DOI: https://doi.org/10.53350/pjmhs22166155

ORIGINAL ARTICLE

Role of Biofilm Formation in Mediation of Drug Resistance in E. Coli

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ABSTRACT

Aim: Measure biofilm formation in clinical isolates of Escherichia coli and compare the survival of bacteria by treatment of ciprofloxacin, ceftriaxone and amoxicillin/clavulanic acid in the presence and absence of biofilm formation.
Methodology: A total of ninety isolates of Escherichia coli were included in the study. All isolates were identified for biofilm production by microtiter assay. Antibiotic susceptibility test for all E. coli strains along with ATCC E. coli 25922 as a control was performed by agar dilution method (CLSI 2016) using three antibiotics e.g. ciprofloxacin, ceftriaxone and amoxicillin/clavulanic acid
Result: Out of ninety E. coli isolates, 18(20%) were strong biofilm formers, 54(60%) were moderate and non-biofilm formers were 18(20%). Resistance to ciprofloxacin, ceftriaxone and amoxicillin/clavulanic acid were 75.5%, 63.3% and 22.2% respectively in all tested isolates. The rate of antibiotic resistance of strong biofilm formers, moderate formers and non-biofilm formers were 83.3%, 74% and 77.7% respectively for ciprofloxacin. Similarly, for ceftriaxone resistance pattern of strong, moderate and non-biofilm former were 66.6%, 66.6% and 50% respectively. Strong, moderate and non-biofilm forming E. coli were 11.1%, 27.7% and 22% respectively resistant to amoxicillin/clavulanic acid.
Conclusion: Biofilm is not essentially involved in mediating drug resistance in E. coli against tested ciprofloxacin, ceftriaxone and amoxicillin/clavulanic acid. Biofilm formers showed more resistance to antibiotics than non-biofilm formers but statistical analysis of both groups against three antibiotics showed that there was no significant difference of resistance observed among them. There may be another molecular and enzymatic mechanism that allows bacteria to mediate resistance.
Keywords: Biofilm formation, antibiotic resistance, minimum inhibitory concentration

INTRODUCTION

Biofilm is a community of bacterial cells that are attached to each other and to surfaces, with the help of self-secreted extracellular polymeric substances[1]. In 17th century, Antoine Von Leeuwenhoek, observed aggregated microorganisms in material from his own teeth that lead to discovery of biofilm[2].The word 'biofilm' was introduced by Costerton in 1978[3]. Biofilm is a major form of bacterial life on earth[4]. Above 80% of all infections are because of biofilm forming bacteria[5].The biofilm formation ability of bacteria helps to survive in unfavorable environment and responsible for chronic infections[6]. Non motile bacteria in biofilm are inherently more resistant to antimicrobial agents than freely moving cell[7]. Extra cellular DNA, exopolysaccharide, cellulose, flagella, protein and amyloid fimbriae are the machinery of biofilm. About 2-5% of biofilm atmosphere is bacterial population and 1-2% extracellular polymeric substances[8]. The exopolysaccharide provides adhesive and structural stability to the matrix and also prevents the entrance of antibiotics[9]. Accessibility of nutrients as well as motility of bacteria towards surface influence biofilm formation[10].

Similarly media composition, underlying organism and quantity of inoculums also boost the process of biofilm formation[1]. The objective of the study is to measure the biofilm formation in clinical isolates of E. coli and compare the survival of bacteria by treatment of ciprofloxacin, ceftriaxone and amoxicillin /clavulanic acid in the presence and absence of biofilm amoxicillin. It is commonly known that biofilm forming bacteria initiate resistance then non- biofilm bacteria. Biofilm formers are considered more powerful than non- formers. So this study is required to access the biofilm formers and non-biofilm formers for the initiation of resistance. This study will add contribution in understanding the resistance associated with bacteria. Outer layers of biofilm are aerobic while inside layers grow to be anaerobic and nutrient deficient[11]. Various antibiotics are effective in oxygen rich part of biofilm[12]. While most antibiotics show activity when bacterial cells are in log phase of growth[13]. Luria Bertani medium (LB) is preferred medium. It is a nutrient rich medium as compared to Tryptic Soya Broth. In LB medium most of the organism were found to be strong and moderate biofilm producers, as compared to TSB medium[14]. There are various methods to detect biofilm production. These include Tissue culture plate method, Tube method, Congo red agar method, bioluminescent assay, piezoelectric sensors and fluorescent microscopic examination[15]. There are several existing models of biofilm formation such as rdar morphotype formation on Congo red plates, adherence of bacteria with walls of test tube and pellicle formation in liquid culture. The rdar morphotype is extensively studied model of biofilm formation in E. coli[16].

The monitoring of rdar expression is done by csgD in Salmonella typhimurium and E. coli[17]. The exopolysaccharide cellulose and amyloid curli fimbriae are two important components of rdar biofilm in E. coli[18]. Rdar refers to the appearance of the colony morphology of a strain expressing the exopolysaccharide cellulose and amyloid curli fimbriae on a low salt agar medium containing the dye Congo red. Under these growth conditions, cellulose producing colonies appear pink (pdar morphotype), whereas curli fimbriae expressing colonies appear brown (bdar morphotype) upon binding Congo red. Congo red binding results in a full rdar morphotype, when both components are expressed. The saw morphotype expresses neither curli nor cellulose[16,17]. There are five stages in development of biofilm [19,20].
Stage 1: Attachment: Connection of bacteria with biotic or abiotic surface by flagella is reversible process that is induced by several environmental signals e. g temperature, nutrient concentration, oxygen supply and pH.
Stage 2: Adherence: After irreversible adherence of bacteria to the epithelial surface, the bacterial cell begins to multiply in aggregates and form micro communities.
Stage 3: Maturation I: In this stage exopolysaccharide development occur that provide structural integrity to the matrix by transcription of certain genes.
Stage 4: Maturation II: Micro communities develop into mature biofilm which thickness reaches more than 100mm.
Stage 5: Dispersion: After maturation when biofilm mass reached to a critical level then some planktonic bacteria get free to settle at new places and again start the cycle.

Biofilm is not only a key virulent factor but also an important factor mediating drug resistance. N. Høiby and J. W. Costerton, were concluded a relation between biofilm and persistent infections, especially Pseudomonas aeruginosa in cystic fibrosis[21]. It is a process in which complex bacterial communities form when bacteria change from unicellular form to multi cellular form[22]. Bacteria within biofilm have the ability to exchange genes through horizontal gene transfer. Escherichia coli a facultative anaerobe and belong to Gram negatives. It act as a normal flora of colon and a good indicator of hygienic quality of water and food processing environments[23]. E. coli is accounted for approximately 75 to 90% of the public-acquire infections and about 30 to 50% of all nosocomial infection[24]. Various virulence factors of E. coli include adherence to receptors on host membranes, cell wall antigen

Received on 24-10-2021
Accepted on 14-05-2022

(O and K) and resistance to phagocytosis predispose to infections[25].The World Health Organization (WHO) has called antibiotic resistance a growing disease. Bacteria may naturally be resistant or may attain resistance to antibiotics[26].

The major concern of the healthcare personnel is the detection of clinical isolates that are challenging to antimicrobial compounds. Routinely performed test e.g. disc diffusion and MIC can identify bacterial fight against antibiotic[27].

The study is planned to explore whether the biofilm production has any impact on sensitive and resistance pattern of E. coli for Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid. This study will also assist in management of patient treatment.

MATERIALS AND METHODS

The study design was quasi experimental conducted in Microbiology Department of University of Health Sciences Lahore, Pakistan.

Sample size: The formula for sample size calculation was

Where $n = \frac{Z^2_{1-\alpha/2} P(1-P)}{d^2}$

$Z^2_{1-\alpha/2}$= for 95% confidence level = 1.96p=anticipated part=73% [28]

d =Margin of error = 9%

n=Sample size = 90 ~ 93.4

Duration: Twelve months after approval of the synopsis

Materials:

Antibiotics:

- Ciprofloxacin (GSK)
- Ceftriaxone (Sami)
- Amoxicillin/clavulanic acid (GSK)

Reference strain: Escherichia coli ATCC 25922 was used as control strain. It was stored in Microbank at -70ºC in the Department of Microbiology, UHS Lahore.

Identification and Processing of isolates:

Subculture: All isolates were inoculated on MacConkey plates and were incubated at 37ºC aerobically for 24 hours. Cultures were examined on next day.

Identification of bacterial isolates: It was done by colony morphology, Gram staining, wet preparation and biochemical testing (Triple sugar iron, urease, citrate, and indole).

Microtiter Plate Assay for Biofilm detection: Biofilm formation was assessed by microtiter plate assay [29]

1. Escherichia coli was cultured on Luria Bertani without salt (LBWS) agar plate and was incubated on 28ºC for 24 hrs.
2. Suspension of organism was made in eppendroff containing 1ml LBWS broth and incubated on 28ºC for 24 hrs.
3. Eppendroff was vortexed after overnight incubation and 1:100 dilution was prepared by taking 10µl from overnight suspension in LBWS eppendroff into fresh 990µl LBWS broth dilution eppendroff and vortex it.
4. One hundred µl from dilution eppendroff was taken and added in 5 wells of microtiter plate leaving 1st square of wells in titer plate as these wells were used for broth sterility controls and these wells were filled with 100µl of LBWS broth and plate was incubate overnight at 28ºC.
5. Following incubation, bacterial cells were dump out by rotating the plate over and quaking away the liquid.
6. Plate was flooded in a small container of clean water. For the removal of free-floating bacteria two times washing was performed.
7. One hundred and twenty-five µl of a 0.1% solution of crystal violet in water was added to each sample well. The microtiter plate was incubated at room temperature for 15 minutes. The plate was rinsed 2-4 times with water by submerge in a tub of water, shake out and blot on a stack of paper towels to rid the plate of all excess cells and dye. The microtiter plate was turned upside down and then dries for 24 hrs.
8. Photos were taken for qualitative assay.
9. In each sample well of the microtiter plate 125µL of 30% acetic acid in water was added to solubilize the crystal violet. Microtiter plate was incubated at 25ºC for 10-15 minutes.
10. Solubilize CV (125µl) was added to a microtiter plate.
11. Optical density of stained biofilm was obtained through spectrophotometer at 546nm.
12. A blank plate was also prepared by accumulation of 125µl of 30% acetic acid in all wells and optical density was taken at 546 nm.

Every organisms were classified into following categories depending upon optical density of tested strains and negative control[30]

- Non-biofilm OD ≤ ODc
- Weak biofilm ODc< OD ≤ 2 OD
- Moderate biofilm 2 ODc< OD ≤ 4 ODc
- Strong biofilm 4 ODc< OD

Minimum Inhibitory Concentration: It is the lowest concentration of antibiotic that inhibited the growth of bacteria after 24 or 48 hours of incubation[13]. MIC of ciprofloxacin, ceftriaxone and amoxicillin/clavulanic acid was done by agar dilution procedure for each of the organism in duplicates according to CLSI guideline (2016).

1- Agar Dilution Procedure: CLSI (2016)

Preparation of stock solution of each antibiotic: An initial stock solution of 10,000 µg/ml of each antibiotic (Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid) was prepared. Weighed 0.1 gram (100mg) of each antibiotic and dissolved in 10 ml distilled water (Ciprofloxacin and Ceftriaxone) and phosphate buffer saline (Amoxicillin/clavulanic acid) to prepare the initial stock solution. The following formula was used to prepare the working solution of the antibiotic:

$M_1V_1 = M_2V_2$

M1= (Desired Conc.) (µg/ml)

V1= (Desired Volume) (ml)

M2= (Stock solution) (µg/ml)

V2= (ml)

Thirteen different testing concentrations of each of three antibiotics (Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid) were prepared and tested against E. coli strains. The ranges of concentration were as follows: 0.25µg/ml, 0.5µg/ml, 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml, 16µg/ml, 32µg/ml, 64µg/ml, 128µg/ml, 256µg/ml, 512µg/ml and 1024µg/ml.

Preparation of antibiotic incorporated MH Agar plates:

1. Mueller Hinton agar was prepared by dissolving 38 grams of Mueller Hinton agar per liter of distilled water by intermittent heating and mixing.
2. Forty ml of the Mueller Hinton medium was poured in volumetric flask of 100 ml capacity. The flasks were autoclaved at 121ºC for 15 minutes.
3. Autoclaved sterile Mueller Hinton agar medium cooled and maintained at 45-55ºC in a water bath.
4. Each tested concentration was labeled in duplicate on the base of the plates (90mm diameter) and flasks containing the medium.
5. The volume of working solution for each antibiotic was then incorporated in to the medium at 50ºC and mixed well.
6. The medium was then poured in pre-labeled plates for each concentration and were allowed to solidify at 25ºC for 10-15 minutes.
7. Plates were kept at 4ºC after setting.

Preparation of Inoculum: The organism was inoculated on Mueller-Hinton agar medium for obtaining separated colonies. After overnight incubation at 37°C, 4-6 isolated colonies were selected for making bacterial suspension and were transferred to a tube of sterile normal saline and vortexed. The bacterial suspension was equal to the 0.5 McFarland standard. The suspension was diluted 1:10 in sterile normal saline. This dilution had made inoculums concentration of 10^7CFU/ml. The suspension was used within 15 minutes of preparation.

Procedure:

1. The plates were dried before performing the test.
2. Multiple inoculator was used for the inoculation of multiple organisms on the prepared plates of multiple dilution.
3. All the tubes were arranged in a rack containing diluted suspensions.
4. The 35 wells were present in the sterile grid and every well was full with 600 µl of each bacterial suspension corresponding to their respective grid number.
5. This instrument has 35 pins each with a diameter of approximately 3mm delivering approximately 3 µl of the suspension per spot. The pointer pin was used for investigating the starting point.
6. The instrument inoculated present study samples simultaneously on a single plate.
7. The plates were leaved at room temperature for proper absorption of the inoculums onto the medium.
8. Plates were incubated on 37ºC for 24 hours without inverting.

Interpretation: Incubation has been done for 24 hrs, the MIC was examined by reading the values against dark background. The

presence of hazy growth of the organism by the inoculum was considered as organism is in inhibition state.

Control strain: ATCC Escherichia coli 25922 was used as a reference strain[31].

Statistical Analysis: The data was entered and analyzed using SPSS 20.0. Biofilm formation was presented as frequency and percentage. Mean±SD was done for quantitative variable e.g. MIC. Independent t-test was done to observe differences in group means (biofilm forming and non biofilm forming).

RESULTS

In this study ninety clinical isolates of Escherichia coli were tested. All isolates were confirmed and processed in Microbiology Department at University of Health Sciences Lahore. Biofilm production and quantification of E. coli strains was done by microtiter plate assay as shown in figure 1 .The results showed that 18(20%) E. coli were strong biofilm producers, 54(60%) were moderate producers and 18(20%) were non-producers as seen in figure 2. Antibiotic susceptibility pattern of E. coli was performed by agar dilution method (CLSI 2016) for Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid Stock solutions (0.1g/10ml) of all three antibiotics were separately prepared as shown in figure 3. A control plate with inoculation of ATCC 25922 E. coli and tested strains of E. coli were also separately tested for each antibiotic as shown in figure 4. ATCC 25922 was inhibited at certain concentration of each antibiotic (<1µg/ml for Ciprofloxacin and Ceftriaxone and 8/4µg/ml for Amoxicillin/clavulanic acid) as described in CLSI 2016. Thirteen different working concentrations (0.25µg/ml, 0.5µg/ml, 1µg/ml, 2µg/ml, 4µg/ml, 8µg/ml, 16µg/ml, 32µg/ml, 64µg/ml, 128µg/ml, 256µg/ml, 512µg/ml and 1024µg/ml) of each antibiotic were tested in duplicate as shown in figure 5.

Table 1: Antibiotic susceptibility pattern of E. coli strains N=90

Antibiotics	% Sensitive	% Resistant
Ciprofloxacin	24.4	75.5
Ceftriaxone	36.6	63.3
Augmentin	77.7	22.2

Table 2: Antibiotic susceptibility pattern of biofilm forming E. coli strains (n=90)

Biofilm formation	**Antibiotics**					
n=90	**Ciprofloxacin**		**Ceftriaxone**		**Augmentin**	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Strong	16.70%	83.30%	33.40%	66.60%	88.90%	11.10%
Moderate	26%	74%	33.40%	66.60%	72.30%	27.70%
Non	22.30%	77.70%	50%	50%	78%	22%

Among all tested E. coli strains resistance to Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid were 75.5%, 63.3% and 22.2% as shown in table 1.The rates of antibiotic resistance of strong, moderate, and non-forming E. coli were 83.3%, 74% and 77.7% for Ciprofloxacin. Similarly, resistance of strong, moderate and non-biofilm forming E. coli was 66.6%, 66.6% and 50% towards Ceftriaxone respectively. Strong, moderate and non-biofilm forming strains of E. coli were 11.1%, 27.7% and 22% resistant to Amoxicillin/clavulanic acid as shown in table 2. Cumulative MIC of Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid against isolates of E. coli was also done as shown in table 3. MIC of Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid for biofilm forming E. coli isolates were shown in table 4. Similarly, MIC of Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid for non-biofilm forming E. coli isolates were shown in table 5.

Independent t-test was computed to observe the mean differences of MIC between biofilm forming and non-biofilm forming groups for all three antibiotics. Statistically no significant difference was observed between biofilm forming group with mean±SD (153.4±30.4) and non-biofilm forming group with mean±SD (107.7±22.7) due to p-value=0.46 for Ciprofloxacin as shown in figure 6. Statistically non-significant value was found in biofilm forming group with mean±SD (138.9±28.6) and non-biofilm forming group with mean±SD (158.9±64.7) due to p-value= 0.76 for Ceftriaxone as shown in figure 7. No significant difference was observed in biofilm forming group with mean±SD (87.1±27.7) and non-biofilm forming group with mean±SD (26.1±5.49) because of p-value= 0.27 for Amoxicillin/clavulanic acid as shown in figure 8. The findings suggest that there was no association of biofilm with antibiotic resistance. Biofilm formers were more antibiotic resistant than non-biofilm formers but statistically no significant results were found. There may be another molecular and enzymatic mechanisms involved.

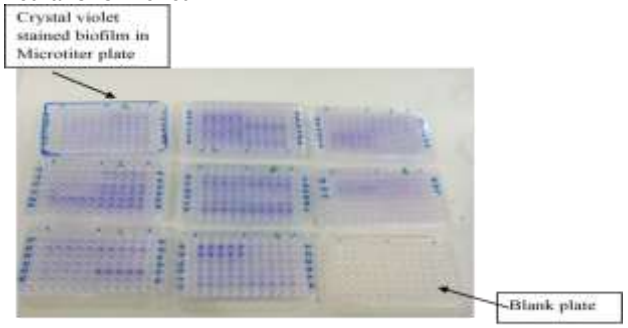

Figure 1: Biofilm detection in Microtiter assay.

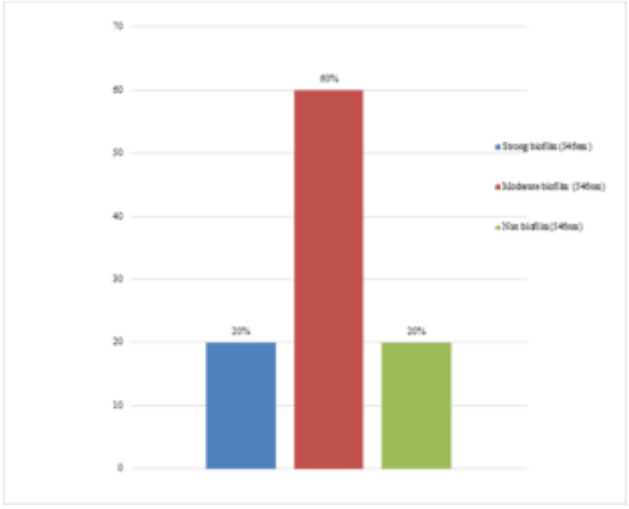

Figure 2: Frequency of strong, moderate and non-biofilm forming E. coli strains n=90

Figure 3: A stock solution of Ciprofloxacin and Ceftriaxone were prepared in water (0.1g/10ml) and of Amoxicillin/clavulanic acid was prepared in phosphate buffer saline (0.1g/10ml).

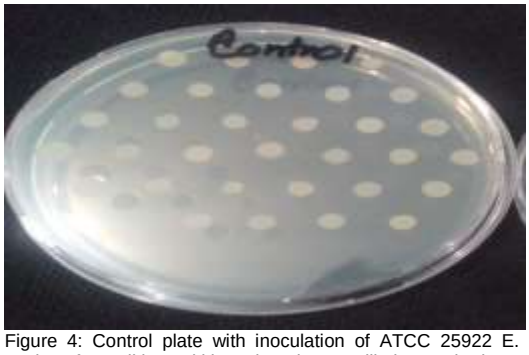

Figure 4: Control plate with inoculation of ATCC 25922 E. coli and test strains of E. coli by multi inoculator in agar dilution method.

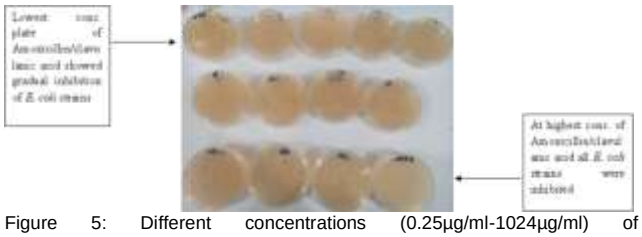

Figure 5: Different concentrations (0.25µg/ml-1024µg/ml) of amoxicillin/clavulanic acid were tested against E. coli and ATCC 25922 E. coli by agar dilution procedure in duplicate.

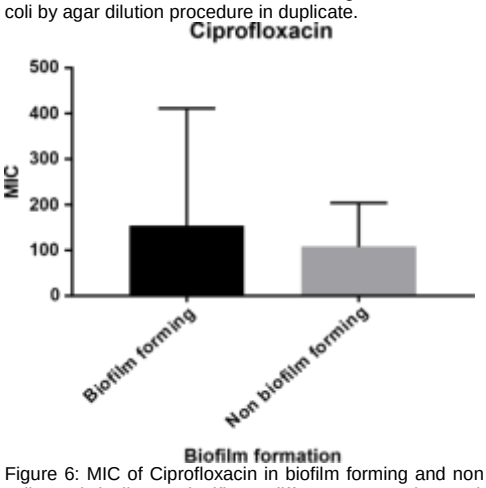

Figure 6: MIC of Ciprofloxacin in biofilm forming and non biofilm forming E. coli. Statistically no significant difference was observed in biofilm forming group with mean ±SD (153.4±30.4) and non biofilm forming group with mean ±SD (107.7±22.7).

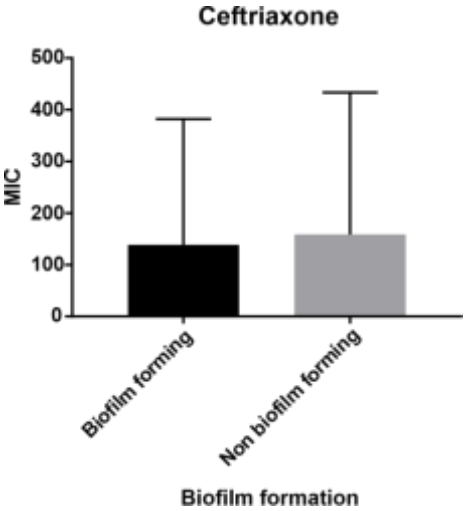

Figure 7: MIC of Ceftriaxone in biofilm forming and non biofilm forming E. coli. Statistically no significant difference was found in biofilm forming group with mean ±SD (138.9±28.6) and non biofilm forming group with mean ±SD (158.9±64.7).

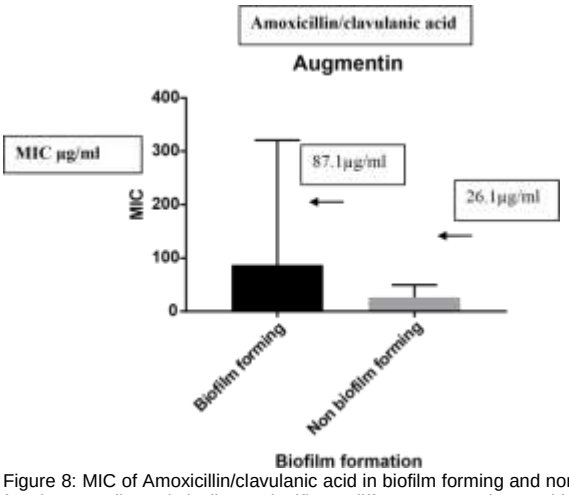

Figure 8: MIC of Amoxicillin/clavulanic acid in biofilm forming and non biofilm forming E. coli. Statistically no significant difference was observed in biofilm forming group with mean ±SD (87.1±27.7) and non biofilm forming group with mean ±SD (26.1±5.49).

DISCUSSION

Microorganisms form small communities of the cells known as biofilm that are irreversibly attach to each other on biotic or abiotic surfaces with the help of extracellular polymeric substances[32]. These substances act as channels to assist in the transfer of enzymes, antimicrobials etc. towards and away from biofilm matrix[33]. Biofilm is formed by several microorganisms like E. coli, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella pneumoniae, Streptococci spp., Staphylococcus aureus, Staphylococcus epidermidis etc[34.] There are numerous mechanisms of antimicrobial resistance; biofilm is one of them[35].The Centers for Disease Control and Prevention (CDC) estimated that above 65% of infections were caused by bacteria growing in biofilm[36]. Various methods are available to detect biofilm e.g. Tissue Culture Plate method, Tube method, Congo Red Agar method etc. Tissue culture plate method is most

common quantitative measure for detection of biofilm. It has the advantage of being simple, reliable, and accurate method. It can be easily modified to analyze the multiple strains within each experiment. Tube method showed variation in outcome by different observer. Congo red agar method generally showed false positive results[37]. Keeping the growing problem of antimicrobial resistance and biofilm development in microorganism in view, the current study was aimed to measure biofilm formation by clinical isolates of Escherichia coli and compares the survival of bacteria by treatment of Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid in the presence and absence of biofilm formation. In the current study ninety clinical strains of Escherichia coli were subjected to biofilm production by microtiter plate assay as this method was more reliable and sensitive one. Present results showed that 18(20%) E. coli isolates were strong biofilm formers, 54(60%) were moderate and 18(20%) were non-biofilm forming E. coli. A study was conducted for assessment of biofilm production in E. coli isolates. Out of fourteen isolates 71.4% were biofilm formers and 28.5% were non-biofilm formers which are reliable with current study as microtiter assay was used to access biofilm production[38]. Another study had found 89.5% biofilm production in E. coli isolates also supported current results[39].

Among hundred E. coli strains tested for biofilm production 6% were strong biofilm formers. It is quite lesser than biofilm forming capability of E. coli found in current and previous studies. It might be due to variation in experimental conditions[40].

Another study was conducted to identify biofilm forming ability in E. coli. Among two hundred and eight E. coli isolates 29%, 31.9% and 23.2% were strong, moderate and non-biofilm producers that were somehow consistent with current study[41].

A study was done to find out the biofilm forming capability in Staphylococcus aureus isolates. Out of fifty-nine isolates, 35.6% were moderate biofilm producers. These results were inconsistent with current study due to decreased number of tested isolates and use of different experimental conditions[42]. In India a study reported 62% Acinetobacter baumanii were biofilm formers and 25.4% were non-biofilm formers among the 55 studied isolates which were comparable with current results[43]. Present study also concluded that 80% strains of E. coli were involved in biofilm related infections. A study tested ninety-nine E. coli isolates in which 3%, 25% and 1% were strong, moderate and non-biofilm producers. Their results were not comparable to current study due to variations in optical density values of biofilm[44]. In the current study, in-vitro biofilm production was 80% by clinical isolates of E. coli. It was supported by other studies conducted in Egypt, India, and Nepal that showed different percentages of biofilm production in E. coli e.g. 63.6%, 60.15% and 51.9%[45] .In current study E. coli showed 75.5%, 63.3% and 22.2% resistance to Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid respectively. Higher resistance of bacteria to these drugs may be due to widespread use and ease of accessibility. Several studies conducted in developing countries also reported higher resistance of microorganisms to various antibiotics. This was due to misuse of antimicrobial agents[46].

A study showed that E. coli was 54.2% and 43.3% resistant to Ciprofloxacin and Ceftriaxone which were consistent with current study[47]. Such higher resistance may be due to various alterations in certain genes and initiation of resistance mechanism of E. coli. Other studies also stated higher resistance of microorganisms to antimicrobial agents. This was due to stimulation of different resistance mechanisms in bacteria on exposure of chemical agents[48].

In contrast, other studies in developed countries stated a large number of E. coli were sensitive to penicillin and cephalosporin[49].

It was reported that E. coli isolates were 69.6% and 53.0% resistant towards Ciprofloxacin and Ceftriaxone also supported current results[50].

There is higher emergence of resistance by overuse of antibiotics, spread of resistant bacteria among patients, health care personnel and community and inappropriate instructions for use of antibiotics[51].

In the present study it was concluded that Ciprofloxacin (75.5%) and Ceftriaxone (63.3%) were most resistant drugs against E. coli. According to geographical locations certain variations were found in resistance pattern of E. coli. As in Bangladesh a study stated that E. coli isolates were 11.5% and 5.5% resistant to Ciprofloxacin and Ceftriaxone which were not consistent with current study[52] ·Ciprofloxacin was most resistant drug in current study and similar results were found in another study[53].

In present study Amoxicillin/clavulanic acid showed only 22.2% resistance to all tested isolates. Amoxicillin/clavulanic acid showed 93.7% resistance which was not consistent to current results. This is an indication of earlier exposure of the isolates to this drug, which may have enhanced resistant development[54]. Another study reported 29.1% and 40.3% resistance to Ceftriaxone and Ciprofloxacin which were not related to current study due to increased number of tested isolates [55].

Amoxicillin/clavulanic acid and Ceftriaxone showed 100% sensitivity to E. coli strains were not comparable to current results due to geographical locations[56].

Another study had tested three hundred and seventy-eight E. coli isolates for susceptibility testing. Results showed 49% and 34% resistance to Ciprofloxacin and Ceftriaxone which were not reliable with current results as different susceptibility methodology was used[57].

It was stated in current study that biofilm formers were more resistant to Ciprofloxacin and Ceftriaxone than non-biofilm formers but statistically there is no significant difference of resistance between them. There may be another genetic and enzymatic mechanism that confers same level of resistance in bacteria[58].

In the present study strong, moderate and non-biofilm forming E. coli showed 83.3%, 74% and 77.7% resistance to Ciprofloxacin. Strong, moderate and non-biofilm forming E. coli were 66.6%, 66.6% and 50% resistant to Ceftriaxone. Similarly, 11.1%, 27.7% and 22% resistance were showed by strong, moderate and non-biofilm forming E. coli against Amoxicillin/clavulanic acid. As stated in current results that biofilm formers were more resistant towards antibiotics than non-biofilm former but statistically no significant difference in resistance was observed between both groups. There may be another mechanism involved in non-biofilm formers that confer same level of resistance as biofilm in biofilm formers. A study was expected that non biofilm formers were more resistant to quinolones as compared to biofilm formers also supported current results. The relation between biofilm and virulence factors in E. coli remains unclear but various studies showed variable conclusions[59].

In current study it was stated that biofilm formers and non-formers were 83.3% and 77.7% resistant to Ciprofloxacin but statistically there was no difference of resistance between both groups. Similarly, 66.6% and 50% resistance were observed for biofilm formers and non-formers against Ceftriaxone and there was also no significant difference of resistance was found. It was found statistically that biofilm formers and non-biofilm formers confer equal resistance to Ciprofloxacin and Ceftriaxone.

Another study stated 42% and 43% resistance to Ciprofloxacin and Amoxicillin Clavulanic acid by biofilm forming E. coli isolates[60]. Biofilm forming Gram negative bacteria showed 95% and 58% resistance towards Ciprofloxacin and Ceftriaxone. While non-biofilm forming bacteria were 50% and 33% resistant to Ciprofloxacin and Ceftriaxone. Phenotypically the resistance was comparable to current results. But biofilm formers were more antibiotic resistant than non-biofilm formers because they tested different clinical samples[5].

Some biofilm-forming isolates of Acinetobacter baumanii showed low resistance to carbapenem and quinolones then non biofilm formers. Possible reason was the capability of some antibiotics to enter the biofilm and slowdown the growth[61] ·It was not necessary that only biofilm production in bacteria plays an important role in resistance. There may be several enzymatic and genetic pathways behind this e.g. efflux pump, enzyme production, reduce permeability and target modification[62].

CONCLUSION

In the present study biofilm is not essentially involved in mediating drug resistance in E. coli against tested Ciprofloxacin, Ceftriaxone and Amoxicillin/clavulanic acid. Biofilm formers showed more resistance to antibiotics than non-biofilm formers but statistical analysis of both groups against three antibiotics showed that there were no significant difference (p value= >0.05) of resistance in them. Statistically both groups confer equal resistance. It was an alarming situation that non biofilm formers were also more antibiotic resistant as biofilm formers. There may be another molecular and enzymatic mechanisms that allow bacteria to mediate resistance. The present study had some limitations e.g. molecular detection of biofilm was not done, less number of isolates were tested, resistance mechanisms in non-biofilm formers were not studied.

The future expansion of this study can be done by genetic analysis and biochemical details of resistance induced by biofilm and other mechanisms to various antibiotics. Improved knowledge about the genetic mechanisms of biofilm formation is vital for the growth of effective treatment. To control biofilm related infection, one will require attempt to build up therapeutic agents that target the biofilm architecture and community signaling that prevent the formation and promote the detachment of biofilm.

Conflict of interest: No author has any kind of conflict of interest at all.
Acknowledgement: This study was supported by the department of Microbiology, University of Health Sciences, Lahore

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