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Multiplex PCR for Detection of Genes Encoding Antibiotic Resistance in *Staphylococcus aureus*

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A B S T R A C T

A study was carried out to evaluate the prevalence of genes encoding antibiotic resistance for commonly used antibiotics in poultry production in *S. aureus* isolated from Chicken meat marketed in Chennai City. A multiplex PCR was used for screening of 50 *S. aureus* isolates for the presence of five genes viz., *aacA-aphD* (aminoglycoside), *tetK* and *tetM (*tetracycline resistance*)*, *erm*(A) and *erm*(C) (macrolide-lincosamide-steptogramin B resistance). The results of the study revealed that of the 50 isolates screened all the isolates (100 per cent) carried either one of these resistance gene. Among the various genes either alone or in combination 48 isolates (96 %) carried *tetK* gene, 44 isolates (88 %) carried *aacA-aphD* gene, 43 isolates (86 %) carried *erm*(C) gene, three isolates (6 %) carried *tetM* gene and none of the isolates carried *erm*(A) gene. It was evident that 37 of the isolates (74 %) carried three resistant genes followed 6 isolates with two resistance genes (12 %) and one isolate with only one gene. The study clearly indicated the prevalence of multidrug resistant *S. aureus* in retail chicken meat and its potential in transference of antibiotic resistance to the consumers. This multiplex PCR can be used as a simple, rapid and accurate assay for identification of antibiotic resistance profile and could be used for surveillance in epidemiological studies.

Introduction

Emergence of antimicrobial resistance in pathogens especially from foods of animal origin has been mainly attributed to widespread and indiscriminate use of antibiotics in control of infection as well as growth promoters (Swartz, 1997). In addition, increase in international travel and trade have facilitated the easy spread of these resistant isolates over different geographical locations even to locations where antibiotics are seldom used. Among the various pathogens of interest, *S. aureus* is an important opportunistic pathogen of greatest concern because of its intrinsic virulence and its capacity to adapt to different environmental condition (Waldvogel, 2000).

Staphylococcus aureus has been one of the most adaptive bacteria in the antibiotic era, which is well documented by its ability to

quickly respond and develop a resistance mechanism to the existing and new antibiotic starting from penicillin, methicillin to the most recent antibiotics used in human and animal therapy (Pantosti *et al.,* 2007). Antibiotic resistance in *S. aureus* is mainly due to acquisition of genes either by horizontal gene transfer or endogenous resistance. The majority of the genes responsible for the resistance to antibiotics are mainly located in the plasmids, transposon or phages. Hence, an understanding of the genes responsible for antibiotic resistance is of public health importance and at present such information is still very limited in India especially with respect to S. *aureus* isolated from meat and me products in India. Hence, the present study was designed with the objective to evaluate the prevalence of genes encoding antibiotic resistance for commonly used antibiotics in poultry production in *S. aureus* isolated from Chicken meat marketed in Chennai City.

Materials and Methods

The approval of the Institutional Biosafety Committee of Tamil Nadu Animal and Veterinary Sciences University, Chennai was obtained for conduct of the study. A total of 50 *S. aureus* isolated from retail Chicken meat marketed in Chennai, Tamil Nadu, India based on standard protocol (ISO standard 6888/1:1999) were used in the present study. The isolates were initially confirmed by biochemical test viz., catalase test, Mannitol fermentation, Coagulase and thermonuclease test as per standard protocol. The final confirmation was carried out based on PCR targeting *nuc* (thermonuclease) gene.

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 are presented in table 1. Multiplex Polymerase chain reaction (m-PCR) for the detection of 5 antibiotic resistance genes was performed according to the methods described by Strommenger *et al.,* (2003). Briefly, amplification reactions were performed in a 25 µL mixture containing 12.5 µL of 2X PCR master mix (Amplicon, Denmark), 5pmol 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: Initial denaturation at 94°C for 3 min was followed by 30 cycles of amplification with 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 sand final extension for 5 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.

Results and Discussion

In the present study it was observed that of the 50 isolates screened all the isolates (100 per cent) carried either one of these resistance gene (Fig. 1). The PCR protocol followed amplified specific products as outlined by Strommenger *et al.,* (2003). Among the various genes studied either alone or in combination 48 isolates (96 %) carried *tetK* gene and amplified 360 bp product and three isolates (6 %) carried *tetM* gene, amplifying 158 bp product. The *tet* genes confers resistance to tetracycline are contained within conjugative transposons that can be transferred horizontally and expressed in both Gram-positive and Gram-negative bacteria. The results of the present study are in concurrence with the findings of Schmitz *et al*., (2001) and Jones *et al*., (2006), who observed higher prevalence of *tetK* gene in

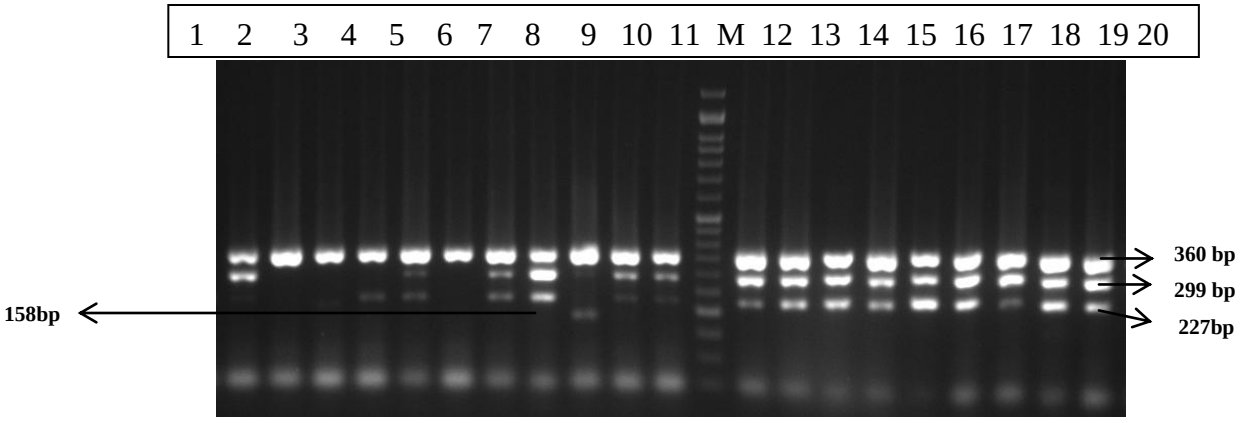
majority of the *S. aureus* isolates (60-100 %). In addition Schmitz *et al*., (2001) opined that among the Methicillin sensitive isolates *tetK* gene was the most prevalent tetracycline resistance determinant whereas in methicillin resistant *S. aureus, tetM* was most frequent gene.

In the present study, 44 isolates (88 %) carried *aacA-aphD* gene and amplified 227 bp product and similarly Nakaminami *et al*., (2008) also observed that *aacA–aphD* gene was the frequently detected resistance gene in both the MRSA (96.1 %) and MSSA (85.8 %) isolates in Japan. The *aacA-aphD*gene encodes resistance to gentamicin–tobramycin–kanamycin or kanamycin–neomycin (Rouch *et al*., 1987).

Table.1 Primers used in this study

Target Gene	Resistance phenotype	Primer sequence (5'-3')	Amplicon size (bp)
aacA-aphD	Gentamicin	TAA TCC AAG AGC AAT AAG GGC	227
		GCC ACA CTA TCA TAA CCA CTA	
erm(A)	Erythromycin, Clindamycin	AAG CGG TAA ACC CCT CTG A	190
		TTC GCA AAT CCC TTC TCA AC	
erm(C)	Erythromycin, Clindamycin	AAT CGT CAA TTC CTG CAT GT	299
		TAA TCG TGG AAT ACG GGT TTG	
tetK	Tetracycline	GTA GCG ACA ATA GGT AAT AGT	360
		GTA GTG ACA ATA AAC CTC CTA	
tetM	Tetracycline	AGT GGA GCG ATT ACA GAA	158
		CAT ATG TCC TGG CGT GTC TA	

Fig.1 PCR for amplification of Antibiotic Resistance Gene in S. aureus isolated from retail Chicken meat in Chennai (tetK- 360bp; erm(C)- 299 bp; aacA-aphD- 227 bp and tetM- 158 bp).



Macrolide resistance in *S. aureus* is mediated by one or more *erm* genes encoding a 23S rRNA methylase. In the present study it was evident that 43 isolates (86 %) carried *erm*(C) gene amplifying 299 bp product, and none of the isolates carried *erm*(A) gene.Similar expression of *erm*(C) gene has been documented by Nicola *et al*., (1998) and Lina *et al*., (1999). The results of the present study were contrary to the findings of Schmitz *et*

al., (2000), who observed that the most prevalent gene was *erm*(A) followed by *erm(C)* in 67 and 23 per cent of the isolates respectively.

The present study clearly indicates that *S. aureus* isolated from the chicken meat marketed in retail outlets of Chennai carries multiple antibiotic resistant genes that confer resistance to commonly used antibiotics in human and animal therapy like Gentamicin, tetracycline and erythromycin. Hence, control strategies with respect to hygiene need to be put in place to prevent the spread of these multidrug resistant pathogens to the consumers.

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