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Investigating the effects of genetic background on the fitness of quinolone resistant mutations in *Escherichia coli*

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ABSTRACT

Antimicrobial resistance (AMR) occurs when microbes become resistant to the antibiotics that are designed to cure them. Genetic interactions or epistasis of AMR mutations play an important role in determining the predictability and the persistence of AMR. Synthetic lethality is an example of genetic interactions, whereby when separated two mutations are viable but when combined are lethal. This validates the putative synthetic lethal/sick interactions of quinolone resistance mutations in 17 conditional knockout *E. coli* strains. The quinolone resistance mutations examined were gyrase A (gyrA) mutations, which include S83L, D87N or S83L-D87N combined. The results showed that 16/17 knockout strains showed reduced phenotypic fitness. Genes whose knockout alleles are synthetically lethal with one or more AMR mutation are potential candidates as drug targets. This study has demonstrated a new therapeutic method to combat AMR mutations by utilizing the concept of synthetic lethal interactions.

INTRODUCTION

Antimicrobial resistance (AMR) has been revealed to be a major threat to public health. Invasive bacterial infections are the leading cause of child mortality and morbidity around the world [1]. With the growing occurrence of AMR, controlling such bacterial infections has become more and more challenging. If action is not taken to manage the situation it is predicated that by 2050 AMR will result in 10 million deaths per year [2]. AMR occurs when bacteria or other microbes resist the effects of an antibiotic, the microbe becomes immune and reduces or eliminates the effectiveness of drugs, chemicals, or other agents that are designed to cure or prevent infections [3]. In general, resistance to antibiotics occurs by chromosomal mutations or horizontal gene transfer of resistance elements [4]. The aim is to validate the putative synthetic lethal/sick interactions of quinolone resistance mutations in conditional knockout strain of *E. coli.*

MATERIALS AND METHODS

Media

Cultures were grown in lysogeny broth (LB; 10 g/l tryptone, 5 g/l yeast extract, 10 g/l NaCl), with various different concentrations and combinations of antibiotics such as, 100mg/ml ampicillin, 50µg/ml Kanamycin, 30µg/mL chloramphenicol and 50ng/mL ciprofloxacin or Super optimal broth + glucose (SOC; 2% tryptone, 0.5% yeast extract, 10 mM NaCl, 2.5 mM KCl, 10 mM MgCl2, 10 mM MgSO4, and 20 mM glucose) overnight at 37°C with shaking at 150 rpm unless otherwise indicated.

Strains and plasmids

The relevant strains used in this study are part of the Keio collection [5] which is a collection made up of about 4000 *E. coli* strains. Each strain has a knockout at every non-essential gene which is replaced by a kanamycin cassette making the strain resistant to kanamycin. The pertinent plasmids used were ASKA plasmids, which come from the ASKA collection [6]. Another plasmid used was pMA7 [7], which is resistant to ampicillin.

The pMA7 plasmids are made up of bet genes and sacB genes to increase methylation of the newly synthesized strands during replication and to allow for plasmid curing by sucrose counter-selection. The gryA mutations used were S83L, D87N and S83L-D87N combined, these mutations are resistant to ciprofloxacin. To insert the mutation into the Keio strain, single stranded oligonucleotides of the three mutations were made.

RESULTS

Gene functionality- there is a validation in the presence of synthetic lethal interactions associated with quinolone resistance mutation in *E. coli* by examining 17 target genes from the Keio collection.

Fitness assessment with IPTG

The lag time, growth rate and maximum density of each double mutated strain were measured through Growth Curves (figure1), but only 4 were measured.

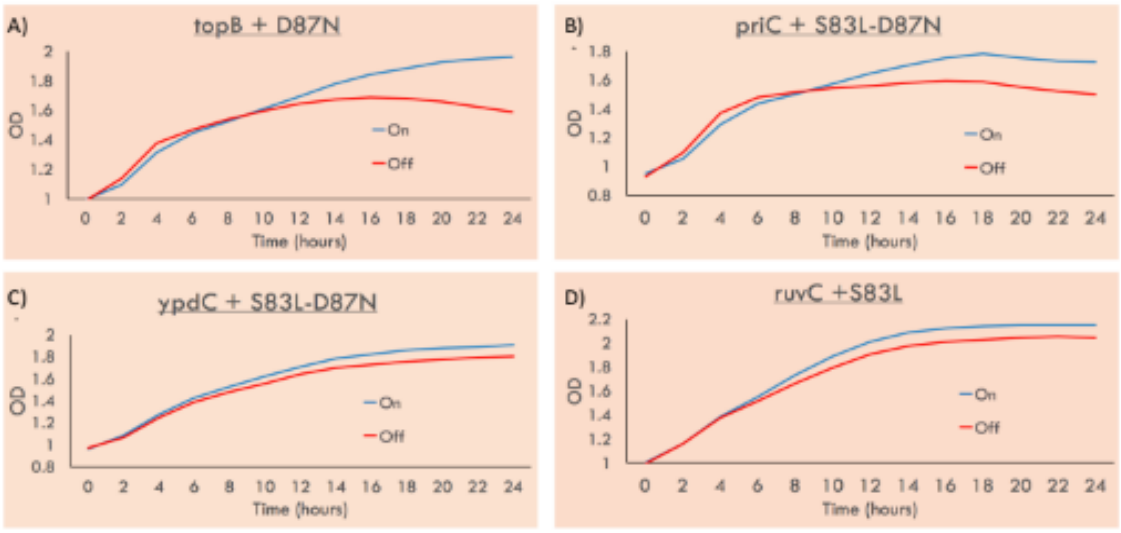

Figure 1. Blue curves represent strains with IPTG(isopropyl beta-D-1 thiogalactopyranoside) and red curves represent those without IPTG. Growth curves were calibrated by measuring the optical density (OD) of each strain every 30 minutes for 24 hours. (A) shows growth curve for topB +D87N mutants while (B) shows growth curve for priC + S83L-D87N. (C) shows curves for ypdC+ S83L-D87N and (D) shows growth of the ruvC+S83L mutant. Hall BG, Acar H, Nandipati A, Barlow M. 2014. (1):232–238.[13]

Table1 shows the relative phenotypic characteristics of the mutated strains in the presence of IPTG, which is a reagent that mimics allolactose in the induction of *E.coli* protein expression. The growth rates of all 17 strains ranged between 0.0311 min-1 for dnaQ to 0.0977 min-1 for rnt, whereas the maximum density ranged from 1.616 (xseA) to 0.762 (mdtK). Considering the presence of IPTG, the lag time of polB averaged at 77.4 minutes and parC averaged at 62.9 minutes. recT lag time had the lowest SD value of 0.4.

Table1. Variation of phenotypic traits among 17 double mutated *E. coli* strains with their respective gyrA mutation. Averaged values for the strain's growth rate, maximum density and lag time in the presence of IPTG. Variables measured in LB, 30µg/mL chloramphenicol and 100mM IPTG. Data means +/- Standard deviation (SD).

Keio strain + GyrA (+) IPTG	Growth Rate (min^{-1})	Max OD_{600}	Lag time (min)
kdpE + S83L-D87N	$0.0947 \pm 2.8x10^{-3}$	$1.104 \pm 5.5x10^{-2}$	54.5 ± 0.9
ruvC + S83L	$0.0593 \pm 4.7x10^{-2}$	$1.205 \pm 5.0x10^{-2}$	56.3 ± 2.9
mtlR + S83L-D87N	$0.0536 \pm 5.0x10^{-2}$	$1.408 \pm 1.1x10^{-2}$	34.2 ± 2.0
parC + S83L-D87N	$0.0648 \pm 4.6x10^{-2}$	$0.828 \pm 1.5x10^{-2}$	62.9 ± 4.7
dnaQ + D87N	$0.0311 \pm 1.2x10^{-2}$	$1.173 \pm 3.6x10^{-2}$	38.6 ± 1.4
recT + S83L-D87N	$0.0963 \pm 5.0x10^{-2}$	$1.077 \pm 3.5x10^{-2}$	26.9 ± 0.4
ompR + S83L-D87N	$0.0676 \pm 4.6x10^{-2}$	$0.983 \pm 6.4x10^{-2}$	78.3 ± 1.8
ypdC + S83L-D87N	$0.0384 \pm 4.7x10^{-2}$	$0.956 \pm 4.3x10^{-2}$	28.7 ± 1.5
xseA + S83L-D87N	$0.0288 \pm 3.7x10^{-2}$	$1.616 \pm 5.7x10^{-2}$	38.8 ± 6.7
Rnt + S83L-D87N	$0.0977 \pm 1.6x10^{-3}$	$0.936 \pm 7.5x10^{-2}$	34.1 ± 6.1
recD + S83L-D87N	$0.0831 \pm 3.8x10^{-2}$	$1.347 \pm 9.4x10^{-3}$	11.6 ± 6.0
mdtK + S83L-D87N	$0.0401 \pm 4.7x10^{-2}$	$0.762 \pm 2.1x10^{-2}$	45.8 ± 3.2
polB + S83L-D87N	$0.0360 \pm 4.2x10^{-2}$	$1.177 \pm 7.3x10^{-2}$	77.4 ± 7.6
yacG + D87N	$0.0442 \pm 4.5x10^{-2}$	$1.029 \pm 6.5x10^{-2}$	14.8 ± 3.8
Tus + S83L-D87N	$0.0932 \pm 4.3x10^{-2}$	$1.209 \pm 3.9x10^{-2}$	27.0 ± 2.4
priC + S83L-D87N	$0.0911 \pm 4.6x10^{-2}$	$0.895 \pm 5.5x10^{-2}$	31.9 ± 7.7
topB + D87N	$0.0144 \pm 3.1x10^{-3}$	$1.013 \pm 7.0x10^{-3}$	38.4 ± 7.9

Fitness assessment without IPTG

Table 2 shows fitness assessments for the mutated strains in the absences of IPTG. The growth rates ranged between 0.0090 min-1 to 0.0727 min-1, whereas the maximum density ranged from 1.376 (xseA) to 0.656 (priC). topB lag time had the lowest SD value of 0.3.

Table 2. Variation of phenotypic traits among 17 double mutated *E. coli* strains with their respective gyrA mutation. Averaged values for the strain's growth rate, maximum density and lag time in the absence of IPTG. Variables were measured in LB and 30µg/mL chloramphenicol. Data means +/- Standard deviation.

Keio strain + GyrA (-) IPTG	Growth Rate (min^{-1})	Max OD	Lag time (min)
kdpE + S83L-D87N	$0.0291 \pm 3.4x10^{-2}$	$1.062 \pm 5.9x10^{-2}$	46.8 ± 2.0
ruvC + S83L	$0.0382 \pm 4.9x10^{-2}$	$1.085 \pm 1.4x10^{-2}$	27.8 ± 6.5
mtlR + S83L-D87N	$0.0677 \pm 5.1x10^{-2}$	$1.261 \pm 2.1x10^{-2}$	17.3 ± 4.8
parC + S83L-D87N	$0.0673 \pm 4.6x10^{-2}$	$0.858 \pm 5.4x10^{-2}$	43.1 ± 0.9
dnaQ + D87N	$0.0301 \pm 3.7x10^{-2}$	$1.065 \pm 2.5x10^{-2}$	33.7 ± 0.9
recT + S83L-D87N	$0.0666 \pm 5.2x10^{-2}$	$0.992 \pm 5.1x10^{-2}$	27.9 ± 7.0
ompR + S83L-D87N	$0.0358 \pm 4.8x10^{-2}$	$1.045 \pm 9.9x10^{-2}$	67.5 ± 8.6
ypdC + S83L-D87N	$0.0370 \pm 4.6x10^{-2}$	$0.835 \pm 8.4x10^{-2}$	56.6 ± 3.2
xseA + S83L-D87N	$0.0345 \pm 4.2x10^{-2}$	$1.376 \pm 4.2x10^{-2}$	48.1 ± 5.9
Rnt + S83L-D87N	$0.0448 \pm 4.5x10^{-2}$	$1.243 \pm 2.9x10^{-2}$	33.8 ± 8.8
recD + S83L-D87N	$0.0679 \pm 4.9x10^{-2}$	$1.282 \pm 3.0x10^{-2}$	67.6 ± 1.7
mdtK + S83L-D87N	$0.0090 \pm 2.1x10^{-3}$	$0.864 \pm 6.7x10^{-2}$	67.9 ± 3.1
polB + S83L-D87N	$0.0092 \pm 1.6x10^{-3}$	$1.176 \pm 1.4x10^{-2}$	65.9 ± 8.4
yacG + D87N	$0.0177 \pm 2.0x10^{-3}$	$1.104 \pm 3.9x10^{-2}$	6.15 ± 1.9
Tus + S83L-D87N	$0.0727 \pm 3.3x10^{-2}$	$1.186 \pm 4.4x10^{-2}$	34.2 ± 0.7
priC + S83L-D87N	$0.0650 \pm 8.1x10^{-3}$	$0.656 \pm 2.8x10^{-3}$	41.3 ± 9.6
topB + D87N	$0.0121 \pm 8.2x10^{-4}$	$0.732 \pm 8.2x10^{-2}$	30.8 ± 0.3

DISCUSSION

Antimicrobial resistance (AMR) occurs when microbes become immune to the antibiotics that are designed to cure or kill it [5]. Resistant mutations continue to persist even in the absence of antibiotics. The most common way for bacteria to become resistant to fluoroquinolones is through mutations in DNA Gyrase and Topoisomerase IV. In *E. coli*, S83L and D87N mutations in GyrA are common in fluoroquinolone resistant clinical isolates, with the S83L/D87N double mutant conferring particularly high levels of resistance. The fitness of AMR mutants can be influenced by epistasis [8]. Epistasis or genetic interaction, refers to the influence that genetic background has on the fitness effects of a mutation. There are three varieties of epistatic interactions: positive epistasis, where the fitness of the double-mutant is higher than expected, negative epistasis, where the fitness of the double-mutant is lower than expected, and sign epistasis which occurs when one mutation has the opposite effect when in the presence of another mutation [9]

Synthetic lethality is an example of genetic interactions, whereby when separated two mutations are viable but when combined are lethal [figure2] [10]. In context of AMR, observing such lethal interactions may suggest drug targets for specific AMR genotypes [11]. Therefore, this study portrayed a therapeutic strategy to target quinolone resistant mutations in *E. coli* in efforts to further advance the controlling of AMR. By having candidate synthetic lethal interactions previously identified in the lab, the focus was on validating the putative SL interactions between gyrA mutations and conditional knockout *E. coli* strains. This was done by transferring quinolone resistant mutations onto Hfr cells. The resistant Hfr strains were isolated and mated with *E. coli* strains with a knockout at non-essential genes, thus allowing for the generation of double mutants. The fitness of the strains was assessed with or without the control of an IPTG inducible promote [12].

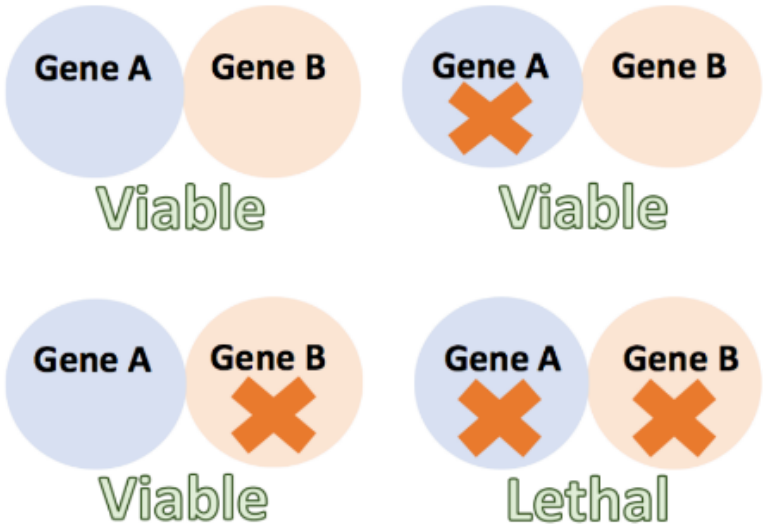

Figure 2. Collateral sensitives of AMR genotypes showing how a synthetic lethal interaction results. Each mutation alone is viable but when combined are lethal.

The fitness of the strains was assessed with or without the control of an IPTG inducible promoter. In the presence of IPTG, the knockout gene is expressed and therefore only the gyrA mutation is assumed present in the strain. Without IPTG, the knockout gene is not expressed and so both mutations are present giving rise to potential SL interactions.

When comparing the data from Table 1 and 2, we can see that topB+D87N had higher growth rates and max OD values with IPTG (0.0144 min-1 and 1.013) than without IPTG (0.0121min-1 and 0.732). Thus, showing signs of synthetic sickness. However, when comparing lag times, it is seen that with IPTG it took the strain 38.4 minutes to eventually start growing, whereas, without IPTG lag time was 8 minutes faster at 30.8 minutes. Whether it was with growth rate, maximum density or lag time, 16/17 strains examined showed synthetic lethal phenotypes in the absence of IPTG. Thus, validating that when combined knockout genes involved with DNA replication and gyrA mutations such as S83L, D87N and S83L-D87N present synthetic lethal/sick interactions.

The one strain that did not show any signs of SL interactions was parC+S83L-D87N.When comparing Tables 1and 2 it is seen that the data was relatively close but with IPTG, the strain showed lower fitness levels.

CONCLUSION

Research focus has shifted to how epistatic interactions of AMR mutations play an important role in the persistence of antimicrobial resistance. This study has demonstrated a new therapeutic method to combat AMR mutations by utilizing the concept of synthetic lethal interactions. Focusing on quinolone resistance mutations and *E. coli* knockout genes we showed that when a knockout mutation is present alongside an AMR mutation, synthetic lethal/sick interactions come to action and effectively reduce the fitness levels of the AMR mutation. The synthetic lethal interactions observed may suggest drug targets for specific AMR genotypes. Therefore, the findings in this report have paved the way for possible clinical application in the future.

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