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Original Research Paper

Investigating The Relationship Between Biofilm Formation and Antibiotic Resistance Patterns of *Escherichia Coli* Isolated from Various Sources

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Abstract: Bacteria can enhance their endurance by joining lifeless surfaces or tissues and introducing multicellular populations encased in a defensive extracellular lattice known as biofilm. Inside this element, microorganisms are exceptionally resistant and impervious to antibiotics. The ongoing work is meant to give more accurate information on the issue by looking at the biofilm-formation ability of *Escherichia coli* isolates in association with antibiotic resistance, as well as a total survey of the subject. Methods: For our situation study, a sum of 240 specimens were gotten from different sources, including urine (74), stool (62), tooth decay (53), and burns (51). The morphology of isolates was observed following 24 hours, and testing for antimicrobial vulnerability was done by the Kirby-Bauer disk dispersion method. Biofilm production of the isolates was identified by the tube methods, microtiter plate (MTP), and Congo red agar. Of the 240 separated isolates, just 20 were *Escherichia coli*, and every one of the 20 (100%) were positive for biofilm creation. Biofilms let microbes improve their defenses, guaranteeing their security from ecological hazards and harmful synthetics. Bacterial biofilms are connected to resistance to antibiotics, as seen by impressively expanded degrees of protection from aztreonam, rifampin, ertapenem, cefixime, ceftazidime, ceftriaxone, and trimethoprim/sulfonamides (P-value: 0.0013). The current study demonstrated that the *E. coli* clinical isolate from sick people in al-Najaf, Iraq, showed a positive link between the antibiotic resistance and biofilm formation; hence, more studying is required to completely understand the connection between antibiotic resistance and biofilm formation, which will help the advancement of imaginative treatment strategies

Keywords: biofilm, E. coli, antibiotics resistance

1.Introduction

negative bacterium, facultative anaerobic, *E. coli* regular habitat is the animal and human digestive system, and it's one of normal flora[1]. Although the way that most *E. coli* strains are innocuous and live in advantageous interaction with their hosts, they can cause extreme and lethal illnesses like urinary tract infections, diarrheal sickness, sepsis, and meningitis at times [2], Infections brought about by pathogenic *E. coli* and other coliforms spp. are much of the time started by binding of the bacteria to the host cell surface by means of bacterial virulence factor that connect from the bacterial surface empowering microbes to stick to host cells [3], Based on both genetic and clinical characteristics, *E. coli* strains are categorized into three main categories the first is commensal strains found in the human and animal gut (lacking specialized virulence factors), second is intestinal pathogenic strains (diarrheagenic) *E. coli* (DEC) , third is extraintestinal pathogenic *E.*

coli (ExPEC) [4], Pathogenic kinds of *E. coli* total together and become problem to eliminated due to biofilm formation, A few investigations have inspected the limit of the *E.coli* strains to create biofilm [5], in which Various microorganisms consolidate together to form miniature networks inside a lattice of extracellular polymeric substances (EPS) which are called biofilm. The capacity of *E. coli* to make biofilm is a significant destructiveness factor and such biofilms are the primary driver of numerous ongoing diseases and improve the capacity of *E.coli* to antibiotics resistance [6], AMR is frequently described as the "Silent Pandemic" and is an issue that requires current attention and should be controlled more effectively, rather than being regarded a future crisis [7], Universally antimicrobial resistance is a serious general wellbeing concern, especially in non-industrial nations where irresistible sicknesses, lack of healthy sustenance, and neediness are endemic [8], Antibiotic resistance principally gets from changes, or transformations, in the DNA of the microorganisms plus to obtaining of antibiotics obstruction genes from other bacterial species through plasmid move. Especially *E. coli* can display a strong capacity to get antibacterial resistance determinants, bringing about not many excess viable treatment choices against its pathogenic variations [9], Microorganisms inside the biofilm act uniquely in contrast to their planktonic partners particularly as far as anti-bacterial, which causes restriction in ordinary antibiotics treatments [10], Biofilm likewise makes cells more impervious to antibiotics by keeping them from penetrating the cells [11], It has been found that microbes inside the biofilm are 10-1000 times more impervious to antimicrobial drugs than the planktonic ones [12]. However, the aim of This study is to investigating antimicrobial susceptibility patterns of *E. coli* isolated from various clinical samples and their correlation to *E. coli* profile of antibiotic susceptibility in al-Najaf city, Iraq.

2.Methodology

Sample Collection

Sampling was achieved during the period from Jule to September 2024. In the current study, 240 samples were collected including 74 isolates taken from the urine and 51 from burn for sick people and 62 isolates from stool, 53 from tooth decay. samples taken from people of different ages between (10 – 60) years and for both sexes (males - females), from AL-Najaf hospitals, including (Al-Sadr Teaching Hospital, Al-Hakim General Hospital, Al-Zahra Teaching Hospital, Burn Center, Central Health Laboratory).

Bacterial Isolation

Sample of urines were gathered by using clean disposal glass containers (5 mL) from patients to stay away from tainting. Also, tooth Declay, stool, and burns isolates were gathered utilizing by using clean cotton swabs. The isolates developed on the culture media will be noticed and characterized in light of their specialist attributes like structure, size, edge, variety, and smell. This information will give helpful experiences about what sort of bacterial type present in the isolates.

Identification of Bacteria

Bacterial samples generally identified by morphologic and biochemical assessments which involvement Motility test and IMVIC test (Voges Proskauer Test, methyl red test, Citrate utilization Test, indole test) and by their phenotypic standards on specific culture media, ultimately characterized by the Vitek 2.

Detection the ability of Escherichia coli to produce biofilm

Congo Red Agar method (CRA)

Congo red agar (CRA) strategy, which is a qualitative measure utilized for the identification of biofilm maker bacteria because of the variety of bacterial colonies that develop on CRA medium, is characterized by Freeman *et al*. The medium is ready by blending 0.8 g of Congo red and 37 g/L of brain heart infusion (BHI) agar with 36 g of sucrose. After the period of incubation of 24 h at 37°C, the shape of bacterial growth that has gone through various colors is separated as biofilm makers or not [13].

Tube method (TM)

Tube methods (TM), which are subjective tests for the identification of biofilm maker bacteria, as a result of the appearance of visible film, are characterized by Christensen *et al* [14], Samples are vaccinated in polystyrene test tubes that contained TSB and incubated for 24 h at 37 °C. After that, planktonic cells are released by washing two times with phosphate-buffered saline (PBS). The sessile detaches of which biofilms shaped on the walls of the polystyrene test tube are stained with crystal violet for one h. Also, at that point, the crystal violet-stained polystyrene test tube is washed two times with PBS to release the stain. After drying the test tube by air, the event of apparent film on the walls and the lower part of the test tube demonstrates biofilm creation [14].

Microtiter Plate Assay

We view this test as the highest quality level strategy for biofilm discovery. 3-4 of the disconnected colonies were suspended in Muller Hinton broth (MHP) with 2% sucrose and afterward brooded for 18 h at 37 °C in a fixed condition. Subsequent to changing the turbidity of bacterial stock to 0.5 McFarland standard, 200 µl of bacterial stock was vaccinated into sterile MTP wells with the exception of the wells of the last segment that was indicated as a negative control containing sterile MHP. The vaccinated plates were brooded for 48 h. The items in wells were emptied, and the residues of microbes in the wells were washed with saline. The microbes in the wells were colored by 150 µl of crystal violet (0.1%) for 15 min at room temperature. The stain was suctioned by pipette and laundered with water. 150 µl of 95% ethanol was pipetted into each well, and the wells were covered to limit evaporation. The outcomes were interpreted by an ELISA reader at 620 nm. The understanding and reviewing (low-moderate-strong) biofilm creation was finished by the measures of Stepanovic *et al* [15, 16].

Antibacterial susceptibility testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar by utilizing the plate dispersion (Kirby Bauer's) method adhering to the CLSI rules, The incorporation measures included patients from the two sexes, from 10 to 60-years of age, with positive microbiological proof of *E. coli* sample from different sources, and consent to partake in the review. Patients who didn't consent to take part in the review were eliminated, the panel of antibiotics was as follows: clindamycin(CD), erythromycin(E15), ceftriaxone(CTX), levofloxacin(LEV), amikacin(AK), nalidixic acid(NA),doxycycline(DO),cefixime(CFM),trimethoprim\sulfonamides(COT),chloramphenicol(C),gentamycin(GEN),ciprofloxacin(CIP,5),minocycline(MI),aztreonam(AT),cefotaxime(CTX),rifampin(RIF),azithromycin(AZM),piperacillin\tazobactam(PIT),imipenem(IPM),ertapenem(ETP),amoxicillin(AMC), ciprofloxacin(5,30).The sensitivity/resistance was interpreted based on the diameter zone of inhibition, inclusive of margins, following the Clinical Laboratory Standards Institute guidelines of CLSI 2023 [17] Table 3.

Statistical analysis

Descriptive investigation (involving means and rates to describe information) was performed utilizing Microsoft Excel 2013 (Microsoft Corp.; Redmond, WA, USA). Extra factual investigations were performed with the IBM SPSS program for Windows 22.0 (IBM Corp, Armonk, NY, USA), utilizing the chi-squared test. P values <0.05 were viewed as important criteria. The understanding of the relationship between the consequences of biofilm creation and antibiotic resistance of E. coli was determined.

3.Results and discussion

from 240 clinical specimen, most the isolates of *E. coli* were acquired from urine sample as 74(31.25%), followed by stool 62(25.8%), tooth Declay53(22.0%), 51burns (21.25%) (Table 1). isolates of *Escherichia coli* were more pervasive in female (59.45%) than in male (40.54%). In urine specimen the occurrence of *E. coli* was more prevalent in females (71,42%) than in males (28.57%). In burns specimen the occurrence of *E. coli* was higher in females (25%) than in male (75%). The distribution of the isolates from other clinical samples in both genders is summarized in Table 1. However, incidence of *E. coli* according to age group is more prevalent in age group (21-30) as 74, then in (31-40) as 48, then (10-20) as 45, and (41-50) as 37, and the last group is (51-60) as show in Table 2.

Table 1: The distribution of *Escherichia coli* segregated from Different Clinical sample in light of Sex**.**

Source Of Isolation	**Total**	**Male**		**Female**	
		No	***E.* coli sample**	**No**	***E.* coli sample**
Urine	**74**	**30**	**2**	**44**	**5**
Stool	**62**	**24**	**2**	**38**	**4**
Tooth Declay	**53**	**33**	**2**	**20**	**1**
Burns	**51**	**29**	**3**	**22**	**1**
Total	**240**	**116**	**9**	**124**	**11**

Table 2: The disruption of Escherichia coli Isolated from Various Clinical Specimens Based on age.

Age	Urine	Stool	Tooth Declay	Burn	Total
10-20	17	9	13	6	45
21-30	25	17	15	17	74
31-40	15	10	12	11	48
41-50	10	12	6	9	37
51-60	7	14	7	8	36
Total	74	62	53	51	240

Antibacterial susceptibility testing

The antibacterial sensitivity pattern of *E. coli* isolates shows as highly sensitive to gentamycin, ciprofloxacin (5)(30) as (100%). piperacillin-tazobactam, doxycycline and chloramphenicol as (95%), (90%) to amikacin and clindamycin, (85%) to azithromycin, erythromycin, minocycline, levofloxacin. and (70%) for imipenem, nalidixic acid as (55%) (Table 3). the resistance rates overall were observed for *E. coli* was resistance for aztreonam, rifampin as (100%), (95%) for ceftriaxone, (90%) for amoxicillin-clavulanic, cefixime, cefotaxime, (75%) for Trimethoprim\Sulfamethoxazole and ertapenem as show in Table (3).

Biofilm formation

In *E. coli* isolates Biofilm was detected by using Congo red agar, tube methods, and microtiter plate (MTP). In tube methods out of 20 *E. coli* isolate shows as biofilm producer (100%), *E. coli* biofilm manufactures were classified into 3 group first was strong and is 7(35%) isolates, second was moderate and is 13 (65%), and the third was weak and is 0(0%). This result is show in Table (4), By using the tube method, the positive result appears as evidence of the formation of biofilms on the inner walls and bottom of the tubes in the form of a purple layer as in the figure (1).

In Congo red agar out of 20 *E. coli* isolate show as biofilm produce (100%), when using the Congo red agar method, the isolates forming biofilms appeared as a dry or shiny black as shown in Figure (2). This is consistent with what many researchers have stated that the isolates of bacteria forming biofilms appeared as a dry or shiny black using the Congo red method, while the bacteria not forming biofilms were described as a pink, red or wine color using the same method, [14, 18].

In MTP methods out of 20 *E. coli* isolate show as biofilm producer (100%) E. coli biofilm producer were branded into 3 groups; weak 0 (0%), moderate 13(65%), and 7(35%) strong biofilm producers, according to the amount of biofilm formed as shown in figure (3).

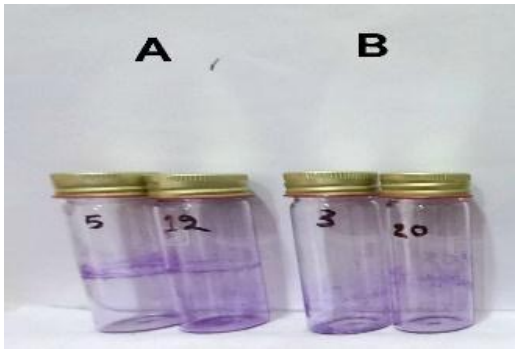

Fig1: biofilm formation in tube methods. A: strong biofilm producer, B: moderate biofilm produced.

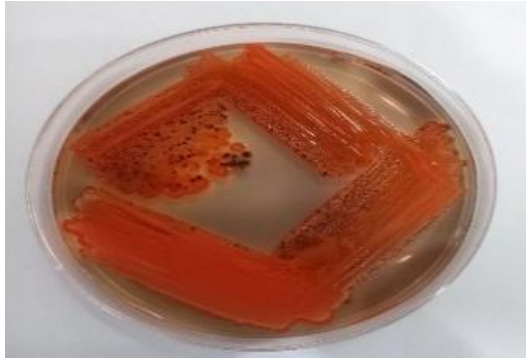

Fig 2: biofilm formation on Congo red agar. the isolates appeared as a dry or shiny black as result of biofilm formation.

Table 3: Antibiotic susceptibility feature of Escherichia coli with (Percent (%) of Resistance), (Percent (%) Of Sensitivity).

The Classes of Antibacterial drugs	Tested antibiotics (Disk content µcg)	Resistance N (%)	Sensitivity N (%)
penicillin's	Amoxicillin-clavulanic acid (AMC) 30mcg	18 (90%)	2 (10%)
	Piperacillin-tazobactam (PIT) 100mcg	1 (5%)	19 (95%)
cephalosporins	Cefixime (CFM) 5mcg	18 (90%)	2(10%)
	Cefotaxime (CTX) 30mcg	18 (90%)	2 (10%)
	Ceftriaxone (CTR) 30mcg	19 (95%)	1 (5%)
aminoglycosides	Gentamicin (GEN) 10mcg	0 (0%)	20 (100%)
	Amikacin (AK) 30mcg	2 (10%)	18 (90%)
Macrolides	Azithromycin (AZM) 15mcg	3 (15%)	17 (85%)
	Erythromycin (E15) 15mcg	3(15%)	17(85%)
Carbapenem	Imipenem (IPM) 10mcg	6 (30%)	14 (70%)
	Ertapenem (ETP) 10mcg	15 (75%)	5 (25%)
monobactam	Aztreonam (AT) 30mcg	20 (100%)	0 (0%)
quinolones	Nalidixic acid (NA) 30mcg	9 (45%)	11 (55%)
fluroquinolones	Ciprofloxacin 30mcg (CIP30)	0 (0%)	100 (100%)
	Ciprofloxacin 5mcg (CIP5)	0 (0%)	100 (100%)
	Levofloxacin (LEV) 15mcg	3 (15%)	17 (85%)
tetracycline	Doxycycline (DO) 30mcg	1(5%)	19 (95%)
	Minocycline (MI) 30mcg	3 (15%)	17 (85%)
phencol	Chloramphenicol (C) 30mcg	1 (5%)	19 (95%)
sulfonamides	Trimethoprim\ Sulfamethoxazole (COT) 25mcg	15 (75%)	5 (25%)
Ansamycin	Rifampin (RIF) 5mcg	20 (100%)	0 (0%)
lincosamides	Clindamycin (CD) 2mcg	2 (10%)	18 (90%)

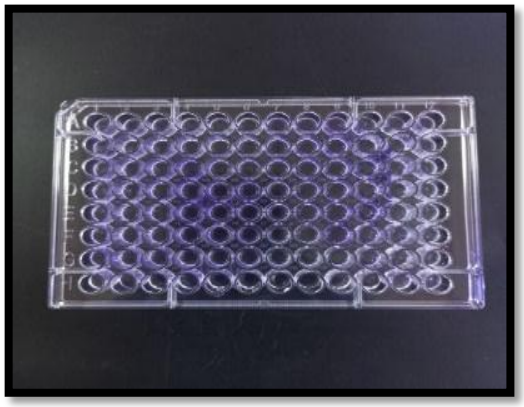

Fig 3: biofilm formation on microtiter plate.

Table 4: Biofilm formation rates in *Escherichia coli* isolates studied by in vitro tube method, Congo red agar and microtiter plate**.**

SAMPLE	Congo red agar	Tube methods	Microtiter plate
1	P	P (+++)	**P (+++)**
2	P	P (+++)	**P (+++)**
3	P	P (++)	**P (++)**
4	P	P (++)	**P (++)**
5	P	P (+++)	**P (+++)**
6	P	P (++)	**P (++)**
7	P	P (+++)	**P (+++)**
8	P	P (++)	**P (++)**
9	P	P (++)	**P (++)**
10	P	P (++)	**P (++)**
11	P	P (+++)	**P (+++)**
12	P	P (+++)	**P (+++)**
13	P	P (++)	**P (++)**
14	P	P (++)	**P (++)**
15	P	P (+++)	**P (+++)**
16	P	P (++)	**P (++)**
17	P	P (++)	**P (++)**
18	P	P (++)	**P (++)**
19	P	P (++)	**P (++)**
20	p	P (++)	**P (++)**

Correlation Between Biofilm Production and Antibiotic Resistance.

Out of the tested isolates, n 240 there are 20 *E. coli* isolates are show as biofilm producer. Regarding piperacillin-tazobactam, gentamycin, ciprofloxacin (5)(30), chloramphenicol, amikacin, doxycycline, clindamycin, azithromycin, erythromycin, levofloxacin, minocycline, imipenem, nalidixic acid, biofilm producer isolates was identified as considerable high levels of antibiotic resistance to aztreonam, rifampin, ertapenem, cefixime, ceftazidime, ceftriaxone, trimethoprim\sulfonamides with significant (P-value: 0.0013) as show in table (Table 5)**.**

Table 5: Correlation Between Biofilm Production and Antibiotic Resistance.

Sample	Rate of Biofilm production	Percentage of resistant to antibiotics
1	0.053	27.27
2	0.058	36.36
3	0.047	50
4	0.048	50
5	0.082	55.54
6	0.040	31.81
7	0.050	40.9
8	0.046	50
9	0.045	40.9
10	0.039	31.81
11	0.058	36.36
12	0.069	36.36
13	0.047	31.81
14	0.049	45.45
15	0.065	40.9
16	0.043	22.72
17	0.036	54.54
18	0.043	22.72
18	0.043	50
20	0.040	36.36

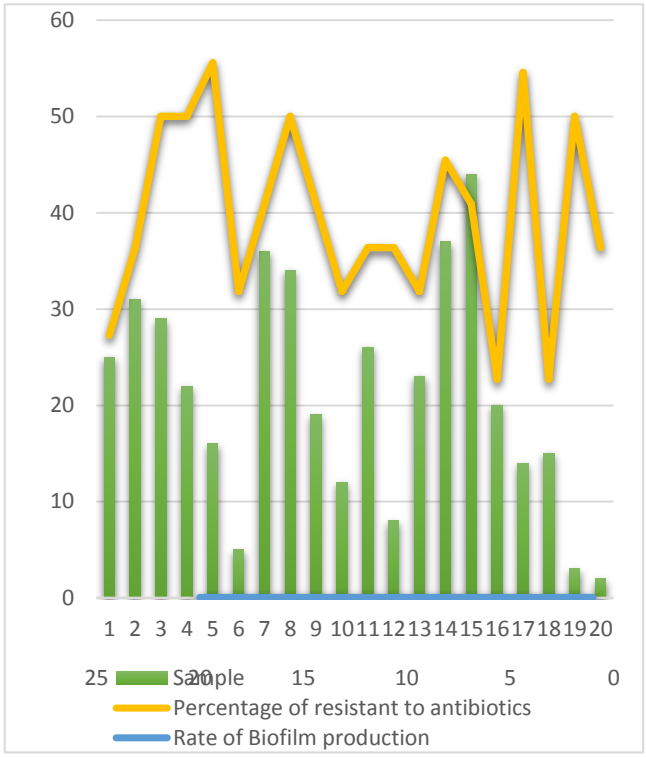

Fig 4: Correlation Between Biofilm Production and Antibiotic Resistance**.**

The specific traits of the *E. coli*, for example, simplicity of dealing with, accessibility of the total genome grouping, and its capacity to develop under the anaerobic and aerobic condition, makes it a significant host living being in biotechnology[19], Nonetheless, a few exceptionally adjusted commensal clones were found to can obtain explicit harmfulness credits and become profoundly destructive and dangerous microorganisms [20]. one of this ferocity factor is

biofilm, bacteria in biofilms can utilize a few methods for surviving to sidestep the host guard frameworks and these variations make the microbes more impervious to antibacterial treatment by inactivating the antimicrobial targets or decreasing the prerequisites of the cell that the antimicrobials slowdown [21]. In our study 20 of bacterial isolates are tested by (MTP), tube methods and Congo red agar to detect the ability of bacteria to produce biofilm and all the bacterial isolates show as biofilm producers and this feature have significant impact on antibacterial resistance patterns that proceed to makes an incredible danger general wellbeing overall and lead to serious medical issues like delayed hospitalization and therapy failure [22]. Hence, this studying intended to identify the antibiotics portability profile of *E. coli* isolate and their associations with biofilm formation from different clinical sources in al-Najaf city, Iraq.

In the current review, a sum of 240 bacterial sample were gathered from different clinical sources. The recurrence of clinical segregates of *E. coli* in urine specimen was higher in females than in males. This outcome is compatible with different investigations revealing a higher pervasiveness of *E. coli* in UTI in females [23], It is assumed that ladies are at high chance for UTI contrasted with men because of their physical and physiological qualities, for instance, the closeness between the genital tract and the urethra/anus in ladies is one potential causes that could work with the auto-transmission of the microorganisms and lead to expanding the pace of UTI in female as well as conduct factors[24], Behavioral factors help microbial uropathogens capitalize on female anatomic weakness for increase rate of spread of pathogenic *E.coli* [25]. These types of UTI are advanced rapidly by lack of cleanliness and low financial status. Change in the vaginal microflora might assume a significant part in empowering the colonization of the vagina with coliforms and this can be related with UTI ,In term of stool isolate the prevalence of *E. coli* is higher in women from men and this is consistent with (H Dela and *et al)* [26], in burns isolate the prevalence of *E.coli* is higher in man than women and is consistent with (A Hakan and *et al)* [27], The reasoning for this might be that men are more presented to the words related dangers to bring in living cash, and subsequently the probability of consumes wounds was higher than women. In our study the prevalence of isolate in tooth Declay are more in man than in women and this is consistent with (B Sehdev and *et al)* study [28], low oral cleaning practice was firmly associated causes with tooth decay. It is hard to make sense of this variety, and further investigations with bigger example sizes are expected to investigate the explanation.

E. coli collected from different clinical sources, showed contrasts in antibiotics sensitivity designs. On case of urine specimens, the antibiotics sensitivity profile showed that *E. coli* samples were very touchy to clindamycin, erythromycin, levofloxacin, amikacin, chloramphenicol, gentamycin, ciprofloxacin (5,30), minocycline, azithromycin, piperacillin-tazobactam, imipenem. and resistant to cefotaxime, cefixime, trimethoprim-sulfonamthaxole, aztreonam, ceftriaxone, rifampin, ertapenem, amoxicillin-clavulanic acid, this outcome is concurrence with past investigations showing that isolates of *E. coli* from urine specimen were intensely resistance to amoxicillin-clavulanic, cefixime, ceftriaxone, and exceptionally delicate to imipenem, clindamycin, ciprofloxacin, amikacin [29]. another investigations discovered that 100 percent of *E. coli* isolate were delicate to gentamicin, piperacillin-tazobactam [30].

E. coli isolates from the stool samples showed sensitive to erythromycin, levofloxacin, amikacin, chloramphenicol, gentamycin, ciprofloxacin (5,30), minocycline, azithromycin, piperacillin-tazobactam, nalidixic acid, doxycycline. and resistance to cefotaxime, cefixime, trimethoprim-sulfonamthaxole, aztreonam, ceftriaxone, rifampin, ertapenem, amoxicillin-clavulanic acid, imipenem. And this result is not compatible with other study conducted in the Kingdom of Saudi Arabia [31]. In other clinical specimens including tooth decay and burns the isolates is sensitive to clindamycin, levofloxacin, amikacin, doxycycline, chloramphenicol, gentamicin, ciprofloxacin (5,30), minocycline, azithromycin, piperacillin-tazobactam, imipenem, ertapenem. and resistance to erythromycin, cefotaxime, cefixime, trimethoprim-sulfonamthaxole, aztreonam, ceftriaxone, rifampin, amoxicillin-clavulanic acid, imipenem. These results are in agreement with study made by (MJ Alwan and *et al*) [32]. There are several reasons to antibacterial resistance that include wrong and abuse of antibiotics agents will definitely selectively press and increment the resistance rate, Patient's incompliance to prescribed treatment was recognized to be one of the reasons for microbial protection from antibiotics, Different patients likewise abuse the antibiotics because of the way that it is promptly accessible in the drug store and they can get it without

having prescription, Additionally the false diagnosis of sicknesses might causes incorrectly prescription of antibiotics to patients prompting the resistance of bacteria [33].

Conclusion

The current study demonstrated that the *E. coli* clinical isolate from sick people in al-Najaf, Iraq, showed a positive link between the antibiotic resistant and biofilm-formation, and the correlation between biofilm-formation and cefixime, ceftriaxone, trimethoprim-sulfonamthaxole, aztreonam, rifampin, ertapenem, amoxicillin-clavulanic acid resistance had been noticing. hence, a standard and local control of biofilm production in *E. coli* isolates and their antibiotics resistance features should be accomplished. And This might assist to elaborate therapeutic strategies against *E. coli* related infections. Additionally, in this study, our specimen's size is a very small and only from one geographic site. Raising the sample size and geographical location in order to casting light on how different geographical area and patient resident participate to the selection of pathogens with various profile of biofilm and its resistance would needs additional study.

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Authors' contributions statement

Hasnawi Z.H. accomplished sample collection and isolation, data acquisition, conception and design, Al-Hilu S.A. contributed in structuring the information and drafted the outcome; all author contributed understanding, correction, and endorsed the last original copy.

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Ethics

The review was endorsed by the ethical committee of the faculty of science University of Kufa, al-Najaf city, Iraq.

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