RESEARCH ARTICLE

Molecular Detection of Multidrug Resistant *Staphylococcus aureus* from Retail Meat Samples of Tirupati Town, Andhra Pradesh

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Abstract

Staphylococcus aureus causes variety of diseases in both animals and humans. Foods of animal origin may be an important source for the transfer of antimicrobial-resistant *S. aureus* and antimicrobial resistance genes to humans. Hence the present study was designed to detect multidrug resistant *S. aureus* among retail meat samples of Tirupati town, Andhra Pradesh (India). A total of 250 samples in the form of meat/swabs (chicken 100, mutton 60, beef 30, pork 30, sea meat 30) were collected from different retail outlets of Tirupati town, and processed for isolation and identification of *S. aureus* by cultural method and molecular characterisation by PCR. *S. aureus* was confirmed targeting *nuc* gene and all the isolates were subjected to antibiotic sensitivity test by disc diffusion method against 10 antibiotics. Results showed that out of 250 meat samples, 200 were positive for *S. aureus* with an overall prevalence of 80.0% (200/250) by cultural method and 68.0% (136/200) of them were confirmed by PCR. Antimicrobial sensitivity test revealed higher resistance to cefoxitin (85.29%) and ceftriaxone (66.91%) compared to other selected antibiotics. They showed high sensitivity to co-trimoxazole (66.91%) but exhibited lower sensitivity to vancomycin (8.82%). This study revealed a high prevalence of *S. aureus* from chicken and sea meat samples by cultural and PCR methods, respectively, which indicates that contamination of *S. aureus* was common in retail meat shops. By enhancing hygienic practices and quality control measures at meat processing facilities the contamination with food borne pathogens can be reduced.

Key words: Antibiotic sensitivity, Food poisoning, Meat samples, *nuc* gene, *S. aureus.*

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Introduction

In recent years, many pathogens have been responsible for food safety issues. *Staphylococcus aureus* is recognized as one of the major food-borne pathogens in fresh and ready-to-eat products and is responsible for various infections around the world (Diep *et al.,* 2006). *S. aureus* is capable of proliferating in a wide range of temperature, pH, and salt concentrations thereby enabling it to grow in wide variety of foods to produce toxins that cause illness (Schmitt *et al.,* 1990). Contamination of food by *S. aureus* results from poor hygiene practices during food processing and storage which involves both human- and animal-associated strains in the infection (Abdalrahman *et al.,* 2015). Parvin *et al.* (2021) stated *S. aureus* as a frequent contaminant of retail chicken due to poor hygienic handling of chicken meat and responsible for Staphylococcal food poisoning (SFP). SFP is mainly caused by the ingestion of toxins of *S. aureus*. Furthermore, the extreme malleability of their genomes and vast potential for adaptability have resulted in increased amounts of antimicrobial-resistant strains of *S. aureus* in all sectors, including those found in food (Peterson and Kaur, 2018). Nowadays, more and more MDR *S. aureus* were reported in food poisoning outbreaks and isolated from food product in previous researches (Papadopoulos *et al.,* 2018). Moreover, in recent years, methicillin resistant *S. aureus* (MRSA) is attracting extensive attention.

Though the conventional microbiological culture based methods are considered as gold standard, they require non-selective enrichment followed by selective enrichment, plethora of biochemical or serological procedures which consumes >48 h for the definitive result (Boukharouba *et al.,* 2022). The demand for rapid, high-resolution and low-cost diagnostic procedures led to detection systems that have significantly shortened final outcome. Polymerase chain

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reaction (PCR) is the most widely accepted molecular tool for pathogens and disease diagnosis. PCR revolutionized diagnostics and allowed definite microbial identification down to species and strain levels (Guenay-Greunke *et al.,* 2022). Genotyping of microbial strains is important to understand how bacteria spread, find a possible source of infection, and identify the dominant types (Farahmand *et al.,* 2020). Several molecular typing methods are widely used for the genetic characterization of *S. aureus* such as multilocus sequence typing, *spa* typing, SCC *mec* typing, and Pulse-field gel electrophoresis (PFGE). Numerous PCR-based studies were carried out targeting the *nuc* gene alone or in combination with the *mecA* gene for rapid identification or recognition of methicillin-susceptible *S. aureus* and methicillin-resistant *S. aureus* (González-Domínguez *et al.,* 2020). These techniques provide means to trace epidemiologically related strains leading to the tracking back to the origin of contamination (Kadariya *et al.*, 2014). The *nuc* gene was known as specific virulence factor for *S. aureus* and it contributed to biofilm formation and immune evasion (Andrade *et al.,* 2021). The PCR amplification of *nuc* gene was used for accurate identification of *S. aureus* (Blaiotta *et al.,* 2004). Hence the present study was designed with the objective of molecular detection of multidrug resistant *S. aureus* isolates using *nuc* gene from meat samples collected from retail outlets of Tirupati town.

Materials and Methods

A total of 250 samples in the form of meat/swabs (chicken 100, mutton 60, beef 30, pork 30, sea meat 30) were collected from different retail outlets of Tirupati town, Andhra Pradesh. All the samples were collected aseptically and labelled. The samples were immediately transferred to laboratory for further processing. As per the procedure described by Farahmand *et al.* (2020), isolation and identification of *S. aureus* was done by using mannital salt agar. All the culturally confirmed *S. aureus* isolates were stored and maintained as nutrient agar slants at 4°C.

PCR Protocol

The culturally confirmed *S. aureus* isolates were subjected to PCR for further confirmation by using primers specific for *nuc* gene (Javid *et al.,* 2018) (Table 1). The DNA was extracted from selective broth cultures by using boiling and snap chilling method (Arora *et al.,* 2006). The PCR protocol was standardized using 25 µL of reaction mixture containing 2 µL of TaqDNA Polymerase (1 unit/ µL), 2.0 µL of DNA template solution, 2.5 µL of 10 x reaction buffer, 0.5 µM of dNTPs, 2 µL each of forward and reverse primers and magnesium chloride ($MgCl_2$). Sterile nuclease free water was added to make up the volume 25 µL of the reaction mixture.

The cycling conditions for PCR included an initial denaturation of DNA at 94°C for 5 min, followed by 35 cycles at 94°C for 60s denaturation, 55°C for 30 sec annealing, extension at 72°C for 1 min, followed by the final extension of 7 min at 72°C and hold at 4°C. In each PCR run, both positive (reference standard DNA) and negative (no template DNA) controls were included to ensure specificity and absence of contamination. The final amplified product was analysed by agarose gel electrophoresis on 2% agarose gel and visualized under gel documentation system (BIO-RAD, USA). The samples that showed the presence of *nuc* gene were considered as positive for *S. aureus* (Fig. 1) (Javid *et al.,* 2018).

Table 1: Primers used for *nuc* gene detection among *S. aureus* isolates

Primer Sequence 5`- 3`	Amplicon size(bp)	Reference
F- CCGATACGCTGCCAATCAGT R- ACGCAGACCGTAGGCCAGAT	270 bp	Javid *et al.* (2018)

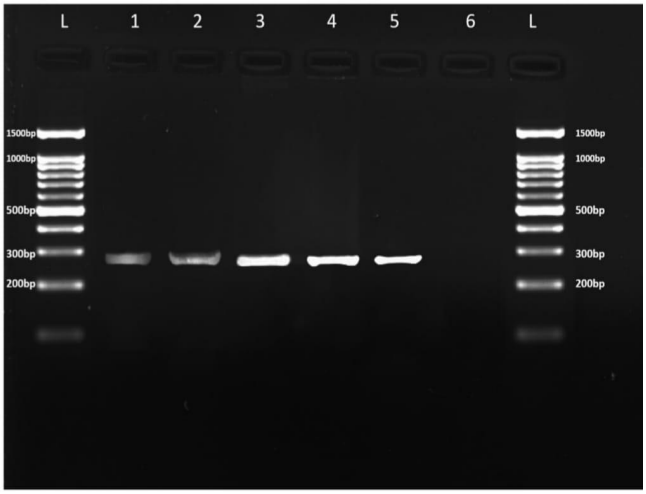

Fig. 1: Detection of the *nuc* gene among *S. aureus.* Lane L: DNA ladder; Lane 1, 2, 3, 4: positive samples with amplification of 270 bp; Lane 5: Positive control; Lane 6: Negative control

All the positive *S. aureus* isolates obtained by PCR from meat samples were subjected to antibiotic sensitivity test by using disc diffusion method against 10 different antibiotics (HI-Media Laboratories) such as Ampicillin (AMP) 10 µg/disc, Ciprofloxacin (CIP) 5 µg/disc, Azithromycin (AZM) 15 µg/disc, Tetracyclin (TE) 30 µg/disc, Amoxacillin (AMX) 10 µg/disc, Co trimaxazole (COT) 25 µg/disc, Ceftriaxone (CTX) 30 µg/disc, Cefoxitin (CX) 30 µg/disc, Vancomycin (VA) 30 µg/disc, Erythromycin (E) 15 µg/disc.

Results and Discussion

Detection of *S. aureus* by Cultural Method

Out of 250 meat samples 200 were found to be positive for *S. aureus* by cultural method, out of which, 80/100 (80%), 51/60 (85%), 23 (76%), 21/30 (70%) and 25/30 (83%) were from chicken, mutton, beef, pork and sea meat, respectively. The highest occurrence of *S. aureus* was noticed from mutton samples with an overall prevalence of 80% (Table 2). Wu *et al.* (2018) reported an average incidence of *S. aureus* as 35.0% (647/1,850) from pooled retail meat samples (raw meat, quick-frozen meat, and ready-to-eat meat) in China, and further they also reported that raw meat is most frequently

contaminated with *S. aureus*, with a prevalence rate of 51.0%. They also reported a higher prevalence of *S. aureus* from raw poultry (67.9%) followed by raw mutton (54.5%), raw beef (50.4%), raw pork (30.1%) and bacon/sausage.

Odetokun *et al*. (2023) reported a prevalence of *S. aureus* ranged from 1.3% in raw cow meat to 72.5% in fresh poultry meat in Nigeria. Muslim (2023) reported 40% prevalence of *S. aureus* from meat swabs collected from super markets of Thi-Qar province, Iraq by using cultural method, whereas Tarabees *et al.* (2016) reported a high prevalence of 70% in minced meat. Pinamonti *et al.* (2025) reported a highest prevalence of presumed *S. aureus* in beef hamburgers (62%), followed by pork sausages (50%) and raw cow milk (41.4%) in Northeastern Italy. Faraz *et al.* (2025) detected the presence of *S. aureus* among 16% of the chicken meat samples (32/200) which included chicken thigh, wing, gizzard and liver and further they also reported highest frequency from chicken thigh samples at 37.5% (12/32), followed by chicken wings at 34.4% (11/32), gizzard at 15.6% (5/32), and liver at 12.5% (4/32).

Detection of *S. aureus* by PCR Targeting *nuc* Gene

All the 200 culturally positive samples were subjected to PCR targeting *nuc* gene for further confirmation of *S. aureus*. Out of 200 samples *nuc* gene encoding the thermonuclease enzyme specific for the *S. aureus* strain was detected in 136 samples, of which 50 (62.5%), 35 (68.6%), 16 (69.6%), 15 (71.4%) and 20 (80%) were from chicken, mutton, beef, pork and sea meat, respectively. Almost similar prevalence was reported as 63% in Georgia (Jackson *et al.,* 2013) and 68% in Poland (Krupa *et al.,* 2014), while a lower detection of *nuc* gene was reported from different countries like 26.31% in Iran (Dehkordi *et al.,* 2017), 27.8% in the United States (Carrel *et al.,* 2017), 32.8% in Japan (Hiroi *et al.,* 2012), 40.38% in Morocco (Ed-Dra *et al.,* 2018), 45% in Ghana (Effah *et al.,* 2018), and 46% in Colombia (Gutierrez *et al.*2017). In contrast to the present study Muslim (2023) reported the presence of *nuc* gene by PCR in all the culturally confirmed isolates (40/40) of meat swabs from supermarkets of Thi-Qar province, Iraq.

Table 2: Detection of *S. aureus* by cultural and PCR method

Type of sample (No.)	No. (%) of samples positive for *S. aureus* by	
	Culture (%)	PCR (%)
Chicken (100)	80 (80.0)	50 (62.5)
Mutton (60)	51 (85.0)	35 (68.6)
Beef (30)	23 (76.0)	16 (69.6)
Pork (30)	21 (70.0)	15 (71.4)
Sea meat (30)	25 (83.0)	20 (80.0)
Total (250)	**200 (80.0)**	**136 (68.0)**

Antibiotic Susceptibilty of *S.aureus* Isolates

The positive *S. aureus* isolates subjected to antimicrobial susceptibility testing using the disk diffusion method (Bauer-Kirby *et al.*, 1966) revealed highly resistant to cefoxitin (85.29%) and ceftriaxone (66.91%) when compared to other selected antibiotics and lowest (8.82%) sensitivity to vancomycin (Table 3). Pinnamonti *et al.* (2025) reported ampicillin resistance among 41.7 % of the meat isolates, which may be due to high frequency of β-lactam prescriptions in veterinary. Further they also reported most frequent resistance among the strains isolated from pork sausages against ampicillin (41.7%, 5/12), followed by cefoxitin (25%, 3/12), tetracycline, levofloxacin (16.7%, 2/12 each), rifampicin and linezolid (8.3%, 1/12 each) and no resistance against netilmicin. Odetokun *et al.* (2023) also reported high level of resistance against β-lactams for *S. sureus* meat isolates from Nigeria. Sanlibaba (2022) reported MDR in 96.87% (93 of 96) of *S. aureus* strains and the frequencies of antibiotic resistance for kanamycin and telithromycin were 83.34% (80 of 96), while it was 78.12% (75 of 96) for penicillin G, 76.04% (73 of 96) for streptomycin, 72.91% (70 of 96) for erythromycin, 62.50% (60 of 96) for cloxacillin.

Wu *et al.* (2018) reported that out of 868 isolates, only 11 isolates (1.26%) were susceptible to all antibiotics, whereas most isolates (821/868, 94.6%) showed resistance or intermediary resistance to more than three or more antibiotics. Of these strains, 104 (12.0%) were resistant to more than 10 antibiotics. However, the most frequent resistance was observed to ampicillin (85.4%), followed by penicillin (84.6%), erythromycin (52.7%), tetracycline (49.3%), kanamycin (45.3%), telithromycin (30.1%), clindamycin (29.6%), streptomycin (21.1%), norfloxacin (20.4%), gentamicin (19.4%), fusidic acid (18.4%), ciprofloxacin (16.9%), chloramphenicol (13.1%), amoxycillin/clavulanic acid (11.0%), and others (<10%). 7.4% of isolates (62/868) were confirmed as methicillin-resistance *S. aureus* (MRSA). Faraz *et al.* (2025) reported that 12.5% (4/32) of the isolates were resistant to only penicillin, but one isolate (1/32; 3%) showed resistance to the antibiotics penicillin, erythromycin, ampicillin, and oxacillin.

Table 3: Antibiotic susceptibility of *S. aureus* isolates

Antibiotic	No. (%) of Isolates		
	Sensitive	Intermediate resistant	Resistant
Ciprofloxacin (CIP5)	48 (35.29)	33 (24.26)	55 (40.44)
Ampicillin (AMP)	20 (14.70)	38 (27.94)	78 (57.35)
Azithromycin (AT15)	38 (27.94)	43 (31.61)	55 (40.44)
Tetracycline (TE30)	58 (42.64)	32 (23.52)	47 (34.55)
Amoxicillin (AMX10)	33 (24.26)	49 (36.00)	49 (36.00)
Co-Trimoxazole (COT25)	91 (66.91)	20 (14.70)	0 (0.0)
Ceftriaxone (CTX30)	0 (0)	28 (20.58)	91 (66.91)
Cefoxitin (CX30)	0 (0)	20 (14.70)	116 (85.29)
Vancomycin (VA30)	12 (8.82)	81 (59.55)	32 (23.50)
Erythromycin (E15)	52 (38.23)	52 (38.23)	32 (23.52)

Conclusion

The study revealed a high prevalence of *S. aureus* from chicken and sea meat samples by cultural and PCR methods, respectively, which indicates common contamination of *S. aureus* in retail meat shops. The presence of *S. aureus* in raw meat samples coupled with varied degrees of antimicrobial resistance from the study area suggests that there may be a possible transmission of AMR from food products to humans.

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