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ASSESSMENT OF THE RELATIVE EFFECTIVENESS OF MULTIPLE ANTIBIOTICS IN INHIBITING ESCHERICHIA COLI GROWTH

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Abstract

Escherichia coli is a Gram-negative, non-spore-forming facultative anaerobe that predominantly inhabits the gastrointestinal tract of humans and warm-blooded animals. Although typically commensal, certain pathogenic strains are responsible for a wide spectrum of infections in both humans and animals, including poultry. This study aimed to isolate and identify E. coli from broiler chickens exhibiting respiratory symptoms and to evaluate the in vitro antibacterial efficacy of Clarithromycin, Moxifloxacin, Linezolid, and Amoxicillin against the isolates. Samples were collected aseptically from the oral and nasal cavities of symptomatic broilers and cultured on nutrient agar, MacConkey agar, and eosin methylene blue (EMB) agar. Isolates demonstrating characteristic colony morphology were further confirmed via Gram staining and standard biochemical assays, including catalase, citrate, and oxidase tests. The confirmed E. coli isolates were subjected to antibiotic susceptibility testing using the agar well diffusion method, with all antibiotics standardized at a concentration of 0.5 mg/ml and results expressed as zones of inhibition. Among the tested antibiotics, Moxifloxacin demonstrated the highest antibacterial activity with a mean inhibition zone of 35.0 mm, indicating strong efficacy. Amoxicillin showed moderate activity (15.1 mm), whereas Clarithromycin and Linezolid exhibited no inhibitory effect, consistent with the known resistance profile of Gram-negative organisms to these antibiotic classes. The findings highlight the importance of targeted antimicrobial therapy based on susceptibility testing and raise concern over increasing resistance patterns in E. coli isolates from poultry. Implementation of antibiotic stewardship and exploration of alternative therapeutic strategies are recommended to mitigate resistance development.

INTRODUCTION

Escherichia coli (*E. coli*) is a Gram-negative, non-spore-forming facultative anaerobe that primarily resides in the intestines of humans and warm-blooded animals. As a key member of the gut microbiota, which can contain up to 10^{11} cells per gram of intestinal content, *E. coli* is the dominant aerobic organism (Berg, 1996). While commonly used as an indicator of fecal contamination in water and sediment, some strains can persist and even grow in external environments under suitable nutrient and temperature conditions.

Belonging to the Enterobacteriaceae family, *E. coli* functions as both a benign commensal and a significant pathogen. In its commensal role, it aids digestion and synthesizes essential compounds such as vitamin K. However, pathogenic strains are responsible for a wide range of infections, including enteritis, urinary tract infections (UTIs), sepsis, and neonatal meningitis (Kaper et al., 2004). In animals, it also causes diarrhea and other clinical conditions. Some strains can survive in diverse environments, including thermal niches such as hot springs (Franz et al., 2008). Reproducing by binary fission, *E. coli* populations can grow rapidly under optimal conditions (Zwietering et al., 1990). Although they typically constitute about 0.1% of the gut flora (Eckburg et al., 2005), their transmission—mainly fecal-oral—is a major public health concern. While many strains survive poorly outside the host, some environmental isolates exhibit prolonged survival (Thompson, 2007; Ishii et al., 2008). Pathogenic *E. coli* share a general infection process: entry, colonization, immune evasion, proliferation, and host damage (Nataro et al., 1998). Transmission routes include contaminated food, water, and direct contact. For example, EHEC O157:H7 is often transmitted from cattle to humans via undercooked meat or unpasteurized milk, with potential for secondary human-to-human spread. Neonatal infections may originate from the maternal birth canal or immediate postnatal environment (Ragione et al., 2009).

Uropathogenic *E. coli* (UPEC) is the leading cause of UTIs globally, accounting for 70–90% of community-acquired and 40% of hospital-acquired cases (Zdziarski et al., 2008). UTIs are more prevalent in women due to anatomical and behavioral factors, with many experiencing recurrent infections. Risk factors include catheter use, diabetes, sexual activity, and recent antibiotic exposure, while protective factors include hydration and cranberry consumption (Foxman et al., 2003).

A critical challenge in managing *E. coli* infections is the escalating problem of antibiotic resistance. Resistance arises via intrinsic mechanisms like efflux pumps or through the acquisition of resistance genes via mobile genetic elements such as plasmids (Nataro et al., 1998). Inappropriate antibiotic use—such as incomplete treatment courses or agricultural overuse—accelerates the selection of resistant strains. Sub-therapeutic antibiotic exposure promotes survival of partially resistant bacteria, enabling full resistance to emerge. Resistant *E. coli* strains are increasingly prevalent in both clinical and agricultural settings. Diarrheagenic *E. coli* show resistance to 37–63% of commonly used antibiotics, and over 89% are multidrug-resistant (MDR). Similar resistance trends are seen in UPEC isolates, particularly in recurrent cases. MDR *E. coli* bloodstream infections are also on the rise, correlating with increased mortality and prolonged hospital stays (Aslani et al., 2008).

This growing resistance underscores the urgency of antibiotic stewardship and the development of alternative therapies, particularly in low-resource settings where access to advanced antimicrobials and public health infrastructure is limited. Keeping in view all facts the currunt study was designed to evaluate and compare the antibacterial efficacy of Clarithromycin, Moxifloxacin, Linezolid, and Amoxicillin against *Escherichia coli* by measuring their zones of inhibition at a standardized concentration

Materials and Methods

Sample Collection

The study commenced with the collection of samples from naturally infected broiler chickens exhibiting respiratory symptoms in various poultry shops across District Kohat, Khyber Pakhtunkhwa. Using sterile cotton swabs, samples were obtained from the oral and nasal cavities of symptomatic birds. Each swab was wrapped in aluminum foil immediately after sampling to prevent contamination during transport. Samples were promptly delivered to the Microbiology Laboratory at Kohat University of Science and Technology for analysis.

Sterilization Procedures

All glassware, culture media, and instruments used in the experimental procedures were sterilized via autoclaving at 121°C under 15 psi pressure for 15–20 minutes. This step was essential to ensure aseptic conditions and prevent interference by environmental contaminants.

Isolation and Cultivation of Bacteria

Nutrient agar was prepared and autoclaved under standard conditions. The sterile medium was poured into Petri dishes within a laminar flow hood. Once solidified, the collected swab samples were streaked onto the agar surface using the streak plate method. Plates were incubated at 37°C for 24 hours, allowing for the development of discrete bacterial colonies.

Selective Isolation of *Escherichia coli*

Colonies grown on nutrient agar were sub-cultured onto MacConkey agar for selective isolation of *E. coli*. The medium, prepared and sterilized under standard conditions, was poured aseptically. Colonies were streaked using sterile wire loops and incubated at 37°C for 24 hours. Light pink colonies indicated lactose fermentation, suggestive of *E. coli*. These colonies were then transferred to Eosin Methylene Blue (EMB) agar plates for further confirmation. Following 24 hours of incubation at 37°C, the appearance of metallic green sheen colonies confirmed the presence of *E. coli* (Figure-1).

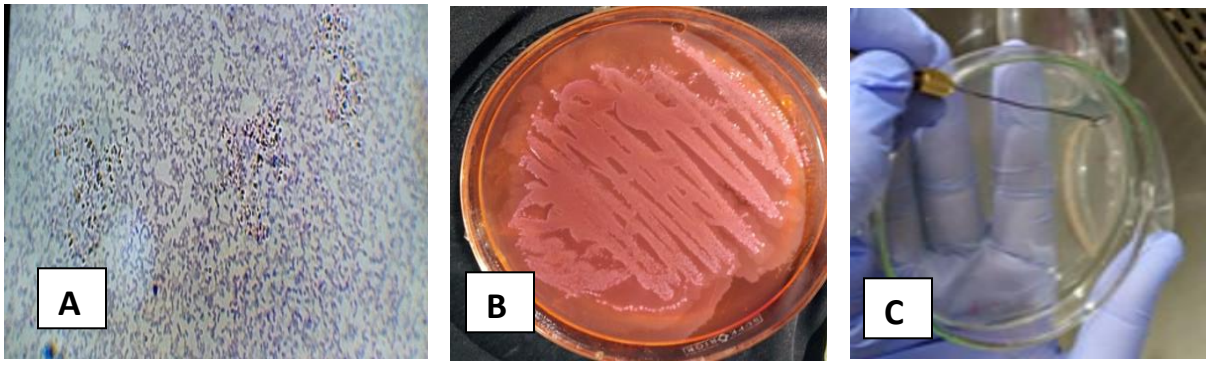


Figure-1: A) Microscopy after Gram Staining, B) *E. coli* colony on Mackonkey Agar, C) Streaking of Bacteria on Agar Plate

Biochemical Identification of *Escherichia coli*

A series of standard biochemical tests were carried out to confirm the identity of the bacterial isolates. Each test was selected based on its relevance to the characteristic metabolic and enzymatic features of *Escherichia coli*.

Gram Staining

Gram staining was used to determine the cell wall characteristics of the isolates. Upon microscopic examination, the stained cells appeared as pink-colored, rod-shaped bacilli. This morphology is typical of Gram-negative bacteria and is consistent with the expected characteristics of *E. coli*.

Citrate Utilization Test

The citrate utilization test was conducted using Simmons' citrate agar slants to assess the ability of the isolates to use citrate as a sole carbon source. After incubation, no bacterial growth or color change was observed, indicating a negative result. This outcome aligns with the known biochemical profile of *E. coli*, which is typically citrate-negative.

Catalase Test

The catalase test was performed by applying hydrogen peroxide to a fresh smear of the bacterial isolate. The rapid formation of oxygen bubbles indicated catalase-positive activity. This reaction confirms the presence of the catalase enzyme, a trait commonly observed in *E. coli* and used to distinguish it from catalase-negative bacteria.

Oxidase Test

The oxidase test was used to determine the presence of cytochrome c oxidase in the bacterial isolates.

Colonies were applied to oxidase reagent–soaked filter paper, and the absence of any color change confirmed a negative result. This finding is characteristic of *E. coli*, which lacks the cytochrome oxidase enzyme.

The table below summarizes the key steps followed for isolating and identifying *Escherichia coli* from respiratory samples of broiler chickens. Each procedure—from sample collection and culturing on selective media to confirmatory biochemical tests—was conducted under sterile conditions to ensure accuracy. Observations such as colony color, morphology, and specific biochemical reactions were used to confirm the presence of *E. coli* (Table-1).

Table-1: Procedural Overview of *E. coli* Isolation and Confirmation

Step	Procedure	Materials Used	Outcome/Observation
Sample Collection	Swabs collected from mouth and nasal cavities of infected broiler chickens	Sterile cotton swabs, aluminum foil	Samples transported to lab aseptically
Sterilization	Autoclaving of all lab materials	Autoclave (121°C, 15 psi, 15–20 min)	Ensured aseptic conditions
Primary Culturing	Swabs streaked on nutrient agar and incubated at 37°C for 24 hours	Nutrient agar, Petri dishes	Bacterial growth observed as distinct colonies
Selective Culturing (MacConkey)	Colonies transferred to MacConkey agar, incubated at 37°C for 24 hours	MacConkey agar	Pink colonies indicating lactose-fermenting *E. coli*
Confirmation on EMB	Colonies streaked on EMB agar, incubated at 37°C for 24 hours	EMB agar	Metallic green sheen colonies confirmed *E. coli*
Gram Staining	Smear prepared, stained with crystal violet, iodine, ethanol, and safranin	Microscope, stains, glass slide	Pink, rod-shaped cells confirmed Gram-negative bacilli
Citrate Test	Simmons' citrate slants inoculated and incubated at 37°C for 24 hours	Simmons' citrate agar	No growth or color change – citrate-negative (confirmed *E. coli*)
Catalase Test	H_2O_2 dropped on bacterial smear	Hydrogen peroxide, glass slide	Bubble formation indicated catalase-positive activity
Oxidase Test	Bacteria applied to oxidase reagent-moistened filter paper	Oxidase reagent, filter paper	No color change indicated oxidase-negative result

Results

Comparative Analysis of Different Antibiotics Against *Escherichia coli*

In this study, a comparative evaluation of the antibacterial efficacy of four different antibiotics—Clarithromycin, Moxifloxacin, Linezolid, and Amoxicillin—was conducted against *Escherichia coli* (*E. coli*), a common Gram-negative bacterium associated with various infections in both humans and animals. Each antibiotic was tested at a uniform concentration of 0.5 mg/ml, and the experiment was performed in triplicates to ensure accuracy and reproducibility of results. The antibacterial activity of each drug was determined by measuring the zone of inhibition, which reflects the extent to which the antibiotic can suppress bacterial growth on an agar plate (Table-2).

Well 1 – Clarithromycin (0.5 mg/ml):

Clarithromycin, a macrolide antibiotic primarily effective against Gram-positive organisms and some Gram-negative respiratory pathogens, showed no zone of inhibition against *E. coli*. This indicates that at the tested concentration, Clarithromycin was ineffective in inhibiting the growth of *E. coli*. This result aligns with existing literature, which suggests that *E. coli*

typically exhibits intrinsic resistance to macrolides due to the permeability barrier posed by the outer membrane of Gram-negative bacteria and the presence of efflux pumps (Figure-2, 3).

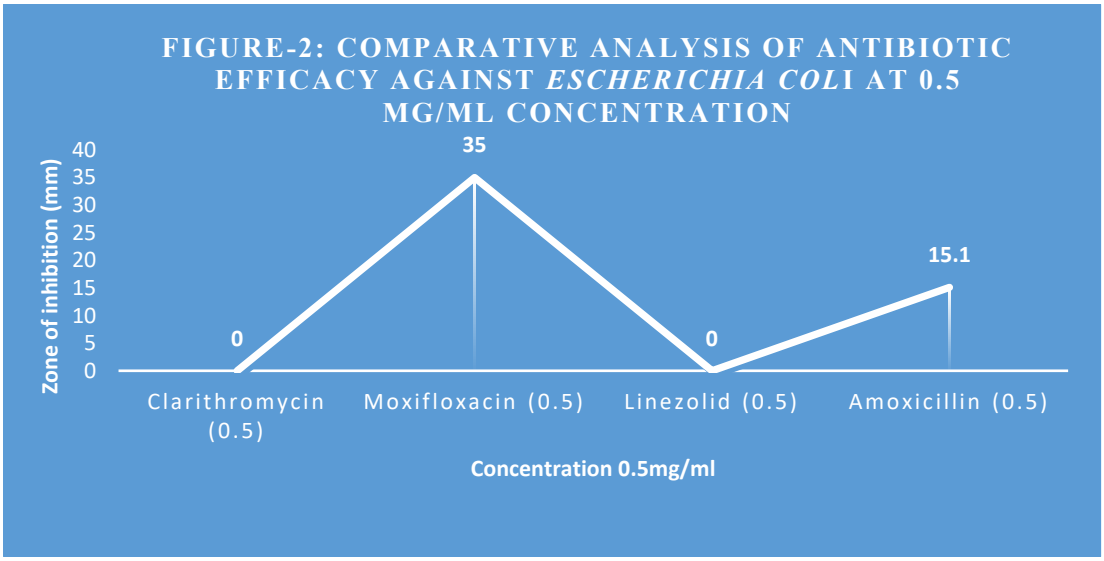
Well 2 – Moxifloxacin (0.5 mg/ml):
Moxifloxacin, a fourth-generation fluoroquinolone, demonstrated the highest antibacterial activity among the four antibiotics tested. It produced an average inhibition zone of 35.0 mm, indicating strong and effective suppression of *E. coli* growth. Fluoroquinolones like Moxifloxacin act by inhibiting bacterial DNA gyrase and topoisomerase IV—enzymes crucial for DNA replication. Due to their broad-spectrum activity and good penetration into Gram-negative bacteria, Moxifloxacin's performance in this experiment was notable and expected (Figure-2, 3).

Well 3 – Linezolid (0.5 mg/ml):
Linezolid, an oxazolidinone-class antibiotic mainly used for treating Gram-positive infections, produced no zone of inhibition against *E. coli*. This confirms that Linezolid lacks significant efficacy against Gram-negative bacteria, likely due to poor permeability through the outer membrane and absence of its molecular targets in Gram-negative organisms. As such, Linezolid was ineffective against *E. coli* at the tested concentration (Figure-2, 3).

Well 4 – Amoxicillin (0.5 mg/ml):
Amoxicillin, a beta-lactam antibiotic belonging to the penicillin class, exhibited a moderate antibacterial effect with an average zone of inhibition of 15.1 mm. Amoxicillin interferes with bacterial cell wall synthesis by inhibiting penicillin-binding proteins. However, its efficacy against *E. coli* can be variable, as many strains produce beta-lactamase enzymes that inactivate beta-lactam antibiotics. The presence of a measurable inhibition zone indicates partial susceptibility, but the relatively smaller diameter compared to Moxifloxacin suggests reduced effectiveness, potentially due to beta-lactam resistance mechanisms in the tested strain (Figure-2, 3).

Table-2: Comparative Analysis of Antibiotic Efficacy Against *Escherichia coli* at 0.5 mg/ml Concentration

Well	Antibiotic	Concentration (mg/ml)	Zone of Inhibition (mm)	Result
1	Clarithromycin	0.5	0.0	No inhibition
2	Moxifloxacin	0.5	35.0	Strong inhibition
3	Linezolid	0.5	0.0	No inhibition
4	Amoxicillin	0.5	15.1	Moderate inhibition



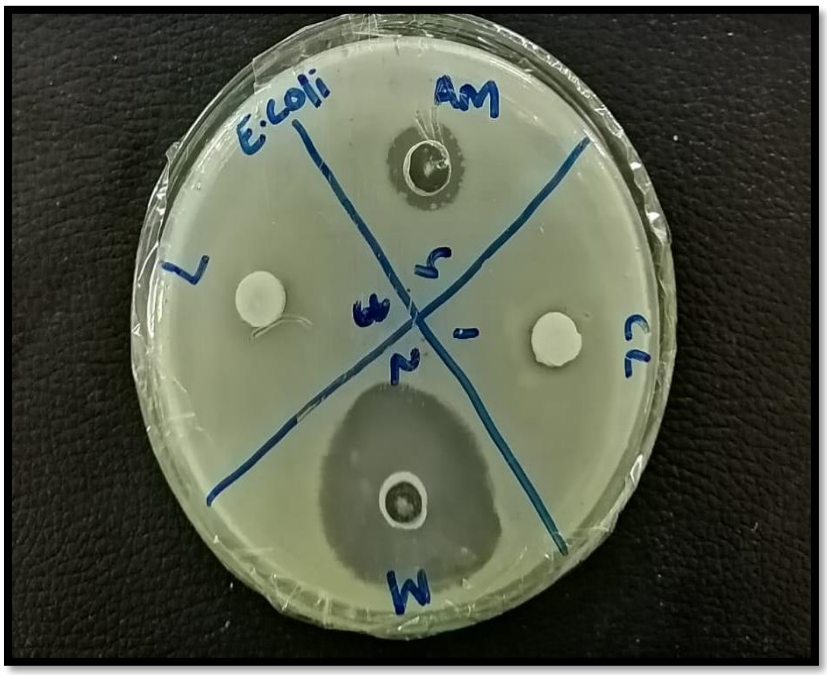


Figure-3: A Comparative Analysis of Different Antibiotics Against *Escherichia coli*

Discussion

Escherichia coli (*E. coli*) is a prominent facultative anaerobic bacterium and the dominant aerobe residing in the gastrointestinal tract (GIT) of humans and other mammals (Hartl & Dykhuizen, 1984). Although it is often a harmless commensal, certain pathogenic strains can cause a range of infections, particularly in poultry, which also pose zoonotic risks to humans.

In the present study, *E. coli* was isolated from naturally infected broiler chickens using sterile cotton swabs taken from the oral and nasal cavities. The bacteria were cultured on MacConkey agar, and colonies displaying characteristic pink coloration were further confirmed using a series of biochemical tests. These included Gram staining, which revealed pink, rod-shaped, Gram-negative bacilli, along with a negative result in the Citrate and Oxidase tests, and a positive result in the Catalase test. This identification protocol aligns with the methodology and findings of Ali Saadi et al. (2017), thereby validating the presence of *E. coli* in the collected samples.

Following isolation and identification, the study aimed to evaluate the efficacy of four different antibiotics—Clarithromycin, Moxifloxacin, Linezolid, and Amoxicillin—against the isolated *E. coli* strain. All antibiotics were tested at the same concentration (0.5 mg/ml) and in triplicates for accuracy. The results indicated a varied response in the bacterial susceptibility profile.

Linezolid, an oxazolidinone-class antibiotic, exhibited complete resistance, with no observable zone of inhibition. This suggests that Linezolid is ineffective against *E. coli*, likely due to its limited permeability through Gram-negative outer membranes or lack of appropriate intracellular targets. This finding is in full agreement with the results reported by Sweeney et al. (2003), who also observed *E. coli* resistance to Linezolid.

Similarly, Clarithromycin, a macrolide antibiotic commonly used for respiratory infections, showed no inhibitory activity against *E. coli*. This resistance can be attributed to the intrinsic limitations of macrolides against Gram-negative bacteria, due to efflux pumps and poor outer membrane penetration. The results here corroborate the earlier findings of Ritchie et al. (1993).

Amoxicillin, a widely used beta-lactam antibiotic, demonstrated moderate activity against *E. coli*, producing an average inhibition zone of 15.1 mm. While some level of inhibition was observed, the relatively small zone suggests partial resistance,

possibly due to beta-lactamase production by the bacteria. This finding aligns with the study by Ritchie et al. (1993), who also reported variable responses of *E. coli* to Amoxicillin.

The most significant finding was the efficacy of Moxifloxacin, a fourth-generation fluoroquinolone, which showed strong antibacterial activity with an average zone of inhibition measuring 35.0 mm. Moxifloxacin targets bacterial DNA gyrase and topoisomerase IV, enzymes essential for DNA replication, and is known for its excellent penetration in both Gram-positive and Gram-negative organisms. This result is consistent with the observations of Rodriguez-Cerrato et al. (2001), who also reported Moxifloxacin's high efficacy against Gram-negative bacteria, including *E. coli*.

Overall, the data clearly indicate the emerging issue of antibiotic resistance among *E. coli* isolates in poultry, which has significant implications for both veterinary and public health. The study emphasizes the importance of performing antibiotic susceptibility testing prior to treatment, rather than relying on empirical therapy. Furthermore, it highlights the need for rational antibiotic use and the implementation of antimicrobial stewardship programs to mitigate resistance development.

Conclusion and Recommendations:

Among the four antibiotics evaluated, Moxifloxacin showed the most potent activity against *Escherichia coli*, suggesting it is highly effective at the tested concentration. Amoxicillin exhibited moderate activity, indicating some effectiveness but also potential resistance. Clarithromycin and Linezolid showed no inhibitory effect, highlighting their limited utility against *E. coli* due to inherent resistance mechanisms typical of Gram-negative organisms. These results emphasize the importance of selecting antibiotics based on specific bacterial susceptibility profiles and reinforce the need for antibiotic sensitivity testing prior to treatment.

Future research should include a broader range of antibiotics and larger sample sizes from diverse locations to better assess resistance patterns. Molecular analysis of resistance genes is recommended to understand the mechanisms behind antibiotic resistance. Monitoring multidrug resistance and evaluating the impact of antibiotic use in poultry farming are also essential. Alternative treatments, such as probiotics or bacteriophages, should be explored to reduce antibiotic reliance. Lastly, a One Health approach involving human, animal, and environmental health sectors is crucial for effective antimicrobial resistance management.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this study.

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