Evaluating the Antibacterial Potency of Erythromycin Against *Escherichia coli*: Minimum Inhibitory Concentration and Zone of Inhibition Analysis

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***Abstract*—Antimicrobial susceptibility testing is essential for evaluating the effectiveness of antibiotics against bacterial pathogens. The present study investigated the in vitro antibacterial activity of erythromycin against *Escherichia coli*, a Gram-negative bacterium, using broth dilution and agar well diffusion assays. A laboratory strain of *E. coli* was cultured and characterized based on its colony morphology and Gram-staining properties. Erythromycin susceptibility was evaluated at concentrations of 2.5, 5, 10, and 20 mg/mL. In the broth dilution assay, visible bacterial growth was observed at 2.5 and 5 mg/mL, whereas no visible turbidity was observed at 10 and 20 mg/mL. Accordingly, the minimum inhibitory concentration (MIC) was determined as 10 mg/mL, representing the lowest concentration that completely inhibited visible growth. Agar well diffusion analysis further demonstrated inhibitory activity of erythromycin against *E. coli*, as evidenced by zones of inhibition surrounding the antibiotic-containing wells. The findings demonstrate that erythromycin possesses measurable in vitro antibacterial activity against the tested *E. coli* strain; however, the relatively high concentration required for growth inhibition suggests limited susceptibility under the experimental conditions. These findings highlight the importance of quantitative susceptibility assessment when evaluating antibiotic activity against Gram-negative bacteria.**

***Index Terms*—Escherichia coli, erythromycin, antimicrobial susceptibility, minimum inhibitory concentration, agar well diffusion, zone of inhibition.**

I. INTRODUCTION

Antimicrobial resistance (AMR) is currently recognized as one of the most serious threats to global public health, affecting healthcare systems, food safety, and economic stability worldwide. The rapid emergence and spread of resistant bacterial strains have significantly reduced the effectiveness of many commonly used antibiotics, making bacterial infections increasingly difficult to treat (World Health Organization [WHO], 2023). The excessive and inappropriate use of antibiotics in human medicine, veterinary applications, and agriculture has accelerated the development of resistant microorganisms, while the discovery and development of new antibiotics have declined considerably over recent decades (Ventola, 2015). As a result, the world is facing a growing crisis where previously manageable infections are becoming more severe and difficult to control.

The COVID-19 pandemic further highlighted the importance of effective antimicrobial therapies and the urgent need to address microbial adaptability and mutation. During the pandemic, increased antibiotic usage and secondary bacterial infections contributed to concerns regarding the acceleration of AMR worldwide. Similar to viral mutation and adaptation, bacteria continuously evolve survival strategies that enable them to resist antibiotic action. These adaptations include enzymatic degradation of antibiotics, modification of target sites, reduced membrane permeability, and activation of multidrug efflux systems, all of which contribute to treatment failure and increased mortality rates (Munita & Arias, 2016).

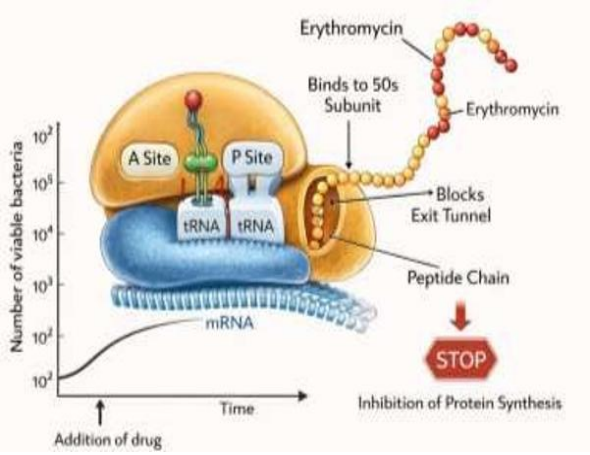

Fig 1: Mechanism of action of erythromycin on bacterial ribosome

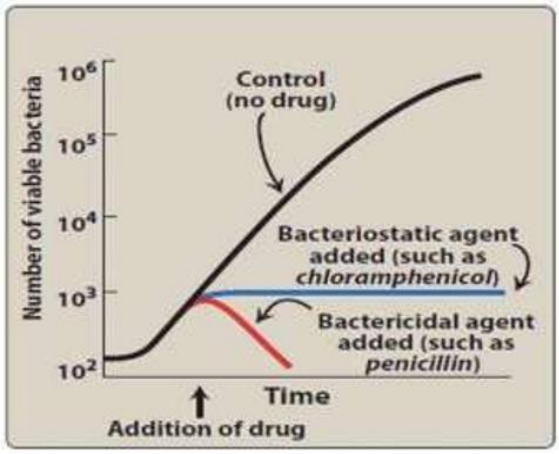

Fig 2: Graph showing bacteriostatic as well as bacteriocidal action

Among resistant bacterial pathogens, Gram-negative bacteria such as Escherichia coli are of particular concern because of their complex cell-envelope structure and efficient resistance mechanisms. E. coli is a widely distributed bacterium commonly found in the intestinal tract of humans and animals; however, pathogenic strains are capable of causing severe gastrointestinal, urinary tract, respiratory, and bloodstream infections (Kaper et al., 2004). The outer membrane of Gram-negative bacteria acts as an effective permeability barrier that limits the penetration of many antimicrobial agents, thereby reducing antibiotic susceptibility. In addition, Gram-negative bacteria possess efflux pumps and plasmid-mediated resistance genes that further enhance their ability to survive antimicrobial exposure (Delcour, 2009).

Erythromycin is a macrolide bacteriostatic antibiotic that has been extensively used in the treatment of various bacterial infections. Structurally, erythromycin contains a large lactone ring attached to sugar moieties that facilitate its interaction with bacterial ribosomes. Its primary mechanism of action involves binding to the 50S ribosomal subunit, where it inhibits bacterial protein synthesis by preventing elongation of the polypeptide chain (Kohanski et al., 2010). By interfering with translation, erythromycin suppresses bacterial growth and reproduction rather than directly killing bacterial cells, which classifies it as a bacteriostatic antibiotic.

Despite its effectiveness against many Gram-positive bacteria, erythromycin exhibits limited activity against Gram-negative organisms such as E. coli. This reduced efficacy is mainly due to the presence of an outer membrane enriched with lipopolysaccharides that restrict the entry of large hydrophobic
molecules like erythromycin. Furthermore, narrow porin channels reduce antibiotic penetration, while active efflux systems expel the antibiotic before it reaches its ribosomal target (Li et al., 2015). Resistance may also arise through ribosomal target modification mediated by plasmid-encoded genes, which further decreases antibiotic binding efficiency. These combined resistance mechanisms make Gram-negative bacteria particularly difficult to treat and emphasize the need for continuous antimicrobial evaluation.

Evaluation of antibiotic efficacy is essential for understanding bacterial susceptibility patterns and guiding effective treatment strategies. One of the most widely used methods for antimicrobial susceptibility testing is the determination of Minimum Inhibitory Concentration (MIC), which is defined as the lowest concentration of an antibiotic capable of inhibiting visible bacterial growth after incubation (Andrews, 2001). MIC determination provides quantitative information regarding the potency of an antimicrobial agent and helps predict appropriate therapeutic dosage levels. Accurate MIC analysis is important in both clinical microbiology and antimicrobial research.

In addition to MIC determination, agar diffusion techniques such as the disc diffusion or zone of inhibition assay are commonly used to evaluate antibacterial activity. These methods are simple, cost-effective, and visually demonstrative, making them particularly suitable for educational and laboratory-based investigations. The size of the inhibition zone produced around an antibiotic disc reflects the susceptibility of the bacterial strain and provides comparative insight into antibiotic effectiveness (Balouiri et al., 2016). Combining MIC analysis with agar diffusion studies offers a more comprehensive understanding of antibiotic potency and bacterial response.

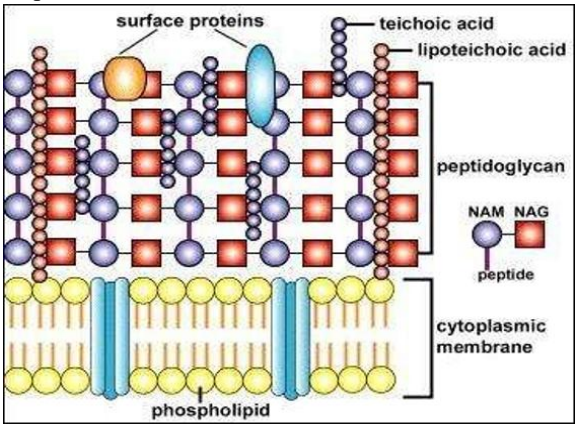

Fig 3: Illustration of gram negative cell envelope structure

With the increasing prevalence of antimicrobial resistance and the reduced susceptibility of Gram-negative bacteria to macrolide antibiotics, further investigation into erythromycin's antibacterial activity remains important. Understanding the inhibitory potential of erythromycin against E. coli may contribute to broader antimicrobial susceptibility studies and support awareness regarding AMR mechanisms and antibiotic effectiveness. Therefore, the present study aims to evaluate the antibacterial potency of erythromycin against Gram-negative Escherichia coli through Minimum Inhibitory Concentration (MIC) determination and agar diffusion-based susceptibility analysis.2. Materials and Methods

2.1. Study Design

The present study was conducted as an in vitro experimental investigation to evaluate the antibacterial potency of erythromycin against Gram-negative Escherichia coli using Minimum Inhibitory Concentration (MIC) determination and agar diffusion-based susceptibility analysis. The experimental design was developed to assess the inhibitory activity of erythromycin through standardized microbiological techniques under controlled laboratory conditions. The study primarily focused on determining the susceptibility pattern of E. coli toward varying concentrations of erythromycin and evaluating the correlation between MIC values and inhibition zone diameters obtained through agar diffusion assays.

2.2. Bacterial Strain and Culture Conditions

A laboratory strain of Escherichia coli was selected as the test organism due to its clinical relevance and increasing involvement in antimicrobial resistance-associated infections. The bacterial culture was maintained on nutrient agar slants at 4 degrees Celsius and subcultured periodically to preserve viability and purity throughout the duration of the experiment. Prior to experimentation, isolated colonies from freshly cultured nutrient agar plates were inoculated into sterile nutrient broth and incubated at 37 degrees Celsius for 18 to 24 hours to obtain actively growing bacterial cultures suitable for antimicrobial susceptibility testing.

The bacterial suspension used for inoculation was standardized corresponding to an approximate bacterial density of 1.5 x 10 to the 8th power CFU per milliliter. Standardization of the inoculum was performed to ensure consistency and reproducibility in antimicrobial susceptibility measurements, as variations in bacterial concentration may significantly influence MIC values and diffusion assay outcomes (Andrews, 2001).

2.3. Preparation of Media and Reagents

Nutrient agar and nutrient broth were prepared and sterilized by autoclaving at 121 degrees Celsius under 15 psi pressure for 15 to 20 minutes. Nutrient agar plates were selected for antimicrobial susceptibility testing because of its reproducible batch composition, satisfactory diffusion characteristics, and recommendation by the Clinical and Laboratory Standards Institute (CLSI) for antibiotic sensitivity assays (CLSI, 2021). Following sterilization, the media were allowed to cool to approximately 45 to 50 degrees Celsius before being aseptically poured

into sterile Petri plates within a laminar airflow chamber to minimize contamination.

Erythromycin used in the study was obtained in standard antibiotic form and prepared in sterile distilled water to obtain different working concentrations for MIC analysis and agar diffusion assays. All glassware, pipettes, and culture instruments used during experimentation were sterilized prior to use to maintain aseptic conditions throughout the study.

2.4. Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration of erythromycin against E. coli was determined using the broth dilution method following standard antimicrobial susceptibility testing guidelines. Serial dilutions of erythromycin were prepared in sterile nutrient broth to obtain a range of antibiotic concentration. Equal volumes of standardized bacterial inoculum were introduced into each dilution tube under sterile conditions. Positive control tubes containing bacterial inoculum without antibiotic and negative control tubes containing sterile broth without bacterial inoculation were included to validate experimental observations.

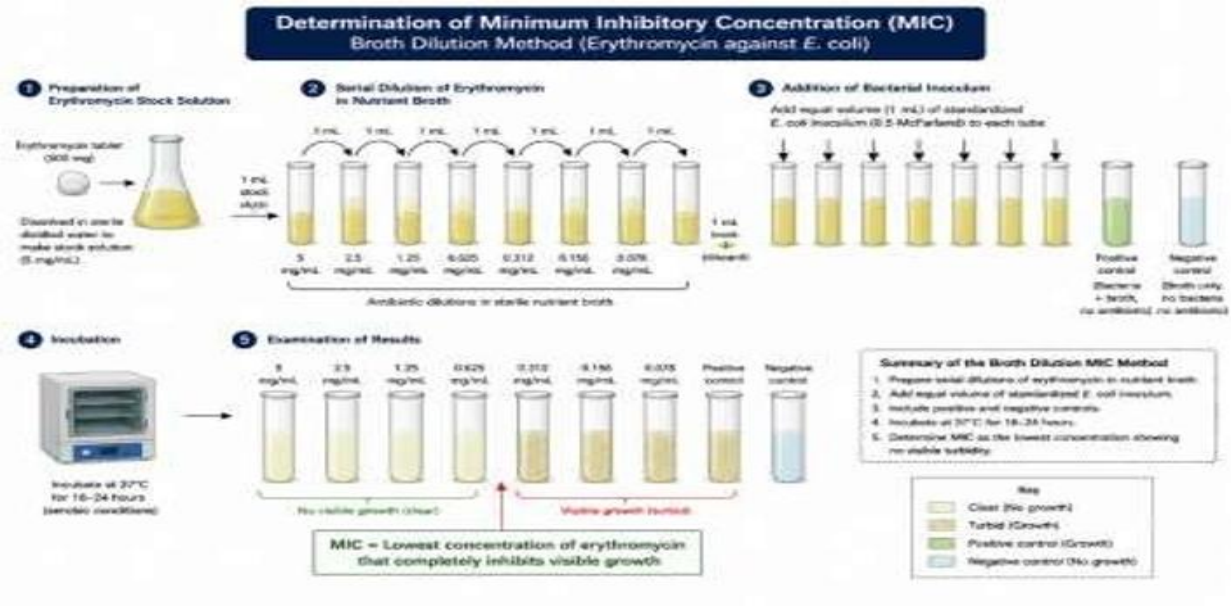

Fig. 4: Determination of the Minimum Inhibitory Concentration (MIC) of Erythromycin against Escherichia coli using the broth dilution method, illustrating serial antibiotic dilution, bacterial inoculation, incubation, and turbidity-based assessment of bacterial growth inhibition.

The inoculated tubes were incubated at 37 degrees Celsius for 18 to 24 hours under aerobic conditions. Following incubation, the tubes were visually examined for bacterial growth based on turbidity formation. The lowest concentration of erythromycin that completely inhibited visible bacterial growth was recorded as the Minimum Inhibitory Concentration (MIC). MIC determination is widely recognized as one of the most reliable quantitative methods for evaluating bacterial susceptibility toward antimicrobial agents and is frequently employed in clinical and microbiological research to guide therapeutic strategies (Andrews, 2001).

2.5. Agar Diffusion Assay

The antibacterial activity of erythromycin was further evaluated using the agar well diffusion method on nutrient agar plates. Sterile agar plates were inoculated uniformly with standardized E. coli suspension using sterile cotton swabs to achieve confluent bacterial growth across the agar surface. Wells of uniform diameter were aseptically created in the agar medium using the tip of a pipette. Different

concentrations of erythromycin solution were carefully dispensed into the wells, ensuring equal volume distribution among all experimental setups.

The inoculated plates were allowed to remain undisturbed for approximately 15 to 20 minutes at room temperature to facilitate radial diffusion of the antibiotic into the agar matrix prior to incubation. Subsequently, the plates were incubated at 37 degrees Celsius for 24 hours under aerobic conditions. Following incubation, clear zones surrounding the wells were observed, indicating inhibition of bacterial growth by erythromycin. The diameters of the zones of inhibition were measured in millimeters, and the antibacterial efficacy of erythromycin was assessed based on the size of the inhibitory zones produced.

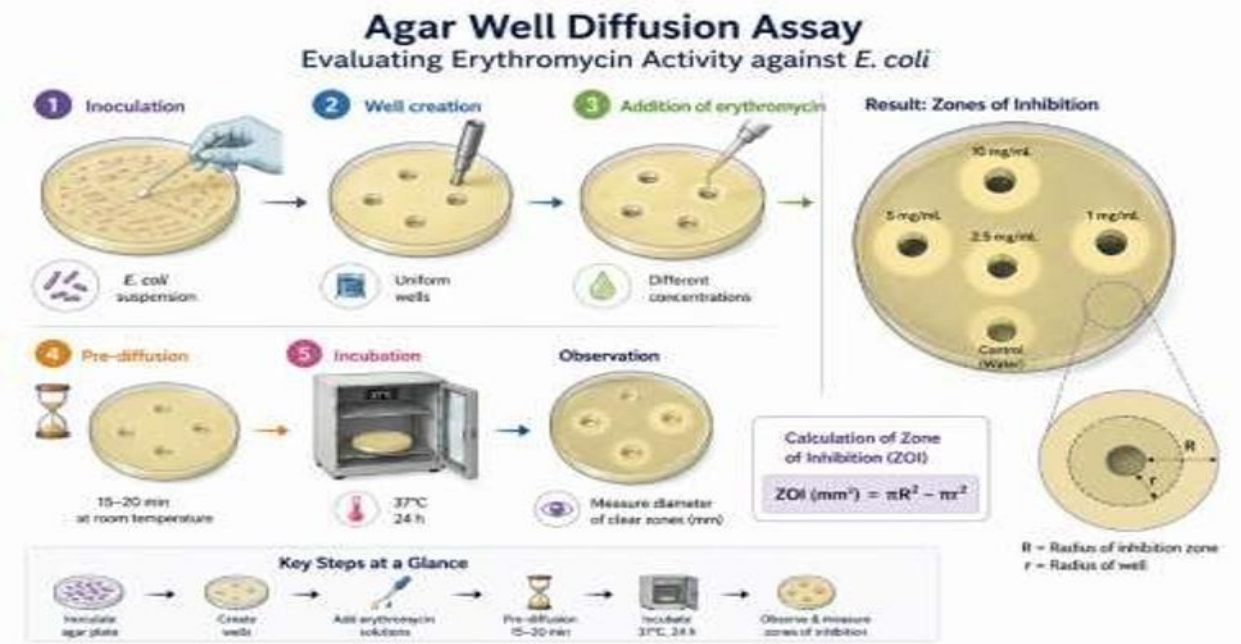

Fig. 5. Illustration of the agar well diffusion assay for evaluating the antibacterial activity of erythromycin against *Escherichia coli*. Antibacterial efficacy was determined by measuring the zone of inhibition formed around wells containing different erythromycin concentrations.

The agar diffusion method is widely utilized in antimicrobial susceptibility studies due to its simplicity, reproducibility, and effectiveness in evaluating the relative potency of antimicrobial agents against bacterial pathogens (Balouiri et al., 2016). The method additionally provides visual confirmation of antibacterial activity, making it suitable for comparative analysis of different antibiotic concentrations.

2.6. Data Analysis

All experimental procedures were performed in triplicate to ensure reliability, reproducibility, and statistical validity of the obtained results. The observed MIC values and zone of inhibition diameters were recorded systematically, and the mean values were calculated along with standard deviation. The antibacterial activity of erythromycin was analyzed descriptively by comparing inhibition zone diameters across varying concentrations. Graphical representations were prepared to illustrate the relationship between erythromycin concentration and bacterial susceptibility.

III. RESULTS AND DISCUSSION

3.1. Isolation and Identification of Escherichia coli

The bacterial isolate was successfully cultured and identified as Escherichia coli based on its characteristic colony morphology and Gram-staining properties. Microscopic examination of the Gram-stained smear revealed pink-colored, short-rod shaped (bacillary) cells, confirming the isolate as a Gram-negative bacterium, which is consistent with the typical morphological characteristics of E. coli (Kaper et al., 2004). The confirmed E. coli culture

was subsequently used for Minimum Inhibitory Concentration (MIC) determination and agar well diffusion assays to evaluate the antibacterial activity of erythromycin.

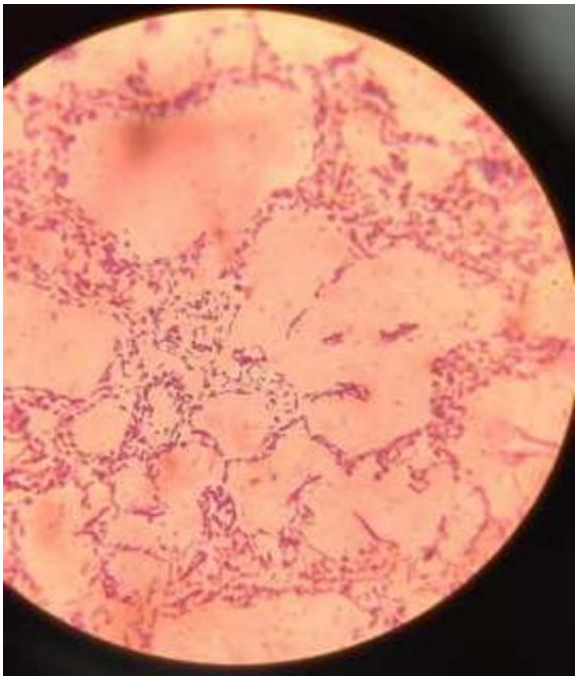

Fig 6: Microscopic image of Gram-stained *Escherichia coli* demonstrating characteristic pink-stained Gram-negative bacilli.

3.2. Minimum Inhibitory Concentration (MIC) Analysis

The antibacterial activity of erythromycin against *Escherichia coli* was evaluated through determination of the Minimum Inhibitory Concentration (MIC) using the broth dilution method. Following incubation, visible turbidity was observed in tubes containing lower concentrations of erythromycin, indicating active bacterial growth, whereas tubes containing higher concentrations showed complete inhibition of visible growth. The MIC of erythromycin against *E. coli* was determined to be 10 mg/ml, as 10 mg/ml was the lowest concentration at which no visible bacterial growth was observed.

The obtained MIC value suggests that erythromycin exhibited measurable inhibitory activity against the tested Gram-negative bacterial strain, although susceptibility was comparatively lower than typically observed in Gram-positive bacteria. The reduced susceptibility of *E. coli* toward erythromycin may be attributed to the structural complexity of the Gram-negative bacterial envelope, particularly the presence of an outer membrane enriched with lipopolysaccharides that restrict antibiotic penetration (Delcour, 2009). In addition, efflux pump systems and porin-associated permeability barriers likely contributed to the observed resistance pattern.

The findings of the present study are consistent with previous reports demonstrating that macrolide antibiotics exhibit limited activity against Gram-negative enteric bacteria due to intrinsic resistance mechanisms (Li et al., 2015). However, inhibitory effects observed at higher erythromycin concentrations indicate that the antibiotic still retains partial antibacterial activity under controlled in vitro conditions. The MIC results therefore provide important insight into bacterial susceptibility and may contribute to understanding the potential limitations of erythromycin when used against Gram-negative pathogens.

3.3. Agar Diffusion Assay

The antibacterial efficacy of erythromycin was further investigated using the agar-well diffusion assay. Clear zones of inhibition surrounding the wells containing erythromycin confirmed the inhibitory effect of the antibiotic against *E. coli*. The measured diameters of inhibition zones increased proportionally with increasing erythromycin concentration, indicating a concentration-dependent antibacterial response.

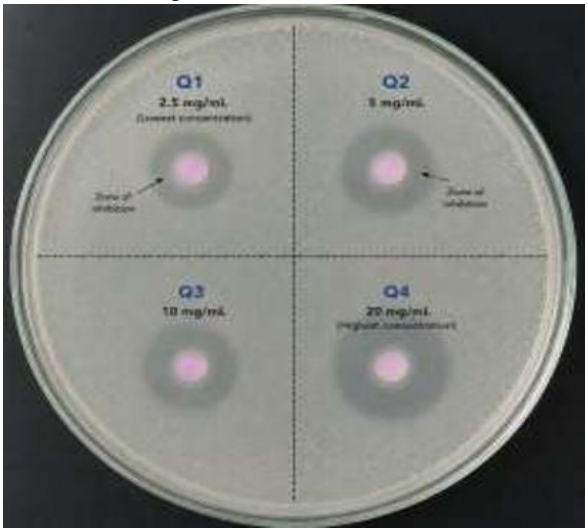

Fig 7: Agar well diffusion assay showing zones of inhibition produced by different concentrations of erythromycin (2.5–20 mg/mL) against *Escherichia coli*.

The recorded inhibition zone diameters are presented in Table 1.

Table 1. Zone of inhibition produced by different concentrations of erythromycin against *Escherichia coli*

Erythromycin Concentration	Zone of Inhibition (cm)
2.5 mg/ml	16.41
5.0 mg/ml	12.63
10 mg/ml	20.63
20 mg/ml	25.29

Table 2. Optical density (OD_{600}) of Escherichia coli cultures treated with different concentrations of Curcuma longa and Azadirachta indica extracts.

Sample	Concentration	OD600
Negative control	—	0.05
Positive control	—	1.10
Curcuma longa extract	25%	0.95
Curcuma longa extract	50%	0.60
Curcuma longa extract	75%	0.28
Curcuma longa extract	100%	0.12
Azadirachta indica extract	25%	1.05
Azadirachta indica extract	50%	0.75
Azadirachta indica extract	75%	0.35
Azadirachta indica extract	100%	0.18

The maximum zone of inhibition observed was 25.29 cm at the concentration of 20 mg/ml, whereas the minimum inhibitory zone recorded was 12.63 cm at the concentration of 5 mg/ml. The presence of

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distinct inhibition zones demonstrates that erythromycin was capable of suppressing bacterial growth despite the inherent resistance characteristics associated with Gram-negative bacteria. Erythromycin exhibited concentration-dependent antibacterial activity against *E. coli*, although relatively high concentrations were required to inhibit bacterial growth.

The observed concentration-dependent increase in inhibition zone diameter aligns with the general principles of antimicrobial susceptibility testing, where higher antibiotic concentrations enhance diffusion and inhibitory interaction with bacterial cells (*Balouiri et al.*, 2016). However, compared with antibiotics specifically targeting Gram-negative organisms, the inhibition zones produced by erythromycin remained relatively moderate, supporting previous findings regarding the limited permeability of Gram-negative bacterial membranes toward macrolide antibiotics.

3.4. Correlation Between MIC and Agar-Diffusion Findings

Comparison of MIC determination and agar-diffusion results demonstrated a direct relationship between erythromycin concentration and antibacterial activity. Lower MIC values corresponded with larger inhibition zones, suggesting that increased antibiotic potency resulted in stronger bacterial growth suppression. Both experimental approaches confirmed that erythromycin possesses detectable antibacterial activity against *E. coli*, although relatively higher concentrations were required to achieve effective inhibition.

The agreement between broth dilution and agar-diffusion findings strengthens the reliability of the present study and supports the effectiveness of combining quantitative and qualitative susceptibility testing methods in antimicrobial evaluation. MIC analysis provided precise information regarding the minimum effective concentration required to inhibit bacterial growth, whereas the agar-diffusion assay visually demonstrated the extent of antibacterial activity.

IV. DISCUSSION

The increasing prevalence of antimicrobial resistance among Gram-negative bacteria has become a major challenge in modern healthcare systems. The present study investigated the antibacterial activity of erythromycin against *Escherichia coli*, a clinically significant Gram-negative pathogen known for its resistance mechanisms and adaptive survival strategies. The results demonstrated that

erythromycin exhibited inhibitory activity against *E. coli*, although relatively higher concentrations were necessary to achieve effective growth suppression.

The comparatively reduced susceptibility observed in this study can be explained by the structural characteristics of Gram-negative bacteria. The outer membrane acts as a selective permeability barrier that restricts the penetration of hydrophobic antibiotics such as erythromycin. Additionally, multidrug efflux pumps actively expel antibiotics from bacterial cells before they can reach their ribosomal targets (*Munita & Arias*, 2016). These combined mechanisms significantly reduce intracellular antibiotic accumulation and contribute to antimicrobial resistance.

Despite these limitations, the measurable inhibitory effects observed in both MIC and agar-diffusion assays indicate that erythromycin retains partial antibacterial potential against *E. coli*. Similar findings have been reported in previous studies investigating macrolide susceptibility among enteric Gram-negative bacteria (*Kohanski et al.*, 2010). The results further highlight the importance of continuous antimicrobial susceptibility monitoring to better understand bacterial resistance trends and optimize therapeutic strategies.

Another significant aspect of the study is the educational and scientific relevance of combining MIC determination with agar-diffusion analysis. While MIC testing provides quantitative assessment of antibiotic potency, agar-diffusion assays offer visual confirmation of bacterial susceptibility, making them particularly useful in microbiological research and academic investigations. The use of both methods together enhances experimental reliability and provides a broader understanding of antimicrobial activity.

The present study also emphasizes the growing need for rational antibiotic use and continued research into antimicrobial resistance mechanisms. Excessive and inappropriate antibiotic consumption remains one of the primary drivers of AMR development worldwide. Therefore, studies evaluating the susceptibility patterns of clinically important bacteria contribute valuable information toward understanding antibiotic effectiveness and promoting responsible antimicrobial usage.

V. CONCLUSION

The findings of the present study demonstrated that erythromycin possesses measurable antibacterial activity against Gram-negative *Escherichia coli*, although its inhibitory effect was moderate compared with its known activity against Gram-positive organisms. Both MIC determination and agar diffusion assays confirmed that increasing concentrations of erythromycin resulted in enhanced bacterial growth inhibition. The reduced susceptibility observed may be associated with the intrinsic resistance mechanisms of Gram-negative bacteria, including outer membrane permeability barriers and antibiotic efflux systems.

Overall, the study highlights the importance of antimicrobial susceptibility testing in evaluating antibiotic effectiveness and understanding bacterial resistance patterns. The combined use of MIC and agar diffusion assays provided reliable and complementary insights into the antibacterial potency of erythromycin against *E. coli*.

VI. FUTURE SCOPE

The present study provides preliminary insight into the antibacterial activity of erythromycin against Gram-negative *Escherichia coli*. Future studies may expand upon this work by evaluating erythromycin against multiple clinically isolated bacterial strains with varying resistance profiles to better understand its spectrum of activity. Additional investigations involving combination antibiotic therapy, synergistic interactions with other antimicrobial agents, and molecular analysis of resistance genes may further contribute to understanding erythromycin resistance mechanisms.

Advanced studies may also incorporate techniques such as time-kill assays, biofilm inhibition studies, and molecular docking approaches to explore the antibiotic's mechanism of action in greater detail. Furthermore, comparative analysis with newer-generation macrolides and alternative antimicrobial compounds could provide valuable information for developing improved therapeutic strategies against antimicrobial-resistant pathogens. The findings of this study may therefore serve as a foundation for future microbiological and antimicrobial resistance research.

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