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Abstract:

Antimicrobial susceptibility testing indicated the highest resistance level in cefoxitin and ceftazidime with resistance of all isolates (100%) followed by doxycycline 95.24%, ceftriaxone 95.24%. However, lower percentages could be obtained for tobramycin 76.19%, imipenim 61.9%, azithromycin 57.14% and penicillin-tazobactam 47.62%. Concerning resistance patterns, the mostly detected pattern was pattern P1, P2, P3 and P9 in 14.8% of all isolates. In the current study, 33 % were considered as XDR, while 52 % as MDR. Diclofenac and dexamethasone significantly decreased the sensitivity of 3 different isolates to ceftriaxone. Molecular investigation included: GDP-mannose 6-dehydrogenase (*algD*), Hemolytic phospholipase C(*plch*) and exotoxin (*toxA*). Realtime PCR studies indicated decreased growth rate in case of ceftriaxone alone to 93.56, 57.91 and 98.77% in case of strains 21, 26 and 28 respectively. Interestingly, diclofenac has decreased the growth rate by 24.57, 57.57 and 31.17%, respectively. In case of silver nano particles, a remarkable high decrease in the growth activity to less than 1.12% could be observed. Realtime PCR indicted a significant down-regulation of *toxA* in presence of diclofenac with ceftriaxone compared with ceftriaxone alone. Nano experiment using green silver particles has proved approximately 100% inhibitory effect in the expression of *algD*, *plch* and *toxA*

Introduction:

Pseudomonas aeruginosa (PA) is a Gram-negative, opportunistic bacterial pathogen which is commonly found in the environment and is known to cause serious illnesses, particularly immunocompromised, which is mainly attributed to the increased common infections caused by multidrug resistant (MDR) strains with limited treatment options. *Pseudomonas aeruginosa* has innate, acquired, and adaptive resistance to a range of antibiotics (1). *P. aeruginosa's* exceptional ability to withstand medications has made its eradication more challenging. *Pseudomonas aeruginosa* strains are characterized by their high levels of inherent and acquired resistance mechanisms, as process that combines biofilm-mediated resistance and the development of multidrug-tolerant cells. However, the phenotypic and genotypic distinctions in antibiotic resistance evolution between biofilm and planktonic growth modes remain poorly known. Because biofilms produce a structured micro environment with spatial gradients in nutrients, oxygen, and antibiotics, bacterial subpopulations can diversify and become less sensitive to antibiotics. Furthermore, spatial features in biofilms may allow resistance mutations to persist, which would otherwise be destroyed by powerful selection sweeps in exposed planktonic populations. All things considered, the biofilm development mode may change the dynamics of antibiotic resistance and mutation fixation (2). Using broad-spectrum antibiotics is one of the main methods for eliminating *P. aeruginosa* infections. One of the medications that could be used is ceftriaxone (CRO), a third-generation cephalosporin. It works by preventing the synthesis of bacterial cell walls. However, a major worry in the treatment of *P. aeruginosa* infections is the emergence of antibiotic resistance, especially resistance to cephalosporins. The release of β -lactamases that hydrolyze the β -lactam ring of CRO, changes in the permeability of bacterial cell walls, and overexpression of efflux pumps are some of the mechanisms that can cause resistance (3). There is a growing demand for the development of alternative approaches or new ways to combat *P. aeruginosa* infections. A number of novel therapeutic methods combating drug-resistant *P. aeruginosa* strains have shown notable efficacy, even primarily at the preclinical stages. This work aimed to evaluate the impact of combination of dexamethasone and declofenac with ceftriaxone and whether this combination in vitro improves or abrogates the activity of these antimicrobial drugs against *P. aeruginosa* strains. For this hypothesis molecular modeling studies were performed for more confirmation. In addition, green silver nanoparticles were examined as alternative to the commonly administered antibacterial.

Material and methods:

Identification of Clinical Isolates

Collection, isolation, and identification of 55 clinical isolates obtained, during January to June 2024 from Mansoura hospitals in Egypt. 21 isolates were identified as *Pseudomonas aeruginosa* by streaking on cetrimide agar plates, which were then incubated for 24 hours at 37°C including: 18 wound wound swaps, 2 blood samples and 1 throat sample. The resulting colonies were examined biochemically and microscopically. Colonies were identified after growth on cetrimide agar, pyocyanin production. Other biochemical tests included: oxidase production, catalase production, and nitrate and citrate utilization. Molecular identification was achieved by 16S gene fragment specific for *P. aeruginosa* using the primer pair listed in Table 1.

Antimicrobial sensitivity testing:

Antimicrobial susceptibility was determined using the disc diffusion method, in accordance with the Clinical and Laboratory Standard Institute (CLSI) for the following antibiotics: azithromycin (AZM, 15 µg), ceftazidime (CAZ), tobramycin (TOB, 10 µg), ceftriaxone (CRO, 30 µg), doxycycline (DOX, 5 µg), imipenim (IMP), ceftazidime (FOX, 30 µg), penicillin-tazobactam (TPZ) (Oxoid, Hampshire, England). As previously documented, multidrug resistance (MDR) is defined as resistance to three or more antimicrobial classes

MIC for different antibiotics in the presence or absence of dexamethasone and tenoxicam

In this study, only 16 *P. aeruginosa* isolates were selected for evaluating the effect of dexamethasone and diclofenac on antibiotic sensitivity and bacterial virulence factors. MICs were determined by the broth microdilution method (CLSI, 2016). Each isolate was cultured in Muller-Hinton broth (MHB, Oxoid, UK) and then incubated at 37°C for 24 hours. The prepared suspension was adjusted to 0.5 McFarland by dilution in MHB to have an approximate cell density of 5×10^6 CFU/mL. In a microtiter plate, two-fold serial dilutions of the antibiotic was prepared in MHB starting concentration was 2000 µg/mL for ceftriaxone, in addition with other 9 dilutions. Positive and negative controls were included for validation. Either dexamethasone (0.0052 µg/mL) or diclofenac (1.4 µg/mL) were included in the previous experiment for broth microdilution together with antibiotic. The plates were incubated at 37°C for 24 hours, then the MIC was determined. Antibacterial activity of nanoparticles was studied using 2 different concentrations 64 and 32 µg/mL.

Polymerase Chain Reaction:

Primers used for detection of the *pseudomonas aeruginosa* virulence genes, namely (*algD*, *plch* and *tox4*) are shown in Table 1. PCR amplification reaction was performed in a volume of 25 µL composed of 7.5 µl water, 12.5 µl taq polymerase master mix, 1 µl of each of forwards and backwards primer (10 µM) and 3 µl sample DNA extract. Each PCR reaction was performed in a thermocycler including: initial denaturation: 94°C for 5 min followed by 35 cycles starting with 94°C for 20 seconds, annealing °C (table 1) for 20 seconds as presented in table 1 and extension for 40 seconds at 72°C. After electrophoresis in a 1 % agarose gel, PCR products were visualized under UV trans-illuminator

Determination of bacterial growth curve:

Three different *Pseudomonas* stains were selected based on the results obtained in table 3. Bacterial growth curve was determined by growth determination each hour for 12 hour. The maximum growth rate was fitted with the exponential stage (4-6 hours) after incubation at 37°C.

Preparation of inocula:

Pure colonies of three bacterial isolates were inoculated into 3 mL broth and left for growth overnight at 37°C with shaking. About 100 µL of each overnight broth culture were sub-cultured into 5 mL of broth containing the calculated amount of antibiotic (ceftriaxone), in addition to the calculated amount of dexamethasone or diclofenac, as presented in Table 1 or silver nanoparticles in clove oil in two different concentrations 7 and 15 µg/mL, followed by incubation at 37°C for 4-5 hours with shaking at 110-115 rpm). Bacterial cells were harvested in the middle of exponential phase OD 630 nm (0.1-0.2).

Bacteria total RNA purification and cDNA synthesis:

RNA extraction was performed by using ABT RNA mini extraction kit (Applied Biotechnology, Egypt) according to the manufacturer's instructions. After RNA extraction, purified RNAs were preserved at -80°C till further use. Using NanoDrop instrument (OPTIZEN NanoQ, Mecasys, Republic of Korea), concentration of purified RNA was determined. First-strand cDNA synthesis was performed according to the manufacturer's instructions using a RevertAid H Minus First-Strand cDNA Synthesis Kit (Thermo Scientific). Synthesized first-strand cDNAs were stored at -80°C until use.

Quantitative Polymerase Chain Reaction:

Realtime PCR reaction was performed using MyGo real-time PCR machine in a reaction mix containing: 12.5 μ L (2x) SYBR Green PCR master mix (Willowfort Co., Birmingham, UK.), 1.5 μ L of each forward and reverse primer (10 μ m each), 3 μ L extracted cDNA and nuclease-free H₂O were added to complete the final volume of 25 μ L. Real-time reactions were carried out under the following conditions: 95 °C for 5 min, followed by 45 cycles of 95 °C for 20 s, annealing temperature (Table 2) for 20 s, and finally 72 °C for 40 s. Ct values and melting curve detection were obtained using MyGo real-time PCR machine software. The relative abundance was calculated using the $2^{-\Delta C_t}$ method (where ΔC_t = the average Ct value of each target - the average Ct value of total bacteria) (4).

Size & Shape of nanoparticles:

Nanoparticles were manufactured at 30 nm size approximately in clove oil by nano green synthesis. Electron microscopes utilize electrons which have short wavelengths and thus allow observation of matters with atomic resolution. In this work, TEM studies were conducted on JEOL JEM-2100 high resolution transmission electron microscope at an accelerating voltage of 200 kV. Samples for TEM were prepared by placing a droplet of colloid suspension in respective solvent on a Formvar carbon-coated, 300-mesh copper grid (Ted Pella) and allowing them to evaporate in air at ambient conditions. Nanoparticles characteristics were identified by Malvern zetasizer Nano ZS-90

Molecular Docking

Molecular docking simulations were performed using MOE software, version 2014 (Chemical Computing Group Inc., Canada). The docking protocol utilized the Triangle Matcher placement method followed by a refinement step, treating the receptor as rigid and the ligands as flexible to allow for conformational adjustments. To validate the docking protocol, the native ligand was re-docked into the receptor's active site. The accuracy of the protocol was confirmed by calculating the Root Mean Square Deviation (RMSD) between the docked pose and the original crystallographic pose. A successful validation is indicated by an RMSD value of ≤ 2.00 Å. For each test compound, 30 distinct binding poses were generated during the docking simulation. The binding affinity was initially evaluated using the affinity dG scoring function, and the top 30 poses with the most favourable scores were retained for further analysis. The final binding affinity for the best pose was reported using the S-Score, where a lower value indicates a stronger interaction.

Results:

Antibiotic sensitivity:

A total of 21 isolates of *P. aeruginosa* were included in the study. All isolates were investigated for the antibiotic susceptibility testing and phenotypic characteristics of antibiotic resistance among 21 isolates are shown in figure 1 and table 2. Antimicrobial susceptibility testing revealed that the highest resistance level could be detected in FOX and CAZ with resistance of all isolates (100%) followed by DOX 95.24%, CRO 95.24%. However, lower percentages could be obtained for TOB 76.19%, IMP 61.9%, AZM 57.14% and TPZ 47.62%. Concerning resistance patterns, the mostly detected pattern was pattern P1, P2, P3 and P9 in 14.8% of all isolates with resistance to (CAZ, TOB, IBM, DO, CRO, AZM, TPZ, FOX), (CAZ, TOB, IBM, DO, CRO, AZM, FOX), (CAZ, TOB, IBM, DO, CRO, TPZ, FOX) and (CAZ, DO, CRO, AZM, FOX). In the current study, most of the isolates were resistant to the tested antimicrobials, in which 33 % were considered as XDR, while 52% as MDR.

Using different antibiotic-drug combinations against resistant *P. aeruginosa* isolates, dexamethasone and diclofenac showed different changes in antimicrobial activity. As shown in Table 3, diclofenac has significantly decreased the sensitivity of 3 different isolates to ceftriaxone. Similarly, dexamethasone has also decreased the sensitivity to the same antibiotic to 3 different isolates, which indicates that the investigated drugs had significantly affected the MICs of the antibiotics in some strains.

Molecular identification of some virulence factors

algD, *plcH* and *toxA* genes were detected at different percentages in the 20 isolates in 90%, 95%, 92%, in case of *algD*, *plch*, *toxA*, respectively. GDP-mannose 6-dehydrogenase (*algD*), Hemolytic phospholipase C(*plch*) and exotoxin(*toxA*).

Nano experiment:

One of the objectives of the study was to test Silver NPs in clove oil, as an alternative therapeutic option. The results revealed susceptibility of all isolates to 15 and 7µg/ml

Down regulation of the expression of virulence genes:

Realtime PCR studies indicated decreased growth rate in case of ceftriaxone alone to 93.56%, 57.91% and 98.77% in case of strains 21, 26 and 28 respectively. Interestingly, diclofenac has decreased the growth rate by 24.57, 57.57 and 31.17%, respectively. In contrast dexamethasone could reduce the

growth of one isolate. In case of silver nano particles, a remarkable high decrease in the growth activity to less than 1.12% could be observed indication efficient inhibition of *Pseudomonas aeruginosa*. Realtime PCR studies have shown a significant down-regulation of *toxA* in the 3 isolates in presence of diclofenac with ceftriaxone compared with ceftriaxone alone, however *algD* was partially decreased. In contrast no remarkable effect could be found in *plch*. In case of dexamethasone, down-regulation was apparently found in *toxA* in the 3 investigated isolates compared with ceftriaxone alone (Fig.2,3). Nano experiment using green silver particles has proved approximately 100% inhibitory effect in the expression of *algD*, *plch* and *toxA* in the 3 different isolates 21, 26 and 28

Molecular Docking

Molecular docking was performed to understand the effect of diclofenac by interaction with LsaR quorum sensing protein in *P. aeruginosa*. Results indicated interaction of 2 oxygen group of the carboxylic groups with Arg 61 and chloride group with Thr 115 (Figure 4). Molecular docking studies were also conducted to understand the molecular interactions of ceftriaxone and declofenac within the carbapenemase receptor (Figures 6, 7, 8 and 9). The docking results revealed varying degrees of binding efficacy, as indicated by their respective S scores (Table 6). Molecular docking analysis revealed the binding interactions of declofenac within carbapenemase protein's allosteric site. Diclofenac formed four direct hydrogen bonds: its carboxyl group with Lys229 (3.05 Å), Arg238 (2.92Å) and Thr232 (2.91Å) and its chloride group with Glu161 (3.73 Å). In addition, the binding interactions of ceftriaxone within penicillinase protein's allosteric site. ceftriaxone formed two direct hydrogen bonds: its carbonyl group with ASN 127 (3.54 Å) and thiazolidine ring with THR 232 (2.84 Å). Ceftriaxone demonstrated a strong binding affinity with an S-score of -6.495 kcal/mol (Figure 5). declofenac showed a notable binding affinity with an S-score of -5.1328 kcal/mol (Figure 6). On the other hand, dexamethasone didn't interact (Figure 7). However the interaction of the main legand tazobactam was indicated in figure 9 and table 6

Discussion:

The effect of commonly used non-antibiotic drugs such as dexamethasone and declofenac in infection treated by ceftriaxone on antibiotic resistance and virulence factors of *P. aeruginosa* was investigated. Concentrations selected were 0.0052 µg/mL for dexamethasone and 1.4 µg/mL for declofenac indicating the peaks of the plasma active metabolite hydroxyl (5, 6). Infectious diseases treatment protocols

suggesting the use of corticosteroids and antimicrobials are commonly adopted by prescribers (7). In such combinations, dexamethasone, a synthetic glucocorticoid with its ability to penetrate the central nervous system is widely used in treatment of infectious diseases, mainly to modulate immune responses triggered by microbial varied toxins, lipopolysaccharide and extracellular DNA (8). Using different antibiotic-drug combinations against resistant *P. aeruginosa* isolates, dexamethasone and diclofenac showed different changes in antimicrobial activity. As shown in Table 3, diclofenac has significantly decreased the sensitivity of 3 different isolates to ceftriaxone. Similarly, dexamethasone has also decreased the sensitivity to the same antibiotic to 3 different isolates, which indicates that the investigated drugs had significantly affected the MICs of the antibiotics in some strains. Molecular docking analysis revealed the binding interactions of declofenac and ceftriaxone within carbapenamase protein's allosteric site which can induce steric hinderance therfore the active compound remains unchanged by carbapenamase increasing its activity. In case of dexamethasone activity, by inhibiting some virulence factors, this glucocorticoid can enhance the antimicrobial effect against *P. aeruginosa* and therefore potentiates its effect against resistant bacteria. Realtime PCR studies indicated decreased growth rate in case of AB alone to 93.56, 57.91 and 98.77% in case of strains 21, 26 and 28 respectively. Interestingly, diclofenac has increased the growth rate by 24.57, 57.57 and 31.17%, respectively. In contrast dexamethasone could reduce the growth of isolate 26. Similarly, in docking studies, dexamethasone did not interact. Other studies demonstrated the effect of drugs on virulence factors of *P. aeruginosa*. In a perivious study (8), impairment antimicrobial activity was detected, when combined with dexamethasone. Results of these studies showed that dexamethasone potentiates drug antimicrobial effect against resistant bacteria, which is related to inhibiting the virulence of the bacteria.

In case of silver nano particles, a remarkable high decrease in the growth activity to 1.12% could be observed indication efficient inhibition of *P. aeruginosa*. Realtime PCR studies have shown a significant down-regulation of *toxA* in the 3 isolates in presence of diclofenac with ceftriaxone compared with ceftriaxone alone, however *algD* was partially decreased. In contrast, no remarkable effect could be found in *plch*. In case of dexamthasone, down-regulation was apperently found in *toxA* in the 3 investigated isolates compared with ceftriaxone alone (Fig. 1). Nano experiment using green silver particles has proved approximately 100% inhibitory effect in the expression of *algD*, *plch* and *toxA* in the 3 different isolates 21, 26 and 28. Silver compounds have been historically used to control microbial proliferation. Docking analysis reveals that the stable binding of diclofenac within the catalytic pocket of betalactamase is directly linked to the mechanism of its inhibition. By occupying this site, a potent

inhibitor physically obstructs the binding of ceftriaxone effectively halting its function and disrupting the antibacterial inhibition.

In this study, NPs when used as an alternative therapeutic option, the results revealed susceptibility of all isolates to silver nanoparticles in clove oil, as due to increased surface area to volume ratio, for this reason that the smaller particle size of NPs the higher their antibacterial activity because of their increased surface reactivity and ion release.

CONCLUSION

In conclusion, the impact of drugs on antibiotic resistance did not show sound results. On the other hand, concerning bacterial counts, promising results were obtained in case of diclofenac when combined with antibiotics with a similar influence on the virulence activity. According to docking studies, this may be attributed to its inhibition of carbapenamase enzyme thus preventing antibiotic inactivation.

ETHICAL STATEMENT:

Ethics approval and consent to participate:

The experimental protocol conducted in this study complies with the ethical guidelines adopted by The Research Ethics Committee, Faculty of Pharmacy, Mansoura University which is in accordance with the Code of Ethics of the World Medical Association (The 1975 Declaration of Helsinki). The experimental protocol was approved by The Research Ethics Committee, Faculty of Pharmacy, Mansoura University

Author contribution:

Conception: MMAE. Supervision: MMAE. Study planning: MMAE. Methodology: **AMR, IEL, AAH, IFA and AAA**. Formal analysis: MMAE. Resources: MMAE. Software: MMAE. Data analysis: **AMR, IEL, AAH, IFA and AAA**. Writing of manuscript: **AMR, IEL, AAH, IFA and AAA**. Validation: MMAE. Reviewing and editing of manuscript: MMAE. All authors read and approved the final version of the manuscript.

Informed consent:

Written informed consent is taken by the hospital

Availability of data and materials:

Available on request from the corresponding author

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Table (1): Different drugs and antimicrobial combinations tested in this study.

No Number	Drug	Antibiotic	Concentration
1	-	Ceftriaxone	6 µg/ml
2	Dexamethasone	Ceftriaxone	0,0052µg/ml, 6 µg/ml
3	Diclofenac	Ceftriaxone	1.4 µg/ml, 6 µg/ml
4	Nano silver	-	15 µg/ml
5	Nano silver	-	7 µg/ml

N: In absence of dexamethasone or tenoxicam, **+E:** In presence of tenoxicam , **+T:** In presence of tenoxicam.

Table (2): Amplification primers used in this study for amplifying different genes among *Pseudomonas aeruginosa* isolates.

Primer name	gene	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Annealing temp.
16srRNA-F		CAAACTACTGAGCTAGAGTACG	215	67
16srRNA-R	16srRNA	TAAGATCTCAAGGATCCCAACGGCT		
algD-F		CGCCGAGATGATCAAGTACA	126	55
algD -R	<i>algD</i>	AGGTTGAGCTTGTGGTCCTG		
plcH -F		GAAGCCATGGGCTACTTCAA	307	55
plcH -R	<i>plcH</i>	AGAGTGACGAGGAGCGGTAG		
toxA -F		GGAGCGCAACTATCCCACT	150	55
toxA -R	<i>toxA</i>	TGGTAGCCGACGAACACATA		

F: forward primer R: reverse primer

Table(3) Antibiotic resistance pattern of different isolates based on their sensitivity to 8 different antibiotics:

Pattern code	Antimicrobial resistance pattern	isolates	Pattern %
P1	CAZ, TOB, IBM, DO, CRO, AZM, TPZ, FOX	380, 103, 27	14.29%
P2	CAZ, TOB, IBM, DO, CRO, AZM, FOX	22, 21, 20	14.29%
P3	CAZ, TOB, IBM, DO, CRO, TPZ, FOX	106, 2, 1	14.29%
P4	CAZ, TOB, DO, CRO, AZM, TPZ, FOX	28	4.76%
P5	CAZ, TOB, IBM, DO, CRO, FOX	4, 29	9.52%
P6	CAZ, TOB, IBM, CRO, TPZ, FOX	8	4.76%
P7	CAZ, TOB, DO, CRO, AZM, FOX	23	4.76%
P8	CAZ, TOB, IBM, DO, AZM, FOX	25	4.76%
P9	CAZ, DO, CRO, AZM, FOX	6, 5, 3	14.29%
P10	CAZ, TOB, DO, CRO, FOX	7	4.76%
P11	CAZ, DO, CRO, TPZ, FOX	30	4.76%
P12	CAZ, DO, CRO, FOX	26	4.76%

Table4 starins classification based on their resistance pattern

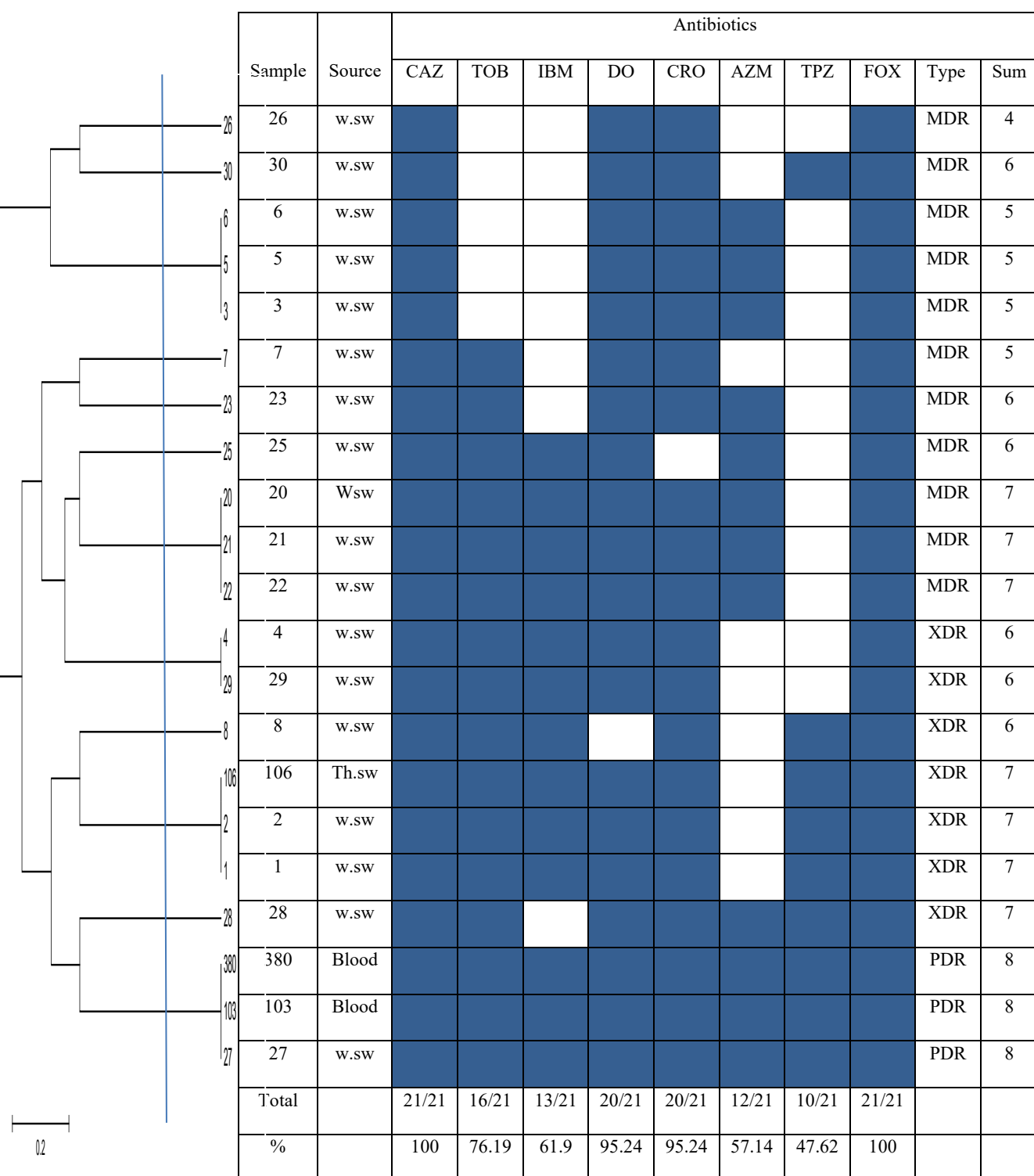


Table5: MIC values of Ceftriaxone for different isolates of *P. aeruginosa* in presence of absence of dexamethasone or diclofenac:

MIC values (µg/ml) Ceftriaxone					
isolate	N	+Di	decrease	+Dx	decrease
4	4000	4000	0	4000	0
26	7	7	0	Sensitive	7
28	125	15	110	15	110
380	4000	4000	0	4000	0
21	4000	2000	2000	4000	0
23	1000	1000	0	1000	0
3	31	15	16	15	16
1	4000	4000	0	4000	0
29	4000	4000	0	4000	0
26	4000	4000	0	4000	0
30	15	15	0	15	0
6	62	62	0	62	0
5	Sensitive	Sensitive	0	Sensitive	0
106	4000	4000	0	4000	0
7	1000	2000	0	1000	0
103	4000	4000	0	4000	0

N: In absence of diclofenac or dexamethasone, +Di: In presence of diclofenac, +Dx: In presence of dexamethasone.

Table 6. Docking results of the two compounds against betalactemase protein

Compound Name	S(Kcal/mol)	RMSD	Hydrogen bond (Distance)	
Ceftriaxone	-6.4956	1.4623	ASN 127 (3.54)	THR 232 (2.84)
Diclofenac	-5.1328	1.5241	GLU 161 (3.73)	THR 232 (2.91)
Dexamethasone	-5.2073	1.3865		
Tazobactam (referance)	-4.84	1.2304	ASN 127 (2.94)	THR 232 (3.07)

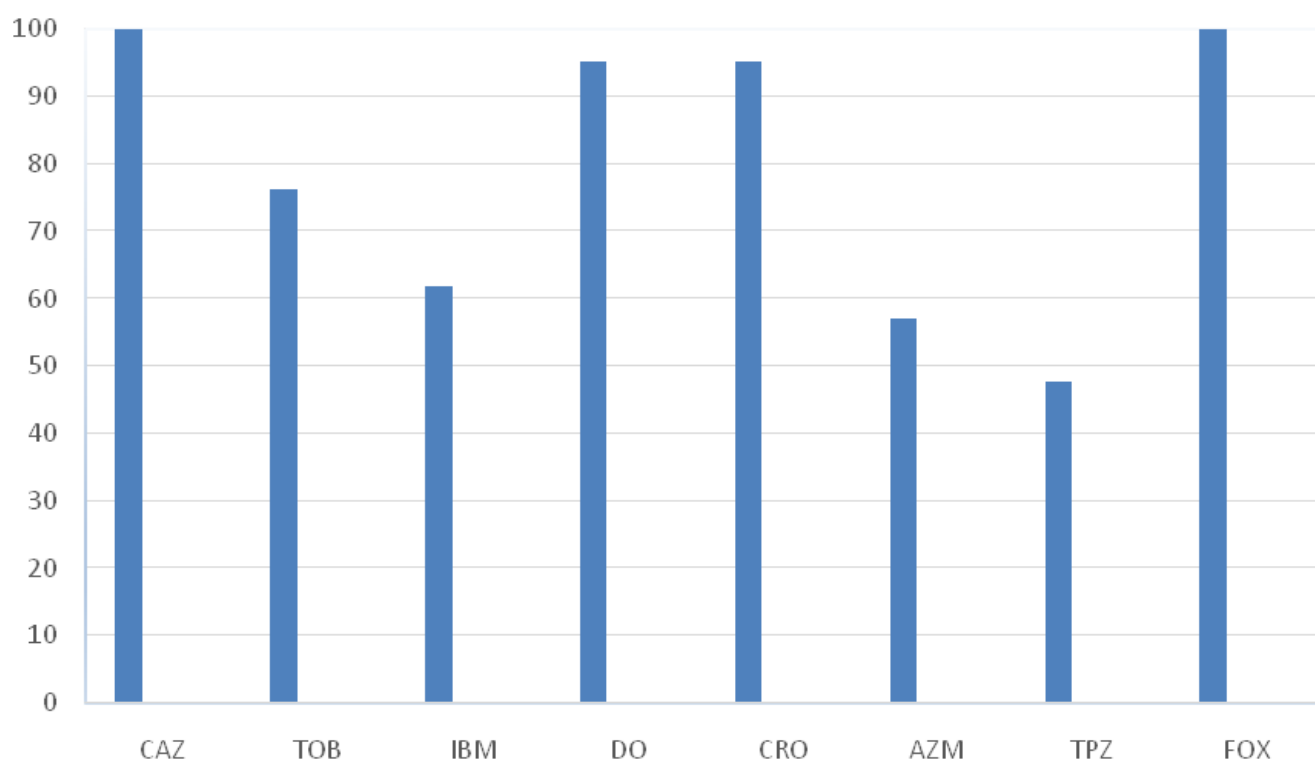


Figure1: antibiotic resistance % of different isolates to 8 different antibiotics

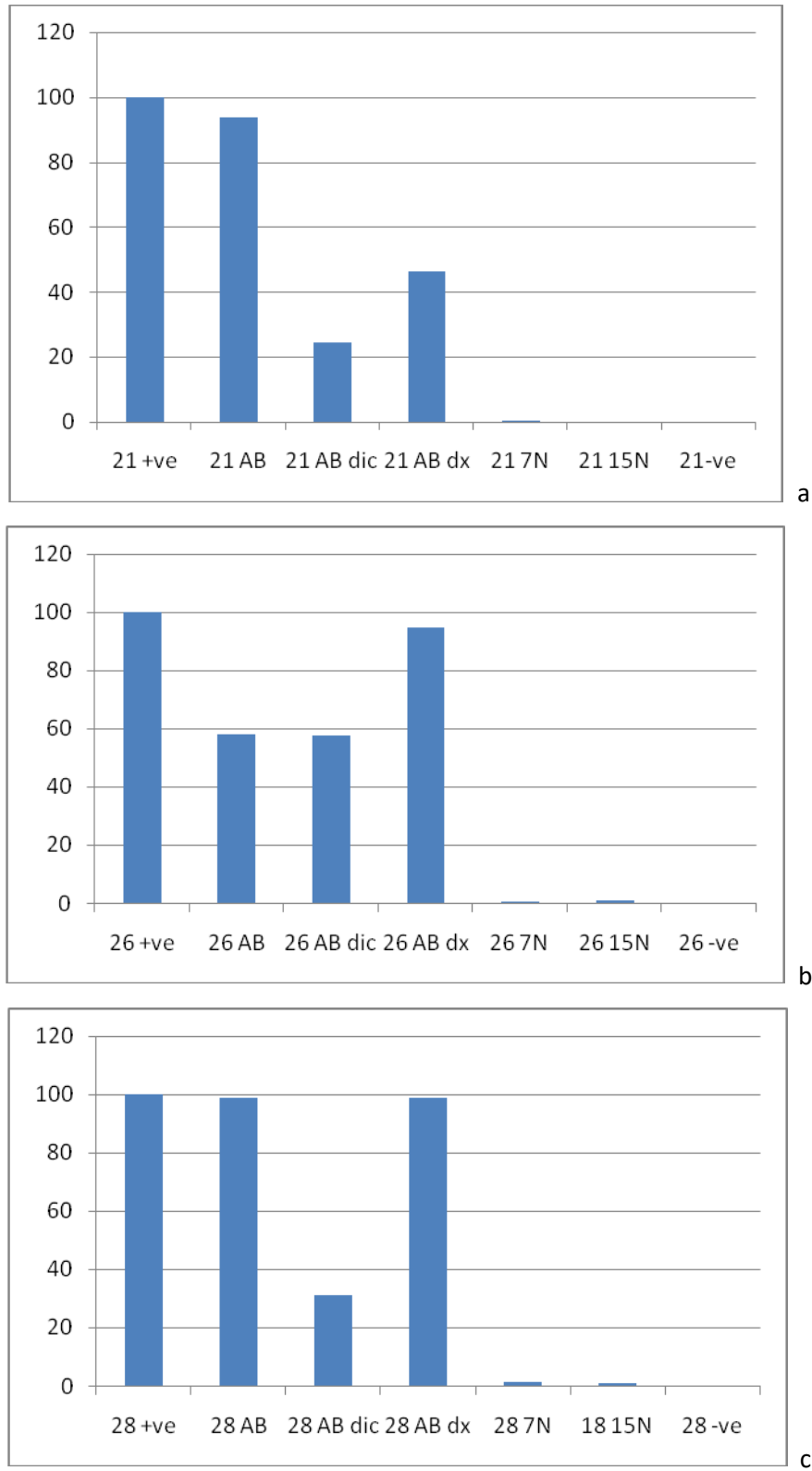
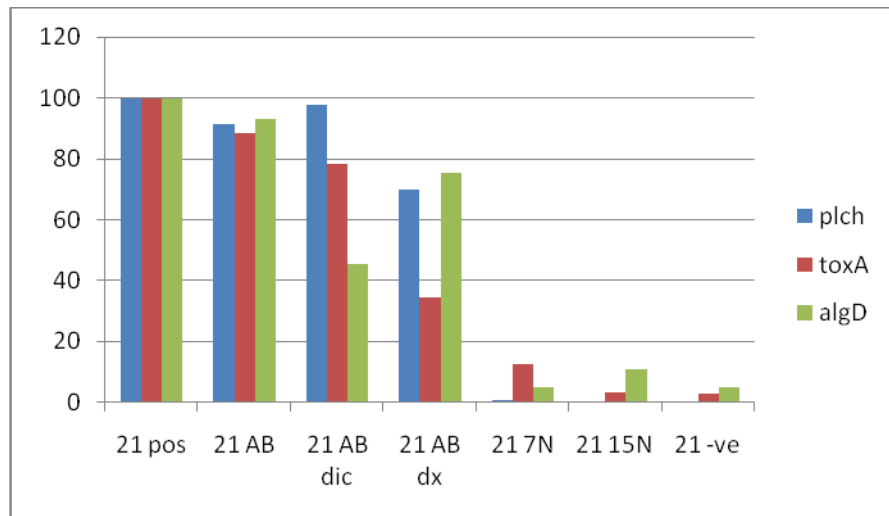
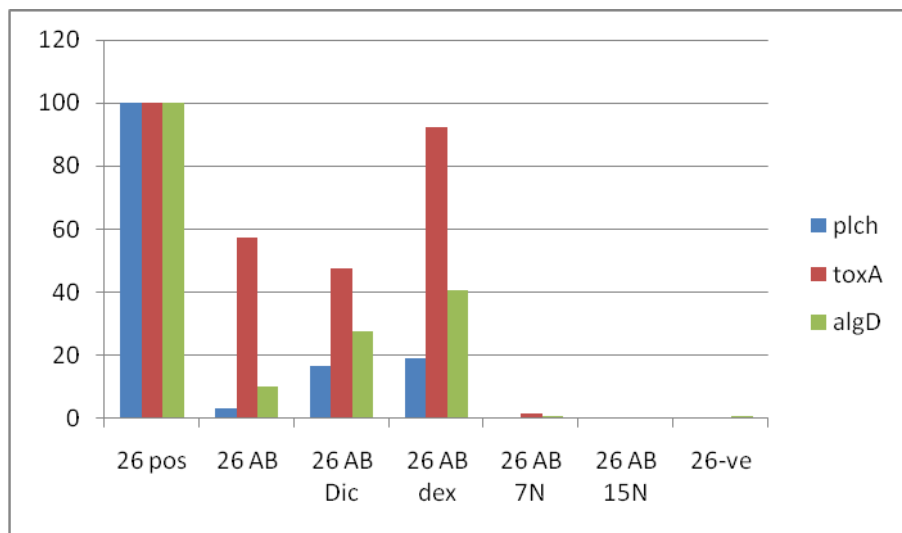


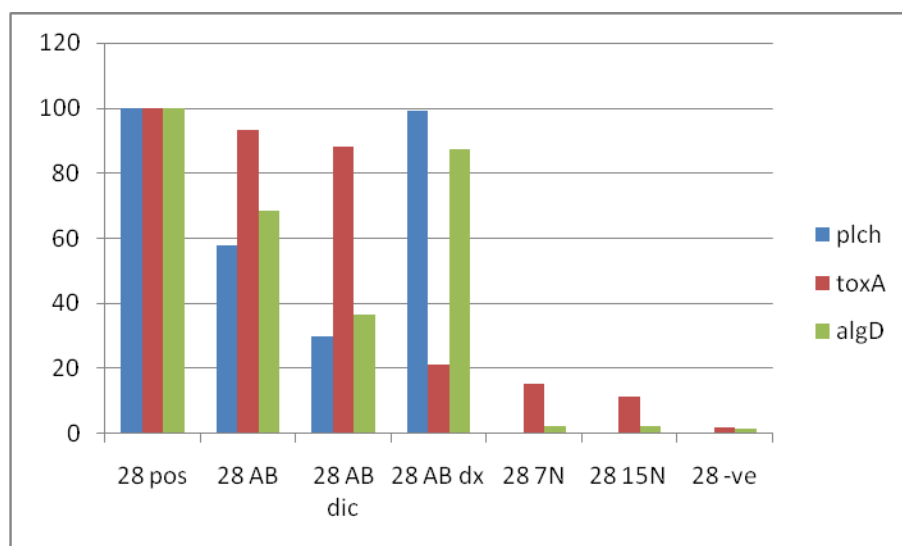
Figure2. Effect of different drugs in addition to ceftriaxone on the growth of *P. aeruginosa*, assessed by real-time PCR for 3 different strains 21, 26 and 28: figures a, b and c respectively.



a



b



c

Figure 3. Effect of different drugs in addition to ceftriaxone on *P. aeruginosa* virulence factors genes *toxA*, *plcH* and *algD*, assessed by real-time PCR for 3 different isolates 21, 26 and 28: figures a, b and c respectively.

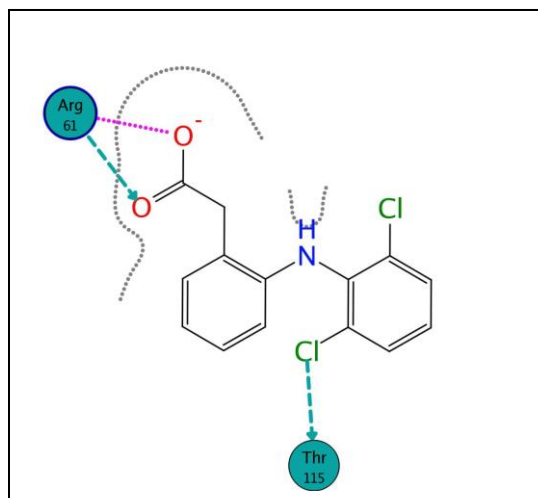
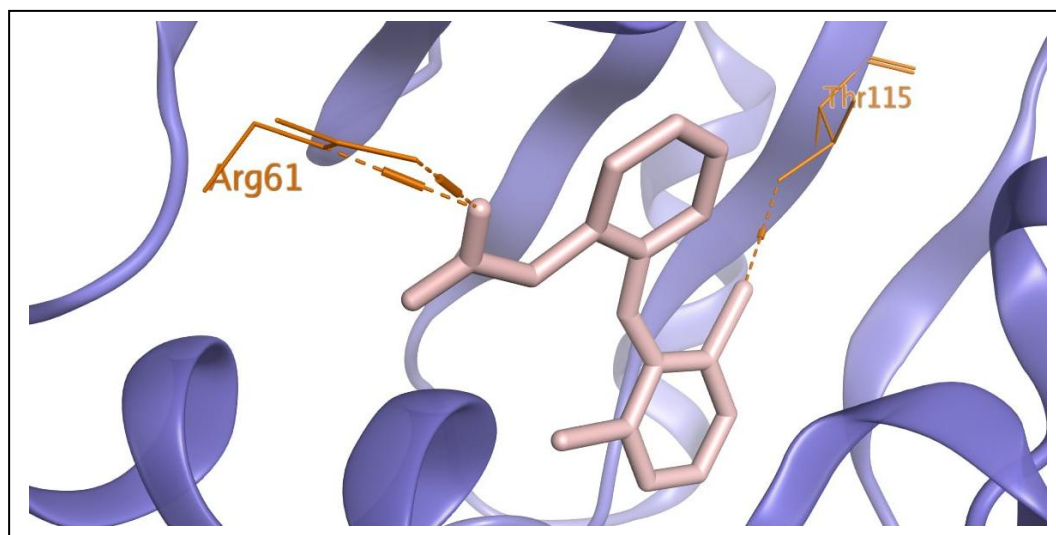


Fig 4. Molecular interactions post docking process of diclofenac with LsaR

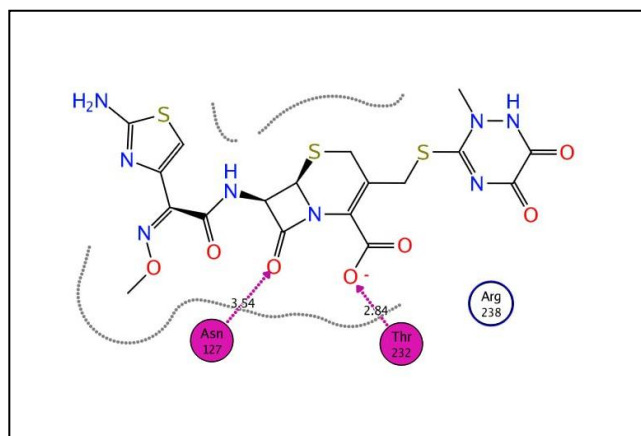
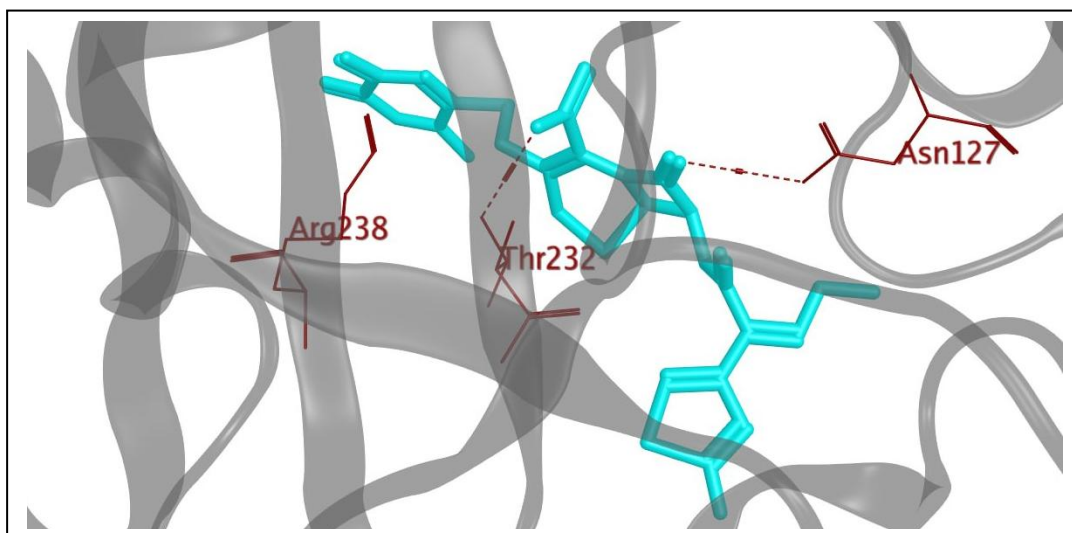


Fig 5. Molecular interactions post docking process of ceftriaxone with Carbapenamase

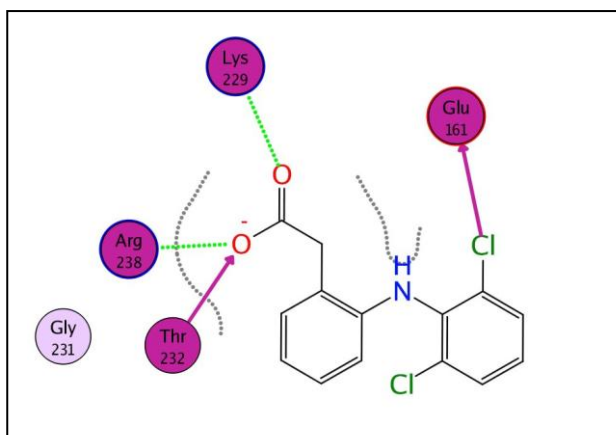
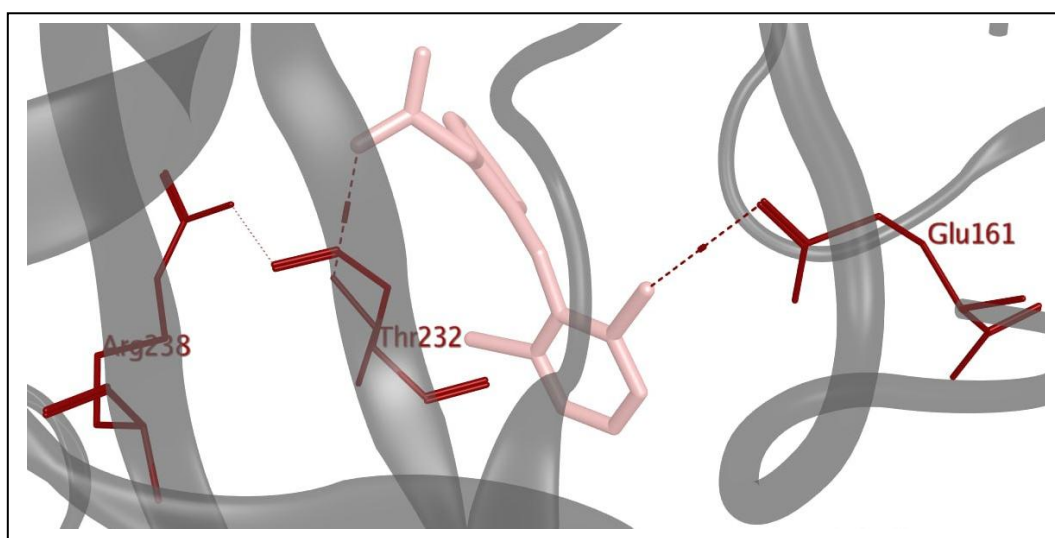


Fig 6 Molecular interactions post docking process of diclofenac with Carbapenamase

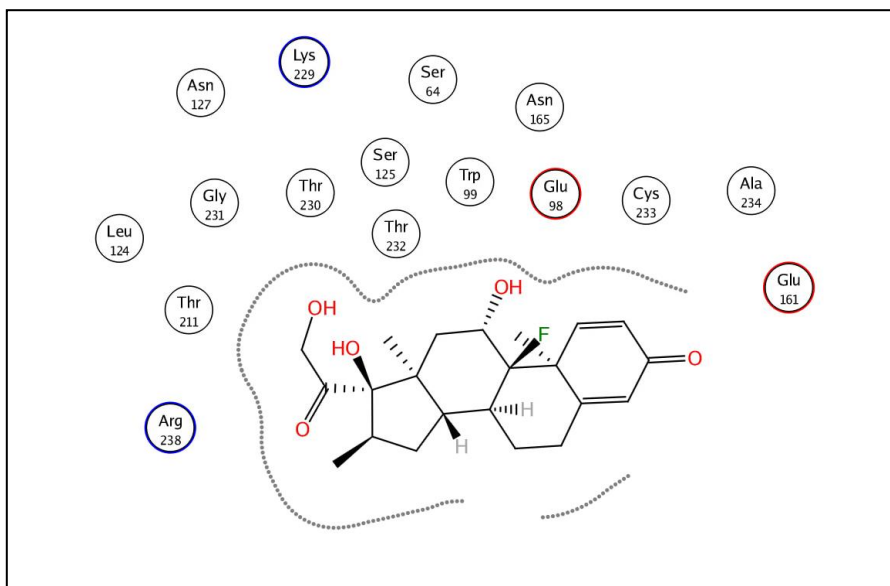


Fig 7 Molecular interactions post docking process of dexamethasone with Carbapenemase

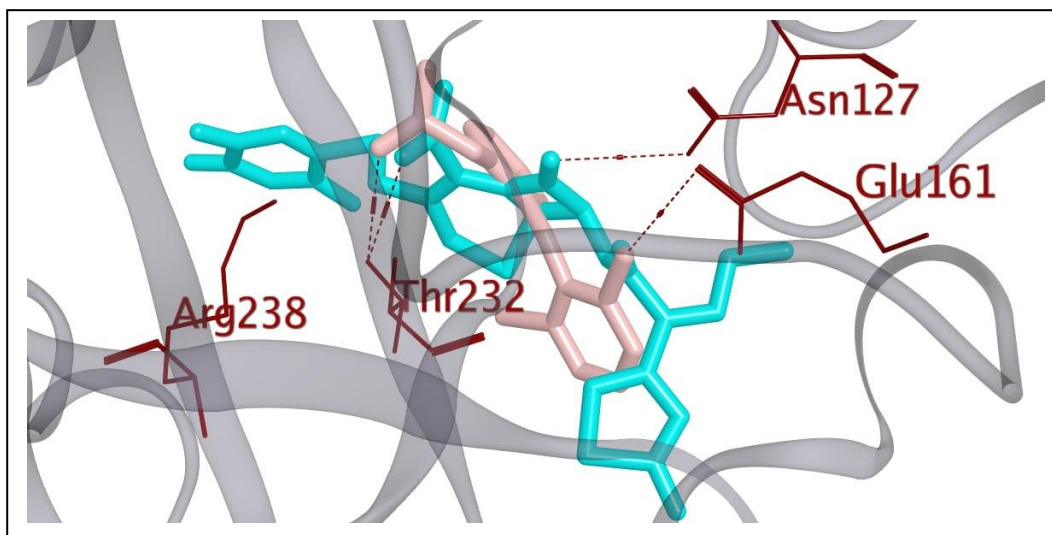


Fig 8 Molecular interactions post docking process of diclofenac and ceftriaxone with Carbapenemase interacting at the same site indicating steric hindrance

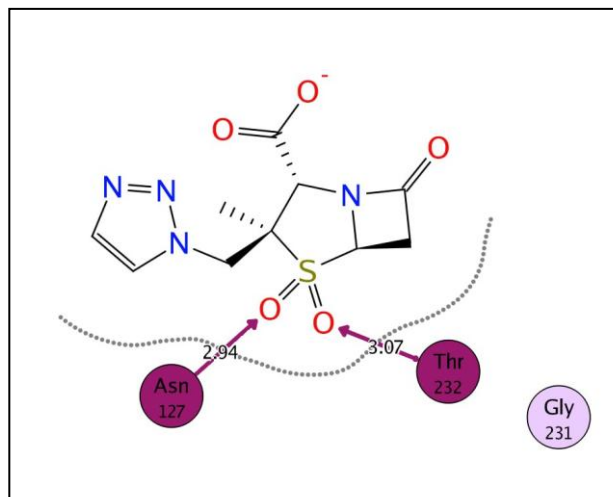
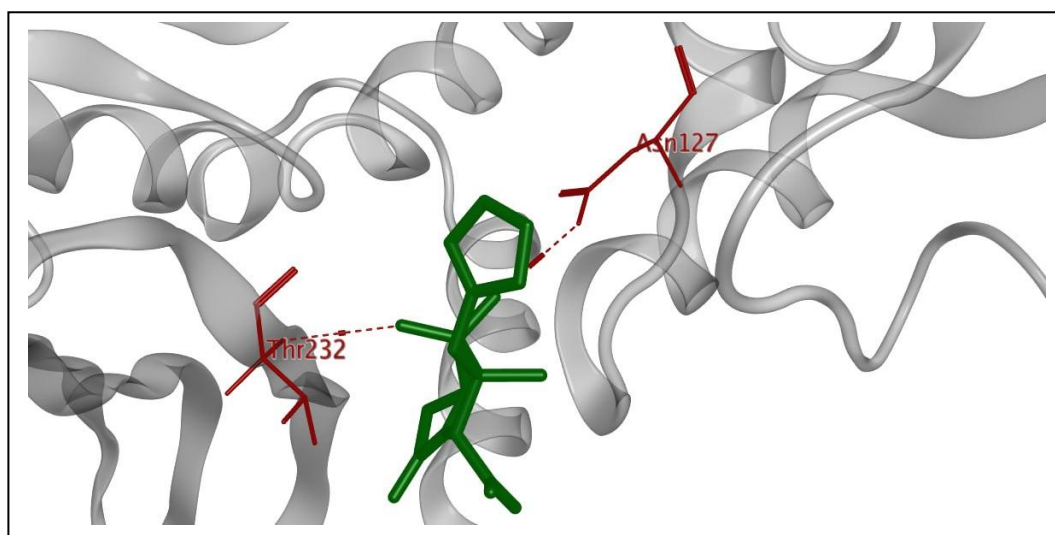


Fig 9. Molecular interactions post docking process of Tazobactam with Carbapenamase