Anti-biofilm activity of caffeine against Uropathogenic *E. coli* is mediated by curli biogenesis.

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Short title – Curli-mediated *E.coli* biofilm inhibition by caffeine.
**Supplementary Table S1: List of primers used for qRT-PCR**

| Gene  | Primer     | Sequence                           | Organism | Amplicon size |
|-------|------------|------------------------------------|----------|---------------|
| csg A | csg A-fwd  | CGGTAATGGTGCGAGATGTGG             | K12      | 192           |
|       | csg A-rev  | TGAGTCACGTTGACGGAGA               |          |               |
| csg A | csg A-fwd  | GGCCAAGTTCTGATGACAGC              | CFT073   | 188           |
|       | csg A-rev  | TTCAAAAGCACAACCTGAGTA             |          |               |
| csg B | csg B-fwd  | TGCCAAACGATGCCAGTATTTTC           | K12,     | 158           |
|       | csg B-rev  | GTTGTGTACGCAGAATAGCC              | CFT073   |               |
| csg C | csg C-fwd  | TATTACTCCTTGCGGCACCTTTC           | K12,     | 156           |
|       | csg C-rev  | TCTGACTTTGCCCCTGACTGC             | CFT073   |               |
| csg D | csg D-fwd  | CGTACCCGACATTGAAAACGT             | K12,     | 183           |
|       | csg D-rev  | GGGCTGATTCCTGCTGTAGTA             | CFT073   |               |
| csg E | csg E-fwd  | TGGCCATGATTTTTACCACAGCC           | K12,     | 223           |
|       | csg E-rev  | CTGCCGACGATTTAGTGCTTC             | CFT073   |               |
| csg F | csg F-fwd  | GCGTGTCAAACATGACGTCAGTAGT         | K12,     | 140           |
|       | csg F-rev  | GGGGCAGCTATTTAAT                  | CFT073   |               |
| csg G | csg G-fwd  | CGAATCCCGCTGCAATCTTT             | K12,     | 187           |
|       | csg G-rev  | GGATCTCGCCGGTACTCACT             | CFT073   |               |
| 16s   | 16s rRNA-fwd | GCTCGTGTTTGTGAAATGTGGG           | K12,     | 222           |
| rRNA  | 16s rRNA-rev | CTTATGAGGTCGCTTGTGCT         | CFT073   |               |
Supplementary Figure S1. Effect of caffeine on CFT073 and K12 MG1655 cells and their biofilms: a) MIC determination of caffeine against CFT073. Similar number of CFT073 cells (1*10^7) were allowed to grow in the presence of various concentrations of caffeine for 24 hrs and optical density was measured at 600nm. b) MIC determination of caffeine against K12 MG1655. c) MBIC determination of caffeine against CFT073. Biofilms were allowed to form in the presence of various concentrations of caffeine for 48 hrs and optical density was measured at 600nm. d) MBIC determination of caffeine against K12 MG1655. Data represent an average of three independent experiments presented as Mean ± SD where * p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001 indicates a statistically significant difference relative to the untreated control (one-way ANOVA).
Supplementary Figure S2. Photographic images. Determination of MIC of caffeine against a) CFT073 inoculated without and with 12mM caffeine; b) K12 MG1655 inoculated without and with 16mM caffeine.

Supplementary Figure S3. Photographic images. Determination of MBIC of caffeine against CFT073 and K12 MG1655 a) CFT073 biofilm with different concentrations of caffeine and b) K12 MG1655 biofilm with different concentrations of caffeine.