ORIGINAL ARTICLE

EXPLORING AMINOPENICILLIN RESISTANCE IN UROPATHOGENIC ESCHERICHIA COLI AND OPTIMIZED LEAD PREDICTION TO OVERCOME RESISTANCE THROUGH NEW GENERATION TOOLS

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ABSTRACT

Introduction: Urinary tract infections (UTIs) are a prevalent condition caused by bacteria such as *Escherichia coli, Klebsiella, Proteus, Staphylococcus, Enterococcus, and Pseudomonas aeruginosa* species. According to the Ministry of Health and Population's 2079/80 annual report, Nepal recorded 5,284 cases of UTI-related morbidity. The growing burden of infectious diseases and antimicrobial resistance poses a significant threat to public health.

Method: A retrospective, descriptive cross-sectional study was conducted at a tertiary care hospital in Kathmandu, Nepal, from July 2023 to January 2024. A purposive sampling technique was employed, and the collected data were analyzed using various standard software, web servers, and statistical modules.

Results: Out of 3,000 urine samples, 246 exhibited significant bacterial growth. Among the isolates, Escherichia coli, the predominant Gram-negative bacterium (63.4%), demonstrated 88.46% resistance to amoxicillin. Similarly, Staphylococcus aureus, the dominant Gram-positive isolate (6.3%), showed 50% resistance to ampicillin, indicating a rising trend of resistance to aminopenicillins.

Conclusion: Amino Penicillin resistance in Escherichia coli species is at high levels, BA-3 and BA-10 emerges as a promising candidate with better physiochemical and pharmacokinetics parameters for future therapeutic development. Experimental validation and exploration of additional resistance mechanisms are recommended for comprehensive treatment strategies.

Key words: ***Antibiotic Resistance, Aminopenicillin, Escherichia coli, Urinary Tract Infections, Molecular Docking, DNA-Gyrase***

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INTRODUCTION

Urinary tract infections (UTIs) occur due to microbial invasion of the urinary system, extending from the renal cortex to the urethral meatus. UTIs are confirmed by the presence of both Gram-negative and Gram-positive bacteria, as well as certain fungi. Uropathogenic Escherichia coli is the most common causative agent, followed by Klebsiella pneumoniae, Staphylococcus saprophyticus, Enterococcus faecalis, Streptococcus species, Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus, and Candida species[1] The clinical symptoms of a urinary tract infection (UTI) typically include a triad of dysuria (painful urination), urgency (a strong urge to urinate), and frequency (increased urination rate[2].

WHO (2017) published a priority list of antibiotic-resistant pathogens that pose the greatest threat to human health. In 2019, there were 0.4 billion global cases of urinary tract infections (UTIs), marking a 60.40% increase since 1990. Alongside this rapid rise in UTI cases, there has been a concerning increase in bacterial antimicrobial resistance (AMR) associated with UTIs [3,4]An estimated 1.27 million deaths were attributable to antimicrobial-resistant infections in 2019 alone, while nearly 5 million deaths were somehow associated with drug-resistant infections, according to a major study published in January 2022[5,6]

Despite the extensive synthesis and testing of β-lactam antibiotics, the growing bacterial resistance necessitates the development of new compounds. Penicillin resistance arises due to structural modifications in penicillin-binding protein (PBP) targets and the production of β-lactamase enzymes [7]

The core structure of penicillin consists of a thiazolidine ring fused with a β-lactam ring. It features a sulfur atom at position 1, geminal dimethyl groups at carbon-2 (C-2), and a carboxylic acid moiety at carbon-3 (C-3)[8]. Amoxicillin, a key member of the β-lactam antibiotic class, is known as 6-[D-α-(p-hydroxyphenyl) acetamido] penicillanic acid. It is an analog of ampicillin, derived from the core penicillin nucleus (9) Amoxicillin, a key member of the β-lactam antibiotic class, is known as 6-[D-α-(p-hydroxyphenyl) acetamido] penicillanic acid. It is an analog of ampicillin, derived from the core penicillin nucleus.

The addition of a hydroxyl group in amoxicillin, compared to ampicillin, enhances its lipid solubility, leading to increased bioavailability, prolonged duration of action, and improved bactericidal activity, as observed in pharmacodynamic studies[10]The spectrum of activity against both Gram-negative and Gram-positive bacteria can be enhanced by modifying the side chain amide functionality with various heterocyclic ring systems, such as 1,2,4-triazoles, pyrroles, oxazoles, thiazoles, indoles, and six-membered rings including piperazine, piperidine, and their derivatives. These compounds serve as promising scaffolds for the development of antimicrobial, anticancer, antiviral, antimalarial, antitubercular, antifungal, anti-inflammatory, and enzyme-inhibiting drugs[11].

Modern drug discovery is mainly based In-silico-chemobiological approach[12] Further, CADD (computer-aided drug design) provides more details and aid to coordinate the information to make the drug design more rational.[13] Molecular docking is a key tool in computer-assisted drug design and molecular biology and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization[14] However, the pace of drug discovery and development in the 21st century struggles to keep up with the rapid and alarming rise in antibiotic resistance. Without a sustained supply of effective antibiotics, it is projected that deaths due to antimicrobial resistance could surpass those caused by cancer [15,16].

How to Cite

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We hypothesize that novel amino penicillin derivatives, designed through in-silico methods, possess enhanced potential to inhibit or modify the DNA gyrase enzyme, acting as effective antibacterial agents to combat antibiotic resistance. This study aims to identify the most promising amino penicillin derivatives for further development.

METHODS

Ethics approval: The study protocol was approved by the Institutional Review Committee of Manmohan Memorial Institute of Health Sciences (MMIHS-IRC) [Reference No: NEHCO/IRC/080/99]. Additionally, permission to collect data at Manmohan Memorial Medical College and Teaching Hospital (MMTH) in Kathmandu was granted by the hospital board [Reference No: 241].

Procedure of Penicillin's Resistance Prevalence Determination A retrospective observational study was conducted at Manmohan Memorial Teaching Hospital (MMTH) in Kathmandu. Data were collected over six months from both inpatient (IPD) and outpatient (OPD) departments through the hospital's medical record department. The study focused on patients who had undergone urine culture and sensitivity tests for urinary tract infections (UTIs). Cases without bacterial isolation were excluded from the analysis. The data were systematically entered into Excel 2019, and descriptive statistics were applied to evaluate socio-demographic variables. Furthermore, the percentage of penicillin-resistant microorganisms among the isolates was determined.

Procedure of lead Optimization through in-Silico methods

Molecular Docking: AutoDock 4.2[17] and chat-GPT [18] was utilized to predict the binding interactions of small molecules, including penicillin, with the DNA gyrase of Escherichia coli, a known target for urinary tract infections (UTIs). In-silico methods were employed to identify potential lead compounds among novel penicillin derivatives.

Preparation of Ligand: Ligands were prepared using a range of computational tools. PubChem[19] was used to obtain SDF files for existing amoxicillin and ampicillin, while Marvin Sketch[20] was employed to design novel penicillin derivatives in SDF format. These files were then converted into PDB format using Discovery Studio and further transformed into PDBQT format via AutoDock software[21] The ligands consisted of amino penicillin acetyl ester derivatives with various scaffolds at positions [R1] and [R2].

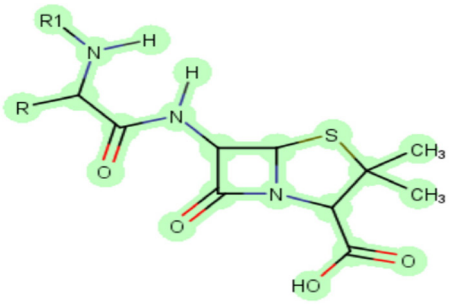
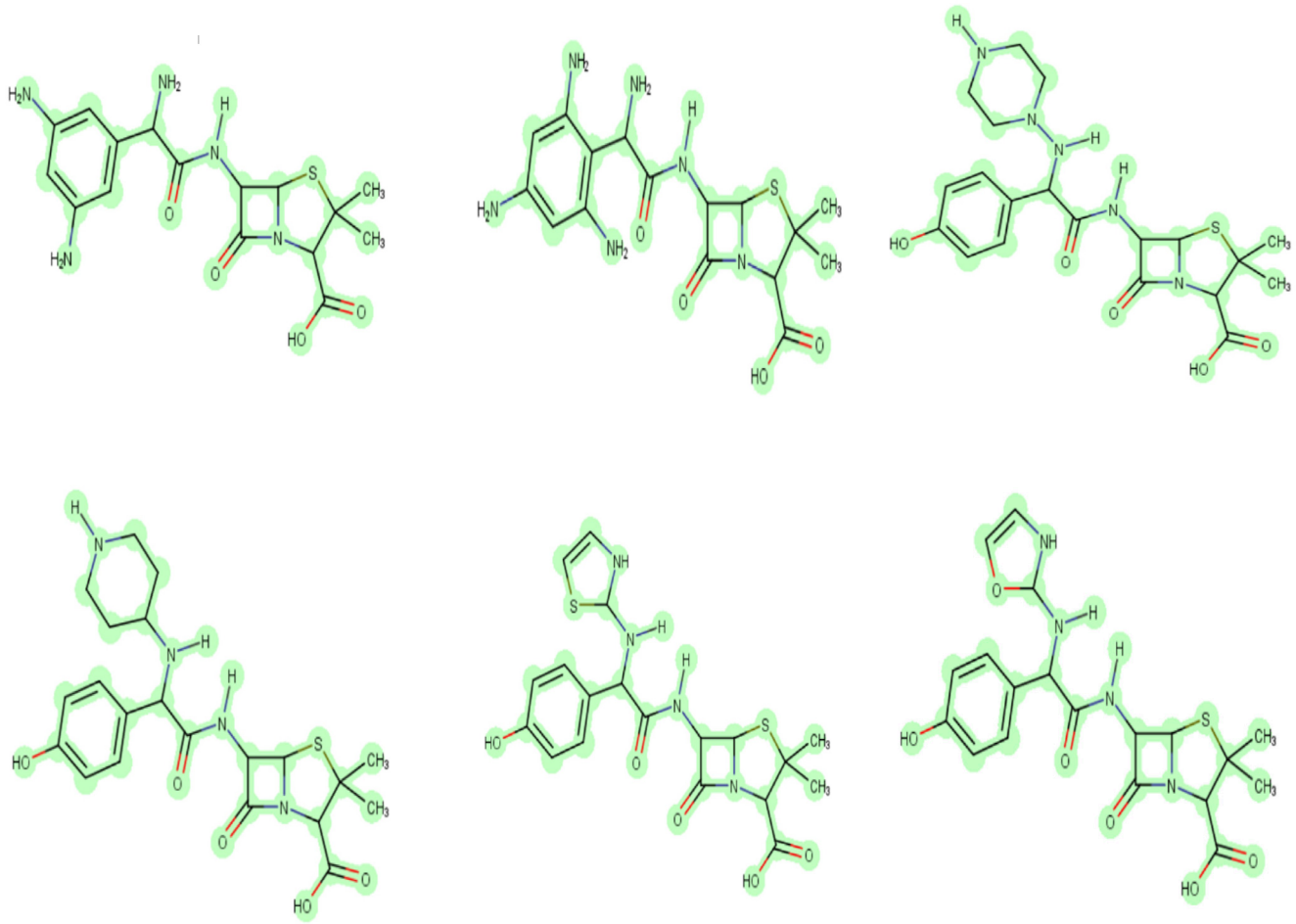


Figure 1: Basic Nucleus of Novel Amino Penicillin Derivatives



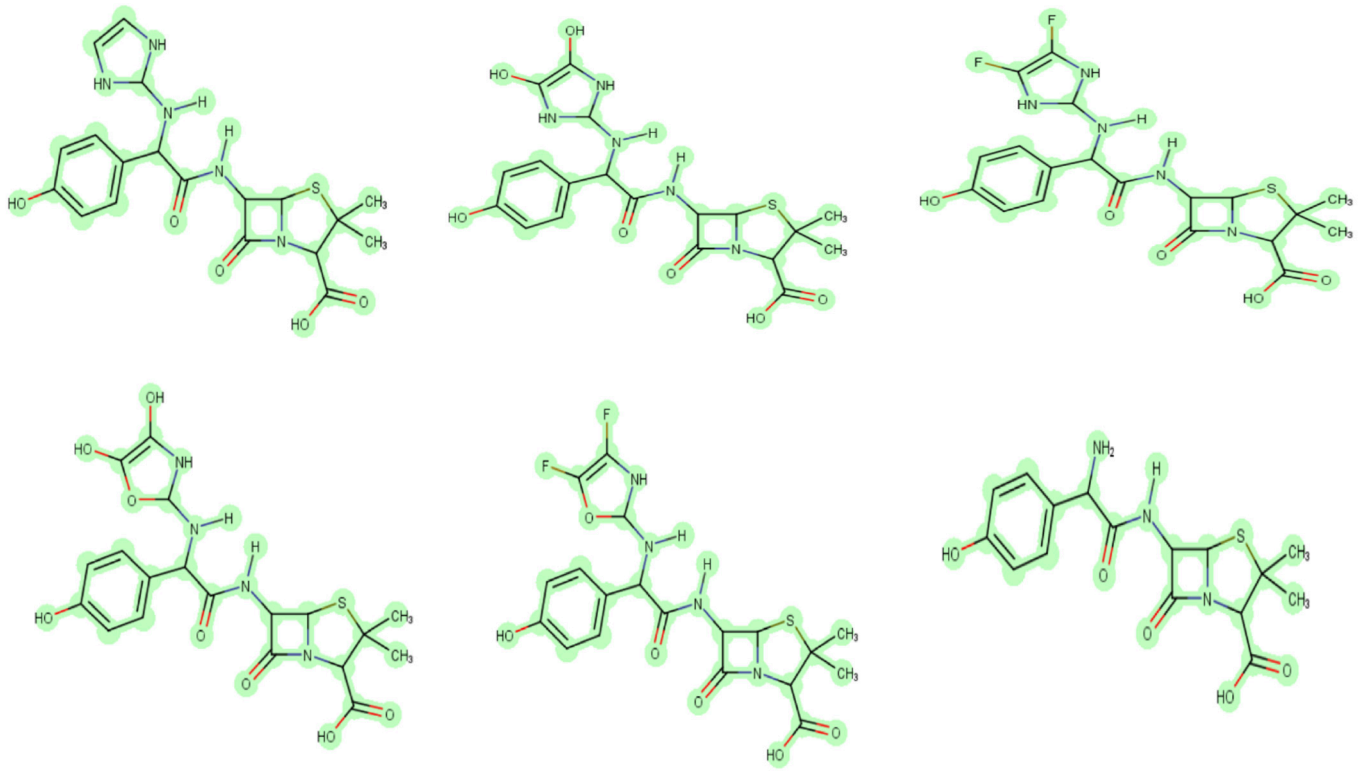


Figure 2: Novel substituted Amino Penicillin Derivatives

Preparation and validation of Protein: The protein structure of the DNA gyrase of Escherichia coli (PDB ID: 4KFG) was obtained from the Protein Data Bank (PDB) (https://www.rcsb.org/)[22]using its designated PDB-ID . The structure was assessed to ensure it met the required parameters, including x-ray diffraction resolution, absence of mutations, and validation through the Ramachandran plot. Optimization involved cleaning the structure, removing irrelevant residues, correcting structural errors, and incorporating polar hydrogen bonds. The protein was validated by inbound ligand. The final refined structure was then saved in PDB format for further docking studies.

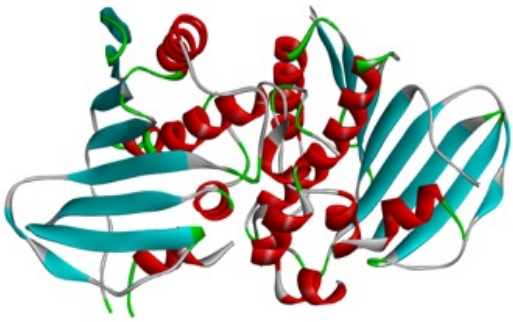


Figure 3: DNA-Gyrase of Escherichia coli (PDB ID: 4KFG)

Identification of Binding Pocket: The active binding site of the protein was determined using the Cast-P server (http://cast.engr.uic.edu)[23], with the amino acid sequence serving as the binding pocket for blind docking. The residues involved in the active site were meticulously analysed and documented in Figure–4 and Table–1.

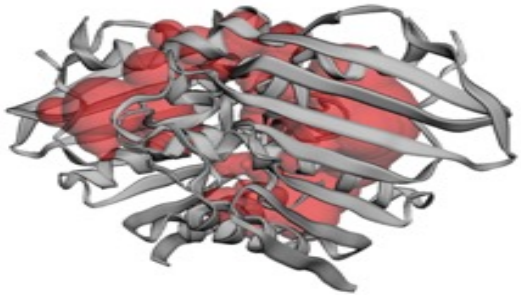


Figure 4: Active binding pockets of DNA-Gyrase of Escherichia coli

Table 1: Active Binding pocket of DNA-Gyrase of Escherichia coli

GLY-15, ALA-18, MET-25, TYR-26, GLU-42, ASP-45, ASN-46, ASP-49, VAL-89, ILE-90, VAL-93, LEU-94, LYS-110, VAL-111, SER-112, GLY-113, GLY-114, LEU 115, HIS-116, GLY-117, VAL-118, GLY-119, VAL-120, SER-121, VAL-122, ARG-190, LEU-197, GLY-24, MET-25, TYR-26, ILE-27, GLY-28, ASP-29, ASP-32, GLY-33, THR-34, HIS-37, HIS-38, PHE-41, GLU-42.

Pharmacokinetic and Toxicity Prediction: The pharmacokinetic properties of all ligands, including gastrointestinal (GI) absorption, distribution, metabolism, and excretion (ADME), were predicted using the SWISS ADME web server (http://www.swissadme.ch/) [24]. Furthermore, their toxicity profiles were assessed using the ProTox-II web server (https://toxnew.charite.de/)[25], facilitating the identification of safe and effective drug candidates.

Biological Activity Prediction: To validate the docking results, the PASS web server (https://www.way2drug.com/passonline/)[26]was

utilized to predict the biological activity of the bioactive compounds, with a focus on antibacterial potential. The analysis revealed that the probability of activity (Pa) exceeded the probability of inactivity (Pi), indicating the compounds' potential antibacterial properties.

Docking Procedure:

Molecular docking studies were conducted using AutoDock Vina to evaluate ligand-receptor interactions. The binding energy was calculated using the formula:

$$\Delta G_{Binding} = \Delta G_{Gauss} + \Delta G_{Repulsion} + \Delta G_{H\text{-}Bond} + \Delta G_{Hydrophobic} + \Delta G_{Tors}$$

Where:

ΔG_{Gauss} → represents the dispersion of two Gaussian functions,
$\Delta G_{Repulsion}$ → accounts for repulsion beyond a threshold distance,
$\Delta G_{H\text{-}Bond}$ → models hydrogen bond interactions,
$\Delta G_{Hydrophobic}$ → is a ramp function for hydrophobic interactions, and
ΔG_{Tors} → is proportional to the number of rotatable bonds.

The protein structure was imported into AutoDock 4.2, converted into PDBQT format, and prepared for docking. Ligands were uploaded, their geometrical energies were minimized to achieve the most stable conformation, and they were subsequently converted into PDBQT format for docking analysis. The docking grid parameters were set with the following values: x: 14.2771 y: 18.6797 z: 13.1865 with dimensions of x: 62.8876 y: 46.4289 z: 61.7872. The obtained conformations were further analysed using Discovery Studio 2023.

RESULT

Among 3,000 patients diagnosed with urinary tract infections (UTIs), 246 samples exhibited significant microbial growth, with each sample containing a single microorganism. Females accounted for a substantial 69.5% of the cases. The highest number of specimens (110; 44.7%) belonged to the 31-60 years age group. The prevalence of Gram-negative bacteria was significantly higher, with 217 (88.2%) cases, compared to Gram-positive bacteria, which were observed in 29 (11.8%) cases. Twelve pathogenic bacteria were identified from 246 urine samples collected from patients. Escherichia coli was the most prevalent isolate, detected in 156 samples (63.4%). Other bacterial species included Staphylococcus aureus in 16 samples (6.5%), Staphylococcus epidermidis in 4 samples (1.6%), and Staphylococcus saprophyticus in 2 samples (0.8%).

Table 2: Socio-demographic and Clinical Characteristics

Variables	Category	Frequency	Percentage
Gender	Male	75	30.5
	Female	171	69.5
Age Group	1-30	81	32.9
	31-60	110	44.7
	61-90	55	22.4
Bacteria Type	Gram Negative	217	88.2
	Gram Positive	29	11.8
Isolated Bacteria	E. coli	156	63.4
	S. Aureus	16	6.5

The resistance pattern of the most common Gram-negative isolates was analysed, focusing on Escherichia coli, which was responsible for 156 infections. The highest resistance was observed against amoxicillin (88.46%), followed by cefixime (55.77%), ceftriaxone (42.31%), cotrimoxazole (41.03%), and ciprofloxacin (30.77%). Lower resistance rates were recorded for nitrofurantoin (11.54%), gentamicin (8.97%), ceftazidime (5.13%), ampicillin (5.13%), amikacin (4.49%), levofloxacin (3.85%), ofloxacin (3.21%), piperacillin (3.21%), piperacillin and tazobactam (2.56%), imipenem (2.56%), and meropenem (1.92%).

Molecular docking analysis

In our study, designed novel substituted amino penicillin derivatives were designed and docked with DNA gyrase of Escherichia coli PDB-ID [4KFG] to evaluate their potential antibacterial activity. The binding energy, number of hydrogen bonds, bond distance, and interacting amino acids are summarized in Table 3. The ligand with the lowest binding energy, higher number of hydrogen bonds, shorter bond distance, and greater amino acid interactions was identified as the most promising candidate for further investigation.

Table 3: Docking result of novel substituted amino penicillin derivatives with binding affinity and number of amino acids interaction.

SN	DNA gyrase of Escherichia coli PDB-ID [4KFG]	
	Binding Affinity	Number of Amino acid with bond length [A°]
AMX	-7.7	ASP45: 2.25, ASN46: 2.55, ASP49: 2.09, LEU115: 3.09
BA-1	-7.9	GLU42: 1.66, ASP45: 2.72, ASN46: 1.84, ASN107: 2.43, LEU115: 2.15
BA-2	-8.1	GLU42:2.78, ASP45: 2.51, ASN46:2.48, ASP49: 2.27, ASP106: 2.64, ASN107: 1.98, LEU115: 2.59, VAL118: 2.21
BA-3	-9.0	GLU42: 2.23, ASN46: 2.45, ILE90: 2.86, LEU115: 2.06, VAL120: 2.24, SER121: 1.91
BA-4	-8.4	LPEU115: 1.87
BA-5	-8.4	GLU42: 1.82, ASN46: 2.74
BA-6	-8.7	ASN46: 2.06
BA-7	-9.1	ASN46: 2.86, LEU115: 2.09
BA-8	-9.0	GLU42: 2.14, ASN46: 1.93, VAL93: 2.52, VAL118: 2.25
BA-9	-8.9	ASN46: 1.81, GLU50: 2.48
BA-10	-9.6	ASN46: 1.80, ASP49: 2.50, LEU94: 2.87, SER108: 2.59, LEU115: 2.66, VAL120: 2.06, SER121: 1.90
BA-11	-9.2	ASN46: 2.06, SER121: 2.47

Biological activity prediction: Docking and pharmacokinetic parameters were further validated using a biological activity prediction tool, which assessed the antibacterial, antibiotic, penicillin-like, and β-lactam-like properties of 11 Nobel substituted amino penicillin derivatives. The probability of activity (Pa) for the Nobel substituted amino penicillin derivatives was as follows: Antibacterial activity: $0.549 < Pa < 0.771$, Antibiotic activity: $0.372 < Pa < 0.598$, Penicillin-like antibiotic activity: $0.398 < Pa < 0.700$, β-lactam-like antibiotic activity: $0.118 < Pa < 0.290$. In all cases, the Pa values were higher than the probability of inactivity (Pi), suggesting that these compounds have potential antibacterial activity.

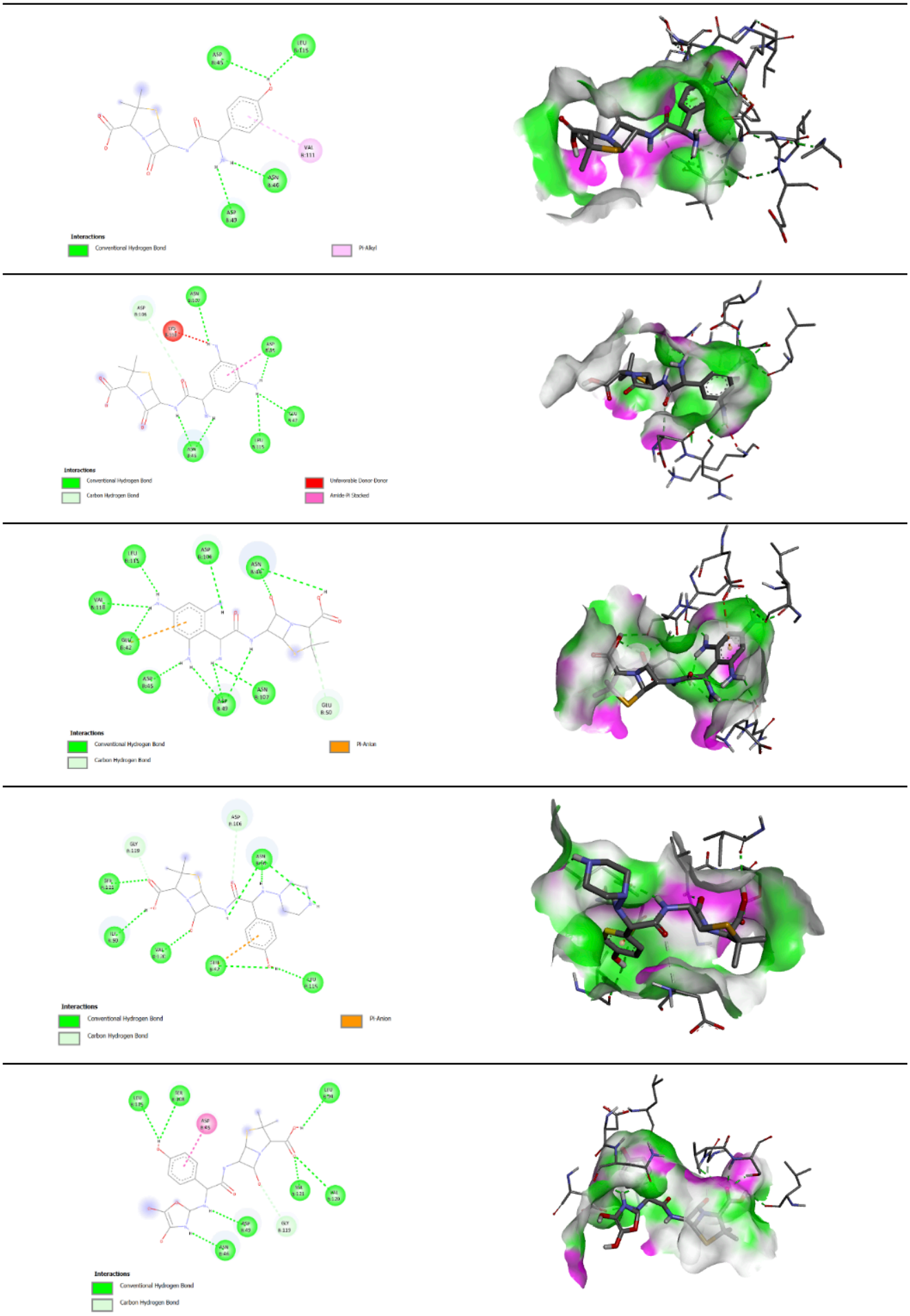


Figure 5: 2D and 3D interaction of [AMX, BA-1, BA-2, BA-3, BA-10] with DNA-Gyrase of Escherichia coli.

Table 4: probability of biological activity of Nobel substituted amino penicillin derivatives

SN	Pa-value	Pi-value	Biological Activities
AMOXICILLIN AMX	0,761	0,003	Antibacterial
	0,581	0,003	Antibiotic
	0,694	0,000	Antibiotic Penicillin-like
	0,278	0,001	Antibiotic beta Lactam-like
BA-1	0,771	0,003	Antibacterial
	0,598	0,003	Antibiotic
	0,691	0,000	Antibiotic Penicillin-like
	0,281	0,001	Antibiotic beta Lactam-like
BA-2	0,764	0,003	Antibacterial
	0,583	0,003	Antibiotic
	0,700	0,000	Antibiotic Penicillin-like
	0,290	0,001	Antibiotic beta Lactam-like
BA-3	0,710	0,004	Antibacterial
	0,496	0,005	Antibiotic
	0,400	0,000	Antibiotic Penicillin-like
	0,144	0,001	Antibiotic beta Lactam-like
BA-8	0,594	0,009	Antibacterial
	0,372	0,009	Antibiotic
	0,459	0,000	Antibiotic Penicillin-like
	0,148	0,001	Antibiotic beta Lactam-like
BA-10	0,664	0,006	Antibacterial
	0,461	0,006	Antibiotic
	0,398	0,000	Antibiotic Penicillin-like
	0,118	0,001	Antibiotic beta Lactam-like

Table 5: Lipinski's Rule and pharmacokinetic analysis

SN	RB	HBA	HBD	Mol. Wt	LogP	LogS	LogKp	BBB	GI-ABs	BA	SA
AMX	5	6	4	365	1.46	-0.70	-9.9	No	Low	0.55	4.17
BA-1	5	5	5	379	0.85	-0.46	-10.3	No	Low	0.55	4.33
BA-2	5	5	6	394	0.87	-0.47	-10.5	No	Low	0.55	4.38
BA-3	7	8	5	449	2.02	-1.11	-10.3	No	Low	0.55	4.86
BA-4	7	7	5	448	2.11	-1.45	-9.9	No	Low	0.55	4.67
BA-5	7	6	5	450	1.49	-1.90	-9.5	No	Low	0.55	4.91
BA-6	7	7	5	434	1.61	-1.46	-9.8	No	Low	0.55	4.87
BA-7	7	6	6	433	1.60	-1.46	-9.8	No	Low	0.55	4.78
BA-8	7	8	8	465	0.81	-1.46	-10.2	No	Low	0.17	4.90
BA-9	7	8	6	469	1.41	-2.06	-9.5	No	Low	0.55	4.90
BA10	7	9	7	466	1.21	-1.46	-10.2	No	Low	0.17	5.00
BA11	7	9	5	470	1.98	-2.06	-9.5	No	Low	0.55	4.98

Lipinski's Rule and Pharmacokinetic parameters analyses:
The docking data are further validated by the drug's physicochemical properties and its absorption, distribution, metabolism, and excretion (ADME) characteristics, as presented in the table-5. All derivatives comply with Lipinski's rule, exhibiting an optimal molecular weight of less than 500 Daltons, no more than 10 hydrogen bond acceptors, and no more than 5 hydrogen bond donors. Additionally, they demonstrate favourable lipophilic properties, low skin permeability, bioavailability and synthetic accessibility.

Toxicity Prediction:
The toxicity assessment of novel amino penicillin derivatives was conducted to evaluate their safety profile. Various toxicological parameters, including hepatotoxicity (liver toxicity), neurotoxicity (nervous system toxicity), nephrotoxicity (kidney toxicity), cardiotoxicity (heart toxicity), cytotoxicity (cell toxicity), and immunotoxicity (immune system toxicity), were carefully examined. The analysis revealed that none of the tested ligands exhibited any signs of these toxic effects, suggesting that the compounds are potentially safe for further pharmacological development. These findings indicate a promising therapeutic profile, supporting the potential use of these derivatives in drug development with minimal risk of systemic toxicity.

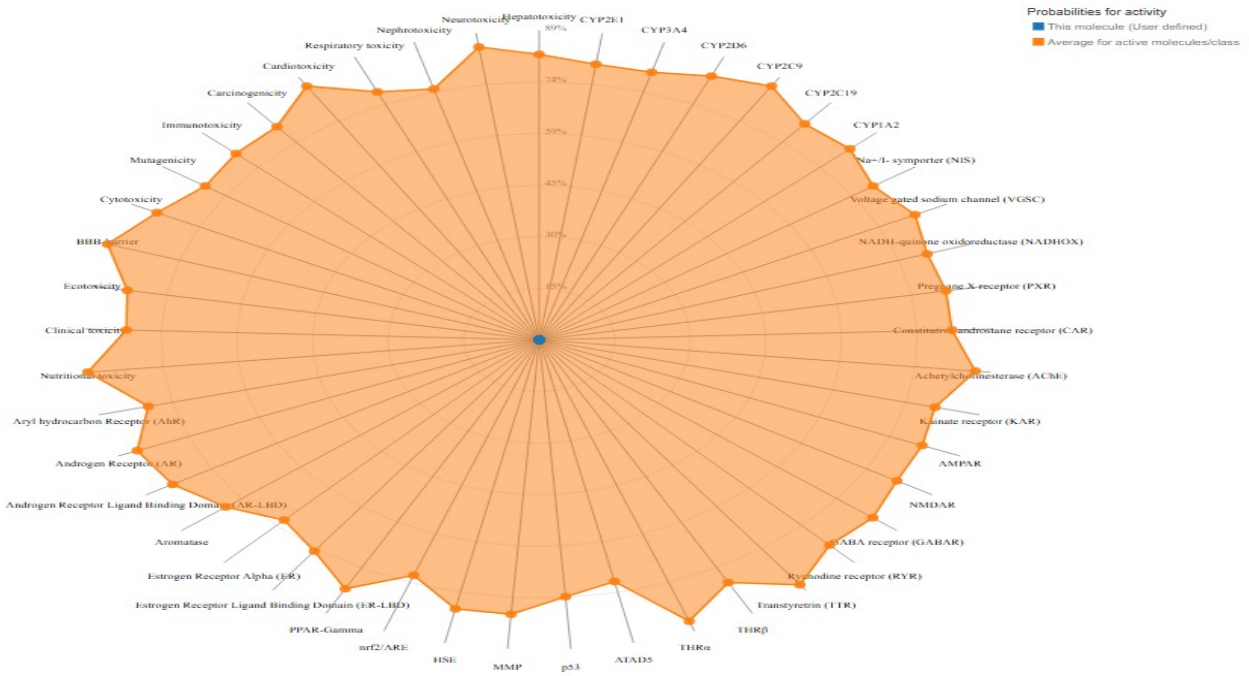


Figure 6: The Toxicological Rader Chart of BA-3

Table 6: Toxicity prediction of standard and potential novel ligands

SN	Hepato-Toxicity	Neuro- Toxicity	Nephro- Toxicity	Cardio-Toxicity	Cyto- Toxicity	Immuno- Toxicity	Toxicity class
AMX	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	6
BA-1	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	5
BA-2	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	5
BA-3	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	6
BA-4	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	6
BA-5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	5
BA-6	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	5
BA-7	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	6
BA-8	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	5
BA-9	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	5
BA10	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	5
BA11	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	5

Note: Class I: fatal if swallowed (LD50 ≤ 5), Class II: fatal if swallowed (5 < LD50 ≤ 50), Class III: toxic if swallowed (50 < LD50 ≤ 300), Class IV: harmful if swallowed (300 < LD50 ≤ 2000), Class V: may be harmful if swallowed (2000 < LD50 ≤ 5000) and Class VI: non-toxic (LD50 > 5000)

DISCUSSION

In this study, we examined the resistance patterns of Uropathogenic Staphylococcus species common causative agents of urinary tract infections (UTIs) and identified potential in-silico lead compounds to overcome penicillin resistance. An analysis of 3,000 clinical cases from July 2023 to January 2024 revealed a culture positivity rate of 8.2% (246 cases). This relatively lower positivity rate, compared to earlier findings by Ghimire et al.[27], shrestha et al.[4] and Subedi et al.[28], may be attributed to regional differences, variations in population demographics, or distinct screening practices, including the testing of asymptomatic individuals or routine screenings prior to surgical procedures[29]

The age-wise distribution indicated a higher prevalence of UTIs among individuals in the reproductive age group, particularly those aged 31–60 years. This trend is likely associated with factors such as increased sexual activity and pregnancy, which can promote the migration of bacteria from the perineal region to the bladder. These observations are consistent with previous studies[30] highlighting the significant role of both biological and behavioural factors in influencing the risk of infection.[27]

In line with established evidence, our study found a higher prevalence of UTIs in females compared to males. This is primarily attributed to anatomical differences, such as the shorter length of the female urethra and its close proximity to the perineal region[31]. Additionally, gram-negative bacteria were dominant in our findings, accounting for 88.2% of isolates. Escherichia coli emerged as the most common pathogen, representing 64.8% of cases, which supports previous studies

highlighting its origin from the intestinal flora[32].

Antibiotic susceptibility testing of Escherichia coli isolates revealed significant resistance to β-lactam antibiotics, with resistance rates of 88.46% to amino penicillin. According to the Shakya et al. observed 80% resistance to amoxicillin/ampicillin in their isolates[15]. The growing resistance to commonly prescribed antibiotics is likely driven by factors such as self-medication, overuse, and the irrational prescribing practices of healthcare providers [33]. This study underscores the ongoing challenge of penicillin resistance among uropathogens and highlights the pressing need for alternative and more effective therapeutic strategies.

Docking analyses of 11 amino penicillin derivatives against DNA-Gyrase of Escherichia coli identified N-piperazine Amoxicillin Derivatives [BA-3] and 4,5 - Di-Hydroxy oxazole Amoxicillin Derivatives [BA-10] found as a better compound, with strong binding affinity [-9.0] and [-9.6] Kcal per mole involving amino acids with bond distance GLU42: 2.23, ASN46: 2.45, ILE90: 2.86, LEU115: 2.06, VAL120: 2.24, SER121: 1.91 and ASN46: 1.80, ASP49: 2.50, LEU94: 2.87, SER108: 2.59, LEU115: 2.66, VAL120: 2.06, SER121: 1.90. demonstrating competitive binding energies for BA-3 and BA-10, which exceed those of traditional inhibitors.

The ADME profiles of the novel amino penicillin derivatives further support their potential as promising drug candidates. All evaluated compounds complied with Lipinski's rule of five, indicating favourable drug-likeness and suitability for oral administration. Notably, all compounds demonstrated low intestinal absorption, Although these derivatives exhibited similar lipophilicity compared to standard aminopenicillins, don't cross BBB and limited skin permeability suggest a controlled systemic distribution, which may reduce the risk of central nervous system toxicity [34]. These results align with previous studies that highlight the significance of selecting compounds with optimal LogP values to ensure a balance between bioavailability and safety [35].

Predictions of biological activity further affirmed the therapeutic promise of these derivatives, demonstrating high Pa/Pi ratios for antibacterial, antibiotic, and β-lactam-like activities. Toxicity evaluations placed the compounds in Class V and VI, suggesting a low risk for hepatotoxicity, Nephrotoxicity, neurotoxicity, cardiotoxicity, cytotoxicity and immunotoxicity. These findings are consistent with previous studies indicating reduced toxicological concerns for newly designed compounds, thereby supporting their potential as safe drug candidates [36]. Among the tested derivatives, all novel amino penicillin derivatives showed the strongest antibacterial activity, supported by favourable hydrogen bonding interactions.

Taken together, the integration of resistance profiling, molecular docking, and ADMET analysis highlights the potential of these novel derivatives to combat penicillin resistance. These results provide a strong foundation for advancing the compounds into preclinical and clinical development.

LIMITATIONS AND FUTURE DIRECTIONS

This study presents several limitations that warrant consideration in future research. Firstly, it relies on retrospective data from a single hospital, which restricts the generalizability of the findings. While molecular docking and ADME analyses revealed promising candidate compounds, the lack of experimental validation such as in vitro or in vivo studies limits their potential for clinical application. Although in-silico toxicity assessments suggested minimal risk, these findings must be confirmed through comprehensive experimental toxicological evaluations. Future research should aim to include multicentre studies across diverse geographic regions and focus on the experimental validation of identified compounds. Additionally, exploring alternative targets, combination therapies, and developing derivatives with broad-spectrum activity against both gram-positive and gram-negative bacteria will be essential. Further efforts should also prioritize thorough pharmacokinetic and toxicological profiling, as well as high-throughput screening of novel chemical scaffolds. Finally, investigating the role of efflux pumps and other antimicrobial resistance mechanisms will be critical to overcoming potential barriers to antibiotic efficacy.

CONCLUSION

This study highlights the critical challenge of penicillin resistance in uropathogenic Escherichia coli, particularly in UTIs. In-silico docking revealed that novel amino penicillin derivatives, N-Piperazine Amoxicillin Derivatives [BA-3] and 4,5 Di-Hydroxy Amoxicillin Derivatives [BA-10], exhibited stronger binding affinities to DNA-Gyrase of Escherichia coli compared to traditional β-lactam antibiotics, demonstrating significant potential as next-generation treatments. While almost all compounds passed ADME and toxicity analyses, requiring further optimization. 4,5-Di-Hydroxy Amoxicillin Derivatives [BA-10], in contrast, exhibited favourable binding energy, minimal toxicity, and promising pharmacokinetic properties, making it a strong candidate for further development. Future research should focus on synthetic scheme, in-vitro and in-vivo evaluations, investigate the role of efflux pumps in resistance, and explore combination therapies to address resistance mechanisms comprehensively.

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CONFLICTS OF INTEREST

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AUTHORS CONTRIBUTION

Sabin Shrestha and Barsha Adhikari: Conceptualization, Supervision, Investigation, Methodology, Formal analysis, Validation, Visualization, Writing – original draft, Writing – review & editing.
Rahi Bikram Thapa and Pharsuram Adhikari: Conceptualization, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing.
Mijala Bajracharya and Usha Giri: Conceptualization, Methodology, Data Curation, Formal Analysis Validation, Visualization, Funding acquisition, Writing – Review & Editing.