OP305

Molecular typing of *Staphylococcus aureus* from fish and ground beef

Fatma ÖZDEMIR, Seza ARSLAN
Department of Biology, Faculty of Arts and Science, Abant İzzet Baysal University, Turkey
ozkardes_f@ibu.edu.tr

Aim of the study:*Staphylococcus aureus* can cause several diseases in humans and animals. The aim of this research was to confirm *S. aureus* from fish and ground beef samples by the species-specific fragment (Sa442) and thermonuclease gene (*nucA*), to characterize the *S. aureus* isolates by polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP) of the coagulase gene (*coa*), and to investigate the presence of toxic shock syndrome toxin gene (*tsst*) and exfoliative toxin genes (*eta*, *etb*), to determine phenotypic virulence determinants such as production of slime and beta-lactamase as well as antimicrobial resistance profiles of *S. aureus* from meat samples.

Material and Methods: Of the 36 *S. aureus* isolates, 19 were from fish samples including freshwater fish (*Oncorhynchus mykiss*) and seawater fish (*Sparus aurata*) and 17 were from ground beef samples. Phenotypically identified *S. aureus* isolates were also confirmed as *S. aureus* using the *S. aureus* specific fragment (Sa442) and *nucA* gene. For molecular typing of all *S. aureus* isolates based on the PCR-RFLP assay, amplification of the *coa* gene of isolates was carried out. The PCR products of the *coa*gene were digested by both *Alu*I and *Hae*III restriction endonuclease enzyme according to manufacturer's instructions. Phylogenetic analysis was performed using NTSYS-pc (version 2.10) software package. Similarity among the isolates was determined using the Dice's similarity and a dendogram was constructed with the unweighted pair group method using arithmetic average (UPGMA) clustering. Some virulence associated genes (*tsst*, *eta*, *etb*) of *S. aureus* were examined using single PCR. The slime production and beta lactamase activity of *S. aureus* isolates were examined using Congo Red Agar and acidometric strip method, respectively. Resistance to various antimicrobial agents of isolates was detected on Mueller Hinton Agar by disk diffusion method.

Results: PCR-RFLP typing based on the polymorphism of the *coa* gene was used to discriminate the *S. aureus* isolates from fish and ground beef samples. The 36 *S. aureus* isolates produced only one amplicon followed by the amplification of the *coa* gene. The PCR product of the *coa*gene revealed five different amplicons in size ranging approximately 442 to 730 bp. In *S. aureus* isolates, seven and six different RFLP profiles were generated by digestion of *Alu*I and *Hae*III restriction enzymes of the *coa*gene, respectively. None of the *S. aureus* isolates from fish and ground beef was found to be positive for *tsst*, *eta* and *etb* gene. All isolates were positive for slime production. Besides, 41.7% of isolates produced beta-lactamase. The highest frequency of resistance to ampicillin (91.7%), followed by tetracycline (22.2%), vancomycin (16.7%) and erythromycin (8.3%) was detected in *S. aureus* isolates. A total of 94.4% of *S. aureus* isolates were resistant to at least one antimicrobial agent and 44.4% of them to at least two or more antimicrobial agents.

Keywords: *Staphylococcus aureus*, *nucA*, Sa442, RFLP, antimicrobial resistance, food.