HRM multiplex real-time PCR detection of *Salmonella* virulence and ESBL resistance genes in food

Miki Hodel, Rajiv Dhital, Azlin Mustapha

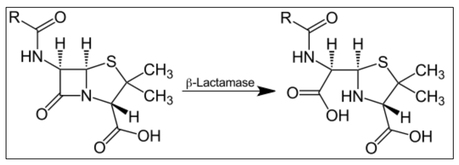
Food Science Program, University of Missouri

Honors College, University of Missouri

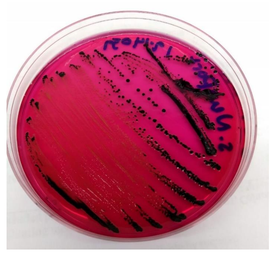
Abstract

Salmonella **is a gram-negative bacterium comprised of two species and 2,500 serotypes. The World Health Organization estimates that each year, there are 78.8 million cases of non-typhoidal salmonellosis caused by contaminated food and water. Currently, several antibiotics are used to treat salmonellosis. However, due to the overuse of antibiotics in human medicine and animal husbandry, and horizontal transfer of antibiotic resistance genes from closely related enteric bacteria, resistance in *Salmonella* has become an emerging public health concern. Extended spectrum beta lactamase is an enzyme that confers resistance to extended spectrum beta-lactam (ESBL) antibiotics, a last resort antibiotic class with high medical value. In MU's Food Science Program, Dr. Mustapha's research group is developing a technique that uses a high resolution melt-curve (HRM) multiplex real-time (RT)-PCR assay to detect ESBL resistance genes in *Salmonella* from contaminated food. HRM RT-PCR is suitable for virulent ESBL-resistant *Salmonella* detection because results can be obtained faster than is achievable by conventional PCR, multiple target genes can be detected in one assay, and there is a low occurrence of false positive results. The HRM RT-PCR assay in development targets nine different genes that includes the virulence genes associated with *Salmonella* infections and ESBL resistance genes. The assay is being applied to detect these genes in a variety of food samples that have been associated with foodborne salmonellosis.**

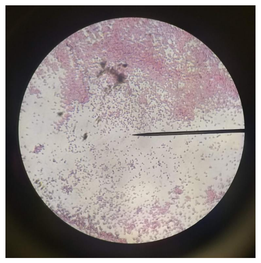
Background



The mechanism of beta-lactamase activity on beta-lactam antibiotics.

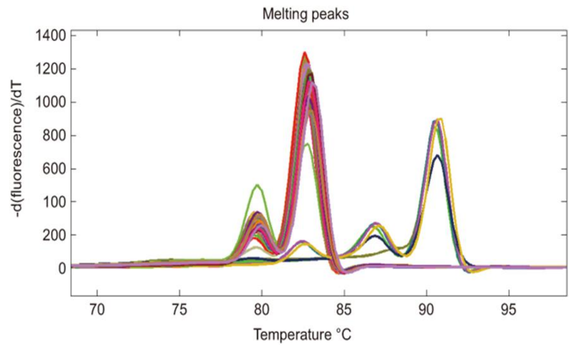


Salmonella **streaked on XDL selective media.**



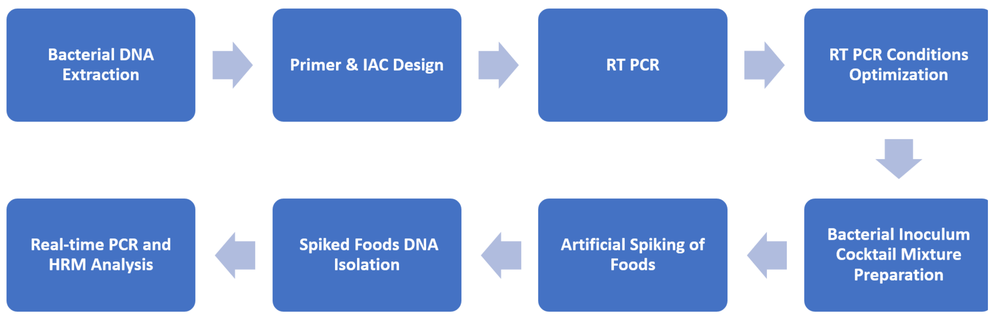
Pink stained cells of *Salmonella* indicate a Gram- negative trait.

Salmonella**, a bacterium that causes food borne illness. Photo by the Centers for Disease Control.**



HRM-multiplex RT-PCR uses multiple primers to rapidly and efficiently amplify multiple genes using differences in melting temperatures.

Experimental Design



Results

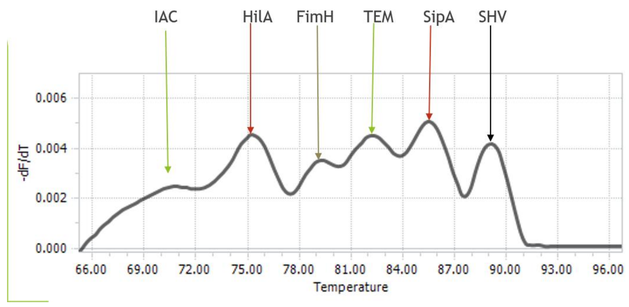
Table 1: Primers used in 1st set assay

Name	Sequence (5'-3')	Amplicon size (bp)	Amplicon Tm (℃)	Concentration used in multiplex PCR
HilA-16F	ACACTATCTCCTTCCGGCTTT	80	75.31	0.38 µM
HilA-16R	TCAGAGGGAACGGATGATGT			
FimH -615F	GGTCGTGGAGTTTGATTTCG	54	79.31	0.43 µM
FimH -615R	CCCGCCTGACTAAATAACGA			
SipA-1097F	CGCGTGTGGATTCGACTA	294	85.49	0.26 µM
SipA-1097R	CCGTCTGGCTTTGCTGTTTA			
blaTEM-212F	AAGTTCTGCTATGTGGTGCG	192	82.26	0.12 µM
blaTEM-212R	AGTTGGCAGCAGTGTTATCA			
blaSHV-309F	GGTCAGCGAAAAACACCTTG	195	89.10	0.35 µM
blaSHV-309R	GCCTCATTCAGTTCCGTTTC			

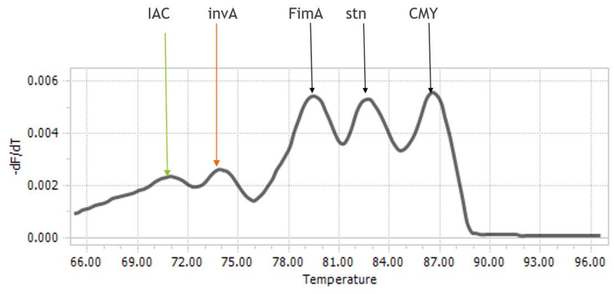
Table 2: Primers used in 2nd set assay

Name	Sequence (5'-3')	Amplicon size (bp)	Amplicon Tm (℃)	Concentration used in multiplex PCR
invA-1227F	ATTTTGATTTGATGCGAGTGG	78	73.98	0.41 µM
invA-1227R	CTTGCTGATGGATTGTTGGA			
FimA-86F	GCGTGAGTGGCGGTACTATT	82	79.46	0.35 µM
FimA-86R	GTTTGATCGGCGGATTTAGT			
stn-103F	AAATCGGAATGGCGGGATTG	113	82.71	0.20 µM
stn-103R	TCAGGTGCGTGAGAAAGTCT			
blaCMY-202F	ATCGCCAATAACCACCCAGT	248	86.54	0.25 µM
blaCMY-202R	GCGGCTTTATCCCTAACGTC			

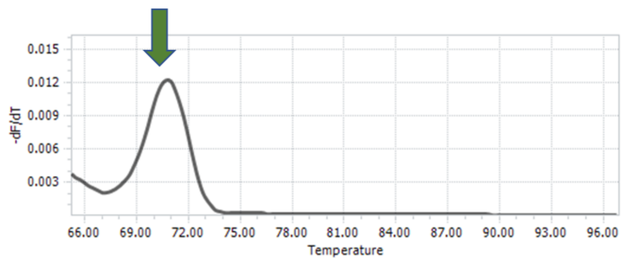
HRM Curve for First Assay



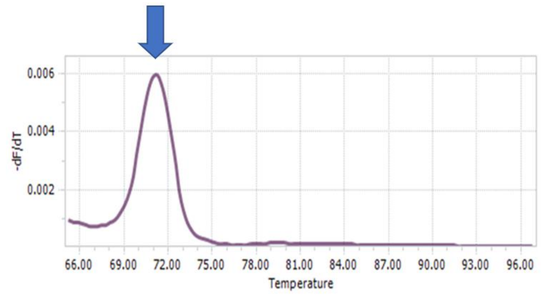
HRM Curve for Second Assay



SHV- Internal Amplification Control (40 fg/µL) No Template Control DNA Sample



stn- Internal Amplification Control (5 fg/µL) No Template Control DNA Sample



Specificity of the multiplex assay on *Salmonella* Strains	First Multiplex	Second Multiplex
# of strains of *Salmonella*	77	77
hil A	75	n/a
fim H	77	75
sip A	77	n/a
bla TEM	7	n/a
bla SHV	6	n/a
inv A	n/a	76
stn	n/a	76
bla CMY	n/a	11

Specificity of the multiplex assay (Non-*Salmonella*)	
Strains fof Non-*Salmonella*	47
Virulence Genes Detected	0
bla TEM ESBL gene	*Proteus mirabilis* 11554, *Klebsiella pneumoniae* 113260, *Klebsiella pneumoniae* 115415, *Klebsiella pneumoniae* KPC CGO2, and *Klebsiella pneumoniae* 111622
bla SHV ESBL gene	*Klebsiella pneumoniae* 113260, *Klebsiella pneumoniae* 115415, *Klebsiella pneumoniae* KPC CGO2, and *Klebsiella pneumonia* e 111622
bla CMY ESBL genes	*Proteus mirabilis* 11554

Conclusions

- **Our studies demonstrate that the use of HRM RT-PCR is both an effective and efficient way to detect *Salmonella* virulence and ESBL resistance genes in food.**

Future of Project

- **The project will be verified with a replication with spiked food samples.**
- **A sister project using solid phase reversible immobilization (SPRI) magnetic beads to measure upstream DNA concentration from food is currently in progress.**
- **Using the results of this project and the sister project, a new HRM multiplex RT-PCR will be designed to detect both foodborne pathogenic bacteria and antibiotic resistance genes.**

Acknowledgments:

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