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Antibiotic Resistance In Biofilm Producing *Escherichia Coli*

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Abstract: Antibiotic resistance poses a significant global threat to public health, with bacterial biofilms being particularly challenging environments where resistance mechanisms are intensified. This study focuses on understanding antibiotic resistance in biofilm-producing *Escherichia coli (E. coli)* through the application of biofilm and crystal violet assays. Biofilm formation is a key survival strategy employed by bacteria, enhancing resistance to antibiotics and host immune responses. The experimental approach involves the utilization of a biofilm assay to assess the biofilm-forming capabilities of *E. coli* strains. The biofilm assay provides insights into the organism's ability to adhere to surfaces and form structured biofilms, simulating conditions encountered during infections. Subsequently, the crystal violet assay is employed to quantify the biomass of the formed biofilms. This method allows for a quantitative assessment of biofilm production, offering a reliable measure of the extent of biofilm formation. The study aims to correlate the observed biofilm formation with antibiotic resistance patterns. Various antibiotics commonly used in clinical settings will be tested against planktonic and biofilm-associated *E. coli* strains. The results will shed light on the enhanced resistance of biofilm-producing *E. coli*, contributing valuable data to the understanding of antibiotic efficacy in biofilm-related infections. The research integrates artificial intelligence (AI) techniques to analyze the complex relationships between biofilm formation, antibiotic resistance, and genetic factors in *E. coli*. AI algorithms will be employed to identify patterns and predict potential resistance mechanisms, enhancing the comprehensiveness of the study. This research employs biofilm and crystal violet assays to explore antibiotic resistance in biofilm-producing *E. coli*. The integration of AI techniques enhances the depth of analysis, providing a holistic understanding of the intricate interplay between biofilm formation and antibiotic resistance. The findings of this study will contribute to the development of targeted strategies for combating biofilm-associated antibiotic resistance in *E. coli* infections.

Index Terms - Antibiotics, Antimicrobial activity, *E. coli*, Antimicrobial resistance, Biofilm, Multidrug resistance.

1. INTRODUCTION

Antimicrobial resistance (AMR) is a persistent global issue. Through a variety of (either chromosomally encoded or plasmid-mediated) resistance mechanisms, pathogenic bacteria can develop resistance to a broad range of chemically unrelated antibiotics. Multidrug resistance (MDR) is defined as non-susceptibility to at least one antimicrobial compound in three or more antimicrobial categories [1]. The resistance of plasmid produced by *Escherichia coli* strains to novel antibiotics is an important study to be conducted.

Most typically, when bacteria are grown in different culture conditions for in vitro research, they are in their planktonic, or free-living, phases. However, bacteria improve their chances of survival in hostile environments or in the living host by adhering to inanimate objects or tissues and appearing as multicellular communities covered in a layer of extracellular matrix known as biofilm.

Biofilm formation is a widespread phenomenon observed in nature and is closely linked to the onset of numerous bacterial infections in humans. Microorganisms produce biofilms as a survival strategy in

challenging environments. These bacterial biofilms consist of bacteria enclosed within a polymeric matrix, providing a protective shield against antibiotics and host defences. This defensive mechanism renders infections challenging to treat and often results in recurring symptoms. Bacteria within biofilms can exhibit a remarkably high level of drug resistance, reaching up to 1000 times the resistance seen in their planktonic counterparts. Exopolysaccharides (EPS), nucleic acids (environmental DNA), proteins, lipids, different ions, and water are the ingredients of biofilms, which are secreted by several bacterial populations and give all embedded bacteria a survival benefit.

Resistance can be attributed to several factors. Firstly, the limited penetration of antibiotics into the biofilm, coupled with the metabolic inactivity of the enclosed organisms, contributes to reduced exposure to antimicrobial agents. Additionally, the degradation of antibiotics by the bacteria within the biofilm further diminishes the efficacy of drug treatments. Biofilms also serve as conduits for the horizontal transfer of drug resistance markers and genes associated with other virulence factors through inter-bacterial interactions.

Escherichia coli, in urinary tract infections, stands out as one of the most prevalent biofilm-producing bacteria. Given its prevalence and biofilm-forming tendencies, *E. coli* poses a substantial challenge in the context of urinary tract infections, where the protective nature of biofilms can complicate treatment and contribute to the persistence of the infection.

This intricate interplay between biofilm formation and antibiotic resistance underscores the need for a comprehensive understanding of these processes. By deciphering the mechanisms behind biofilm-mediated resistance, researchers can develop targeted strategies to combat these persistent and challenging infections, offering new avenues for the effective treatment of bacterial infections associated with biofilm formation [1].

The formation of biofilms, which frequently results in resistant, chronic infections (such as those linked to catheter use, skin and soft tissue infections, and dental cavities), is a significant concern in addition to the "classical" resistance-determinants produced by bacteria. Antibiotics are ineffective against metabolically inactive or dormant bacteria, also known as small-colony variants, and biofilms shield bacteria from the penetration and accumulation of various toxic substances and antibiotics in effective concentrations [2].

The drugs, widely recognized for their antibiotic efficacy, have recently emerged as a versatile group with diverse therapeutic potential. Beyond their conventional role in combating bacterial infections, contemporary research has unveiled a spectrum of additional effects within this drug class. These effects span neuroprotective, antioxidant, analgesic, and immunomodulatory capabilities.

This overview seeks to provide a concise exploration of the antibiotic effects. Subsequently, the focus will shift to the non-antibiotic effects within three distinct subfamilies: penicillin's, cephalosporins, and beta-lactamase inhibitors. Each subfamily exhibits unique molecular structures and pharmacokinetics, opening avenues for diverse clinical applications [3].

1.1 Bacterial Species

***Escherichia coli (E. coli)*:**

The most common commensal habitant of the gastrointestinal tracts of humans and other warm-blooded animals, *E. coli* is a member of the Enterobacteriaceae family of bacteria and one of the most significant pathogens. It rarely spreads illness because it coexists with hosts in a mutually beneficial relationship called a commensal. Nonetheless, due to its wide range of disease-causing potential, it is also one of the most prevalent viruses for both humans and animals. Because of its unique properties, including its ease of handling, the availability of the entire genome sequence and its capacity to grow in both aerobic and anaerobic environments, *Escherichia coli* is a valuable host organism in biotechnology. Numerous applications of *E. coli* exist, both in the industrial and medical area and it is the most used microorganism [4].

Compared to other faecal coliforms, *E. coli* is a more specific indication of faecal contamination and belongs to the faecal coliform group. The discovery that some faecal coliforms were not faecal in origin and the advancement of *E. coli* testing techniques are the two main factors that have contributed to the trend toward the use of *E. coli* as the preferred indicator for the detection of faecal contamination, not only in drinking water but also in other matrices. The definition of faecal coliforms has also been updated to better align with the genetic composition of its constituents and incorporates recently discovered environmental species. Consequently, thermotolerant coliforms are a term that is increasingly being used to describe faecal coliforms.

The greatest bacterial indicator of faecal contamination in drinking water now seems to be *E. coli*. This is because thermotolerant (faecal) coliforms are more common in temperate environments than *E. coli* is, that *E. coli* is more common in human and animal feces than other thermotolerant coliforms, and that there are inexpensive, quick, sensitive, specific, and simple to use *E. coli* detection methods available [5].

2. REVIEW OF LITERATURE

Antimicrobial resistance (AMR) in biofilm-producing *Escherichia coli (E. coli*) is a complex and pressing issue that poses significant challenges in healthcare settings. Biofilms, protective structures formed by microorganisms, contribute to persistent infections and increased resistance to antibiotics. A comprehensive review of the existing literature reveals the intricate mechanisms, clinical implications, and potential strategies for tackling AMR in biofilm-producing *E. coli*.

Biofilm Formation and Mechanisms of Antimicrobial Resistance:

Biofilm formation is a natural survival strategy for bacteria, allowing them to adhere to surfaces and produce a matrix that shields them from external threats. In the context of *E. coli*, a common pathogen in community and hospital-acquired infections, biofilm formation contributes to the chronicity of infections and the development of resistance.

The mechanisms underlying antimicrobial resistance in biofilms are multifaceted. Limited antibiotic penetration into the biofilm matrix is a significant factor, hindering the drugs' access to bacterial cells. The extracellular polymeric substances (EPS) within the biofilm matrix create a physical barrier that prevents antibiotics from reaching their targets effectively. Additionally, the metabolic inactivity of bacteria within biofilms reduces their susceptibility to antibiotics that target actively dividing cells.

A study [20] delves into the molecular mechanisms of biofilm formation, highlighting the role of quorum sensing and the regulation of adhesion factors. Understanding these mechanisms is crucial for developing targeted interventions to disrupt biofilm formation and enhance antibiotic efficacy.

Clinical Significance and Impact on Infections:

The clinical significance of AMR in biofilm-producing *E. coli* is particularly evident in the context of chronic and recurrent infections. Biofilm-associated infections are challenging to treat due to the resilience of bacteria within the matrix. This is exemplified in urinary tract infections (UTIs), where *E. coli* is a leading cause.

A study [21] emphasizes the association between biofilm formation and uropathogenic *E. coli* (UPEC), indicating that biofilm-producing strains contribute to the persistence of UTIs. The ability of *E. coli* to form biofilms in the urinary tract exacerbates the challenges in eradicating infections completely, leading to recurrent episodes and necessitating prolonged or repeated courses of antibiotics.

Horizontal Gene Transfer and Dissemination of Resistance:

Biofilms serve as hotspots for horizontal gene transfer, facilitating the exchange of genetic material, including antibiotic resistance genes, among bacterial populations. This phenomenon contributes to the dissemination of resistance in both clinical and environmental settings.

A comprehensive review [22] explores the role of biofilms in horizontal gene transfer, emphasizing the implications for the spread of antibiotic resistance. The interconnectedness of bacteria within biofilms creates an environment conducive to genetic exchange, accelerating the evolution of antibiotic-resistant strains.

Innovative Approaches and Therapeutic Strategies:

Addressing AMR in biofilm-producing *E. coli* requires innovative approaches to disrupt biofilm formation and enhance the efficacy of existing antibiotics. Various studies have explored potential strategies, including the use of biofilm-disrupting agents, combination therapies, and the development of novel antimicrobial compounds.

A study [23] investigates the potential of antimicrobial peptides as alternatives to traditional antibiotics, emphasizing their efficacy against biofilm-forming bacteria. Additionally, [24] explores the use of bacteriophage therapy to target biofilm-associated infections, providing insights into a promising avenue for future interventions.

The literature on antimicrobial resistance in biofilm-producing *E. coli* underscores the urgency of addressing this complex issue. Biofilm formation contributes significantly to the persistence and recurrence of infections, necessitating a multidimensional approach to combat AMR. Understanding the molecular mechanisms, clinical implications, and potential therapeutic strategies is essential for guiding future research and interventions [21-24].

3. MATERIALS and METHODOLOGY

3.1 Materials:

3.1.1 Media & Reagents:

Luria Bertani Broth & Luria Bertani Agar:

Luria-Bertani (LB) broth stands as a widely employed and versatile liquid medium within the field of microbiology, particularly finding extensive use in bacterial culture and molecular biology experiments. Comprising peptone, yeast extract, and sodium chloride dissolved in water, LB broth provides a rich nutrient base essential for bacterial growth. Peptone and yeast extract deliver crucial nutrients, amino acids, and vitamins, while sodium chloride ensures the osmotic balance necessary for microbial proliferation. The well-balanced composition of LB broth makes it amenable to supporting the growth of both Gram-negative and Gram-positive bacteria.

Its solid counterpart, Luria-Bertani Agar, more commonly known as LB agar, plays a pivotal role in microbiological laboratories, serving as a solid growth medium for bacterial cultivation. The formulation of LB agar encompasses peptone, yeast extract, sodium chloride, and agar. Peptone and yeast extract continue to provide the requisite nutrients for bacterial growth, while sodium chloride maintains osmotic equilibrium. Agar, extracted from seaweed, acts as a solidifying agent, enabling the medium to solidify into a gel-like structure suitable for the cultivation of bacteria on solid surfaces.

This dual combination of LB broth and LB agar provides microbiologists with essential tools for a spectrum of applications, ranging from routine bacterial propagation to sophisticated molecular biology experiments. The versatility of these media makes them foundational components in microbial research and experimentation [6].

Tryptic Soy Broth:

Tryptic Soy Broth (TSB) is a versatile and widely used liquid medium in microbiology, providing a nutrient-rich environment for the cultivation of a broad range of microorganisms. The formulation of TSB typically includes enzymatic digests of casein and soybean meal, providing a complex mixture of amino acids, peptides, and other nutrients essential for microbial growth. Sodium chloride is often added to maintain osmotic balance. TSB's composition makes it suitable for supporting the growth of both Gram-positive and Gram-negative bacteria [7].

Crystal Violet:

Crystal violet, a synthetic dye with a significant history in microbiology, has found diverse applications, particularly in bacterial staining and identification. In microbiological staining methods, crystal violet plays a crucial role as the primary stain in the Gram staining procedure, a foundational technique used to classify bacteria based on their distinct cell wall characteristics. This dye effectively attaches to the peptidoglycan layer of bacterial cell walls, aiding in the differentiation between Gram-positive and Gram-negative bacteria. This differentiation is vital for a comprehensive understanding of bacterial morphology, overall structure, and, specifically within the context of Gram staining, the broader characteristics associated with bacterial cell walls [8].

3.1.2 Antibiotics:

Cepodem – XP 325 mg
Norflox – 200 mg
Niftax – 50 mg
Taxim-o – 200 mg
Erythromycin – 500 mg
Metronidazole – 400 mg
Azithromycin (Aziken) – 500 mg
Amoxycilin & potassium clavalanate tablets (Amoxyclav) – 625 mg
Cefixime (CEFIX) – 200 mg

3.2 Methods:

3.2.1 Sample Collection:

E. coli sample was collected from Rajarajeshwari Dental College as part of our research work.

3.2.2 Sub culturing:

Sub culturing is a fundamental technique in microbiology that involves the transfer of microorganisms from an existing or primary culture to a new growth medium. This process is essential for maintaining and propagating specific strains of microorganisms. The sub culturing process typically begins with the selection of a well-developed colony or a portion of an existing culture. Aseptic techniques are rigorously applied to avoid contamination during the transfer. The selected microorganisms are then introduced into a fresh medium, which could be a different agar plate, broth, or other suitable growth media, depending on the specific requirements of the experiment or study.

Sub culturing is crucial for the long-term preservation of microbial strains. In microbiological laboratories, especially in studies involving the maintenance and characterization of bacterial, fungal, or other microbial cultures [9].

3.2.3 Disc Diffusion Assay:

The disc diffusion assay is a widely used microbiological technique designed to evaluate the susceptibility of microorganisms to various antimicrobial agents. This method provides valuable information about the effectiveness of specific antibiotics against a given bacterial strain, aiding in the determination of antibiotic sensitivity.

In the disc diffusion assay, paper discs containing a known concentration of an antibiotic are placed on the surface of an agar plate inoculated with the target microorganism. As the antibiotic diffuses into the agar, a concentration gradient is established. If the microorganism is susceptible to the antibiotic, an inhibition zone (zone of inhibition) will form around the disc, indicating the area where bacterial growth is inhibited.

The size of the inhibition zone is measured to determine the sensitivity of the microorganism to the tested antibiotic. This method is essential in clinical microbiology for guiding antibiotic therapy and in research settings to assess microbial resistance patterns [10].

3.2.4 Biofilm Assay:

The biofilm assay is a laboratory technique employed to study the formation and characteristics of biofilms produced by microorganisms. Biofilms are complex communities of microorganisms encased in a self-produced extracellular matrix, adhering to surfaces. This assay provides insights into the adhesive and cohesive properties of microbial communities, helping to understand their role in various contexts, such as medical device-associated infections and environmental processes.

In a typical biofilm assay, microorganisms are allowed to adhere and form biofilms on a specific substrate, often in multi-well plates or other suitable platforms. After a designated incubation period, the biofilms are quantified using various methods, such as crystal violet staining, which allows for the measurement of biofilm biomass. Other techniques may involve the use of fluorescent dyes or microscopy for visualizing biofilm structure. Understanding biofilm formation is crucial for developing strategies to prevent or control infections related to biofilm-producing microorganisms. It has applications in medical research, environmental microbiology, and industrial settings [11, 12].

Two different techniques were employed to study the formation of biofilm:

3.2.4 (A) EPS Analysis by phenol sulphuric acid method at 480 nm:

The analysis of Extracellular Polymeric Substances (EPS) through the phenol-sulfuric acid method at 480 nm is a widely employed technique in microbiology to assess the polysaccharide levels in biofilms. EPS, a crucial component of the biofilm matrix, significantly influences the structure and stability of biofilms.

In this method, EPS is initially extracted from the biofilm matrix using established procedures. The extracted polysaccharides undergo treatment with phenol, followed by the addition of sulfuric acid. This chemical reaction between phenol and carbohydrates forms a colored complex, and the color intensity is directly proportional to the polysaccharide concentration. The spectrophotometric measurement of absorbance at 480 nm provides a reliable indication of the polysaccharide content in EPS.

The selection of 480 nm as the analysis wavelength is based on the maximum absorbance of the color complex, ensuring an accurate measure of polysaccharide concentration in EPS. This method proves valuable for comprehending the composition and quantity of EPS in biofilms, contributing to the characterization of biofilm architecture and its implications in various processes [13, 14].

3.2.4 (B) Crystal Violet Assay:

The crystal violet assay is a widely used method in microbiology for studying bacterial biofilm formation. This technique assists in assessing the adherence and growth of bacteria on surfaces, providing insights into the biofilm-forming capabilities of microorganisms. The assay is particularly valuable for understanding bacterial adhesion, a critical step in the initiation of biofilm formation.

In the crystal violet assay, a culture of bacteria is allowed to adhere and form a biofilm on a substrate. After a specific incubation period, the biofilm is stained with crystal violet, a synthetic dye that binds to cellular components and extracellular polymeric substances within the biofilm matrix. Excess stain is then washed away, and the dye bound to the biofilm is solubilized. The optical density of the solubilized crystal violet, measured spectrophotometrically, provides a quantitative measure of biofilm formation.

This method allows researchers to compare the biofilm-forming abilities of different bacterial strains or test the impact of various experimental conditions on biofilm development. The crystal violet assay is instrumental in biofilm-related studies in fields such as microbiology, environmental science, and medical research. [15].

4. RESULT AND DISCUSSION

4.1 Disc Diffusion Assay:

Initially 12 different *E. coli* samples were collected from Rajarajeshwari Dental College.

Table 1: 12 different *E. coli* sample ID's

Sl. No.	*E. coli* Sample ID's
01	525
02	3835
03	651
04	656
05	636
06	631
07	728
08	591
09	3454
10	650
11	850
12	574

Out of these 12 different *E. coli* samples only 6 *E. coli* were further examined using disc diffusion method (574, 728, 591, 631, 651, and 850).

The results for disc diffusion assay involves assessing the zones of inhibition around the antibiotic discs placed to determine the sensitivity of microorganisms to the tested antibiotics. The size of the inhibition zones reflects the effectiveness of the antibiotics in inhibiting bacterial growth. At the outset, we collected 12 distinct clinical samples specifically from dental sources, all originating from *E. coli*.

Out of the initial 12 *E. coli* samples collected from dental sources, we proceeded to conduct a detailed examination on a subset of six samples. This subset, comprising *E. coli* sample ID's 574, 728, 591, 631, 651, and 850, was subjected to testing against six distinct antibiotics. (Cepodem – XP 325 mg, Norflox – 200 mg, Niftax – 50 mg, Taxim-o – 200 mg, Erythromycin – 500 mg and Metronidazole – 400 mg). The objective was to observe and analyse the zones of inhibition resulting from the interaction between these specific *E. coli* samples and the respective antibiotics.

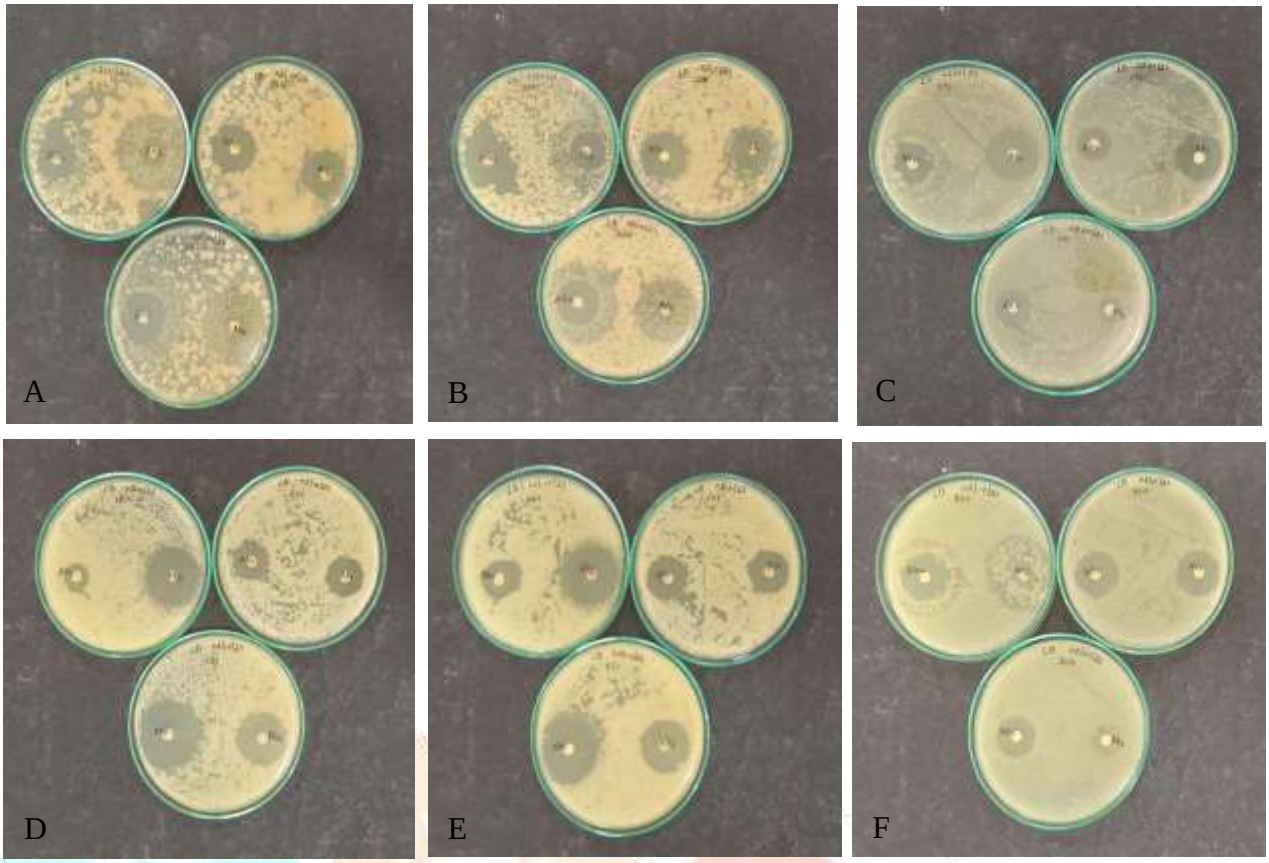

Figure 1: Respective plates (*E.coli* sample ID's) A. 574, B. 728, C. 591, D. 631, E. 651, F. 850, showing inhibition zone of LAB culture supernatants treated with different antibiotics against *E.coli*.

Table 2: Different antibiotics (Cepodem, Norflox, Niftax, Taxim, Erythromycin, Metronidazole) showing different zone of inhibition.

Sample ID's	Zone of inhibition					
	Ab1	Ab2	Ab3	Ab4	Ab5	Ab6
574	2.4 cm	1.3 cm	2.5 cm	2.1 cm	2.5 cm	0
728	2.2 cm	2 cm	2.5 cm	3 cm	2.4 cm	0
591	2.5 cm	2.8 cm	1.5 cm	2 cm	2.9 cm	1 cm
631	2 cm	3 cm	2 cm	1.4 cm	3.4 cm	2.3 cm
651	2.2 cm	3 cm	2.2 cm	2 cm	3.2 cm	2.5 cm
850	2.3 cm	1 cm	2.3 cm	2.5 cm	2 cm	1.5 cm

Again, the plates, which showed resistance where further, examined, specifically samples 574 and 728. Then examined these two *E. coli* samples more closely by using three different antibiotics (Azithromycin (Aziken) – 500 mg, Amoxicillin & potassium clavalanate tablets (Amoxyclav) – 625 mg & Cefixime (CEFIX) – 200 mg).We tested them by placing discs with the above mentioned antibiotics on the samples.

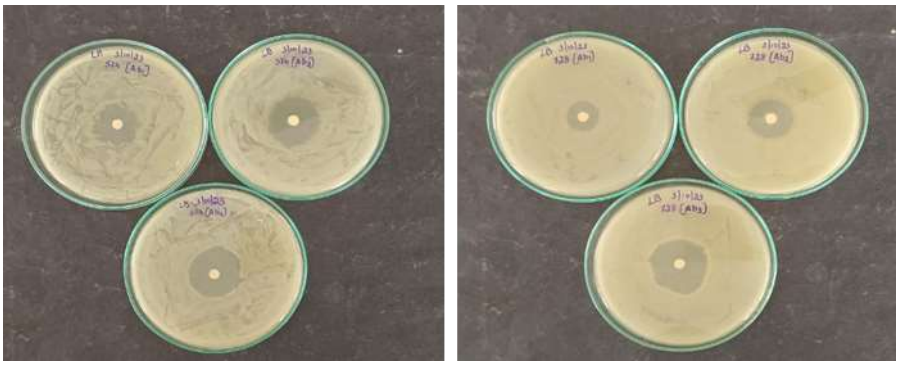

Figure 2: Respective plates (*E. coli* sample ID's) A. 574, B. 728 showing inhibition zone of LBA culture supernatants treated with different antibiotics against *E. coli*.

Table 3: Different antibiotics (Azithromycin, Amoxycav, Cefime) showing different zone of inhibition.

Sample ID's	Zone of inhibition		
	Ab7	Ab8	Ab9
574	2.2 cm	2.6 cm	2.7 cm
728	1.6 cm	2.2 cm	3 cm

A B

4.1.2 Biofilm Assay:

4.1.2 (A) Findings from the analysis of Extracellular Polymeric Substances (EPS) using the phenol-sulphuric acid method at 480 nm are as follows:

Interpreting the results of Extracellular Polymeric Substances (EPS) analysis before incubation involves assessing the quantity and characteristics of EPS in the microbial environment. This analysis provides insights into the baseline EPS production by microorganisms before any incubation or growth period [16].

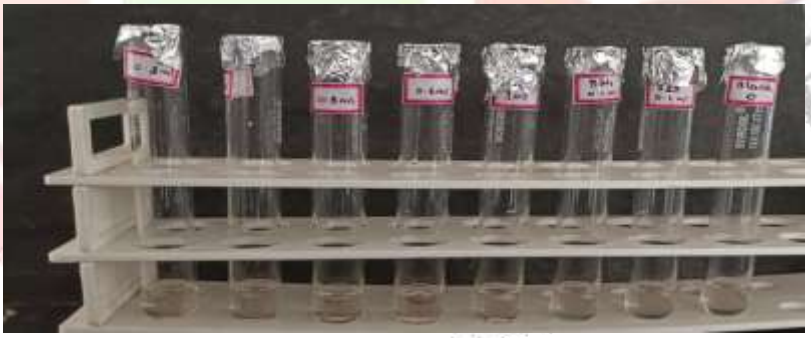

Figure 3: EPS Analysis using phenol-sulphuric acid method at 480 nm before incubation.

Analysing Extracellular Polymeric Substances (EPS) after incubation involves assessing any changes in quantity and characteristics following a period of microbial growth. This examination provides insights into the dynamic nature of EPS production during incubation and its potential impact on biofilm development [16].

Figure 4: EPS Analysis using phenol-sulphuric acid method at 480 nm after incubation.

Interpreting the results of EPS analysis at OD 480 nm involves assessing the optical density measurements at its specific wavelength. The optical density at 480 nm provides insights into the concentration of Extracellular Polymeric Substances (EPS) within the sample [16].

Table 4: Observation table of Phenol-sulphuric acid assay.

Sl. No.	Sample	Working standard (ml)	Distil water (ml)	5% phenol solution	Sulphuric acid	Allow to stand for 10 minutes. Shake the contents thoroughly and place it in boiling water bath for 20 minutes.	Absorbance at 490 nm
1		0.2 ml	0.8 ml	1 ml	1 ml		0.155
2		0.4 ml	0.6 ml				0.163
3		o.6 ml	0.4 ml				0.169
4		0.8 ml	0.2 ml				0.177
5		1 ml	0.0 ml				0.181
6a	728	0.1 ml	0.9 ml				0.311
6b	574	0.1 ml	0.9 ml				0.404
7	Blank	0	1 ml				0

4.1.2 (B) Crystal Violet Assay:

Interpreting the results of a Crystal Violet assay involves understanding the degree of biofilm formation by microorganisms. Crystal Violet is commonly used to stain biofilms, and the intensity of staining is proportional to the amount of biofilm present [17].

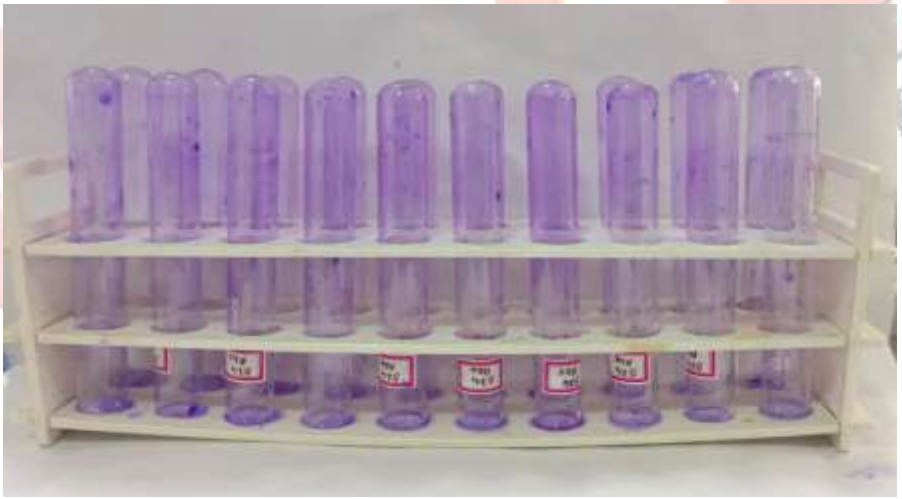

Figure 5: Test tubes showing results for Crystal Violet Assay.

4.2 Discussion:

4.2.1 Disc Diffusion Assay:

In the initial phase of the study, we collected 12 *E. coli* samples from dental sources. To check its antibiotic susceptibility, we selected a subset of six samples, namely *E. coli* samples with ID numbers 574, 728, 591, 631, 651, and 850. These samples underwent testing against six distinct antibiotics - Cepodem (XP 325 mg), Norflox (200 mg), Niftax (50 mg), Taxim-o (200 mg), Erythromycin (500 mg), and Metronidazole (400 mg). Our aim was to observe and analyse the zones of inhibition, providing insights into the effectiveness of these antibiotics against the specific *E. coli* strains.

Upon identifying samples 574 and 728 as exhibiting higher resistance, we conducted a more focused examination. These two *E. coli* samples were subjected to testing with three different antibiotics - Azithromycin (Aziken) at 500 mg, Amoxicillin & potassium clavulanate tablets (Amoxyclav) at 625 mg,

and Cefixime (CEFIX) at 200 mg. Discs containing these antibiotics were placed on the samples, aiming to assess their response and resistance patterns.

This information allows us to gain valuable information about the antibiotic sensitivity and resistance profiles of the selected *E. coli* samples. Such insights are essential for guiding clinical decision-making and understanding microbial responses to antibiotic treatments.

4.2.2 Biofilm Assay:

4.2.2 (A) EPS Analysis by phenol sulphuric acid method at 480 nm:

In this method, EPS is extracted from the biofilm matrix, and the extracted polysaccharides undergo a reaction with phenol and sulfuric acid. The resulting colored complex is quantified spectrophotometrically at 480 nm, where the color exhibits maximum absorbance. The intensity of the absorbance at this wavelength is directly proportional to the concentration of polysaccharides present in the EPS.

4.2.2 (B) Crystal Violet Assay:

The dye binds to the biofilm biomass and extracellular polymeric substances, allowing quantification of biofilm formation.

5. SUMMARY & CONCLUSION

5.1 Summary:

Antibiotic resistance in biofilm-producing *Escherichia coli (E. coli)* is a critical and challenging aspect in microbial research. Biofilms, protective structures formed by microorganisms, play a significant role in bacterial infections, and their resistance to antibiotics poses a serious threat to effective treatment. *E. coli*, a common pathogen, is known for its ability to form biofilms, further complicating antibiotic therapy.

The biofilm matrix shields bacteria from antibiotics and host defences, leading to persistent infections. The high level of drug resistance exhibited by bacteria within biofilms, often up to 1000 folds, is attributed to factors such as limited antibiotic penetration, metabolic inactivity of organisms, and enzymatic degradation of antibiotics. Additionally, biofilms can facilitate the horizontal transfer of drug resistance genes among bacteria.

Especially concerning is the association of biofilm-producing *E. coli* with urinary tract infections, where the recurrent nature of symptoms is linked to the resilience of these bacteria within biofilms. This phenomenon underscores the urgent need for a deeper understanding of biofilm-associated antibiotic resistance and the development of targeted therapeutic strategies.

For an in-depth exploration of this topic, relevant literature provides valuable insights into the mechanisms and challenges associated with antibiotic resistance in biofilm-producing bacteria [18, 19].

5.2. Conclusion:

Antibiotic resistance in biofilm-producing *Escherichia coli (E. coli)* stands as a formidable challenge in the landscape of microbial infections, necessitating a comprehensive understanding of the mechanisms involved and prompting the exploration of innovative therapeutic strategies. The biofilm matrix, a protective and complex structure formed by microorganisms, plays a pivotal role in the persistence of *E. coli* infections and their resistance to antibiotic treatments.

Biofilms characterized by bacteria enclosed in a polymeric matrix, present a unique set of challenges for effective antibiotic action. The limited penetration of antibiotics into the biofilm, coupled with the metabolic inactivity of the microorganisms within, creates an environment conducive to heightened resistance. The degradation of antibiotics by biofilm-embedded bacteria further compounds the difficulty in treating infections associated with *E. coli* biofilms.

Antibiotic resistance in biofilm-producing *E. coli* is the low amount of antibiotics reaching the bacteria within the biofilm. This limitation, along with the metabolic dormancy of biofilm-enclosed bacteria, creates a scenario where conventional antibiotic therapies struggle to exert their full efficacy. The bacteria within biofilms can exhibit resistance levels up to 1000 folds higher compared to their planktonic counterparts, emphasizing the urgent need for targeted interventions.

Biofilms have been identified as potential hotspots for the horizontal transfer of drug resistance markers and genes associated with virulence factors. The inter-bacterial interactions within biofilms create an environment conducive to the exchange of genetic material, contributing to the dissemination of antibiotic resistance among bacterial populations. This phenomenon not only exacerbates the challenges in treating *E. coli* infections but also raises concerns about the broader implications for the spread of antibiotic resistance.

The clinical significance of biofilm-producing *E. coli* is particularly evident in urinary tract infections (UTIs), where *E. coli* is a prevalent causative agent. Biofilm formation by *E. coli* in the urinary tract can lead to persistent and recurrent infections. The protective biofilm matrix shields the bacteria from host defences and antibiotics, making these infections challenging to eradicate completely. The recurrent nature

of UTIs associated with biofilm-producing *E. coli* highlights the need for tailored therapeutic approaches that address the unique challenges posed by biofilm-associated antibiotic resistance.

In addressing the complex issue of antibiotic resistance in *E. coli* biofilms, research has played a crucial role in unravelling the underlying mechanisms and exploring potential avenues for intervention. Pioneering work [18] has provided foundational insights into bacterial biofilms, emphasizing their role as a common cause of persistent infections. Additionally, the contributions [19] have shed light on the survival mechanisms of clinically relevant microorganisms within biofilms, shaping our understanding of the challenges posed by these microbial communities.

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