



Inactivation of UmuC Protein Significantly Reduces Resistance to Ciprofloxacin and SOS Mutagenesis in *Escherichia coli* Mutants Harboring Intact *umuD* Gene

Razieh Pourahmad Jaktaji ¹,* and Sayedeh Marzieh Nourbakhsh Rezaei ¹

¹Department of Genetics, Faculty of Science, Shahrekord University, Shahrekord, Iran

*Corresponding author: Department of Genetics, Faculty of Science, Shahrekord University, Shahrekord, Iran. Tel/Fax: +98-3832324419, Email: raziehtjaktaji@gmail.com

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Abstract

Background: Ciprofloxacin induces SOS response and mutagenesis by activation of UmuD₂C (DNA polymerase V) and DinB (DNA polymerase IV) in *Escherichia coli*, leading to antibiotic resistance during therapy. Inactivation of DNA polymerase V can result in the inhibition of mutagenesis in *E. coli*.

Objectives: The aim of this research was to investigate the effect of UmuC inactivation on resistance to ciprofloxacin and SOS mutagenesis in *E. coli* mutants.

Methods: Ciprofloxacin-resistant mutants were produced in a *umuC* genetic background in the presence of increasing concentrations of ciprofloxacin. The minimum inhibitory concentration of *umuC* mutants was measured by broth dilution method. Alterations in the rifampin resistance-determining region of *rpoB* gene were assessed by PCR amplification and DNA sequencing. The expression of SOS genes was measured by quantitative real-time PCR assay.

Results: Results showed that despite the induction of SOS response (overexpression of *recA*, *dinB*, and *umuD* genes) following exposure to ciprofloxacin in *E. coli umuC* mutants, resistance to ciprofloxacin and SOS mutagenesis significantly decreased. However, rifampicin-resistant clones emerged in this genetic background. One of these clones showed mutations in the rifampicin resistance-determining region of *rpoB* (cluster II). The low mutation frequency of *E. coli* might be associated with the presence and overexpression of *umuD* gene, which could somehow limit the activity of DinB, the location and type of mutations in the β subunit of RNA polymerase.

Conclusions: In conclusion, for increasing the efficiency of ciprofloxacin against Gram-negative bacteria, use of an inhibitor of *umuC*, along with ciprofloxacin, would be helpful.

Keywords: DinB, Mutagenesis, RpoB, UmuDC, *Escherichia coli*

1. Background

Fluoroquinolones are broad-spectrum antibiotics against Gram-positive and Gram-negative bacteria. In these bacteria, DNA topoisomerase IV and DNA gyrase are the main targets of fluoroquinolones, such as ciprofloxacin (1, 2). Ciprofloxacin inhibits the progression of DNA replication and transcription, producing DNA breaks and R-loops (RNA-DNA hybrids) in Gram-negative bacteria, such as *Escherichia coli* (3, 4). These changes induce the SOS response by promoting the RecA-dependent self-cleavage of LexA, as the repressor of SOS genes, including *recA*, *dinB*, and *umuCD*; the last two genes encode SOS DNA polymerases, namely DNA polymerase IV (pol IV) and DNA polymerase V (pol V). Pol V, as a complex

enzyme, consists of UmuC and UmuD₂ dimers. These proteins are encoded by *umuCD* operon and activated after self-cleavage and dimerization of UmuD (4, 5).

SOS DNA polymerases participate in error-prone mutagenic DNA repair processes, such as translation synthesis (TLS), to restart replication forks that are stalled at damage sites or to repair gaps that have left the replication forks behind. Pol IV acts before pol V in the replisome region, and by increasing the concentrations of UmuD₂ and UmuD₂C, the access of pol IV to the replisome regions is prevented (6). Moreover, pol IV participates in error-prone double-strand break repair and transcription-coupled TLS. Therefore, DinB interacts with RecA and NusA during strand exchange activities and gap repair, respectively (7). The gap produced in the transcribed strand, opposite to a lesion

in the non-transcribed strand, prevents the progression of the transcription complex. A component of this complex, which is a modulator of RNA polymerase activity (NusA), recruits DinB to fill the gap (8).

Activation of DNA pol IV and pol V confers resistance to ciprofloxacin through accumulation of several mutations in the target genes, encoding the subunits of DNA gyrase and topoisomerase IV, as well as regulatory genes (*marR* and *acrR*), inhibiting the overexpression of the AcrAB-TolC efflux pump (4, 9). The overexpression of this pump causes multi-drug resistance to structurally irrelevant antibiotics, such as tetracycline and rifampin. Rifampin (also called rifampicin), a derivative of rifamycin, is one of the most potent and broad-spectrum antibiotics, which binds to RNA polymerases (RNAP) and inhibits the transcription process (10, 11). The acquired mutation in genes, encoding the subunits of RNAP, especially *rpoB* gene that encodes the β subunit of enzyme, confers resistance to rifampin. These mutations in the rifampin resistance-determining region of *rpoB*, which mainly consist of cluster I and cluster II amino acid residues (located deep within the DNA-RNA channel of RNAP), reduce the affinity of enzymes for rifampin and affect the elongation and termination steps of transcription.

The rifampin naphthyl moiety contacts β residues, including Val146, Leu511, Ser513, Arg529, Ser531, Leu533, Gly534, Asn568, and Ile572. On the other hand, the antibiotic ansa moiety contacts other β residues, including Arg143, Glu510, Leu511, Leu512, Phe514, Asp516, Thr525, His526, Pro564, and Pro564 (11). Mutations in these sites are associated with certain phenotypes, such as slow growth, cold sensitivity, and stringent response mimicry phenotypes. Nonetheless, the last phenotype may not confer rifampicin resistance. Therefore, depending on the site of mutation, the minimum inhibitory concentration (MIC) for rifampin varies.

Substitutions at three sites, that is, Asp516, His526, and Ser531, cause high levels of resistance to rifampin and are associated with little or no loss of fitness (11). These mutations have been frequently detected in the clinical isolates of Rif-resistant (Rif^r) bacteria. For example, the Ser531-to-Leu substitution has been frequently detected in the clinical isolates of *Mycobacterium tuberculosis*. Moreover, other mutations in *rpoA*, *rpoB*, and *rpoC* genes, encoding α (RpoA), β (RpoB), and β' (RpoC) subunits of RNAP, respectively, have been also found in clinical isolates. Most of the secondary mutations in RpoC have been identified in the region interacting with RpoA (12). Important regions in the β subunit of RNAP remain conserved in bacteria (13).

In Gram-negative bacteria, *umuC* and *umuD* are mostly

located in the same operon and are overexpressed differently late in SOS response (level of UmuD is higher than UmuC). However, levels of UmuD2 and UmuC are kept to a minimum through their proteolysis by the Lon protease in undamaged cells. In damaged cells, UmuD' in UmuD/D' heterodimer is rapidly degraded by the ClpXP protease (14, 15). On the other hand, *umuD* is not found in Gram-positive bacteria, such as *Streptococcus* and *Staphylococcus* species. Therefore, unlike *E. coli*, DNA pol V is not the main mutagenic polymerase in these bacteria following exposure to ciprofloxacin. Ciprofloxacin induces mutagenesis in *Streptococcus* species through *umuC*-dependent or *umuC*-independent mechanisms (5). The independent mechanism of *S. pneumonia* and *S. uberis* depends on acquiring mutations in the rifampicin resistance-determining region of *rpoB* gene in wild type strain and *umuC* (Δ *umuC*) mutant (16). It is not clear whether ciprofloxacin treatment of *E. coli* mutants lacking active *umuC* can cause rifampin-resistant mutations.

2. Objectives

We aimed to investigate whether the formation of rifampin-resistant mutations in *E. coli umuC* mutants after exposure to ciprofloxacin would compensate for the mutagenesis defect.

3. Methods

3.1. Bacterial Strains and Growth

The bacterial strains used are listed in Table 1. Among them *Escherichia coli* BW25113 and a knockout mutant JW11731 (BW25113 Δ *umuC*:Kan^r) were obtained from the Keio collection (*E. coli* Genetic Stock Collection (CGSC), USA) (17). Clones with higher levels of resistance to ciprofloxacin were generated from JW11731 in presence of increasing amounts of ciprofloxacin (Sigma, USA) up to 2 μ g/mL ciprofloxacin (18).

3.2. Minimum Inhibitory Concentration

Minimum inhibitory concentration of ciprofloxacin and rifampin (Merck, Germany) for *E. coli* strains and mutants were determined by the broth dilution method in accordance to Clinical and Laboratory Standards Institute (CLSI) guidelines (19). Briefly, Luria-Bertani (LB) inoculated with 5×10^7 - 5×10^8 CFU/mL of bacteria was used. Depending on bacterial genetic background, serial dilutions of ciprofloxacin (from 5 ng/mL to 2 μ g/mL) and rifampin (from 10 ng/ml to 60 μ g/mL), were added to cultures and

Table 1. Bacterial Strains and Mutants

Strain/Mutant	Genotype	MIC, $\mu\text{g/mL}$		Source/Reference
		Cip	Rif	
MG1655	Wild type	0.035	0.4	A gift from Prof. RG Lloyd
BW25113	Parent strain	0.04	0.4	Keio collection
JW11731	BW25113 $\Delta umuC$:Kanr	0.04	0.4	Keio collection
M2	<i>gyrA</i> (Ser83 \rightarrow Leu) <i>marOR</i> (20 bp duplication) <i>acrAB</i> overexpression	100	100	Pourahmad Jaktaji and Psand 2016
MN1	JW11731 $\Delta umuC$:Kanr	0.3	1	This work
MN2	JW11731 $\Delta umuC$:Kanr	2	16	This work
MN3	JW11731 $\Delta umuC$:Kanr	2	16	This work
MN4	JW11731 $\Delta umuC$:Kanr	2	30	This work
MN5	JW11731 $\Delta umuC$:Kanr	2	50	This work

Abbreviations: Cip, ciprofloxacin; MIC, minimum inhibitory concentration; Rif, rifampin.

the cultures were incubated for 16 - 24 h at 37°C. The experiments were conducted three times.

3.3. PCR Amplification and DNA Sequencing

The rifampin resistance-determining region of *rpoB* gene (containing cluster I and II of the β subunit of RNA polymerase) was amplified by colony PCR method. The following primers were used for *rpoB*: forward primer, 5'-TGATCAACGCCAAGCC-3' and reverse primer, 5'-TACAACACCGTCGGTC-3'. The amounts of PCR ingredients were: 2 μL of DNA, 5 μL of PCR buffer, 0.15 μL of Taq polymerase (genfanavar, Iran), 0.5 μL of each primer, 0.5 μL of MgCl_2 and 0.5 of dNTP. The final volume of reaction mixture was reached to 25 μL with sterile distilled water. The thermal condition was: pretreatment 95°C for 3 min, amplification steps, 94°C for 45 s, 52°C for 30 s and 72°C for 1 min, for 30 cycles and final extension 72°C for 4 min. The sizes of amplified segments should be 323 base pairs. Then PCR products were visualized by gel electrophoresis, then both strands of PCR products were sequenced by DNA sequencing with the forward and reverse primers and the nucleotide sequence of them was compared to that of MG1655 strain, which is retrieved from NCBI. The sequence of cluster I and II of *rpoB* gene was presented previously (10, 11).

3.4. Ciprofloxacin-induced Mutagenesis

Ciprofloxacin-induced rifampin resistance assays was conducted as described previously (20). Three independent exponentially growing cultures of MG1655, *umuC* clones (carrying the *umuC* mutation) and M2 in LB without and with ciprofloxacin ($0.1 \times \text{MIC}$) incubated for 4 hours

at 37°C with shaking (250 rpm). The bacterial cells were collected by centrifugation (for 10 min at 6000 rpm) in centrifuge (Sigma, Germany). The pellet was resuspended in fresh LB broth and incubated overnight at the same conditions described above. Viable cells were determined after preparing appropriate dilutions and plating on LB agar with and without rifampin (20 $\mu\text{g/mL}$); and incubated overnight at 37°C. The numbers of colonies were counted and the frequency of mutations, the ratio of resistant cells to viable cells, was calculated.

3.5. RNA Sample Preparation

Fresh cultures in LB broth containing sub-inhibitory concentration of ciprofloxacin were prepared. Cultures were grown to a mid-logarithmic phase (OD600 of 0.5 - 0.6). Appropriate volumes from each of cultures were mixed with 2 volumes of RNA protect reagent (Qiagen, Germany) and were centrifuged. Cell pellets were used for RNA extraction using an RNeasy Mini Kit (Qiagen, Germany). The quality and concentration of RNA was determined using the Ultrospec 1100 spectrophotometer (Japan).

3.6. Real Time PCR (qRT-PCR)

RNase-free DNase (Fermentas, USA) enzyme was used to eliminate DNA from RNA samples based on the manufacturer's guideline. Then RNA samples were repurified using an RNeasy Mini kit (Qiagen, Germany). To detect possible DNA contamination RNA samples were amplified by PCR and visualized by gel electrophoresis. The cleaned RNA samples were used for cDNA synthesis. Reverse transcription was performed using the RevertAid Reverse Transcriptase Kit (Thermo scientific, USA), random hexamer and pu-

rified total RNA (500 ng). To test the quality of cDNAs, they were amplified by PCR reaction with specific primers.

Primers for *recA*, *umuD*, and *gapA* are described previously (18). Primers for *dinB* were as follows: Forward primer, 5'-CTCGCGCTACACCTCG-3' and reverse primer, 5'-ACGCCGTCA GTTGACG-3'. To determine gene expression by real time PCR, Rotor Gene 6000 thermocycler (Corbett Research, Australia) and SYBR Green kit (Takara, Japan) were used. Thermal cycling conditions were described previously unless for annealing temperature, which differs from one gene to another (18). Melting curve analysis at 60°C - 95°C with continuous fluorescence reading was conducted. Relative gene expression was calculated based on Pfaffl method (21). All data on gene expressions are the average of duplicate analyses.

3.7. Statistical Analysis

Statistically significant differences in gene expression were determined by Student's *t*-test (two paired samples, with two tailed distribution), using SPSS version 16 software.

4. Results

4.1. Generation of Ciprofloxacin Resistant Clone

Clones, MN1-MN5 with higher resistance to ciprofloxacin were generated from JW11731 in the presence of increasing amounts of ciprofloxacin (Table 1). Exposure to more than 2 µg/mL ciprofloxacin, did not result in generating higher resistant clones.

4.2. Susceptibility of Strains and Mutants to Ciprofloxacin and Rifampin

Minimum inhibitory concentration of ciprofloxacin and rifampin for *E. coli* strains and mutants were presented in Table 1. MN5 showed about 50-fold higher MIC than the parental strain (JW11731) to ciprofloxacin and rifampin. Based on this result, MN5 showed intermediate level of resistance to ciprofloxacin and high level of resistance to rifampin based on previously reported data (22). However, M2 exhibited approximately 100-fold increases in MIC for rifampin as compared with wild type strain MG1655, due to overactivation of AcrAB-TolC efflux pump. As expected, MG1655 and M2 indicated low and high level of resistance to rifampin. Moreover, MN1 and MN2-MN4 showed low and high levels of resistance to rifampin, respectively. Therefore, it was possible to produce clones with high levels of resistance to rifampin from rifampin-sensitive *umuC* mutant strain.

4.3. PCR Amplification and DNA Sequencing

To determine the location of possible mutation in rifampin resistant clones (MN1-MN5), the rifampin resistance-determining region of *rpoB* gene (containing cluster I and II) was amplified by PCR method and the result of gel electrophoresis can be seen in Figure 1. Then, PCR products were sequenced by DNA sequencing. Although MN2-MN4 were resistant to rifampin, the results of DNA sequencing and comparison with sequence of *rpoB* gene of MG1655 (ID: 948488) did not showed any change in clusters I and II of RpoB subunit of RNA polymerase (data not shown). However, MN5 had alternation in this region (Figure 2). Ile572 (ATC) was changed to Leu (CTC). Ile572 is a conserved amino acid in rifampin resistance-determining region of *rpoB* gene among Gram positive and negative bacteria (11). Ile and Leu are hydrophobic amino acids and have similar characteristics. Other clusters, including N and III might have mutation in these clones.

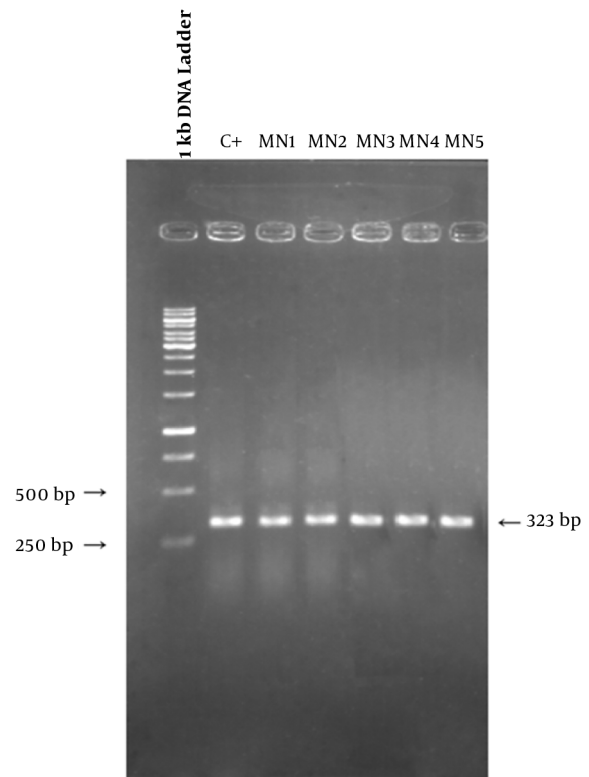


Figure 1. Gel electrophoresis of *rpoB* gene PCR products. Lanes from left to right show 1 kb DNA ladder, positive control, and MN1-MN5 PCR products.

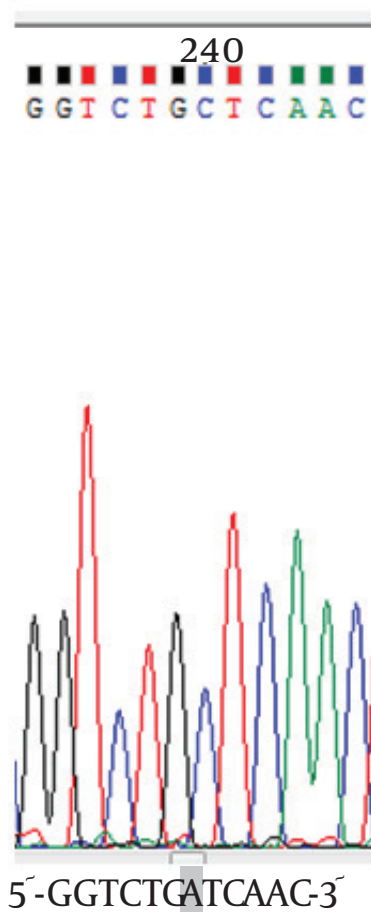


Figure 2. Partial sequence of *rpoB* gene containing the site of mutation in MN5 clone. Corresponding partial sequence in MG1655 was shown below. The site of mutation was highlighted (A → C).

4.4. Ciprofloxacin-induced Mutagenesis in *umuC* Null Mutant

To see whether ciprofloxacin induces mutagenesis in *E. coli umuC* mutant, strains MG1655 (wild type), JW11731 (*umuC*) and M2 (ciprofloxacin resistant clone with overactivated AcrAB-TolC efflux pump) were used. Addition of ciprofloxacin ($0.1 \times \text{MIC}$) resulted in less than 10 and approximately 100-fold increases in frequency of rifampin resistant mutants in MG1655 and M2, respectively (Figure 3). Interestingly, MN5 produced rifampin resistant clones, but relatively less than MG1655. Thus, ciprofloxacin induces mutagenesis even in the absence of active UmuC.

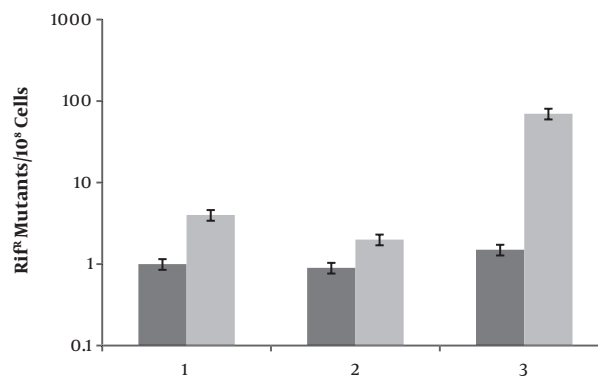


Figure 3. Frequencies of rifampin resistant (Rif^R) mutants emerging from different *E. coli* strains. The ratio of Rif^R cells were determined in cultures that either untreated (black bar) or treated with ciprofloxacin (grey bar). Lanes 1, 2 and 3 are wild type, MG1655, *umuC* mutant and M2. Values are the means of three independent experiments \pm SD.

4.5. Expression of SOS Genes

Figures 4-6 show amplification and melting curves of *recA*, *umuD*, and *dinB* genes, respectively. Table 2 shows the relative expression of these genes in different mutants. *recA* gene was not overexpressed in MN1 with low resistance to ciprofloxacin and rifampin (Table 2). However, this gene was overexpressed in MN2 and MN5 ($P < 0.05$) with high level of resistance to rifampin (Table 2). Moreover, the expression of *umuD* and *dinB* was increased in MN2 and MN5 ($P < 0.05$), but not in MN1 (Table 2). *umuD* gene is located upstream of *umuC* in an operon. Therefore, overexpression of *umuD* alone did not lead to production of UmuD²C.

5. Discussion

In *E. coli*, the mutagenic response to ciprofloxacin is dependent on TLS pathways through SOS-induced DNA polymerases, including pol IV and pol V (UmuD²C). Inactivation of *umuCD* inhibits the mutagenic response to ciprofloxacin and prevents the emergence of ciprofloxacin-resistant mutants (3, 23). Despite its important role in mutagenesis and its regulatory effect on the UmuC and pol IV activities, the active *umuD* gene is not found in the genome of Gram-positive bacteria, including *S. pneumonia* and *S. aureus* (5, 14). However, these Gram-positive bacteria show increased mutation frequency in the presence of sub-inhibitory concentrations of ciprofloxacin (16, 22, 24). The mechanism of mutagenesis in these bacteria relies on acquiring mutations in *rpoB* gene. In this study, we aimed to identify possible

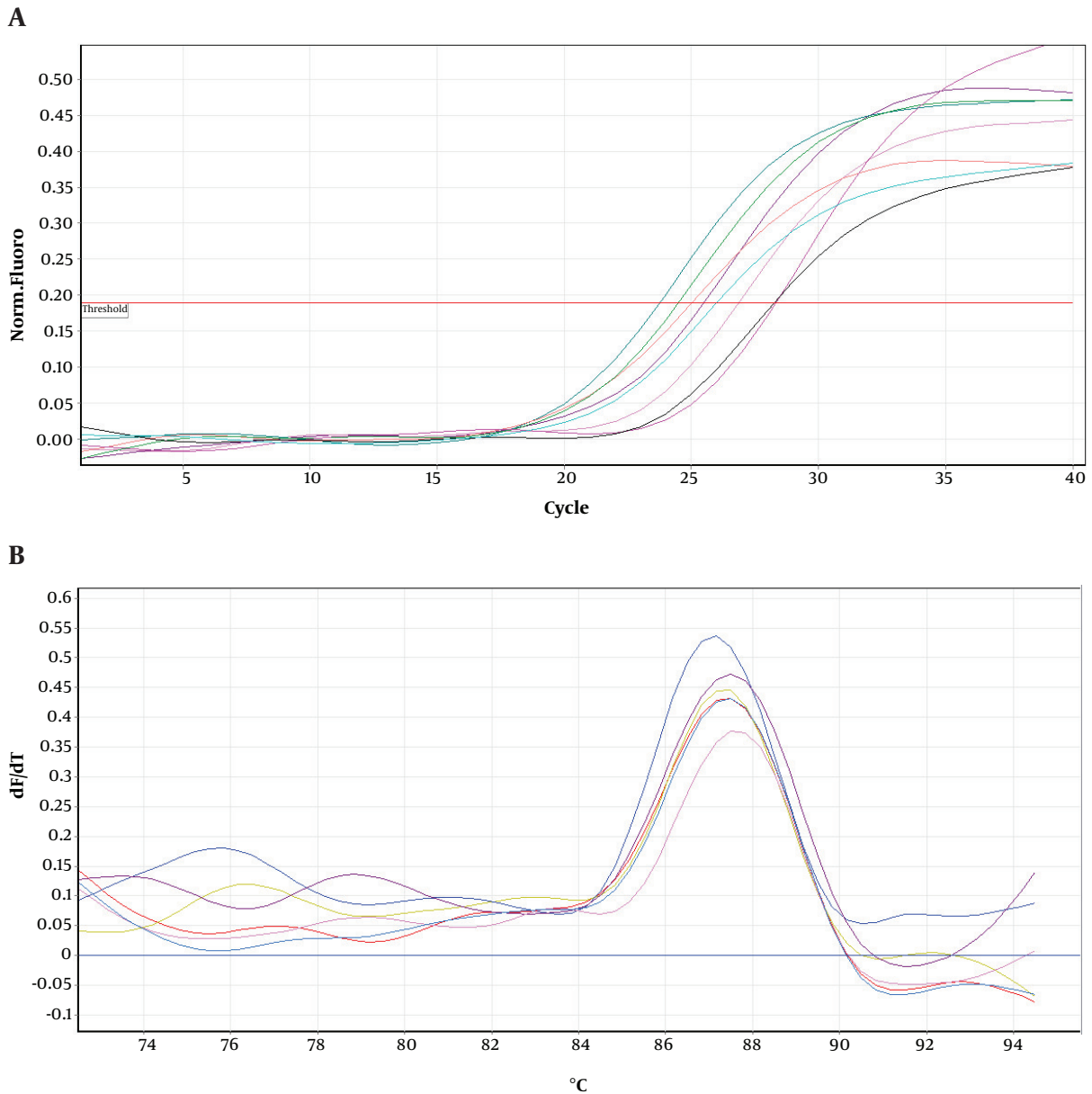


Figure 4. Amplification and melting curves of *recA* gene in wild type and mutants.

alternative mechanisms of mutagenesis to ciprofloxacin in the absence of UmuC in *E. coli*. We detected rifampin-resistant *umuC* null mutants, although they were not as common as those in Gram-positive bacteria in the absence of UmuC-like proteins (5, 16, 22). Therefore, the emergence of rifampin-resistant mutations in *umuC* mutants did not lead to increased mutagenesis following exposure to ciprofloxacin. Also, the low level of mutagenesis in

umuC null mutants hinders the formation of clones with high levels of resistance to ciprofloxacin, as we could not produce clones with MICs above 2 $\mu\text{g}/\text{mL}$ for ciprofloxacin in this genetic background. However, prolonged contact with ciprofloxacin increased resistance to rifampin, as MN5 showed more resistance to rifampin than MN2-MN4, and additional mutation was found in RpoB.

On the other hand, resistance to ciprofloxacin emerges

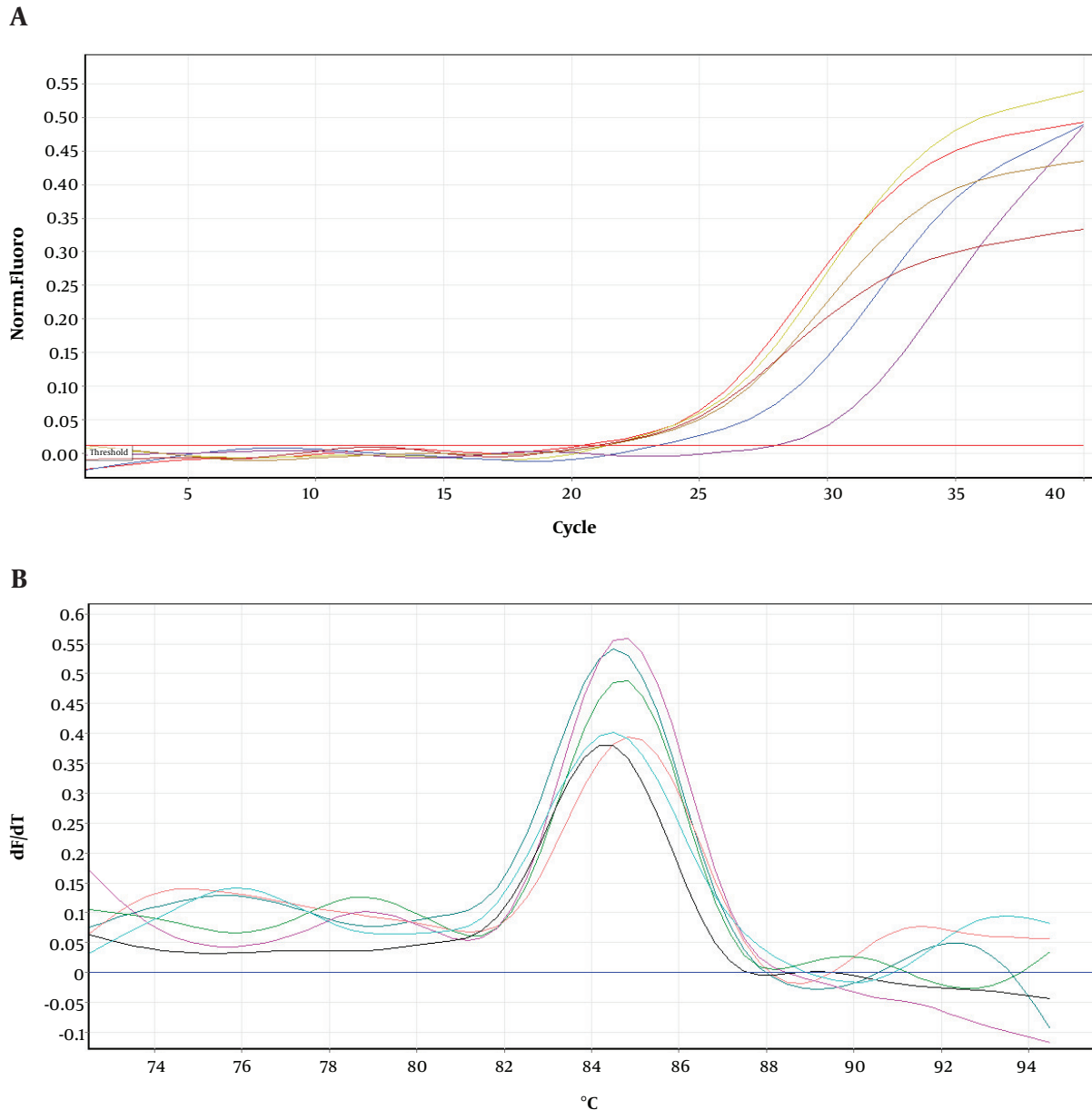


Figure 5. Amplification and melting curves of *umuD* gene in wild type and mutants.

rapidly in *Staphylococcus* species, considering its marginal susceptibility to ciprofloxacin. A single mutation in *grlA* gene (encoding DNA topoisomerase IV subunit A) is sufficient to confer resistance to ciprofloxacin. However, resistance to ciprofloxacin in *E. coli*, with a significantly higher intrinsic susceptibility to ciprofloxacin, requires multiple mutations, involving the drug target (*gyrA* and *gyrB*) and drug accumulation (overactivation of the AcrAB-TolC efflux

pump) (9, 25). Activation of the AcrAB-TolC efflux pump occurs after the induction of mutagenic SOS DNA pol V and pol IV (18). Therefore, high levels of resistance to rifampin are expected following the overactivation of the efflux pump, as it was found for the M2 mutant. However, few rifampin-resistant mutants were formed in the *umuC* background in the absence of DNA pol V. It seems that the presence of other genetic factors and the site of mutation

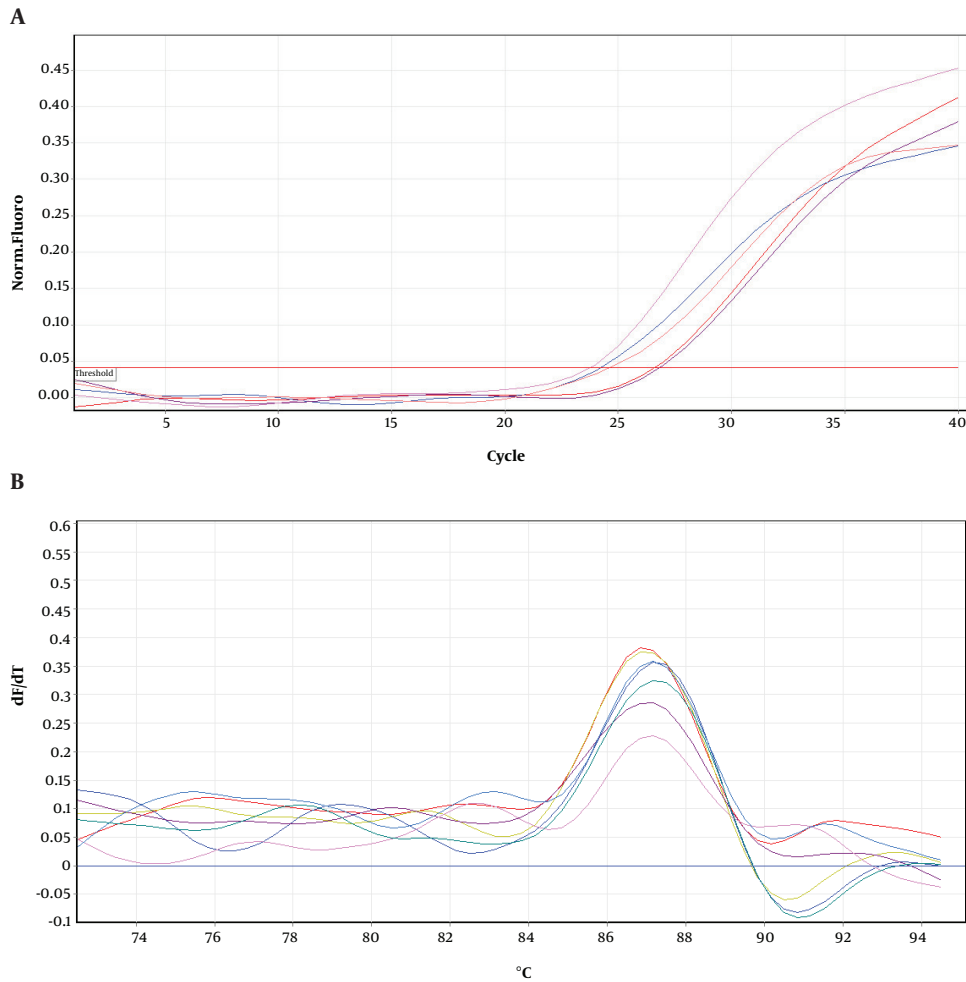


Figure 6. Amplification and melting curves of *dinB* gene in wild type and mutants.

Table 2. Relative Expression of SOS Genes^a

Strain/Mutant	Expression of Genes					
	<i>recA</i>	P-Value	<i>umuD</i>	P-Value	<i>dinB</i>	P-Value
MG1655	1	-	1	-	1	-
M2	7.1	0.02	3.54	0.03	9.4	0.01
MN1	1.2	0.5	1	-	1	-
MN2	2.5	0.05	2	0.05	2	0.05
MN5	5.4	0.02	3.8	0.02	2.5	0.05

^aValues represent fold change (mean of two sets with two samples) in comparison with wild type strain (MG1655). In all cases the standard deviation was less than 10% of mean. Values more than 2 were considered overexpression.

in the β subunit of RNAP are important. This genetic factor may not be MutSL, as rifampin resistant mutants were generated in *mutS* and *mutL* genetic backgrounds after ex-

posure to ciprofloxacin (22).

Rifampin interferes with the elongation step of transcription. Besides transcription, progression of DNA repli-

cation is inhibited by damage caused by ciprofloxacin. This blockage eventually causes double-strand breaks (DSBs) through formation of either R-loops or single-stranded DNA (4). Generally, DSBs are recognized as potent inducers of SOS response. Adequate amounts of DSBs or R-loops may be found in the *umuC* clone (MN5), leading to the overexpression of *recA*, *umuD*, and *dinB*. Therefore, formation of RNA polymerase mutants could facilitate the termination of transcription so that the RNA polymerase would not act as a roadblock for DNA replication. Moreover, the overexpression of *umuD* and *dinB* genes did not enhance mutagenesis in *umuC* mutants, as the active UmuD₂C was not present, and the possible overproduction of UmuD₂ or UmuD₂' prevented the access of pol IV to replisome (6). Therefore, DNA pol IV could not act as the main pathway of mutagenesis in *E. coli umuC* mutants. However, there is no *umuD* in *Streptococci* species, such as *S. uberis* and *S. pneumonia*, while *dinB* is present (almost 42% identical to that of *E. coli*). Arg38 and Cys66, as conserved residues in DinB, are involved in interactions with RecA (Figure 7) (7). It is possible that DinB acts as the main mutagenic DNA polymerase in these Gram-positive bacteria.

In the present study, we found that most ciprofloxacin-induced mutations in RpoB were not located in cluster I and II, except one at Ile572 (cluster II) in MN5. However, the higher level of resistance to rifampin in comparison with other mutants (MN1-MN4) may indicate that these mutants had acquired a primary mutation either in other clusters of RpoB (clusters N and III) or in other subunits of RNAP (RpoA and RpoC). Also, Ile572 was converted to Leu in MN5. Pourahmad Jaktaji and Nourbakhsh recorded this mutant protein in the National Center for Biotechnology Information (NCBI) database (protein ID: AWH12965.1). Ile and Leu are hydrophobic amino acids with similar characteristics. Replacement of Ile572 with Thr and Asn, as polar amino acids, has been reported in the literature (13). The Ile572Thr and Ile572Asn mutations are categorized as mutations that decrease the transcriptional slippage efficiency (yielding one protein product), relative to wild type RNAP, and also influence the slippage directionality by inhibiting insertions more than deletions. Therefore, mutations at position 572 of RNAP do not seem to result in acquiring different mutant phenotypes.

On the other hand, Pro564Leu mutation increased the slippage efficiency (yielding more than one protein product) and also increased the tendency of RNAP toward insertions rather than deletions (13). This finding was confirmed by Varhimo et al. (16), as they found a variety of mono- to tetra-nucleotide insertions at positions 512, 515, 516, 531,

532, and 564 of RpoB, which might cause diverse mutant phenotypes due to the production of different RNAP mutant enzymes. Therefore, it seems that mutations in clusters I and II of β subunit of RNAP affect (either increase or decrease) the mutation rate in the elongation step of transcription and may affect the interaction of RNAP with proteins, involved in transcription elongation and termination. The substitution of Ile572 with Phe was also detected in RpoB, which might be associated with the suppression of termination defect in *nusA* mutants (11). It was also found that other mutations, including Ser522Phe, Δ Ala523 (deletion of Ala523), Leu524Pro, and Thr563Pro, are suppressors of *nusA* mutant phenotype (11, 26). It was also found that position 522 in cluster I of RNAP β subunit is prone to acquiring insertion mutations (mono- to tetra-nucleotide insertions) in *S. uberis* (16), and the slippage phenotypes increased in *E. coli* (13). Therefore, it seems that there is a relationship between the transcription slippage efficiency of RNAP (mutation rate) and NusA activity.

Moreover, it was found that the single *nusAII* (*dinB*⁺) mutant contains wild type *E. coli* level of mutagenesis to UV light, indicating that SOS induction and DNA pol V are functional (27). Also, double *nusA-dinB* mutants exhibit the same level of mutagenesis, suggesting the essential presence of intact NusA, since in the absence of DinB, NusA can interact with DNA pol V. However, it is not clear whether a mutation in *nusA* or *dinB* would decrease the mutation rate in *S. uberisumuC* mutants. It was proposed that NusA interacts physically with DinB (28). The C terminal 263 amino acids of NusA (from amino acid 233 to amino acid 495), which bind to RNAP, seem to be important for interaction with DinB; however, the residues of DinB for the interaction with NusA are not clear (8). There are differences in NusA between *E. coli* and *Streptococci* species (*S. uberis* and *S. pneumonia*). Generally, NusA of *E. coli* is longer than that of *Streptococci* species (Figure 8). However, there are conserved residues among them, which may interact with DinB; we did not evaluate the sequence of *nusA* gene for possible mutations. Wild type NusA recruits DinB to sites where RNAP is stalled by a gap in the transcribed strand. If DinB cannot be recruited due to the presence of UmuD₂ or UmuD₂' , the presence of intact or mutant *nusA* may not be important. However, the question arises as to how mutations in cluster I and II RpoB can help bacterial cells rescue stalled RNAP.

5.1. Conclusions

In conclusion, to increase the efficiency of ciprofloxacin against Gram-negative bacteria, it is nec-

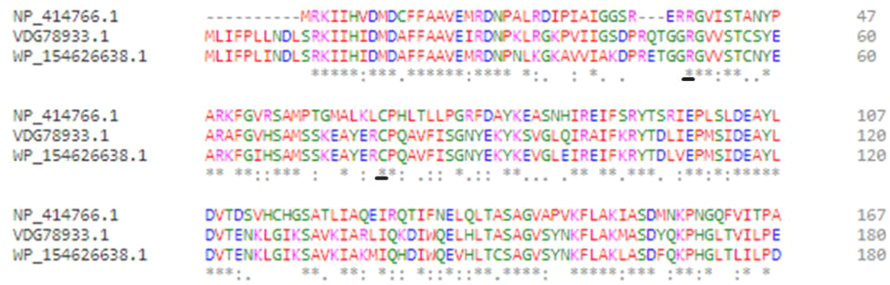


Figure 7. Partial N terminal amino acid sequence alignment of *dinB* gene from MG1655 (NP-414766.1), *Streptococcus pneumonia* (VDG78933) and *S. uberis* (WP-154626638.1). The conserved residues for interaction with *RecA* are underlined.

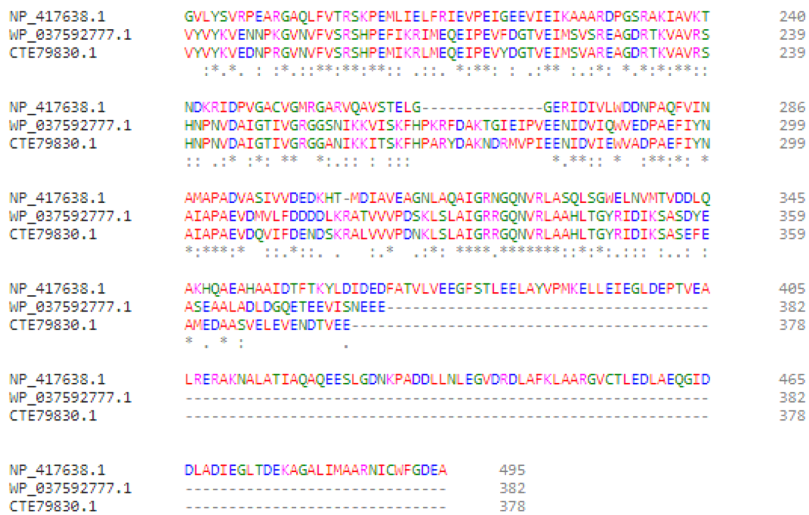


Figure 8. Partial C terminal amino acid sequence alignment of *nusA* gene from MG1655 (NP-417638.1), *S. uberis* (WP-037592777) and *Streptococcus pneumonia* (CTE79830).

essary to inactivate *umuC* gene. Inactivation of *umuC* did not inhibit the generation of rifampin resistant mutants. This did not increase mutagenesis. Thus, finding an inhibitor of *umuC*, but not an inhibitor of the *umuCD* operon is suggested. It can be used as an adjuvant along with ciprofloxacin to eliminate infection caused by *E. coli*.

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Footnotes

Authors’ Contribution: Razieh Pourahmad Jaktaji conceived and designed the study and analyzed the data. Razieh Pourahmad Jaktaji and Sayedeh Marzieh Nourbakhsh Rezaei performed experiments. Razieh Pourahmad Jaktaji wrote the manuscript. The authors have read and approved the final manuscript.

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