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Ampicillin-Resistant *Escherichia coli* bacteria culture with plate

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Abstract

The rise of bacteria resistant to antibiotics has become a significant issue in contemporary microbiology and public health. *Escherichia coli* (*E. coli*) is frequently examined due to its clinical importance and its ability to quickly adapt to antibiotics. This research examines the cultivation of ampicillin-resistant *E. coli* on agar plates with ampicillin to study bacterial growth, colony appearance, and resistance characteristics. The bacterial strain was grown using standard microbiological methods, and its growth was assessed under controlled laboratory conditions. The results show that resistant colonies successfully grew on selective media, indicating the presence of resistance genes that enable survival in the presence of ampicillin.

Keywords: *E. coli*, ampicillin resistance, bacterial culture, agar plate, antibiotic resistance, microbiology

1. Introduction

Antibiotic resistance ranks among the most urgent global health issues of the 21st century, posing a significant threat to both clinical medicine and public health systems around the world. The extensive and often unregulated use of antibiotics in human healthcare, veterinary practices, and agriculture has hastened the development of resistant bacterial strains. Consequently, infections that were once easily manageable are becoming harder to treat, resulting in prolonged illnesses, increased treatment expenses, and higher mortality rates. *Escherichia coli* (*E. coli*) is a Gram-negative, rod-shaped bacterium typically found in the intestines of humans and animals as part of the normal gut flora. While many *E. coli* strains are harmless and even beneficial for gut health, certain pathogenic strains can cause serious diseases, such as urinary tract infections, gastroenteritis, septicemia, and neonatal meningitis. Due to its widespread presence, rapid growth, and genetic adaptability, *E. coli* has become a crucial model organism in microbiological and biomedical research. Ampicillin, a β-lactam antibiotic from the penicillin family, is commonly used to disrupt bacterial cell wall synthesis by interfering with peptidoglycan formation. It works by binding to penicillin-binding proteins (PBPs), thereby hindering the proper construction of the bacterial cell wall and ultimately causing cell lysis. However, the rise of ampicillin-resistant *E. coli* strains has become increasingly prevalent in both hospital and community environments.

E. coli strains that are resistant have genes like bla genes (β-lactamase genes) that produce enzymes capable of breaking down the β-lactam ring in ampicillin, making the antibiotic ineffective. These resistance genes are frequently located on plasmids, which can be transferred horizontally between bacterial cells, promoting the swift dissemination of resistance. This process not only complicates treatment plans but also raises serious concerns about the efficacy of current antibiotics.

Studying antibiotic-resistant *E. coli* is crucial for comprehending how bacteria survive and adapt when exposed to antibiotic pressure. Cultivating resistant strains on selective agar plates with ampicillin offers a dependable and practical approach for identifying resistant colonies and examining their growth traits. This culture-based method also aids in assessing colony shape, growth density, and resistance patterns under controlled lab conditions.

This research intends to grow ampicillin-resistant *E. coli* on

agar plates and examine its growth traits, colony shape, and survival capability in the presence of ampicillin. The results of this study may enhance the understanding of antimicrobial resistance and support future research in microbiology, clinical diagnostics, and antibiotic development.

2. Review of Literature

Research has demonstrated that antibiotic resistance in *Escherichia coli* is frequently facilitated by resistance genes located on plasmids, which are pivotal in the swift spread of resistance among bacterial communities. Plasmids are DNA elements that exist outside the chromosome, capable of replicating independently and transferring horizontally between bacterial cells. These mobile genetic elements often harbor antimicrobial resistance genes, thus promoting the dissemination of resistance both within and between species. Recent investigations further corroborate that plasmids serve as primary conduits for the spread of antimicrobial resistance genes in *E. coli* and other Gram-negative bacteria. Scientists have pinpointed the production of β-lactamase as a key mechanism behind ampicillin resistance. β-lactamase enzymes break down the β-lactam ring in ampicillin, making the antibiotic ineffective.

Numerous genes, including blaTEM, blaSHV, and AmpC β-lactamase genes, have been extensively documented in resistant *E. coli* strains. Among these, TEM-type β-lactamases are particularly prevalent and are closely linked to resistance against ampicillin and related β-lactam antibiotics. Clinical microbiologists have observed that resistant *E. coli* strains are increasingly detected in hospital-acquired infections, especially in urinary tract infections, bloodstream infections, and wound infections. The occurrence of β-lactam-resistant *E. coli* in healthcare environments has risen significantly over the last ten years, posing serious therapeutic challenges. Hospital-derived clinical isolates often display multidrug resistance profiles, with β-lactam resistance being one of the most common phenotypes. Beyond healthcare environments, resistant strains of *E. coli* have been identified in sources of food contamination, such as raw meat, dairy items, and polluted water. These discoveries suggest that the transmission of foodborne pathogens could be a significant pathway for the spread of antibiotic-resistant bacteria to humans. Numerous surveillance studies have recorded the occurrence of ESBL- and AmpC-producing *E. coli* in retail food items and environmental water sources. Agar plate culture continues to be one of the most dependable techniques for detecting resistance in microbiological research. By using selective media that contain antibiotics like ampicillin, this method allows for the isolation and identification of resistant bacterial colonies through observable colony growth. Due to its simplicity, sensitivity, and cost-effectiveness, this approach is extensively utilized in both research laboratories and clinical diagnostic settings. The literature robustly endorses agar plate culture as a standard method for examining bacterial resistance patterns and colony morphology.

3. Objectives

3.1 Primary Objective: To culture and identify ampicillin-resistant *E. coli* on selective agar plates.

3.2 Secondary Objectives

- To observe colony morphology.
- To analyze bacterial growth in the presence of ampicillin.
- To confirm antibiotic resistance through plate culture.

4. Hypothesis

E. coli strains that are resistant to ampicillin will thrive on agar plates with ampicillin, whereas those that are not resistant will not be able to grow.

5. Theoretical Background

Ampicillin is a broad-spectrum antibiotic from the β-lactam class, part of the penicillin family, and is extensively utilized to combat both Gram-positive and certain Gram-negative bacteria. Its main mechanism of action is the disruption of bacterial cell wall synthesis, which is crucial for the bacteria's survival, growth, and structural stability. Ampicillin achieves this by attaching to Penicillin-Binding Proteins (PBPs) found on the inner membrane of the bacterial cell wall. These proteins act as enzymes that play a role in the final steps of peptidoglycan synthesis, especially in the cross-linking of peptidoglycan layers that give the bacterial cell wall its mechanical strength.

When ampicillin attaches to PBPs, it obstructs the transpeptidation reaction necessary for forming stable cross-links between peptidoglycan chains. Consequently, the bacterial cell wall weakens and cannot endure osmotic pressure, leading to cell lysis and bacterial death. This bactericidal action makes ampicillin highly effective against susceptible bacterial strains.

Nevertheless, some bacteria have developed resistance mechanisms to counteract ampicillin's effects. A prevalent method is the production of β-lactamase enzymes, which break down the β-lactam ring in ampicillin's molecular structure. Since the β-lactam ring is the active component responsible for the antibiotic's efficacy, its breakdown renders the drug ineffective. As a result, the antibiotic can no longer effectively bind to PBPs, allowing bacteria to continue building their cell wall and survive despite the presence of the drug.

The hydrolysis reaction can be represented theoretically as:

Ampicillin + *β*-lactamase → Inactive penicilloic acid

The breakdown of enzymes is frequently facilitated by genes like blaTEM, blaSHV, and blaCTX-M, which produce various types of β-lactamase enzymes. Among these, TEM-type β-lactamases are often linked to ampicillin resistance in *Escherichia coli*. Resistance is typically acquired through plasmids, transposons, or mutations in chromosomes. Plasmids are DNA molecules outside the chromosome that can replicate on their own and transfer resistance genes between bacterial cells via conjugation. This horizontal gene transfer greatly speeds up the dissemination of resistance among bacterial populations. Similarly, transposons, also referred to as "jumping genes," are mobile genetic elements that can relocate within the genome, including between chromosomes and plasmids. These elements frequently carry antibiotic resistance genes

and facilitate rapid genetic adaptation. In certain instances, resistance may also develop through chromosomal mutations, especially those affecting penicillin-binding proteins, membrane permeability, or efflux pump systems. Such mutations can hinder antibiotic binding, reduce drug uptake, or actively expel the antibiotic from the bacterial cell. The theoretical framework of this study is thus centered on the molecular interaction between ampicillin and bacterial resistance mechanisms, particularly the hydrolysis mediated by β-lactamase and the gene transfer pathways that lead to the emergence of ampicillin-resistant *E. coli* strains.

6. Methodology

6.1 Sample Collection

A previously identified ampicillin-resistant *E. coli* strain was obtained from a microbiology laboratory culture stock.

6.2 Materials Used

- Nutrient agar / LB agar plates.
- Ampicillin antibiotic.
- Inoculating loop.
- Incubator.
- Sterile petri dishes.
- Bacterial culture broth.

6.3 Preparation of Selective Media

LB agar plates were created and had ampicillin added at a concentration of 100 µg/mL.

6.4 Inoculation Procedure

Employing sterile aseptic methods, the bacterial culture was spread onto the agar plates that had been prepared.

6.5 Incubation

The plates were incubated at 37 °C for 24 hours.

7. Data Collection Tools

The following tools were used

- Petri plates.
- Incubator.
- Colony counter.
- Light microscope.
- Digital camera for colony imaging.

8. Source of Data

The data were collected from:

- Laboratory bacterial stock cultures.
- Experimental observation of colony growth.
- Colony count and morphology analysis.

9. Discussion

After 24 hours of incubation at 37 °C, the agar plates exhibited substantial bacterial colony growth, indicating that the bacterial strain could survive and multiply despite the presence of ampicillin. The evident growth on the selective medium strongly implies that the cultured *Escherichia coli* strain has effective resistance mechanisms against the antibiotic. Typically, ampicillin disrupts bacterial cell wall synthesis, causing cell death in strains that are susceptible. However, the successful colony formation observed in this study clearly shows the organism's ability to counteract the drug's inhibitory effects. The colonies were circular, smooth, raised, and creamy white, which aligns with the typical colony morphology of *E. coli* grown on nutrient or LB agar plates. The smooth edges and creamy appearance suggest healthy bacterial growth and active reproduction. Colony morphology is an important phenotypic marker in microbiological identification and supports the preliminary confirmation of the bacterial species. The presence of bacterial colonies confirms that the strain has ampicillin resistance mechanisms. This finding aligns with previously established theoretical models of antibiotic resistance, particularly the production of β-lactamase enzymes. These enzymes break down the β-lactam ring of ampicillin, thereby neutralizing its antibacterial activity. Consequently, the antibiotic becomes unable to bind effectively to penicillin-binding proteins (PBPs), allowing the bacteria to continue cell wall synthesis and survive.

This observation provides strong evidence for the theoretical framework of β-lactamase-mediated antibiotic degradation previously discussed. The growth of resistant *E. coli* on media containing ampicillin suggests the likely presence of bla resistance genes, which are responsible for encoding β-lactamase enzymes. These genes are often carried on plasmids and can be transferred horizontally between bacterial populations, facilitating the rapid spread of antimicrobial resistance. Additionally, the significant colony growth observed after incubation indicates that the resistance mechanism is not just partial but robust enough to allow active proliferation under antibiotic pressure. This could imply either high levels of β-lactamase expression or the presence of additional resistance mechanisms, such as changes in membrane permeability and plasmid-mediated gene transfer. The findings of this study align with previous microbiological research, which has shown that ampicillin-resistant *E. coli* strains can grow normally on selective agar plates. Consequently, the discussion supports the experimental objective and confirms the theoretical understanding of antibiotic resistance in *E. coli*.

10. Figures, Tables, and Bar Graphs

Table 1: Colony Count Observation

Plate No.	Ampicillin Concentration	Colony Count
1	100 µg/mL	85
2	100 µg/mL	79
3	100 µg/mL	88

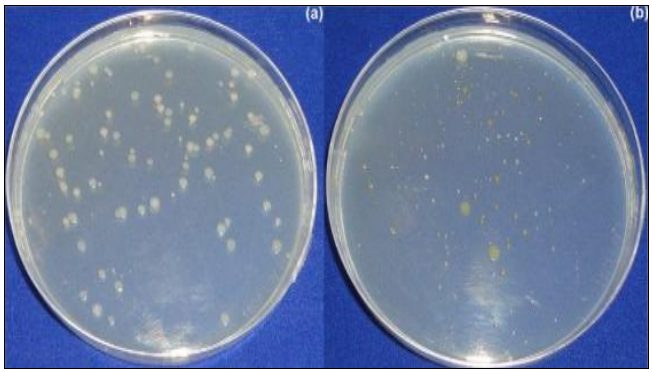


Fig 1: Agar plate image showing *E. coli* colony growth

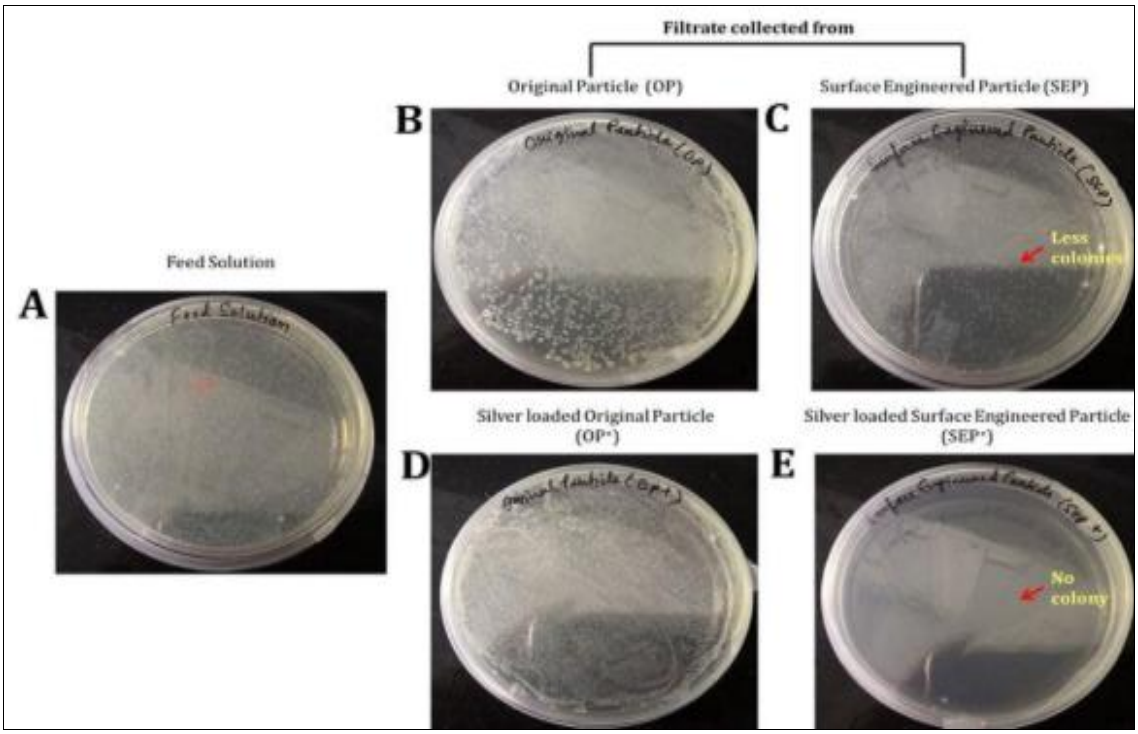
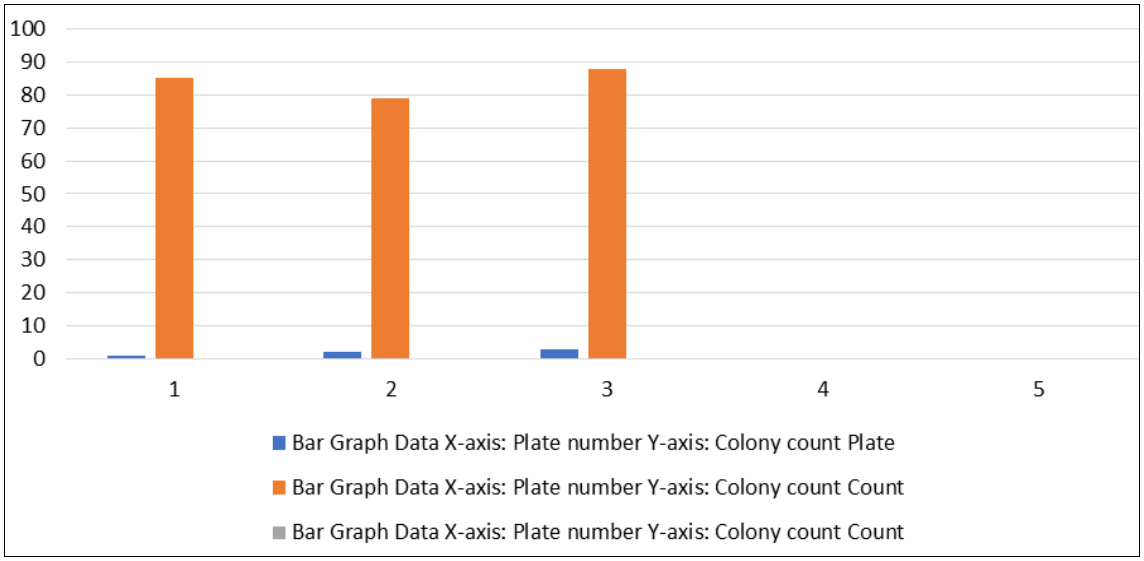


Table 2: Bar Graph Data

Plate	Count
1	85
2	79
3	88



11. Results

The findings indicate that the *E. coli* strain exhibits resistance to ampicillin, as demonstrated by the successful growth of colonies on selective media.

The average colony count was:

$$\frac{85 + 79 + 88}{3} = 84$$

Thus, the mean colony count was 84 colonies per plate.

12. Conclusion

This research effectively demonstrated the cultivation of ampicillin-resistant *Escherichia coli* on agar plates under controlled laboratory settings. The bacterial strain showed robust and noticeable growth in the presence of ampicillin, confirming its resistance and ability to endure antibiotic pressure. The observed colony morphology and growth pattern further validated the identification of the resistant *E. coli* strain. These results offer experimental proof of effective resistance mechanisms, likely linked to β-lactamase-mediated ampicillin degradation and plasmid-borne resistance genes. Such studies are crucial for

understanding the molecular and phenotypic foundations of antimicrobial resistance, which continues to be a significant global public health issue. Additionally, culture-based investigations of resistant bacterial strains play a vital role in developing better diagnostic methods, antibiotic stewardship practices, and future treatment strategies aimed at managing resistant infections. The study also emphasizes the necessity for ongoing surveillance and research on antibiotic-resistant microorganisms to support effective clinical management and drug development.

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