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ORIGINAL ARTICLE

Identification of Antibacterial Efficacy of Cb6 (E)-1-(4-fluorophenyl)-3-Phenylprop-2-en-1-one against Escherichia Coli

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ABSTRACT

Objective of the study: Escherichia coli bacteria with increased antibiotic resistance represent a serious threat to public health. The aim of this study was to assess Cb6 (E)-1-& effectiveness against microorganisms (4-fluorophenyl), determine the effectiveness of Cb6 (E)-1-(4-fluorophenyl)-3-phenyl prop-2-en-1-one against anti-fungal agents and -3 -phenylprop-2-en-1-one against E. coli (Curvularia).

StudyDesign: Study was designed by sample collection, culturing, biochemical tests, sensitivity assay and qRT-PCR.

Place and Duration: The prospective study was carried out at the University of Agriculture in Faisalabad, Pakistan from 1st February 2019 to 18th June 2019.

Methodology: Through horizontal and vertical gene transfer, Escherichia coli acquired resistance genes and developed resistance against many antibiotic classes. The following genes were examined in the current study in E. coli: Van B, ermC, Iva-A, Qnr-A, Qnr-B, tet M, tet O, and tet W resistance genes. To assess the effectiveness of the antibacterial and antifungal agents, antibiotic sensitivity testing was carried out.

Results: E.coli chalcone resistance genes, have displayed highly significant (P 0.0001) expression in the patient sample when compared to the control.

Conclusion: It was concluded that the chalcones are highly resistant to E coli in prediction. Van B, erm C, Iva-A, Qnr-A, Qnr-B, tet M, tet O, and tet Ware resistant genes of the Escherichia coli against the Cb6 (E)-1-(4-fluorophenyl)-3 -phenyl prop-2-en-1-one and it was showed the high expression level.

Keywords: Chalcones Resistant gene, Antibacterial action, Antibiotic sensitivity, Expression.

INTRODUCTION

E. coli is a flagella organism and somatic antigen that is based on capsular (K). About 700 serotypes of E. coli have been known previously. The E. coli that produced the food poison in humans are also biotype as sorbitol non-fermentative bacteria. This biotype results from an inclusive series of clinical indicators, as well as inconsequential illness, nausea, the unpredicted beginning of diarrhea, and death (Shahzadet al., 2013). The pathogenic strain of E. coli spread various intestinal infections, urinary infections, and some pathogenic E. coli related to mild gastric diseases (Croxenet al., 2013). Shiga toxins or cytotoxic are produced by the strains of E. Colicalled STEC (Gavin et al., 2004). More than 400 serotypes are known as STEC, some subgroups associated with human infections (Hussein, 2007).

Instant increase of antibiotic resistance regarding microbes is a challenging issue for living organisms. Bacteria have a natural resistance against antibiotics obtained by mutation. Resistance genes are present on chromosomes, plasmids, or transposons (Davison et al., 2000). Antibiotic resistance genes survive in the atmosphere for the long term since antibiotics are known as clinical mediators (D'Costaet al., 2011). An Antibiotic is an organic compound that produces its effects by preventing or killing microorganisms through contact with a bacterial target (Davies and Davies, 2010). The process by which microorganisms develop resistance against the antibiotics which are used for the treatment of infections is defined as antibiotics resistance (Zhi Li et al., 2015).

Resistance against antibiotics was not established as a fact, because the molecular mechanism of resistance and certain other factors in bacteria were not studied completely. But now it has been proven that microbes develop rapidly by mutation (Hoeket al., 2011). Novel antimicrobial discovery and combination antibiotic therapy are being pursued to combat emerging antibiotic-resistant bacteria. Combination therapy reduces the adverse effect of antibiotics and increases the potency of antimicrobial agents against resistant pathogens (Khameneh et al., 2016). Some bacterial species express a high intrinsic resistance to numerous antibiotics. Acquired resistance develops through genetic modifications in the bacteria by horizontal transfer of resistance genes placed in many mobile DNA elements.When Resistance occurs at a low level that may create the genetic development for higher and more complex resistance levels (Normark and Normark, 2002).

Under the response, mutations and genetic exchanges within the cell increase for damaged DNA to be repaired and the cell to survive. Because of these genetic manipulations, any gene sequence, including those for antibiotic targets can be altered, providing the possibility for the evolution of resistance (Yuet al., 2005).

Chalcones have antimicrobial activity against bacteria. Numerous chalcones have vital action against Gram-positive bacteria. The antibacterial activity can be obtained by comparison of the result related to structures such as the presence of a C-40 oxygenated substituent or a C-30 isoprenoid side chain and a C-4 hydroxyl group. Chalcones have an inhibitory effect on human pathogenic microorganisms that relate to the substitution patterns of the aromatic rings (Ávila et al., 2008). With the versatile characteristics of coumarin and chalcone, coumarin-chalcone hybrids have received much attention in recent years (Xueet al., 2018).

In this study, a series of chalcone-imide derivatives 5a-g were synthesized, and their anticancer activity against C6 rat glioma was characterized. Due to resistant bacteria, many infectious diseases are untreatable. Therefore, need to identify new synthetic compounds that would be effective against resistant bacteria like Cb6 (E)-1-(4-fluorophenyl)-3 -phenyl prop-2-en-1-one. Scientists' approaches should be used to search for new compounds to encounter antimicrobial resistance (Patilet al., 2010). The main objective of this study is to identify of antibacterial efficacy ofCb6 (E)-1-(4-fluorophenyl)-3-phenyl prop-2-en-1-one against Escherichia Coli.

METHODOLOGY

Study location: The prospective study was carried out at the University of Agriculture in Faisalabad, Pakistan from 1st February 2019 to 18th June 2019.

Study area and sample collection: One hundred and sixty-seven E. coli from urine and sepsis samples of UTI patients were collected from Allied hospital, Faisalabad.

Sterilization of equipment and work environment: Glasswares were washed with detergent in tap water and purified by autoclaving at 121ºC for 15 minutes. Swab sticks were brought sterile and used once for each specimen.

Preparation of culture media for isolation of bacteria: Suspended McConkey agar 37.5g in 1 liter of distilled water and it was boiled to dissolve completely. Then it was sterilized by autoclaving at 121ºC for 15 minutes. Let it cool to 60ºC and shook the media to oxidize one of the ingredients. Finally, it was suspending the precipitate to give it its appropriate shape in the agar plate.

Storage Conditions and Shelf Life: The dehydrated medium was stored at 10-30 ºC and use before the expiry date on the label. Prepared plates were stored at 2-80 ºC away from light.

In the laboratory, each species sample was inoculated into a MacConkey agar plate under class-II laminar airflow for aerobic plate count by swabbing. The plates were incubated at 37 ^{0} C for 24 hrs. Distinctive colonies on MacConkey agar were sub-cultured by streaking. The plates were incubated at 37 ^{0}C for 24 hrs for the growth of bacteria.

Table 1: Biochemical Test for Confirmation of E. coli

Isolation and identification of E. coli: Gram Staining	Negative
Oxidase Test	Negative
Catalase Test	Positive
H_2S Production	Negative
Gas	Positive
Glucose Fermentation	Positive
Nitrate Reduction	Positive

Gram Staining: Gram stain was a variance method used for determination to categorize the bacteria. There are two major types of bacteria (gram-negative and gram-positive). For this technique, 3 different strains were used. These include Crystal violet, Iodine, Safranin, and Alcohol.

Catalase Test: The microorganism produced catalase which is an enzyme that presents in an oxygenated situation to deactivate the toxicity of oxygen metabolites; H_2O_2. The bactericidal impact of hydrogen peroxide can be protected and neutralized by the catalase enzyme.

TS ITest: Lactose, sucrose and glucose in the concentration of 10:10:1 (i.e., part Lactose (1%), 10-part sucrose (1%) and 1-part glucose (0.1%). TSI is like Kligler's iron agar (KIA), except that Kligler's iron agar contains only two carbohydrates: glucose (0.1%) and lactose (1%).

gDNA Isolation: Genomic DNA was isolated for analysis of microbiota.

Molecular detection of Escherichia coli:PCR -based methods for the detection of E. coli in animal fecal samples and enriched meat samples have been extensively described in the literature. The inclusion of positive and negative reference strains and process control to detect inhibition of the PCR reaction by the sample matrix are required for all molecular E. coli detection methods.

Primer's list

Sr. #	Genes	Primer Sequence v
1	vanB-(F)	GTAGGCTGCGATATTCAAAGC
	vanB-(R)	GCCGACAATCAAATCATCCTC
2	tet - W(F)	CGGATTGTGGCATTTGT
	tet -W(R)	GCATAGAGGGTGAAGGAG
3	tet-M (F)	CGA ACA AGAGGAAAG CAT AAG
	tet-M (R)	CAA TAC AAT AGGAGCAAG C
4	tet-O (F)	AACTTAGGCATTCTGGCTCAC
	tet-O (R)	TCCCACTGTTCCATATCGTCA
5	Qnr-A (F)	ATTTCTCACGCCAGGATTTG
	Qnr-A (F)	GATCGGCAAAGGTTAGGTCA
6	Qnr-B (F)	GGMATHGAAATTCGCCACTGC
	Qnr-B (R)	TTTGCYGYYCGCCAGTCGAAC

Statistical analysis: Statistical data were evaluated by one-way ANOVA and DMR (Duncan's Multiple Range) techniques by using graph pad prism version 6 statistical software.

RESULTS

The research trial was performed at the Institute of Pharmacy Physiology and Pharmacology, University of Agriculture, Faisalabad. The purpose of the study was Identification and culturing of E. colon MacConkey agar medium and identification of different resistance gene expressions.

To investigate the 'genes' capacity to confer chalcones resistance against the E. coli host, different concentrations of expression inducers were added to MacConkey growth media used for chalcone susceptibility using the Kirby-Bauer disk diffusion method was done according to the Clinical Standards Institute guidelines (Hombachet al., 2013). Samples were swabbed on the required media for the growth of bacteria near the heat. After swabbing, plates were placed in an incubator at 37^{0}C for one night. Pinkish growth of E. coli appeared on media. In the isolated colonies of Escherichia coli, its growth diameter is 2-3mm with little propensity to confluent growth, exhibiting the pinkish shine by transmitted light. Catalase Test is performed if the test is positive, it shows the immediate effervescence (bubble creation). No bubble creation shows the catalase negative test. Gram staining was also performed to confirm the Escherichia coli present in the sample. Gram Staining confirms Escherichia coli presence by giving negative results of staining. After the gram staining, TSI tests were also performed.

Table2: TSI Test

Organism Name	Butt	Slant	H_2S	Gas
Escherichia Coli	Acid (A)	Acid (A)	(+)	(+)

Morphological Examination:

Color and texture: An E. coli colony is pinkish with a shiny texture. It looked like mucus and a Fixed margin. Steady growth pattern and creating concentric growth rings. I notice the ring under the microscope.

Sensitivity Assay: Escherichia coli showed a 0mm zone of inhibition against cb6. Standards were also applied for the confirmation of bacterial sensitivity for comparison. Different antibiotics were used to determine the zone of inhibition such as Ciproflaxicin, Team, CeftinandLevofloxacin.E. coliis highly resistant to these antibiotics except in Vietnam.

Sensitivity Assay against fungus: Chalcone cb6 was also applied on fungus (Curvularia). Chalcones cb6 has not any antifungal effects as shown in figure 4.10.

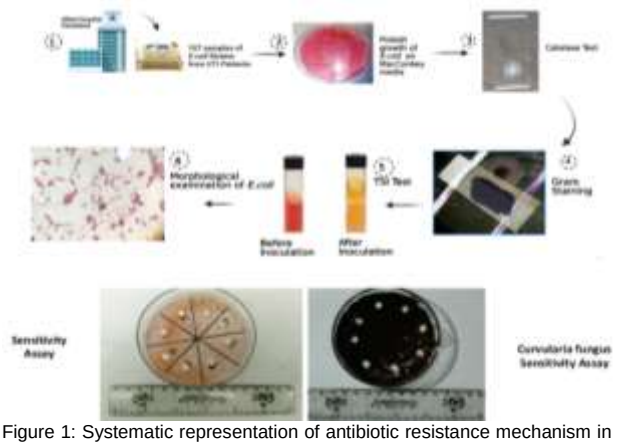


Figure 1: Systematic representation of antibiotic resistance mechanism in E. coli

qRT -PCR Results:

qRT- PCR was performed to check the expression level of genes and determine the absolute quantification of genes in the control V/s clinical sample.

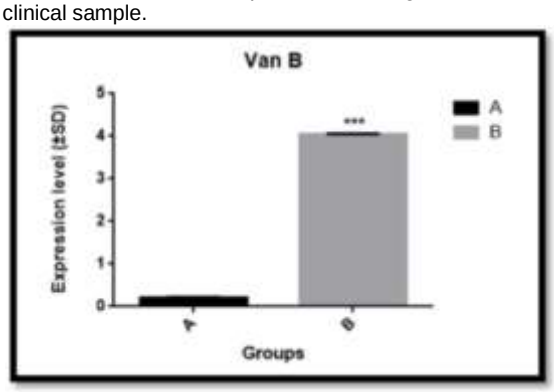
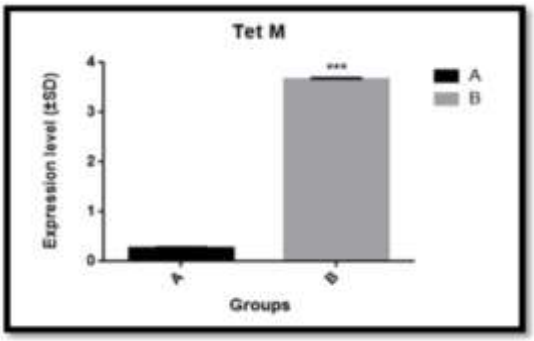
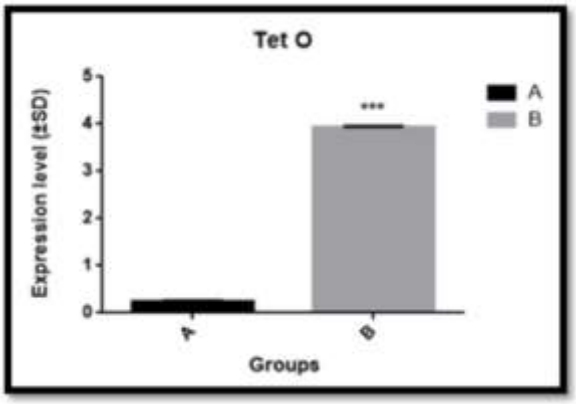


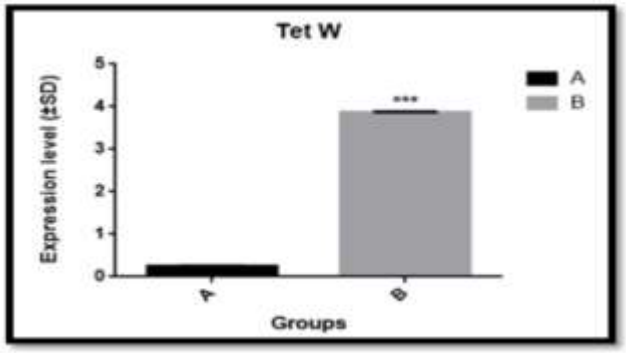
Figure is showing high value of Van B gene expression level in patient as compared to control sample (P-value <0.0001) and table represents different values of Van B gene, mean, standard deviation of error of mean in control as well as in patient. Values in the graph and table are showing the highly resistance expression level of Van B gene and it is up-regulated.



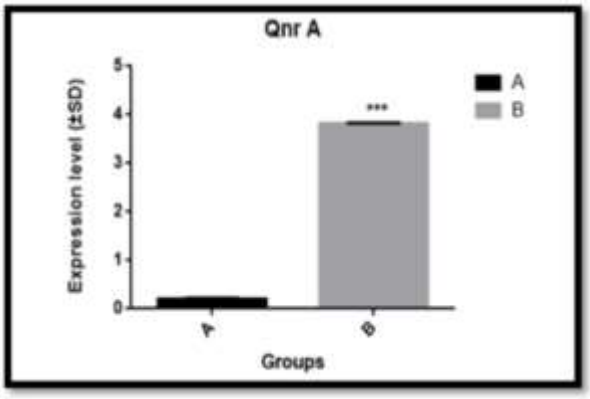
(A) Control sample of non-pathogenic Escherichia coli and (B) Patient sample of pathogenic Escherichia coli.Low expression level of Tet M gene in control sample as compared to patient sample. Graph is showing high value of Tet M gene expression level in patient as compared to control sample (p values <0.0001) and table represents different values of tet M gene, mean, standard deviation of error of mean in control as well as in patient.Values in the graph and table are showing the highly resistance expression level of tet M gene and it is up-regulated.



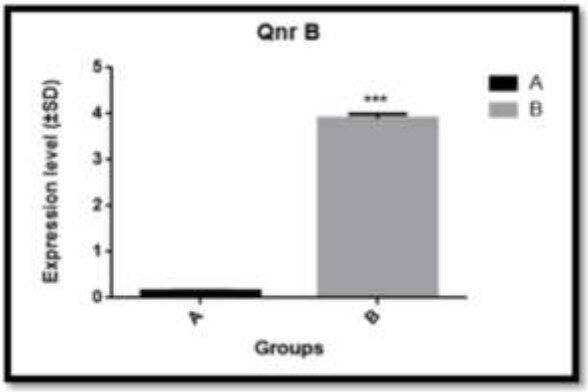
(A) Control sample of non-pathogenic Escherichia coli and (B) Patient sample of pathogenic Escherichia coli.Low expression level of Tet W gene in control sample as compared to patient sample. Graph is showing high value of Tet W gene expression level in patient as compared to control sample (P-value <0.0001). The table represents different values of tet W gene, mean, standard deviation of error of mean in control as well as in patient. Values in the graph and table are showing the highly resistance expression level of tet W gene and it is up-regulated.



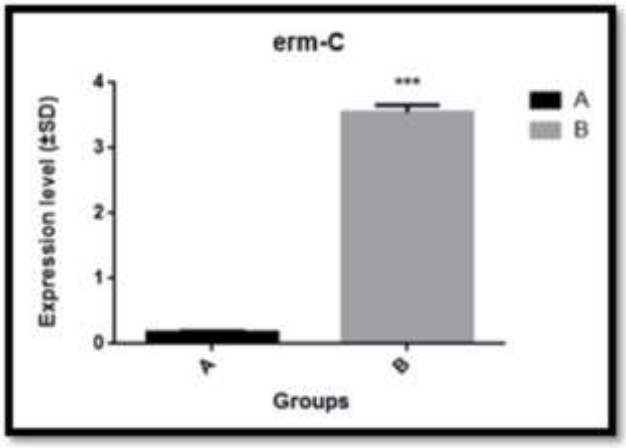
(A) Control sample of non-pathogenic Escherichia coli and (B) Patient sample of pathogenic Escherichia coli.Low expression level of Tet O gene in control sample as compared to patient sample. Graph is showing high value of Tet O gene expression level in control as compared to patient sample (P values <0.0001). Above table represents different values of tet O gene, mean, standard deviation of error of mean in control as well as in patient. Values in the graph and table are showing the highly resistance expression level of tet O gene and it is up-regulated.



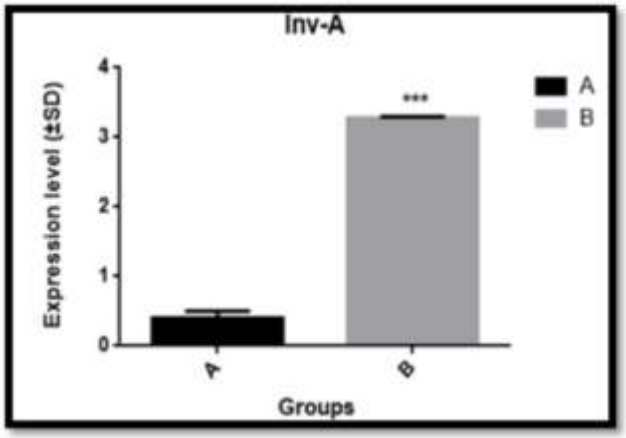
(A) Control sample of non-pathogenic Escherichia coli and (B) Patient sample of pathogenic Escherichia coli.Low expression level of Qnr A gene in control sample as compared to patient sample. Graph is showing high value of Qnr A gene expression level in control as patient as compared to control sample (p values <0.0001).And the above table represents different values of Qnr A gene, mean, standard deviation of error of mean in control as well as in patient. Values in the graph and table are showing the highly resistance expression level of Qnr A gene and it is up-regulated.



(A) Control sample of non-pathogenic Escherichia coli and (B) Patient sample of pathogenic Escherichia coli.Low expression level of Qnr B gene in control sample as compared to patient sample. Graph is showing high value of Qnr B gene expression level in patient as compared to control sample (p values<0.0001). Above table represents different values of Qnr B gene, mean, standard deviation of error of mean in control as well as in patient. Values in the graph and table are showing the highly resistance expression level of Qnr B gene and it is up-regulated.



(A) Control sample of non-pathogenic Escherichia coli and (B) Patient sample of pathogenic Escherichia coli. Low expression level of emr-C in control sample as compared to patient sample. Graph is showing high value of emr-C gene expression level in patients as compared to control sample (p values <0.0001). Above table represents different values of erm- C gene, mean, standard deviation of error of mean in control as well as in patient. Values in the graph and table are showing the highly resistance expression level of erm- C gene and it is up-regulated.



(A) Control sample of non-pathogenic Escherichia coli and (B) Patient sample of pathogenic Escherichia coli. Low expression level of Inv-A gene in control sample as compared to patient sample. Graph is showing high value of Inv-A gene expression level in patient as compared to control sample (p values <0.0001).Above table represents different values of Inv- A gene, mean, standard deviation of error of mean in control as well as in patient. Values in the graph and table are showing the highly resistance expression level of Inv- A gene and it is up-regulated.

DISCUSSION

Bacterial resistance is developed by the over and misuse of antibiotics against the microbes. This study concludes that the antimicrobial susceptibility configuration of bacteria isolated from the patient sample(Bassetti et al., 2022) It is understood that magicians are always conscious of the new antibiotic resistance steps before using the new antibiotics against bacterial infections in humans (Zhanelet al., 2006).Human illness is brought on by the Shiga toxin-producing E. coli O157. The pathogen's main reservoir is cattle. Ethiopia has limited information on the connection between contaminated beef consumption and human diarrheal diseases(Desissa Gutema, 2021).Bacterial resistance against the chalcones has not been reported yet to be explained in any study although there separate some correlative studies with inadequate standards that indicate the beginning of resistant bacteria and antibiotics from many sources (Ferreira da silva al., 2007; Lefkowitz and Duran, 2009).

The strains of E. coli are not harmful, some strains causes the diseases in humans like vomiting, diarrhea(Mutuku et al., 2022). The pathogenic strain of E. coli that spread the various intestinal infections, urinary infection and some pathogenic E. coli related with the mild gastric diseases(Taha and Deghmane, 2022).More than 400 serotypes are known as STEC, some sub group associated with human infections. E.coli is the microbe that mostly cause human infection and a universal colonized present in human and animal intestinal tract. Antibiotic resistance in human may associated with the selection and use of antibiotics(Blanco et al., 2004).

Inclusion of positive and negative reference strains and process control to detect inhibition of the PCR reaction by the sample matrix are required for all molecular E. coli detection methods. Quantitative real-time polymerase chain reaction (qRT-PCR) is influential apparatus to evaluate the tools original biosynthetic ways of substances, qRT-PCR was used for precise studies of transcript levels owing to its quickness, sensitivity and consistency which depend on the expression stability of the reference genes used for data normalization. Confirming the reliability of gene expression investigation qRT-PCR was used. Through, the precision of qRT-PCR consequences relies on multiple aspect, counting the preliminary RNA quality and quantity, primer specificity, reverse transcription effectiveness, amplification efficacy, PCR circumstances, and transcript standardization. Regularization of qRT-PCR record with appropriate interior reference gene(s) is one technique to gain true and reliable outcomes of qRT-PCR.One primer only amplify a common sequencing in the all microorganisms genome. For amplification effectiveness, primer use for amplification process should be optimum with preferred properties like temperature of melting, content of GC. For the synthesis of secondary structures such as self-dimer and loop of heparin.The design of primer is according to the requirements that will suitable for the template and give the desired results (Maheuxet al., 2009) DNA region of the all species. Sequencing of each bacteria should be known for more than one species identification.In this study in the early phase of the cycle maximum quantification indicates the presence of the excess amount of the target gene. All Van B, Tet W, Tet M, Tet O, Qnr A, Qnr B, erm C, and Inv-A genes showed high resistance expression levels.

CONCLUSION

There is a high prevalence of infection among patients in the Faisalabad region. Different Bacteria show different resistant diversity against the different antibiotics. But E. coli is the dominant bacteria that shows high resistance against the chalcones. Van B, erm C, Iva-A, Qnr-A, Qnr-B, tet M, tet O, and tet Ware resistant genes of the Escherichia coli against the Cb6 (E)-1-(4-fluorophenyl)-3 -phenyl prop-2-en-1-one and it was showed the high expression level.

Abbreviations:
E.coli: Escherichia coli
UTI: Urinary tract infection
qRT- PCR: Quantitative real-time PCR
gDNA: genomic DNA

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