Changes in Gene Expression Induced by RNA Polymerase Inhibitors in *Shigella flexneri*

Jin, Q., Yuan, Z., Xu, J., Wang, Y., Shen, Y., Lu, W., … Yu, J. (2002). Genome sequence of *Shigella flexneri* 2a: insights into pathogenicity through comparison with genomes of Escherichia coli K12 and O157. Nucleic Acids Research, 30(20), 4432–4441.

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Outline

I. Shigella infection can be controlled by antibiotics but drug resistance is a growing issue.
II. 72 total samples were created with 0.5 and 1 x MIC concentrations of each drug at different time intervals and assayed.
III. Figures and tables show similar trends in expression changes between RX and RP that are dependent on concentration and time.
IV. This study upheld findings of previous studies while expanding on prior research.
V. Implications of this study include testing how pathogenesis is enhanced during antibiotic treatment with RNA synthesis inhibitors.

Shigella flexneri can be controlled by antibiotics but drug resistance is developing quickly.

- 4 known species of *Shigella*
  - gram negative bacillus
  - intracellular pathogen
  - genes required to invade and spread inside cell are encoded by a virulence plasmid (VP)
- Infection controlled by rifamycin antibiotic
  - Pros to rifamycin: low absorption, low toxicity, low interaction
  - 2 compounds used: rifampin (RP) and rifaximin (RX)
  - Bind to RNA and inhibit synthesis

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Samples were created through treatment with 0.5 and 1 x MIC of each drug at different time intervals.

- S. flexneri 2a strain (Sf301) grown at 37°C in Mueller-Hinton broth
- RP and RX MIC were determined
- Sf301 inoculated to optimum density
  - RP and RX dissolved in methanol to concentrations of 0xMIC, 0.25MIC, 0.5MIC, 1xMIC, 2xMIC, and 4xMIC
- Drugs were added to create 0.5 x MIC and 1 x MIC with 0.25% methanol
- Control cultures were created for each sample with only methanol (same concentration)
- After 10, 30, and 60 minute increments samples were collected and washed
  - Each experiment was independently performed 3 times (72 samples)

cDNA was spotted and hybridized and resulting microarray data was analyzed.

- RNA reverse transcribed to prepare a copy of the cDNA
  - Control was labeled with Cy3-dCTP
  - Drug treated samples Cy5-dCTP
- Purified amplified products of ORF-specific primer pairs readjusted to 100 ng/µl and spotted onto the slides
- Followed the protocol for hybridization of cDNA to DNA microarrays
  - can be found at http://www.ifr.ac.uk/safety/microarrays/protocols.html#Hybridisations

QRT-PCR was used to verify the microarray data.

- qRT-PCR is a powerful tool for the quantitative detection of RNA levels
  - It is frequently used in the expression analysis of single or multiple genes, and expression patterns for identifying infections and diseases.
- The mean Cy5/Cy3 ratios of the gene were calculated to provide further analysis into changes in gene expression
  - Significant changes in expression is anything greater than a twofold change
- Quantitative real-time Polymerase Chain Reaction was performed to verify microarray data
  - used ABI 7000 instrument using Power SYBR Green Universal Master Mix
- Gene-specific primers were designated using the Primer Premier 5.0 software

Gene specific primers for the quantitative real-time PCR

Gene	Sense Primer Sequence	Antisense Primer Sequence
rpoB	5′ACCTGGTAACTTGCCGTAGCA3′	5′CGGTTGGCGTCATCGTG3′
mutM	5′CCATTCTTCATGCGGTGGTG 3′	5′TGGTTGATCGCTTAATCGGTAG 3′
shiA	5′TCCGACGCCTTGCTCAAC 3′	5′ACCTGCCGCCAGTCCTTT 3′
tdk	5′GCATGGTGCTGCGTCTTGA 3′	5′CGTGGCGATGCCTTTCCT 3′
virF	5′AAAGGTGTTCAATGACGGTTAGC3′	5′TGTCAAGGCTTATAATCTCAAATGG3′
mxiA	5′TGATAGCGATAATATGGGACGTAAC3′	5′GCCAAGGCAAGAGCTGATGT3′
CP0002	5′AATTGTCCACCGTCTGTCAGTAC3′	5′ATACCGTGAACCCTCTGAAAATC3′
ushA	5′TTCCAGAATAAAGGCAAAGCAC3′	5′CAGACGTCCGTTCACAACCC3′
rhoL	5′ATGCGAAGTGAACAGATTTCTGG 3′	5′CAGAACTGAAACGACAAGACGGA3′
rplY	5′CGTAAAGAGCAGGGTAAGGGTG3′	5′CGATGGTTAGAACTTCGCTGTAGA3′
cspA	5′ATCACTCCTGACGATGGCTCTA3′	5′GCCGCTTTCGATGGTGAA3′
rimM	5′GGGTTCGTCTTACGGTATTCG3′	5′TGTCCTGATTGTGGTGCTTCC3′
ompC	5′ACGGCTTCGCAACCTACC3′	5′TCATAAGTAATAGAACCGCCAACG3′
groEL	5′TGGACCCAACCAAAGTAACCC3′	5′CATCAGGCCAGCCACAGAA3′

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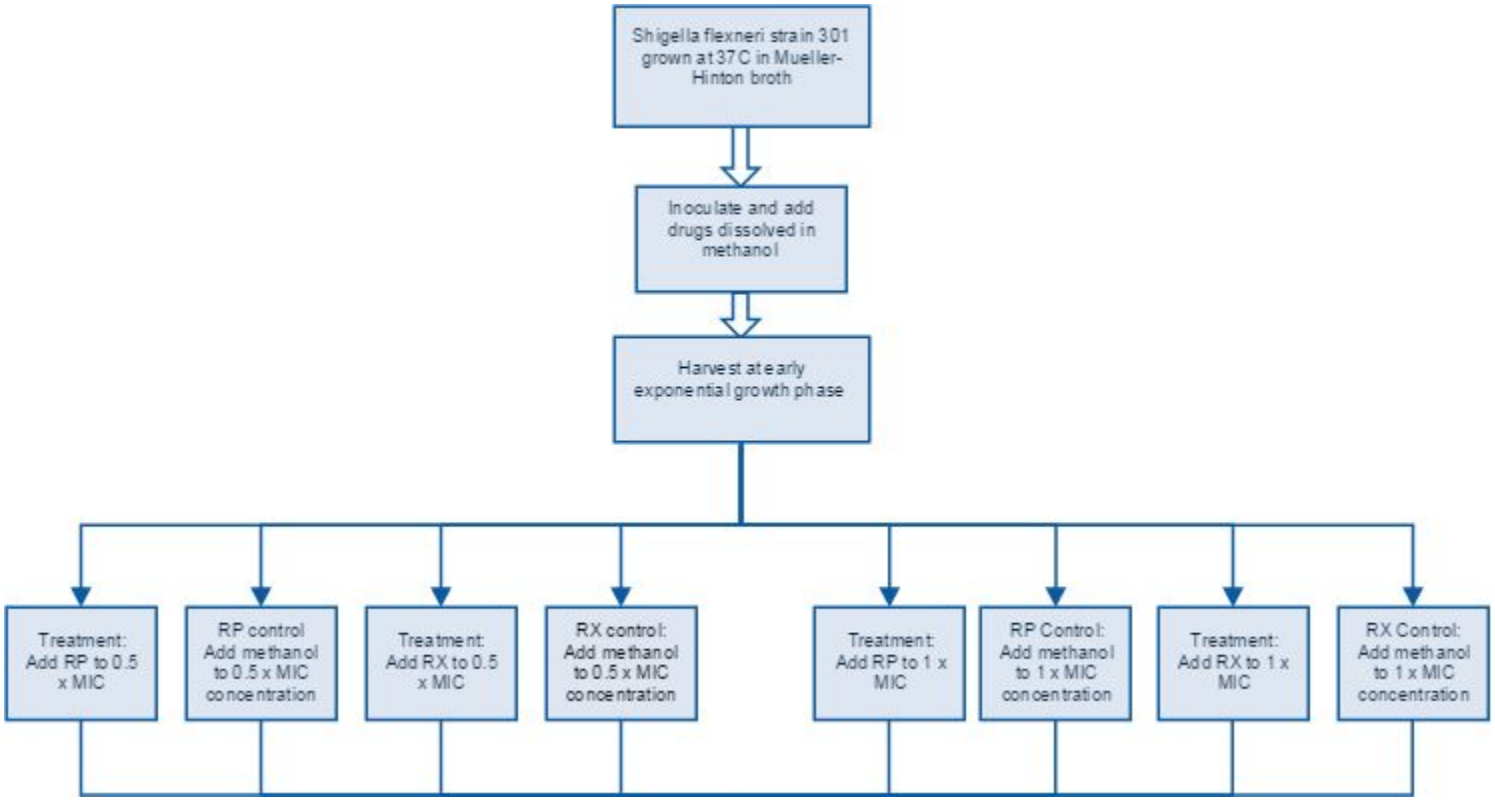
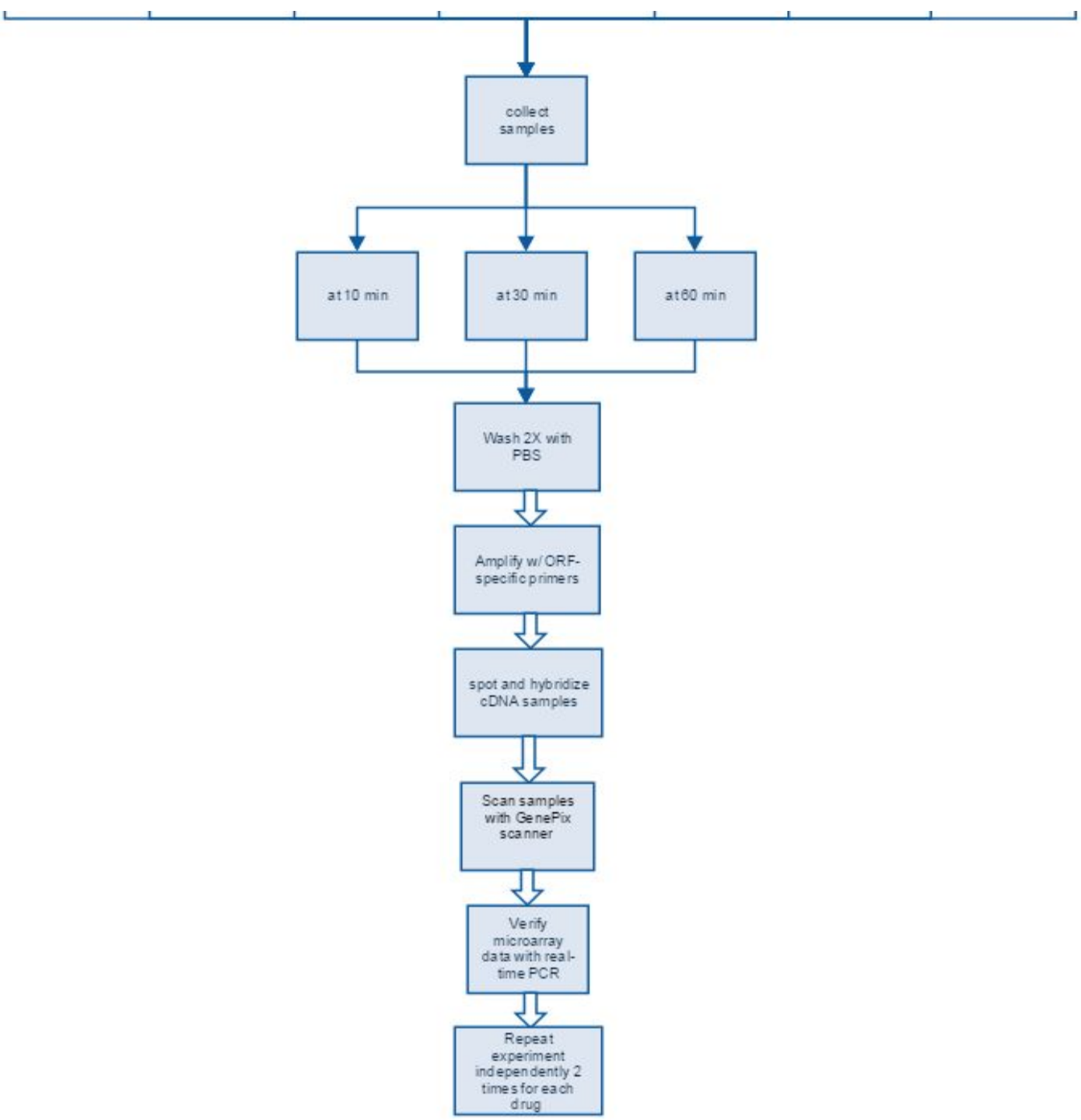
Outline of Experimental Methods




collect samples
at 10 min
at 30 min
at 60 min
Wash 2X with PBS
Amplify w/ ORF-specific primers
spot and hybridize cDNA samples
Scan samples with GenePix scanner
Verify microarray data with real-time PCR
Repeat experiment independently 2 times for each drug

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Antimicrobial activity of RP and RX are concentration and time-dependent.

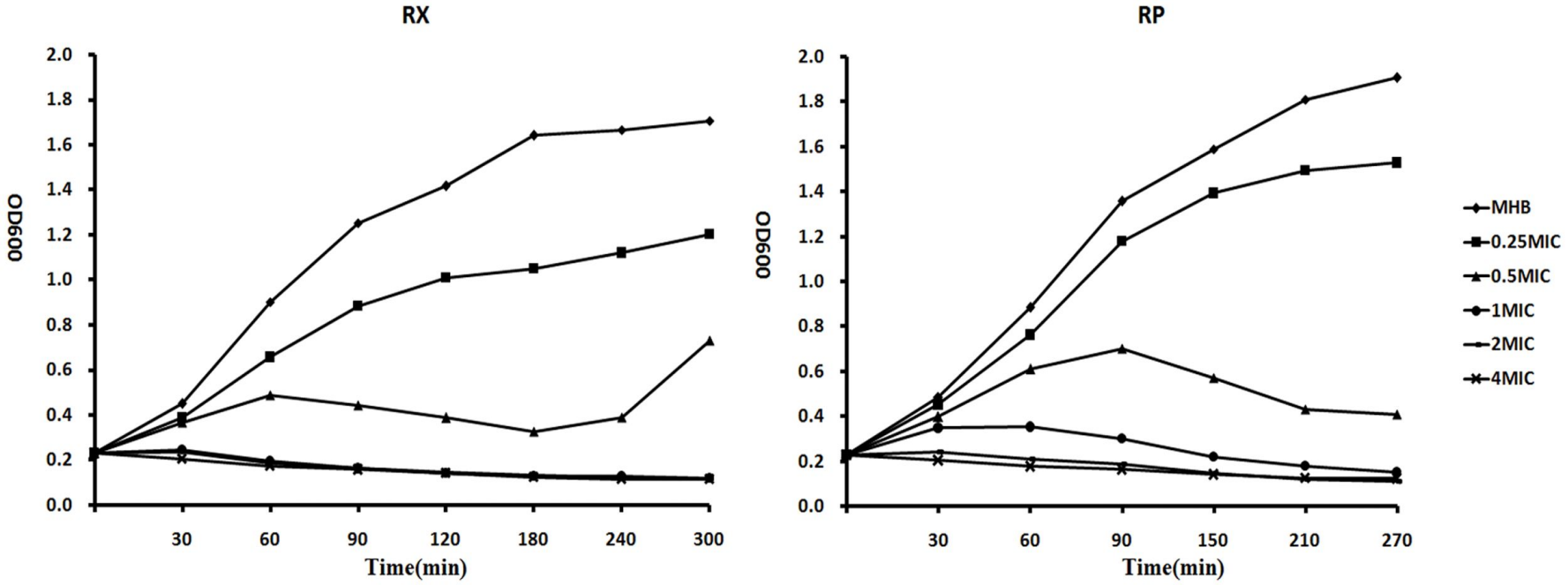


Figure 1

- RX more effective at inhibiting growth 90 min+
- Growth was severely inhibited above 1 x MIC

RX and RP displayed similar trends in up-regulation and down-regulation.

- Experimental criteria quantified by a twofold change
  - RX: 535 genes substantially altered
  - RP: only 367 genes substantially altered
- More genes displayed reduced expression

<u>Commonly Induced</u>

- *osp*: Type III secretion system (TTS) genes
- *mxi*: TTS assembly proteins
- other plasmid genes
- genes involved in heat shock early on

<u>Commonly Repressed</u>

- cell growth genes
- metabolism genes
- *rho* genes

RX and RP displayed similar trends in up-regulation and down-regulation (cont'd).

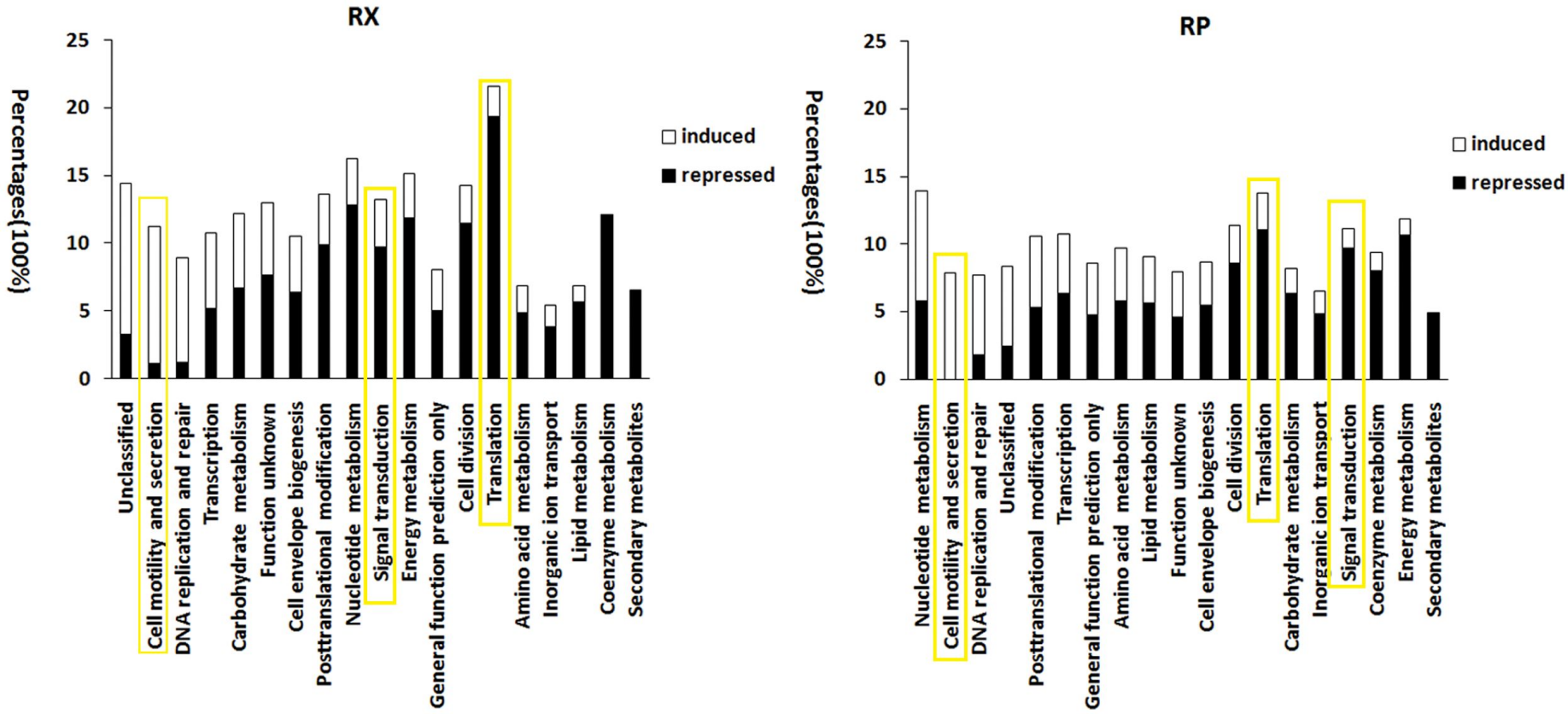
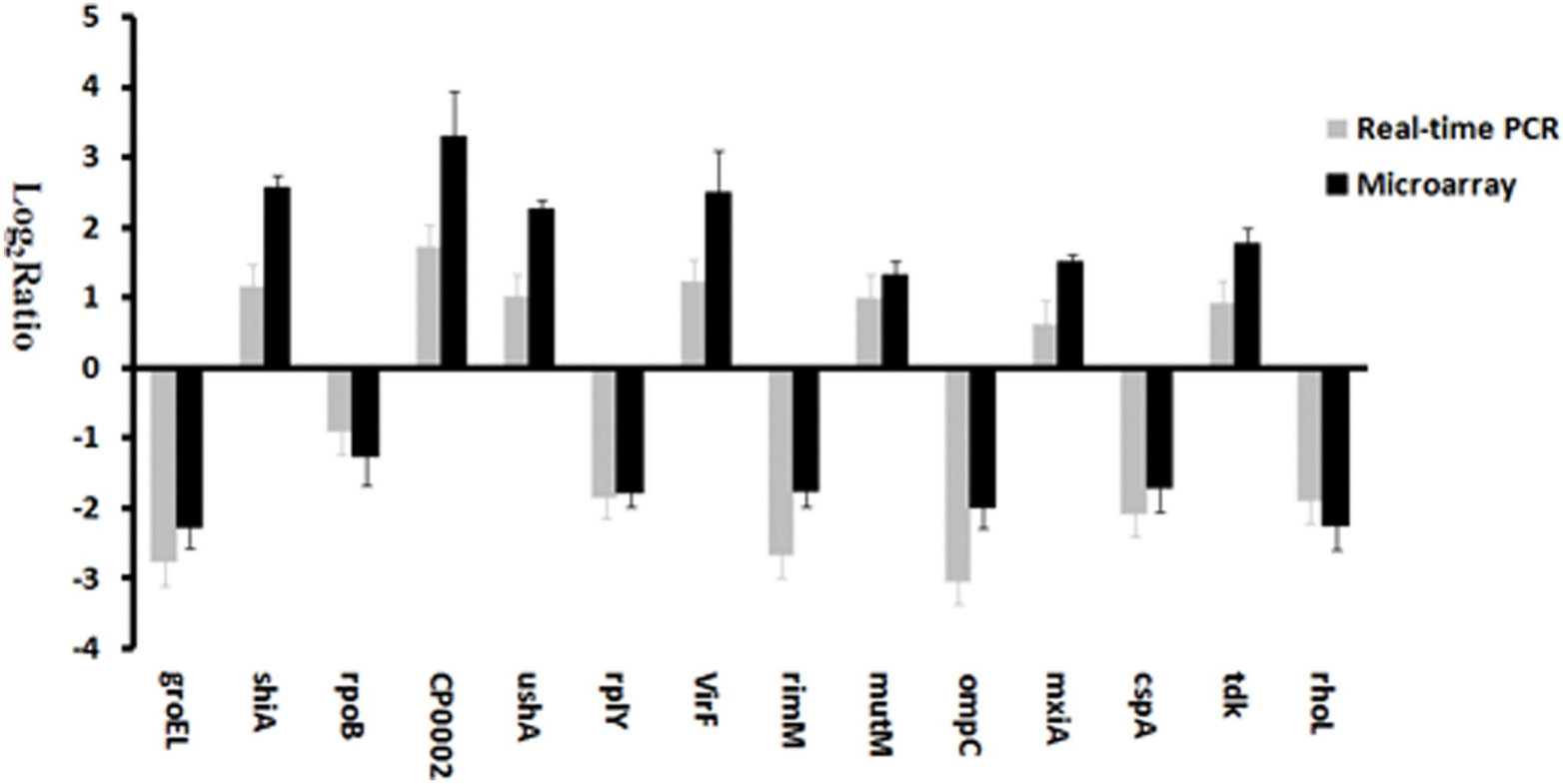


Figure 2

QRT-PCR validated the microarray data based upon a select 14 genes.



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This study expanded on research of previous studies and found similar results.

- *Rho* gene continuously down-regulated by both drugs
- Virulence-plasmid encoded genes induced by both drugs
  - Larger percentage than previous studies found (possibly related to virulence)
  - Encode a gene cluster (island)
- Ribosomal protein genes rapidly repressed
- RX and RP induced heat shock, down-regulated cold-shock
  - Previous studies have shown inhibitors regulate thermal stress

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RX and RP induce RNA synthesis and translation to cease while virulence genes may be enhanced.

- RX and RP cause RNA synthesis and translation
- Many induced genes included virulence genes from the plasmid
  - Pathogenic genes not involved in invasion but in intracellular movement of intercellular dissemination
- Implications
  - Predict if something can act on RNA polymerase
  - Test if genes are shared by other inhibitors
  - Shigella pathogenesis

Acknowledgments

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