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Prevalence of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* in retail buffalo meat in Anand, India

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Abstract
A total of 150 carabeef samples were collected from retail meat shops in and around Anand city and isolation and identification of *Staphylococcus aureus* was performed. Systematic bacteriological examination resulted in the isolation of 83 Staphylococci isolates, among these 42 isolates were identified as *S. aureus* based on cultural, morphological, biochemical and molecular characteristics. PCR detection of *nuc* gene was performed simultaneously for the detection of *S. aureus* species. All the 42 presumptive *S. aureus* isolates amplified *nuc* gene (385 bp) and 3/42 (7.14%) of the isolates carried *mecA* gene (533 bp). The higher prevalence of *S. aureus* and MRSA indicates the lack of hygiene practices in the retail outlets in Anand and could causes several infections related to *S. aureus* in the consumers.

Keywords: Buffalo meat, MRSA, prevalence, *Staphylococcus aureus*

Introduction
Meat of animal origin is the primary source of protein and other micronutrients making them essential for growth, repair, and maintenance of body cells. Buffalo meat (*Bubalus bubalis*) is low in calories and cholesterol and is more healthy meat among the red meats known for human consumption. The high level of bacterial growth in meat is due to the high slaughter contamination rate and the long storage at room temperature and the poor sanitary conditions of slaughterhouses and meat markets, so numerous outbreaks of food-borne diseases caused by the consumption of contaminated raw or undercooked meat have been reported in various parts of the world.

Food-borne diseases are a major public health concern all over the world. Food-borne diseases are widespread in developing countries due to poor food handling and sanitation practices (Haileselassie *et al*, 2013) [13]. Although animal tissue is sterile, microorganisms from the exterior or interior environments may contaminate the tissue during slaughter (Beyene *et al.*, 2017) [5].

Staphylococcus aureus is a gram-positive, facultative anaerobic, coagulase positive, coccal bacterium that about 25% of people and animals harbored it on their skin and in their nose. *S. aureus* is a common organism found in the environment, including the air, water, milk, sewage, and milking equipment (Bergdoll, 1991) [4]. *S. aureus*, which is part of the normal flora of human and animal skin and mucous membranes, is an opportunistic pathogen with numerous virulence factors that can cause serious infections.

Staphylococcus aureus is carried asymptomatically by humans (20–40%) and animals. It is associated with a wide range of risks such as minor skin infections, septicemia, endocarditis, toxic shock, and pneumonia in humans; and wound infections, mastitis, udder impetigo, bumblefoot, tick pyemia, bumblefoot, and botryomycosis in food animals (sheep, goat, pig and buffalo) and birds. This foodborne pathogen is one of the world's leading causes of foodborne disease outbreaks, responsible for a variety of manifestations and diseases. (Jamali *et al*., 2015) [14].

In the last decade, Methicillin-resistant *Staphylococcus aureus* (MRSA) has been a major epidemiological and clinical problem worldwide (Mimica *et al*., 2007) [18]. MRSA is a strain of *Staphylococcus aureus* resistant to beta lactam antibiotics such as methicillin as well as more common antibiotics such as oxacillin, penicillin, and amoxicillin. In 2011, the CDC estimated that there were 80,461 MRSA infections and 11,285 related deaths in the United States (CDC, 2016) [6].

MRSA has been identified as a significant nosocomial pathogen and has been linked to food-borne diseases (Fetsch *et al*., 2011) [2].

Based on epidemiological distribution and molecular typing, MRSA is divided into three types: hospital associated MRSA (HA-MRSA), livestock-associated MRSA (LA-MRSA) and community-associated MRSA (CA-MRSA) (Dahms *et al*., 2014) [7]. MRSA has a morbidity rate that is 100 times that of tuberculosis (TB), but a mortality rate that is greater than that of HIV/AIDS (Peterson & Diekema, 2010) [19].

In view of raising the public health awareness regarding food safety and quality, knowledge on prevalence of *S. aureus* as well as MRSA in food animals is of great significance. The present study was conducted to evaluate the prevalence of *S. aureus* and to detect the presence Methicillin resistance (*mecA*) gene in buffalo meat marketed in retail outlets in Anand by conventional and molecular methods.

Materials and Methods

Isolation and identification of *Staphylococcus aureus*

A total of 150 carabeef samples were collected from retail meat shops in and around Anand city. Systematic bacteriological examination was carried out of all the collected samples resulted in the isolation of 83 staphylococci isolates on the selective agar BPA and MSA. Among these, 42 isolates (50.6%) were identified as *S. aureus* based on Gram staining showing Gram positive cocci in bunch of grapes, Catalase Positive, VP test positive, Coagulase positive and Oxidase negative.

Isolation of *Staphylococcus aureus* was done as per the standard procedure (ISO standard 6888/1: 1999) [13]. Enrichment of all the samples was carried out in Peptone Water (PW) enrichment broth. Ten gm of meat sample was homogenized with 90 ml sterile PW and then was incubated at 37 ºC for 24 h in a bacteriological incubator.

The selective media used for isolation of *S. aureus* was Mannitol Salt Agar (MSA) and Baird Parker Agar (BPA). A loopful of inoculum was taken from enrichment broth and was streaked on MSA and on BPA medium supplemented with 50% Egg Yolk Emulsion and 1% Potassium Tellurite solution. The inoculated plates were then incubated aerobically for 24-48 hrs at 37 ºC. Yellow colored colonies on MSA and characteristic jet-black colonies surrounded by a white halo zone on BPA were considered presumptive to *S. aureus*. Gram's staining, Methyl red test, Voges - Proskauer test, Catalase test, Oxidase test and Coagulase test were performed for further morphological confirmation of *S. aureus* following the standard procedures in practice.

DNA extraction

The DNA was extracted by boiling method. In a sterilized micro centrifuge tube 100µl of DNAse and RNAse free milliQ water and a loopful of pure culture was suspended. The suspension was vortexed and then heated at 95˚C for 10 mins in a thermal cycler and then centrifuged at 6269g for 7 mins. The cell debris settle down as sediment and the supernatant separates. The upper supernatant was transferred to another PCR tube, and this was used as a DNA template for PCR.

nuc and *mecA* genes detection by PCR

The isolates presumptive for *S. aureus* were later subjected to PCR by species specific thermonuclease (*nuc*) gene as given by Shylaja *et al*. (2010) [22] and *mecA* gene as given by Merlino *et al.* (2002) [17] with slight modifications. The primer sequence and product size are mentioned in table 1 and PCR conditions are mentioned in the table 2.

Results and Discussion

All the isolates yielded desired amplified product of approximately 385 bp similar to that of reference strain of *S. aureus* using the primer pair for *nuc* gene (Fig. 1). According to Hu *et al*., (2013) [12] detection of nuc gene by using PCR amplification was considered as a gold standard method and is most useful for rapid diagnosis of *S. aureus* in clinical and food samples.

The prevalence of *S. aureus* in carabeef (buffalo meat) was 28% (42/150) in the present study which was similar to the findings of Raji *et al*. (2016) [20] and Thapaliya *et al*. (2017) [23] who reported 25% and 27.8% of *S. aureus* prevalence in raw retail meat samples. The findings of the present study i.e., 28% (42/150) prevalence were also similar with Aziz *et al*. (2019) [3] and Zehra *et al*. (2019) [25] who reported a prevalence of 24% (66/275) and 25.27% (46/182) respectively. Martínez-Vázquez *et al*. (2021) [16] reported a higher prevalence of 44.3% (47/106), higher than the current study.

Out of total 42 *S. aureus* isolates 3 were positive for *mecA* yielding a desired amplified product of 533 bp simila to the reference strain of MRSA (Fig. 2), indicating a prevalence of 7.14% (3/42) of MRSA is similar reports were given by Weese *et al*. (2010) [24] as 5.6%. Lower reports were given by Lim *et al*. (2010) [15], Hanson *et al*. (2011) [11], Agersø *et al*. (2012) [1] and Ge *et al*. (2017) [9] as 1%, 1.2%, 1.4% and 1.9% respectively. In contrast to this study, higher prevalence was reported by De Boer *et al*. (2009) [8] 10.6% and Sadiq *et al*. (2020) [21] 63%.

Table 1: Details of Oligonuleotide primers

Target-gene	Primer sequence (5' — 3')	Product Size (Base pairs)	Reference
nuc	F-GCTGGCATATGTATGGCAATT R-GCTTCAGGACCATATTTCTCTAC	385 bp	Shylaja *et al*. (2010) [22]
mecA	F-AAAATCGATGGTAAAGGTTGGC R-AGTTCTGCAGTACCGGATTTGC	533 bp	Merlino *et al*. (2002) [17]

Table 2: Steps and conditions of thermal cycling

Primers (Forward and Reverse)	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension
nuc(F) *nuc*(R)	94 ºC 5 min	94 ºC 30 sec	55 ºC 30 s	72 ºC 30 sec	72 ºC 5 min
		Repeated for 30 cycles			
mecA(F) *mecA*(R)	94 ºC 2 min	94 ºC 30 s	55 ºC 30 s	72 ºC 1 min	72 ºC 5 min
		Repeated for 35 cycles			

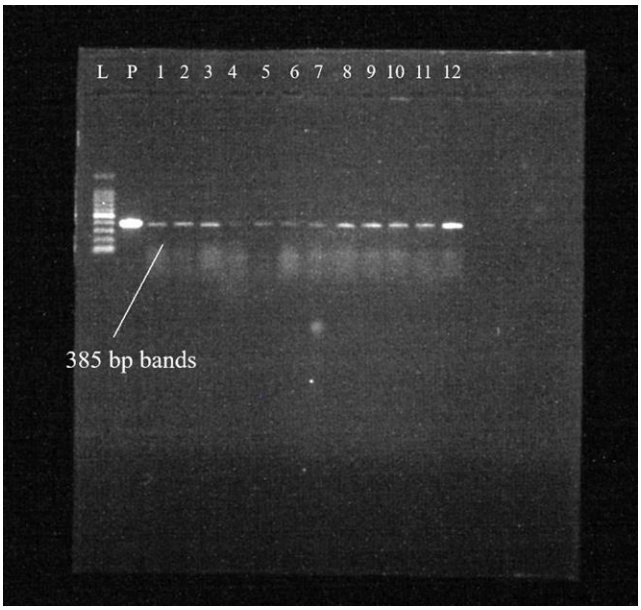

Fig 1: Agarose gel Electrophoresis of PCR products for detection of *nuc* gene (L – DNA Ladder (100bp), P-Positive control (*Staphylococcus aureus* MTCC 737), N- Negative Control, Lane 1-12: Isolates positive for *nuc* gene.

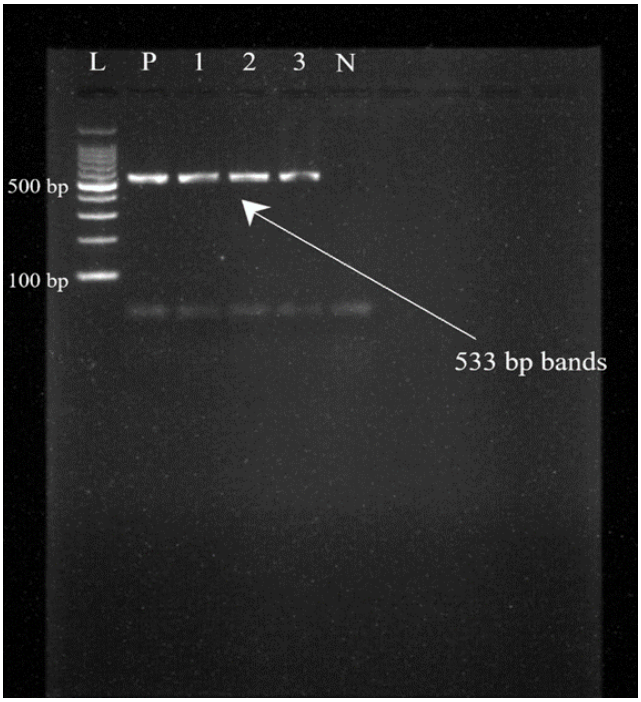

Fig 2: Agarose gel Electrophoresis of PCR products for detection of *mecA* gene (L – DNA Ladder (100bp), P-Positive control (MRSA ATCC 43300), N- Negative Control, Lane 1-3: Isolates positive for *mecA* gene.

Conclusion

The present study indicates a moderate prevalence of *S. aureus* and methicillin resistance *S. aureus* in carabeef. This indicates that sanitary and hygienic practices in meat handling must be improved.

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