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Detection of antibiotic resistance gene of *Staphylococcus aureus* isolates derived from milk, meat and egg

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Abstract
The purpose of this study was to examine the genes associated with antibiotic resistance in *S. aureus* that was isolated from milk, meat, and eggs purchased from retail establishments in Udaipur, Rajasthan. To achieve this, between July 2018 and October 2018, 120 samples-40 each for milk, meat, and eggs were chosen at random from supermarkets, stores, and a few dairy farms in the city of Udaipur. Using morphological (Gram staining), biochemical testing, *S. aureus* was extracted and positively identified. The *ermC, tetK,* and *aacA-aphD* genes of *S. aureus* were detected using a standardised PCR technique. *ermC, tetK,* and *aacA-aphD* gene prevalence were calculated to be respectively 13%, 26%, and 21.7%.

Keywords: Antibiotic resistant gene, *ermC, tetK, aacA-aphD*

Introduction
Staphylococcus aureus is a Gram-positive bacterium that frequently lives innocuously in a variety of habitats, including environmental samples and the skin and mucous membrane of people and other animals. In both people and dairy cattle, *Staphylococcus aureus* is a significant opportunistic infection. One of the most common causes of clinical infections worldwide is *Staphylococcus aureus* (Kwon *et al.*, 2006) [11]. In the dairy food industry, contamination from *S. aureus* in dairy cows and raw milk is still an issue. *S. aureus* can cause severe cases of mastitis, arthritis, and urinary tract infections in animal species, including ruminants. It can also cause sub-clinical mastitis (Sutra *et al.*, 1994) [19]. The numerous outbreaks of food-borne illness connected to contaminated dairy products highlight the significance of *S. aureus* for public health importance. (McMillan *et al*., 2016) [9].
S. aureus is commonly found in milk, dairy products, raw meat/meat products, eggs/egg products, and aquatic products (Can *et al.*, 2017) [8]. Meat is one of the most important food stuff associated with Staphylococcal foodborne diseases (Hanson *et al*., 2011) [7]. Animal-derived Staphylococci contain a wide range of antimicrobial resistance (AMR) genes (Argudin *et al*., 2017) [2].
Eggs are truly a low-cost, high-nutritional-value food that can be considered a nutritious formula in the diet for people of all ages and stages of life. Eggs contain 18 vitamins and minerals as well as zinc, selenium, retinol, and tocopherols (El-Kholy *et al*., 2020) [4]. Poor handling and storage in unsanitary conditions in poultry farms or shops endangers egg quality and may have an impact on human health (Pyzik and Marek, 2012) [16].

Materials and Methods
A total of 120 samples, 40 each of milk, eggs, and meat were randomly gathered from various locations throughout the city of Udaipur. The samples were taken in sampling vials aseptically, and were transferred to the lab on ice packs maintained at 4^0 C until the time of processing.

Molecular Characterization
Isolation of DNA from pure culture was undertaken using by Nucleo-pore gDNA fungal/bacterial mini kit by following the manufacturer's instructions supplied along with the kit. Genomic DNA isolated from *S. aureus* isolates were used in the PCR. Published primers were used for the detection of *ermC, tetK,* and *aacA-aphD* genes in *S. aureus* isolates are described in Table No.1.

Table 1: Primers used for detection of *ermC*, *tetK*, and *aacA-aphD gene*

S. No	Oligo Name	Sequence(5^1-3^1)	Size of amplified product(bp)	Reference
1.	*aacA-aphD*	F-TCCAAGAGCAATAAGGGC R-CACACTATCATAACCACTA	227bp	Strommenger *et al.*, (2003) [18].
2.	*ermC*	F-ATCGTCAATTCCTGCATGT R-ATCGTGGAATACGGGTTTG	299bp	Strommenger *et al.*, (2003) [18].
3	*tetK*	F- AGCGACAATAGGTAATAGT R-AGTGACAATAAACCTCCTA	360bp	Strommenger *et al.*, (2003) [18].

F=Forward, R=Reverse

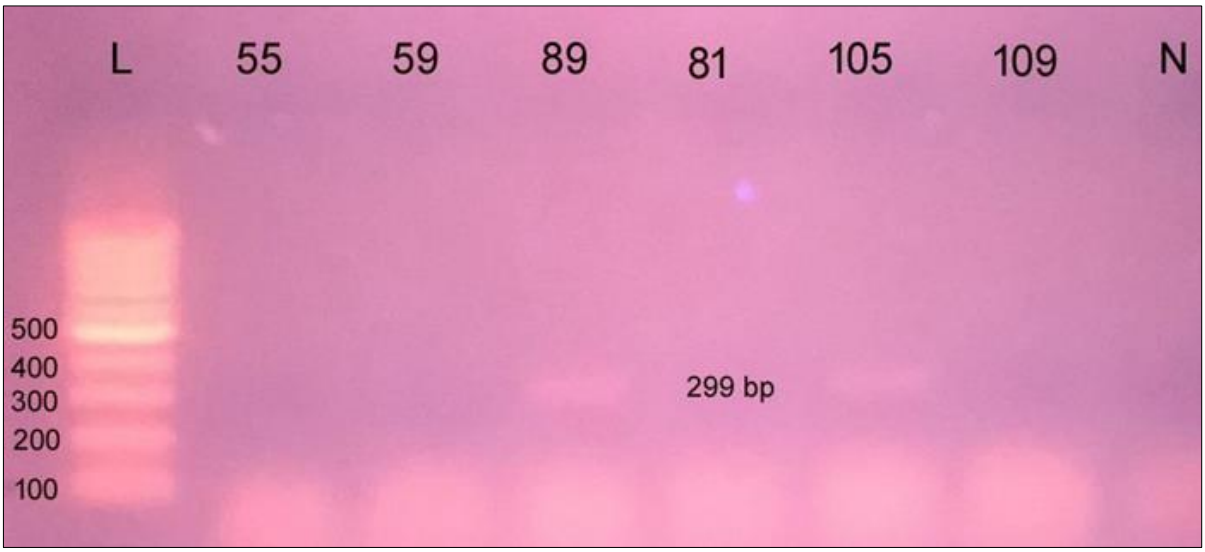
The PCR procedure to screen the *ermC, tetK*, and *aacA-aphD genes* in S. aureus isolates was standardized as described by Strommenger *et al.*, (2003) [18] with certain modifications. Followed by preliminary trials, the reaction mixture was optimized to contain 12.5µl 2X PCR master mix, 10nmol of each forward and reverse primer, 10.5 µl nuclease free water and 1 µl of DNA template. The reaction was performed in the thermal cycler with pre-heated lid (lid temp=105^0 C). The cycling conditions of *ermC, tetK*, and *aacA-aphD gene* were comprised of 30 cycles of denaturation, annealing and extension which are described in Table No 2.

Table 2: Steps and conditions of thermal cycling for different primer pairs in PCR

Primers (Forward and Reverse)	Cycling conditions				
	Initial denaturation	Denaturation	Annealing	Extension	Final Extension
aacA-aphD(F) *aacA-aphD*(R)	94 ^{0}C for 5 minutes	94 ^{0}C for 1 minutes	55 ^{0}C for 1 minutes	72 ^{0}C for 1 minutes	72 ^{0}C for 5 minutes
ermC(F) *ermC*(R)					
tetK(F) *tetK*(R)					
Repeated for 30 cycles					

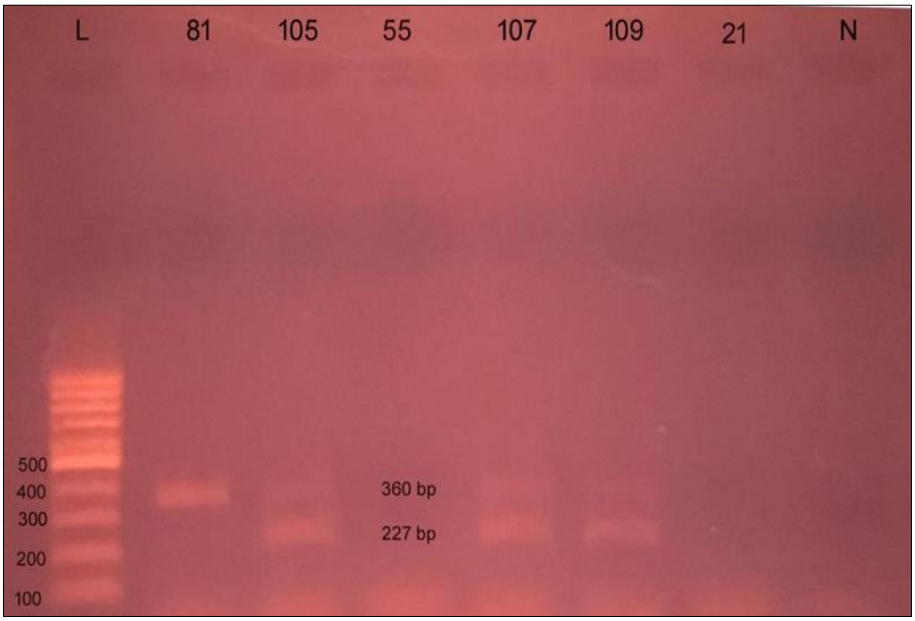
Results and Discussion

Out of the 120 samples analysed *Staphylococcus aureus* was recovered from 23 samples of foods of animal origin (milk, egg, and meat) based on cultural and biochemical tests. The *ermC*, *aacA-aphD*, and *tetK* genes, which are associated with antibiotic resistance, were detected as positive by PCR in 13.04 percent (4/23), 21.73 percent (5/23) and 26.08 percent (6/23) of the samples, respectively. The results of our investigation, which found that the ermC gene was 13 percent prevalent (Fig-1), were consistent with earlier research by Parvizi *et al* (2012) [15]. Lower prevalence rates were disclosed by Zmantar *et al*., (2011) [20] in which 6 percent prevalence was identified, but higher prevalence rates were shown by Ghanbari *et al*., (2016) [6] and Lim *et al* (2012) [12] where presence of ermC gene was determined to be 44.4 percent and 21 percent respectively.



L–1kb DNA Ladder N – Negative Control, Positive samples (55, 59, 89, 105, 109)

Fig 1: Agarose gel showing PCR amplified product (299bp) for *ermC* gene in *S. aureus* isolates



L-1kb DNA Ladder N – Negative Control, Positive samples (81,105, 55, 107,109,21)

Fig 2: Agarose gel showing PCR amplified product (227bp) for *aacA-aphD* and (360bp) for *tetK* gene in *S. aureus* isolates

The incidence of the *aacA-aphD* gene was nearly consistent with Kumar *et al*. (2010) [10]. In comparison to our study, Monecke and Ehricht (2005), Achek *et al*. (2018), and Ruban *et al*. (2017) [14, 1, 17] reported prevalences of 29 percent, 30.76 percent, and 88 percent, respectively, whereas Monecke *et al*. reported a lower prevalence of (2.4 percent) (2016) [13]. According to Emaneini *et al*. (2013) [5] and Lim *et al*. (2012) [12], who reported prevalence rates of 17.2 percent and 21 percent, respectively, the prevalence of the tetK gene in the current study (26 percent) was consistent with their findings. While Dehkordi *et al*. (2017) [3] study found a greater prevalence rate (72.97 percent) and Monecke *et al*. (2016) [13] studies found a lower prevalence rate (4.8 percent) in (Fig.2).

Conclusion

The study concludes by showing that the high level of S. aureus contamination has contributed to the varying level of prevalence. Milk, eggs, and meat all contain S.aureus, which is enough to induce food poisoning and is a major cause of gastroenteritis. Therefore, contamination can be decreased by treating milk properly, maintaining clean meat shops and poultry farms, and practising good hygiene.

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