ANALYSIS OF CLINICAL SAMPLES FOR E. COLI ALONG WITH MOLECULAR DOCKING OF PBP1B WITH CEFOPERAZONE AND CTX-M-14 WITH CEFTRIAXONE

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ABSTRACT

Objective: To isolate E. coli from various clinical specimens, to analyze its prevalence and antimicrobial susceptibility pattern. Implementation of molecular docking to observe binding orientation of drug molecules with bacterial proteins.

Study Design: Prevalence study.

Place and Duration of Study: Microbiology Laboratory, Department of Microbiology, Shaheed Benazir Bhutto Women University Peshawar, from July 2014 to September 2014.

Methodology: Microbiological analysis of 80 clinical specimens comprising of urine, blood, pus, and sputum samples were undertaken and collected aseptically in a sterile plastic containers. Blood agar, MacConkey agar and CLED (Cysteine Lactose Electrolyte Deficient) agar were used as growth media for the culturing of microorganisms. Micro-or¬ganisms were identified through Gram staining followed by citrate utilization, urease, and TS! (triple sugar iron) tests. The isolates were then subjected to test antimicrobial susceptibility pattern. Bioinformatics tool such as docking was applied to understand the structure-activity association of bacterial proteins and antibiotics.

Results: Out of 80 samples, 12 (15%) were substantiated as E. coli with higher prevalence rate in females (10%) than males (5%) frequently in young age (up to 30 years). Amikacin (83.3%), cefoperazone (66.6%), and meropenen (66.6%) appeared to be more sensitive while erythromycin (100%), ceftazidime (83.3%), and ceftriaxone (75%) showed resistance than others. Among cephalosporins, cefoperazone was considerably active drug while ceftriaxone exhibited resistance. These drugs when docked using hex server demonstrated a number of interactions. Amino acid residue GLU166 is responsible for positioning cefoperazone to active site of PBP1b and restricting the bacterial cell to cause infection. Whereas, amino acid residues ARG 153, THR149, GLY146, and ASP163 of beta-lactamase (CTX-M-14) make important contacts with ceftriaxone in the receptor-ligand complex, which inhibited the drug from binding to sensitive proteins in E. coli.

Conclusion: The study concluded that among other infections E. coli existed more in UT's (20.9%) predominantly in females frequently in young age. Hence, molecular docking is suspected to be applicable for recognizing the active sites of molecules for enhancing the activity and designing more potent and versatile drugs.

Keywords: E. coli, analysis, antimicrobial susceptibility, molecular docking.

INTRODUCTION

Escherichia coli belonged to the family Enterobacteriaceae and genus Escherichia (Kaper et al., 2004) abbreviated as E. coli is gram-negative, rod shaped bacterium generally observed in the lower intestine of warm-blooded organisms (Vogt and Dippold., 2005). This organism possesses dual characteristics. It act as a commensal which inhabits gastrointestinal track of humans, on the other hand it could also possess pathogenic characters which can be harmful to humans (Yoo et al., 2009). In microbiology, approximately all strains of E. coli are derivative of E. coli B strains or E. coli K-12 strains (Perna et al., 2001). The best-known strain that produces Shiga toxin is O157:H7 that has the ability to cause severe disease or even human loss (Eisenstein et al., 2000).

Nosocomial infections are majorly caused by E. coli (Jafri et al., 2014) that is linked to persistent medical instruments or surgical procedures (Peleg et al., 2010). Furthermore the microbe is well-known for causing community acquired infections (Jafri et al., 2014). The infection is also transmitted by ingestion of contaminated food and water (Chin, 2000). Clinical infections like bloodstream infections, enteritis, and UTIs are predominantly caused by this bacterium (Allocati et al., 2013). For identification of E. coli, the culturing of biological specimen is routinely done in microbiology laboratory (Croxall et al., 2011). The distribution and

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severity of infections vary according to time and environment (Prakash and Saxena., 2013).

In humans, more than 80% of infections caused by E. coli include UTIs (Watts et al., 2010) which is a substantial economic and societal burden a formidable public health issue. Individuals suffered from UTI experience significant pain, infertility, while certain infections lead to more severe conditions which compel the usage of antibiotics (Sivick and Mobley., 2010). Comparatively, females are a lot of infected than males, with high proportion of infection in fruitful cohort (Debnath et al., 2014).

Antibiotics that are counseled to treat infections caused by E. coli embody penicillin, cephalosporins, trimethoprim-slufamethoxazole, fluoroquinolones, and nitrofurantoin but attributable to abuse and misuse of those antibiotics, in depth resistance of microorganisms to those antibiotics has developed (Kenechukwu et al., 2006). Microorganism adaptation to cut back flow through porins is an increasing obstacle worldwide that contributes, alongside outflow systems, to the appearance and dissemination of antibiotic resistance (Pages et al., 2008). β-lactamase production in E. coli is the most vital intermediator of resistance to broad spectrum. β-lactamase represent a good category of enzymes, that are typically encoded on plasmid. These enzymes grant resistance to penicillins and cephalosporins which is one of the rising reasons for multidrug resistance in gram-negative microorganisms. Many differing kinds of β-lactamases are represented (Poirel., 2012) like CTX-M producing enzymes and AMPC β-lactamases. Novel mixtures of antibiotics are being employed within the community and broad spectrums like carbapenems are being employed progressively as emperical treatment for severe infections (Pallett and Hand, 2010). However the increasing prevalence of infections caused by antibiotic resistant bacterium makes the empirical treatment of UTIs tougher (Arsalan et al., 2005).

Molecular docking is a method in which interaction of receptor with ligand is done (Mukesh and Rakesh., 2011). The objective is to find out prime binding site(s) of the ligand with 3D structure of proteins. Therefore docking could be key tool in structural biological science and computer-assisted drug style (Morris and Lim-Willey., 2008).

MATERIALS AND METHODS

The research work was conducted at microbiology laboratory of Khyber Teaching Hospital KTH), Peshawar, Pakistan from July to September 2014 to isolate E. coli from various clinical samples and perform their antibiogram analysis. During the period of two months clinical samples comprising of urine, pus, blood and sputum were acquired from patients including both males and females of all age groups ranging from 5 to 60 years. Samples were obtained from various wards without any distinction of symptomatic or asymptomatic, complicated or uncomplicated, acute or chronic. Factors including age, gender, and health condition of patients were taken into account for data study. A total of 80 samples were collected from government hospitals, among which 43 urine, 25 pus, 10 blood and 2 of them were sputum samples (see table 1). Assemblage with respect to sexual category included 45 female and 35 male under trials (see table 2). The specifics of patients under trial were recorded. Samples were collected aseptically, transfer to sterile disposable plastic containers. The media used for sample processing were MacConkey, blood, and CLED agar. Broth utilized was nutrient broth. To check antibiotic susceptibility, Muller-hinton agar (MHA) was used. Preparation was done according to standard techniques to use microbial free media for further culturing. For sample processing, culturing was carried out under controlled conditions. For urine samples, culturing on CLED, MacConkey, and blood agar plate was done in which 1 µl (micro-litre) of urine was streaked on media using a calibrated standard loop. For pus, blood, and sputum samples, culturing on MacConkey and blood agar plates were carried out. Colony morphology was ensured by scrutinizing color, shape, size and texture of isolates. Gram staining followed by biochemical tests (citrate utilization, urease, and TSI tests) were carried out on the colonies to substantiate organisms isolated. Effectiveness of antimicrobial agents against E. coli isolates was measured (see table 4). Antibiotic sensitivity was confirmed by Kirby-Bauer disc diffusion method. The antibiotic discs utilized were amikacin, augmentin, erythromycin, aztreonam, ceftazidime, cefoperazone, ceftriaxone, cefepime, moxifloxacin, norfloxacin, ciprofloxacin, and meropenem. Sensitive and resistant antibiotics against E. coli were specified. Their structures were obtained from drug bank (www.drugbank.ca). To download 3D structures of drugs, the file was first converted into MOLFILE (mol*) and then saved in PDB format, which was viewed in discovery studio visualizer. Similarly, sensitive and resistant proteins of E. coli were determined and selected. Their accession code was copied from uniprot (www. uniprot.org) and pasted in rcsb (www.rcsb.org). From there the structures were downloaded in pdb(gz) format and file was seen in discovery studio visualizer. Both the ligand (drug) and receptor (protein) were uploaded online on software named hex server (http://hexserver.loria.fr/), which were then studied in discovery studio visualizer (see Figure 1, 2). Their interaction was examined and details were recorded (see table 5).

RESULTS

E. coli was identified in clinical specimens, significantly in urine sample. Pus samples also contained a small number of E. coli in them, while no E. coli was found in blood and sputum samples. A total of 80 specimens were processed, among which E. coli was seen in 12 of all the isolates. Distribution of

Table 1: Sample wise distribution

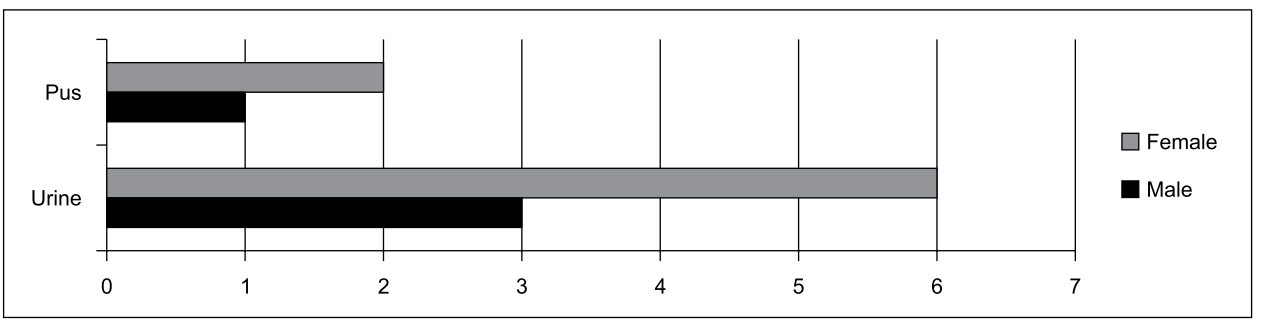
Biological Samples	Samples processed	Organism isolated	Samples identified	Percentage identification
Urine	43	E. coli	9	20.9%
Blood	10		0	0%
Pus	25		3	12%
Sputum	2		0	0%
Total	80		12	15%

Table 2: Gender wise distribution

Sample processed				Sample identified				
Sample	No. of samples	Female	Male	Organism	Female	Male	Female %	Male %
Urine	43	24	19	E. coli	6	3	13.9%	6.9%
Blood	25	15	10		-	-	0	0
Pus	10	5	5		2	1	80%	4%
Sputum	2	1	1		-	-	0	0
Total	80	45	35		8	4	10%	5%

Table 3: Age wise distribution

Sample processed							
Samples	No. of samples	Organism	No. of samples identified	Below 10 years	11-30 years	31-50 years	Above 50 years
Urine	43	E. coli	9	2	3	1	3
Blood	25		0	-	-	-	-
Pus	10		3	-	2	1	-
Sputum	2		0	-	-	-	-
Total	80		12	2	5	2	3



Graph 1: Number of E. coli isolates in male and female

different samples and their percentage identification is elaborated in Table 1.

Results showed that both the genders were infected by E. coli. The isolation rate of E. coli from biological samples demonstrated that this organism prevailed more in females than in males (see Table 2).

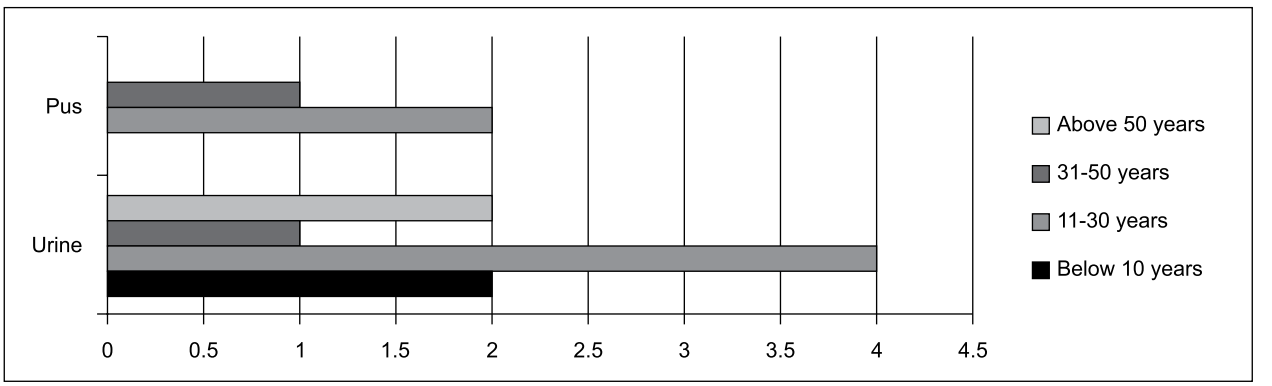
The ratio of females was greater than males in both the samples as shown in graph 1. Every age factor including children, adults, and elderly individuals confirmed the presence of E. coli irrespective of gender. But individuals with age above 11 and below 30 years were more prone to this organism (see Table 3).

Table 4: Antimicrobial susceptibility of E. coli isolates

Antimicrobial drugs	Disc used (µg)	Standard growth inhibiting zone (mm)			No. of samples processed = 12		
		R	MS	S	R (%)	MS (%)	S (%)
Aminoglycoside							
Amikacin (AK)	30	≤14	15-16	≥ 17	0(0%)	2 (16.6%)	10 (83.3%)
Aminopenicillin							
Augmentin (AMC)	30	≤ 13	14-17	≥ 18	3 (25%)	2(16.6%)	7 (58.3%)
Macrolide							
Erythromycin (E)	15	≤ 13	14-22	≥ 23	12 (100%)	0(0%)	0(0%)
Monobactam							
Aztreonam (ATM)	30	≤ 15	16-21	≥ 22	3(25%)	4 (33.3%)	5(41.6%)
Cephalosporin							
Ceftazidime (CAZ)	30	≤ 14	15-17	≥ 18	10(83.3%)	0(0%)	2 (16.6%)
Cefoperazone (CFP)	75	≤15	16-20	≥ 21	2(16.6%)	2 (16.6%)	8(66.6%)
Ceftriaxone (CRO)	30	≤ 13	14-20	≥ 21	9 (75%)	2 (16.6%)	1 (8.3%)
Cefepime (FEP)	30	≤18	19-24	≥25	1 (8.3%)	9 (75%)	2 (16.6%)
Fluoroquinolone							
Moxifloxacin (MXF)	05	≤14	15-17	≥18	7 (58.3%)	3 (25%)	2 (16.6%)
Norfloxacin (NOR)	10	≤12	13-16	≥17	8 (66.6%)	2 (16.6%)	2 (16.6%)
Ciprofloxacin (CIP)	05	≤15	16-20	≥21	3 (25%)	5 (41.6%)	4 (33.3%)
Carbapenem							
Meropenem (MEM)	10	≤19	20-22	≥23	1 (8.3%)	2 (16.6%)	8 (66.6%)

Table 5: Docking of proteins of E. coli with cefoperazone and cefriaxone

Drug	Protein	Protein code	Protein residue	Residue atom	Hetatom	Drug atom	Distance
Cefoperazone	PBP1b	3FWL	GLU166	O		H47	1.535
Ceftriaxone	Beta-lactamase CTX-M-14	1YLT	ARG 153			S6	1.573
			THR149		HOH2055	O11	2.310
			GLY146				2.773
						S18	0.920
						N20	2.561
			ASP163	OD1	HOH2187		2.704
						O11	1.840
						N21	2.656
						O36	2.176
						H41	2.459



Grpah 2: Number of E. coli isolates in different age groups

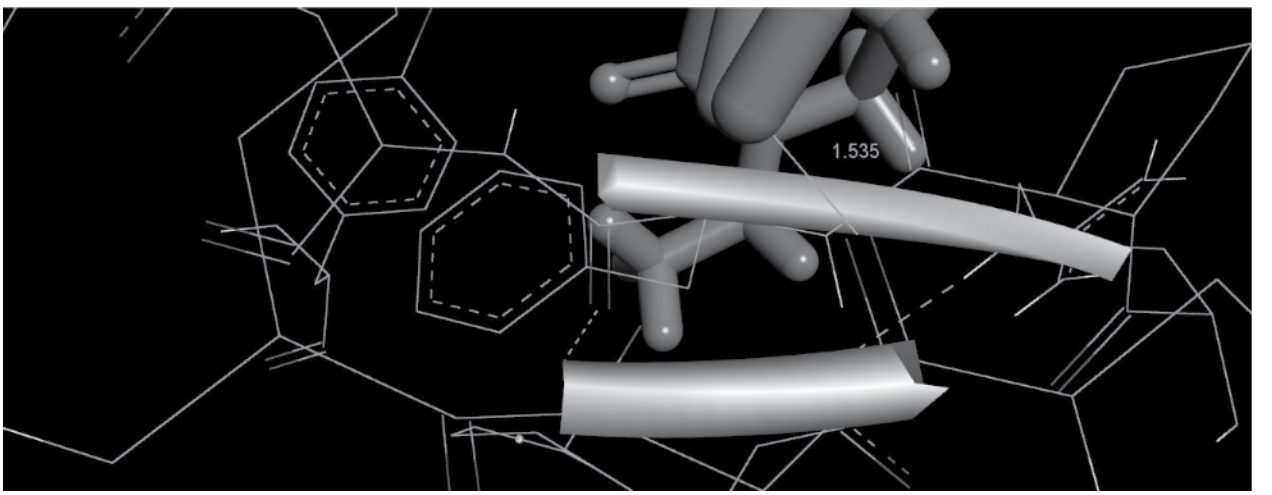


Figure 1: Interaction of cefoperazone with PBP1b

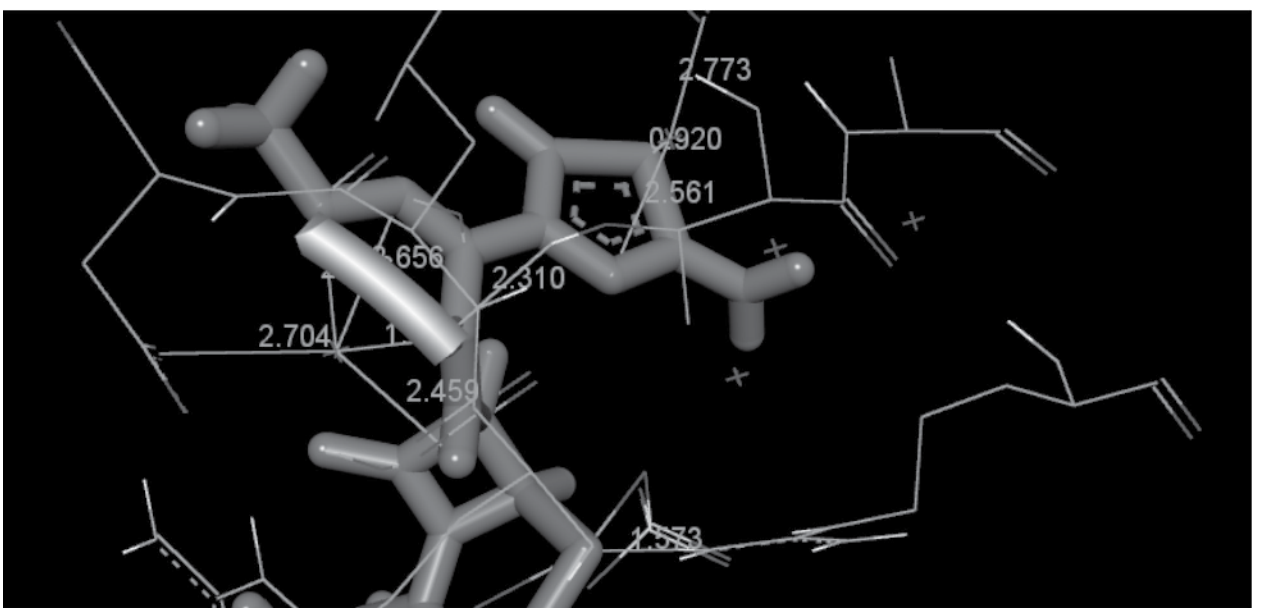


Figure 2: Interaction of ceftriaxone with beta lactamase CTX-M-14

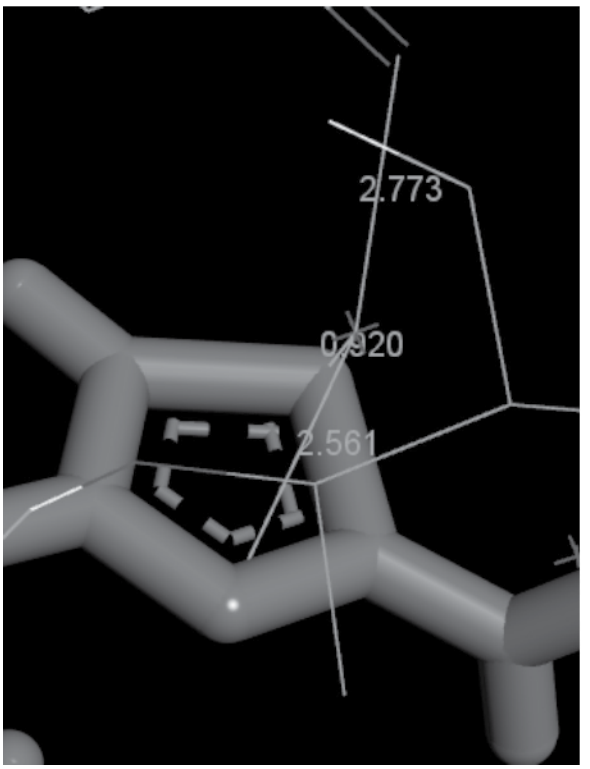


Figure 3a: GLY146 connected to S18 and N20 through HOH2055

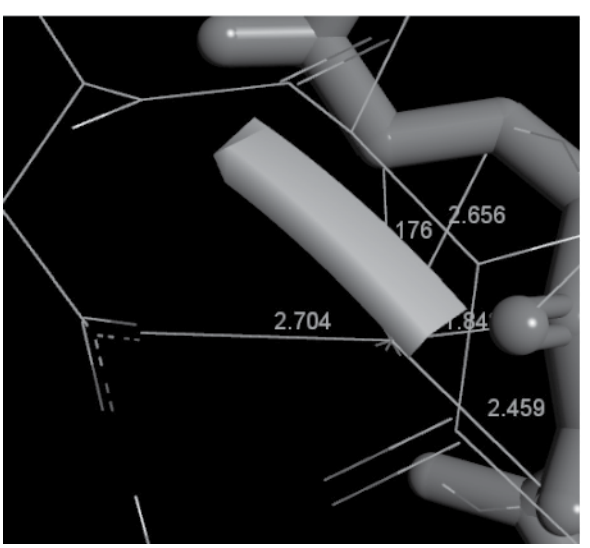


Figure 3b: ASP163 connected to 011, N21, O36, and H41 through HOH2187

Specimens when cultured on MacConkey agar showed stupendous growth. Pink colored, small, circular colonies with elevated smooth margins confirmed fermentation of lactose and indicated growth of Gram negative organism. Processing of cultures on CLED agar indicated the existence of Gram negative bacteria by appearance of yellow colored, small, circular, lactose fermenting colonies with elevated smooth margins. Primary isolation of E. coli from urine and pus samples was carried out on blood agar which revealed β-hemolytic activity on the agar plate. Gram reaction of all those isolates was viewed under microscope which confirmed the emergence of pink colored, scattered rod shaped bacilli as they retained secondary stain and revealed to be gram-negative.

After morphological characterization of samples, they were subjected to biochemical analysis of E. coli. Different biochemical tests including TSI, Simmons citrate, and urease tests were performed, which confirmed that organism isolated was E. coli. Urease test presented negative result. No growth of organism was found on the agar slant which indicated that organism does not hydrolyze urea. Hence, no production of enzyme (urease) occurred and color of slant remained yellow. Citrate test showed to be negative as no color change was observed and agar slant remained green. So, the organism does not utilize citrate as sole carbon source. All the 12 isolates showed positive result for TSI test as colour was changed from pink to orange while H2S production was not taken place. Appearance of orange color on slope indicated that bacteria consumed the sugars, and form acid. Appearance of cracks or bubbles on slope or butt indicated the production of gas.

Isolates were subjected to antibiotic sensitivity testing after the detection of E. coli by several biochemical tests. The method used for checking the activity of antimicrobial drugs was disc diffusion technique. Amikacin, augmentin, cefoperazone, and meropenem showed highly sensitive results where as cefepime showed moderate sensitivity against E. coli. Erythromycin, ceftriaxone, ceftazidime, and norfloxacin were seen as resistant drugs in majority of the isolates while ciprofloxacin, aztreonam, and moxifloxacin showed intermediate results as illustrated in Table 4.

Molecular docking was performed to explore the susceptibility of drugs against E. coli. Cefoperazone; sensitive drug against this pathogen was selected and docked with sensitive protein (PBP1b) of E. coli. GLU166 amino acid at position O showed interaction with drug atom H47 and created a distance of 1.535Å (see figure 1). Resistant drugs against E. coli were enlisted by observing the results of antibiotic sensitivity test, from which ceftriaxone was picked. Docking of drug with beta lactamase CTX-M-14 (resistant protein of E. coli against ceftriaxone) was carried out (see table 5). Strong interactions, both direct and indirect were detected between drug and pathogen protein. Figure 2 displayed that protein residue ARG153 showed interaction with drug atom S6 at position HH22 by making distance 1.573Å while THR149 at position HG1 showed interaction with drug atom O11 making distance of 2.310Å.

It was also monitored that drug showed interaction with protein residues through water molecules. The residues that were seen to have indirect interaction with

drug atoms through water molecules included GLY146 and ASP163 as shown in table 5. GLY146 at position O interacted with hetatom HOH2055 creating a distance of 2.77Å which in turn interacted with drug atoms S18 and N20 making distance of 0.920Å and 2.561Å respectively (see figure 3a). Similarly, amino acid ASP163 indirectly interacted with drug atoms O11, N21, O36, and H41 at position OD1 through hetatom HOH2187 making distance of 2.704Å which in turn interacted with drug atoms at distances 1.840Å, 2.656Å, Å2.176, and 2.459Å respectively as shown in figure 3b.

DISCUSSION

The aim of the study was to isolate E. coli from biological specimens. Colony morphology appeared to be small in size, circular in shape, smooth regular margins with lactose fermentation. Pink colored rods with scattered arrangement were seen under the microscope authenticating the emergence of gram-negative bacteria. Biochemical reactions showed negative results for Simmons citrate and urease agar tests as citrate was not utilized and urease was not produced by the microbe. TSI test showed positive result by changing the color of slant from pink to yellow with production of gas. These above results were in accordance with Kumar et al., (2014).

In our study, it was estimated that 15% of E. coli exist in biological samples among which percentage of this microbe was highest in urine samples (20.9%) followed by pus samples (12%). So E. coli was concluded as the major cause of UTI among other infections, which is in accordance with Rastogi et al., (2011). No growth was seen in blood and sputum samples. One of the reasons could be due the development of resistance described by Arsalan et al., (2005), who stated that extensive use of antibiotic could lead to high level of resistance in the body. Thus, antibiotic resistant bacteria cause high prevalence of infections which makes empirical treatment more complicated. In out of 12 isolates both male and female were present but the number differed, that is 4 and 8 respectively (see table 2). So, it was observed that isolation rate of E. coli was high in women in comparison to men. Percentage with respect to gender comprises of 10% females and 5% males. Ratio of E. coli in female urine samples was comparatively high, which is in accordance with Daoud and Afif., (2011). Females are more prone to cause UTIs due to their anatomy as their urethra size is much shorter, so bacteria are more liable to penetrate the urethra and proliferate to initiate infection. Hence, females are at increased hazard of originating UTI than males. According to Ahmed et al., (2014) E. coli prevailed mostly in young age (more than 20 and less than 30 years) as compared to other age groups which is in accordance with our results, shown in table 3. In the study, isolates of E. coli were observed in individuals of every age group ranging from children to elderly individuals. But the young age group till 30 years was largely infected by the pathogen followed by children and elders of other ages. Among this age group, the most infective gender was of female patients. This is because women within reproductive age group are more susceptible to infection.

The susceptibility pattern for E. coli strains was checked to establish the efficiency of specified drugs for the treatment of infections. Drugs from diverse families like aminoglycoside, macrolide, monobactam, cephalosporin, fluoroquinolone, and carbapenem were used. Amikacin, meropenem, and augmentin showed maximum sensitivity that is 83.3%, 66.6%, and 58.3% respectively. The result is in accordance with Kurtoglu et al., (2008) who stated that E. coli was sensitive against the drugs; amikacin (96%), meropenem (100%), and augmentin (50%). The difference in percentages of results is due to the difference in number of isolates processed and identified in both the researches. It can be considered that most gram-negative bacteria particularly E. coli strains are susceptible towards aminoglycosides, carbapenems, and aminopenicillin drugs. The current study was according to the work performed by Mos et al., (2010) who revealed that fourth generation cephalosporins were active against E. coli. In the contemporary research it was detected that cefepime was moderate sensitive against 75% of the isolates and the percentage of highly sensitive result was 16.6% whereas only one isolate was seen to be resistant against cefepime.

Cephalosporins are beta-lactam antibiotics that bind to PBP in case of E. coli. In our research, many third generation cephalosporin drugs were used among which, cefoperazone (66.6%) was most effective drug. The study showed comparable results with Rizvi et al., (2014) who disclosed that sensitivity of cefoperazone was ore than 50% against E. coli. It is a broad spectrum antibiotic which is used for treating gram-negative as well as gram-positive bacteria. Our results expressed less sensitivity towards some of the cephalosporin drugs. The research conducted by Mos et al., (2010) accomplished that attenuated susceptibility of cephalosporins is because of the production of beta-lactamases in strains of E. coli. The principal source of resistance in beta-lactam antibiotics is due to expanding production of beta-lactamases (enzymes) among gram-negative microbes. However, rate of drug resistance is not static, because dispersion and susceptibility of microbes changes from time to time and area to area as denoted by Ahmed et al., (2014).

Ciprofloxacin and azteonam showed intermediate results. Some of the isolates were sensitive against these drugs while some of them exhibited resistance. Both the drugs were noticed to have 25% resistance. The researchers Arsalan et al., (2005) and Pakzad et al., (2011) concluded in their study that the drugs ciprofloxacin (26.6%) and aztreonam (28%) manifested resistance, respectively. Both the evaluations appeared

to be consistent with ours. Hence, it can be speculated that these drugs were not highly resistant against E. coli; however their overuse could be one of the aspect in rising emergence of antibiotic resistance.

In our present study, E. coli was resistant against some of the drugs. Erythromycin was inactive against all of the isolates, and showed 100% resistance. In Kibret and Abera, (2011) research, the highest resistance rate was also seen in erythromycin (89.4%). The antibiotics moxifloxacin (58.3%) and norfloxacin (66.6%) belonging to fluoroquinolones were resistant as well in almost half of the isolates. It can be assumed that the resistance rate is more than 50% in fluoroquinolones. According to Verma et al., (2012) the resistance rate of above mentioned drugs was lower than their research that is moxifloxacin (86.6%) and norfloxacin (84%). This is because the number of samples differs in both the cases. Some cephalosporin drugs that included ceftazidime (83.3%) and ceftriaxone (75%) were also resistant in majority of isolates which is in accordance with Shehriar et al., (2010) who reported ceftazidime (83.75) and ceftriaxone (87.5%) as highly resistant drugs in his study.

After microbiological techniques, computational methods were applied to ensure the activity and affinity of antibiotics where cephalosporin drugs were taken into account to implement molecular docking. The drug and bacterial protein structures were docked using PDB structures. After perceiving the ligands, their interaction with receptors was observed using hex server which is comparable with Paul and Bagchi, (2011). In their review docking was carried out on protein molecules against drugs by operating hex server to monitor interaction of target proteins. Analysis of docked structures was performed using Discovery Studio (Accelyrs) as done by Skakil and Khan, (2010).

Cefoperazone was docked with PBP1b which revealed interaction with one of its binding site that is GLU166 amino acid attached with drug atom H47 and created a distance of 1.535Å. The strong interaction showed that PBP of bacterial cell was sensitive to beta-lactam antibiotic which resulted in killing of bacterial cell. In case of cefrtiaxone, beta-lactamase (CTX-M-14) showed bonding directly and indirectly with important residues at multiple binding sites. ARG153 showed interaction with drug atom S6 at position HH22 by making distance 1.573Å while THR149 at position HG1 showed interaction with drug atom O11 making distance of 2.310Å. GLY146 interacted with hetatom HOH2055 which in turn interacted with drug atoms S18 and N20 making distance of 0.920Å and 2.561Å correspondingly. Similarly, amino acid ASP163 indirectly interacted with drug atoms O11, N21, O36, and H41 through hetatom HOH2187 making distance of 2.704Å which in turn interacted with drug atoms at distances 1.840Å, 2.656Å, Å2.176, and 2.459Å respectively as shown in table 12. Thus, amino acid residues ARG 153, THR149, GLY146, and ASP163 were found crucial in stabilizing the complexes through hetatoms or without hetatoms. It was observed that cefrtiaxone was bonded strongly with resistant proteins (CTX-M-14) so that they cannot bind to sensitive proteins (PBPs) and thus the killing of bacterial cell was inhibited. Docking results led to explore the binding patterns of molecules. Key interactions between the ligands and binding site residues of protein molecules were discovered. Hence, molecular docking is suspected to be applicable for recognizing the active sites of molecules or further innovations and drug designing.

CONCLUSION

The current study revealed that existence of E. coli is more in UTI among other infections with high prevalence rate in females frequently in young age. In vitro, susceptibility results of amikacin, cefoperazone, and meropenem appears to be more sensitive while erythromycin, ceftazidime, and ceftriaxone showed resistance than others. Docking studies disclosed that the amino acid residue GLU166 of PBP1b make important contact with cefoperazone which resulted in the killing of bacterial cell. While amino acid residues ARG 153, THR149, GLY146, and ASP163 are responsible for positioning ceftriaxone into the active site of CTX-M-14. Thus, researchers are advised to utilize this knowledge for better recognizing the binding interactions of drugs with bacterial proteins to enhance the activity as well as designing of more potent drugs.

REFERENCES

1. Ahmed, K., Raja, I., Hussain, I., Jan, M., Nafees, M. A., Jahan, Z., ... & Latif, A. (2014). Prevalence of Escherichia coli in Suspected Urinary Tract Infected Patients and Their Sensitivity Pattern Against Various Antibiotics in Gilgit-Baltistan, Pakistan. Pakistan J. Zool, 46(6), 1783-1788.
2. Allocati, N., Masulli, M., Alexeyev, M. F., & Di Ilio, C. (2013). Escherichia coli in Europe: An Overview. International journal of environmental research and public health, 10(12), 6235-6254.
3. Arslan, H., Azap, Ö. K., Ergönül, Ö., & Timurkaynak, F. (2005). Risk factors for ciprofloxacin resistance among Escherichia coli strains isolated from community- acquired urinary tract infections in Turkey. Journal of Antimicrobial Chemotherapy, 56(5), 914-918.
4. Chin, J. (2000). Control of communicable diseases manual.
5. Croxall, G., Weston, V., Joseph, S., Manning, G., Cheetham, P., & McNally, A. (2011). Increased human pathogenic potential of Escherichia coli from polymicrobial urinary tract infections in comparison to isolates from monomicrobial culture samples. Journal of medical microbiology, 60(1), 102-109.
6. Daoud, Z., & Afif, C. (2011). Escherichia coli isolated from urinary tract infections of lebanese patients between 2000 and 2009: epidemiology and profiles of resistance. Chemotherapy research and practice, 2011.

7. Debnath, J., Das, P. K., Debnath, M., & Haldar, K. K. (2014). Aetiological Profile and Antibiotic Susceptibility Pattern in Patients with Urinary Tract Infection in Tripura. Journal of clinical and diagnostic research: JCDR, 8(8), DL01.

8. Eisenstein, B., & Zaleznik, Dori. (2000). Enterobacteriiaceae, in Mandell, Douglas, & Bennett's. Principles and practice of infectious diseases, 5th ed. 206, 2294-2310.

9. Jafri, S. A., Qasim, M., & Masoud, M. S. (2014). Antibiotic resistance of E. coli isolates from urine samples of Urinary Tract Infection (UTI) patients in Pakistan. Bioinformation, 10(7), 419.

10. Kaper, J. B., Nataro, J. P., & Mobley, H. L. (2004). Pathogenic Escherichia coli. Nat Rev Microbiol 2: 123–140.

11. Kenechukwu, M., Chinekwu, O., Davidson, N., & Golibe, U. O. (2006). Antibiotic Sensitivity Patterns in Urinary Tract Infections at a Tertiary Hospital. Proteus, 13, 1-6.

12. Kibret, M., & Abera, B. (2011). Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia. African health sciences, 11(3), 40-45.

13. Kumar, D., Singh, A. K., Ali, M. R., & Chander, Y. (2014). Antimicrobial Susceptibility Profile of Extended Spectrum β-Lactamase (ESBL) Producing Escherichia coli from Various Clinical Samples. Infectious diseases, 7, 1.

14. Kurtoğlu, M. G., Bozkurt, H., Tuncer, O., Kesli, R., & Berktas, M. (2008). Distribution, optimum detection time and antimicrobial susceptibility rates of the microorganisms isolated from blood cultures over a 4-year time period in a Turkish university hospital and a review of the international literature. Journal of International Medical Research, 36(6), 1261-1272.

15. Morris, G. M., & Lim-Wilby, M. (2008). Molecular docking. In Molecular Modeling of Proteins (pp. 365-382). Humana Press.

16. Moş, I., Micle, O., Zdrâncă, M., Mureşan, M., & Vicaş, L. (2010). Antibiotic sensitivity of the Escherichia coli strains isolated from infected skin wounds.Farmacia, 58(5), 637-644.

17. Mukesh, B., & Rakesh, K. (2011). Molecular docking: A review. Int. J. Res. Ayurveda Pharm, 1746-1751.

18. Pagès, J. M., James, C. E., & Winterhalter, M. (2008). The porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. Nature Reviews Microbiology, 6(12), 893-903.

19. Pakzad, I., Ghafourian, S., & Taherikalani, M. (2011). Qnr prevalence in extended spectrum beta-lactamases (ESBLs) and none-ESBLs producing Escherichia coli isolated from urinary tract infections in central of Iran. Iranian journal of basic medical sciences, 14(5), 458.

20. Pallett, A., & Hand, K. (2010). Complicated urinary tract infections: practical solutions for the treatment of multiresistant Gram-negative bacteria. Journal of antimicrobial chemotherapy, 65(suppl 3), iii25-iii33.

21. Paul, K., & Bagchi, P. (2014). IDENTIFICATION OF POTENTIAL ONCOGENE AND TRANSCRIPTION FACTOR INHIBITORS USING IN-SILICO METHODS.

22. Peleg, A. Y., & Hooper, D. C. (2010). Hospital-acquired infections due to gram-negative bacteria. New England Journal of Medicine, 362(19), 1804-1813.

23. Perna, N. T., Plunkett, G., Burland, V., Mau, B., Glasner, J. D., Rose, D. J., ... & Blattner, F. R. (2001). Genome sequence of enterohaemorrhagic Escherichia coli O157: H7. Nature, 409(6819), 529-533.

24. Poirel, L., Bonnin, R.A., & Nordmann, P. (2012). Genetic support and diversity of acquired extended-spectrum beta-lactamases in Gram-negative rods. Infect. Genet. Evol. 12, 883–893.

25. Prakash, D., & Saxena, R. S. (2013). Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of Meerut City, India. ISRN microbiology, 2013.

26. Rastogi, C., Zahera, M., Singh, P., Iram, S., Khalid, S., & Kushwaha, A. (2011). Isolation, identification and characterization of Escherichia coli from urine samples and their antibiotic sensitivity pattern. Eur J Exp Biol, 1, 118-24.

27. Rizvi, M., Ahmed, M., Naqvi, B., Abbas, A., & Sabah, A. (2014). Comparision of in vitro antibacterial activity of cefoperazone and levofloxacin against different clinical isolates.

28. Shahriar, M., Hossain, M. A. H. B. O. O. B., & Kabir, S. (2010). A Survey on Antimicrobial Sensitivity Pattern of Different Antibiotics on Clinical Isolates of Escherichia coli Collected from Dhaka City, Bangladesh. Journal of Applied Sciences and Environmental Management, 14(3).

29. Shakil, S., & Khan, A. U. (2010). Interaction of CTXM15 enzyme with cefotaxime: a molecular modelling and docking study. Bioinformation, 4(10), 468.

30. Sivick, K. E., & Mobley, H. L. (2010). Waging war against uropathogenic Escherichia coli: winning back the urinary tract. Infection and immunity, 78(2), 568-585.

31. Verma P., Chandrakar V., & Chitra. (2012). Antibiotic sensitivity treatment for gram negative bacteria isolated from pus sample. International Journal of Pharmacy and Biological Sciences, 2(3).

32. Vogt, R. L., Dippold, L . (2005). Escherichia coli O157:H7 outbreak associated with consumption of ground beef, June–July 2002". Public Health Rep, 120 (2), 174–8.

33. Watts, R. E., Hancock, V., Ong, C. L. Y., Vejborg, R. M., Mabbett, A. N., Totsika, M., ... & Schembri, M. A. (2010). Escherichia coli isolates causing asymptomatic bacteriuria in catheterized and noncatheterized individuals possess similar virulence properties. Journal of clinical microbiology, 48(7), 2449-2458.

34. Yoo, S. H., Jeong, H., Kwon, S. K., & Kim, J. F. (2009). Genomics, Biological Features, and Biotechnological Applications of Escherichia coli B: "Is B for better?!". Springer: Berlin, Germany.