Molecular Characterization of *Staphylococcus aureus* Isolated from Foods of Animal Origin by Targeting Virulence and Antibiotic Resistance Genes

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**ABSTRACT**

Meat, milk and egg are a major component in the human diet and milk is an important food for vegetarian class, but it also serves as a very good medium for the growth of many microorganisms including pathogenic bacteria. In the present study, samples of milk, egg and meat were collected from different areas of Udaipur city. PCR assay was standardized for the detection of species specific genes (16SrRNA gene, virulence gene (*tsst* gene) and antibiotic resistance gene (*ermC*, *tetK*, and *aacA-aphD*) of *S. aureus*. The prevalence of *S. aureus* was found to be 37.5%, 5%, and 15% in milk, egg and meat respectively. The prevalence of 16SrRNA, *tsst*, *ermC*, *tetK*, and *aacA-aphD* genes were recorded as 100%, 21.73%, 13%, 26% and 21.7% respectively.

**Keywords**

Milk, Meat, Egg, PCR, Virulence gene and antibiotic resistance gene

**Article Info**

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**Introduction**

In developing countries, food borne diseases are one of the major causes of concern resulting in several deaths annually along with lots of economic burden. As per WHO, due to food borne pathogens approximately 600 million people are getting infected and around 4,20,000 die annually. Most cases of food borne outbreak which are caused by *Staphylococcus aureus*, *Salmonella* spp, *Escherichia coli* etc. had been reported worldwide (WHO, 2015). *Staphylococcus aureus* is an important opportunistic pathogen which is found both in humans and in dairy cattle. *Staphylococcus aureus* is one of the most prevalent causes of clinical infections globally (Kwon *et al.*, 2006).

In humans, *S. aureus* can cause a varied range of diseases relatively from minor skin infections to life-threatening infections such as endocarditis, pneumonia, and sepsis. In dairy cattle, this pathogen is considered as one of
the most common causative agents of mastitis (Haran et al., 2012). The presence of S. aureus or its enterotoxins is generally an indication of poor sanitation of food processing equipment.

Meat is one of the important food items which is consumed worldwide, and is commonly contaminated by antibiotic resistant strains of S. aureus which pose a great risk to public health (Herve and Kumar, 2017).

Eggs are one of the most wholesome and economical foods worldwide and are rich in proteins, fats, vitamins, and minerals (Kralik and Kralik, 2017). Poor handling and storage under unhygienic conditions in the poultry farms or shops poses a risk to egg quality and may consequently affect human health (Pyzik and Marek, 2012).

In last few decades, excessive application of antibiotics in animal husbandry as preservative have led to the occurrence of drug resistance in microorganisms (Durbin 1956).

The indiscriminate use of antibiotics in food animals for therapeutic purposes or as growth promoters is a primary factor in production of antimicrobial-resistant bacterial pathogens (Barber et al., 2003). Methicillin resistant S. aureus (MRSA) has emerged as a major concern for public health. MRSA has been found in several species of meat-producing animals, including pigs (Khanna et al., 2008; Smith et al., 2009), chickens (Nemati et al., 2008) and cattle (Hasman et al., 2010). During the past years, the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) has increased in many parts of the world (Witte, 1999).

Therefore, in the present study an attempt was made to characterize Staphylococcus aureus from foods of animal origin by targeting virulence and antibiotic resistance genes.

Materials and Methods

Collection of samples

A total of 120 samples comprising of milk (n=40), egg (n=40), and meat (n=40) were collected from different areas of Udaipur city. The samples were collected aseptically in sterile sampling vials and transported on ice packs to the laboratory under chilled condition.

Isolation and identification

After collection of samples, 1ml/1gm of the milk, egg and meat sample was inoculated in 9ml of buffered peptone water and incubated at 37°C for 24 hrs. Then, a loopful of inoculum was streaked on selective media i.e. mannitol salt agar (MSA) and incubated at 37°C for 24 hrs. After 24 hrs, the plates were observed for the presence of yellow colored colonies. Suspected colonies were further confirmed by biochemical tests viz., Gram’s staining, catalase, coagulase, haemolysis pattern, and motility.

Molecular characterization of S. aureus

Staphylococcus aureus isolates were subjected to PCR for finding out the presence of the 16S rRNA, tsst, aacA-aphD, ermC and tetK gene. The primers designed by Loveseth et al., (2004) (F-5’GTAGGTGGCAAGCGTTATCC3’; R-5’CGCACATCAGCGTCAG3’) were used for the detection of 16S rRNA gene for confirmation of S. aureus. The primers used in the present study for detection of tsst gene (F-5’GCTTGCGACAACTGCTACAG3’; R-5’TGGATCCGTCATTCATTGTTAT3’) were designed by Loveseth et al., (2004). While, primers for aacA-aphD (F-5’TAATCCAAGAGCAATAAGGCC3’; R-5’GCCACACTATCATAACCACCTA3’) were designed by Loveseth et al., (2004). While, primers for ermC (F-5’AATCGTCAAATCCCTGCTAG3’; R-5’TAATCGTGAATACGGGGT3’;
and \textit{tetK} genes (F-5’GTAGCGACAATAGGTAATAGT3’) (R-5’GTAGTGACAATAAACCTCCTA3’) were designed by Strommenger et al., (2003).

**Standardization of PCR for the detection of 16S rRNA, \textit{tsst}, \textit{ermC}, \textit{aacA-aphD} and \textit{tetK} genes**

The PCR procedure to screen the \textit{16S rRNA} gene and \textit{tsst} gene was standardized as described by Loveseth et al., (2004) with certain modifications. The cycling conditions of \textit{16S rRNA} were comprised of an 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 min and final extension at 72°C for 5 minutes. While for \textit{tsst} gene, the cycling conditions were comprised of 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 min and final extension at 72°C for 5 minutes. The PCR procedure to screen the antibiotic resistance genes viz., \textit{ermC}, \textit{aacA-aphD} and \textit{tetK} was standardized as described by Strommenger et al., (2003). The cycling conditions were comprised of 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 min and final extension at 72°C for 5 minutes. The PCR procedure to screen the \textit{16S rRNA} primer, which resulted in 100% positivity of the gene in all the presumptive isolates. As far as the result of \textit{tsst} gene (Fig. 2) is concerned, our result was in accordance with Alni et al., (2018).

However, the highest prevalence rates were revealed in the study conducted by Koosha et al., (2016) and Loveseth et al., (2004) in which \textit{tsst} gene prevalence was found to be 68% and 38% respectively, while a lower prevalence rate of 3.5%, 4.5% and 0% were also recorded for \textit{tsst} gene by Aung et al., (2017), Shylaja et al., (2018) and Nemati et al., (2013) respectively. The prevalence of \textit{ermC} gene (13%) (Fig. 3) in our study the findings were in accordance with the earlier studies conducted by Parvizi et al., (2012). Higher rates of prevalence were revealed by Ghanbari et al., (2016) and Lim et al., (2012) in which presence of \textit{ermC} gene was found to be 44.4% and 21% respectively, while lower prevalence rate was revealed by Zmantar et al., (2011) in which 6% prevalence was found. The prevalence of \textit{aacA-aphD} gene (Fig. 4) was almost in accordance with Kumar et al., (2010). Monecke and Ehrlich (2005), Achek et al., (2018) and Ruban et al., (2017) showed 29%, 30.76% and 88% prevalence which was higher than our study, while lower prevalence (2.4%) was reported by Monecke et al., (2016).
**Fig. 1** Agarose gel showing PCR amplified product (228bp) for *16S rRNA* gene in *S. aureus* isolates

L – 1kb DNA Ladder  
N – Negative Control

**Fig. 2** Agarose gel showing PCR amplified product (559bp) for *tsst* gene in *S. aureus* isolates

L – 1kb DNA Ladder  
N – Negative Control
**Fig. 3** Agarose gel showing PCR amplified product (299bp) for *ermC* gene in *S. aureus* isolates

L – 1kb DNA Ladder  
N – Negative Control

**Fig. 4** Agarose gel showing PCR amplified product (227bp) for *aacA-aphD* and (360bp) for *tetK* gene in *S. aureus* isolates

L – 1kb DNA Ladder  
N – Negative Control
The prevalence of \textit{tetK} gene (Fig. 3) in the present study (26\%) was in accordance with the findings of Emaneini \textit{et al.}, (2013) and Lim \textit{et al.}, (2012) who reported the prevalence as 17.2\% and 21\%, respectively. While higher rate of prevalence (72.97\%) was revealed in the study conducted by Dehkordi \textit{et al.}, (2017), along with lower rate of prevalence (4.8\%) which was reported in the study conducted by Monecke \textit{et al.}, (2016).

In conclusion, the study reveals that variable level of prevalence has been due to high level of contamination of \textit{S. aureus} in milk, egg and meat which is sufficient to produce food poisoning and leading cause of gastroenteritis. So proper treatment of milk, hygiene and clean environment of meat shop and poultry farm can reduce the contamination of \textit{S. aureus} pathogen.

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\textbf{References}


Ghanbari, F., Ghajavand, H., Havaei, R., Jamì, M.S., Khademi, F. and Heydari, L. (2016). Distribution of \textit{erm} genes among \textit{Staphylococcus aureus} isolates with inducible resistance to clindamycin in...
Rocchetti, T.T., Martins, K.B., Martins, P.Y.F.,


World Health Organization (WHO), Food safety, Fact sheet N 399, December 2015.


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