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Molecular characterization of *Staphylococcus aureus* isolated from animal foods using the virulence gene

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Abstract
The ability of foodborne pathogens to spread through animals and their capacity to produce poisons can result in fatal illnesses. There are now more than 50 species of Staphylococcus. In many animal species, including humans, these tiny, resilient bacteria live normally on the skin and mucous membranes; they are also pervasive in the environment. The primary habitat is milk and milk-derived products, meat and egg. The microbial ecosystems are extremely complicated by staphylococcal enterotoxins. Milk, egg, and meat samples were collected from various locations throughout Udaipur for this study. The PCR assay was developed to detect species-specific genes *16SrRNA* gene and Virulence gene (*tsst* gene). *S. aureus* was found to be present in 37.5 percent of milk, 5% of eggs, and 15% of meat, respectively. The prevalence of the *16SrRNA* and *tsst* genes was recorded as 100% and 21.73% respectively.

Keywords: *Staphylococcus aureus*, 16S rRNA gene, tsst gene

Introduction
Food-borne disease, including food-borne intoxications and food-borne infections, is a major public health concern worldwide. (Balaban and Rasooly, 2000) [5]. According to the Foodborne Disease Burden Epidemiology Reference Group (FERG) Report published in 2010, an estimated 3-5 billion people are affected by food-borne associated disease, with nearly 1.5 million deaths occurring each year. (Havelaar *et al*., 2015) [10].

Food-borne diseases are primarily caused by a diverse range of pathogens, including bacterial species such as *Salmonella* spp., *Vibrio* spp., *Clostrodium* spp., *Campylobacter* spp., and *Staphylococcus* spp. (Scallan *et al*., 2011) [18]. *Staphylococcus aureus* is a major causative agent of food-borne associated disease, and most sporadic food-borne diseases are linked to *Staphylococcus aureus* in different parts of the world. (Bennet *et al.*, 2013) [6]. *Staphylococcus aureus* is commonly found in food, where it grows and produces endotoxins, contaminating the food. (Hennekinne *et al*, 2012; Kedariya *et al*, 2014; Umeda *et al*, 2017) [11, 12, 23]. The ubiquitous Staphylococcus species have been found in a wide range of food materials, including vegetarian and non-vegetarian items, ranging from raw, cooked, and ready-to-eat foodstuff, which increases the risk for all consumers and can affect societal financial burden. (Sofos, 2008; Syne *et al*, 2013) [21, 22].

The higher survival probability range of *Staphylococcus aureus* has been observed, which can survive in low to high temperature ranges and grow in different pH ranges ranging from acidic to alkaline medium. (Chaibenjawong and Foster, 2011) [8]. There are only a few laboratory practises available to evaluate and confirm *Staphylococcus aureus* for commercial diagnostic purposes. Misdiagnosis, a lack of standard medical attention, and improper handling of specimens all increase the risk of misinterpretation of *Staphylococcus aureus* prevalence. (Guerrant *et al*, 2001; Scallan *et al*, 2006; Argudin *et al*, 2010) [9, 18, 3].

As a result, the current study attempted to characterise *Staphylococcus aureus* from animal-derived foods by targeting virulence genes.

Materials and Methods
A total of 120 samples, including 40 each of milk, eggs, and meat, were gathered from various locations within the city of Udaipur. The samples were taken aseptically in sterile sampling vials and brought to the lab in a refrigerated state using ice packs. 1ml/1gm of the milk, egg, and meat samples were infected in 9ml of buffered peptone water and then incubated at 37 ^{0}C for 24 hours after sample collection. A loopful of inoculum was then streaked over mannitol

salt agar (MSA), a selective medium, and cultured there for 24 hours at 37°C. The presence of colonies with a yellow tint was checked on the plates after 24 hours. Biochemical tests, such as Gram's staining, catalase, coagulase, haemolysis pattern, and motility, were used to confirm suspected colonies. To determine the presence of the *16S rRNA* and *tsst gene* in *Staphylococcus aureus* isolates, PCR was used. The *16S rRNA* gene was utilised to validate the presence of *S. aureus* using the primers created by Loveseth *et al*. (2004) [14] (F-5'GTAGGTGGCAAGCGTTATCC3'; R-5'CGCACATCAGCGTCAG3'). The primers (F-5'GCTTGCGACAACTGCTACAG3'; R-5'TGGATCCGTCATTCATTGTTAT3') utilised in the current investigation to detect the *tsst* gene were created by Loveseth *et al.* (2004) [14].

Standardization of PCR for the detection of *16S rRNA*, and *tsst* genes

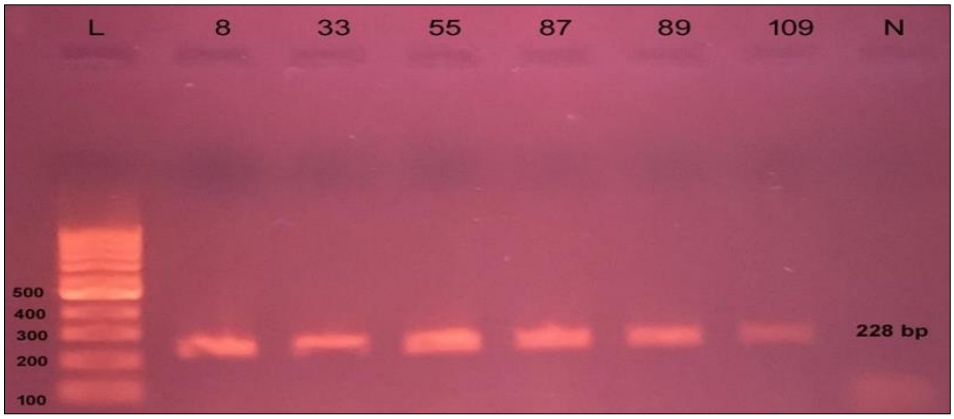
The *16S rRNA* gene and the *tsst* gene were screened using a standardised PCR technique that was modified slightly from that provided by Loveseth *et al*. (2004) [14]. In order to cycle *16S rRNA*, the following conditions were used: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 54°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes. The cycling conditions for the *tsst* gene included an initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 1 minute, annealing at 55 °C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes.

Results and Discussion

Staphylococcus aureus was recovered from 23 samples of foods of animal origin (milk, egg, and meat) out of the 120 samples examined using culture and biochemical testing. In 23 out of 23 isolates that underwent molecular analysis, the *16S rRNAgene* (Fig. A) was found. In 21.73 percent (5/23) of the isolates, the pathogenic *tsst* gene was detected.

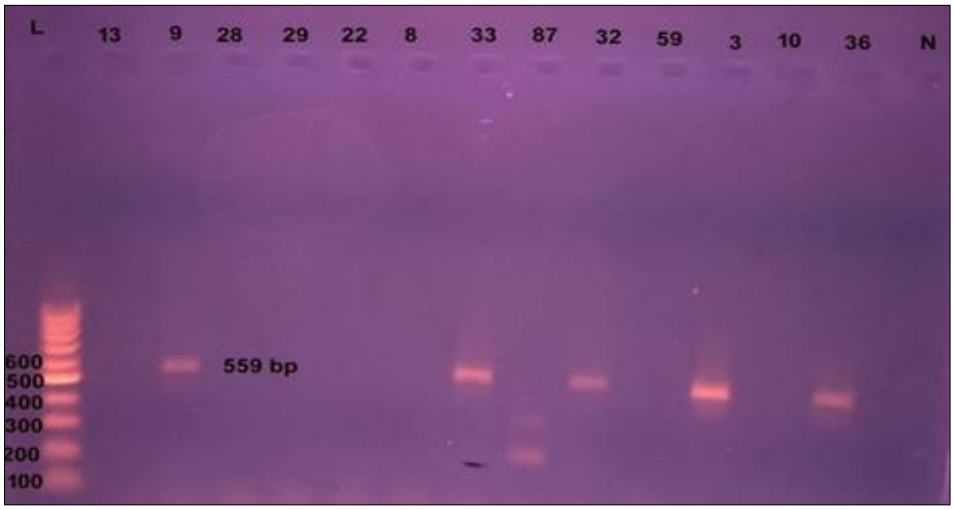
All of the biochemically tested isolates in the current investigation were found to be positive for the *16S rRNA gene*, which is consistent with studies by Loveseth *et al*. (2004) [14], Mukherjee *et al*. (2012), Wada *et al*. (2010), Bunnoeng *et al*. (2014), Al-Alak and Qassim (2016), and Roochetti *et al*. (2018) [15, 24, 7, 1, 17]. According to the results of the current study, all phenotypically positive isolates were verified by PCR using a *16S rRNA* primer, which produced 100% positivity for the gene in all presumed isolates.

In terms of the *tsst* gene result (Fig. B), our findings were consistent with those of Alni *et al*. (2018) [2]. However, the studies by Koosha *et al.* (2016) [13] and Loveseth *et al.* (2004)[14] revealed the highest prevalence rates for the *tsst* gene, which were found to be 68 percent and 38 percent, respectively. Conversely, Aung *et al*. (2017), Shylaja *et al*. (2018), and Nemati *et al*. (2013) [4, 20, 16] reported lower prevalence rates for the *tsst* gene of 3.5 percent, 4.5 percent, and 0 percent, respectively.



L-1kb DNA ladder, N- Negative Control, Positive samples (8,33,55,87,89,109)

Fig A: Agarose gel showing PCR amplified product (228bp) for *16S rRNA* Gene in *S.aureus* isolates



L-1kb DNA ladder, N- Negative Control, Positive samples (13,9,28,29,22,8,33,87,32,59,3,10,36)

Fig B: Agarose gel showing PCR amplified product (559bp) for *tsst* gene in *S. aureus isolate*s

Conclusion

Major human pathogen *Staphylococcus aureus* causes a number of food-borne illnesses, nosocomial infections, and community-acquired infections. The study's findings indicate that the varying levels of prevalence have been caused by the high levels of *S. aureus* contamination in milk, eggs, and meat, which is enough to induce food poisoning and is a major cause of gastroenteritis. So good milk handling, hygienic conditions, and a clean atmosphere in the butcher shop and poultry farm can lessen S. aureus pathogen contamination. Molecular-based approaches have a strong chance of overcoming the limitations of identification processes linked to outcomes based on biochemical traits.

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