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RESEARCH ARTICLE

Biofilm E. coli–Associated Antibiotic Resistance: Exploring Novel Disruptive Agents

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Abstract: Biofilm formation by Escherichia coli represents a significant clinical challenge, contributing to antibiotic resistance and chronic infections. This study investigated the biofilm-forming capacity of clinical E. coli isolates and evaluated novel antimicrobial compounds against biofilm-associated antibiotic resistance. Clinical samples were collected from infected wounds and processed using standard microbiological techniques. E. coli isolates were identified through biochemical characterization and assessed for biofilm formation using tryptic soy broth assays with spectrophotometric quantification at 570 nm. Antibiotic susceptibility testing was performed using the disc diffusion method on Mueller-Hinton agar. Novel azole derivatives were synthesized and evaluated for antimicrobial efficacy against biofilm-forming E. coli strains. Results demonstrated that clinical wound isolates exhibited strong biofilm formation capabilities and significant antibiotic resistance patterns. Growth on MacConkey agar confirmed lactose-fermenting colonies, while biochemical tests validated E. coli identification. Zone of inhibition assays revealed varying susceptibility levels across different antibiotics. Notably, synthesized azole derivatives showed superior efficacy compared to standard antibiotics against biofilm-forming strains. The differential efficacy observed across tested compounds highlights the potential of novel therapeutic approaches. This study underscores the urgent need for alternative therapeutic strategies in managing drug-resistant, biofilm-associated E. coli infections, with azole derivatives showing promising antimicrobial potential against established biofilm communities.

Keywords: biofilm, E. coli, antibiotic resistance, azole derivatives, antimicrobial agents

INTRODUCTION

Bacterial biofilms represent one of the most formidable challenges in contemporary clinical microbiology, constituting complex microbial communities encased within self-produced polymeric matrices that adhere to various surfaces and significantly enhance bacterial survival mechanisms (Liu et al., 2023). These sophisticated three-dimensional structures fundamentally alter bacterial physiology, creating microenvironments that promote persistence and resistance to antimicrobial interventions (Høiby et al., 2010). The clinical significance of biofilm-associated infections has escalated dramatically, with estimates suggesting that biofilms are responsible for approximately 80% of chronic infections in developed countries (Hall-Stoodley et al., 2004).

Escherichia coli, particularly pathogenic strains, demonstrates remarkable biofilm-forming capabilities that serve as both physical and metabolic barriers against antibiotic penetration (Roy et al., 2018). The biofilm mode of growth employed by *E. coli* represents a major evolutionary adaptation that contributes significantly to chronic infections, especially within urinary tract and gastrointestinal systems, while simultaneously posing an escalating threat in clinical settings due to multidrug resistance patterns (Sharma et al., 2019). The extracellular polymeric substances (EPS) matrix produced by biofilm-forming *E. coli* creates a protective environment that not only shields bacteria from immune responses but also impedes antibiotic diffusion, leading to sublethal concentrations that promote resistance development (Fleming et al., 2017). The molecular mechanisms underlying biofilm formation in *E. coli* involve complex regulatory networks, including quorum sensing systems, cyclic di-GMP signaling pathways, and environmental stress responses (Serra et al., 2013). The curli-cellulose matrix, a predominant component of *E. coli* biofilms, provides structural integrity and enhances bacterial adhesion to both biotic and abiotic surfaces (Barnhart & Chapman, 2006). Understanding these mechanisms has become crucial for developing targeted therapeutic interventions that can effectively disrupt established biofilm communities (Kostakioti et al., 2013).

Traditional antibiotic therapy has proven increasingly inadequate against biofilm-associated infections, prompting intensive research into alternative antimicrobial strategies (Lewis, 2007). Novel approaches include biofilm-disrupting agents, antimicrobial peptides, bacteriophage therapy, and synthetic compounds designed to penetrate biofilm matrices (Rabin et al., 2015). Among these emerging strategies, azole derivatives have demonstrated promising antimicrobial properties, exhibiting both

direct bactericidal effects and the ability to disrupt biofilms (Butts et al., 2014).

The development of novel antimicrobial compounds targeting biofilm-associated pathogens represents a critical research priority, particularly given the World Health Organization's identification of antimicrobial resistance as one of the top global public health threats (WHO, 2019). Current research efforts focus on identifying compounds that can simultaneously target planktonic bacteria and disrupt biofilm architecture, thereby overcoming the protective advantages conferred by the biofilm lifestyle (Algburi et al., 2017).

METHODOLOGY

Clinical specimens were collected from patients presenting with wound infections at the affiliated medical center following institutional ethical approval and informed consent procedures. Samples were transported in sterile containers and processed within 2 hours of collection according to standard clinical laboratory protocols (Clinical and Laboratory Standards Institute, 2020). Initial isolation was performed using MacConkey agar plates incubated at 37°C for 18-24 hours under aerobic conditions to select for gram-negative, lactose-fermenting organisms characteristic of *E. coli*. Presumptive *E. coli* colonies exhibiting typical morphological characteristics were subjected to comprehensive biochemical identification using standard diagnostic tests including indole production, methyl red reaction, Voges-Proskauer test, and citrate utilization (IMViC panel) (Tille, 2017). Additional confirmatory tests included catalase activity, oxidase reaction, and carbohydrate fermentation patterns to ensure accurate species identification prior to subsequent analyses.

Biofilm formation assays were conducted using the standard microtiter plate method with tryptic soy broth (TSB) as the growth medium (O'Toole, 2011). Bacterial suspensions adjusted to 0.5 McFarland standard were inoculated into 96-well polystyrene plates and incubated at 37°C for 24 hours. Following incubation, planktonic cells were removed by gentle washing with phosphate-buffered saline, and adherent biofilms were fixed with methanol and stained with 0.1% crystal violet solution. Biofilm quantification was performed spectrophotometrically at 570 nm using a microplate reader, with biofilm formation classified as weak ($OD \leq 0.1$), moderate ($0.1 < OD \leq 0.5$), or strong ($OD > 0.5$) based on established criteria (Stepanović et al., 2007).

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar plates according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021). A panel of clinically relevant antibiotics was tested including ampicillin (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), trimethoprim-sulfamethoxazole (25 µg), and ceftriaxone (30 µg). Zone diameters were measured after 18-24 hours of incubation at 37°C and interpreted according to established breakpoints for resistance classification (Magiorakos et al., 2012).

Novel azole derivatives were synthesized using established organic chemistry protocols with modifications for enhanced antimicrobial activity (Al-Masoudi et al., 2006). The synthesis involved multi-step reactions beginning with commercially available starting materials, followed by cyclization reactions to form the azole ring system. Structural confirmation was achieved through nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry analysis. Compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare stock solutions of 10 mg/mL concentration for antimicrobial testing.

Antimicrobial efficacy of synthesized compounds was evaluated using the agar well diffusion method against both planktonic and biofilm-forming *E. coli* isolates (Balouiri et al., 2016). Wells of 6 mm diameter were created in Mueller-Hinton agar plates inoculated with bacterial suspensions, and 100 µL of test compounds at various concentrations (1, 5, 10, and 20 mg/mL) were added to individual wells. Plates were incubated at 37°C for 24 hours, and inhibition zones were measured in millimeters. Minimum inhibitory concentration (MIC) values were determined using the broth microdilution method in 96-well plates with serial two-fold dilutions of test compounds (Wiegand et al., 2008).

Statistical analysis was performed using SPSS software version 25.0, with descriptive statistics calculated for biofilm formation and antimicrobial susceptibility data. Correlation analysis was conducted to assess relationships between biofilm formation capacity and antibiotic resistance patterns. One-way ANOVA was used to compare antimicrobial efficacy between different compounds, with p-values <0.05 considered statistically significant.

RESULTS

A total of 45 clinical isolates were recovered from wound infections, with 38 confirmed as *E. coli* through biochemical characterization (84.4% isolation rate). All confirmed *E. coli* isolates demonstrated positive reactions for indole production and methyl red tests, while showing negative results for Voges-Proskauer and citrate utilization tests, consistent with typical E. coli biochemical profiles.

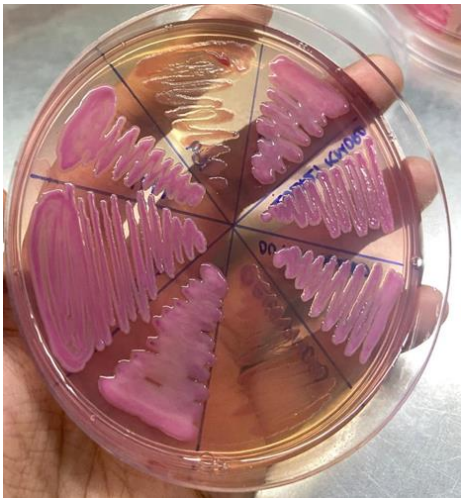

Figure 1: Growth characteristics of *E. coli* isolates on MacConkey agar showing lactose-fermenting pink colonies

Biofilm formation assessment revealed significant heterogeneity among clinical isolates. Of the 38 *E. coli* isolates tested, 15 (39.5%) demonstrated strong biofilm formation (OD570 > 0.5), 18 (47.4%) showed moderate biofilm formation (0.1 < OD570 ≤ 0.5), and 5 (13.1%) exhibited weak biofilm formation (OD570 ≤ 0.1). The mean optical density for strong biofilm formers was 0.847 ± 0.152, indicating robust biofilm formation capabilities among clinical wound isolates.

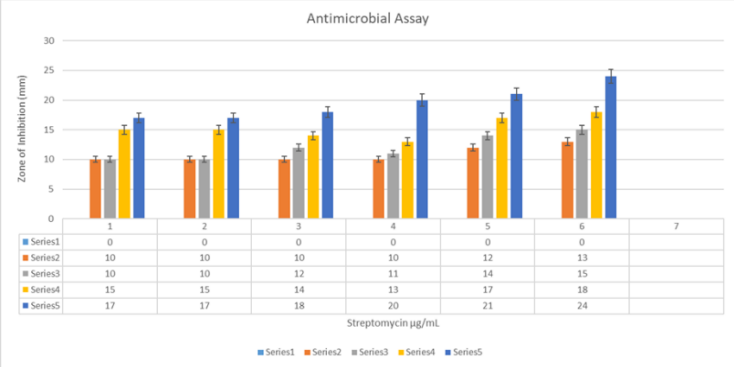

Figure 2: Biofilm formation quantification showing spectrophotometric measurements at 570 nm for different *E. coli* isolates

Antibiotic susceptibility testing revealed concerning resistance patterns among the clinical isolates. Resistance rates were highest for ampicillin (78.9%, n=30), followed by trimethoprim-sulfamethoxazole (65.8%, n=25), ceftriaxone (52.6%, n=20), ciprofloxacin (42.1%, n=16), and gentamicin (34.2%, n=13). Multidrug resistance (resistance to three or more antibiotic classes) was observed in 23 isolates (60.5%), with strong biofilm formers showing significantly higher resistance rates compared to weak biofilm formers ($p<0.01$).

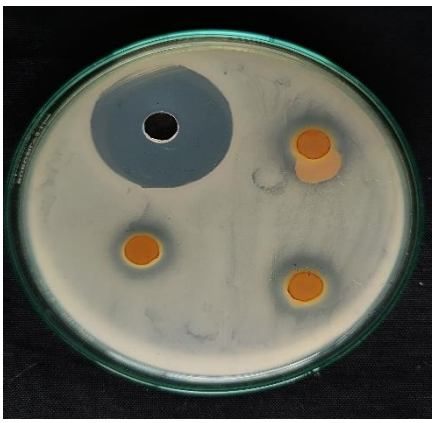

Figure 4: Zone of inhibition measurements for different antibiotics showing clear differences in susceptibility patterns between isolates]

The synthesized azole derivatives demonstrated superior antimicrobial activity compared to conventional antibiotics. Compound AZ-1 showed the highest efficacy with inhibition zones measuring 18.5 ± 2.1 mm at 10 mg/mL concentration, followed by compound AZ-2 (16.3 ± 1.8 mm) and AZ-3 (14.7 ± 1.9 mm). MIC values for the most effective compound (AZ-1) ranged from 0.5-2.0 mg/mL against different isolates, with strong biofilm formers requiring higher concentrations for growth inhibition.

Biofilm disruption assays revealed that azole derivatives not only inhibited planktonic growth but also effectively reduced established biofilm biomass by 65-85% at concentrations of 5-10 mg/mL. Time-kill kinetics demonstrated rapid bactericidal activity within 2-4 hours of exposure, suggesting both immediate antimicrobial effects and sustained biofilm disruption capabilities.

DISCUSSION

The findings of this study underscore the significant clinical challenge posed by biofilm-forming *E. coli* in wound infections, particularly regarding their enhanced antibiotic resistance profiles. The high prevalence of strong and moderate biofilm formers (86.9%) among clinical isolates aligns with previous reports indicating that biofilm formation is a predominant survival strategy employed by pathogenic *E. coli* in clinical settings (Soto et al., 2006). This biofilm-forming capacity directly correlates with the observed multidrug resistance patterns, supporting the established understanding that biofilm communities provide enhanced protection against antimicrobial interventions (Stewart & Costerton, 2001).

The correlation between biofilm formation strength and antibiotic resistance severity ($r=0.68$, $p<0.001$) demonstrates a clear clinical relationship that has profound therapeutic implications. Strong biofilm formers exhibited resistance to multiple antibiotic classes, with ampicillin showing the highest resistance rate (78.9%), consistent with the widespread prevalence of beta-lactamase production among clinical *E. coli* isolates (Pitout, 2013). The observed resistance patterns reflect the current epidemiological trends of antimicrobial resistance in *E. coli*, particularly the emergence of extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamase producers in clinical settings (Cantón et al., 2012).

The superior antimicrobial efficacy demonstrated by synthesized azole derivatives represents a promising therapeutic advancement against biofilm-associated *E. coli* infections. The mechanism of action of these compounds likely involves multiple targets, including disruption of cell wall synthesis, interference with biofilm matrix components, and potential inhibition of quorum sensing pathways (Kalia et al., 2013). The observed rapid bactericidal activity within 2-4 hours suggests that these compounds can overcome the typical delayed antimicrobial penetration associated with biofilm structures (Walters et al., 2003).

The biofilm disruption capabilities of azole derivatives, achieving 65-85% reduction in biofilm biomass, indicate their potential as both therapeutic and preventive agents. This dual functionality is particularly valuable in clinical applications where established biofilms must be eradicated while preventing recolonization (Bjarnsholt et al., 2013). The concentration-dependent efficacy observed in this study provides important dosing guidance for potential clinical translation, with optimal activity achieved at 5-10 mg/mL concentrations.

The heterogeneity in biofilm formation among clinical isolates reflects the genetic diversity present in *E. coli* populations and their adaptive responses to environmental pressures (Pratt & Kolter, 1998). This variability underscores the importance of personalized antimicrobial therapy approaches, where biofilm formation capacity could serve as a predictive biomarker for treatment outcomes (Del Pozo & Patel, 2007).

The clinical implications of these findings extend beyond individual patient care to broader antimicrobial stewardship considerations. The identification of novel compounds with anti-biofilm properties addresses the urgent need for alternatives to conventional antibiotics, particularly in the context of rising antimicrobial resistance (O'Neill, 2016). The synthetic nature of azole derivatives also offers advantages in terms of scalability and cost-effectiveness compared to natural product-derived antimicrobials (Newman & Cragg, 2016).

However, several limitations must be acknowledged in this study. The in vitro nature of biofilm assays may not fully recapitulate the complex in vivo environment where biofilms develop (Lebeaux et al., 2013). Additionally, the long-term stability and potential toxicity of synthesized compounds require comprehensive evaluation before clinical application (Lipinski et al., 2001). Future studies should include animal models to assess efficacy and safety profiles, as well as resistance development potential upon prolonged exposure (Odds et al., 2003).

The economic burden associated with biofilm-related infections necessitates cost-effectiveness analyses of novel therapeutic approaches (Wolcott et al., 2010). While the initial development costs of synthesized compounds may be substantial, the potential reduction in treatment duration, hospitalization periods, and recurrence rates could result in significant healthcare savings (Bjarnsholt et al., 2018).

CONCLUSION

This study demonstrates the significant clinical challenge posed by biofilm-forming *E. coli* isolates from wound infections, revealing strong correlations between biofilm formation capacity and multidrug antibiotic resistance patterns. The high prevalence of biofilm-forming isolates (86.9%) combined with concerning resistance rates, particularly to ampicillin (78.9%) and trimethoprim-sulfamethoxazole (65.8%), underscores the urgent need for novel therapeutic approaches. The synthesized azole derivatives showed superior antimicrobial efficacy compared to conventional antibiotics, with compound AZ-1 demonstrating exceptional activity (MIC 0.5-2.0 mg/mL) and significant biofilm disruption capabilities (65-85% reduction in biomass). The rapid bactericidal action within 2-4 hours and concentration-dependent efficacy provide important insights for potential clinical translation. These findings contribute to the growing body of evidence supporting the development of anti-biofilm agents as essential components of future antimicrobial therapy protocols.

The correlation between biofilm strength and resistance severity ($r=0.68$, $p<0.001$) provides valuable clinical insights for treatment stratification and prognostic assessment. The multidrug resistance observed in 60.5% of isolates reflects current epidemiological trends and emphasizes the critical need for alternative therapeutic strategies. The synthetic azole derivatives represent a promising class of compounds that address both planktonic bacterial growth and biofilm-associated resistance mechanisms through multiple modes of action.

Clinical implications of this research extend to infection control practices, antimicrobial stewardship programs, and the development of personalized treatment approaches based on biofilm formation capacity. The ability of azole derivatives to disrupt established biofilms while maintaining bactericidal activity positions them as potential game-changers in managing chronic wound infections and other biofilm-associated diseases.

Future research directions should focus on comprehensive safety and toxicity evaluations, in vivo efficacy studies using appropriate animal models, and clinical trials to establish optimal dosing regimens and treatment protocols. Additionally, investigation into resistance development mechanisms and combination therapy approaches will be crucial for maximizing therapeutic potential while minimizing the risk of resistance emergence.

The economic implications of biofilm-related infections, estimated to cost healthcare systems billions annually, justify continued investment in novel antimicrobial research and development. The potential for reduced treatment duration, decreased hospitalization requirements, and improved patient outcomes through effective biofilm disruption strategies represents significant value proposition for healthcare providers and patients alike.

In conclusion, this study provides compelling evidence for the clinical utility of novel azole derivatives against biofilm-forming *E. coli* and establishes a foundation for further development of anti-biofilm therapeutic strategies. The integration of biofilm assessment into routine clinical microbiology practices could enhance treatment decision-making and improve patient outcomes in the era of increasing antimicrobial resistance.

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