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**S Wilfred Ruban**  
Associate Professor and Head,  
Department of Livestock  
Products Technology, Veterinary  
College, Hebbal, Bangalore- 560  
024, India

**R Narendra Babu**  
Professor, Department of  
Livestock Products Technology  
(Meat Science), Madras  
Veterinary College, Chennai,  
India.

**K Porteen**  
Assistant Professor, Department  
of Veterinary Public Health and  
Epidemiology, Madras  
Veterinary College, Chennai,  
India

**P Raja**  
Assistant Professor, Department  
of Animal Biotechnology,  
Madras Veterinary College,  
Chennai, India

**Govind Vemala**  
PhD Scholar, Department of  
Livestock Products Technology  
(Meat Science), Madras  
Veterinary College, Chennai,  
India

**V Appa Rao**  
Professor and Head, Department  
of Livestock Products  
Technology (Meat Science),  
Madras Veterinary College,  
Chennai, India

**Robinson JJ Abraham**  
Retired Professor & Head,  
Department of Livestock  
Products Technology (Meat  
Science), Madras Veterinary  
College, Chennai, India

**Correspondence**  
**S Wilfred Ruban**  
Associate Professor and Head,  
Department of Livestock  
Products Technology, Veterinary  
College, Hebbal, Bangalore- 560  
024, India

## Retail meat as a potential source of Panton valentine Leukocidin (*pvl*) positive methicillin resistant *S. aureus*

**S Wilfred Ruban, R Narendra Babu, K Porteen, P Raja, Govind Vemala, V Appa Rao and Robinson JJ Abraham**

### Abstract

Panton–Valentine leukocidin (PVL), is an exotoxin encoding gene and has been associated with most CA-MRSA (Community Associated Methicillin Resistant *S. aureus*) strains which causes severe skin infections and necrotizing pneumonia in human. The present study was carried out to evaluate the prevalence of Panton Valentine Leukocidin (*pvl*) gene in *S. aureus* isolated from mutton marketed in retail outlets of Chennai, India. A total of 40 isolates (16 MRSA and 24 MSSA) from mutton samples, were screened based on PCR specific for *pvl* gene. It was evident that the 15 of the 40 isolates carried *pvl* gene, indicating an overall prevalence of 37.50 per cent. The prevalence of *pvl* gene in MRSA was 31.25 per cent (5/16) and in MSSA was 41.66 per cent (10/24). The results of the present study indicates that the prevalence of Community associated *S. aureus* in retail chicken meat, clearly indicating that the major source of *S. aureus* contamination in meat is human handlers and retail meat is a potential vehicle in transmission of MRSA to handlers as well as consumers.

**Keywords:** *S. aureus*, mutton, retail outlet, *pvl* gene, MRSA

### 1. Introduction

*S. aureus* is a commensal pathogen and natural inhabitant of the skin and nasal cavity of both human as well as animals and contamination of meat and meat products by this pathogen is inevitable since they enter the food chain through contaminated water, improper hygiene during slaughter as well as infected meat handlers [1]. The prevalence of *S. aureus* in retail meat and meat products have been documented by several workers and data suggests that the rate of prevalence is much higher in developing countries compared to that of developed countries indicating the lack of hygiene during slaughter and further processing. The rate of prevalence varies with the type of meat as well as the geographical location of sampling, with pork being the most implicated meat compared to Chicken, mutton and beef [2, 3]. In India the reported prevalence of *S. aureus* in different retail meats ranged from 10 to 100 per cent [4-6]. *S. aureus* is extraordinarily adaptable pathogen with a proven ability to develop resistance to antibiotics [7] and prevalence of methicillin resistant *Staphylococcus aureus* (MRSA) in humans as well as in foods of animal origin has been increasing worldwide. In 1932, Panton and Valentine first associated the leukotoxin with skin and soft tissue, long before penicillin resistant *Staphylococcus aureus* (PRSA) and methicillin resistant *Staphylococcus aureus* (MRSA) were of clinical concern. PVL gene may be found in both methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains [8].

In addition, *pvl* gene has been considered as a marker for differentiation of CA-MRSA (Community Associated MRSA) strains which causes severe skin infections and necrotizing pneumonia in human from that of HA-MRSA (Hospital acquired MRSA) [9].

*S. aureus* and MRSA have been documented in human, food-producing animals and meat sold in retail outlets in various countries [2, 10, 11, 12]. However, data on prevalence of *pvl* positive *S. aureus* in India have been documented in clinical settings, whereas no such documentation had been carried out from retail meat. Hence, the present study has been carried out to document the prevalence of *pvl* (virulence factor) genes and to validate the potential of retail meat in transfer of antibiotic resistance to meat handlers and consumers through the food chain.

## 2. Materials and Methods

The protocol and methodology used in the present study for isolation and characterization of *S. aureus* from mutton was carried out with approval from the Institutional Biosafety Committee of Tamil Nadu Animal and Veterinary Sciences University, Chennai (No.0880/DFBS/B/2015/20.04.2015). A total of 40 *S. aureus* isolates characterized by biochemical and molecular methods were used in the present study. Among the 40 isolates 16 were methicillin resistant isolates (characterized by *mecA* PCR) and 24 isolates were methicillin sensitive (negative for *mecA* gene).

**2.1 Polymerase Chain Reaction:** The genomic DNA was extracted by using DNA extraction kit (Qiagen) and the primers were custom synthesized. The sequences of the primers used for gene amplification was F-ATCATTAGGTAAAATGCTGGACATGATCCA and R-GCATCAACTGTATTGGATAGCAAAAGC. All oligonucleotide primers were custom synthesized by M/s. Eurofins, Bangalore. Polymerase chain reaction (PCR) for the detection *pvl* genes was performed according to the methods described by Lina *et al.* [8]. Briefly, amplification reactions were performed in a 25 µL mixture containing 12.5 µL of 2X PCR master mix (Amplicon, Denmark), 10 pmol of each primers and 2 µL of DNA template and the final volume was adjusted to 25 µL by adding nuclease free water. Amplification reactions were performed using a DNA thermal cycler (Master Cycler Gradient, Eppendorf, Germany) with the following program: denaturation for 10 minutes at 95 °C, followed by 30 cycles of denaturation for 3 seconds at 94 °C, annealing for 30 seconds at 55 °C and extension for one

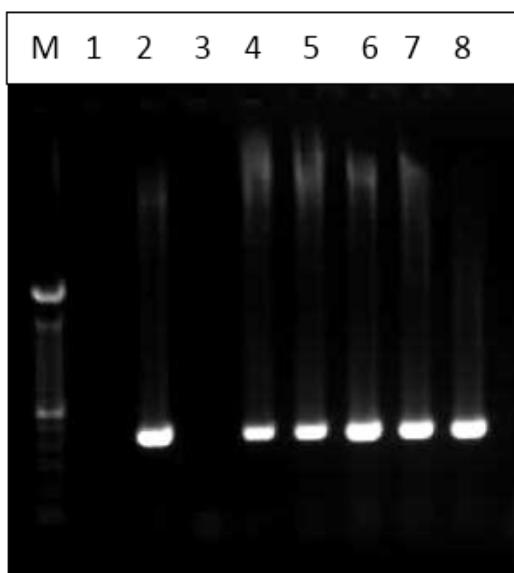
minute at 72 °C and final extension for 10 minutes at 72 °C. The PCR products were stained with 1% solution of ethidium bromide and visualized under UV light after gel electrophoresis on 2.0% agarose gel. Nuclease free water was used as the negative control.

## 3. Results and Discussion

In the present study all the 40 isolates (16 MRSA and 24 MSSA) were screened for the presence of Panton–Valentine leukocidin (*pvl*) gene, a marker for Community Associated MRSA (CA-MRSA) based on PCR and it was observed that 15 of the 40 isolates carried *pvl* gene and amplified 433 bp product (Fig. 1) specific for *pvl* gene as described by Lina *et al.* [8], indicating an overall prevalence of 37.50 per cent (Table 1). The prevalence of *pvl* gene in MRSA was 31.25 per cent (5/16) and in MSSA was 41.66 per cent (10/24). The prevalence of *pvl* gene in *S. aureus* isolated from retail meat has been documented by several researchers in different countries. The results were in line with the findings of Abdalrahman *et al.* [13] who observed that 66.7 per cent MRSA isolates obtained from chicken meat carried *lukS-lukF* gene (*pvl*). Similar findings have been recorded by O’Brein *et al.* [14] who examined the prevalence of *S. aureus* on retail pork and observed that 19.2 per cent of the methicillin resistant isolates from pork in Iowa, Minnesota and New Jersey carried *pvl*, a genotype epidemiologically associated with increased virulence. Jackson *et al.* [15] evaluated the prevalence of *pvl* gene in *S. aureus* isolated from retail beef and handlers and opined that majority of the human isolates were *pvl* positive as compared to only one isolate from beef.

**Table 1:** Prevalence of Panton Valentine Leukocidin (*pvl*) gene among MRSA and MSSA isolated from mutton

Isolate Type	MRSA (n=16)	MSSA (n= 24)	Overall Prevalence (%)
<i>pvl</i> Positive			
Numbers (%)	5 (31.25)	10 (41.66)	37.50
<i>pvl</i> Negative			
Numbers (%)	11 (68.75)	14 (58.34)	62.50



**Fig 1:** PCR amplification of *pvl* (433 bp) gene in *S. aureus* isolated from mutton marketed in Chennai city

Lane M: 100 bp DNA ladder, Lane 1: Negative Control, Lane 2: *pvl* reference strain (MVCMS27), Lane -3: *pvl* negative Methicillin resistant *S. aureus* isolates from mutton Lane 4-6: *pvl* positive Methicillin resistant *S. aureus* isolates from mutton, Lane 7-8: *pvl* positive Methicillin resistant *S. aureus* isolates from mutton

In the Indian context, no literature was available with reference to prevalence of *pvl* positive *S. aureus* in retail meat and this is the first report of such prevalence in India. However, prevalence of *pvl* positive isolates has been documented in *S. aureus* isolated from clinical settings. Bhutia *et al.* [16] observed that 94.44 and 5.55 per cent of MRSA and MSSA clinical isolates of *S. aureus* from India carried *pvl* gene and they opined that MRSA is an important reservoir of *pvl* gene and is now being slowly acquired by MSSA strains. In similar line Kaur *et al.* [17] generated a baseline data on the extent of MRSA infections and the frequency of PVL-positive *S. aureus* in Belgaum, South India and observed that the overall prevalence of PVL positive *S. aureus* was 62.85 per cent and prevalence of *pvl* gene in MRSA and MSSA were 85.1 and 48.8 per cent respectively. In the present study it was evident that there was not much significant difference in prevalence of *pvl* gene among the

MRSA and MSSA isolates suggesting that the *pvl* gene may be found in both methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) strains [8].

#### 4. Conclusion

The results clearly indicates that the isolates of *S. aureus* from retail mutton samples carry the *pvl* gene, which encodes exotoxin responsible for virulence of these strains and with the ability to causes severe skin infections in human and person in contact with such contaminated meat. Hence, a detailed study on the other virulence factors viz., enterotoxin gene profiles and an antibiotic resistance gene and pattern of resistance of these isolates needs to be ascertained to elucidate the potential of these isolates in causing infection in meat handlers and consumer.

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