Supplementary materials for the article:
Kamran M. et al. Activity of Fluoroquinolones and Proton Pump Inhibitors against Resistant Oral Bacterial Biofilms, *in silico* and *in vitro* Analysis.
Pol J Microbiol. 2024, Vol. 73, No 3, 329–342

Supplementary data

Methodology

DNA extraction. The DNA was extracted through a method (phenol/chloroform method) with slight modification (Cheng and Jiang 2006). Here in this case the lysis step was skipped in which SDS/lysozyme or proteinase K and lysed the cells directly through phenol. First of all in order to extract the DNA from positive bacteria, 1 ml cell suspension was centrifuged at 8,000 *g* for 2 minutes. After the completion of the centrifugation the supernatant was removed, the isolated cells were then washed twice with STE buffer (100 mM NaCl, 10 mM Tris/HCl, 1 mM EDTA, pH 8.0). After that the isolated pallets were re-suspended in 200 µl TE buffer (10mM Tris/HCl, 1 mM EDTA, pH 8.0). Then 100 µl of the Tris-saturated phenol (pH 8.0) was added to theses followed by a vortex-mixing step of 60s for bacteria to lyse cells. Then the samples were centrifuged at 13,000 *g* for 5 minutes to separate the organic phase from the aqueous phase. Then from the aqueous phase 160 µl were taken into a clean 1.5 ml tube. TE buffer 40 µl was added to make the volume 200 µl and mixed 100 µl chloroform and centrifuged again for the time duration of 5 minutes at the rate of 13,000 *g* at 4°C. chloroform extraction was adopted to purify the lysate. The chloroform extraction procedure was repeated 3 times. Then the upper aqueous phase was transferred to the 1.5 ml clean tubes in the volume of 160 µl. In order to digest the RNA 40 µl and 5 µl of the RNase (10 mg/ml) and stored at 37°C for 10 minutes. Then to the tubes 100 µl of the chloroform was added and centrifuged at 13,000 *g* at 4°C. The upper aqueous phase (150 µl) was then added to the 1.5 ml clean tube. Finally the aqueous phase composed of the purified DNA was stored at −20°C and can be used directly as such.

DNA quantification. The quantity of DNA obtained was determined using the Qubit® 2.0 Fluorometer and the Qubit® dsDNA HS Assay Kit (Invitrogen™; Thermo Fisher Scientific, Inc., USA), and the minimum amount that could be detected was 10 pg/l. To prevent any possible contamination, each PCR was conducted with and without cleaning the master mix. Four extraction controls (EC1-4) and two blank PCR controls (known as NT1 and NT2, which had no template) were employed. In order to eliminate any potential contamination, the master mix was decontaminated using the ArcticZymes® PCR Decontamination Kit (ArcticZymes Technologies ASA, Norway) which contained a double strand specific DNase and DTT. To achieve this, 0.5 µl of double strand DNase and 0.5 µl of DTT per 20 µl reaction (adjusted for 50 endpoint reactions) were added to the master mix solutions, along with primers and probes for qPCR. The mixtures were then incubated at 37°C for 20 minutes for double strand DNase activation and 20 minutes at 60°C for double strand DNase inhibition in accordance with the manufacturer's guide lines. To

prevent heat-related volume changes, the decontaminated master mix solutions were then cooled on ice for two minutes. (Stinson et al. 2018)

16S rRNA gene qPCR. The bacterial DNA levels in dsDNase-treated and crude extractions and PCR controls were compared using real-time PCR. The specific primers 891F (5′-TGGAGCATGTGGTTTAATTCGA-3′) and 1033R (5′-TGCGGGACTTAACCCAACA-3′) were used to amplify the V6 region of the 16S rRNA gene, as represented earlier by Stinson et al. in 2018. The reaction mixture consisted of a 20 µl volume, which included 5 µl of either blank extraction control or nuclease-free water from Integrated DNA Technologies, Inc. (USA), 1 × TaqMan™ Fast Advanced Master Mix for qPCR (Applied Biosystems™; Thermo Fisher Scientific, Inc., USA), 0.9×10^{-6} mol/l of each forward and reverse primers, 0.25×10^{-6} mol/l of probe (5′-FAM-CAC-GAGCTGACGACARCCATGCA-TAMRA-3′), and 4.2 µl of water. The ViiA™ 7 Real-Time PCR System (Applied Biosystems™; Thermo Fisher Scientific, Inc., USA) using TaqMan™ Fast settings was used to the sample and for the amplification 40 cycles were performed. It was noted that all the loaded samples were duplicated (Stinson et al. 2018).

16S rRNA gene sequencing. The Agencourt AMPure XP Reagent was used to purify the PCR products in according to the guidelines of the manufacturer. The purified amplicons were suspended in Low TE buffer, and the Ion Plus Fragment Library Kit (Ion Torrent™; Thermo Fisher Scientific, Inc., USA) was used to create the NGS library as per the manufacturer's guidelines. End Repair Enzyme Mix was used to blunt-end the already purified PCR products (amplicon). After that at the same time while doing nick repairing the amplicon was attached with Ion Xpress Barcodes as well as Ion P1 adaopter. Agencourt AMPure XP reagent was used to purify the batteries. After the completion of the purification process these were amplified by using the Platinum™ PCR SuperMix High Fidelity (Invitrogen™; Thermo Fisher Scientific, Inc., USA) and Library Amplification. Specific thermal cycling conditions were adapted to mix the primer. The provided thermal conditions included denaturation for 5 minutes at 95°C. Then it was followed by 5 cycles of denaturation at 95°C and 58°C for fifteen seconds respectively and then at 70°C for the time period of only one minute. At this stage Qubit® double strand DNA HS assay kit was used for measuring after the cleaning of the libraries properly using the reagent addressed previously in this experiment. The range of each library was adjusted to 100×10^{12} mol/l in the Low TE Buffer to guarantee the even representation of each barcoded library in the sequencing reaction. The untreated samples were added in equal parts (13:1) to the final pool in equimolar concentration. Templates were prepared automatically by using the Ion Chef™ System with Ion 520™ & Ion 530™ ExT Kit-Chef (Ion Torrent™; Thermo Fisher Scientific, Inc., USA) and load chips using isothermal amplification technology from Thermo Fisher Scientific. Initially for templating onto ISPs and loading on an Ion 530™ a 50 µl of the pooled library sample with the concentration of 100×10^{-12} mol/1 was loaded onto the ion S5 Ext Reagent cartridge. Finally, the Ion S5 ExT Sequencing Kit on an Ion S5 Sequencer with Torrent Suite™ Software 5.2.2 (Ion Torrent™; Thermo Fisher Scientific, Inc., USA) using default calibration from Thermo Fisher Scientific was used to sequence the loaded hip for 1,300 flows (Stinson et al. 2018).

Table SI

Identification of strains based on 16S rRNA gene sequence published in DNA database.

Strain No.	Strain ID	Number of nucleotides of 16S rRNA gene	Closely related validly published taxa	Sequence accession number of closely related species	Similarity % age of 16S rRNA gene sequence with closely related species	No. of closely related species having > 97% (> 98%) similarity of 16S rRNA gene sequence
1.	U7(1)-pink	402	*Staphylococcus epidermidis* (NCTC 11047(T)	UHDF01000003	99.75	> 30
2.	NCCP-2093_27F-981 (U6)	981	*Staphylococcus aureus* subsp. *aureus* (DSM 20231T)	AMYL01000007	99.39	6(5)
3.	U7(red) small	412	*Staphylococcus aureus* strain S33 (ATCC® 12600™)	NR_037007.2	99.51	> 30

Table SII
Antimicrobial resistance pattern of strains.

Bacterial strain	Ciprofloxacin	Moxifloxacin	Gentamicin	Ceftriaxone	Ceftazidime	Cefepime	Amoxicillin	Coamoxiclav	Imipenem	Meropenem	Aztreonam	Metronidazole	Azithromycin	Ofloxacin	Tetracycline
Staphylococcus aureus[1]	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Staphylococcus epidermidis[1]	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Staphylococcus aureus[2]	S	R	R	S	S	S	S	S	S	S	R	R	S	S	S
Pseudomonas aureginosa[2]	S	R	R	S	S	S	S	S	S	S	R	R	S	S	S

[1] – clinical strain

[2] – reference strain (adopted from our previously published data from Rafey et al. 2022)

R – resistant, S – sensitive (CLSI 2020)

Supplementary information

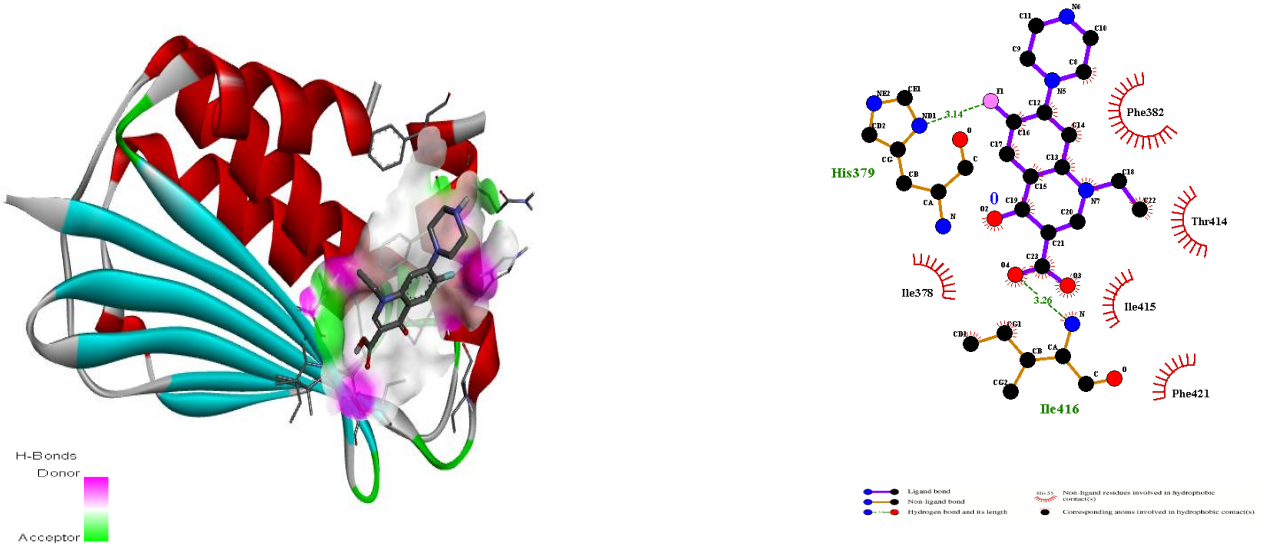

Fig. S1. 3D interaction and H, non-H bonding interactions of norfloxacin inside binding sites of transcriptional regulator 4BXI with pose 2.

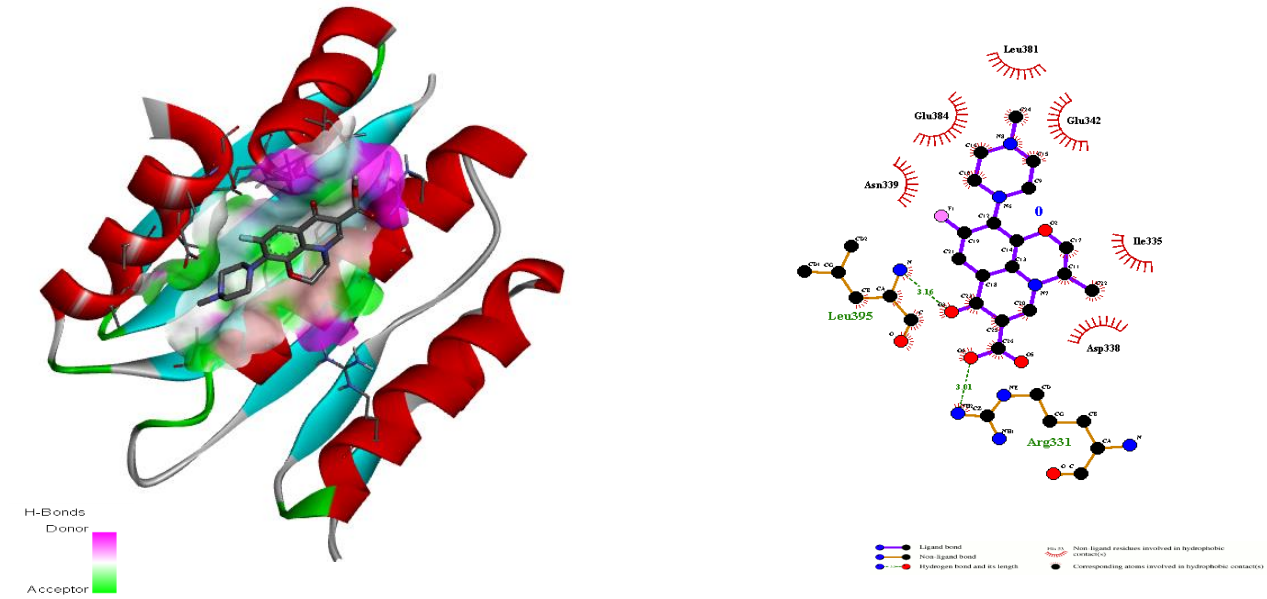

Fig. S2. 3D interaction and H, non-H bonding interactions of ofloxacin inside binding sites of transcriptional regulator 4BXI with pose 4.

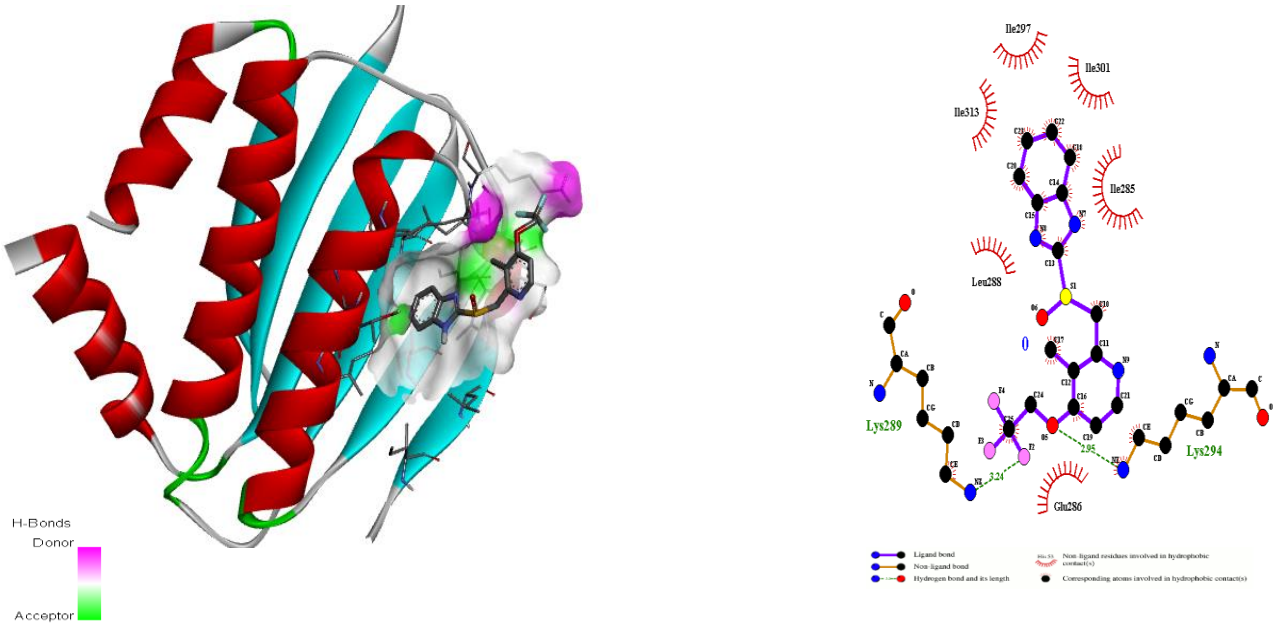

Fig. S3. 3D interaction and H, non-H bonding interactions of dexlonsprazole inside binding sites of transcriptional regulator 4BXI with pose 2.

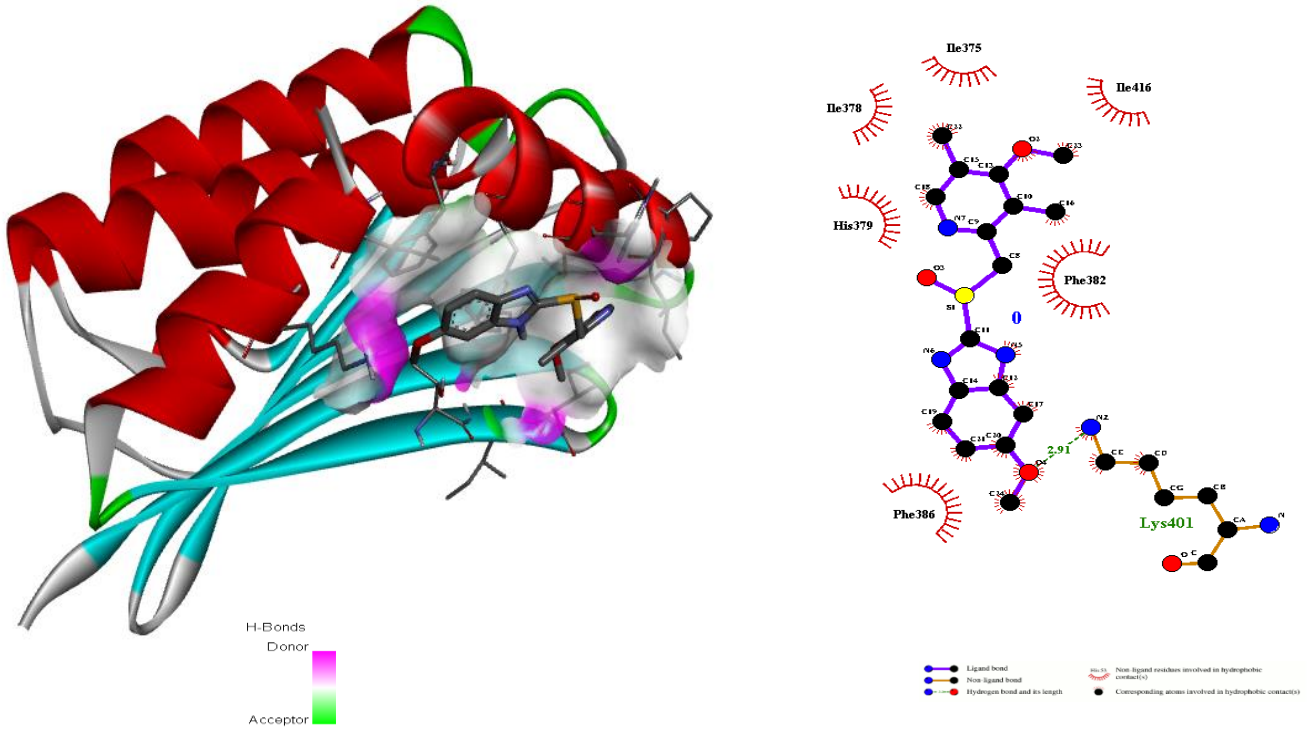

Fig.S4. 3D interaction and H, non-H bonding interactions of esomeprazole inside binding sites of transcriptional regulator 4BXI with pose 1.

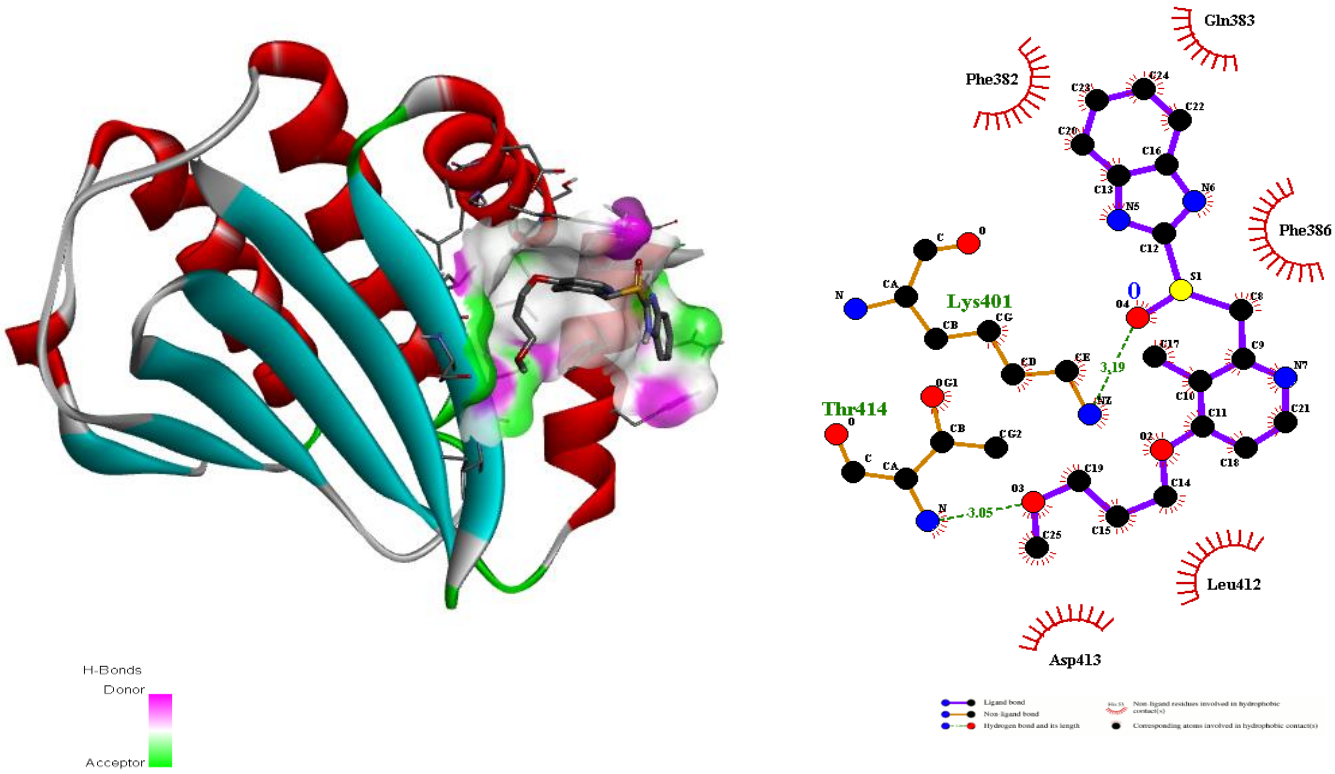

Fig. S5. 3D interaction and H, non-H bonding interactions of rabeprazole inside binding sites of transcriptional regulator 4BXI with pose 2.

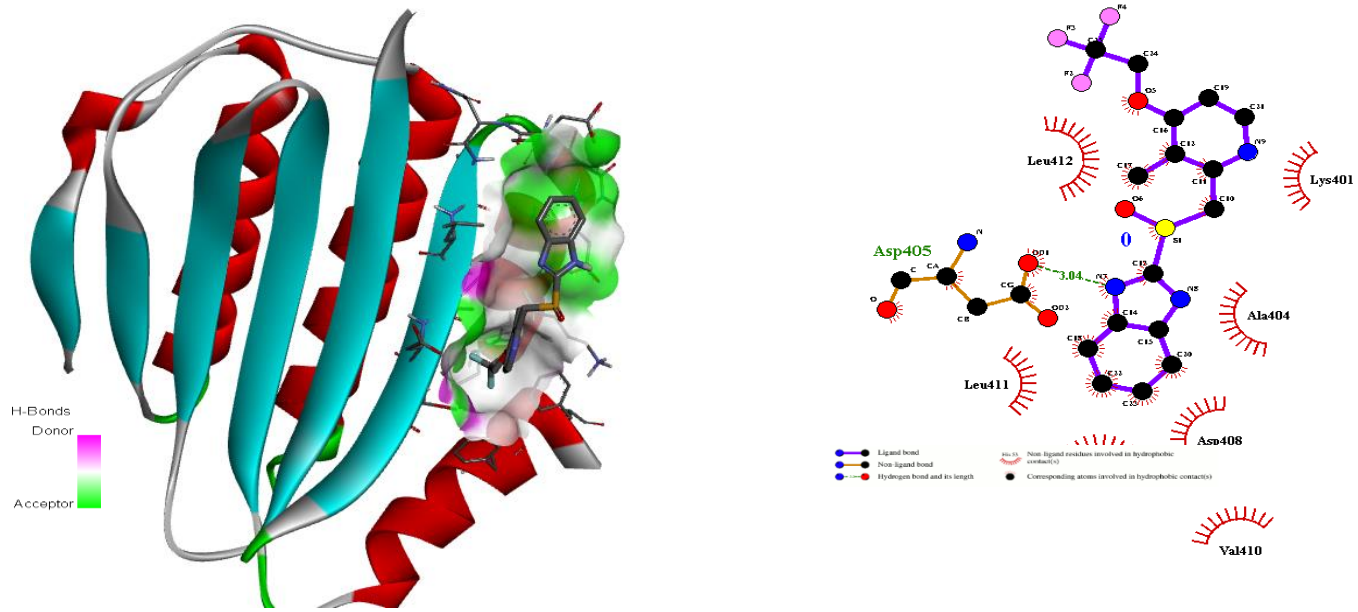

Fig. S6. 3D interaction and H, non-H Bonding interactions of norfloxacin inside binding sites of transcriptional regulator 3QP1 with pose 6.

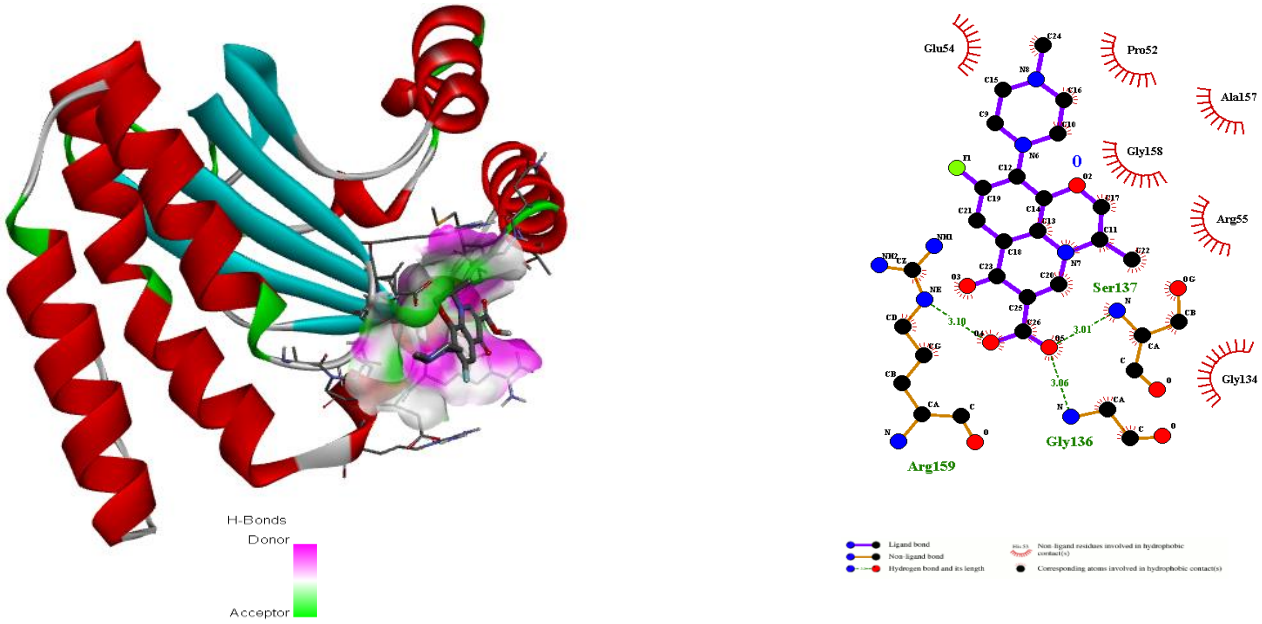

Fig. S7. 3D interaction and H, non-H bonding interactions of ofloxacin inside binding sites of transcriptional regulator 3QP1 with pose rank 2.

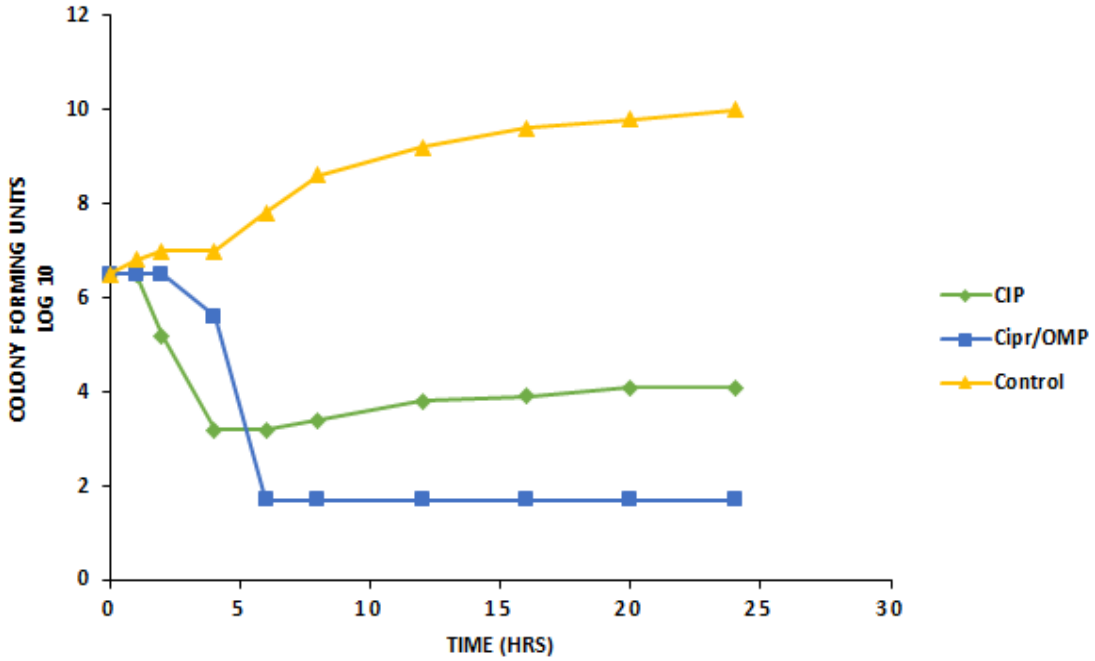

Fig. S8. Time-kill kinetics study of ciprofloxacin and its combinations towards *Staphylococcus aureus*.

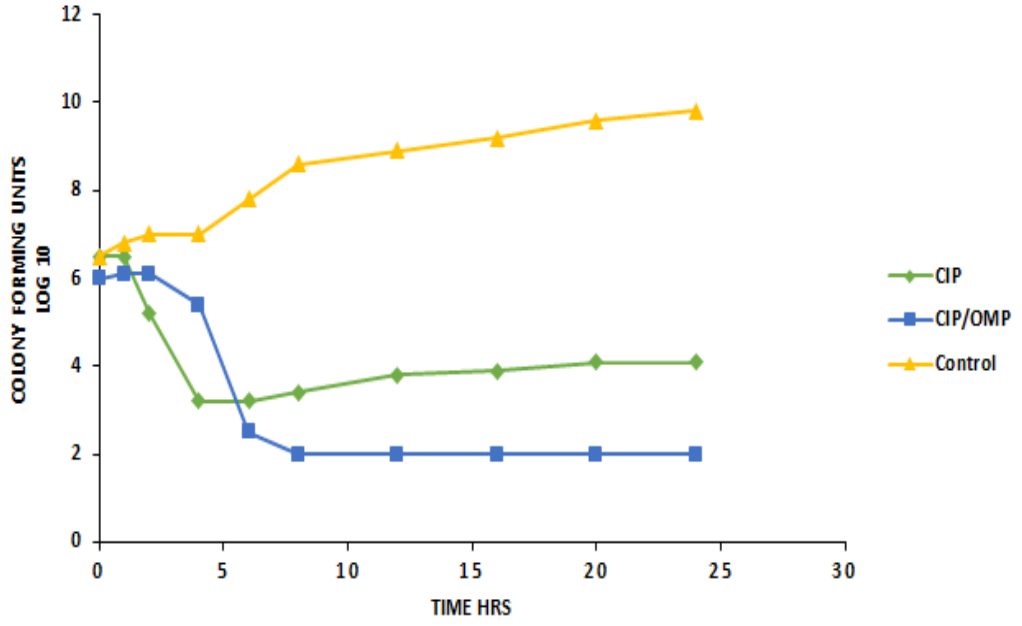

Fig. S9. Time-kill kinetics study of ciprofloxacin and its combinations towards *Staphylococcus epidermidis*.

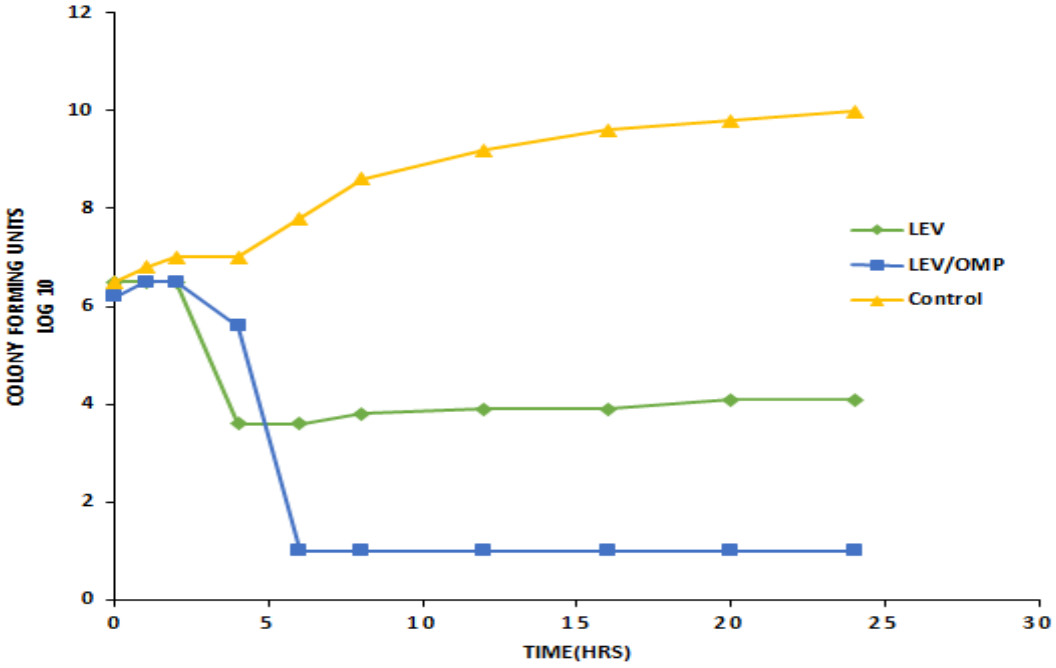

Fig. S10. Time-kill kinetics study of levofloxacin and its combinations towards *Staphylococcus aureus*.

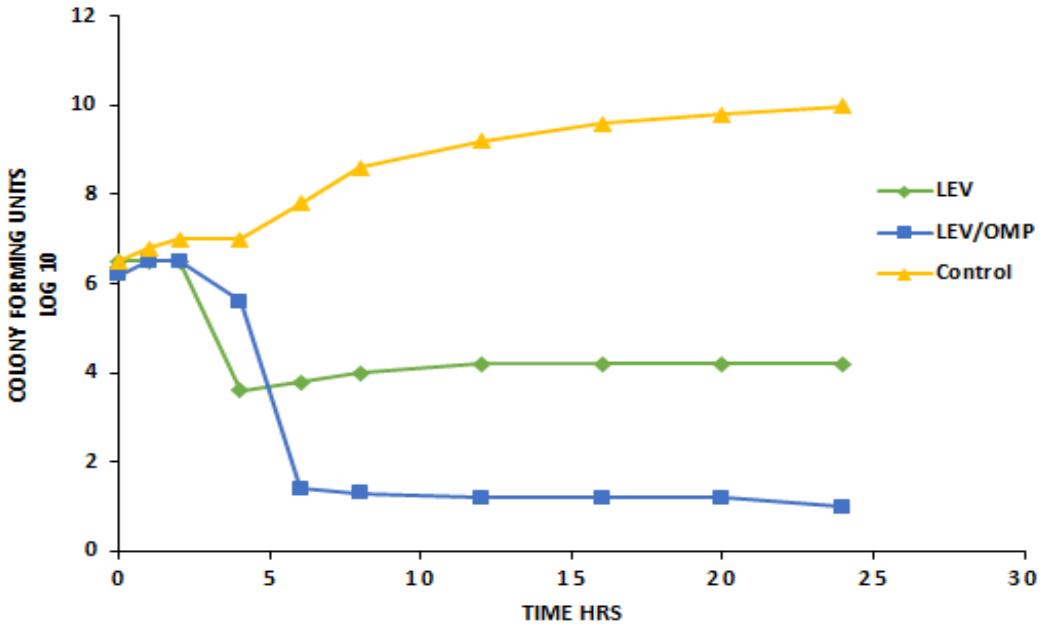

Fig. S11. Time-kill kinetics study of ciprofloxacin and its combinations towards *Staphylococcus epidermidis*.