

Activity of Fluoroquinolones and Proton Pump Inhibitors against Resistant Oral Bacterial Biofilms, *in silico* and *in vitro* Analysis

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Abstract

Oral bacterial infections are a great health concern worldwide especially in diabetic patients. Emergence of antimicrobial resistance with reference to biofilms in oral cavity is of great concern. We investigated antibiotics combination with proton pump inhibitors against oral clinical isolates. The strains were identified as *Staphylococcus epidermidis* and *Staphylococcus aureus* by the 16S rRNA gene sequencing. In molecular docking, ciprofloxacin, levofloxacin, and omeprazole best fit to active pockets of transcriptional regulators 4BXI and 3QP1. None of the proton pump inhibitors were active against *S. epidermidis*, whereas omeprazole showed significant inhibition (MIC 3.9 µg/ml). Fluoroquinolones were active against both *S. epidermidis* and *S. aureus*. In combination analysis, a marked decrease in minimum inhibitory concentration was noticed with omeprazole (MIC 0.12 µg/ml). In antiquorum sensing experiments, a significant inhibitory zone was shown for all fluoroquinolones (14–20 mm), whereas among proton pump inhibitors, only omeprazole (12 ± 0.12 mm) was active against *Chromobacterium violaceum*. In combination analysis, a moderate increase in antiquorum sensing activity was recorded for ciprofloxacin, ofloxacin, and proton pump inhibitors. Further, significant *S. aureus* biofilm eradication was recorded using of ciprofloxacin, levofloxacin, and omeprazole combination (78 ± 2.1%). The time-kill kinetic studies indicated a bactericidal effect by ciprofloxacin: levofloxacin: omeprazole combination over 24 hrs. It was concluded that fluoroquinolone combined with omeprazole could be an effective treatment option for eradicating oral bacterial biofilms.

Key words: antiquorum sensing, molecular docking, synergism, fluoroquinolones, proton pump inhibitors

Introduction

Oral bacterial infections, including periodontitis, are generally considered a significant public health problem that is associated with chronic inflammatory conditions that finally lead to teeth loss (Petersen and Ogawa 2012) and have serious long-term complications, including cardiovascular diseases (Genco et al. 2005; Sravani et al. 2015). The disease is characterized by an overwhelming

growth of bacteria in the dental plaque, thus activating a substantial immune response (Sudhakara et al. 2018). Above 90% of the world's population is affected by its general form (gingivitis) (Pihlstrom et al. 2005), whereas 10% are affected by chronic periodontitis (FDI World Dental Federation 2014). Staphylococci have long been accepted as a vital member of the oral flora. However, due to their transient membership in the oral cavity, their role in oral infections is debatable

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(McCormack et al. 2015). Despite this, *Staphylococcus aureus* occurrence rate in patients with dentures is 24–84% (Ohara-Nemoto et al. 2008). Various studies have also confirmed its role in various oral infections, including staphylococcal mucositis, angular cheilitis, and parotitis are caused by this microorganism (Kronström et al. 2001; Rokadiya and Malden 2008), which could be the result of cross infections from diverse sources (Petti and Polimeni 2011). Similarly, statistics have confirmed the prevalence of *Staphylococcus epidermidis* in the oral cavities of 27.3% of patients with periodontal disease (Loberto et al. 2004) and 41% of orally healthy individuals (Ohara-Nemoto et al. 2008).

Oral bacteria generally produce biofilms, bind irreversibly on various surfaces in the oral cavity, and generate extracellular polymers to boost matrixes that change bacterial phenotypes (Donlan 2001). This modification facilitates the development of resistance to antibiotics (Tan et al. 2014). Researchers have confirmed that the cell-to-cell communication mechanism or quorum sensing in certain bacteria results in biofilm formation (Pena et al. 2019). Fluoroquinolones are broad-spectrum antibacterials generally used against various microorganisms, including periodontal bacteria (Zechiedrich and Cozzarelli 1995). These antibiotics inhibit the enzymes involved in the synthesis of bacterial DNA, both of which are DNA topoisomerases (DNA gyrase and topoisomerase IV) that facilitate bacterial DNA replication (Hooper 2001). The proton pump inhibitors (PPIs) are used for the treatment of patients with acid-related diseases, including gastroesophageal reflux disease (GERD), duodenal ulcers, and gastric ulcers (Shin and Sachs 2008). Studies have proven that the combination therapy of omeprazole and antibiotics can favor bacterial eradication because omeprazole not only has antibacterial properties (Sjöström et al. 1996) but also facilitates macromolecular transport by widening the intra-epithelial spacing and increasing the permeability of gastric mucosa (Hopkins et al. 2002), that may result in enhanced antibacterial activity. Keeping in mind the limitations of current therapeutic regimens, we aimed to analyze the effectiveness of proton pump inhibitors and fluoroquinolone against resistant oral bacterial biofilms using *in silico* and *in vitro* methods.

Experimental

Materials and Methods

Bacterial strains, growth media, and chemicals.

The bacterial strains, including *Chromobacterium violaceum* (CV) (DSM 30191), were acquired from the German Collection of Microorganisms and Cell Cultures (DSMZ). The *S. epidermidis* and *S. aureus* were isolated from female diabetic patients with periodonti-

tis. The bacterial culture media, which include Lauria-Bertani broth (LB), triple sugar iron agar (TSIA), eosin methylene blue (EMB), Tryptic soya broth (TSB), and nutrient agar were purchased from HiMedia Laboratories Pvt. Ltd. (India). Dr. Asif Nawaz from ADDCL Gomal Centre of Pharmaceutical Sciences of the Gomal University Dera Ismail Khan kindly donated all standard compounds.

Bacterial identification. The dental plaques were collected in sterilized vials with the help of a qualified dentist and placed in culture media immediately in the lab. Preliminary purification and identification were performed using various differential media, including TSIA, EMB, and MacConkey agar. The purified bacterial cultures were identified through the 16S rRNA sequencing, where several procedures including DNA extraction, DNA quantification, the 16S rRNA gene qPCR, and the 16S rRNA gene sequencing, were performed. Finally, the strains were identified as *S. epidermidis* and *S. aureus* (Supplementary data).

Molecular docking studies. The X-ray crystallographic structures of the transcriptional regulators are required for conducting molecular docking studies 4BXI (Khayat et al. 2022) and 3QP1 (Chen et al. 2011) for quorum sensing were acquired from the Protein Data Bank (PDB). The molecular docking process was carried out through the Lamarckian Genetic Algorithm integrated within the AutoDock v 4.2.1 software (Trott and Olson 2010). The most favorable docked structures were selected for additional analysis, and this selection was based on the binding energy scores (ΔG). Software tools such as Accelrys DS Visualizer 2.0 (Accelrys, Inc., USA), The PyMOL Molecular Graphics System, Version 3.0 (Schrödinger, LLC) and LigPlot+ (Laskowski and Swindells 2011) were used to calculate the hydrogen bonding and hydrophobic interactions between the ligand and protein.

Determination of resistance patterns. The resistance patterns of *S. epidermidis* and *S. aureus* were determined using diverse antibiotics with a disc diffusion assay (CLSI 2020).

Minimum inhibitory concentration (MIC). The minimum inhibitory concentration (MIC) assay was performed using a resazurin-based 96-microwell plate assay (Marques-da-Silva et al. 2022). Briefly, 50 μ l of the bacterial strain (adjusted with MacFarland 0.5×10^5 CFU/ml) to each well of 96-microwell plates, followed by the addition of test samples (50 μ l; 2–125 μ g/ml) was added in each well of 96-microwell plate. The plates were then incubated at 37°C for 24 hours. Afterwards, 40 μ l of indicator solution (resazurin 0.015%) was added to each well and incubated at 37°C for 60 minutes. A 96-microplate reader (Hippo MPP-96; SIA Biosan, Latvia) was used to record the colorimetric readings. Ciprofloxacin was included as a positive control. All results were compared with CLSI standards (CLSI 2020).

Antibiofilm activity. The antibiofilm potential of test samples was analyzed by standard procedure (Bazargani and Rohloff 2016). Briefly test sample (100 μ l; sub-MIC values) was added the already prepared bacterial culture (100 μ l adjusted with MacFarland 0.5×10^6 CFU/ml) in 96-microwell plate and incubated at 37°C (for 24 hrs). Afterwards that the cell growth in the 96-microwell plates was measured at 592 nm. Further biofilm quantification was performed by using crystal violet staining assay. Finally, the following formula was used to calculate the percent inhibition:

$$\text{inhibition \%} = \left(1 - \frac{A_1}{A_2}\right) \times 100$$

where A_1 , A_2 are absorbance of sample and control, respectively.

Antiquorum sensing. The antiquorum sensing activity of the test sample was determined by using the bio-marker strain *C. violaceum*, as described earlier (Koh and Tham 2011). An overnight-grown broth culture of *C. violaceum* was briefly loaded onto LB agar and sterilized filter paper discs were placed on each plate. After 20 min, an aliquot of 15 μ l of the test sample (4–128 μ g/ml) was added to each disc and allowed to dry (at least 30 min). Afterwards the plates were incubated at 30°C for 24–48 hrs. The inhibition zone (mm) was measured and results were recorded.

Violacein quantification assay. The violacein quantification was performed using the standard method (McLean et al. 2004). Briefly a 24-hour-old culture of *C. violaceum* containing 200 μ l (optical density 0.4 at 600 nm) was added to the micro plates that contained different concentration of the test compounds (4–128 μ g/ml). The loaded microplates were then stored at 30°C for the time period of 24 hrs. At last, the reduction in the violacein dye was measured at 585 nm. The percent inhibition of the violacein dye was by using the following equation:

$$\text{violacein inhibition \%} = \left(1 - \frac{A_1}{A_2}\right) \times 100$$

where A_1 , A_2 are absorbance of sample and control, respectively.

Time-kill kinetic studies. Time-kill kinetic studies were performed by using the standard method (Appiah et al. 2017). Briefly, test samples were cultured with *S. epidermidis* and *S. aureus* (3×10^6 CFU/ml) and incubated at 37°C. After incubation, the sample were taken on different time intervals (0 to 24 hrs), streaked onto Muller-Hinton agar plates, and incubated further at 30°C for 24 hrs. A control sample (without a test sample) was taken and processed in parallel. The results were expressed graphically by plotting log CFU/ml against time interval (0–24 hrs).

Statistical analysis. All biological activity experiments were performed in three independent experiments and data was expressed as $\pm$ SD. One-way ANOVA followed by post-hoc Tukey test with $p < 0.05$ was also used for calculations.

Results

Molecular Docking studies. Among fluoroquinolones, ciprofloxacin showed best fit ($-5.7 \Delta G$ (kJ mol $^{-1}$)) in the active pocket of transcriptional regulator 4BXI. The ciprofloxacin (pose 3) showed strong H-bonding interaction with 2 amino acid residues including Ile416, His379 (Table I). The neighboring amino acids, including Phe421, Thr414, Ile415, Phe382, Ile378, showed van der Waal's, halogen, and pi-alkyl interactions (Fig. 1). Similarly, moxifloxacin also showed interaction ($-6.4 \Delta G$ (kJ mol $^{-1}$)) in binding pocket with pose 3 (Table I). Ala404 and Thr414 were the two amino acids with which the moxifloxacin forms a strong H-bonding interaction. The neighboring amino acids included Asp413, Lys401, Leu412, Asp405, Val410, and Leu411, showing van der Waal's and pi-alkyl interactions (Fig. 1). In case of levofloxacin, the pose 1 ($-5.7 \Delta G$ (kJ mol $^{-1}$)) (Table I) was observed with 2 H-bonding interaction (Ile416, His379) and 5 hydrophobic interactions with neighboring amino acids (Phe421, Ile378, Phe382, Ile415, and Thr414,) (Fig. 1).

Ciprofloxacin formed a very strong fitting in the binding site at pose rank 7 with binding free energy $-5.6 \Delta G$ (kJ mol $^{-1}$) (Table I) in the transcriptional regular 3QP1. The ciprofloxacin showed strong H-bonding interaction with 3 amino acid residues including Ser53, Glu54, and Ala57 (Fig. 2). The neighboring amino acids included Pro52, Arg159, Arg163, Gly158, Arg55 that showed van der Waal's and pi-alkyl interactions. Similarly, the moxifloxacin also presented a nice fit in binding pocket with pose 2 and free binding energy $-6.3 \Delta G$ (kJ mol $^{-1}$) (Table I). Moxifloxacin showed strong H-bonding interaction with 3 amino acid residues including Ser161, Leu10, and Glu11 (Fig. 2). The neighboring amino acids included Glu160, Pro52, Ala157, Arg55, Met135, and Gly134 showing van der Waal's and pi-alkyl interactions. The PPI were also docked on transcriptional regulator 4BXI to see possible interactions. Omeprazole showed best fit ($-5.4 \Delta G$ (kJ mol $^{-1}$)) in the active pocket of transcriptional regulator 4BXI. Among poses, omeprazole pose 7 showed strong H-bonding interaction with 2 amino acid residues including Leu412, Thr414 (Table II). The neighboring amino acids included Val410, Asp405, Ala404, Phe382, Leu397, Lys397, Lys401, Leu411 showing van der Waal's, halogen, and pi-alkyl interactions (Fig. 3). Similarly, esomeprazole also showed interaction ($-5.8 \Delta G$

Table I
Docking score, H and non H-bonding interactions of fluoroquinolones compounds.

Compound	Binding free energy ΔG (kJ mol ⁻¹)	Pose rank	No. of H bonds	H bond interaction residues	Other interaction residues
4BXI					
Ciprofloxacin	-5.7	3	2	Ile416, His379	Phe421, Thr414, Ile415, Phe382, Ile378
Levofloxacin	-5.7	1	2	Ile416, His379	Phe421, Ile378, Phe382, Ile415, Thr414,
Moxifloxacin	-6.4	3	2	Ala404, Thr414	Asp 413, Lys401, Leu412, Asp405, Val410, Leu411
Norfloxacin	-6.3	2	3	His379, Ile416	Ile378, Phe382, Thr414, Ile415, Phe421
Ofloxacin	-6.6	4	2	Arg331, Leu395	Asn339, Glu384, Leu381, Glu342, Ile335, Asp338
3QP1					
Ciprofloxacin	-5.6	7	3	Ser53, Glu54, Ala57	Pro52, Arg159, Arg163, Gly158, Arg55
Levofloxacin	-6.5	2	2	Gly105, Arg10	Met9, Pro176, Gln180, His177, Ala144
Moxifloxacin	-6.3	2	3	Gly136, Ser137, Arg159	Glu160, Pro52, Ala157, Arg55, Met135, Gly134,
Norfloxacin	-5.4	6	3	Arg159, Ser137, Gly134	Arg55, Glu54, Ser53, Pro52, Gly158,
Ofloxacin	-6.3	2	3	Arg159, Gly136, Ser137	Gly134, Arg55, Gly158, Ala157, Pro52, Glu54

4BXI – structure of the *Pseudomonas aeruginosa* LasR ligand-binding domain bound to its autoinducer
3QP1 – crystal structure of CviR ligand-binding domain bound to the native ligand C6-HSL

Table II
Docking score, H and non H-bonding interactions of tested PPI's.

Compound	Binding free energy ΔG (kJ mol ⁻¹)	Pose rank	No. of H bonds	H bond interaction residues	Other interaction residues
4BXI					
Dexlansoprazole	-6.1	4	2	Lys289, Lys294	Glu286, Leu288, Ile313, Ile297, Ile301, Ile285
Esomeprazole	-5.8	1	1	Lys401	Phe386, His379, Ile378, IL3375, Ile416, Phe382
Lansoprazole	-6.5	2	1	Asp405	Lys401, Ala404, Asp408, Val410, Ala407, Leu411, Leu412, Leu 397
Omeprazole	-5.4	7	2	Leu412, Thr414	Val410, Asp405, Ala404, Phe382, Leu397, Lys397, Lys401, Leu411
Pantoprazole	-6.0	1	1	His 379	Phe386, Lys401, Phe382, Ile378, Phe421, Ile416
Rabeprazole	-5.7	2	2	Thr414, Lys401	Phe382, Gln383, Phe386, Leu412, Asp413
3QP1					
Dexlansoprazole	-5.7	8	3	Glu112, Ser137, Thr131	Lys 124, Asn116, Phe 115, Ile127, Gly136, Arg159
Esomeprazole	-5.9	3	4	Gln95, Arg101, Leu72, Asn64	Ala94, Ile69, Leu100, Gln70, Arg71, Gln68
Lansoprazole	-6.2	1	1	Met30	Glu26, Ile34, Glu39, His33, His29
Omeprazole	-6.0	2	4	Arg101, Leu72, Gln95, Asn64	Gln68, Ile69, Leu100, Ala94, Gln70, Arg71,
Pantoprazole	-6.2	1	4	Leu72, Arg101, Asn64, Gln95	Gln70, Ala94, Ile69, Leu100,
Rabeprazole		2	3	Arg101, Asn64, Gln95	Gln66, Ile69, Leu72, Leu100, Ala94, Gln68

4BXI – crystal structure of ATP binding domain of AgrC from *Staphylococcus aureus*
3QP1 – crystal structure of CviR ligand-binding domain bound to the native ligand C6-HSL

(kJ mol⁻¹)) in binding pocket with pose 1 (Table II). H-bonding interaction with Lys401 amino acid residue and neighboring amino acids involved Phe386, His379, Ile378, IL3375, Ile416, and Phe382 that presented van der Waal's and pi-alkyl interactions (Fig. 1). Likewise, pantoprazole pose 1 (-6.0 ΔG (kJ mol⁻¹)) showed only 1 H-bonding interaction with His 379 and hydrophobic interactions with Phe386, Lys401, Phe382, Ile378,

Phe421, and Ile416 (Table II, Fig. 2). Other interactions are shown in supplementary data (Fig. S1 and S2).
In the transcriptional regulator 3QP1, among PPI's, the esomeprazole showed a fine fit in binding pocket with pose rank 2 and free binding energy -5.9 ΔG (kJ mol⁻¹) (Table II). The esomeprazole showed strong H-bonding interaction with 4 amino acid residues including Gln95, Arg101, Leu72, Asn64 (Fig. 4). The

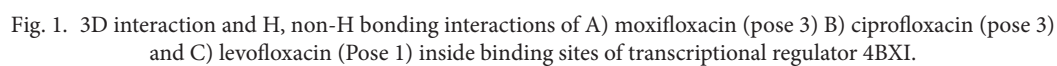


Fig. 1. 3D interaction and H₂ non-H bonding interactions of A) moxifloxacin (pose 3) B) ciprofloxacin (pose 3) and C) levofloxacin (Pose 1) inside binding sites of transcriptional regulator 4BXI.

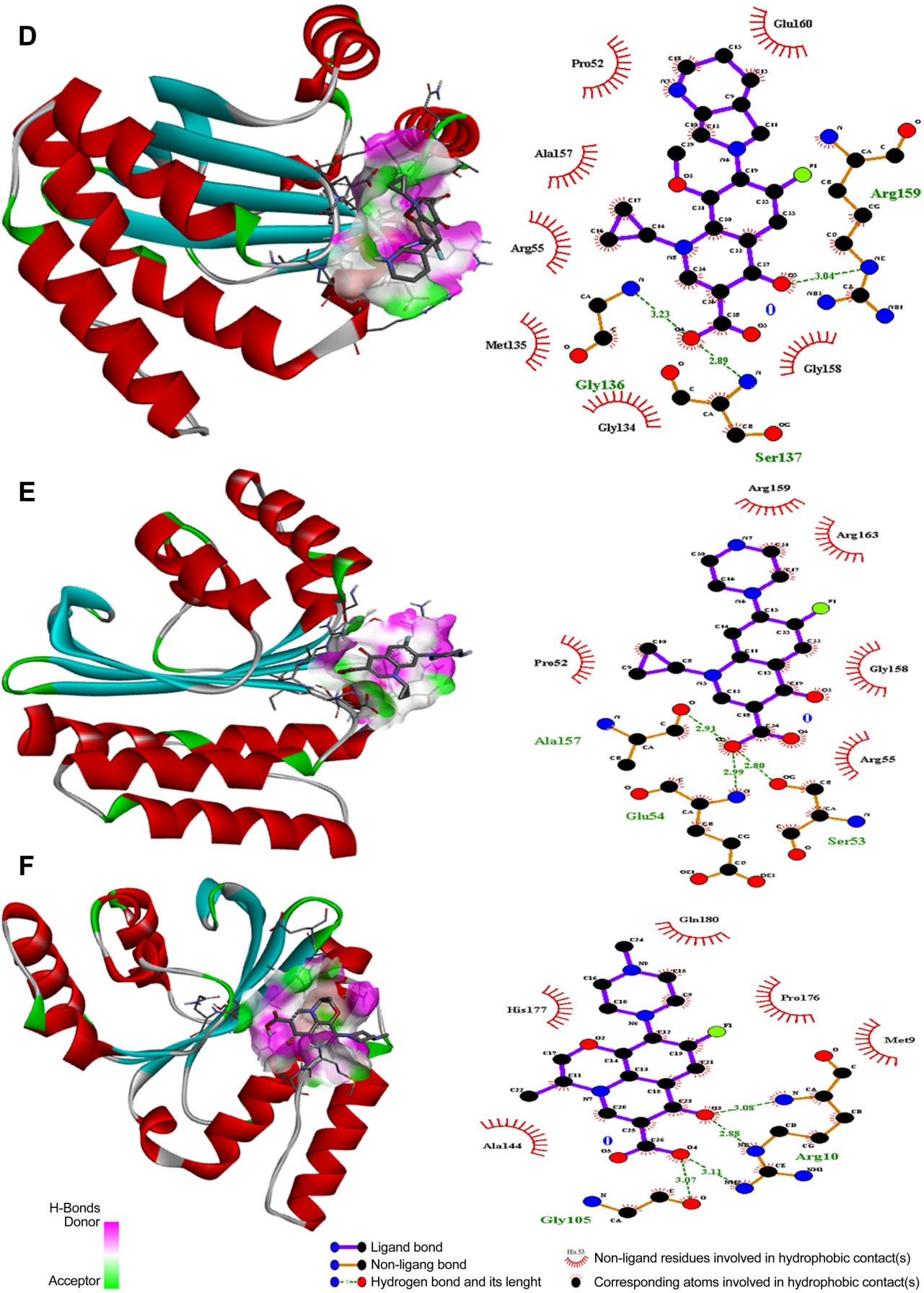


Fig. 2. 3D interaction and H, non-H bonding interactions of D) moxifloxacin (pose 2) E) ciprofloxacin (pose 8) and F) levofloxacin (Pose 2) inside binding sites of transcriptional regulator 3QP1

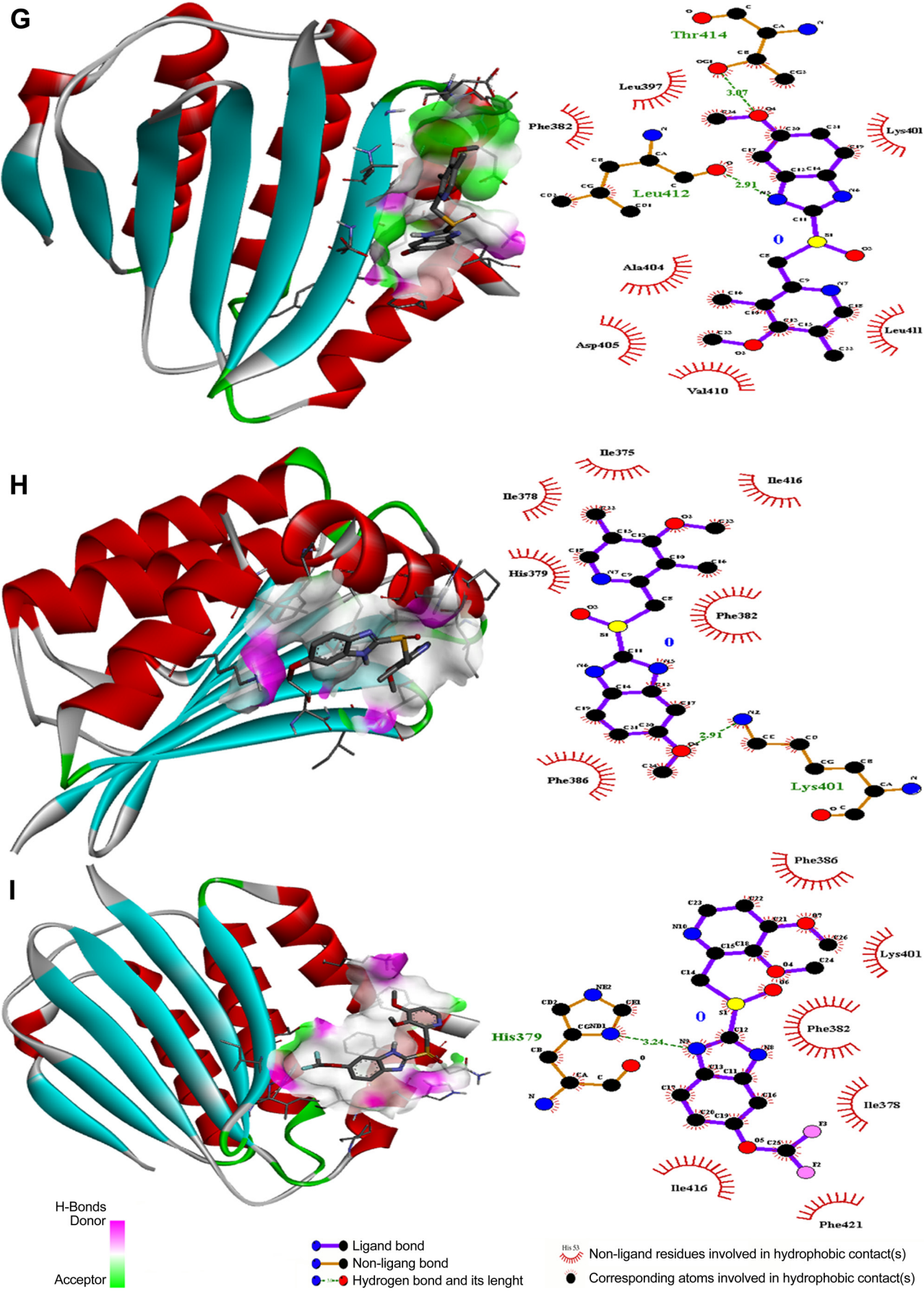


Fig. 3. 3D interaction and H, non-H bonding interactions of G) omeprazole (pose 1) H) esomeprazole (pose 1) and I) pantoprazole (Pose 1) inside binding sites of transcriptional regulator 4BXI.

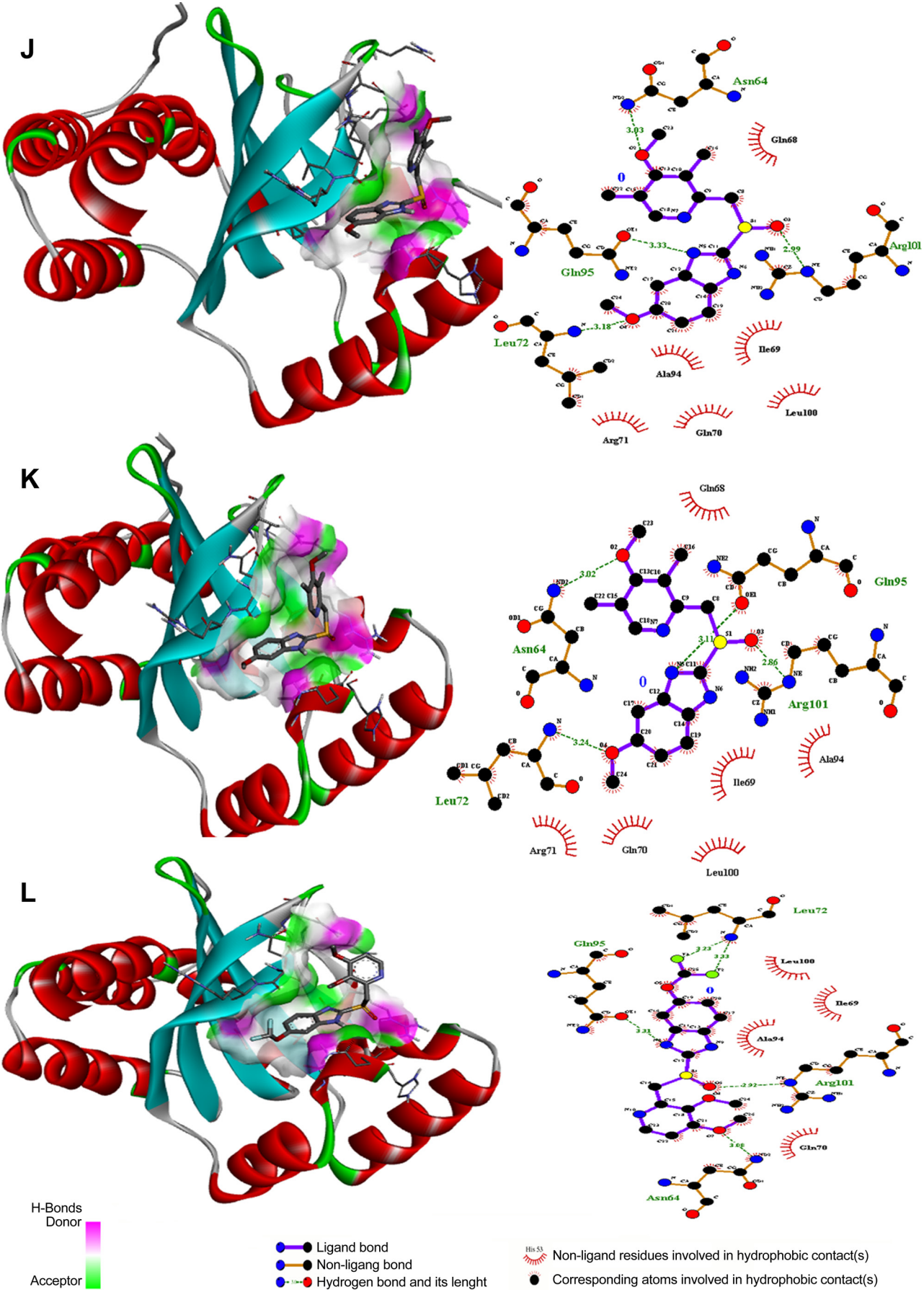


Fig. 4. 3D interaction and H, non-H bonding interactions of J) omeprazole (pose 5) K) esomeprazole (pose 3) and L) pantoprazole (Pose 1) inside binding sites of transcriptional regulator 3QP1.

neighboring amino acids included Ala94, Ile69, Leu100, Gln70, Arg71, Gln68 that showed van der Waal's and pi-alkyl interactions. Similarly, the omeprazole also presented a nice fit in binding pocket with pose 2 and free binding energy $-6.0 \Delta G$ (kJ mol⁻¹) (Table II). The omeprazole showed strong H-bonding interaction with 3 amino acid residues including Arg101, Leu72, Gln95, and Asn64 (Fig. 4) and neighboring amino acids included Gln68, Ile69, Leu100, Ala94, Gln70 and Arg71 showed van der Waal's and pi-alkyl interactions.

Antimicrobial studies. The resistance pattern of antibiotics was determined against clinical strains of *S. epidermidis* and *S. aureus*. It was evident that isolated strains were resistant to most tested antimicrobial agents compared to CLSI guidelines (CLSI 2020). The resistance pattern is presented in supplementary information (Table SII). PPI's were screened for antimicrobial analysis against isolated clinical strains *S. epidermidis* and *S. aureus* (Table III). For *S. epidermidis* none of the PPI's were able to show any inhibition within prescribed range (64 µg/ml). However, in the case of *S. aureus*, a higher inhibition (3.9 µg/ml) was recorded for omeprazole, and none of the other tested PPIs could show any inhibition within the set range (64 µg/ml). The higher inhibition of *S. aureus* was recorded (MIC 1.5 µg/ml), however comparably lesser was seen in the case of *S. epidermidis* (MIC 3.9 µg/ml). It was interesting to note that combination of PPI's with fluoroquinolones has not shown any synergistic or antagonistic effect (Table III). Omeprazole although have shown promising individual antibacterial inhibition (MIC 3.9 µg/ml), a further decrease in MIC (MIC 3.9 µg/ml) was seen in combination with fluoroquinolone. Ciprofloxacin amongst all fluoroquinolones was most active and having shown promising inhibition of both *S. aureus* (MIC 0.24 µg/ml) and *S. epidermidis* (MIC 0.24 µg/ml) (Table III). In combination analysis with PPI's, a marked decrease in MIC was noticed with omeprazole (MIC 0.12 µg/ml).

Likewise, levofloxacin was also able to show promising results *S. aureus* (MIC 0.24 µg/ml) and *S. epidermidis* (MIC 0.24 µg/ml) (Table III). Also upon combination with omeprazole a significant change in the MIC value (MIC 0.12 µg/ml) against *S. epidermidis* and *S. aureus* (MIC 0.48 µg/ml) was observed. A further slight reduction in MIC with other PPIs clearly reflected a synergistic effect.

Antiquorum sensing activities. In antiquorum sensing experiments, omeprazole showed a significant inhibitory zone (12 mm) against *C. violaceum* and $56 \pm 1.4\%$ inhibition of violacine pigment. The esomeprazole and dexlansoprazole showed only mild inhibition (2 ± 0.11 mm). None of other tested PPI's were able to show activity in tested concentration (Table IV). In case of fluoroquinolones a significant inhibition was seen by levofloxacin (20 ± 0.63 mm), followed by nor-

floxacin, moxifloxacin (18 ± 0.23 mm), ciprofloxacin, and ofloxacin (14 ± 1.00 mm) (Table IV).

Despite excellent activity of levofloxacin (20 ± 0.63 mm), during combination analysis with PPI's, no marked increase or decrease in activity was seen. In combination analysis, however, a moderate increase in antiquorum sensing activity was recorded for ciprofloxacin (increase in a zone of inhibition from 14 ± 0.63 mm to 19 ± 1.10 mm) and ofloxacin (increase in the zone of inhibition from 16 ± 1.00 mm to 19 ± 1.10 mm) combination with omeprazole. The violacine inhibition assays for all tested compounds were performed and a moderate inhibition ($56 \pm 1.4\%$) was observed in case of omeprazole and none of other tested PPI's showed any inhibition (Table IV). However, in case of fluoroquinolones, significant inhibition of violacine was recorded (62–72%). Further, during combination analysis (68–76%) inhibition was observed (Table IV).

Antibiofilm assay. Antibiofilm assays were performed with drug combination that presented marked synergism in earlier assays. This assay was performed against isolated biofilm producer strains of *S. aureus* and *S. epidermidis*. Ciprofloxacin combination with omeprazole presented a moderate increase in antibiofilm activity against *S. aureus* ($67 \pm 1.23\%$ to $78 \pm 2.1\%$) and *S. epidermidis* ($65 \pm 2.1\%$ to $75 \pm 0.48\%$). Similarly, the levofloxacin: omeprazole combination recorded a significant increase in activity (Table V).

Time kill kinetics. For time-kill kinetic studies, ciprofloxacin, levofloxacin, and their combination with omeprazole were used. According to the previous literature if CFU/ml decrease is $\geq 3 \log_{10}$, then, the antibacterial compound is considered as bactericidal (99.9%). On the other hand, if decrease of CFU/ml is < 3 , then, the antibacterial compound is reflected as bacteriostatic (less than 99.9%) (Petersen et al. 2007). The combination of ciprofloxacin and omeprazole was reported to have promising activity against *S. aureus*, with an eradication rate of more than 90%. Almost similar results were recorded in case of *S. epidermidis* (Supplementary information). In the case of levofloxacin/omeprazole combination, significant results were recorded with 99.5% inhibition for *S. aureus* and *S. epidermidis* (Supplementary information).

Discussion

Oral hygiene is important in overall well-being (Hassan 2022) and is a global health concern. Among Asian countries, about 60% of the Pakistani population is suffering from oral hygiene issues, including dental caries (Siddiqui et al. 2021). The latest WHO statistics are quite alarming that indicate heavy prevalence rates of periodontitis, dental caries, and edentulism

Table III
Determination of minimum inhibitory concentration
MIC (µg/ml) of tested compounds.

Sample	<i>Staphylococcus epidermidis</i>	<i>Staphylococcus aureus</i>
Omeprazole	> 64	3.9
Esomeprazole	> 64	> 64
Pantoprazole	> 64	> 64
Dexlansoprazole	> 64	> 64
Lansoprazole	> 64	> 64
Rabeprazole	> 64	> 64
Ofloxacin	3.9	1.9
Ofloxacin/Omeprazole	3.9	3.9
Ofloxacin/Esomeprazole	3.9	3.9
Ofloxacin/Pantoprazole	3.9	3.9
Ofloxacin/Dexlansoprazole	3.9	3.9
Ofloxacin/Lansoprazole	3.9	3.9
Ofloxacin/Rabeprazole	3.9	3.9
Norfloxacin	7.8	3.9
Norfloxacin/Omeprazole	3.9	3.9
Norfloxacin/Esomeprazole	3.9	3.9
Norfloxacin/Pantoprazole	3.9	3.9
Norfloxacin/Dexlansoprazole	3.9	3.9
Norfloxacin/Lansoprazole	3.9	3.9
Norfloxacin/Rabeprazole	3.9	3.9
Moxifloxacin	3.9	3.9
Moxifloxacin/Omeprazole	3.9	3.9
Moxifloxacin/Esomeprazole	3.9	3.9
Moxifloxacin/Pantoprazole	3.9	3.9
Moxifloxacin/Dexlansoprazole	3.9	3.9
Moxifloxacin/Lansoprazole	3.9	3.9
Moxifloxacin/Rabeprazole	3.9	3.9
Ciprofloxacin	0.24	0.24
Ciprofloxacin/Omeprazole	0.12	0.12
Ciprofloxacin/Esomeprazole	0.24	0.24
Ciprofloxacin/Pantoprazole	0.12	0.24
Ciprofloxacin/Dexlansoprazole	0.24	0.24
Ciprofloxacin/Lansoprazole	0.24	0.24
Ciprofloxacin/Rabeprazole	0.24	0.24
Levofloxacin	1.2	1.2
Levofloxacin/Omeprazole	0.12	0.48
Levofloxacin/Esomeprazole	0.24	15.6
Levofloxacin/Pantoprazole	0.24	31.25
Levofloxacin/Dexlansoprazole	0.48	31.2
Levofloxacin/Lansoprazole	0.24	31.2
Levofloxacin /Rabeprazole	0.24	31.2

Ratio of antibiotics and PPI = 1 : 1, max tested concentration = 64 µg/ml

(WHO 2022), which are mainly attributed to poor oral hygiene (Shah et al. 2011). Further, the development of resistance in patients with poor hygiene makes treatment options very limited (Batool et al. 2023). Thus,

Table IV
Determination of antiquorum sensing activity
of tested compounds.

Sample	Anti QS (mm)	% Violacine inhibition
Omeprazole	12 ± 0.12	56 ± 1.4
Esomeprazole	2 ± 0.11	0
Pantoprazole	0	0
Dexlansoprazole	2 ± 0.00	0
Lansoprazole	0	0
Rabeprazole	0	0
Ofloxacin	14 ± 1.00	65 ± 1.2
Ofloxacin/Omeprazole	19 ± 1.1	74 ± 1.24
Ofloxacin/Esomeprazole	18 ± 1.2	65 ± 0.56
Ofloxacin/Pantoprazole	16 ± 0.14	65 ± 1.45
Ofloxacin/Dexlansoprazole	18 ± 0.12	70 ± 1.41
Ofloxacin/Lansoprazole	19 ± 0.25	69 ± 1.6
Ofloxacin/Rabeprazole	19 ± 0.25	68 ± 1.4
Moxifloxacin	18 ± 1.1	62 ± 1.2
Moxifloxacin/Omeprazole	19 ± 0.56	70 ± 1.02
Moxifloxacin/Esomeprazole	18 ± 0.42	68 ± 0.78
Moxifloxacin/Pantoprazole	18 ± 0.00	66 ± 0.63
Moxifloxacin/Dexlansoprazole	18 ± 0.47	65 ± 1.6
Moxifloxacin/Lansoprazole	20 ± 0.63	72 ± 2.2
Moxifloxacin/Rabeprazole	20 ± 0.00	70 ± 1.7
Norfloxacin	18 ± 0.23	72 ± 0.63
Norfloxacin/Omeprazole	18 ± 0.45	68 ± 2.4
Norfloxacin/Esomeprazole	18 ± 0.63	65 ± 1.7
Norfloxacin/Pantoprazole	20 ± 1.1	68 ± 1.3
Norfloxacin/Dexlansoprazole	18 ± 0.63	70 ± 1.21
Norfloxacin/Lansoprazole	18 ± 0.42	72 ± 1.36
Norfloxacin/Rabeprazole	18 ± 0.12	70 ± 1.24
Ciprofloxacin	14 ± 0.63	60 ± 1.64
Ciprofloxacin/Omeprazole	19 ± 1.1	75 ± 0.46
Ciprofloxacin/Dexomeprazole	16 ± 1.3	68 ± 1.27
Ciprofloxacin/Pantoprazole	19 ± 1.1	76 ± 0.61
Ciprofloxacin/Omeprazole	16 ± 0.45	66 ± 2.5
Ciprofloxacin/Esomeprazole	19 ± 0.62	76 ± 1.4
Levofloxacin	20 ± 0.63	72 ± 1.26
Levofloxacin/Omeprazole	20 ± 0.45	76 ± 1.09
Levofloxacin/Esomeprazole	20 ± 0.25	70 ± 1.6
Levofloxacin/Pantoprazole	20 ± 0.13	74 ± 0.23
Levofloxacin/Dexlansoprazole	20 ± 0.45	72 ± 1.56
Levofloxacin/Lansoprazole	20 ± 0.12	74 ± 1.20
Levofloxacin/Rabeprazole	20 ± 0.63	76 ± 1.36

Ratio of antibiotics and PPI = 1 : 1, max tested concentration = 64 µg/ml

a significant need exists to look for newer strategies to address the challenge of oral bacterial resistance. We investigated the combination of fluoroquinolones and proton pump inhibitors for the possible eradication of

Table V
Antibiofilm assay of fluoroquinolones and PPI's.

Sample	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>
Ciprofloxacin ^a	67 ± 1.23%	65 ± 2.1%
CIP-OMP	78 ± 2.1%	75 ± 0.48%
LEV ^b	74 ± 1.83%	68 ± 1.4%
LEV-OMP	82 ± 2.4%	76 ± 1.87%

^a – 0.48 µg/ml, ^b – 1.4,
CIP – ciprofloxacin, OMP – omeprazole, LEV – levofloxacin

biofilms. Initially, molecular docking was performed to elucidate possible molecular mechanisms of interactions amongst tested drugs and receptors 4BXI and 3QP1. Receptor 4BXI represents the *S. aureus* TCS system (PDB ID: 4BXI), which plays a crucial role in staphylococci biofilm and quorum sensing. It was evident that all tested antibiotics and PPI, including omeprazole, esomeprazole, and lansoprazole, showed stable H-bonding interactions with target sites. Polar interaction with Ile416 and His379 was commonly observed in docking fluoroquinolones with 4BXI, which supports strong H-H bonding on the target site, as evidenced by previous investigations.

Further, pi-sigma, carboxy side chain, and N-H interactions were involved in stabilizing interactions (Nicod et al. 2014; Mahdally et al. 2021). On the other hand, in the case of 3QP1 (CviR ligand-binding domain bound to the native ligand C6-HSL) interaction with fluoroquinolones, diverse H-bonding and hydrophobic interactions were observed in multiple binding modes. The Hydrogen H-bonding interactions are considered important since they potentiate multiple cellular activities by assisting several molecular interactions (Chen et al. 2016).

Molecular docking studies of PPI's and 4BXI target sites also revealed interaction mainly with Leu412 and Thr414 (in the case of omeprazole) with strong H-bonding and hydrophobic interactions with amino acid residues. The binding pattern of omeprazole and rabeprazole was alike; however, a diverse interaction was observed in the case of hydrophobic interactions, where strong support existed in the case of omeprazole. Likewise, in interaction with 3QP1, esomeprazole, omeprazole, lansoprazole, and pantoprazole presented nearly similar H-binding interaction patterns with Gln95, Arg101, Leu72, and Asn64, which supports a similar mechanism of action for stabilizing the docking complex and enzyme catalytic reactions (Chaudhary et al. 2009).

All tested drug molecules were further screened for antimicrobial activity against clinically isolated *S. aureus* and *S. epidermidis* (Rafey et al. 2021). Omeprazole was the only active drug among PPIs against

S. aureus, whereas no activity was observed in the case of *S. epidermidis*. This could be due to structural conformations of the tested drug (Matysiak et al. 2019) and the formation of the sulfenamide derivative (Sjöström et al. 1996). Ciprofloxacin, amongst fluoroquinolones, was significantly active compared to all tested antibiotics. Antibacterial assay of moxifloxacin against tested strains revealed significant results (MIC 3.9 µg/ml). However, no synergistic or antagonistic effect was recorded. A similar effect was seen in the case of pantoprazole: ciprofloxacin combination assay, where a decrease in the MIC value was observed (MIC 0.12 µg/ml) for *S. aureus*. In the case of all other combinations, no synergistic or antagonistic effect was observed. Fluoroquinolones are quite diverse due to the presence of nitrogen and fluorine atoms, which are mainly responsible for providing stronger antibiotic action and increased spectrum of activity (Ball 2000). They mainly act as potent inhibitors of bacterial type II topoisomerases responsible for cellular processes, including DNA replication (Webber et al. 2013).

Bacterial quorum sensing (cell-to-cell signaling) is used by both Gram-negative and Gram-positive bacterial species to regulate their pathogenesis. Several auto-inducers like acyl-homoserine lactones (AHLs), LuxR-AHL occur in Gram-negative bacteria (Wolska et al. 2016; Hegazy et al. 2020) quorum sensing systems in Gram-positive bacteria employ cytoplasmic transcription factors and sensor kinase receptors to detect oligopeptides, which in turn control the activation of virulence genes (LaSarre and Federle 2013). In this project, only omeprazole showed antiquorum sensing activity amongst PPI, whereas all fluoroquinolones showed significant inhibition of quorum sensing. Considering the excellent activity of fluoroquinolones and omeprazole (the only active PPI among all tested drugs), a combination analysis was performed to determine synergistic activity. Interestingly, in combination with ciprofloxacin with all PPI, an increase in the inhibition zone was recorded (14 mm to 19 mm). In the case of omeprazole, it could be due to the antiquorum sensing activities of omeprazole, whereas no possible explanation can be given in the case of other PPIs. The violacine inhibition assays for all tested compounds and a moderate inhibition (56%) was observed in the case of omeprazole, and none of the other tested PPIs showed any inhibition. However, in the case of fluoroquinolones, significant inhibition of violacine was recorded (62–72%). Further, during combination analysis, 68–76% inhibition was observed. Therefore, fluoroquinolone: PPI combination may have moderate synergistic effects.

Oral biofilms are the main reason for eminent bacterial virulence, resulting in increased resistance to antimicrobials (Kuang et al. 2018). This is because developed biofilm needs more drugs for complete eradication since

the biofilm matrix limits the entry of antimicrobials in deep cell layers (Xiao et al. 2012; Benoit and Koo 2016). Still, various antimicrobials, including chlorhexidine, effectively eradicate bacterial biofilms; however, the continuous removal of biofilm remains a significant health concern (Dziedzic et al. 2015). In continuation with previous experiments, all effective drug combinations were employed for antibiofilm assays.

In the case of *S. aureus*, ciprofloxacin presented significant antibiofilm activity that increased up to 78% upon usage in combination with omeprazole, which may be due to a synergistic effect. Similarly, in the case of *S. epidermidis*, a marked increase in biofilm inhibition (75%) was seen in the case of a combination (ciprofloxacin and omeprazole) compared to a single drug assay.

Since levofloxacin alone was highly active, antibiofilm activities were performed on this combination to determine any possible effect on bacterial biofilms. Upon analysis against *S. aureus*, the levofloxacin showed significant inhibition of biofilm (74%) and the effect was enhanced upon the analysis in combination (82%). A similar trend was recorded for *S. epidermidis*.

The combination of ciprofloxacin and omeprazole was found to be a highly effective (Supplementary information) therapy against *S. aureus* with an eradication rate of more than 90%, due to the fact that this combination resulted in a decrease of CFU/ml $> 3 \log_{10}$ over a throughout 24 hrs (Petersen et al. 2007). Very nearly similar results were documented for *S. epidermidis* (Supplementary information). Levofloxacin and omeprazole in combination, provided even better results were recorded compared to ciprofloxacin combinations in *S. aureus* since the decrease of CFU/ml was $> 3 \log_{10}$ over a period of 24 hrs (Supplementary information). It was confirmed that this combination resulted in 99.5% killing with bactericidal effects. Nearly similar results were recorded in case of *S. epidermidis*.

Conclusions

Staphylococci are considered an integral part of the oral flora since they are transient members in the oral cavity. Their role in oral infections is essential since they are mainly prevalent in patients wearing dental implants and periodontitis. Due to the development of biofilms, eradication of such bacteria is crucial in clinical settings. We investigated proton pump inhibitors and fluoroquinolones alone and in combination against clinical isolates of *S. aureus* and *S. epidermidis*. It was observed that all tested antibiotics and PPI, including omeprazole, esomeprazole, and lansoprazole, presented stable H-bonding interactions with the target. Only omeprazole was active in antimicrobial assays, whereas all fluoroquinolones presented significant

inhibition. Combined, fluoroquinolones and PPIs presented significant antibiofilm and antiquorum sensing activities. Thus, ciprofloxacin or levofloxacin combined with omeprazole can be an effective treatment option for eradicating oral biofilms produced by transient members. Further detailed mechanistic studies are proposed with more diverse combinations.

Ethical statement

The ethical approval for the project was obtained from ethical review board (970/QEC/GU) and an informed consent was obtained as stated in ethical review.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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