International Journal of Scientific Advances
ISSN: 2708-7972
Volume: 5 | Issue: 6 | Nov – Dec 2024 Available Online: www.ijscia.com
DOI: 10.51542/ijscia.v5i6.42

The Potential of Alcohol and Triclosan in Inhibiting *E. Coli* Biofilm Formation

Christopher Surya Lodianto[1], Agung Dwi Wahyu Widodo[2*], Muhammad Vitanata Arfijanto[3], Nurul Wiqoyah[4]

[1]Faculty of Medicine, Airlangga University, Surabaya, Indonesia

[2]Department of Clinical Microbiology, Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital. Surabaya, Indonesia

[3]Department of Internal Medicine, Faculty of Medicine, Airlangga University, Dr. Soetomo General Hospital. Surabaya, Indonesia

[4]Department of Microbiology, Faculty of Medicine, Airlangga University

E-mail: christopher.surya.lodianto-2021@fk.unair.ac.id; agungimunologi@gmail.com; vitanatadr@gmail.com; nurul-w@fk.unair.ac.id

*Corresponding author details: Agung Dwi Wahyu Widodo; agungimunologi@gmail.com

ABSTRACT

Escherichia coli (*E. coli*) is a facultative anaerobic, gram-negative bacillus bacterium. Most strains of *E. coli* are harmless but some strains can cause various diseases. Additionally, *E. coli* can form biofilms, which are complex structures consisting of bacterial cells surrounded by an extracellular matrix and are much more difficult to treat compared to their planktonic counterpart. Biofilms formed by pathogenic strains of *E. coli* can cause various diseases, such as diarrhea, neonatal meningitis, septicemia, UTIs, bile duct infections, and catheter-associated cystitis. This study aims to clarify the potential of alcohol and triclosan's ability to inhibit *E. coli* biofilm formation.

Keywords: *E. coli*; biofilm formation inhibition; biofilm inhibition; alcohol, triclosan.

INTRODUCTION

In recent decades, biofilm has been increasingly recognized as a major contributor to the pathogenesis of chronic infections [1]. Biofilm-forming microorganisms can attach themselves to various medical devices, such as urinary catheters, implants, etc., contributing to increased mortality and morbidity and transforming infections into chronic diseases [2]. Biofilms have unique characteristics not found in planktonic cells, including protection from external interference such as changes in pH and temperature, ultraviolet radiation, dryness, oxidation, metal ions, or biocides [3]. Out of many biofilm-forming microorganisms, *E. coli* is one of them [4].

Currently, despite many studies to prevent *E. coli* infections, cases of infections caused by *E. coli* still occur frequently and endanger human health. In the United States, about 1 in 3 women will experience at least one UTI requiring antibiotic treatment in their lifetime, and about 11% will experience at least one UTI each year [5]. In Indonesia, UTI is a relatively common disease in all ages ranging from infants to the elderly.

The prevalence of UTI increases significantly from 5%-10% at the age of 70 years to 20% at the age of 80 years [6]. Urologic *Escherichia coli* (UPEC), a type of extra-intestinal pathogenic *E. coli* (ExPEC), is the main pathogen causing UTI in the community (80-90%) and in hospitals (30-50%) [7].

The emergence and spread of resistance to antimicrobial agents is considered one of the major health threats worldwide, especially among bacteria. In this context, biofilms play an important role [8]. A study showed that 72% of *E. coli* extended-spectrum beta-lactamase (ESBL) isolates were multidrug-resistant, with the prevalence of *E. coli* ESBL in bacteriuria episodes increasing from 17% to 24% between 2014 and 2020. Resistance to common antibiotics has increased. Resistance to ciprofloxacin increased from 3% to 17% between 2000 and 2010. Trimethoprim-sulfamethoxazole (TMP-SMX) resistance increased from 0.8% to 1.6% during the same period [9]. Biofilms also increase resistance to treatment by 100 to 1000-fold compared to planktonic cells. In addition, biofilms can evade innate and adaptive immune defenses, making treatment and eradication of biofilms extremely difficult [8].

In Indonesia, antiseptic products that are often used among the public are Antiseptic A. Antiseptic A contains alcohol with the active ingredient triclosan. Alcohol is an antimicrobial agent that works by denaturing proteins and shows decent in vitro antimicrobial activity against Gram-positive and Gram-negative vegetative bacteria and various strains of fungi [10]. Triclosan is a broad-spectrum antibacterial agent that has a mechanism of inhibiting fatty acid synthesis. A study showed, that an effective dose of triclosan can reduce the growth of S. aureus and *E. coli* by 99% [11], Triclosan is also often used to disinfect medical equipment to prevent potential contamination [12].

This literature review aims to understand the potential of alcohol and triclosan in inhibiting *E. coli* biofilm formation by analysing recent study findings. By analysing recent study findings, this review clarifies the potential inhibitory abilities of alcohol and triclosan against the formation of *E. coli* biofilm.

REVIEW CONTENT

1. *E. coli* Bacteria

1.1 *Strain*

Although *E. coli* is a normal flora of the human gut, there are some strains of *E. coli* that have specific virulence factors that allow them to become pathogenic to humans and cause various diseases. These pathogenic strains can cause infections in the gut that can result in diarrheal diseases. In addition to the gut, *E. coli* can also cause extra-intestinal infections, such as UTI, sepsis, and meningitis [13]. In general, enteric *E. coli* pathotypes consist of:

A. enterotoxigenic *E. coli* (ETEC), which is associated with Traveler's diarrhea [14];
B. enteropathogenic *E. coli* (EPEC), which is associated with pediatric diarrhea [14];
C. enterohaemorrhagic *E. coli* (EHEC), which belongs to the Shiga toxin-producing *E. coli* (STEC) serogroup, associated with hemorrhagic colitis and Hemolytic Uremic Syndrome (HUS) in humans [14];
D. enteroaggregative *E. coli* (EAEC), which is associated with persistent diarrhea in humans [14];
E. diffuse-adherent *E. coli* (DAEC), which is associated with acute diarrhea, especially in children [15];
F. enteroinvasive *E. coli* (EIEC), which is associated with intestinal invasive infections and dysentery in humans and various animals [14];[16];
G. adherent-invasive *E. coli* (AIEC), which is associated with Crohn's disease [17];
H. whereas extra-intestinal pathogenic *E. coli* (ExPEC) consists of:
    a. uropathogenic *E. coli* (UPEC), which is associated with UTI [18];
    b. neonatal meningitic *E. coli* (NMEC), which is associated with meningitis in newborns [19];
    c. bloodborne strains of *E. coli* (BBEC), which are associated with septicemia in humans and animals [20].

1.2 *Pathophysiology*

The pathophysiology of UTI caused by *E. coli*, especially UPEC, involves a complex set of interactions between the bacteria and the host urinary tract. Urinary tract infections often begin with periurethral contamination by UPEC strains that commonly originate from the gastrointestinal tract. These bacteria use fimbriae (or pili) to adhere to the uroepithelial cells lining the urinary tract, with type 1 fimbriae playing an important role in adhesion, allowing the bacteria to attach to the urethra and bladder effectively. Once attached, *E. coli* can migrate from the urethra to the bladder, where they can proliferate. This migration process is facilitated by flagella-mediated motility, which allows the bacteria to swim against the urine stream [21]. After colonization, some *E. coli* are able to invade bladder epithelial cells and form intracellular bacterial communities, which help to evade host immune responses and persist within the urinary tract. In addition, UPEC also uses various strategies to evade host defenses, including the production of toxins that damage host tissues, synthesis of factors that inhibit cytokine responses, and biofilm formation that protects the bacteria from immune cell and antibiotic attack [22].

2. Biofilm

2.1 *Definition*

Biofilms are one/multiple types of microorganisms enclosed in an Extracellular Polymeric Substance (EPS) matrix attached to a surface. Biofilms can adhere to a wide variety of surfaces, such as living tissue and medical devices. The nature of biofilms can vary depending on the environment to which they are attached, for example, biofilms on medical devices consist of a single coccus organism and associated EPS matrix [23].

2.2 *Biofilm Formation*

Planktonic bacteria will carry out the process of biofilm formation when the bacteria capture signals from environmental conditions that become triggers to initiate attachment to a surface. This trigger signal is different for different types of bacteria. *P. aeruginosa* and *P. fluorescens* will form biofilms with any conditions that allow growth. Meanwhile, bacteria such as *E. coli* K-12 and *Vibrio cholerae* will only form biofilms if there are sufficient amino acids. In addition to the nutritional components provided by the medium, other things such as temperature, pH, osmolarity, oxygen, and Fe can affect biofilm formation [24].

Biofilm formation begins with the attachment of cells to a surface, either an abiotic surface such as tissue, or an abiotic surface such as a medical device. This cell-to-surface attachment step has two important factors, namely the substratum and the cell surface. The substratum or surface of the bacterial cell attachment has a great impact on the speed and degree of cell attachment. In general, the more hydrophobic and rougher the substrate, the more the speed of biofilm formation increases [25].

Cells that have irreversibly attached will then undergo mitosis, form microcolonies, and produce EPS that form biofilms [26]. Oftentimes, cells will detach themselves from the biofilm due to food limitations or inadequate biofilm environmental conditions [24].

2.3 *Biofilm Formation Inhibition*

Inhibition of biofilm formation is essential to control microbial growth in various environments, including healthcare and industrial applications. Some effective strategies in inhibiting biofilm formation based on recent research include surface modification, chemical inhibition, physical disruption, enzymatic, and nanotechnology [27]; [28]; [29].

Surface modification can be done by changing the physiochemical characteristics of a surface, such as hydrophobicity and smoothness, which can prevent microbial attachment. This strategy involves coating the surface with antimicrobial materials or modifying the surface so that conditions are created that make biofilm formation difficult [27]. Chemical inhibition can be performed using antimicrobial agents such as the combination of modified green tea polyphenol, EGCG-S, with antibiotics that have been shown to exhibit significant inhibition of biofilm formation on various bacterial strains. In addition, chemical inhibitors that can disrupt signaling pathways that regulate bacterial communication (QS) can also prevent biofilm maturation, including targeting molecules such as N-acyl-homoserine lactones (AHLs) that are important for biofilm development [29].

Physical disruption can be performed by applying external forces such as ultrasound techniques or mechanical agitation can physically disrupt the already-formed biofilm and promote bacterial cell detachment. This method can be combined with chemical inhibition to increase effectiveness [27]; [28]. Enzymatic approach strategies can also be carried out, namely by using enzymes to decompose EPS. Enzymes such as glycoside hydrolase can damage the polysaccharides in EPS [29]. The use of nanoparticles is also an effective strategy to inhibit biofilm formation, for example, using nanoparticles to deliver antimicrobial agents directly into the biofilm will improve treatment efficacy while minimizing systemic exposure and the potential for side effects [29].

3. Alcohol

The most commonly used alcohols as antimicrobial agents are ethyl alcohol or isopropyl alcohol. Ethyl alcohol (ethanol) or isopropyl alcohol in an aqueous solution is a cheap and accessible disinfectant. Dilute solutions containing 70 to 92% alcohol concentration are rapid-acting and bactericidal against Gram-negative bacteria, Gram-positive bacteria, and germs for most viruses, fungi, and other pathogens. Due to their broad spectrum of activity, alcohols are often found as ingredients in various antiseptics and disinfectants. For its mechanism, alcohol has a bactericidal effect by denaturing proteins, changing the protein structure from an active form to an inactive form. This denaturation occurs because alcohol breaks hydrophobic and hydrogen bonds between protein molecules, so the protein loses its biological function. Alcohol also causes the coagulation of bacterial proteins, which is the formation of protein aggregates that can no longer function [30].

Ethanol with higher concentrations, such as 40% (v/v), disrupts bacterial cell membranes and affects gene expression related to biofilm formation. It has been observed to reduce bacterial motility, which is crucial for the initial attachment and formation of biofilms. Additionally, ethanol may alter the production of curli fibers, which are essential for adhesion and biofilm stability in *E. coli* [31]. However, research indicates that ethanol can also upregulate biofilm production in *E. coli*. This occurs through the initiation of a global stress response, which leads to significant changes in gene transcription profiles, including those related to curli protein production. Ethanol exposure has been shown to increase the expression of curli fibers, thereby enhancing biofilm formation under certain conditions. This indicates that while lower concentrations can promote biofilm formation, higher concentrations can disrupt and reduce existing biofilms [32].

4. Triclosan

Triclosan (TCS), or 5-chloro-2-(2,4-dichlorophenoxy) phenol, is a synthetic broad-spectrum antimicrobial developed since the 1960s. As a polychlorinated bisphenolic compound, TCS is slightly soluble in water and has a fairly strong odor. TCS dissolves well in organic solvents such as ethanol, dimethylsulfoxide, and methanol [33]. TCS inhibits an enzyme that functions in fatty acid biosynthesis, the enzyme enoyl-Acyl Carrier Protein (ACP) reductase, by imitating its natural substrate. According to the study, mutated or over-expressed ACP, encoded by the fabI gene, was shown to confer bacterial resistance to TCS. Thus, these findings prove ACP is a specific subcellular TCS target [34].

Triclosan exhibits varying effects on bacterial biofilm formation depending on concentration and species. At subinhibitory concentrations, triclosan can promote biofilm formation and adherence in Streptococcus mutans by upregulating genes involved in these processes [35]. However, at higher concentrations, triclosan effectively inhibits biofilm formation in several urinary tract pathogens, including *Escherichia coli* and *Klebsiella pneumonia* [36]. In aquatic environments, triclosan exposure impairs bacterial biofilm development, alters community composition, and reduces bacterial diversity. This impairment affects the biofilm's ability to stabilize sediments, potentially impacting sediment dynamics and pollutant dispersal [37].

CONCLUSIONS

The emergence of biofilms and their antimicrobial-resistant nature have been one of the many major health threats worldwide. Studies have shown alcohol and triclosan on their exhibit a decent potential in inhibiting *E. coli* biofilm formation. However, the concentration of alcohol and triclosan should be considered, as studies have shown that sub-inhibitory concentration may enhance biofilm formation instead. Further research is required to validate their clinical use.

ACKNOWLEDGEMENT

The author would like to thank the healthcare professionals, researchers, and academic mentors who have helped the completion of this literature review.

REFERENCES

[1] Perry EK, Tan MW. Bacterial biofilms in the human body: prevalence and impacts on health and disease. Front Cell Infect Microbiol. 2023;13. Available from: https://doi.org/10.3389/fcimb.2023.1237164.

[2] [2] Ballén V, Sosa E, Alvarez R, et al. Clinical Escherichia coli: From biofilm formation to new antibiofilm strategies. Microorganisms. 2022;10:1103. Available from: https://doi.org/10.3390/microorganisms10061103.

[3] Lerch TZ, Chenu C, Dignac MF, Barriuso E, Mariotti A. Biofilm vs. planktonic lifestyle: Consequences for pesticide 2,4-D metabolism by Cupriavidus necator JMP134. Front Microbiol. 2017;8:904.

[4] Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. Nat Rev Microbiol. 2004;2(2):123–40. Available from: https://doi.org/10.1038/nrmicro818.

[5] Kaye KS, Gupta V, Mulgirigama A, et al. Antimicrobial resistance trends in urine Escherichia coli isolates from adult and adolescent females in the United States from 2011 to 2019: Rising ESBL strains and impact on patient management. Clin Infect Dis. 2021;73(11):1992–9. Available from: https://doi.org/10.1093/cid/ciab560.

[6] [6] Ni Made Susilawati, Marni Tangkelangi, Dorotia Masi Daen. Prevalensi infeksi saluran kemih pada penderita diabetes melitus di RSUD Prof. Dr. W. Z Johannes Kupang tahun 2021. Jurnal Kesehatan Dan Kedokteran. 2022;1(3):19–23. Available from: https://doi.org/10.56127/jukeke.v1i3.290.

[7] Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol. 2010;7(12):653–60.

[8] González JF, Hahn MM, Gunn JS. Chronic biofilm-based infections: Skewing of the immune response. Pathog Dis. 2018;76(3):fty023.

[9] Ku J, Bruxvoort K, Salas SB, et al. Multidrug resistance of Escherichia coli from outpatient uncomplicated urinary tract infections in a large United States integrated healthcare organization. Open Forum Infect Dis. 2023;10(7). Available from: https://doi.org/10.1093/ofid/ofad287.

[10] Ochwoto M, Muita L, Talaam K, et al. Anti-bacterial efficacy of alcoholic hand rubs in the Kenyan market, 2015. Antimicrob Resist Infect Control. 2017;6(1). Available from: https://doi.org/10.1186/s13756-017-0174-3.

[11] Tauanov Z, Zakiruly O, Baimenova Z, et al. Antimicrobial and antiviral properties of triclosan-containing polymer composite: Aging effects of pH, UV, and sunlight exposure. Polymers (Basel). 2023;15(5):1236. https://doi.org/10.3390/polym15051236.

[12] Zeng W, Xu W, Xu Y, et al. The prevalence and mechanism of triclosan resistance in Escherichia coli isolated from urine samples in Wenzhou, China. Antimicrob Resist Infect Control. 2020;9(1). Available from: https://doi.org/10.1186/s13756-020-00823-5.

[13] Bekal S, Brousseau R, Masson L, Prefontaine G, Fairbrother J, Harel J. Rapid identification of Escherichia coli pathotypes by virulence gene detection with DNA microarrays. J Clin Microbiol. 2003;41:2113–25.

[14] Kai A, Konishi N, Obata H. Diarrheagenic Escherichia coli. Nihon Rinsho. 2010;68(1):203–7.

[15] Scaletsky ICA, Fabbricotti SH, Carvalho RLB, et al. Diffusely adherent Escherichia coli as a cause of acute diarrhea in young children in northeast Brazil: A case-control study. J Clin Microbiol. 2002;40(2):645–8.

[16] Van den Beld MJC, Reubsaet FAG. Differentiation between Shigella, enteroinvasive Escherichia coli (EIEC), and noninvasive Escherichia coli. Eur J Clin Microbiol Infect Dis. 2012;31(6):899–904.

[17] Barnich N, Darfeuille-Michaud A. Adherent-invasive Escherichia coli and Crohn's disease. Curr Opin Gastroenterol. 2007;23:16–20.

[18] Terlizzi ME, Gribaudo G, Maffei ME. Uropathogenic Escherichia coli (UPEC) infections: Virulence factors, bladder responses, antibiotic, and non-antibiotic antimicrobial strategies. Front Microbiol. 2017;8:1566.

[19] Wijetunge DSS, Gongati S, Debroy C, et al. Characterizing the pathotype of neonatal meningitis-causing Escherichia coli (NMEC). BMC Microbiol. 2015;15(1):211.

[20] Mokady D, Gophna U, Ron EZ. Virulence factors of septicemic Escherichia coli strains. Int J Med Microbiol. 2005;295(6–7):455–62.

[21] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: Epidemiology, mechanisms of infection, and treatment options. Nat Rev Microbiol. 2015;13(5):269–84. Available from: https://doi.org/10.1038/nrmicro3432.

[22] Nielubowicz GR, Mobley HL. Host–pathogen interactions in urinary tract infection. Nat Rev Urol. 2010;7(8):430–41. Available from: https://doi.org/10.1038/nrurol.2010.101.

[23] Donlan RM, Costerton JW. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin Microbiol Rev. 2002;15:167–93. Available from: https://doi.org/10.1128/CMR.15.2.167-193.2002.

[24] O'Toole GA, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol. 2000;54(1):49–79. Available from: https://doi.org/10.1146/annurev.micro.54.1.49.

[25] Characklis WG, Cooksey KE. Biofilms and microbial fouling. Adv Appl Microbiol. 1983;29:93–138.

[26] Evans LV. Biofilms: recent advances in their study and control. CRC Press. 2000:167–87.

[27] Yin W, Xu S, Wang Y, et al. Ways to control harmful biofilms: prevention, inhibition, and eradication. Crit Rev Microbiol. 2021;47(1):57–78. Available from: https://doi.org/10.1080/1040841x.2020.1842325.

[28] Shrestha L, Fan HM, Tao HR, Huang JD. Recent strategies to combat biofilms using antimicrobial agents and therapeutic approaches. Pathogens. 2022;11(3):292. https://doi.org/10.3390/pathogens11030292.

[29] Srivastava A, Verma N, Kumar V, Apoorva P, Agarwal V. Biofilm inhibition/eradication: exploring strategies and confronting challenges in combatting biofilm. Arch Microbiol. 2024;206(5). https://doi.org/10.1007/s00203-024-03938-0.

[30] Spann CT, Taylor SC, Weinberg JM. Topical antimicrobial agents in dermatology. Clin Dermatol. 2003;21(1):70–7.

[31] Sissons CH, Wong L, Cutress TW. Inhibition by ethanol of the growth of biofilm and dispersed microcosm dental plaques. Arch Oral Biol. 1996;41(1):27–34. Available from: https://doi.org/10.1016/0003-9969(95)00103-4.

[32] Lim JY, May JM, Cegelski L. Dimethyl sulfoxide and ethanol elicit increased amyloid biogenesis and amyloid-integrated biofilm formation in Escherichia coli. Appl Environ Microbiol. 2012;78(9):3369–78. Available from: https://doi.org/10.1128/aem.07743-11.

[33] Schaffner RM. A meta-analysis of the published literature on the effectiveness of antimicrobial soaps. J Food Prot. 2011;74(11):1875–82.

[34] Alfhili MA, Lee MH. Triclosan: An update on biochemical and molecular mechanisms. Oxid Med Cell Longev. 2019;2019:1–28. https://doi.org/10.1155/2019/1607304.

[35] Bedran TBL, Grignon L, Spolidorio DP, Grenier D. Subinhibitory concentrations of triclosan promote Streptococcus mutans biofilm formation and adherence to oral epithelial cells. PLoS One. 2014;9(2):e89059. Available from: https://doi.org/10.1371/journal.pone.0089059.

[36] Jones GL, Muller C, O'Reilly M, Stickler DJ. Effect of triclosan on the development of bacterial biofilms by urinary tract pathogens on urinary catheters. J Antimicrob Chemother. 2006;57(2):266–72.

[37] Lubarsky HV, Gerbersdorf SU, Hubas C, et al. Impairment of the bacterial biofilm stability by triclosan. PLoS One. 2012;7(4):e31183. https://doi.org/10.1371/journal.pone.0031183.