneous detection of *Salmonella* spp., *Listeria monocytogenes*, and *Escherichia coli* in meat samples. Journal of Food Protection. 68(3): 551-556.
- Lowy, F. D. 1998. *Staphylococcus aureus* infection. The New England Journal of Medicine. 339: 520–532.
- Moura, T. M., F. S. Campos, and Azevedo, P. A. D. 2012. Prevalence of enterotoxin-encoding genes and antimicrobial resistance in coagulase-negative and coagulase-positive *Staphylococcus* isolates from black pudding in Southern Brazil. Revista da Sociedade Brasileira de Medicina Tropical. 45: 579-585.
- Nagarajappa, S., M. S. Thakur, and Manonmani, H. K. 2012. Detection of enterotoxigenic staphylococci by Loop-mediated isothermal amplification method. Journal of Food Safety. 32: 59-65.
- Normanno, A. G., S. Firinu, G. Virgilio, A. Mula, A. Dambrosio, L. Pogggiu, R. Decastelli, S. Mioni, G. Scuota and Bolzoni, E. 2005. Coagulase-positive *Staphylococci* and *Staphylococcus aureus* in food products marketed in Italy. International Journal of Food Microbiology. 98(1): 73-79.
- Piette, A., and Verschraegen, G. 2009. Role of coagulase-negative Staphylococci in human disease. Veterinary Microbiology. 134: 45–54.
- Raji, A., L. A. Waddell, J. M. Sargeant, S. Read, J. Farber, M. J. Firth, and Chambers, A. 2007. An overview of microbial food safety programs in beef, pork and poultry from farm to processing in Canada. Journal of Food Protection. 70(5):1286-1294.

How to cite this article:

Shathish Sharma, B.J., S. Rathnapraba, S. Meignanalakshmi, S. Manoharan, S. Saranya and Vijayarani, K. 2018. Incidence and Molecular Characterization of *Staphylococcus aureus* Isolated from Meat Products. *Int.J.Curr.Microbiol.App.Sci*. 7(09): 3163-3169.
doi: <https://doi.org/10.20546/ijcmas.2018.709.394>