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College of Medicine

Study The Effect Of Some Selected Antibiotic Used To Inhibit The Growth And Biofilm Formation Of *Escherichia Coli* Isolated From Clinical Sources

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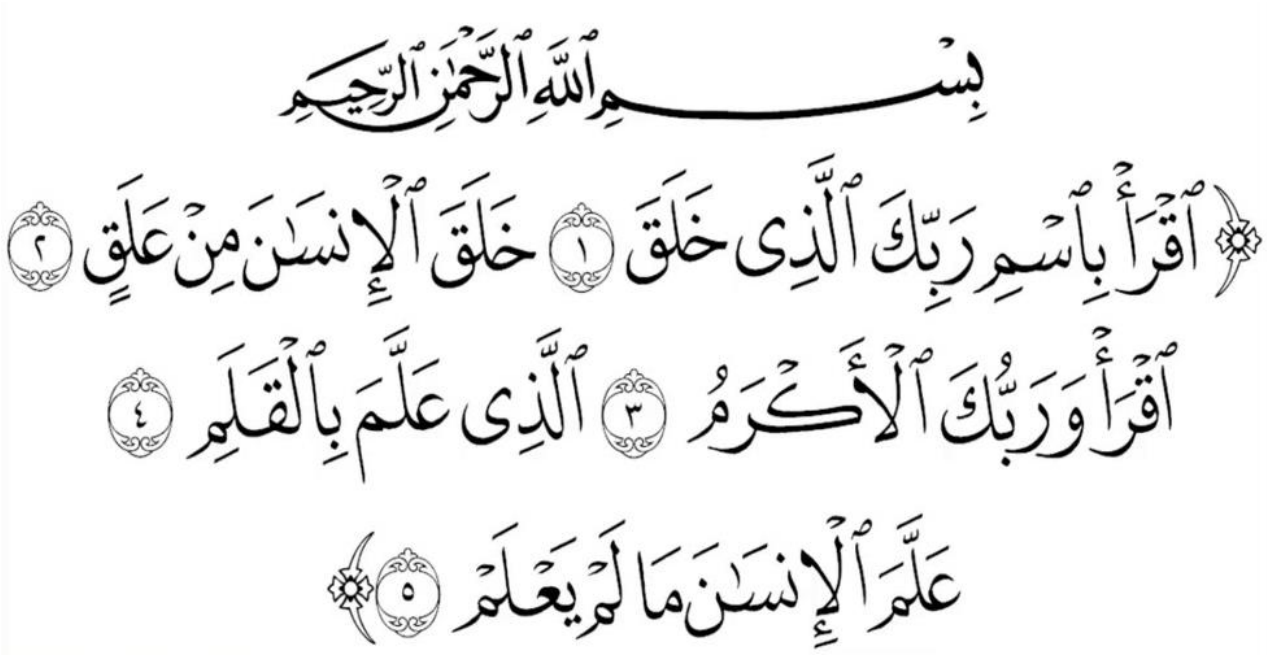
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صَدَقَ اللهُ العَظِيمُ

سورة العلق/ (٩٦)

السيد رئيس لجنة بحوث تخرج الطلبة المحترم ....

م/ تسليم بحث تخرج

تحية طيبة

يرجى التفضل بالموافقة على استلام بحث تخرج طالب المرحلة السادسة (فاطمة ساجد عزيز) للعام الدراسي ٢٠٢٤-٢٠٢٥ الموسوم

Study the effect of some selected antibiotic used to inhibit the growth and biofilm formation of *Escherichia coli* isolated from clinical sources

لإكمال كافة متطلباته ..

مع فائق الاحترام والتقدير ...

مشرف الطالب
م.د عدنان ياس خضير

Dedication

I present this research to our God Almighty who showed us his blessings in everyday lives , also present this work to our home country and to our dear university / college of medicine and to the professor and doctors of the college who have done so much to get us to this place . I present this research to our families who have given everything they have to make them proud of us .

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List of content

Paragraph number	Title	Page number
	Introduction	١
	Literature Review	٢
1	*Escherichia coli*	٢
2	Normal microbiota	٢
3	Role in disease	٣
4	Antibiotic resistance	**4**
5	Biofilm formation	**4**
	Materials and Methods	**6**
2	Materials	**6**
2.1	Equipment , Apparatuses , Identification Kits , Antibiotics	**6**
3	Methods	**6**
3.1	3.1 Preparation of Culture Media	**6**
٣,٢	Sample Collection	**7**
٣,٣	Isolation of Escherichia coli	**7**
٣,٤	Identification of bacteria	**7**
٣,٤,١	Cultural Examination	**7**
٣,٤,٢	Microscopical Examination	**7**
٣,٤,٣	Biochemical Tests	**8**
٣,٤,٣,١	Catalase Test	**8**
٣,٤,٣,١	Identification Using Vitek 2 System	**8**
٢,٥	Maintenance of bacterial isolates	**8**
٢,٥,١	Short Term Storage	**8**
٢,٦	Antibacterial Susceptibility Test	**8**
2.7	Biofilm formation	**9**
٢,٨	Minimum inhibitory concentration	**9**
٢,٩	Minimum inhibitory concentration against biofilm formation	**9**
	Results and Discussion	
3.1	Isolation of bacteria	**10**
3.2	Identification of bacterial isolates	**10**
3.2.1	Cultural and Microscopical Characterization	**10**
٣,٢,٢	Biochemical Tests	**10**
٣,٢,٣	Identification Using Vitek 2 System	**11**
٣,٣	Antibacterial Susceptibility Test	**12**
٣,٤	Biofilm formation	**14**
٣,٥	Minimum inhibitory concentration	**15**
٣,٦	Minimum inhibitory concentration against biofilm formation	**16**
	Conclusions and Recommendation	
	Conclusions	**19**
	Recommendation	**19**
	References	**20**

List of Table

Table number	Title	Page number
1	Equipment , Apparatuses , Identification Kits and Antibiotics used in this study .	**6**
2	Culture media used in this study .	**7**
3	Sensitivity of bacterial isolates isolated from feces and urine to antibiotics	**13**
4	Biofilm formation ability of *E.coli* isolates isolated from urine and stool samples	**15**
5	Minimum inhibitory concentration of Trimethoprim to *E.coli* isolates isolated from urine and stool samples	**16**
6	Percentage of inhibition of biofilm formation by using trimethoprim	**17**

List of Figures

Figure number	Title	Page number
1	*Escherichia coli* on Macconkey agar (24h /37°C).	**10**
2	*Escherichia coli* on citrate agar (24h /37°C).	**11**
3	Percentages of *Escherichia coli* isolates isolated from stool and urine samples	

Summery

A complete study was accomplished to identify and characterize *Escherichia coli* (*E. coli*) Isolates from diverse clinical specimens .From thirty specimens , only 25 bacterial isolates were recovered and recognize as *E. coli* isolates via numerous tests , including microscopic examination , biochemical tests , and VITEK 2 automated microbial identification system .The findings indicated that 17 (68%) isolates were obtained from urine specimens and 8 (32%) isolates from stool specimens.

Antimicrobial susceptibility testing was done by disc diffusion technique . Most of the isolates of *E. coli* exhibited sensitivity to tigecycline and resistance to trimethoprim-sulfamethoxazole and ampicillin . Also , the biofilm- forming capacity of the *E. coli* isolates was assessed, with a notable 64% (16/25) of the isolates testing positive for biofilm production with 11 out of 17 isolates from urine samples positivity , whereas 4 out of 8 from stool samples .

The minimum inhibitory concentration (MIC) of Trimethoprim was determined , and the results revealed that Trimethoprim was capable to inhibit *E. coli* isolates with MIC between 25-50 μg/ml . Microtiter plates were utilized to estimate the ability of Trimethoprim to inhibit biofilm formation and the results showed that Trimethoprim could inhibit biofilm formation in a concentration- dependent manner with 100% inhibition observed at 200 μg/ml.

Generally , the study confirmed the need of identifying and characterizing *E. Coli* isolates from clinical specimens and the studying antibiotic susceptibility testing to recognize treatment choices . The results also highlight Trimethoprim potential as a treatment for *E. Coli* infections, especially those caused by strains that produce biofilms.

Chapter One

Introduction

Introduction

Biofilm formation is complex process occurs when bacteria stick to a surface and create a matrix of protective polymers. In addition to allowing bacteria to survive in settings, this biofilm increases antibiotic resistance. Four phases make up the formation process: adhesion , growth , maturation , and maturity **(1)** . Bacteria first adhere to the surface, then quickly proliferate and multiply. The biofilm becomes more complex as it ages , which increases its resistance to host immune responses and drugs . Biofilm production is influenced by a number of variables , such as solid surfaces , ideal temperatures, humidity , nutrients , and chemicals **(2)** . Biofilm production has serious negative effects , including tissue inflammation , persistent infections , antibiotic resistance, and malfunctioning medical devices . Effective methods for preventing the production of biofilms include the use of anti-biofilm chemicals , thorough cleaning and disinfection , antibiotic therapy , and environmental factor control. For the development of novel bacterial infection prevention and treatment approaches , an understanding of biofilm formation is essential **(3)** .

The Gram-negative bacterium *Escherichia coli* (*E. coli*) plays two important roles in human health. Pathogenic strains of *E. coli* cause diseases such as urinary tract infection , gastroenteritis and pneumonia ,while commensal strains live in the gut without causing any harm. Because of its genetic diversity, *E. coli* can adapt to a variety of conditions, which contributes to its adaptability **(4)** .Toxins ,adhesins and invasins are examples of virulence factors that certain strains create to facilitate colonization and infection. Useful strains of *Escherichia coli* ,on the other hand , support gut health via vitamin synthesis , food metabolism ,and immune system regulation .To effectively treat and prevent *E. coli*- related diseases, it is crucial to understand the intricate relationships that *E. coli* has with its host **(5)** .

Therefore, the goal of this study was to identify and choose the optimal antibiotic dose to prevent *E. coli* from growing and forming biofilms.

Chapter two

Literature Review

Literature Review

1- *Escherichia coli*

The Gram-negative bacterium *Escherichia coli* (*E. coli*) , is frequently exit in the guts of human and other animals .Pathogenic strains cause diseases like sepsis , pneumonia, gastroenteritis , and urinary tract infections (UTIs) . Because of its genetic diversity , *E. coli* can adapt to a variety of conditions , which makes colonization and infection easier **(6)** . In order to facilitate host cell attachment and invasion , pathogenic strains generate virulence factors such as adhesins , invasins , and Shiga toxins . By producing vitamins K and B , breaking down nutrients, controlling the immune system, and preserving intestinal homeostasis, beneficial strains of *Escherichia coli* play a vital role in gut health . Antibiotics , stress , and food all have an impact on the symbiotic connection between E. coli and its host. Diseases and illnesses can result from dysbiosis, or imbalance **(7)** .

Contaminated food , water, and interpersonal contact are the main ways that *E. coli* infections are spread. The location and intensity of the infection affect the symptoms . PCR, serotyping, and culture are used in the diagnosis process . Antibiotics, supportive care, and hospitalization are available forms of treatment for severe instances **(8)** . Since *E. coli* strains are becoming resistant to several drugs, antibiotic resistance is becoming a bigger problem . This highlights the need to develop vaccines, antibiotics use sparingly , and improve cleaning . Understanding the pathophysiology of *E .coli* , developing innovative treatments and exploring the use of probiotics are major areas **(9)** .

2- Normal microbiota

E. coli is a common intestinal bacterium that is necessary for huma . By generating vitamins , such as vitamin K and B vitamins , beneficial *E. coli* strains , such *E. coli* Nissle 1917, support a healthy gut flora . In addition , these bacteria control the immune system , help break down and absorb nutrients, and preserve the integrity of the intestinal epithelial barrier **(10)** . Additionally , dangerous bacteria can be outcompeted by beneficial strains of *Escherichia coli*, lowering the chance of infection. Furthermore, *E. Coli* has been utilized as a probiotic to treat a number of gastrointestinal

conditions , including diarrhea , inflammatory bowel disease , and irritable bowel syndrome (IBS) . All things considered , *E. Coli's* advantageous functions emphasize how crucial it is to keep the gut microbiota balanced for optimum health **(11).**

Beyond the gut , *Escherichia coli* has positive benefits on general health and wellbeing. To assist control the gut microbiota and avoid infections, *E. coli*, for example, produces antimicrobial peptides such colicins . Short- chain fatty acids , which give colonic epithelial cells energy and support a healthy gut environment, are also produced as a result of *E. coli's* metabolic activities **(12)** . The immune system growth and operation are also influenced by *E. coli*, especially in the gut- associated lymphoid tissue (GALT) . *E. coli's* capability to colonize the gut and stimulate immunological responses has also led to studies investigating its potential as a delivery system for therapeutic compounds ,including vaccines and antibodies .*E. Coli* numerous advantages underscore its significance as a vital component of the human gut microbiome **(13)** .

3- Role in disease

Numerous illnesses in humans can be brought on by *Escherichia coli* , or *E. coli*. Adhesins , invasins , toxins , and siderophores are only a few of the virulence characteristics that pathogenic *E. coli* strains have that allow them to infiltrate and infect host tissues. Urinary tract infections (UTIs), one of the most prevalent illnesses brought on by *E. coli*, can result in serious side effects like sepsis and pyelonephritis **(14)** . Another common cause of gastroenteritis , especially in young infants and those with weakened immune systems , is *E. coli*. Some *E. coli* strains , such as O157:H7 can produce shiga toxins that can lead to hemolytic uremic syndrome (HUS) and other life-threatening conditions including pneumonia, meningitis, and septicemia **(15)** .

Antibiotic- resistant microorganisms make treatment choices more difficult , demanding the development of new treatment strategies and preventative actions. To develop effective vaccines and therapies , understanding the molecular pathways that underlie *E. coli* pathogenesis is important . Also, the rise of *E. Coli* strains that are resistant to medicines emphasizes the need for better infection control techniques and the wary use of antibiotics in order to slow the spread of these diseases **(16)** .

4- Antibiotic resistance

Antibiotic resistance to *Escherichia coli* (*E. coli*) has become a serious public health issue worldwide. The spread and emergence of resistance *E. coli* was accelerated by widespread use and misuse of antibiotics . By producing enzymes that render antibiotics inactive, altering target sites , and removing drugs from the cell through efflux pumps , these bacteria have evolved a number of defense strategies against the effects of antibiotics **(17)** . *E. Coli* has become resistant to a number of antibiotics , such as aminoglycosides ,beta-lactams ,and fluoroquinolones .The most alarming development is the rise of extended- spectrum beta- lactamase- producing *E. coli* (ESBL-*E. coli*) and carbapenem- resistant *E. coli* (CRE) ,which are resistant to the majority of the antibiotics on the market .Horizontal gene transfer , which permits bacteria to exchange resistance genes , has contributed to the proliferation of *E. Coli* strains that are resistant to antibiotics **(18)** . Antibiotic- resistant *E. coli* infections can result in higher rates of morbidity, death , and medical expenses , which has important ramifications for human health .Adopting a multimodal strategy that includes prudent antibiotic usage , enhanced infection control procedures , and the production of new medications and treatment approaches is required to the fight against antibiotic resistance. Additionally, in order to track the development of resistant strains and inform public health strategies , surveillance and monitoring of antibiotic resistance patterns are essential **(19)** .

5- Biofilm formation

Escherichia coli (*E. coli*) can form biofilms on many surfaces , including medical equipment, catheters and intestinal epithelial cells .Adhesion , growth , maturation , and dissemination are all steps in in the complex biofilm development process . Flagella, pili ,and adhesins , which promote surface attachment , are some of the components that mediate *E. coli's* capacity to create biofilms **(20)** . After adhering , *E. coli* generates extracellular polymeric substances (EPS), such as proteins, polysaccharides, and nucleic acids , which give the biofilm structural support and defense . *E. coli* may withstand antibiotic treatments , withstand environmental changes , and elude human immune responses by forming biofilms .Biofilms can also operate as harbors for bacteria that are

resistant to antibiotics , which helps resistance genes proliferate **(21)** . To effectively prevent and treat illnesses linked to biofilms , it is essential to comprehend the mechanisms underlying *E. coli* biofilm development . To stop biofilm formation , research has concentrated on finding substances that inhibit biofilm formation , creating innovative antimicrobial treatments , and enhancing surface coatings . Furthermore , knowledge of the function of regulatory networks and quorum sensing in biofilm formation can aid in the creation of focused therapeutic approaches **(22)** .

Chapter three

Materials and Methods

Materials and Methods

2 Materials

2.1 Equipment , Apparatuses , Identification Kits and Antibiotics

Table (1) : Equipment , Apparatuses , Identification Kits and Antibiotics used in this study .

Equipment and Apparatuses	Manufacturing company and origin	
Autoclave	GallenKamp (UK)	
Compound light microscope	Olympus (Japan)	
Hot plate with Magnetic Stirrer	Labcco (Germany)	
Incubator	Memmert (Germany)	
Micropipettes	Oxoid (England)	
Safety cabinet hood	Fume Hood (Turkey)	
Sensitive Balance	Labcco (Germany)	
Vitek 2 Compact autoanalyzer	Bio Merieux (France)	
Kit	**Company (origin)**	
Vitek 2 GP Kit	Biomerieux (France)	
Antibiotics Disk	**Symbol**	**(µg / disk)**
Amikacin	Amk	30
ciprofloxacin	CIP	10
Imipenem	IMP	10
Tigecycline	TGC	10
Nitrofurantoin	NIT	30
Gentamicin	GEN	10
Piperacillin - Tazobactam	TZP	20/10
Cefepime	FEP	30
Levofloxacin	LVX	5
Trimethoprim - Sulfamethoxazole	SXT	25
Ampicillin	AMP	10

3 Methods

3.1 Preparation of Culture Media

All media were prepared in a laboratory in accordance with scientific references. media , with a few exceptions , were autoclave sterilized for 15 minutes at 121 ^{o}C (15 Ib/inch2).

Table(2) : Culture media used in this study .

Medium	Manufacturing company and origin
Nutrient broth	Hi-Media (India)
Nutrient agar	Hi-Media (India)
Brain Heart Infusion Broth	Oxoid (England)
Macconkey agar	Hi-Media (India)
Muller-Hinton Agar	Oxoid (England)

3.2 Sample Collection

Various clinical specimens were obtained from the Al-Batool Hospital in the province of Diyala, placed in sterile containers, and then transported to the laboratory for bacterial isolation under refrigerated circumstances.

3.3 Isolation of *Escherichia coli*

Each sample was put to nutrient broth and cultured for 24 hours at 37°C in an anaerobic environment in order to isolate bacteria . After incubation, a series of dilutions (10^{-1},10^{-2},10^{-3},10^{-4}) were prepared using regular saline. After that , 0.1 ml of the fourth dilution was transferred onto Macconkey agar plates and incubated anaerobically for 24 hours at 37 °C. For additional purification , isolated colonies were sub cultured on Macconkey agar and incubated for 24 hours at 37°C in an anaerobic environment **(23)** .

3.4 Identification of bacteria

3.4.1 Cultural Examination

Shape, color, texture, size, opacity, and colony margin are among the morphological characteristics on nutritional agar and mannitol salt agar that were used to identify the isolates.

3.4.2 Microscopical Examination

Microscopic analysis can be utilized to detect and distinguish between shape , arrangement, and gram stain reactivity, according to Garvie **(24).**

3.4.3 Biochemical Tests

3.4.3.1 Catalase Test

A loopful of a 20-hour-old bacterial colony on a clean microscope slide was treated with one drop of 3% H2O2. The development of gaseous bubbles within 20-30 seconds suggests a successful positive result **(25)** .

3.4.3.1 Identification Using Vitek 2 System

A pure culture of the *Esherichia coli* grown on nutrient agar plates at 37ºC for 24 h suspended in a sterile saline solution to a McFarland standard of 0.5-1.0. The sample is then loaded into the instrument, along with an identification card specific to the type of microorganism being identified . The instrument incubates the sample for a specified period, usually 2-12 hours , and then reads the results to provide an identification . In accordance with industry guidelines (Biomerieux) , the VITEK 2 system evaluates the data obtained and uses colorimetric tests (biochemical reactions) to identify the tested bacteria.

2.5 Maintenance of bacterial isolates

2.5.1 Short Term Storage

To keep the bacterial isolates alive for a few weeks, they were inoculated in nutrient broth and incubated for 24 hours at 37°C. The bacterial broth is then streaked on a nutrient agar slant and incubated for 24 hours at 37 °C. Sub- cultured each month and kept in the refrigerator **(26)** .

2.6 Antibacterial Susceptibility Test

A Muller-Hinton agar plate is inoculated with a bacterial suspension , and antibiotic discs are applied to the agar surface. The diameter of the zone of inhibition surrounding each antibiotic disc is then measured after the plate has been incubated for 16-18 hours at 35-37°C **(27)** .Greater susceptibility to the antibiotic is shown by broader zones of inhibition, which are used to assess the bacteria's susceptibility . In order to categorize the bacteria as susceptible , intermediate , or resistant , the Clinical and Laboratory Standards Institute (CLSI) offers breakpoints for interpreting the results **(28)** .

2.7 Biofilm formation

Prepare Congo Red Agar plates according to the manufacturer's instructions. Inoculate the plates with the test bacteria using a sterile loop or swab. The plates incubated at 37°C for 24-48 hours. Observe the results : Observe the plates for the presence of red or pink colonies, which indicate biofilm formation **(29)** .

2.8 Minimum inhibitory concentration

Mueller-Hinton agar plates containing antibiotic Trimethoprim concentrations (200-100 - 50- 25- 12.5 μg/mL) are prepared for this purpose . Next , to create a bacterial inoculum, a pure culture of the test organism is suspended in saline solution until the concentration reaches 1-2 x 10^8 CFU/ mL. Then , using a sterile loop or swab , the inoculum is transferred to the agar plates. The plates are incubated at 35-37°C for 16-20 hours , and the findings are read by looking for growth on the plates **(30)** . By determining the lowest antibiotic concentration that prevents discernible growth , the MIC is calculated.

2.9 Minimum inhibitory concentration against biofilm formation

To inhibit biofilm formation , antibiotics such as ciprofloxacin can be used . The process begins with preparing stock solutions of the antibiotics, which are then diluted to create a series of concentrations (200-100 - 50- 25- 12.5 μg/mL). A bacterial inoculum is prepared by suspending a pure culture of the test organism in saline solution , and 20 μl then inoculated into 80 μl brain heart infusion broth in flat bottomed Microtitration plates . The antibiotic solutions 100 μl are added to the mixture at different concentrations , and the mixture is incubated at 35-37°C for 24-48 hours . After incubation , the biofilm is stained using a staining method such as crystal violet or Congo red , and the biofilm formation is measured using a microplate reader or Spectrophotometer . The percentage of inhibition of biofilm formation was calculated by applying the equation **(31).**

$$\text{Percentage of inhibition of biofilm formation} = 1 - \frac{\text{Optical density (O.D) in the presence of}}{\text{Optical density (O.D) of control treatment}} \times 100\%$$

Chapter four

Results and Discussion

Results and Discussion

3.1 Isolation of bacteria

Thirty samples of various clinical specimens yielded a total of 25 bacterial isolates. After undergoing numerous identification tests , it was determined that these isolates belonged to *Escherichia coli* species.

3.2 Identification of bacterial isolates

3.٢.1 Cultural and Microscopical Characterization

Colonies of *Escherichia coli* were observed on the surface of Macconkey agar . Results showed that all isolates form small pink to red , round , convex colonies while microscopic examination showed that all isolates were gram negative , non -spore forming , rod-shaped occurring as a single or pairs **(32)** .



Figure (1): *Escherichia coli* on Macconkey agar (24h /37℃).

3.2.2 Biochemical Tests

According to biochemical tests , all isolates were able to ferment lactose , which produced acid and turned the media red or yellow. They were also able to use citrate as a carbon source, which turned green citrate agar to blue. In the oxidase and catalase tests, *E. coli* tested positive for catalase and negative for oxidase **(33)** .

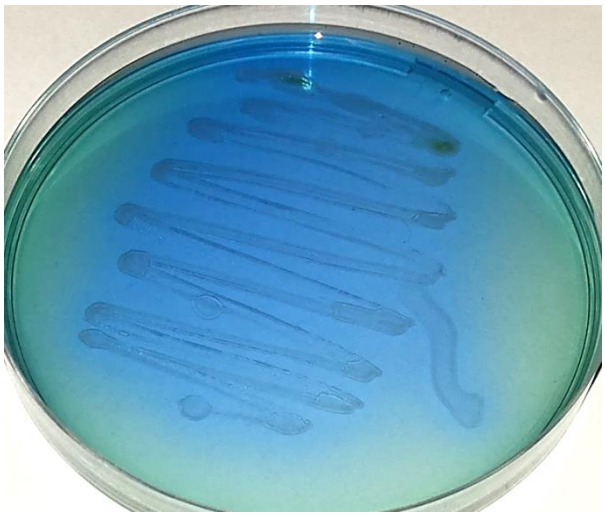


Figure (2): *Escherichia coli* on citrate agar (24h /37°C).

3.2.3 Identification Using Vitek 2 System

VITEK 2 is a widely used automated microbial identification system produced by bioMérieux. It's a powerful tool for identifying bacteria, yeast, and other microorganisms in laboratory and clinical settings . The results showed that 25 bacterial isolates were belonged to *Escherichia coli* species , and distributed as follows: 17 isolates were belong to urine samples and 8 isolates belonged to stool samples. The results above indicates the dominance of *E.coli* isolates isolated from urine samples, which gave (17) isolates (68%) , followed by (8) isolates (32%) from stool .

The essential diagnostic technique for the detection of gastrointestinal and urinary tract infections (UTI) is the isolation of *E. coli* from urine and stool samples . With a sensitivity of 95.5% , MacConkey agar has been shown to be the most successful medium for the separation of *E. coli* from stool and urine samples . In order to isolate *E. coli* from clinical samples, a selective medium like MacConkey agar was necessary **(34)**. *E. coli* accounts for approximately 85 percent of community acquired UTIs and 50 percent of hospital acquired UTIs. Different factors like age, gender, immuno-repression and urological instruments may affect frequency of UTIs. Catheter- associated UTIs is one of the most dangerous health risks contributing 34% of all health care associated infections **(35)** . A total of 150 *E. coli* isolates were obtained from urine samples collected from patients at the Kalyan Nagar Medical Diagnostic Centre in Bangalore over four months November 2022 to February 2023. Isolation and screening of

pathogenic *E. coli* was performed using Eosin methylene blue agar and Nutrient Agar, followed by identification by Gram- positive staining and biochemical characterization **(36)** .

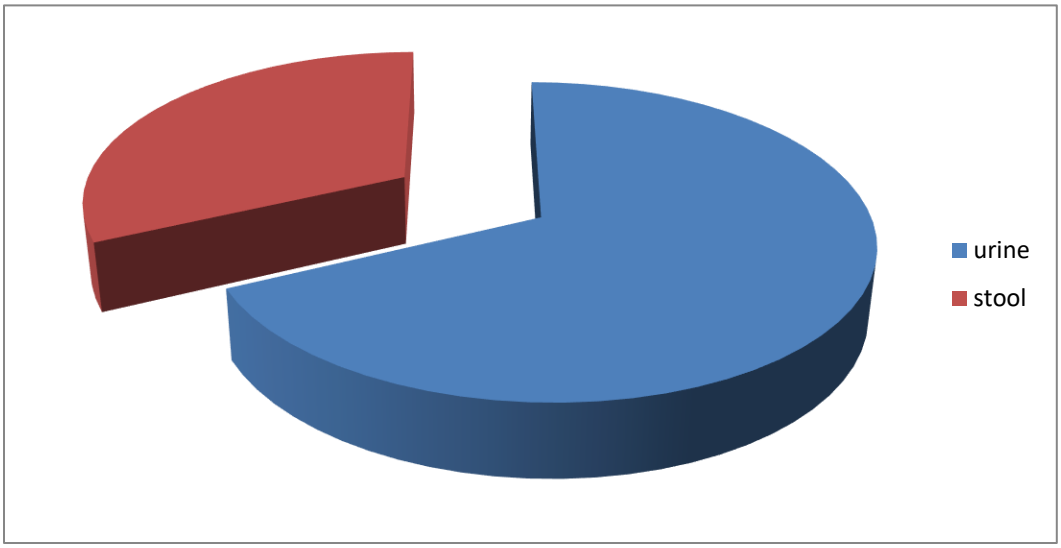


Figure (3): Percentages of *Escherichia coli* isolates isolated from stool and urine samples

3.3 Antibacterial Susceptibility Test

The disc diffusion method , also known as the Kirby-Bauer test , is a widely used technique for determining the antimicrobial susceptibility of microorganisms , such as bacteria and fungi . In this method , the sensitivity of the bacterial isolates were tested against (11) type of antibiotics . According to the diameter of inhibition zone around the antibacterial disc. The isolates were divided in to three groups : resistant , intermediate and sensitive . The results shown in Table (3) showed that there is a variation in the resistance of the isolates under study to the antibiotics used, as most *E.coli* isolates were resistant to antibiotics Trimethoprim – Sulfamethoxazole and Ampicillin , While it was sensitive to antibiotic Tigecycline , and its sensitivity to other antibiotics used varied.

Around the world , there is growing concern over the rise of *E. coli* strains that are resistant to antibiotics. Ampicillin and trimethoprim are two popular antibiotics used to treat *E. coli* infections. The bacterium has become resistant to these antibiotics , though, which makes therapy more difficult . Research has indicated that isolates of *E. coli* exhibit variable degrees of resistance to trimethoprim and ampicillin **(37)** . Trimethoprim

resistance was found in 48.95% of *E. Coli* isolates from UTIs, according to a study. An further study found that 35.6% of *E. Coli* isolates from bloodstream infections had ampicillin resistance **(38)** .

The frequency and molecular characterization of trimethoprim and ampicillin resistance in *E. coli* isolates from UTIs were examined in a study that was published in Antimicrobial Agents and Chemotherapy . Using disk diffusion and PCR , the study examined 150 *E. coli* isolates and discovered that 51.3% of them were resistant to trimethoprim and 41.3% to ampicillin **(39) .** Another study that examined 200 *E. Coli* isolates from blood, stool, and urine samples discovered that 60% of them were resistant to trimethoprim, 50% to ampicillin, and 30% to both antibiotics. **(40)** .

Table (3):Sensitivity of bacterial isolates isolated from feces and urine to antibiotics

Bacterial isolates	**antibiotics**										
	Amk	**CIP**	**IMP**	**TGC**	**NIT**	**GEN**	**TZP**	**FEP**	**LVX**	**SXT**	**AMP**
Escherichia coli (U_1)	S	R	S	S	S	S	R	R	I	R	R
Escherichia coli (U_2)	S	S	S	S	S	S	S	S	S	R	R
Escherichia coli (U_3)	S	I	S	S	S	S	S	S	I	R	R
Escherichia coli (U_4)	R	R	S	S	S	R	S	I	R	R	R
Escherichia coli (U_5)	R	R	S	S	R	R	S	I	R	R	R
Escherichia coli (U_6)	S	R	S	S	S	S	R	R	R	R	R
Escherichia coli (U_7)	S	R	S	S	S	S	S	S	R	R	R
Escherichia coli (U_8)	S	S	S	S	S	S	S	S	S	R	R
Escherichia coli (U_9)	R	R	S	S	R	R	S	S	S	R	R
Escherichia coli (U_{10})	S	R	S	S	S	S	R	R	I	R	R
Escherichia coli (U_{11})	R	R	S	S	R	R	R	S	R	R	R
Escherichia coli (U_{12})	R	R	S	S	R	R	S	I	R	R	R
Escherichia coli (U_{13})	S	R	S	S	S	S	R	R	R	R	R
Escherichia coli (U_{14})	S	R	S	S	S	S	S	S	R	R	R
Escherichia coli (U_{15})	S	S	S	S	S	S	S	S	S	R	R
Escherichia coli (U_{16})	R	R	S	S	R	R	S	S	S	R	R
Escherichia coli (U_{17})	S	R	S	S	S	S	R	R	I	R	R
Escherichia coli (S_1)	S	S	S	S	S	S	S	S	S	R	R
Escherichia coli (S_2)	S	R	S	S	S	S	S	S	I	R	R
Escherichia coli (S_3)	S	S	S	S	S	S	S	S	S	R	R
Escherichia coli (S_4)	S	S	S	S	S	S	S	S	I	R	R

Escherichia coli (S_5)	S	R	S	S	S	S	R	R	I	R	R
Escherichia coli (S_6)	S	S	S	S	S	S	S	S	S	R	R
Escherichia coli (S_7)	S	R	S	S	S	S	S	S	I	R	R
Escherichia coli (S_8)	S	S	S	S	S	S	S	S	S	R	R

Amikacin : Amk **Ciprofloxacin** : CIP **Imipenem** : IMP

Tigecycline : TGC **Nitrofurantoin** : NIT **Gentamicin** : GEN

Piperacillin - Tazobactam : TZP **Cefepime** : FEP **Levofloxacin** : LVX

Trimethoprim – Sulfamethoxazole : SXT **Ampicillin** : AMP

Resistances : R **Sensitive** :S **Intermediate** : I

Urine : U **Stool** : S

3.4 Biofilm formation

The ability of *E.coli* isolates isolated from urine and stool samples to form biofilm was tested using solid Congo red medium. A positive result for biofilm formation was based on the production of dark black colonies with a dry crystalline density, while pink colonies were considered a negative result for this test . The results showed that (16) out of (25) isolates of *E.coli* bacteria had the ability to form a biofilm and distributed as (11) out of (17) isolates isolated from urine samples and (4) out of (8) isolates isolated from stool samples .

Antimicrobial agents, such as antibiotics and disinfectants, cannot penetrate *E. Coli* biofilms. The existence of persisting cells, the protective effects of the EPS matrix , and the expression of genes linked to antibiotic resistance are some of the causes of this resistance. Effective methods for preventing and treating infections linked to biofilms require an understanding of the mechanisms behind *E. coli* biofilm production and maintenance. **(41)** .

A study examining the function of quorum sensing in *E. coli* biofilm formation was published in the Journal of Bacteriology. Combining genetic and biochemical methods, the researchers showed that the AI-2 system regulated the expression of genes involved in the creation of biofilms , including those that produce extracellular polymeric compounds **(42)** . According to a different study, biofilms were generated by 75% of *E. coli* isolates from UTIs , and 60% of these biofilms were antibiotic -resistant .

Additionally, the study discovered that 40% of the isolates of *E. coli* produced extended-spectrum beta-lactamases (ESBLs), which are enzymes that provide antibiotic resistance **(43)** .

Table (4) :Biofilm formation ability of *E.coli* isolates isolated from urine and stool samples

Isolates	Biofilm formation	Isolates	Biofilm formation
Escherichia coli (U_1)	+	*Escherichia coli* (U_{14})	-
Escherichia coli (U_2)	+	*Escherichia coli* (U_{15})	+
Escherichia coli (U_3)	+	*Escherichia coli* (U_{16})	-
Escherichia coli (U_4)	+	*Escherichia coli* (U_{17})	-
Escherichia coli (U_5)	-	*Escherichia coli* (S_1)	+
Escherichia coli (U_6)	-	*Escherichia coli* (S_2)	+
Escherichia coli (U_7)	+	*Escherichia coli* (S_3)	-
Escherichia coli (U_8)	+	*Escherichia coli* (S_4)	+
Escherichia coli (U_9)	+	*Escherichia coli* (S_5)	-
Escherichia coli (U_{10})	+	*Escherichia coli* (S_6)	+
Escherichia coli (U_{11})	-	*Escherichia coli* (S_7)	-
Escherichia coli (U_{12})	+	*Escherichia coli* (S_8)	+
Escherichia coli (U_{13})	+		

positive result : + **negative result** : -

Urine : U **Stool** : S

3.5 Minimum inhibitory concentration

The minimum inhibitory concentration of Trimethoprim was determined to *E.coli* Isolates isolated from urine and stool samples . The results showed the ability of Trimethoprim to inhibit *E.coli* isolates with MIC between (25-50 μg/mL) .

The bacteria *Escherichia coli* (*E. coli*), which frequently causes sepsis, pneumonia, and urinary tract infections , has become resistant to several antibiotics , including trimethoprim . Several strategies have been established by *E. coli* to withstand trimethoprim, such as horizontal gene transfer , dihydrofolate reductase gene alterations, and excessive dihydrofolate reductase synthesis **(44)** . Consequently, infections brought on by resistant strains of *E. coli* may result in extended hospital admissions , higher rates of morbidity and mortality , and treatment failures . In order to tackle trimethoprim

resistance, monitoring minimum inhibitory concentrations (MICs) is crucial for directing antibiotic treatment **(45)** .

For *E. coli* isolates , the minimum inhibitory concentration (MIC) of trimethoprim varied greatly; 55.6% of isolates had a MIC of ≤ 1 μg/mL , which indicates sensitivity. But 18.0% of isolates had a MIC of > 8 μg/mL , which indicated full resistance , and 26.4% had a MIC of 2-4 μg/mL , which indicated intermediate resistance . Given that almost 20% of *E. coli* isolates show trimethoprim resistance, these results imply that the antibiotic's resistance is becoming a bigger problem **(46)** . According to a different study, 31.5% of isolates displayed intermediate resistance to trimethoprim , with a MIC of 1-2 μg/mL, whilst 42.1% were sensitive at a MIC of ≤ 0.5 μg/mL . Remarkably , 26.4% of isolates had a MIC of > 32 μg/mL, indicating high-level resistance **(47)** .

Table (5) : Minimum inhibitory concentration of Trimethoprim to *E.coli* isolates isolated from urine and stool samples

Isolates	Minimum inhibitory concentration mg/ml	Isolates	Minimum inhibitory concentration mg/ml
Escherichia coli (U_1)	50	*Escherichia coli* (U_{14})	25
Escherichia coli (U_2)	50	*Escherichia coli* (U_{15})	50
Escherichia coli (U_3)	50	*Escherichia coli* (U_{16})	25
Escherichia coli (U_4)	50	*Escherichia coli* (U_{17})	25
Escherichia coli (U_5)	25	*Escherichia coli* (S_1)	50
Escherichia coli (U_6)	25	*Escherichia coli* (S_2)	50
Escherichia coli (U_7)	50	*Escherichia coli* (S_3)	25
Escherichia coli (U_8)	50	*Escherichia coli* (S_4)	50
Escherichia coli (U_9)	50	*Escherichia coli* (S_5)	25
Escherichia coli (U_{10})	50	*Escherichia coli* (S_6)	25
Escherichia coli (U_{11})	25	*Escherichia coli* (S_7)	25
Escherichia coli (U_{12})	50	*Escherichia coli* (S_8)	50
Escherichia coli (U_{13})	50		

3.6 Minimum inhibitory concentration against biofilm formation

Microtiter plates were used to examine how well trimethoprim inhibited the formation of biofilms in *E. coli* isolates obtained from urine and stool samples . The best

and most precise technique for identifying bacterial adherence and biofilm formation is the use of microtiter plates. The results showed the ability of trimethoprim on inhibiting the formation of biofilm of *E.coli* isolates that varied with different concentration , the percentage were ranged between 12.5 (29% - 35%) , 25 µg/ml (40% - 47%) , 50 µg/ml (59%-67%) , 100 µg/ml (88%-91%) 200 µg/ml (100%) .

Table (6) : Percentage of inhibition of biofilm formation by using trimethoprim

Isolates	**Minimum inhibitory concentration**				
	12.5 µg/ml	**25 µg/ml**	**50 µg/ml**	**100 µg/ml**	**200 µg/ml**
Escherichia coli (U_1)	32 %	44%	60%	98%	100%
Escherichia coli (U_2)	35%	40%	65%	95%	100%
Escherichia coli (U_3)	29%	42%	59%	97%	100%
Escherichia coli (U_4)	36%	44%	63%	94%	100%
Escherichia coli (U_7)	33%	43%	65%	96%	100%
Escherichia coli (U_8)	31%	45%	65%	92%	100%
Escherichia coli (U_9)	35%	43%	60%	96%	100%
Escherichia coli (U_{10})	30%	41%	64%	91%	100%
Escherichia coli (U_{12})	35%	40%	61%	93%	100%
Escherichia coli (U_{13})	30%	46%	66%	96%	100%
Escherichia coli (U_{15})	31%	40%	63%	93%	100%
Escherichia coli (S_1)	35%	46%	60%	97%	100%
Escherichia coli (S_2)	31%	45%	60%	98%	100%
Escherichia coli (S_4)	37%	47%	63%	98%	100%
Escherichia coli (S_6)	29%	41%	59%	88%	100%
Escherichia coli (S_8)	30%	45%	67%	92%	100%

Trimethoprim , a widely used antibiotic, has been shown to inhibit biofilm formation in *E. coli* , Studies have demonstrated that Trimethoprim can effectively inhibit biofilm formation in *E. coli* **(48)** . One study found that Trimethoprim concentrations as low as 12.5 µg/mL could inhibit biofilm formation by 40%, while higher concentrations of 200 µg/mL could inhibit biofilm formation by up to 98% . These findings suggest that Trimethoprim's ability to inhibit biofilm formation is concentration- dependent , with higher concentrations resulting in greater inhibition **(49)** . The mechanisms by which Trimethoprim inhibits biofilm formation in *E. coli* are not fully understood . However, it is thought that Trimethoprim may interfere with the production of extracellular

polymeric substances (EPS) , which are essential components of the biofilm matrix . Additionally , Trimethoprim may inhibit the expression of genes involved in biofilm formation, such as those encoding adhesins and biofilm-associated proteins **(50)** .

While other researches showed that Ciprofloxacin (10 μg/mL) was the most effective , inhibiting biofilm formation by 85% , followed by Gentamicin (5 μg/mL) which inhibited biofilm formation by 75% . In contrast Ampicillin (50 μg/mL) had no significant effect , with only 10% inhibition, and Tetracycline (20 μg/mL) had a limited effect, with 20% inhibition . These findings suggest that the choice of antibiotic can significantly impact the treatment of biofilm-associated infections **(51).**

Conclusions

and

Recommendation

Conclusions and Recommendation

Conclusions

1- According to the results , 25 bacterial isolates were correctly identified as *E. coli* by the VITEK 2 technology, with isolates from urine samples accounting for 68% of the total and isolates from stool samples for 32%.

2- The majority of isolates exhibited resistance to ampicillin , trimethoprim - sulfamethoxazole, which suggesting a possible public health issue. Tigecycline, on the other hand, was generally sensitive to the isolates , indicating that it might be used as a treatment. Antimicrobial susceptibility testing is crucial for guiding treatment decisions and preventing antibiotic resistance because of the varying sensitivity to various medications.

3- Most *E. Coli* isolates from stool and urine samples were able to form biofilms, and the ability to do so was more common in urine isolates than in stool isolates.

4- Trimethoprim's potential efficacy against *E. coli* isolates was indicated by its minimum inhibitory concentration (MIC), which varied between 25 and 50 μg/ml.

5- Additionally , the results of the microtiter plate biofilm inhibition assay demonstrated that trimethoprim had an inhibitory effect on biofilm formation that was concentration- dependent , meaning that higher doses resulted in greater inhibition. In particular, the biofilm inhibition percentage varied from 29–35% at 12.5 μg/mL to 100% at 200 μg/ml .

Recommendation

1- Molecular identification and genetic study for all bacterial isolates .

2- Study the other characteristics of virulence factor .

3- Determination the minimum inhibitory concentration (MIC) and antibiotic susceptibility test of wide range of antibiotic .

4- Further studies are needed to confirm these results and to investigate the clinical efficacy of Trimethoprim against biofilm-associated *E. coli* infections.

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