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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.

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 item 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 37^0C for 24 hrs. Then, a loopful of inoculum was streaked on selective media i.e. mannitol salt agar (MSA) and incubated at 37^0C 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'TAATCCAAGAGCAATAAGGGC3'; R- 5'GCCACACTATCATAACCACTA3';) *ermC*(F-5'AATCGTCAATTCCTGCATGT3'; R- 5'TAATCGTGGAATACGGGTTTG'3;)

and *tetK* genes (F-5'GTAGCGACAATAGGTAATAGT3') (R-5'GTAGTGACAATAAACCTCCTA3') were designed by Strommenger *et al.,* (2003).

Standardization of PCR for the detection of *16S rRNA*, *tsst*, *ermC*, *aacA-aphD andtetK* genes

The PCR procedure to screen the *16S rRNA*gene and *tsst* gene was standardized as described by Loveseth *et al.,* (2004) with certain modifications. The cycling conditions of *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 *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., *ermC, aacA-aphD* and *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.

Results and Discussion

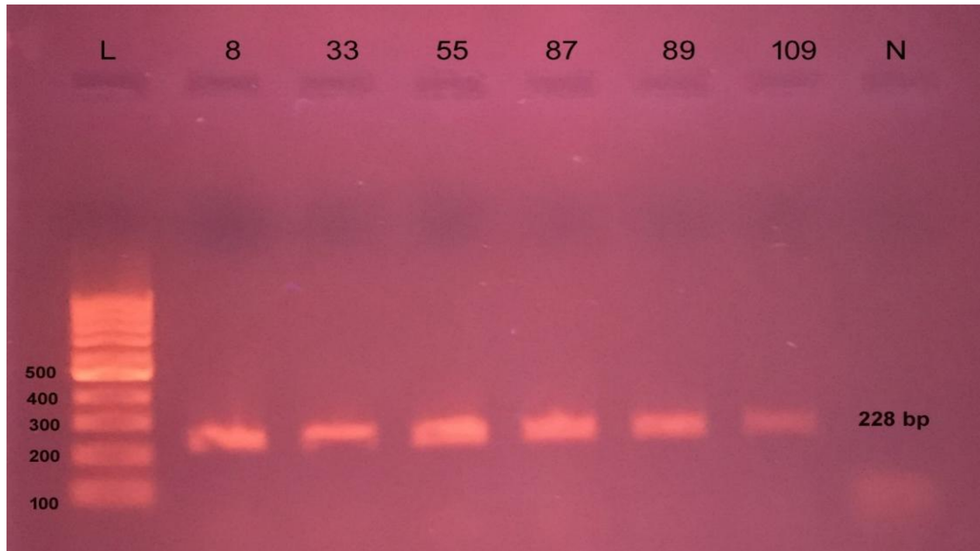
Out of the 120 samples screened, *Staphylococcus aureus* was isolated from 23 samples of foods of animal origin (milk, egg and meat) based on cultural and biochemical tests. On molecular analysis, *16S rRNA*gene (Fig. 1) was detected in 100% (23/23) isolates. Detection of virulent *tsst* gene was found positive in 21.73% (5/23) of the isolates. PCR assay for antibiotic resistance gene *ermC*, *aacA-aphD* and *tetK* genes were found positive in 13.04% (4/23), 21.73% (5/23) and 26.08% (6/23) samples, respectively.

In the present study, all the biochemically tested isolates were found positive for *16S rRNA* gene, which was in accordance to the study conducted by Loveseth *et al.,* (2004), Mukherjee *et al.,* (2012), Wada *et al.,* (2010), Bunnoeng *et al.,* (2014), Al–Alak and Qassim (2016) and Roochetti *et al.,* (2018).

The present study revealed that all the phenotypically positive isolates were confirmed by PCR using *16S rRNA* primer, which resulted in 100% positivity of the gene in all the presumptive isolates. As far as the result of *tsst* gene (Fig. 2) is concerned, our result was in accordance with Alni *et al.,* (2018).

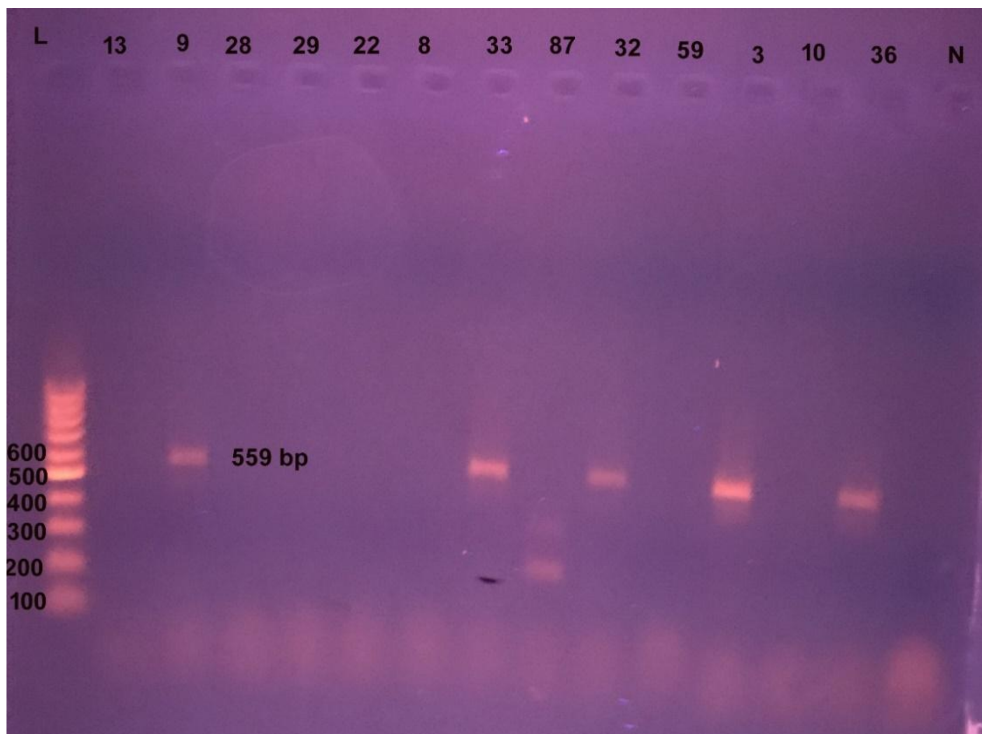
However, the highest prevalence rates were revealed in the study conducted by Koosha*et al.,* (2016) and Loveseth *et al.,* (2004) in which *tsst* gene prevalence was found to be 68% and 38% respectively, while a lower prevalence rate of 3.5%, 4.5% and 0% were also recorded for *tsst* gene by Aung *et al.,* (2017), Shylaja *et al.,* (2018) and Nemati *et al.,* (2013) respectively. The prevalence of *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 *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 *aacA-aphD* gene (Fig. 4) was almost in accordance with Kumar *et al.,* (2010). Monecke and Ehricht (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



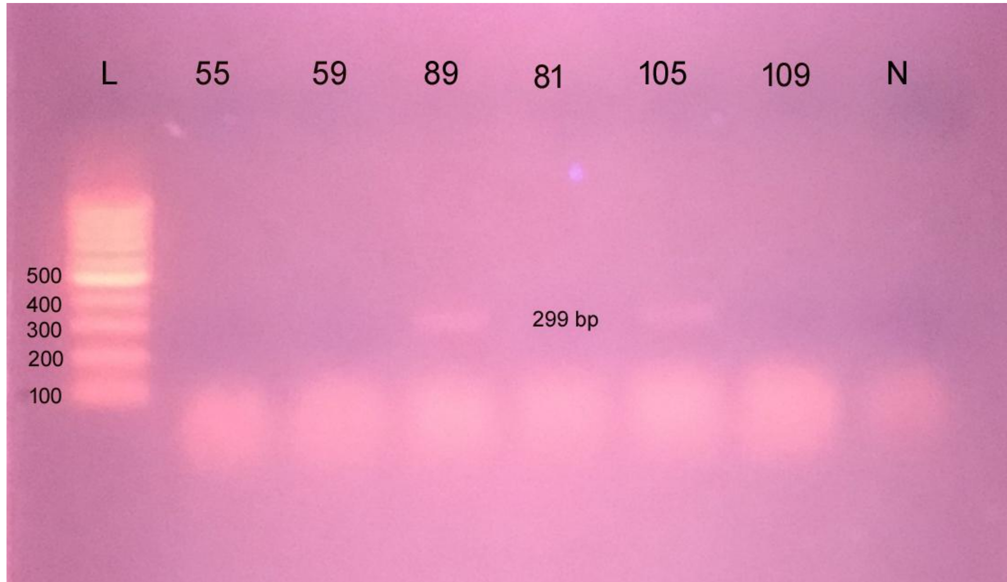
L–1kb DNA Ladder
N – Negative Control

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



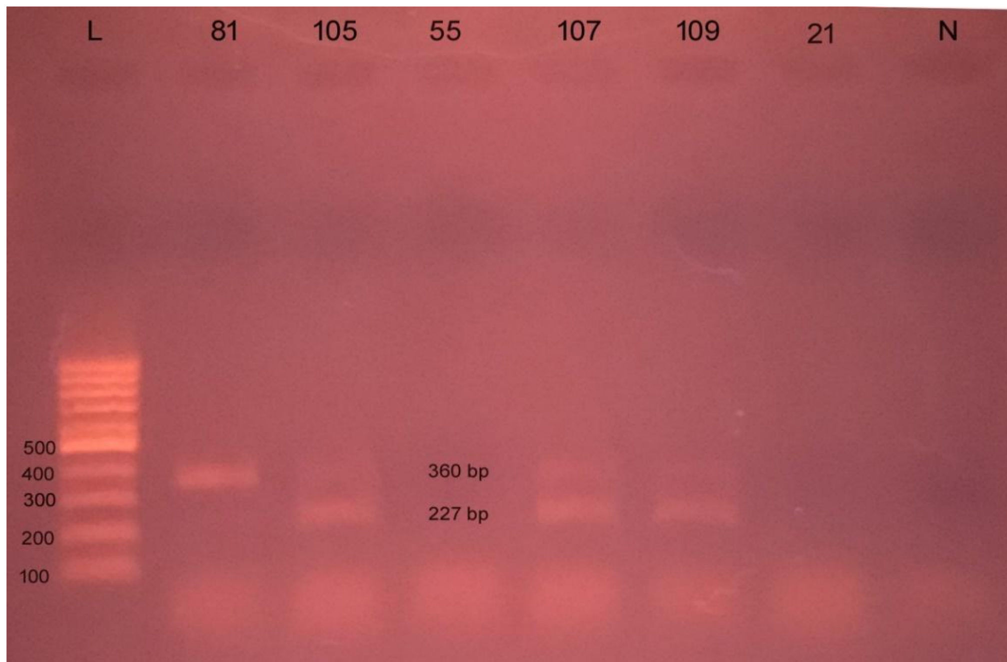
L–1kb DNA Ladder
N – Negative Control

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 *tetK* gene (Fig. 3) in the present study (26%) was in accordance with the findings of Emaneini *et al.,* (2013) and Lim *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 *et al.,* (2017), along with lower rate of prevalence (4.8%) which was reported in the study conducted by Monecke *et al.,* (2016).

In conclusion, the study reveals that variable level of prevalence has been due to high level of contamination of *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 *S. aureus* pathogen.

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