PCR-Based Identification of Streptomycin Resistance in Salmonella Serovars Using a Novel Primer Set

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Streptomycin has been used to treat bubonic plague and tularemia and, as a second line of antibiotic drug of choice, to treat tuberculosis. Streptomycin is also used as a pesticide to control bacteria, fungi, and algae in zoonotic and plant diseases. Extensive use of antibiotics has resulted in the emergence of antibiotic resistance in bacterial pathogens. Sheng Chen and colleagues (2004) have shown that 73 of 133 Salmonella isolates obtained from retail meats in the U.S. and China were resistant to at least one antimicrobial agent, and 61% of them were resistant to streptomycin. To understand antimicrobial resistance in Salmonella, it is important to develop molecular and biophysical tools to study streptomycin resistance.

In this study, we have designed and used novel primers specific for aadA gene coding for aminoglycoside adenyltransferase (required for streptomycin resistance) in the top 20 Salmonella serovars of human foodborne outbreak origins. Primers (aadA-F: 5'-TCCACCTTCCAGGGAATGC-3'; aadA-R: 5'-GGGTATACCAGATACGCAG-3') were designed by using the Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Salmonella cultures were grown for three hours to extract total DNA by boiling method. PCR amplicon of size 287 bp was obtained from Salmonella serovars, while non-Salmonella serovars did not yield any amplification. In the bacterial growth inhibition assay, eleven Salmonella serovars exhibited significant growth ($A_{595} > 0.4$) in the presence of streptomycin (100 µg/ml) cultured in a 96-well microtiter plate (Microtest U-Bottom, BD), indicating strong antibiotic resistance. The nine remaining serovars did not show considerable growth ($A_{595} < 0.4$) as monitored by a spectrophotometer, indicating their sensitivity to streptomycin. All non-Salmonella cultures tested were also sensitive to streptomycin. This study lays the foundation for a future objective, to examine expression of aminoglycoside adenyltransferase in the Salmonella serovars that carried the aadA gene but failed to grow in the presence of streptomycin.

Research advisor Arun Bhunia and postgraduate Atul K. Singh explain, “Antibiotic resistance in human pathogens poses a serious public health risk. Developing molecular methods, including PCR assays targeting resistance genes, will help scientists understand the genotypic and phenotypic relationships in antibiotic resistance. Exposure of students, especially those in the early stage of their careers, to such global challenges helps develop skills required for investigative solutions.”