

IntechOpen

IntechOpen Series
Infectious Diseases, Volume 41

Escherichia coli
From Normal Intestinal Bacteria
to Lethal Microbes

Edited by Ping Zeng



Escherichia coli - From
Normal Intestinal Bacteria
to Lethal Microbes

Edited by Ping Zeng

Published in London, United Kingdom

Escherichia coli - From Normal Intestinal Bacteria to Lethal Microbes

<http://dx.doi.org/10.5772/intechopen.1008048>

Edited by Ping Zeng

Contributors

Fayez S. Bahwerth, Gayatree Nayak, Hui Chen, Ivo Ganchev, Kavita Gupta, Lanxi Zhang, Monalisa Mohanty, Omar B. Ahmed, Ping Zeng, Punyatoya Kar, Qasemi Ali, Rahimi Fateh, Tianran Zhao, Yaqi Zhao, Yuge Liu, Zixuan Yan

© The Editor(s) and the Author(s) 2025

The rights of the editor(s) and the author(s) have been asserted in accordance with the Copyright, Designs and Patents Act 1988. All rights to the book as a whole are reserved by INTECHOPEN LIMITED. The book as a whole (compilation) cannot be reproduced, distributed or used for commercial or non-commercial purposes without INTECHOPEN LIMITED's written permission. Enquiries concerning the use of the book should be directed to INTECHOPEN LIMITED rights and permissions department (permissions@intechopen.com).

Violations are liable to prosecution under the governing Copyright Law.



Individual chapters of this publication are distributed under the terms of the Creative Commons Attribution 4.0 Unported License which permits commercial use, distribution and reproduction of the individual chapters, provided the original author(s) and source publication are appropriately acknowledged. If so indicated, certain images may not be included under the Creative Commons license. In such cases users will need to obtain permission from the license holder to reproduce the material. More details and guidelines concerning content reuse and adaptation can be found at <http://www.intechopen.com/copyright-policy.html>.

Notice

Statements and opinions expressed in the chapters are those of the individual contributors and not necessarily those of the editors or publisher. No responsibility is accepted for the accuracy of information contained in the published chapters. The publisher assumes no responsibility for any damage or injury to persons or property arising out of the use of any materials, instructions, methods or ideas contained in the book.

First published in London, United Kingdom, 2025 by IntechOpen

IntechOpen is the global imprint of INTECHOPEN LIMITED, registered in England and Wales, registration number: 11086078, 167-169 Great Portland Street, London, W1W 5PF, United Kingdom

For EU product safety concerns: IN TECH d.o.o., Prolaz Marije Krucifikse Kozulić 3, 51000 Rijeka, Croatia, info@intechopen.com or visit our website at intechopen.com.

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library

Escherichia coli - From Normal Intestinal Bacteria to Lethal Microbes

Edited by Ping Zeng

p. cm.

This title is part of the Infectious Diseases Book Series, Volume 41

Topic: Bacterial Infectious Diseases

Series Editor: Alfonso J. Rodriguez-Morales

Topic Editors: Tomas Jarzembowski and Katarzyna Garbacz

Print ISBN 978-1-83635-298-3

Online ISBN 978-1-83635-297-6

eBook (PDF) ISBN 978-1-83635-299-0

ISSN 2631-6188

If disposing of this product, please recycle the paper responsibly.

IntechOpen

intechopen.com

Built by scientists, for scientists



[Explore all IntechOpen books](#)

IntechOpen Book Series

Infectious Diseases

Volume 41

Aims and Scope of the Series

This series will provide a comprehensive overview of recent research trends in various Infectious Diseases (as per the most recent Baltimore classification). Topics will include general overviews of infections, immunopathology, diagnosis, treatment, epidemiology, etiology, and current clinical recommendations for managing infectious diseases. Ongoing issues, recent advances, and future diagnostic approaches and therapeutic strategies will also be discussed. This book series will focus on various aspects and properties of infectious diseases whose deep understanding is essential for safeguarding the human race from losing resources and economies due to pathogens.

Meet the Series Editor



Dr. Alfonso J. Rodriguez-Morales received his MD from Universidad Central de Venezuela in Caracas and his MSc in Protozoology/Parasitology from Universidad de Los Andes in Trujillo, Venezuela. He received his Diploma in Tropical Medicine & Hygiene (DT-M&H) from Universidad Peruana Cayetano Heredia, Lima, Peru, and the University of Alabama at Birmingham, Alabama, USA. He also holds a DipEd. Dr. Rodriguez-Morales is a fellow of the Royal Society for Tropical Medicine & Hygiene (FRSTMH), London, United Kingdom; of the Faculty of Travel Medicine (FFTM) of the Royal College of Physicians and Surgeons of Glasgow (RCPSG), Glasgow, Scotland, United Kingdom; of the American College of Epidemiology (FACE), USA; and the International Society for Antimicrobial Chemotherapy (FISAC). He has a HonDSc from Universidad Privada Franz Tamayo (UniFranz), Cochabamba, Bolivia. He is the President of the Latin American Society for Travel Medicine (SLAMVI) (2023–2025) and the Past President of the Colombian Infectious Diseases Association (2021–2023). He is a member of the Council (2020–2026) of the International Society for Infectious Diseases (ISID). He is a senior researcher at Colciencias (2015–2027) and a professor at the Fundación Universitaria Autónoma de las Américas in Pereira, Risaralda, Colombia, as well as at the Universidad Científica del Sur (UCSUR) in Lima, Peru. He is a visiting professor at multiple national and international universities.

Meet the Volume Editor



Dr. Ping Zeng is currently an associate professor at the Qilu Institute of Technology. Dr. Zeng completed his PhD at the Hong Kong Polytechnic University. After that, he was awarded a Postdoctoral Fellowship from the School of Pharmacy at the Chinese University of Hong Kong. His research focuses on the potential application of peptide-based molecules as novel antibacterial agents. Dr. Zeng has published more than 40 papers in renowned journals including *Advanced Functional Materials*, *Advanced Healthcare Materials*, *Biotechnology Advances*, etc. Now, he serves as the associate editor of *Frontiers in Nutrition*, a youth editor board member of *Biomaterials Translational* and a guest editor of *Pharmacological Research*.

Contents

Preface	XV
Section 1	
Overview of <i>Escherichia coli</i>	1
Chapter 1	3
Dual Nature of <i>Escherichia coli</i> : Useful Biological Tool versus Notorious Pathogen by Hui Chen, Yaqi Zhao, Zixuan Yan, Tianran Zhao, Yuge Liu, Lanxi Zhang and Ping Zeng	
Chapter 2	21
From Gut Flora to Global Threat: The Evolutionary Story of <i>Escherichia coli</i> by Kavita Gupta, Monalisa Mohanty, Punyatoya Kar and Gayatree Nayak	
Section 2	
The Current Situation of Drug Resistance of <i>Escherichia coli</i>	37
Chapter 3	39
The Prevalence of Antibiotic-Resistant Genes in <i>Escherichia coli</i> Isolated from the Intestines of Imported Sheep by Omar B. Ahmed and Fayez S. Bahwerth	
Section 3	
Advanced Therapeutics against <i>Escherichia coli</i>	53
Chapter 4	55
Therapeutic Advances against Uropathogenic <i>E. coli</i> : From Beta-Lactamase Inhibition to Anti-Biofilm Agents by Rahimi Fateh and Qasemi Ali	
Chapter 5	75
Catabolite Repression of Dual-Species Biofilm Formation by Participation of <i>Escherichia coli</i> Strains by Ivo Ganchev	

Preface

Escherichia coli is a bacterium of profound duality. As a commensal, it is an indispensable resident of our gut, providing essential vitamins and helping maintain microbial balance. As a scientific tool, it has revolutionized biology and medicine. However, specific pathogenic strains lead to severe illness, ranging from mild diarrhea to life-threatening complications. Understanding this difference between harmless gut residents and dangerous pathogenic variants is crucial.

This book explores these phenomena, revealing how *Escherichia coli* mirrors the complexities of life. Experts from all over the world shared their latest insights and views. Section 1 contains two chapters which provide an overview of this bacterium. For humans, *Escherichia coli* is far more than a passive resident. Within hours of birth, it colonizes the infant's gut, becoming a cornerstone of the intestinal microbiome. This facultative anaerobe adapts effortlessly to oxygen-rich and oxygen-poor environments, showcasing metabolic versatility that sustains its host.

Yet, lurking within this benign majority are rogue variants. When *Escherichia coli* acquires virulence genes, often through plasmids or phages, it morphs into a formidable pathogen. The World Health Organization recognizes different pathotypes with distinct mechanisms of harm. Worse still, misuse of antibiotics makes them resistant and more difficult to eradicate. The content on drug resistance of *Escherichia coli* can be found in section 2 of this book.

Of course, scientists have never given up the fight against pathogens. From killing planktonic *Escherichia coli* cells to destroying stubborn biofilm, new therapeutics are constantly being proposed. This also gives us confidence in winning the war against notorious germs. Section 3 describes existing approaches and also looks at future prospects.

The academic editor would like to express sincere gratitude to Prof. Xing Gao (Qilu Institute of Technology), Prof. Jibin Song (Beijing University of Chemical Technology) and Prof. Sharon Shui Yee Leung (The Chinese University of Hong Kong) for their generous guidance and assistance during book editing.

Ping Zeng
Qilu Institute of Technology,
College of Biological and Chemical Engineering,
Jinan, China

Section 1

Overview of *Escherichia coli*

Chapter 1

Dual Nature of *Escherichia coli*: Useful Biological Tool versus Notorious Pathogen

Hui Chen, Yaqi Zhao, Zixuan Yan, Tianran Zhao, Yuge Liu,
Lanxi Zhang and Ping Zeng

Abstract

This introductory chapter provides a comprehensive overview of *Escherichia coli* (*E. coli*), highlighting its dual nature as both a nonpathogenic and pathogenic microorganism. Nonpathogenic strains are crucial components of the intestinal microbiota. Moreover, as a model species in modern biology, *E. coli* plays a central role in diverse biomedical fields, such as genetic and molecular research, recombinant protein expression, and metabolic engineering. In addition to its significance in the health sector, *E. coli* possesses substantial biotechnological potential, with diverse applications spanning industrial synthesis, energy production, and environmental remediation, including power generation and wastewater treatment. On the contrary, by investigating the epidemiological and clinical implications of pathogenic strains, this chapter also underscores the significant public health threat posed by these strains and highlights the urgent need to develop innovative therapeutic strategies to combat resistance mechanisms, including drug-resistant plasmids. Overall, this chapter elucidates the dual nature of *E. coli* and underscores its significance in advancing our understanding of bacteriology, as well as in tackling global health and sustainability challenges.

Keywords: intestinal flora, nonpathogenic strain, pathogenic microorganism, drug resistance, novel therapeutics

1. Introduction

E. coli is an important model organism in modern biology, offering a range of advantages and widespread applications [1]. As a gram-negative bacterium, *E. coli* has a simple cellular structure and a rapid reproduction rate. Its small genome, which has been completely sequenced and annotated, facilitates genetic and physiological research [2]. *E. coli* is widely used in the study of gene expression regulation, translation processes, protein folding and modification, and can also help in the study of microbial metabolic pathways and their regulation [3]. *E. coli* is a commonly

used host bacterium, capable of introducing foreign genes to produce therapeutic proteins or industrial enzymes [4]. The composition of the culture medium for *E. coli* is simple and inexpensive, and the culture and experimental techniques are easy to master, with high safety of the experimental strains. With these advantages, *E. coli* is indispensable in various fields of modern biology and has effectively promoted the progress of life sciences [5].

E. coli has a dual nature, including nonpathogenic and pathogenic aspects. Nonpathogenic strains are members of normal flora of the human and animal intestines, which can maintain the balance of intestinal microecology, participate in the digestion and absorption of food, and synthesize vitamins [6]. For instance, vitamin K and some B vitamins produced by *E. coli* are essential for normal physiological functions. Vitamin K participates in the blood coagulation process, and B vitamins play an important role in energy metabolism and nervous system function. Furthermore, nonpathogenic strains enhance intestinal immune function, thus preventing pathogenic bacteria from colonization [7].

E. coli is a commonly host bacterium in genetic engineering and is a gene engineering vector. Its genome is relatively small, which would be easy to perform genetic manipulations and introduce foreign genes into *E. coli*, facilitating the expression of required proteins, such as insulin, growth hormone, interferon, and other biopharmaceuticals [8]. Moreover, *E. coli* cells are able to synthesize target proteins quickly in large quantities. By optimizing expression system, it can achieve the expression of different types of proteins, providing strong support for structure and function research and vaccine development [9]. These could provide important means for the treatment of diseases.

Since *E. coli* exists in large quantities in the intestines of humans and animals and has a relatively short survival time in the environment, it is often used as an indicator bacterium for fecal contamination of water bodies and soil. By detecting the number of *E. coli* in environmental samples, we can indirectly analyze whether the environment has been contaminated by feces and the extent of pollution, thereby providing a basis for environmental quality monitoring and public health protection [10].

On the contrary, pathogenic strains have special virulence factors, such as producing toxins or having structures such as flagella, which can help them colonize, reproduce, and cause disease in the host body [11]. Pathogenic strains can be divided into enterohaemorrhagic, enteropathogenic, enteroinvasive, and other types, leading to various diseases. In severe cases, it can result in dehydration, electrolyte disturbances, and even life-threatening conditions [12]. Additionally, pathogenic *E. coli* strains can also cause extraintestinal infections, such as urinary tract, sepsis, meningitis, especially in people with weakened immune systems [13].

In summary, *E. coli* plays a crucial role in modern biological research. The study of *E. coli* has been instrumental in elucidating fundamental principles of bacterial physiology and gene regulation. For instance, *E. coli* research has provided a framework for understanding bacterial gene regulation through operons, allostery, and environmental sensing, while also shedding light on physiological adaptations in metabolism, stress response, and host interactions. With the continuous deepening of future life science research, the functions of *E. coli* will be further explored through synthetic biology and multi-omics technologies. For example, engineered metabolic pathways allow *E. coli* to produce non-native high-value compounds, and comparative multi-omics quantifies strain-specific variations to match phenotypes with industrial needs.

The application prospects of *E. coli* in various fields will be broader, and the research and utilization of *E. coli* will continue to promote the development of human health and biotechnology industries, bringing more innovation to medical treatment, green manufacturing, and other fields.

2. Nonpathogenic *E. coli*: Biology and applications

2.1 Role in gut microbiota and gastrointestinal colonization

E. coli is widely distributed in the intestinal tracts of humans and animals and can be classified into pathogenic and nonpathogenic strains based on its virulence. Nonpathogenic *E. coli* was once overlooked, but in recent years, it has emerged as a research hot spot due to its critical functions and potential in multiple fields [14]. In-depth exploration of nonpathogenic *E. coli* contributes to a better understanding of gut microbiota ecology, genetic and molecular mechanisms, and the development of industrial applications.

The gut microbiota is a complex microbial community that symbiotically inhabits the human intestinal tract and plays a crucial role in host health. As a key component of the normal microbiota, nonpathogenic *E. coli* helps suppress harmful microorganisms and maintains the dominance of beneficial bacteria [15]. Adhesion factors, like fimbriae on the bacterial surface, could facilitate its attachment to intestinal epithelial cells, thus enabling colonization. Once established, it forms a biological barrier against pathogen invasion while its metabolic activities stimulate intestinal epithelial development and enhance mucosal immunity [16]. Studies indicated that nonpathogenic *E. coli* can induce epithelial cells to secrete antimicrobial peptides and cytokines, thereby strengthening immune defenses [17].

2.2 Applications as a model organism

Due to diverse growth phenotypes and adaptability of *E. coli*, it serves as an ideal biological model for studying responses to varying growth conditions and ecological niches. The genome of nonpathogenic *E. coli* is compact with well-characterized regulatory mechanisms, facilitating genetic manipulation. Its simple culture requirements, rapid reproduction (doubling time ~ 20 minutes), and well-established genetic tools, like gene knockout systems, make it a powerful platform for functional genomics research [18].

As a widely used host for recombinant protein production, engineered *E. coli* can efficiently synthesize biopharmaceuticals such as insulin. Strategies including codon optimization and chaperone co-expression enhance protein yield and solubility [19]. Additionally, genetic editing redirects metabolic fluxes to synthesize high-value compounds like biodiesel from fatty acid metabolism and food-grade amino acids [20]. As a biocatalyst, *E. coli* enables sustainable synthesis of natural products under mild conditions, reducing reliance on hazardous chemical processes.

In medical application, genetically engineered *E. coli* is designed for targeted delivery of anticancer drugs. Such kind of “bacterial robot” can accurately gather in the tumor hypoxia area to release therapeutic agents [21]. There are also studies that use *E. coli* as a living diagnosis system to generate color signals by sensing specific metabolic markers, so as to realize early non-invasive diagnosis of diseases [22].

Recently, an engineered probiotic *E. coli* was reported to deliver an α PD-L1-PE38 fusion toxin directly to tumors [23]. Another work designed an *E. coli*-quantum dots biohybrid system for direct converting glycerol to H₂ production with a high rate [24]. These advancements demonstrate the wide-ranging application of *E. coli* in various fields.

2.3 Industrial applications

Industrially, engineered *E. coli* produces diverse compounds, such as human milk oligosaccharides including 2'-fucosyllactose, 3'-sialyllactose, and 6'-sialyllactose, with scalable and automatable fermentation processes [25]. Under anaerobic conditions, *E. coli* oxidizes organic matter to generate electrons and protons, producing electric current. Optimizing strains and reactor designs improves power output [26]. In the field of biohydrogen production, genetic modifications enhance hydrogen yield and substrate utilization efficiency, supporting scalable clean energy generation [27].

E. coli can be also applied to environmental remediation, including degrading organic pollutants in wastewater by reducing the ratio of chemical oxygen demand and biochemical oxygen demand (COD/BOD) and absorbing nitrogen and phosphorus to mitigate eutrophication. In addition, for soil remediation, modulating microbial communities to degrade organic pollutants and immobilizing heavy metals by *E. coli* will be beneficial for improving fertility and structure. Engineered strains show improved degradation of recalcitrant contaminants [28].

Collectively, nonpathogenic *E. coli* holds immense value in gut ecology, molecular research, and industrial biotechnology. However, challenges such as unclear microbial interactions in the gut and suboptimal production efficiency in industrial settings remain unsolved. Future research should integrate advanced technologies like multi-omics and synthetic biology to expand its applications, offering solutions for health, energy, and environmental sustainability.

3. Pathogenic *E. coli*: Prevalence and challenges

3.1 Overview of important strains

Pathogenic *E. coli* is capable of causing various diseases in humans and animals. Certain pathogenic strains carrying specific virulence factors may cause intestinal or extraintestinal infections through different mechanisms [29]. Based on their pathogenic mechanisms, clinical manifestations, and epidemiological characteristics, pathogenic *E. coli* in the gut can be primarily categorized into six types: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteroaggregative *E. coli* (EAEC), and diffuse adherent *E. coli* (DAEC). Extraintestinal infections are mainly caused by uropathogenic *E. coli* (UPEC) and neonatal meningitis-causing *E. coli* (NMEC) [30]. These pathogens spread through contaminated water sources, food, or human-to-human contact, causing mild diarrhea to severe complications such as hemolytic uremic syndrome (HUS). In recent years, the emergence of drug-resistant pathogenic *E. coli* has further exacerbated public health challenges. This section will systematically explore the virulence factors, pathogenic mechanisms, epidemiological characteristics, and clinical challenges of three most iconic pathogenic *E. coli* strains, EHEC, ETEC, and UPEC.

3.1.1 EHEC

EHEC is a type of pathogenic *E. coli* that produces Shiga toxin (Stx), primarily causing hemorrhagic colitis and HUS. The most representative serotype is O157:H7, which has been the most common serotype responsible for human disease both domestically and internationally in recent years [31]. However, other serotypes such as O26:H11, O104:H4, O45:H2, O103:H2, O111:H8, and O121:H2 can also cause disease.

Stx, as a protein toxin and also an exotoxin, possesses enterotoxicity, cytotoxicity, and neurotoxicity. It is one of the most potent toxins known today and plays a crucial role in the pathogenesis of EHEC [32]. The toxin can be divided into two categories: Stx1, which can be neutralized by anti-Stx, and Stx2, which cannot be neutralized by anti-Stx. Among them, Stx2 has a much higher cytotoxicity to human glomerular microvascular endothelial cells than Stx1, with its half lethal dose (LD₅₀) being only 1/400 that of Stx1. In the development of hemorrhagic enteritis and hemolytic uremic syndrome, the role of Stx2 is even more critical [33]. Stx-induced cytotoxicity begins with ribosomal inactivation and free A-subunit toxicity, progressing to endothelial damage in the gut and kidneys [34].

Due to the close association between Stx and EHEC pathogenesis, in scientific literature, when discussing related strains, the terms Shiga toxin-producing *E. coli* (STEC) and EHEC are frequently used interchangeably [35]. However, although they appear similar, there are fundamental differences between the two. According to the authoritative definition by the Food and Agriculture Organization of the United Nations, STEC refers to strains containing the Stx gene (one or more) encoding Stx but not necessarily carrying other virulence genes. EHEC, on the other hand, is defined as strains that contain both the Stx gene and an adhesion factor [36]. In most cases, once the human body ingests EHEC strains, it will trigger a series of symptoms. When the condition is mild, it manifests as bloody diarrhea and hemorrhagic colitis. If the condition worsens, it can even develop into a potentially fatal HUS [37].

3.1.2 ETEC

ETEC is one of the most common causes of diarrhea. It frequently occurs in the colon of both domestic and wild animals, even humans, leading to the death of newborn calves and piglets due to severe neonatal diarrhea, meanwhile posing a significant health threat to people in low-income and middle-income countries [38].

ETEC produces various adhesins, such as CFA/I and CFA/II, which are flagellum-like structures. These adhesins can specifically recognize and bind to receptors on the surface of small intestinal epithelial cells, allowing bacteria to adhere tightly to the intestinal mucosa [39]. This process helps ETEC resist the flushing action of intestinal peristalsis and digestive fluids, enabling it to colonize the small intestine and create conditions for subsequent pathogenic processes.

Exotoxins are crucial for the ability of ETEC to proliferate within the host and spread to other potential hosts. Different ETEC strains have multiple combinations of exotoxins, which determine their overall virulence, with the most significant being heat-labile toxins and heat-stable toxins [40]. Heat-labile toxins bind to receptors on the surface of small intestinal epithelial cells, activating adenylate cyclase, increasing intracellular cyclic adenosine monophosphate levels, inhibiting the absorption of sodium, chloride, and water by the intestinal mucosa, promoting secretion of intestinal fluid, and causing diarrhea. Heat-stable toxins bind to guanylate cyclase receptors

on small intestinal epithelial cells, increasing intracellular 3'-5'-cyclic guanosine monophosphate levels, affecting ion transport, promoting secretion of intestinal fluid, and triggering diarrhea [41].

3.1.3 UPEC

UPEC belongs to the extraintestinal pathogenic *E. coli*, rather than the traditional intestinal pathogenic *E. coli*. UPEC is generally recognized as the primary pathogen causing urinary tract infections [42].

The pili of UPEC assists in adhering to epithelial cells, allowing the bacteria to firmly attach to urinary tract tissues and avoid being washed away by urine, thus achieving colonization in the urinary system. Subsequently, periodic morphological changes occur, such as filamentation. This process not only helps evade the body's immune system but can also lead to false-negative test results, playing a crucial role in the development of urinary tract infections [43]. Additionally, UPEC has the ability to secrete extracellular polysaccharides and other substances, which can aggregate to form a biofilm that tightly encapsulates the bacteria. The microenvironment created by the biofilm provides excellent protection for the bacteria, enhancing their resistance to the host's immune defense system, making it difficult for immune cells to approach and eliminate them. On the other hand, the biofilm can impede antibiotic penetration, preventing the complete eradication of bacteria, ultimately leading to chronic infections with frequent recurrence, significantly increasing the difficulty of treatment [44].

The virulence factors of UPEC mainly include α -hemolysin and cytotoxin-associated protein. The α -hemolysin is encoded by the *hlyA* gene and secreted outside the cell via the Type I secretion system. It acts as a pore-forming toxin by recognizing and binding to host cell membrane receptors. It then inserts into the cell membrane to form transmembrane pores, cause the leakage of intracellular ions and small molecules, disrupt the ion and osmotic balance within the cell, and finally lead to cell swelling and rupture [45].

Cytotoxin-associated protein consists of three subunits: CdtA, CdtB, and CdtC. Among these, CdtB is the active subunit with nuclease activity. After binding to surface receptors on the host cell, it enters the cell through endocytosis, and CdtB is transported to the nucleus where it cleaves host DNA, inducing double-strand breaks in DNA, activating signaling pathways that lead to cell cycle arrest and apoptosis. It also disrupts the urinary tract mucosal barrier, interferes with immune cell function, and facilitates the survival and proliferation of UPEC within the host [46].

3.2 Epidemiology and health effects of pathogenic *E. coli*

Pathogenic *E. coli* is widely prevalent globally, with its primary sources of infection being carriers and patients. These individuals excrete large amounts of bacteria through their feces, contaminating water sources, food, and everyday items. Foodborne transmission is the most common route. For instance, consuming undercooked contaminated meat, eggs, dairy products, and other items can easily lead to infection [47]. Additionally, close contact between people or touching contaminated objects also facilitates the spread of pathogenic *E. coli*. Children, the elderly, and individuals with weakened immune systems, are more susceptible and at higher risk of infection [48]. Some strains may also be more likely to infect certain genders.

For example, UPEC, due to anatomical factors such as a shorter urethra which facilitates bacterial entry into the bladder, women have a significantly higher risk of urinary tract infections [49].

Pathogenic *E. coli* is a type of intestinal bacterium that poses a serious threat to human health. Based on their pathogenic mechanisms, they can be categorized into several types, each causing varying degrees of health issues. In addition to the three strains highlighted earlier, there are many other pathogenic *E. coli* strains. For example, EIEC can invade the intestinal mucosal epithelial cells, proliferate extensively within the cells, and destroy them, triggering inflammatory responses. Patients may experience fever, tenesmus, abdominal pain, bloody stools, and other symptoms similar to those of bacterial dysentery, severely affecting normal intestinal function [50]. EAEC grows in clusters within the intestine, producing toxins and invasive proteins that damage the microvilli structure of the intestine. They could impact nutrient absorption and cause persistent diarrhea, vomiting, dehydration, and long-term infection that can lead to delayed growth and development in children [51]. DAEC can adhere to intestinal epithelial cells in a diffuse manner, potentially associated with chronic diarrhea and urinary tract infections. Some strains may also cause bacteremia, affecting the entire body [52].

Such infections exhibit significant differences across regions. In developing countries, ETEC, EPEC, and EAEC strains often cause severe diarrhea in infants and young children, which can be fatal without timely medical intervention, whereas cases in developed countries typically have milder symptoms and are easier to manage [53]. Notably, recent trends show a new distribution of infection types, with EHEC and EAEC gradually becoming the primary pathogenic strains. Infections caused by these pathogens are significantly associated with foodborne disease outbreaks in industrialized nations [54].

3.3 Clinical challenges of pathogenic *E. coli*

Pathogenic *E. coli* presents numerous challenging issues in clinical practice. In terms of diagnosis, due to the varied pathogenic mechanisms and clinical manifestations of different types of pathogenic *E. coli*, watery diarrhea caused by enterotoxigenic *E. coli* can resemble symptoms of other viral infections. Hemorrhagic diarrhea caused by enterohemorrhagic *E. coli* is also easily confused with other intestinal diseases [55].

Moreover, some strains grow slowly or are difficult to culture under routine conditions, making rapid and accurate diagnosis extremely challenging. In treatment, antibiotics were once the primary means of therapy, but in recent years, the problem of antibiotic resistance has become increasingly severe, especially with the emergence of extended-spectrum β -lactamase (ESBL) and carbapenemase, which render common antibiotics ineffective [56]. The misuse of antibiotics may also induce enterohemorrhagic *E. coli* to release more toxins, increasing the risk of severe complications such as hemolytic uremic syndrome. Once cross-infection occurs in public places like hospitals, it can spread rapidly. Additionally, some patients carry the bacteria for extended periods without showing symptoms, making asymptomatic carriers difficult to detect, all of which pose significant challenges in controlling the source of infection [57].

Evaluating how to use vaccines to control *E. coli* would not be an easy task. Specific toxins and colonization factors may lead to ineffective prevention [58]. Accurately identifying enterotoxins and colonization factors is hard to achieve mainly due to the

extensive antigenic diversity of pathogenic strains and host-specific variations, which complicate broad-spectrum protection [59].

4. Emergence of drug-resistant *E. coli*

4.1 Global threat of carbapenem-resistant *E. coli*

Carbapenem antibiotics, such as imipenem and meropenem, are crucial in treating infections caused by multidrug-resistant Gram-negative bacteria, which were often considered the “last line of defense” due to their broad-spectrum activity [60]. However, the global dissemination of carbapenem-resistant *Enterobacteriaceae* began following the initial detection of New Delhi metallo- β -lactamase (NDM)-producing bacteria in India in 2009. The rapid spread of the NDM gene via mobile genetic elements, such as plasmids, has led to a surge in resistant strains within healthcare settings [61]. The proliferation of carbapenem-resistant *E. coli* has compelled clinicians to increasingly rely on alternative agents, including polymyxins and tigecycline. However, resistance to these drugs is also escalating, potentially leading to a “no-drug-available” scenario [62].

Resistance genes like *bla*_{NDM} and *bla*_{OXA-48} are typically located on transferable plasmids which spread to different bacterial species through direct bacterial contact. NDM requires zinc ions for activity and rapidly degrades the structure of carbapenems, penicillins, and other drugs, rendering them ineffective [63]. These genes can even be transmitted from animals to humans via environmental vectors such as flies and sewage. Furthermore, NDM genes are constantly mutating (*bla*_{NDM-1} to *bla*_{NDM-9} for instance), making bacteria more resistant to newly developed antibiotics and increasing the difficulty of treatment [64].

Within hospital settings, intensive care units and surgical wards represent high-risk areas. The transmission of antimicrobial-resistant bacteria occurs through medical devices, hand contact by healthcare personnel, or patient transfer [65]. *E. coli* cells readily accumulate multiple resistance genes, serving as a primary transmission vector [66]. Currently, variants of the NDM gene have been identified in many countries worldwide, and international travel, medical activities, and food trade accelerate the transnational spread of resistant bacteria, posing a threat to both human and animal health [67].

Carbapenem-resistant *E. coli* infections lead to prolonged hospital stays and increased treatment costs. To address these challenges, a global antimicrobial resistance gene surveillance system should be established to track transmission pathways using genomic sequencing technologies [68]. In addition, developing inhibitors which target NDM enzymes [69] or utilize novel therapies like phage, antibacterial peptides would also be alternative strategies [70].

4.2 Mechanisms of antimicrobial resistance

The primary mechanism of bacterial resistance to carbapenem antibiotics involves the production of carbapenemases. These enzymes are categorized into three main types. Type 1 enzymes (KPC, IMI) exhibit resistance to inhibition by clavulanic acid or tazobactam, but are susceptible to novel inhibitors such as boronic acid derivatives. These enzymes are prevalent in the United States, Europe, and China. Type 2 enzymes are metallo- β -lactamases (NDM, VIM, IMP) that require zinc ions for activity. They hydrolyze carbapenems but do not affect aztreonam. For instance, NDM-1

has spread extensively in India and Southeast Asia, while VIM is common in several European countries. Type 3 enzymes, exemplified by OXA-48, are predominantly found in Turkey and the Middle East. These enzymes demonstrate robust activity against penicillins and are generally insensitive to most inhibitors [71].

Antimicrobial resistance (AMR) genes are horizontally transferred between bacterial species via mobile genetic elements. For instance, plasmids, such as IncF and IncP1 types, carrying genes like *bla*_{KPC-2} and *bla*_{NDM-1}, facilitate direct bacterial conjugation [72]. Global surveillance indicates that NDM-producing strains have disseminated through international travel and healthcare activities.

4.3 Innovative therapies and strategies

A recent study in Chinese neonatal intensive care units has revealed that *E. coli* exhibits an uppermost 56.36% resistance rate to β -lactam antibiotics, primarily due to the widespread dissemination of *bla*_{CTX-M} and *bla*_{TEM} genes [73]. Consequently, stringent restrictions on the overuse of carbapenems are warranted, alongside the promotion of antimicrobial stewardship programs.

Researchers are actively developing adjunctive therapies to augment the efficacy of existing antibiotics. For example, nitazoxanide enhances the bactericidal effect of polymyxin B against resistant *E. coli* through the inhibition of bacterial fimbriae formation [74]. Furthermore, novel β -lactamase inhibitors, such as ebselen derivatives, in combination with meropenem, significantly improve the clearance of NDM-1-producing *Enterobacteriaceae* strains [75]. New strategies such as antimicrobial peptides, fecal transplantation, and CRISPR-Cas system-mediated drug-resistant gene knock-out have also entered the preclinical research stage [76].

Broad-spectrum antibiotics disrupt the normal gut microbiota, predisposing individuals to colonization by carbapenem-resistant *Enterobacteriaceae*. Research indicates that probiotic supplementation or modulation of gut microbiota may mitigate resistant *E. coli* proliferation [77]. Probiotics were believed to increase short-chain fatty acids, thereby inhibiting *E. coli* replication. Animal studies suggest that microbiome therapy is able to reduce the abundance of antibiotic-resistant bacteria in the gut, offering novel strategies for infection prevention [78].

Finally, enhanced surveillance and stewardship are crucial for controlling the dissemination of antimicrobial resistance. Whole-genome sequencing (WGS) can track the transmission pathways of resistance genes. During the outbreak of EHEC O104:H4 in Germany in 2011, WGS technology determined the source of the epidemic in a short time, which provided a key basis for the formulation of prevention and control measures [79]. The concept of “One Health” emphasizes the coordinated intervention of human beings, animals, and the environment, which is particularly important for controlling the spread of drug-resistant *E. coli*.

5. Conclusion

As a model organism of microbiology research, *E. coli* shows unique dual characteristics in nature and host. In the intestines of healthy mammals, symbiotic *E. coli* plays an indispensable ecological function. These strains participate in the biosynthesis of various vitamins, competitively inhibit the colonization of pathogenic microorganisms, and promote the development and maturity of the host immune system through continuous antigen stimulation [80]. They also participate in the energy

metabolism process of the host, helping to decompose complex polysaccharides and produce beneficial metabolites such as short-chain fatty acids [81]. In the field of biotechnology, *E. coli* maintains the status of “microbial workhorse”. After decades of optimization, its protein expression system has become the preferred platform for industrial production of recombinant proteins. In recent years, more complex proteins such as antibody fragment, vaccine antigen, and enzyme preparation have also been industrialized [82]. Additionally, by introducing exogenous metabolic pathways, engineered strains can produce high value-added compounds like amorphaadiene, greatly reducing the production cost of this antimalarial drug [83].

The environmental adaptability of *E. coli* is an important basis for its dual characteristics. It can survive in a variety of environmental conditions, and this metabolic flexibility makes it an ideal model for studying bacterial environmental adaptation [84]. On the other hand, this adaptability has also brought severe public health challenges. Pathogenic *E. coli* strains with specific virulence factors can lead to serious infectious diseases. Among them, EHEC O157:H7 and UPEC are of greatest concern, because of their high prevalence rate and mortality rate. The emergence and spread of resistant *E. coli* strains has become a global health threat [85]. Through horizontal gene transfer, *E. coli* can quickly acquire new characters. The evolution of EHEC is a typical example, whose virulence genes mainly come from phage and virulence plasmid. This genomic plasticity not only explains its pathogenic variation, but also makes it an ideal platform for genetic engineering and synthetic biology research [86].

In the future, research regarding *E. coli* will focus on in-depth analysis of pathogenic mechanism with the help of single-cell sequencing and spatial transcriptomics, and the development of safer tools for gene manipulation. By leveraging cutting-edge technologies, research can transition to dynamic, spatially resolved, and single-cell mechanistic maps of *E. coli* pathogenesis, accelerating precision antimicrobials and host-directed therapies. Particularly, protein design assisted by artificial intelligence will optimize enzyme activity produced by *E. coli*. Moreover, engineered *E. coli* may become a “living drug”, which colonize the intestine and continuously secrete therapeutic proteins [87].

To sum up, the dual characteristics of *E. coli* bring both challenges and opportunities. Global cooperation is needed to monitor drug resistance. While we should also continue to tap its potential in biotechnology. Interdisciplinary integration will promote its greater contribution in the fields of environment, health, and industry (Table 1).

Useful biological tool	A key component of the normal microbiota
	Biological model for studying responses to varying growth conditions and ecological niches
	Biopharmaceuticals synthesis
	Biocatalyst for biodiesel production
	Targeted delivery of drugs
	Environmental remediation
Notorious pathogen	Intestinal or extraintestinal infection
	Urinary tract infection
	Severe complications such as hus

Table 1.
Dual nature of E. coli.

Acknowledgements

We thank the financial support by the Research Program of Qilu Institute of Technology (Grant No. QIT25TP001).

Conflict of interest

The authors declare no conflict of interest.

CRedit authorship contribution statement

Hui Chen: conceptualization and supervision. Yaqi Zhao: writing—original draft of Section 4. Zixuan Yan: writing—original draft of Section 3. Tianran Zhao: writing—original draft of Section 1. Yuge Liu: writing—original draft of Section 5. Lanxi Zhang: writing—original draft of Section 2. Ping Zeng: conceptualization, writing—review & editing, supervision, and funding acquisition.

Author details

Hui Chen^{1*}, Yaqi Zhao^{1†}, Zixuan Yan^{1†}, Tianran Zhao^{1†}, Yuge Liu^{1†}, Lanxi Zhang^{1†} and Ping Zeng^{1,2*}

1 College of Biological and Chemical Engineering, Qilu Institute of Technology, Jinan, China

2 School of Pharmacy, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, Hong Kong

*Address all correspondence to: 13162892216@163.com; pingzeng@qlit.edu.cn and pingzeng@cuhk.edu.hk

† These authors contributed equally to this chapter.

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Vila J, Saez-Lopez E, Johnson JR, Romling U, Dobrindt U, Canton R, et al. *Escherichia coli*: An old friend with new tidings. FEMS Microbiology Reviews. 2016;**40**(4):437-463
- [2] Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal *Escherichia coli*. Nature Reviews. Microbiology. 2010;**8**(3):207-217
- [3] Last D, Hasan M, Rothenburger L, Braga D, Lackner G. High-yield production of coenzyme F(420) in *Escherichia coli* by fluorescence-based screening of multi-dimensional gene expression space. Metabolic Engineering. 2022;**73**:158-167
- [4] Mahalik S, Sharma AK, Mukherjee KJ. Genome engineering for improved recombinant protein expression in *Escherichia coli*. Microbial Cell Factories. 2014;**13**:177
- [5] Blount ZD. The unexhausted potential of *E. Coli*. eLife. 2015;**4**:e05826
- [6] Ye Z, Shi B, Huang Y, Ma T, Xiang Z, Hu B, et al. Revolution of vitamin E production by starting from microbial fermented farnesene to isophytol. Innovations. 2022;**3**(3):100228
- [7] Leech J, Golub S, Allan W, Simmons MJH, Overton TW. Non-pathogenic *Escherichia coli* biofilms: Effects of growth conditions and surface properties on structure and curli gene expression. Archives of Microbiology. 2020;**202**(6):1517-1527
- [8] Niazi SK, Magoola M. Advances in *Escherichia coli*-based therapeutic protein expression: Mammalian conversion, continuous manufacturing, and cell-free production. Biologics. 2023;**3**(4):380-401
- [9] Du F, Liu YQ, Xu YS, Li ZJ, Wang YZ, Zhang ZX, et al. Regulating the T7 RNA polymerase expression in *E. Coli* BL21 (DE3) to provide more host options for recombinant protein production. Microbial Cell Factories. 2021;**20**(1):189
- [10] Holcomb DA, Knee J, Sumner T, Adriano Z, de Bruijn E, Nala R, et al. Human fecal contamination of water, soil, and surfaces in households sharing poor-quality sanitation facilities in Maputo, Mozambique. International Journal of Hygiene and Environmental Health. 2020;**226**:113496
- [11] Denamur E, Clermont O, Bonacorsi S, Gordon D. The population genetics of pathogenic *Escherichia coli*. Nature Reviews. Microbiology. 2021;**19**(1):37-54
- [12] Braz VS, Melchior K, Moreira CG. *Escherichia coli* as a multifaceted pathogenic and versatile bacterium. Frontiers in Cellular and Infection Microbiology. 2020;**10**:548492
- [13] Sora VM, Meroni G, Martino PA, Soggiu A, Bonizzi L, Zecconi A. Extraintestinal pathogenic *Escherichia coli*: Virulence factors and antibiotic resistance. Pathogens. 2021;**10**(11):1355
- [14] Roy A, Miyai Y, Rossi A, Paraswar K, Desai UR, Saijoh Y, et al. Metabolic engineering of non-pathogenic *Escherichia coli* strains for the controlled production of low molecular weight heparosan and size-specific heparosan oligosaccharides. Biochimica et Biophysica Acta - General Subjects. 2021;**1865**(1):129765
- [15] Sonnenborn U, Schulze J. The non-pathogenic *Escherichia coli* strain Nissle 1917—Features of a versatile probiotic.

Microbial Ecology in Health and Disease. 2009;**21**(3-4):122-158

[16] Govindarajan DK, Viswalingam N, Meganathan Y, Kandaswamy K. Adherence patterns of *Escherichia coli* in the intestine and its role in pathogenesis. *Medicine in Microecology*. 2020;**5**:100025

[17] Zargar A, Quan DN, Carter KK, Guo M, Sintim HO, Payne GF, et al. Bacterial secretions of nonpathogenic *Escherichia coli* elicit inflammatory pathways: A closer investigation of interkingdom signaling. *MBio*. 2015;**6**(2):e00025

[18] Yu D, Banting G, Neumann NF. A review of the taxonomy, genetics, and biology of the genus *Escherichia* and the type species *Escherichia coli*. *Canadian Journal of Microbiology*. 2021;**67**(8):553-571

[19] Lynch JP, Goers L, Lesser CF. Emerging strategies for engineering *Escherichia coli* Nissle 1917-based therapeutics. *Trends in Pharmacological Sciences*. 2022;**43**(9):772-786

[20] Wang X, Zhang X, Zhang J, Xiao L, Zhou Y, Wang F, et al. Metabolic engineering of *Escherichia coli* for efficient production of linalool from biodiesel-derived glycerol by targeting cofactors regeneration and reducing acetate accumulation. *Industrial Crops and Products*. 2023;**203**:117152

[21] Lou X, Chen Z, He Z, Sun M, Sun J. Bacteria-mediated synergistic cancer therapy: Small microbiome has a big hope. *Nano-Micro Letters*. 2021;**13**(1):37

[22] Tanniche I, Behkam B. Engineered live bacteria as disease detection and diagnosis tools. *Journal of Biological Engineering*. 2023;**17**(1):65

[23] Li X, Wang Y, Wang Y, Xie H, Gong R, Wu X, et al. Anti-tumor activity of an alphaPD-L1-PE38 immunotoxin delivered by engineered Nissle 1917. *International Journal of Biological Macromolecules*. 2025;**295**:139537

[24] Lin S, Tao Z, Li Z, Li S, Wang X, Liu X, et al. Enhancing photocatalytic hydrogen production from engineered *Escherichia coli*-biohybrid system via intracellular electron redirection. *Chemical Engineering Journal*. 2024;**499**:156488

[25] Hu M, Zhang T. Expectations for employing *Escherichia coli* BL21 (DE3) in the synthesis of human milk oligosaccharides. *Journal of Agricultural and Food Chemistry*. 2023;**71**(16):6211-6212

[26] Liu X, Ueki T, Gao H, Woodard TL, Nevin KP, Fu T, et al. Microbial biofilms for electricity generation from water evaporation and power to wearables. *Nature Communications*. 2022;**13**(1):4369

[27] Goveas LC, Nayak S, Kumar PS, Vinayagam R, Selvaraj R, Rangasamy G. Recent advances in fermentative biohydrogen production. *International Journal of Hydrogen Energy*. 2024;**54**:200-217

[28] Sharma S, Pathania S, Bhagta S, Kaushal N, Bhardwaj S, Bhatia RK, et al. Microbial remediation of polluted environment by using recombinant *E. Coli*: A review. *Biotechnology Environmental*. 2024;**1**(1):8

[29] Pakbin B, Bruck WM, Rossen JWA. Virulence factors of enteric pathogenic *Escherichia coli*: A review. *International Journal of Molecular Sciences*. 2021;**22**(18):9922

[30] Kaper JB, Nataro JP, Mobley HL. Pathogenic *Escherichia coli*.

Nature Reviews. Microbiology. 2004;**2**(2):123-140

[31] Zeng P, Yi L, Cheng Q, Liu J, Chen S, Chan K-F, et al. An ornithine-rich dodecapeptide with improved proteolytic stability selectively kills gram-negative food-borne pathogens and its action mode on *Escherichia coli* O157:H7. International Journal of Food Microbiology. 2021;**352**:109281

[32] Melton-Celsa AR. Shiga toxin (Stx) classification, structure, and function. Microbiol Spectrum. 2014;**2**(4):EHEC-0024-2013

[33] Basu D, Tumer NE. Do the a subunits contribute to the differences in the toxicity of Shiga toxin 1 and Shiga toxin 2? Toxins. 2015;**7**(5):1467-1485

[34] Lee KS, Jeong YJ, Lee MS. *Escherichia coli* Shiga toxins and gut microbiota interactions. Toxins. 2021;**13**(6):416

[35] Hunt JM. Shiga toxin-producing *Escherichia coli* (STEC). Clinics in Laboratory Medicine. 2010;**30**(1):21-45

[36] Monteiro R, Ageorges V, Rojas-Lopez M, Schmidt H, Weiss A, Bertin Y, et al. A secretome view of colonisation factors in Shiga toxin-encoding *Escherichia coli* (STEC): From enterohaemorrhagic *E. Coli* (EHEC) to related enteropathotypes. FEMS Microbiology Letters. 2016;**363**(16):fnw179

[37] Goldwater PN, Bettelheim KA. Treatment of enterohemorrhagic (EHEC) infection and hemolytic uremic syndrome (HUS). BMC Medicine. 2012;**10**:12

[38] von Mentzer A, Connor TR, Wieler LH, Semmler T, Iguchi A, Thomson NR, et al. Identification of enterotoxigenic *Escherichia coli* (ETEC)

clades with long-term global distribution. Nature Genetics. 2014;**46**(12):1321-1326

[39] Duan Q, Pang S, Feng L, Liu J, Lv L, Li B, et al. Heat-labile enterotoxin enhances F4-producing enterotoxigenic *E. Coli* adhesion to porcine intestinal epithelial cells by upregulating bacterial adhesins and STb enterotoxin. Veterinary Research. 2022;**53**(1):88

[40] Liu J, Jokiranta TS, Carlin N, Stroup S, Zhang JX, Sjostrand B, et al. Use of a TaqMan Array card for identification of enterotoxins and colonization factors directly from stool samples in an enterotoxigenic vaccine study. Microbiology Spectrum. 2025;**13**(3):e01870-e01824

[41] Rodriguez-Martinez R, Ochoa SA, Valle-Rios R, Jaimes-Ortega GA, Hernandez-Castro R, Mancilla-Rojano J, et al. Genome sequencing and assembly of enterotoxigenic *Escherichia coli* E9034A: Role of LngA, CstH, and FliC in intestinal cell colonization and the release of the proinflammatory cytokine IL-8. Microorganisms. 2025;**13**(2):374

[42] Sharma K, Dhar N, Thacker VV, Simonet TM, Signorino-Gelo F, Knott GW, et al. Dynamic persistence of UPEC intracellular bacterial communities in a human bladder-chip model of urinary tract infection. eLife. 2021;**10**:e66481

[43] Fang YW, Tao S, Chen HM, Xu Y, Chen LY, Liang W. Influence of bacterial morphotype on urine culture and molecular epidemiological differences in harboring bacterial morphotype-induced urinary tract infections. Microbiology Spectrum. 2025;**13**(4):e00980-e00924

[44] Naziri Z, Kilegolan JA, Moezzi MS, Derakhshandeh A. Biofilm formation by uropathogenic *Escherichia coli*: A complicating factor for treatment and

recurrence of urinary tract infections. The Journal of Hospital Infection. 2021;117:9-16

[45] Colautti A, Orecchia E, Comi G, Iacumin L. Lactobacilli, a weapon to counteract pathogens through the inhibition of their virulence factors. Journal of Bacteriology. 2022;204(11):e00272-e00222

[46] Carlini F, Maroccia Z, Fiorentini C, Travaglione S, Fabbri A. Effects of the *Escherichia coli* bacterial toxin cytotoxic necrotizing factor 1 on different human and animal cells: A systematic review. International Journal of Molecular Sciences. 2021;22(22):12610

[47] Yi L, Zeng P, Liu J, Wong K-Y, Chan EW-C, Lin Y, et al. Antimicrobial peptide zp37 inhibits *Escherichia coli* O157:H7 in alfalfa sprouts by inflicting damage in cell membrane and binding to DNA. LWT. 2021;146:111392

[48] Bonten M, Johnson JR, van den Beggelaar AHJ, Georgalis L, Geurtsen J, de Palacios PI, et al. Epidemiology of *Escherichia coli* bacteremia: A systematic literature review. Clinical Infectious Diseases. 2021;72(7):1211-1219

[49] Guglietta A. Recurrent urinary tract infections in women: Risk factors, etiology, pathogenesis and prophylaxis. Future Microbiology. 2017;12(3):239-246

[50] Pasqua M, Michelacci V, Di Martino ML, Tozzoli R, Grossi M, Colonna B, et al. The intriguing evolutionary journey of enteroinvasive *E. Coli* (EIEC) toward pathogenicity. Frontiers in Microbiology. 2017;8:2390

[51] Boisen N, Osterlund MT, Joensen KG, Santiago AE, Mandomando I, Cravioto A, et al. Redefining enteroaggregative *Escherichia coli* (EAEC): Genomic characterization

of epidemiological EAEC strains. PLoS Neglected Tropical Diseases. 2020;14(9):e0008613

[52] Javadi K, Mohebi S, Motamedifar M, Hadi N. Characterization and antibiotic resistance pattern of diffusely adherent *Escherichia coli* (DAEC), isolated from paediatric diarrhoea in shiraz, Southern Iran. New Microbes New Infections. 2020;38:100780

[53] Sneha K, Singh T, Dar SA, Haque S, Ramachandran VG, Saha R, et al. Typical and atypical enteropathogenic *Escherichia coli* in children with acute diarrhoea: Changing trend in East Delhi. Biomedical Journal. 2021;44(4):471-478

[54] Gambushe SM, Zishiri OT, El Zowalaty ME. Review of *Escherichia coli* O157:H7 prevalence, pathogenicity, heavy metal and antimicrobial resistance, African perspective. Infection and Drug Resistance. 2022;15:4645-4673

[55] Rani A, Ravindran VB, Surapaneni A, Mantri N, Ball AS. Review: Trends in point-of-care diagnosis for *Escherichia coli* O157:H7 in food and water. International Journal of Food Microbiology. 2021;349:109233

[56] Chen C, Cai J, Shi J, Wang Z, Liu Y. Resensitizing multidrug-resistant gram-negative bacteria to carbapenems and colistin using disulfiram. Communications Biology. 2023;6(1):810

[57] Akbar A, Naeem W, Liaqat F, Sadiq MB, Shafee M, Gul Z, et al. Hospital acquired pathogenic *Escherichia coli* from clinical and hospital water samples of Quetta Balochistan. Journal of Tropical Medicine. 2022;2022:6495044

[58] Khalil I, Walker R, Porter CK, Muhib F, Chilengi R, Cravioto A, et al. Enterotoxigenic *Escherichia coli* (ETEC) vaccines: Priority activities

to enable product development, licensure, and global access. Vaccine. 2021;**39**(31):4266-4277

[59] Kowarik M, Wetter M, Haeuptle MA, Braun M, Steffen M, Kemmler S, et al. The development and characterization of an *E. Coli* O25B bioconjugate vaccine. Glycoconjugate Journal. 2021;**38**(4):421-435

[60] Chinemerem Nwobodo D, Ugwu MC, Oliseloke Anie C, Al-Ouqaili MTS, Chinedu Ikem J, Victor Chigozie U, et al. Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. Journal of Clinical Laboratory Analysis. 2022;**36**(9):e24655

[61] Fu B, Xu J, Yin D, Sun C, Liu D, Zhai W, et al. Transmission of Bla(NDM) in enterobacteriaceae among animals, food and human. Emerging Microbes & Infections. 2024;**13**(1):2337678

[62] Li Y, Zhang Y, Sun X, Wu Y, Yan Z, Ju X, et al. National genomic epidemiology investigation revealed the spread of carbapenem-resistant *Escherichia coli* in healthy populations and the impact on public health. Genome Medicine. 2024;**16**(1):57

[63] Xia C, Yan R, Liu C, Zhai J, Zheng J, Chen W, et al. Epidemiological and genomic characteristics of global blaNDM-carrying *Escherichia coli*. Annals of Clinical Microbiology and Antimicrobials. 2024;**23**(1):58

[64] Lin YC, Kuroda M, Suzuki S, Mu JJ. Emergence of an *Escherichia coli* strain co-harboring mcr-1 and Bla(NDM-9) from a urinary tract infection in Taiwan. Journal of Global Antimicrobial Resistance. 2019;**16**:286-290

[65] Korzeniewska E, Korzeniewska A, Harnisz M. Antibiotic resistant

Escherichia coli in hospital and municipal sewage and their emission to the environment. Ecotoxicology and Environmental Safety. 2013;**91**:96-102

[66] Szmolka A, Nagy B. Multidrug resistant commensal *Escherichia coli* in animals and its impact for public health. Frontiers in Microbiology. 2013;**4**:258

[67] Acman M, Wang R, van Dorp L, Shaw LP, Wang Q, Luhmann N, et al. Role of mobile genetic elements in the global dissemination of the carbapenem resistance gene Bla(NDM). Nature Communications. 2022;**13**(1):1131

[68] Diallo OO, Baron SA, Abat C, Colson P, Chaudet H, Rolain JM. Antibiotic resistance surveillance systems: A review. Journal of Global Antimicrobial Resistance. 2020;**23**:430-438

[69] Wang T, Xu K, Zhao L, Tong R, Xiong L, Shi J. Recent research and development of NDM-1 inhibitors. European Journal of Medicinal Chemistry. 2021;**223**:113667

[70] Zeng P, Wang H, Zhang P, Leung SSY. Unearthing naturally-occurring cyclic antibacterial peptides and their structural optimization strategies. Biotechnology Advances. 2024;**73**:108371

[71] Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. Clinical Microbiology Reviews. 2007;**20**(3):440-458

[72] Fang L, Chen R, Li C, Sun J, Liu R, Shen Y, et al. The association between the genetic structures of commonly incompatible plasmids in gram-negative bacteria, their distribution and the resistance genes. Frontiers in Cellular and Infection Microbiology. 2024;**14**:1472876

- [73] Zhou J, Zhou J, Chen M, Lu P, Jiang C. Prevalence of beta-lactam antibiotic resistance of *Escherichia coli* isolated from a neonatal intensive care unit. *BMC Pediatrics*. 2025;**25**(1):95
- [74] Li W, Ji B, Li B, Du M, Wang L, Tuo J, et al. Nitazoxanide inhibits pili assembly by targeting BamB to synergize with polymyxin B against drug-resistant *Escherichia coli*. *Biochimie*. 2025;**233**:47-59
- [75] Jin WB, Xu C, Cheng Q, Qi XL, Gao W, Zheng Z, et al. Investigation of synergistic antimicrobial effects of the drug combinations of meropenem and 1,2-benzisoxaselenazol-3(2H)-one derivatives on carbapenem-resistant enterobacteriaceae producing NDM-1. *European Journal of Medicinal Chemistry*. 2018;**155**:285-302
- [76] Bikard D, Euler CW, Jiang W, Nussenzweig PM, Goldberg GW, Duportet X, et al. Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nature Biotechnology*. 2014;**32**(11):1146-1150
- [77] Mousa WK, Mousa S, Ghemrawi R, Obaid D, Sarfraz M, Chehadeh F, et al. Probiotics modulate host immune response and interact with the gut microbiota: Shaping their composition and mediating antibiotic resistance. *International Journal of Molecular Sciences*. 2023;**24**(18):13783
- [78] Caprarulo V, Turin L, Hejna M, Reggi S, Dell'Anno M, Riccaboni P, et al. Protective effect of phytogetic plus short and medium-chain fatty acids-based additives in enterotoxigenic *Escherichia coli* challenged piglets. *Veterinary Research Communications*. 2023;**47**(1):217-231
- [79] Karch H, Denamur E, Dobrindt U, Finlay BB, Hengge R, Johannes L, et al. The enemy within us: Lessons from the 2011 European *Escherichia coli* O104:H4 outbreak. *EMBO Molecular Medicine*. 2012;**4**(9):841-848
- [80] Kamada N, Seo SU, Chen GY, Nunez G. Role of the gut microbiota in immunity and inflammatory disease. *Nature Reviews. Immunology*. 2013;**13**(5):321-335
- [81] Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. *Nature Reviews. Immunology*. 2016;**16**(6):341-352
- [82] Huleani S, Roberts MR, Beales L, Papaioannou EH. *Escherichia coli* as an antibody expression host for the production of diagnostic proteins: Significance and expression. *Critical Reviews in Biotechnology*. 2022;**42**(5):756-773
- [83] Shukal S, Chen X, Zhang C. Systematic engineering for high-yield production of viridiflorol and amorphadiene in auxotrophic *Escherichia coli*. *Metabolic Engineering*. 2019;**55**:170-178
- [84] Karve SM, Bhawe D, Nevgi D, Dey S. *Escherichia coli* populations adapt to complex, unpredictable fluctuations by minimizing trade-offs across environments. *Journal of Evolutionary Biology*. 2016;**29**(12):2545-2555
- [85] Gati NS, Temme IJ, Middendorff-Bauchart B, Kehl A, Dobrindt U, Mellmann A. Comparative phenotypic characterization of hybrid Shiga toxin-producing / uropathogenic *Escherichia coli*, canonical uropathogenic and Shiga toxin-producing *Escherichia coli*. *International Journal of Medical Microbiology*. 2021;**311**(7):151533
- [86] Rodriguez-Rubio L, Haarmann N, Schwidder M, Muniesa M, Schmidt H.

Bacteriophages of Shiga toxin-producing *Escherichia coli* and their contribution to pathogenicity. *Pathogens*. 2021;**10**(4):404

[87] Hashemi A, Basafa M, Behravan A. Machine learning modeling for solubility prediction of recombinant antibody fragment in four different *E. Coli* strains. *Scientific Reports*. 2022;**12**(1):5463

From Gut Flora to Global Threat: The Evolutionary Story of *Escherichia coli*

*Kavita Gupta, Monalisa Mohanty, Punyatoya Kar
and Gayatree Nayak*

Abstract

Escherichia coli (*E. coli*), earlier considered a harmless resident of gut microbiota, has evolved into a global threat. It typically establishes itself in the gastrointestinal tract shortly after birth, forming part of the normal intestinal flora. Commensal *E. coli* reside in the mucus layer of the colon. However, under certain circumstances, such as in the presence of foreign bodies, anatomical or functional abnormalities, or a large and diverse bacterial load, these otherwise harmless strains can contribute to extraintestinal infections. The acquisition of virulence factors over time has led to the advent of various pathotypes of *E. coli*, such as intestinal and extraintestinal pathogenic *E. coli* (InPEC and ExPEC). InPEC is mainly responsible for foodborne illness, and ExPEC causes urinary tract infections, sepsis, and neonatal meningitis. The rise of antimicrobial-resistant strains is an alarming aspect of the evolution of *E. coli*, complicating treatment and increasing mortality. Food contamination, agricultural antibiotic overuse, and transcontinental travel facilitate its spread. Genomic research, antibiotic stewardship, continuous AMR surveillance, vaccines, promotion of alternative treatment approaches (e.g., bacteriophage, CRISPR-based therapy, probiotics, etc.), and hand hygiene and sanitation practice are the cornerstones in curbing its impact.

Keywords: commensal *Escherichia coli*, intestinal and extraintestinal pathogenic *E. Coli* (InPEC and ExPEC), AMR surveillance, genomic research, new treatment approaches

1. Introduction

Escherichia coli (*E. coli*), a forerunner of the human gut and often colonizes newborns shortly after birth. *E. coli* is almost exclusively a nonpathogenic bacterium, and it constitutes the intestinal microbiome of over 90% of individuals [1]. It is a facultative anaerobe and may help in reducing oxygen levels inside the mucosa of the gastrointestinal (GI) tract, mucosal, and thus it creates a friendly environment for colonization by anaerobic bacteria. *Escherichia coli* is one of the most extensively

explored bacterial species. *Escherichia coli* (*E. coli*), once a harmless commensal of gut microbiota, has evolved into a global health threat through genetic adaptation and acquisition of antimicrobial resistance (AMR). However, the acquisition of virulence factors over time has led to the emergence of various pathotypes of *E. coli*, such as intestinal and extraintestinal pathogenic *E. coli* (InPEC and ExPEC). While InPEC is mainly responsible for foodborne illness, ExPEC causes meningitis among neonates, sepsis, and urinary tract infections. The rise of antimicrobial-resistant strains (ESBL, CRE) is another alarming aspect of the evolution of *E. coli*, complicating treatment and increasing mortality. Food contamination, agricultural antibiotic overuse, and transcontinental travel facilitate its spread. Genomic research, antibiotic stewardship, continuous AMR surveillance, vaccines, promotion of alternative treatment approaches (e.g., bacteriophage, CRISPR-based therapy, probiotics, etc.), hand hygiene, and sanitation practice are the cornerstones in curbing its impact. Strangely, there is a paucity of information about the dynamics of the evolution of *E. coli* available in the literature.

2. *E. coli* residency in human gut

The human gastrointestinal tract contains approximately 10^{14} microorganisms consisting of more than 10^3 bacterial species, viruses, and fungi that form our gut microbiota. The complex interaction between these organisms maintains the integrity of gut health. In the human gut microbiota, the *Enterobacteriaceae* family commonly constitutes less than 1%. Different bacteria, such as *Escherichia*, *Enterobacter*, *Klebsiella*, etc., are present in the family, among which *E. coli* is shown to be, by far, the most dominant *Enterobacteriaceae* in healthy humans [2]. *E. coli* is the first bacterial species that colonizes the infant intestine. It starts appearing in the feces of newborns only after a few days of birth, indicating the establishment of the intestinal microflora. The source of *E. coli* is typically the maternal fecal flora during delivery; other plausible sources may be the maternity ward—particularly through nursing staff during high loads of patients—and the formula feed given to the infant. Some of these *E. coli* strains remain in the intestine of individuals for months to years and behave as resident strains, while the transient strains disappear within a few weeks [3]. The inhabitant *E. coli* strains have certain properties that help them to survive in the gut, such as expression of P fimbriae and adherence capabilities to the lumen of the intestine. The structure of P fimbriae comprises a fimbrial rod with an adhesin tip of one of three varieties, namely papG classes I, II, and III. The class II variety is linked to intestinal persistence. In addition, the inhabitant *E. coli* strains more commonly possess other virulent genes like capsular K1 and K5, aerobactin, and hemolysin compared to the transient flora. The symbiosis between the resident microflora of the GI tract and the host leads to several important consequences for human health and physiology. On one end, the positive interactions can have beneficial nutritional, developmental, and immunological effects, while on the other hand, various conditions such as cystic fibrosis, Crohn's disease (CD), metabolic diseases like diabetes, obesity, etc. cause dysbiosis in the gut microbiota leading to various detrimental effects on host like expansion of pathogenic strains of *E. coli* [4].

2.1 Spectrum of *E. coli* in human host/pathotypes

The widespread presence of *E. coli* and commensalism in gut microbiota often leads to clinical ambiguity. While it typically exists symbiotically within the

gastrointestinal tract, it is one of the prominent etiological agents responsible for human and animal infections. Pathogenic strains of *E. coli* are implicated in various intestinal disorders acute watery and/or bloody diarrhea in both adult and pediatric groups, persistent diarrhea, colitis, and hemolytic uremic syndrome (HUS). Approximately 11 different diarrheagenic strains of *E. coli*, such as Enterotoxigenic *E. coli* (ETEC), Enteropathogenic *E. coli* (EPEC), Enterohemorrhagic *E. coli* (EHEC), Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), Diffuse adhering *E. coli* (DAEC), and Adherent-invasive *E. coli* (AIEC). It also causes numerous extraintestinal diseases such as urinary tract infections (UTI), attributed to uropathogenic *E. coli* (UPEC), bacteremia and sepsis, neonatal meningitis, and is one of the foremost causes of both community and healthcare-acquired invasive bacterial disease. Details of the pathotypes causing intestinal and extraintestinal disease manifestations are depicted in **Table 1** and **Figure 1** [5]. Apart from this, the presence of thermogenic *E. coli* in the water samples is a biological indicator of fecal contamination while testing the quality of the supply water. *E. coli* is also commonly used as a surrogate organism

Pathotypes	Genetic markers/virulence markers	Clinical features
Intestinal pathogenic <i>E. coli</i> (IPEC/InPEC); diarrheagenic <i>E. coli</i> (DEC)		
Enterotoxigenic <i>E. coli</i> [ETEC]	Heat-labile enterotoxin (LT) and/or heat-stable enterotoxin (ST) (mechanism is similar to cholera toxin); Different colonization factors (Example- CFA/I, CS30, CS6, etc.)	Travelers' diarrhea, mild to severe diarrhea, is frequently seen in children in developing countries.
Enteropathogenic <i>E. coli</i> [EPEC]	Locus for enterocyte effacement (LEE)—intimin (<i>eae</i>); Bundle-forming pilus (BFP)—present in typical EPEC (tEPEC); absent in atypical EPEC (aEPEC)	Lesions are described as attaching and effacing on the surfaces of intestinal epithelial cells; diarrhea is frequently associated with fever, vomiting, and dehydration, mainly in children.
Enteroinvasive <i>E. coli</i> [EIEC]	Invasion plasmid pINV, Enterotoxin present on the chromosomal pathogenicity island, Shiga toxin	Highly invasive; causes bacillary dysentery/shigellosis is associated with fever, damages the walls of the intestine, and may lead to hemolytic uremic syndrome (HUS).
Enterohemorrhagic <i>E. coli</i> (EHEC), Verocytotoxin-producing <i>E. coli</i> (VTEC), Shiga toxin-producing <i>E. coli</i> (STEC)	Shiga toxin 1 or 2 (<i>stx1</i> or <i>stx2</i>), LEE (in very few lineages)	Hemorrhagic colitis (mild to bloody diarrhea) along with fever, vomiting, or abdominal cramps, can result in HUS.
Enteroaggregative <i>E. coli</i> (EAEC)	Virulence plasmids carrying pAA genes for aggregative adherence fimbriae (AAF)	Persistent diarrhea, which may be watery, mucoid, or sometimes bloody, can be accompanied by other symptoms like nausea, abdominal pain, and low-grade fever (may progress to HUS). Travelers' diarrhea may occur. Aggregative adhesion lesion is described as a stacked-brick adherence pattern in HEp-2 cells.
Diffuse adhering <i>E. coli</i> (DAEC)	Afa/Dr. adhesins	Persistent watery diarrhea with abdominal pain, fever, and dehydration. It may be associated with Crohn's disease (CD) and ulcerative colitis (UC).

Pathotypes	Genetic markers/virulence markers	Clinical features
Adherent-invasive <i>E. coli</i> (AIEC)	Inconsistent virulence factors	Able to adhere to and invade epithelial cells; able to survive and replicate within macrophages. More often associated with chronic inflammatory bowel disease, particularly CD and UC.
Extraintestinal pathogenic <i>E. coli</i> (ExPEC)		
Uropathogenic <i>E. coli</i> (UPEC);	No specific virulence factors have been found for any ExPEC pathotype. However, some virulence factors have been suggested to play a role in disease manifestations, like PapGII and PapGIII adhesins in UPEC, K1 capsule and pS88 antigen in NMEC, fimbrial adhesin H (FimH), P fimbriae, hemolysin A, Cnf, etc.	UTIs, cystitis, pyelonephritis, prostatitis
Neonatal meningitis <i>E. coli</i> (NMEC, NEMEC); Meningitis-associated <i>E. coli</i> (MAEC);		Meningitis in newborns
Avian pathogenic <i>E. coli</i> (APEC);		Colibacillosis, septicemia, and cellulitis in poultry
Sepsis-associated <i>E. coli</i> (SEPEC);		bacteremia/sepsis
Unspecified ExPEC		Cutaneous and soft tissue infections, ventilator-associated pneumonia

Table 1.
Pathotypes of E. coli and characteristics.

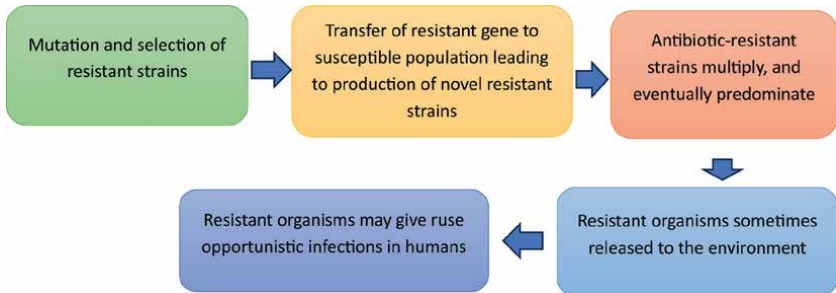


Figure 1.
Effect of exposure of antibiotics on commensal flora.

of Gram-negative bacteria for testing of antibiotic susceptibility, and to evaluate the antibiogram pattern in a given location and time under a surveillance program.

2.2 Gut colonization of antimicrobial-resistant *E. coli*

Escherichia coli (*E. coli*) has received increasing attention due to the emergence and spread of antimicrobial resistance (AMR), contributing to substantial morbidity and fatality [6]. The genetic makeup of commensal *Escherichia coli* is influenced by a complex interplay between host and environmental factors. The presence of virulent genes may help the bacteria adapt to the gut environment, while selection pressures like antibiotic use and microbial competition may incidentally foster the development of virulent traits as well as antimicrobial resistance (AMR), thereby establishing commensal *E. coli* strains as reservoirs for virulence and resistance determinants [6]. Global data indicate that insufficient sanitation, contaminated water, overcrowded

housing, and a lack of hygiene contribute to the rise and transmission of antibiotic-resistant bacteria in humans [7].

2.2.1 Colonization mechanisms

Only a small proportion of microbes ingested through food establish themselves in the gut. Effective colonization relies on several factors, such as the ecological interactions between the resident gut microbiota and the potential colonizing pathogen. Some of the gut bacteria actively secrete toxic compounds that inhibit the growth of other microbes, showing the intense microbial competition within the gut environment. Multidrug-resistant *E. coli* strains, in particular, can exploit a wide array of carbohydrate sources, allowing them to outcompete and displace commensal *E. coli*, emphasizing the critical role of resource competition in colonization success.

The effect of exposure to antibiotics on commensals is depicted in the following flow chart (**Figure 1**) [8].

Lina et al. mentioned that biofilm formation by MDR *E. coli* can be a factor supporting the colonization of the same. Subpopulations of *E. coli* carrying the biofilm-forming genetic locus (*pgaABCD*) and the toxin-antitoxin system (*HipAB*), which appeared after antibiotic treatment, have the ability to withstand the harmful effects of antibiotics, and eventually they become a dominant subspecies in opposition to *E. coli* strains that did not carry *pgaABCD* and *HipAB*.

Theories also say that the human gut acts as a habitat for numerous bacteriophages. As evidenced by animal studies, the process of transduction significantly contributes to the genetic variability of *E. coli* strains and the emergence of antibiotic resistance in gut microbes. Gut dysbiosis has been associated with various metabolic disorders like obesity, hepatic steatohepatitis, and insulin resistance in type 2 diabetes [9].

3. Challenges and implications in clinical settings

Genetic changes increase the virulence of *E. coli* strains. Genetic mutations facilitate immune evasion and contribute to the persistence of infection by allowing the pathogen to acclimate to the host's defense system. The resistant gut colonizers are also associated with the development of hospital-acquired infections influenced by factors such as hospital environment, patient age, sex, and underlying comorbid conditions.

3.1 Therapeutic strategies

- Judicial use of antimicrobial agents
- Use of prebiotics, probiotics, and symbiotics
- Drug repurposing
- Microbiota-directed drug delivery systems like nanoparticles, nanoemulsions.

The integrated use of precision gut supplements, innovative drug delivery technologies, and rational polypharmacy offers a significant potential for effective microbiome management in clinical settings.

4. Genomic evolution of drug resistance in *Escherichia coli*

Antimicrobial resistance (AMR) is the capability of bacterial pathogens to resist the bactericidal properties of antibiotics. This resistance arises from genetic or biochemical changes in bacteria that reduce the effectiveness of antibiotics, chemical agents, or other therapeutic measures used to control infections [10]. The evolutionary trajectory of *E. coli* indicates its potential to become highly pathogenic through the acquisition of fitness advantages, including antimicrobial resistance (AMR) and a range of virulence factors [11]. Currently, multidrug-resistant pathogenic *Escherichia coli* has been categorized as an “urgent threat” by the Centers for Disease Control and Prevention (CDC) and a “critical priority” by the World Health Organization (WHO) [12, 13]. The molecular evolution of resistance traits in the *E. coli* genome represents a major reason for treatment failure in critically ill patients. The emergence and speedy spread of AMR within *E. coli* populations have been driven by complex evolutionary processes, including horizontal gene transfer, spontaneous mutations, and selective pressures exerted by antimicrobial use in human medicine, veterinary practice, and agriculture.

The genetic structure of commensal *Escherichia coli* is formed by a complex host-environment interface. Selective pressures within these environments may incidentally foster the development of virulence factors, alongside AMR, that have transformed commensal *E. coli* strains as key reservoirs for both pathogenic and resistance traits. The extensive use of antimicrobials results in the development of antibiotic resistance in commensal populations.¹ Long-term use, misuse, and abuse of drugs are the basis of resistant mechanisms that ultimately cause difficulties in the prevention as well as treatment of pathogens. The prevalence of AMR was ranked according to the percentage of *E. coli* carrying AMR [14]. The dissemination of antimicrobial resistance genes within bacterial communities is largely facilitated by the interaction of different types of mobile genetic elements (MGEs) [15].

4.1 Antimicrobial resistance

Bacteria have developed several strategies to evade the action of antimicrobial agents. Understanding these resistance mechanisms is essential for the development of effective antimicrobial therapies and for implementing strategies to combat the growing threat of antimicrobial resistance.

The mechanism may be limiting uptake of the drug (decrease antibiotic influx by modifying membrane permeability) or modifying the target of the drug (acquire mutations or enzymatic modifications in antimicrobial targets) or inactivating the drug (production of enzymes which inactivate antimicrobial agents through chemical modification or degradation) or active efflux of the drug (efflux pump in cell membrane actively expels antimicrobial agents from the cell). In multidrug-resistant organisms, more than one mechanism can be seen [16].

4.1.1 Intrinsic vs. acquired resistance

These mechanisms may be either *intrinsic* or *acquired*:

- *Intrinsic resistance*

It is chromosomally mediated and is an important characteristic shared by all members of a bacterial species. It is typically expressed constitutively (at a constant,

usually low level), but in some cases, expression can be upregulated in response to exposure to specific antimicrobial agents, leading to inducible resistance.

- *Acquired resistance*

This results from genetic exchange through horizontal gene transfer mechanisms, such as transformation, transduction, or conjugation. Mobile genetic elements (MGEs) such as plasmids, integrons, and transposons are the most common vectors for transferring resistance genes between bacteria.

4.1.2 Antimicrobial resistance in *E. coli*

Evolutionary paths of *Escherichia coli* reveal that the emergence of highly pathogenic lineages is driven by the acquisition of fitness-enhancing traits such as antimicrobial resistance (AMR) traits and virulence factors, primarily *via* horizontal gene transfer (HGT). Although *E. coli* is intrinsically susceptible to most of the antibiotics but it can also accumulate AMR genes [17]. WHO has selected the extended-spectrum β -lactamase (ESBL)-producing *E. coli* as an indicator for “One Health” AMR surveillance [18].

4.2 Evolutionary dynamics of resistance

- *Role of environmental and clinical factors*

The environment, particularly animal populations, serves as a significant reservoir of clinically relevant antimicrobial resistance (AMR) and offers a crucial context for understanding the ecological consequences of human-driven antimicrobial resistance [19]. **Figures 1** and **2** describe the potential pathways of dissemination of drug resistance in the triad of human, animal, and environment (**Figure 3**) [20, 21].

The association of heavy metals in AMR in *E. coli* is established by two theories, co-resistance and cross-resistance. Mobile genetic elements (MGEs), including

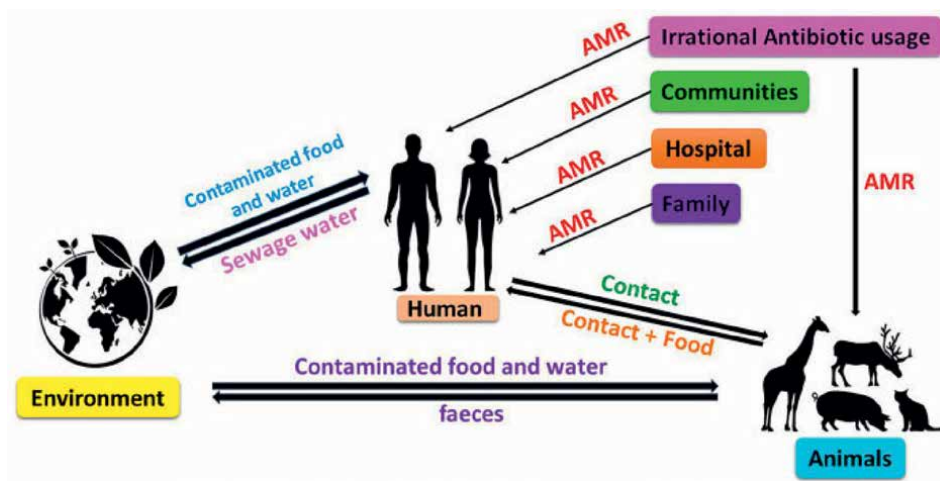


Figure 2.
 Diagrammatic representation for various pathways for the spread of antibiotic-resistant bacteria across reservoirs in humans, animals, and the environment.

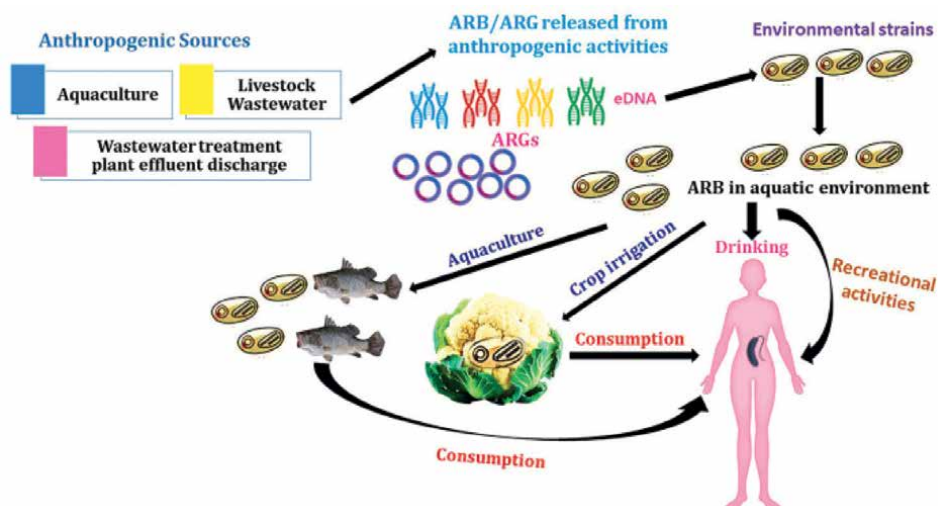


Figure 3. Antibiotic resistant genes (ARGs) and antibiotic-resistant bacteria (ARB) involved in the transmission of antibiotic resistance in the environment.

integrons, transposons, and plasmids, frequently serve as carriers for co-resistance. These elements function as physical vectors that facilitate the horizontal transfer of resistance genes among microbial populations, primarily through the process of conjugation.

4.2.1 Mutation rates and selective pressures

Through successive evolutionary adaptations, *E. coli* has acquired several genotypic and phenotypic characteristics that enable it to survive under selective pressure, including misuse or overuse of antibiotics and environmental factors. The traits such as (i) enzyme-mediated degradation of available nutrient sources, (ii) synthesis of antimicrobial toxins (bacteriocins) limits the intrusion of by other microorganisms, (iii) transitioning into a dormant or stress-resistant states to withstand hostile environments, and (iv) combined activity of diverse resistance genes and virulence determinants has played a key role in expression of a highly adapted and successful pathogen [11].

4.2.2 Clonal expansion and genetic diversification

The widespread use of antibiotics in communities is a major contributor to the emergence of antibiotic-resistant bacteria. According to the prevailing theory, antibiotic treatment eliminates susceptible bacterial strains but allows the resistant ones to survive. This facilitates the clonal expansion of resistant strains, leading to the rise of antibiotic resistance within the community [22]. The most suitable example is *Escherichia coli* sequence type 131 (ST131), which appeared as the most prevalent extraintestinal pathogenic lineage on a global scale. This multidrug-resistant (MDR) strain's strong association with resistance to fluoroquinolones and extended-spectrum cephalosporins (harboring ESBL, CTX-M gene) poses substantial challenges to the effective treatment of *E. coli* infections [23].

4.3 Timeline of notable resistance with genetic evolution

In the last 20 years, there has been a substantial rise in the emergence and spread of MDR strains of *E. coli* showing resistance to drugs belonging to the fluoroquinolone, aminoglycoside, nitrofurantoin, and the fosfomycin group (**Figure 4**).

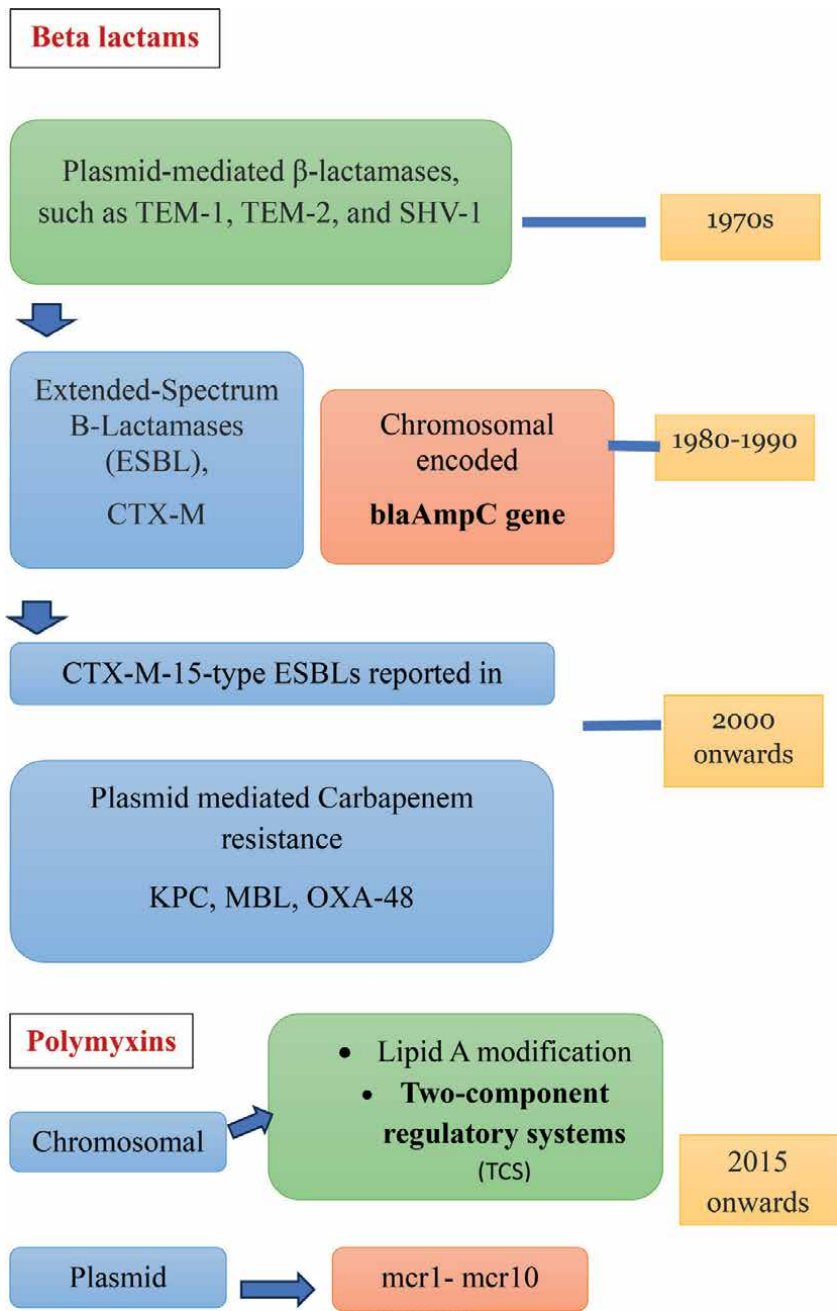


Figure 4.
Timeline of Notable Resistance with genetic evolution.

5. Novel approaches to combat drug resistance in *E. coli*

The rise in incidence of MDR strains of *E. coli* across the globe has rendered the available antimicrobials ineffective, leading to a frequent rate of hospitalizations, longer duration of illnesses, and higher mortality rates. The limited availability of newer drugs due to the slower development of new antibiotics, overuse, and misuse of existing antibiotics in human medicine, animal husbandry, and agricultural sectors have escalated the development of resistance in *E. coli* through different mechanisms such as beta-lactamase production, efflux pumps, and genetic exchange over the past decades. Furthermore, the ability of biofilm production by the pathogenic strains of *E. coli* worsens the situation by contributing to chronic and recurrent infections, especially UTIs and medical device-associated infections [24]. Therefore, different novel approaches have been trialed and are in the pipeline to combat drug resistance in *E. coli*. A few of them are described briefly in this chapter as follows.

5.1 Phage therapy

Bacteriophages infect bacteria and can be genetically modified as a substitute for antibiotics. The continuous ascent of antibiotic resistance compels the exploration of bacteriophages for managing infections caused by MDR superbugs. The mechanism of action of bacteriophages against bacteria is described in **Figure 1**. The first step is the adsorption of bacteriophage to a bacterial cell surface, followed by the release of phage genetic material into the host cell. Lytic phages undergo replication and produce lytic enzymes that degrade the peptidoglycan layer of the bacterial cell wall. However, lysogenic phages can be genetically altered to express certain proteins, peptides, toxins, or enzymes, altering the normal metabolism of bacteria. Phage therapy is being thoroughly investigated worldwide for the advancement of an ideal single phage or combination of phages. Tailored phages can specifically target *E. coli* strains without disrupting normal host microbiota (**Figure 5**).

5.2 Antimicrobial peptides (AMPs)

Antimicrobial peptides (AMPs) are naturally available or synthetically designed peptides with broad-spectrum antibacterial activities (mostly bactericidal) in conjunction with a low likelihood of developing resistance. These are one of the few emerging alternatives to treat MDR pathogens. AMPs are capable of destroying the bilayer structures of both Gram-negative (polysaccharide layer) and Gram-positive (lipoteichoic layer) bacterial cell walls. Many of them have excellent activities against MDRs, but the main problem is their easy degradation and denaturation in the intestine by several proteolytic enzymes and acidic environment when administered as an oral formulation. Some of the AMPs exhibit hemolytic activities that need to be monitored. Hence, studies have been carried out on its oral formulation being modified in the form of microcapsulation with alginate, and using nanotechnology without affecting the antimicrobial activity. To date, the FDA has approved several AMPs to treat MDR infections, while additional AMPs are still under clinical trials [25, 26]. Few examples of FDA-approved AMPs used in MDR *E. coli* infections are polymyxins, colistins. Few examples of AMPs that have proven clinical activities against MDR *E. coli*, but are yet to get FDA approval, are Pseudin-2 (PSE-T2), AMPR-11, and the synthetic peptide SAAP-148.

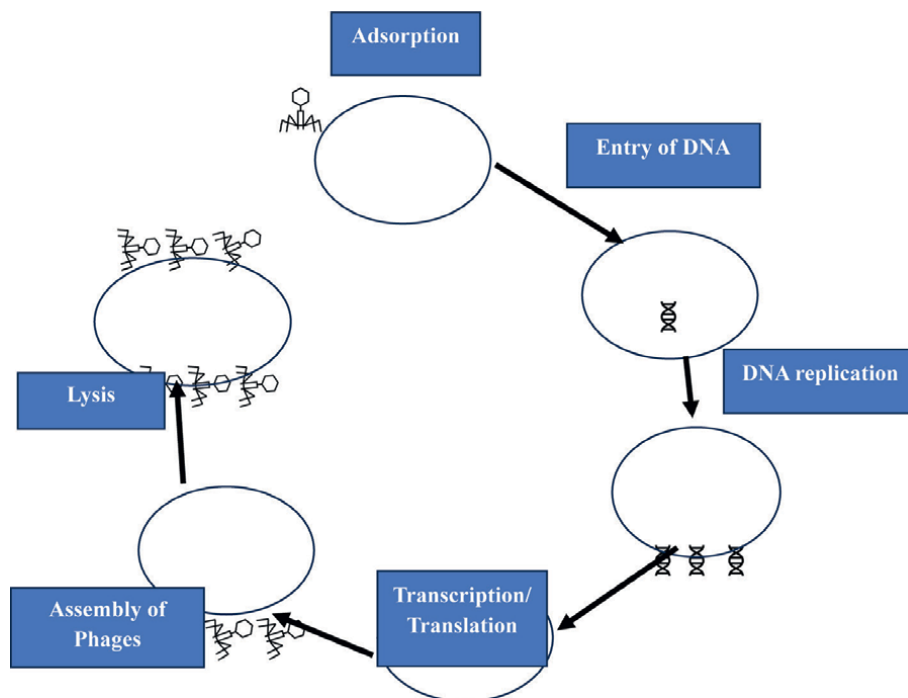


Figure 5.
 Antimicrobial mechanism of action of bacteriophage. Note: Diagram of a bacteriophage infecting the bacterial cell (adsorption to bacterial cell surface, DNA replication, transcription and translation, assembly of new phages, and bacterial cell lysis).

5.3 Monoclonal antibodies (MABs)

Over the past few years, MABs have made an impression as one of the promising therapeutic interventions due to their high specificity and efficacy. They have proven usefulness in dealing with inflammatory disorders, autoimmune disorders, and cancers. Though data are scarce regarding its efficacy in treating MDR pathogens, research studies have been going on evaluating the role of MABs in this field. Few examples of MABs under trials in treating MDR *E. coli* are AII24, a humanized MAB targeting the O25b O antigen of *E. coli* responsible for infections in immunocompromised patients and those undergoing abdominal surgeries [27].

5.4 Efflux pump inhibitors (EPIs)

One of the typical resistance strategies of *E. coli* is mediated through efflux pumps that expel antibiotics and decrease their concentration at the target sites. Inhibitors targeting these efflux pumps, especially the AcrAB-TolC multidrug efflux pump, from *E. coli* emerge as a compelling, innovative, and appealing method that can restore sensitivity of antibiotics and decrease resistance [28].

5.5 CRISPR-Cas systems

CRISPR-Cas systems are one of the important gene-editing tools that can be employed to mitigate AMR by removing antibiotic-resistant genes. CRISPR-Cas9 can

target and eliminate colistin resistance genes, reimposing carbapenem susceptibility in bacteria. However, variations in the CRISPR loci among different bacterial species can affect the efficacy of CRISPR-Cas systems [29].

5.6 Nanoparticle-based drug delivery

Nanocarriers can provide better antibiotic delivery, increase bioavailability, improve drug uptake, and reduce off-target effects. Silver, gold, zinc oxide, and lipid-based nanoparticles have proven efficacy against different MDR organisms like *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and MRSA [30].

5.7 Probiotics and modulation of microbiota

Probiotics exhibit beneficial effects when administered in adequate amounts. They can restore healthy gut microbiota by replacing resistant *E. coli* strains. Currently, probiotics, prebiotics, or fecal microbiota transplantation (FMT) are being explored to decrease colonization of MDR bacteria [31].

5.7.1 Future directions

Recent technologies like Dual RNA sequencing, CRISPR-Cas9 help in detecting genes for survival of pathogens in the gut. Study of metabolomics and metaproteomics helps in deciphering the complexity of microbiomes in the gut environment. The transmission of antimicrobial resistance genes (ARGs) between different metagenomes is crucial, and further research is necessary to comprehend the intricate relationship between microorganisms and pathogens. The influence of various environmental factors that affect the dynamics of antimicrobial resistance (AMR) remains an unexplored domain and warrants further investigation. In resource-limited countries, where inadequate provision of healthcare services and suboptimal sanitation infrastructure exist, the development of effective AMR control strategies requires additional research and innovation.

Author details

Kavita Gupta^{1*}, Monalisa Mohanty², Punyatoya Kar³ and Gayatree Nayak⁴

1 Department of Microbiology, Postgraduate Institute of Child Health, Noida, India

2 Department of Microbiology, IMS & SUM Hospital- Campus 2, Bhubaneswar, India

3 Department of Microbiology, IMS & SUM Hospital, Bhubaneswar, India

4 Department of Microbiology, Kalinga Institute of Medical sciences, Bhubaneswar, India

*Address all correspondence to: drkavitagupta2010@gmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal *Escherichia coli*. *Nature Reviews. Microbiology*. 2010;**8**(3):207-217. DOI: 10.1038/nrmicro2298
- [2] Moreira de Gouveia MI, Bernalier-Donadille A, Jubelin G. *Enterobacteriaceae* in the human gut: Dynamics and ecological roles in health and disease. *Biology (Basel)*. 2024;**13**(3):142. DOI: 10.3390/biology13030142
- [3] Nowrouzian F, Hesselmar B, Saalman R, Strannegard IL, Aberg N, Wold AE, et al. *Escherichia coli* in infants' intestinal microflora: Colonization rate, strain turnover, and virulence gene carriage. *Pediatric Research*. 2003;**54**(1):8-14. DOI: 10.1203/01.PDR.0000069843.20655.EE. Epub 2003 Apr 16
- [4] Martinson JNV, Walk ST. *Escherichia coli* residency in the gut of healthy human adults. *EcoSal Plus*. Sep 2020;**9**(1):1-19. DOI: 10.1128/ecosalplus.ESP-0003-2020
- [5] Geurtsen J, de Been M, Weerdenburg E, Zomer A, McNally A, Poolman J. Genomics and pathotypes of the many faces of *Escherichia coli*. *FEMS Microbiology Reviews*. 2022;**46**(6):fuac031. DOI: 10.1093/femsre/fuac031
- [6] Smit CCH, Lambert M, Rogers K, Djordjevic SP, Van Oijen AM, Keighley C, et al. One health determinants of *Escherichia coli* antimicrobial resistance in humans in the community: An umbrella review. *International Journal of Molecular Sciences*. 2023;**24**:17204. DOI: 10.3390/ijms242417204
- [7] Rodrigo L. *E. Coli* Infections—Importance of Early Diagnosis and Efficient Treatment [Internet]. London, UK: IntechOpen; 2020. DOI: 10.5772/intechopen.80139
- [8] Lin D, Chen K, Guo J, Ye L, Li R, Chan EWC, et al. Contribution of biofilm formation genetic locus, *pgaABCD*, to antibiotic resistance development in gut microbiome. *Gut Microbes*. 2020;**12**(1):1842992. DOI: 10.1080/19490976.2020.1842992
- [9] Dongre DS, Saha UB, Saroj SD. Exploring the role of gut microbiota in antibiotic resistance and prevention. *Annals of Medicine*. 2025;**57**(1):2478317. DOI: 10.1080/07853890.2025.2478317. Epub 2025 Mar 17
- [10] Boolchandani M, D'Souza AW, Dantas G. Sequencing-based methods and resources to study antimicrobial resistance. *Nature Reviews Genetics*. 2019;**20**(6):356-370
- [11] Saini P, Bandsode V, Singh A, Mendem SK, Semmler T, Alam M, et al. Genomic insights into virulence, antimicrobial resistance, and adaptation acumen of *Escherichia coli* isolated from an urban environment. *MBio*. 2024;**15**(3):e0354523. DOI: 10.1128/mbio.03545-23. Epub 2024 Feb 20
- [12] Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services; 2019 Available from: <https://www.cdc.gov/antimicrobial-resistance/data-research/threats/index.html>
- [13] World Health Organization. WHO Bacterial Priority Pathogens List, 2024. Geneva: World Health Organization; 2024. Available from: <https://iris.who.int/bitstream/handle/10665/376776/9789240093461-eng.pdf>

- [14] Leekitcharoenphon P, MHK J, Munk P, Malorny B, Skarżyńska M, Wadepohl K, et al. Genomic evolution of antimicrobial resistance in *Escherichia coli*. Scientific Reports. 2021;**11**(1):15108. DOI: 10.1038/s41598-021-93970-7
- [15] Partridge SR, Kwong SM, Firth N, Jensen SO. Mobile genetic elements associated with antimicrobial resistance. Clinical Microbiology Reviews. 2018;**31**:e00088-e00017
- [16] Arbab S, Ullah H, Wang W, Zhang J. Antimicrobial drug resistance against *Escherichia coli* and its harmful effect on animal health. Veterinary Medicine and Science. 2022;**8**(4):1780-1786. DOI: 10.1002/vms3.825. Epub 2022 May 24
- [17] Gomi R, Matsumura Y, Yamamoto M, Tanaka M, Komakech AJ, Matsuda T, et al. Genomic surveillance of antimicrobial-resistant *Escherichia coli* in fecal sludge and sewage in Uganda. Water Research. 2024;**248**:120830. DOI: 10.1016/j.watres.2023.120830. Epub 2023 Nov 3
- [18] World Health Organization. One health. 2021. Available from: <https://www.who.int/news-room/q-a-detail/one-health>
- [19] Chetri S. *Escherichia coli*: An arduous voyage from commensal to antibiotic-resistance. Microbial Pathogenesis. 2025;**198**:107173. DOI: 10.1016/j.micpath.2024.107173. Epub 2024 Nov 27
- [20] Baker-Austin C, Wright MS, Stepanauskas R, McArthur JV. Co-selection of antibiotic and metal resistance. Trends in Microbiology. 2006;**14**(4):176-182
- [21] Ondon BS, Li S, Zhou Q, Li F. Sources of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) in the soil: A review of the spreading mechanism and human health risks. Reviews of Environmental Contamination and Toxicology. 2021;**256**:121-153. DOI: 10.1007/398_2020_60
- [22] Keenan JD, Klugman KP, McGee L, Vidal JE, Chochua S, Hawkins P, et al. Evidence for clonal expansion after antibiotic selection pressure: Pneumococcal multilocus sequence types before and after mass azithromycin treatments. The Journal of Infectious Diseases. 2015;**211**(6):988-994. DOI: 10.1093/infdis/jiu552. Epub 2014 Oct 6
- [23] Stoesser N, Sheppard AE, Pankhurst L, De Maio N, Moore CE, Sebra R, et al. Evolutionary history of the global emergence of the *Escherichia coli* epidemic clone ST131. MBio. 2016;**7**:1-15. DOI: 10.1128/mbio.02162-15
- [24] Berger I, Loewy ZG. Antimicrobial resistance and novel alternative approaches to conventional antibiotics. Bacteria. 2024;**3**(3):171-182. DOI: 10.3390/bacteria3030012)
- [25] Roque-Borda CA, da Silva PB, Rodrigues MC, Azevedo RB, Di Filippo L, Duarte JL, et al. Challenge in the discovery of new drugs: Antimicrobial peptides against WHO-list of critical and high-priority bacteria. Pharmaceutics. 2021;**13**(6):773. DOI: 10.3390/pharmaceutics13060773
- [26] Xuan J, Feng W, Wang J, Wang R, Zhang B, Bo L, et al. Antimicrobial peptides for combating drug-resistant bacterial infections. Drug Resistance Updates. 2023;**68**:100954. DOI: 10.1016/j.drug.2023.100954
- [27] Guachalla LM, Hartl K, Varga C, Stulik L, Mirkina I, Malafa S, et al. Multiple modes of action of a monoclonal antibody against

multidrug-resistant *Escherichia coli* sequence type 131-H30. Antimicrobial Agents and Chemotherapy. 2017;**61**:1-11. DOI: 10.1128/aac.01428-17

[28] Alenazy R. Drug efflux pump inhibitors: A promising approach to counter multidrug resistance in gram-negative pathogens by targeting AcrB protein from AcrAB-TolC multidrug efflux pump from *Escherichia coli*. Biology (Basel). 2022;**11**(9):1328. DOI: 10.3390/biology11091328

[29] Ahmed MM, Kayode HH, Okesanya OJ, Ukoaka BM, Eshun G, Mourid MR, et al. CRISPR-Cas systems in the fight against antimicrobial resistance: Current status, potentials, and future directions. Infection and Drug Resistance. 2024;**17**:5229-5245. DOI: 10.2147/IDR.S494327

[30] Brar B, Marwaha S, Poonia AK, Koul B, Kajla S, Rajput VD. Nanotechnology: A contemporary therapeutic approach in combating infections from multidrug-resistant bacteria. Archives of Microbiology. 2023;**205**(2):62. DOI: 10.1007/s00203-023-03404-3

[31] Carvalho FM, Mergulhão FJM, Gomes LC. Using lactobacilli to fight *Escherichia coli* and *Staphylococcus aureus* biofilms on urinary tract devices. Antibiotics (Basel). 2021;**10**(12):1525. DOI: 10.3390/antibiotics10121525

Section 2

The Current Situation of Drug Resistance of *Escherichia coli*

The Prevalence of Antibiotic-Resistant Genes in *Escherichia coli* Isolated from the Intestines of Imported Sheep

Omar B. Ahmed and Fayez S. Bahwerth

Abstract

Intestinal bacteria are among the most important natural bacteria present in animals especially sheep, forming part of the “microbiome”. This chapter aimed to assess the prevalence of antibiotic-resistant genes in *Escherichia coli* isolated from the intestines of imported sheep from the Western region of the Kingdom of Saudi Arabia (KSA). A total of 68 fecal (or rectal swab) samples were cultured to identify *E. coli* bacteria and their antibiotic susceptibility, and antibiotic resistance genes. High resistance was observed against ampicillin (88.5%), trimethoprim-sulfamethoxazole (80.8%), and cefuroxime (65.4%). Resistance to cefepime (61.5%) and levofloxacin (57.7%) was also significant. Molecular analysis of the antibiotic resistance genes in *E. coli* isolates revealed a high prevalence of *sul2* (96.2%), followed by *aadA1* (73.1%) and *tet_A* (65.4%). This study highlights the high prevalence of antibiotic-resistance genes in *E. coli* isolated from the intestines of sheep. The detection of multiple resistance genes, particularly those encoding sulfonamide and tetracycline resistance, suggests widespread antibiotic resistance. Additionally, the significant association between extended-spectrum beta-lactamase (ESBL) production and resistance to third- and fourth-generation cephalosporins underscores the need for stringent antibiotic stewardship in livestock management. There should be a strict monitoring of antibiotic use in animal farming and development of strategies to control the spread of resistant infections to ensure long-term food safety and human health. It is important to raise awareness and educating farmers, consumers, and health workers about the dangers of the overuse of antibiotics and the importance of maintaining food safety through good hygiene practices.

Keywords: antibiotic resistance, resistant genes, *Escherichia coli*, intestine, sheep

1. Introduction

The normal flora (or microbiota) in animals consists of a diverse community of microorganisms, including bacteria, fungi, viruses, and protozoa, that naturally

inhabit various body surfaces such as the skin, gastrointestinal tract, respiratory tract, and urogenital system. These microorganisms play essential roles in health, digestion, and immunity. Naturally occurring microorganisms, a diverse group of microorganisms that naturally inhabit animals, play a significant role in maintaining animal health and assisting in digestion processes [1]. In animals such as sheep, these bacteria are found in various parts of the body, particularly in the digestive system (intestines), skin, and mucous membranes. They include *Lactobacillus* spp., *Bacteroides* spp., *Clostridium* spp., *Escherichia coli* (commensal strains), and *Ruminococcus* spp. Sheep (*Ovis aries*) are one of the oldest domesticated animals and serve multiple purposes in agriculture, industry, and scientific research. Their versatility makes them valuable in various sectors, including food production, the fiber industry, and even medical research. Their meat is tender and widely consumed (lamb or mutton), more flavorful, and commonly eaten in many parts of the world, including the Middle East, Asia, and Africa. Sheep hold great significance in Islamic culture, religion, and daily life. Sheep are deeply woven into the religious, economic, and cultural fabric of the Islamic world [2]. Their role in sacrificial rituals, food, trade, and traditional customs makes them one of the most valued livestock animals in Muslim communities. *E. coli* are among the most important natural bacteria present in sheep, forming part of the “microbiome” (the natural microbial community). Their role includes improving the health of sheep by aiding digestion, where bacteria in the intestines and rumen help break down fibers and starches, enhancing nutrient absorption efficiency and boosting sheep health [3]. They also enhance immunity, acting as a first line of defense against harmful organisms by competing for resources and space, and contribute to the production of vitamins that improve the overall health of sheep. Moreover, the balance between different bacteria helps prevent the spread of pathogenic bacteria [4]. The introduction of antimicrobial agents in animal husbandry, whether for treatment, prevention, or growth promotion, inadvertently contributed to the selection and persistence of antibiotic-resistant bacteria [5]. The overuse and misuse of antibiotics in livestock create a favorable environment for the development and spread of bacterial resistance genes. Consequently, these genes can be transferred between bacteria, promoting the emergence of multidrug-resistant strains, posing a significant public health threat, and potentially affecting sheep health and productivity. These genes make it difficult to treat infections with conventional antibiotics, necessitating the use of more effective antibiotics or combinations of several types [6]. Resistance of intestinal bacteria to antibiotics is a pressing concern in both animal and public health. The resistance of intestinal bacteria in sheep to antibiotics is a complex problem that requires serious attention from farmers and veterinarians. Managing the prudent use of antibiotics and adopting comprehensive health strategies can help reduce the spread of resistance and preserve the efficacy of antibiotics for the future [7].

The presence of antibiotic-resistance genes in gram-negative intestinal bacteria in sheep poses a significant challenge to both animal and human health [8]. These genes complicate the treatment of infections using conventional antibiotics, necessitating the use of more potent antibiotics or combinations of antibiotics. The diversity of antibiotic-resistance genes in intestinal bacteria indicates that these bacteria can resist a wide range of antibiotics, complicating treatment and increasing the risk of transferring these resistant bacteria to humans or other environments. Controlling the spread of these genes requires optimal antibiotic use in animal husbandry, alongside rigorous and continuous monitoring programs and preventive measures to avoid bacterial contamination in sold meat [9].

The spread of bacterial resistance genes in the intestines of animals is a major concern due to its potential impact on public health, as it can lead to the transfer of resistance to human pathogens [10]. Horizontal gene transfer of resistance genes between different bacterial species can result in the creation of more challenging strains. If resistant bacteria from meat enter the human body, they may cause infections that are difficult to treat with conventional antibiotics, significantly limiting treatment options for bacterial infections. The presence of resistance genes in the normal flora of sheep also has a major impact on the environment. Resistant bacteria from sheep can be introduced into soil, water, and wildlife, leading to the spread of antimicrobial resistance across ecosystems. Therefore, monitoring and detecting outbreaks of infectious diseases is crucial in protecting consumers and highlighting critical points during both production and consumption stages. Studying the prevalence of bacterial resistance genes in the intestines of animals is essential for protecting public health, ensuring food safety, and promoting the responsible use of antibiotics in both animal and human healthcare [11]. This chapter aimed to assess the prevalence of antibiotic-resistance genes in *E. coli* isolated from the intestines of imported sheep in the Western region of KSA.

2. Methodology

2.1 Sample collection and bacterial isolation

A total of 68 fecal (or rectal swab) samples were previously collected from imported sheep and stored at -70°C in the microbiology laboratory of the Custodian of the Two Holy Mosques Institute of Hajj and Umrah Research, Umm Al-Qura University, Saudi Arabia. Bacterial isolation was performed using selective and differential media, and gram-negative strains were identified based on standard microbiological techniques.

2.2 Antibiotic susceptibility testing

Antibiotic resistance patterns of *E. coli* isolates were determined using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. A panel of antibiotics representing different classes was tested, including beta-lactams, aminoglycosides, fluoroquinolones, and sulfonamides.

2.3 Molecular detection of antibiotic resistance genes

PCR analysis was conducted to detect antibiotic-resistance genes in *E. coli* isolates. DNA extraction was performed using modified boiling methods [13], and specific primers targeting resistance genes (*sul1*, *sul2*, *sul3*, *blaSHV*, *blaTEM*, *tet_A*, *tet_C*, *aadA1*, *strA_strB*, *qnr*, and *cat*) were used [14–21]. PCR products were analyzed *via* gel electrophoresis to confirm gene presence.

2.4 Statistical analysis

Pearson's chi-square test was applied to assess the statistical significance between ESK production and antibiotic resistance. A *p*-value of <0.05 was considered statistically significant.

3. Results

Table 1 summarizes the key findings from the study. A total of 49 Gram-negative bacterial strains were isolated from the intestines of sheep. *E. coli* was the most frequently isolated species, accounting for 53.1% (26/49) of the total isolates. Other bacterial species included *Enterobacter cloacae* (30.6%), *Klebsiella pneumoniae* (4.1%), *Salmonella paratyphi A* (2%), *Acinetobacter haemolyticus* (2%), *Klebsiella intermedia* (2%), *Enterobacter aerogenes* (2%), *Aeromonas caviae* (2%), and *Tatumella* (2%) as shown in **Figure 1**. The antibiotic susceptibility testing of the *E. coli* isolates revealed varying resistance levels (**Figure 2**). High resistance was observed against ampicillin (88.5%), trimethoprim-sulfamethoxazole (80.8%), and cefuroxime (65.4%). Resistance to cefepime (61.5%) and levofloxacin (57.7%) was also significant. Notably, no resistance was detected against amoxicillin-clavulanate and piperacillin-tazobactam. ESBL production was identified in 50% (13/26) of the *E. coli* isolates. Molecular analysis of the antibiotic resistance genes in *E. coli* isolates revealed a high prevalence

	Result	No	(%)
Types of isolated strains (gram-negative)	<i>Escherichia coli</i>	26	53.1
	<i>Enterobacter cloacae</i>	15	30.6
	<i>Klebsiella pneumoniae</i>	2	4.1
	<i>Salmonella paratyphi A</i>	1	2
	<i>Acinetobacter haemolyticus</i>	1	2
	<i>Klebsiella intermedia</i>	1	2
	<i>Enterobacter aerogenes</i>	1	2
	<i>Aeromonas caviae</i>	1	2
	<i>Tatumella</i>	1	2
	Total	49	100
Antibiotic resistance of isolated <i>Escherichia coli</i>	Amikacin	8	30.8
	Amoxicillin/clavulanic acid	0	0.0
	Ampicillin	23	88.5
	Aztreonam	1	3.8
	Cefazolin	4	15.4
	Cefepime	16	61.5
	Cefotaxime	1	3.8
	Cefoxitin	8	30.8
	Ceftazidime	1	3.8
	Cefuroxime	17	65.4
	Gentamicin	8	30.8
	Imipenem	1	3.8
	Levofloxacin	15	57.7
	Piperacillin/tazobactam	0	0.0
	Sulfamethoxazole/trimethoprim	21	80.8
	ESBL	13	50

	Result	No	(%)
The prevalence rate of antibiotic resistance genes in <i>Escherichia coli</i>	<i>strA_strB</i>	8	30.8
	<i>aadA1</i>	19	73.1
	<i>sul1</i>	12	46.2
	<i>sul2</i>	25	96.2
	<i>sul3</i>	11	42.3
	<i>tet_A</i>	17	65.4
	<i>tet_B</i>	0	0
	<i>tet_C</i>	4	15.4
	<i>blaTEM</i>	1	3.8
	<i>blaSHV</i>	9	34.6
	<i>cat</i>	4	15.4
	<i>qnr</i>	0	0

Table 1.
The results of laboratory testing of the isolated bacteria.

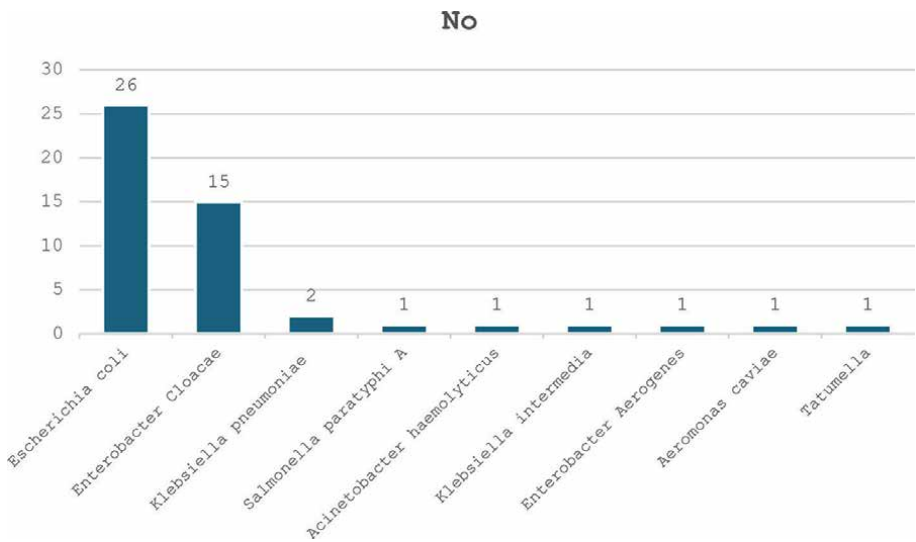


Figure 1.
Types of isolated strains (gram-negative bacteria).

of *sul2* (96.2%), followed by *aadA1* (73.1%) and *tet_A* (65.4%), as shown in **Figures 3** and **4**. The *sul1* and *sul3* genes were detected in 46.2% and 42.3% of isolates, respectively. The *blaSHV* gene was present in 34.6%, while *strA_strB* was detected in 30.8%. Other genes, including *tet_C* (15.4%), *cat* (15.4%), *blaTEM* (3.8%), and *qnr* (0%), were found at lower frequencies. **Table 2** shows the distribution of antibiotic resistance gene multiplicity in *E. coli* isolates, which reveals significant variability. Only one isolate (3.8%) carried no resistance genes, while two isolates (7.7%) carried two genes. Three and four gene combinations were observed in 19.2 and 7.7% of isolates, respectively. Notably, 50% of isolates carried five resistance genes, and 11.5%

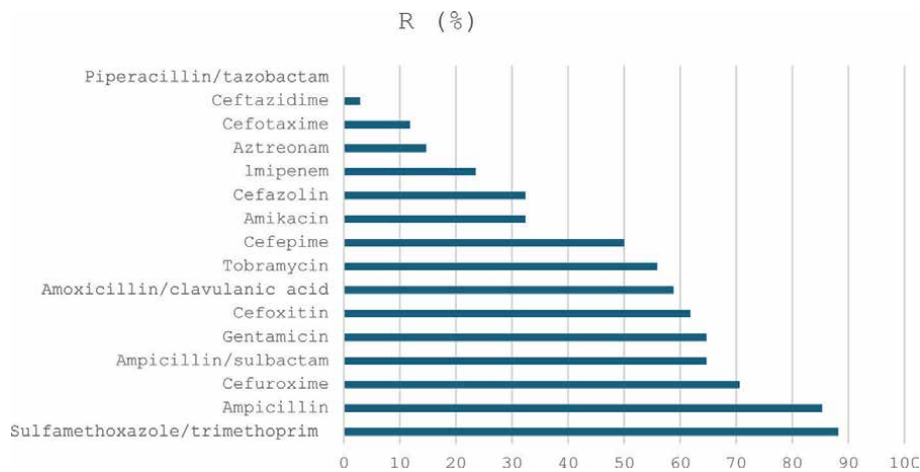


Figure 2.
Antibiotic resistance of Escherichia coli to the commonly used antibiotics.

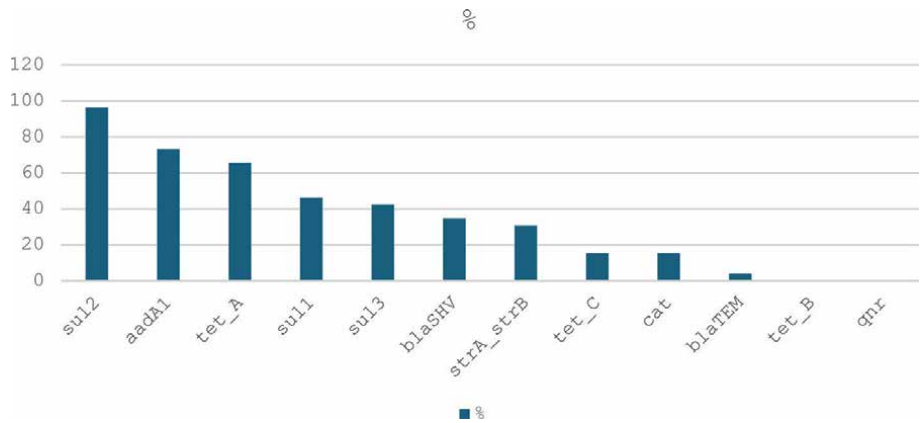


Figure 3.
The prevalence of antibiotic resistance genes in Escherichia coli isolated from the intestines of live livestock.

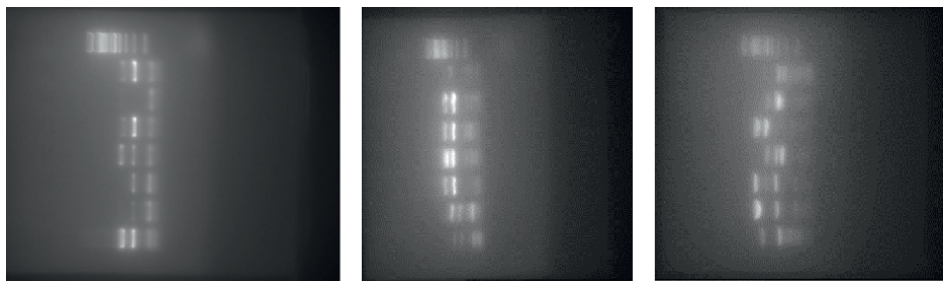


Figure 4.
PCR analysis results of antibiotic resistance genes in Escherichia coli isolated from the intestines of live livestock. Figures showing strains carrying resistance genes, whether single, dual, or multiple.

Gene multiplicity	No	%
Zero gene	1	3.8
Two genes	2	7.7
Three genes	5	19.2
Four genes	2	7.7
Five genes	13	50.0
Six genes	3	11.5
Total	26	100

Table 2.
The gene multiplicity in Escherichia coli isolated from sheep intestines.

(Pearson chi-square)	Antibiotic	p-value
Significance of resistance to antibiotics and ESBL production in <i>Escherichia coli</i>	Aztreonam	0.020
	Cefazolin	0.014
	Cefepime	0.00
	Cefotaxime	0.269
	Cefoxitin	0.369
	Ceffazidime	0.020

Table 3.
The statistical significance between resistance to certain antibiotics and ESBL production in Escherichia coli isolated from the intestines of animals.

harbored six resistance genes, indicating a high level of genetic resistance diversity. Statistical analysis using Pearson’s chi-square test showed significant associations between ESBL production and resistance to specific antibiotics (**Table 3**). Resistance to cefepime ($p = 0.000$), cefazolin ($p = 0.014$), aztreonam ($p = 0.020$), and ceftazidime ($p = 0.020$) were significantly correlated with ESBL production. However, no significant association was found with resistance to cefotaxime ($p = 0.269$) and cefoxitin ($p = 0.396$).

4. Discussion

Sheep are among the most widely consumed types of meat in the world in addition to their nutritional benefits [22]. The gut microbiota of sheep naturally harbors resistant bacteria, which can spread within livestock populations and potentially transfer to humans. While normal flora plays a vital role in maintaining gut health and digestion, the spread of resistance genes can contribute to the emergence of antibiotic-resistant bacteria, which can be transferred to humans, animals, and ecosystems.

The findings of chapter demonstrate a substantial prevalence of *E. coli* among gram-negative bacterial isolates from sheep intestines. The dominance of *E. coli*

(53.1%) is consistent with previous studies highlighting its widespread presence in livestock intestines [23, 24]. The significance of *E. coli* lies in the fact that some strains, such as *E. coli* O157, can be pathogenic and cause food poisoning if transmitted to humans [24]. The presence of other bacterial species, such as *Enterobacter cloacae* and *Klebsiella pneumoniae*, further indicates the diverse microbial community within the animal gut, some of which can act as opportunistic pathogens. These results were consistent with global studies confirming that *E. coli* is the most prevalent intestinal bacteria in sheep [25, 26]. The aforementioned percentages reflect the natural presence of bacteria in the intestines; however, some can pose a public health risk if they become pathogenic or resistant to antibiotics.

The high resistance rates observed in *E. coli*, particularly against ampicillin (88.5%) and trimethoprim-sulfamethoxazole (80.8%), raise significant concerns regarding the efficacy of commonly used antibiotics. The high resistance levels to these antibiotics are concerning because they are commonly used to treat a wide range of bacterial infections, and this resistance may affect both animal and public health. This was similar to a study conducted in Qatar, where the highest resistance in *E. coli* was to ciprofloxacin (69.4%) and trimethoprim/sulfamethoxazole (45.8%) [27]. A global study also indicated that 13 types of gram-negative bacteria isolated from goats and sheep intestines were resistant to various antibiotics, including penicillin, ampicillin, amoxicillin, chloramphenicol, and others [28]. Resistance to beta-lactam antibiotics, especially third- and fourth-generation cephalosporins like cefepime (61.5%) and cefuroxime (65.4%), suggests the emergence of extended-spectrum beta-lactamase (ESBL)-producing strains. These findings highlight the potential risk of antimicrobial resistance transmission from animals to humans through direct contact or food consumption. Thus, antibiotic resistance in these intestinal bacteria poses a complex challenge that requires serious attention from farmers and veterinarians, and comprehensive health strategies need to be adopted to reduce the spread of resistance and maintain the effectiveness of antibiotics in the future.

The molecular analysis further reinforces these concerns, as the widespread presence of resistance genes, such as *sul2* (96.2%) and *aadA1* (73.1%), indicates an extensive genetic basis for resistance. The presence of antibiotic-resistance genes in the normal flora of sheep is a growing concern with serious implications for animal health, human health, and environmental sustainability. The transmission of resistant bacteria through direct contact, food, and environmental contamination highlights the urgent need for better antibiotic stewardship, hygiene measures, and surveillance programs to reduce the impact of antimicrobial resistance. A similar study found an increase in resistance in bacteria isolated from livestock feces, with common genes such as *sul3*, *cat*, *qnr*, *qnrB*, and *tet(A)* [29]. The problem is that these genes enable bacteria to resist various antibiotics, complicating the treatment of related infections. Sulfonamide resistance genes (*sul1*, *sul2*, *sul3*) are widespread in bacteria and make them resistant to sulfonamides such as sulfamethoxazole [30]. Aminoglycoside-modifying enzymes (AMEs), such as *aadA1*, are among the most prevalent genes in intestinal bacteria, rendering antibiotics like gentamicin, tobramycin, and amikacin ineffective. Likewise, tetracycline resistance genes (*tet(A)*, *tet(B)*, *tet(M)*), beta-lactamase genes (*SHV*), and chloramphenicol resistance genes (*cat*) are also widespread [29, 31]. The presence of these resistance genes in gram-negative bacteria in the intestines of sheep and lambs presents a significant challenge to public health. These genes make it difficult to treat infections with traditional antibiotics, and controlling their spread requires strict policies on antibiotic use in livestock farming, along with continuous monitoring programs. The detection of ESBL-related genes

(*blaSHV* in 34.6% and *blaTEM* in 3.8% of isolates) suggests that some strains have acquired mechanisms to hydrolyze a wide range of beta-lactam antibiotics. Notably, the absence of *qnr* genes suggests that resistance to fluoroquinolones may be mediated by alternative mechanisms.

The gene multiplicity analysis highlights a concerning trend, with half of the *E. coli* isolates carrying five resistance genes. This suggests that a significant proportion of the bacterial population possesses multiple resistance determinants, increasing the likelihood of multidrug resistance. The detection of isolates with up to six resistance genes further emphasizes the genetic adaptability of *E. coli* and its potential to evade multiple antibiotic classes. This high level of gene accumulation poses a serious threat to antimicrobial efficacy, as bacteria carrying multiple resistance determinants can spread resistance traits more effectively within microbial communities. The presence of multiple antibiotic-resistance genes complicates treatment and increases the risk of transmitting resistant bacteria to humans or other environments, especially due to the widespread use of antibiotics in sheep farming [32]. The health and environmental challenges posed by multiple resistance genes make bacterial infections harder to treat, and multi-resistant bacteria can spread easily between animals and humans through the food chain or direct contact. This could reduce the effectiveness of antibiotics in agriculture, leading to higher animal mortality rates and potential public health risks [33, 34]. Bacteria with multiple resistance genes can survive even with the use of multiple antibiotics, making treatment more difficult. Also, the spread of bacteria carrying multiple resistance genes poses a risk of transferring these genes to other bacteria, in the environment or other living organisms, including humans, through the food chain or direct contact. The treatment challenges also include the reduced availability of common antibiotics, requiring veterinarians to resort to less common or more expensive antibiotics, which may have greater side effects on animals [35].

The statistical correlation between ESBL production and resistance to third- and fourth-generation cephalosporins (cefepime, cefazolin, aztreonam, and ceftazidime) aligns with global reports on the increasing prevalence of ESBL-producing *E. coli* in both human and veterinary medicine. This finding emphasizes the need for improved surveillance and stricter antimicrobial usage policies in livestock production to mitigate the further spread of resistance.

Overall, these results underscore the urgent need for antimicrobial stewardship programs targeting livestock to curb the dissemination of resistant bacteria. Future research should focus on alternative strategies, such as probiotics and phage therapy, to reduce reliance on conventional antibiotics while ensuring animal health and food safety. In general, this chapter highlights the high prevalence of antibiotic-resistance genes in *E. coli* isolated from the intestines of sheep. The detection of multiple resistance genes, particularly those encoding sulfonamide and tetracycline resistance, suggests widespread antibiotic resistance. Additionally, the significant association between ESBL production and resistance to third- and fourth-generation cephalosporins underscores the need for stringent antibiotic stewardship in livestock management.

5. Conclusion

The presence of multiple antibiotic-resistance genes in intestinal bacteria isolated from sheep livestock represents a significant challenge to both animal and human health. These results highlight a growing issue of multidrug resistance among bacteria isolated from sheep intestines. The high prevalence of strains carrying multiple

resistance genes underscores the urgent need for effective measures to reduce the spread of resistance and promote the rational use of antibiotics in agriculture and livestock farming. Alternative therapeutic strategies must be developed to preserve the effectiveness of antibiotics. There should be strict monitoring of antibiotic use in animal farming and the development of strategies to control the spread of resistant infections to ensure long-term food safety and human health. It is important to raise awareness and educate farmers, consumers, and health workers about the dangers of overuse of antibiotics and the importance of maintaining food safety through good hygiene practices.

Acknowledgements

The researchers would like to express their sincere thanks to the Custodian of the Two Holy Mosques Institute for Hajj and Umrah Research for supporting this project No (103/24).

Author details

Omar B. Ahmed^{1*} and Fayez S. Bahwerth²

1 Department of Environmental and Health Research, The Custodian of the Two Holy Mosques Institute of Hajj and Umrah Research, Umm Al-Qura University, Saudi Arabia

2 King Faisal Hospital, Ministry of Health, Makkah, Saudi Arabia

*Address all correspondence to: abuaglah1@hotmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Hugenholtz P, Pace NR. Identifying microbial diversity in the natural environment: A molecular phylogenetic approach. *Trends in Biotechnology*. 1996;**14**(6):190-197
- [2] Rout PK, Behera BK, Rout PK, Behera BK. Goat and sheep farming. In: *Sustainability in Ruminant Livestock: Management and Marketing*. Singapore: Springer Nature; 2021. pp. 33-76
- [3] Rudolph KM, Hunter DL, Rimler RB, Cassirer EF, Foreyt WJ, DeLong WJ, et al. Microorganisms associated with a pneumonic epizootic in Rocky Mountain bighorn sheep (*Ovis canadensis canadensis*). *Journal of Zoo and Wildlife Medicine*. 2007;**38**(4):548-558
- [4] Yang G, Zhang S, Li Z, Huang J, Liu Y, Liu Y, et al. Comparison between the gut microbiota in different gastrointestinal segments of large-tailed Han and small-tailed Han sheep breeds with high-throughput sequencing. *Indian Journal of Microbiology*. 2020;**60**:436-434
- [5] Sazykin IS, Khmelevtsova LE, Seliverstova EY, Sazykina MA. Effect of antibiotics used in animal husbandry on the distribution of bacterial drug resistance. *Applied Biochemistry and Microbiology*. 2021;**57**:20-30
- [6] Cabello FC, Godfrey HP. Even therapeutic antimicrobial use in animal husbandry may generate environmental hazards to human health. *Environmental Microbiology*. 2016;**18**(2):311-313
- [7] Helliwell R, Morris C, Raman S. Antibiotic stewardship and its implications for agricultural animal-human relationships: Insights from an intensive dairy farm in England. *Journal of Rural Studies*. 2020;**78**:447-456
- [8] Khalifa HO, Oreiby A, Abd El-Hafeez AA, Abd El Latif A, Okanda T, Kato Y, et al. High β -lactam and quinolone resistance of *Enterobacteriaceae* from the respiratory tract of sheep and goat with respiratory disease. *Animals*. 2021;**11**(8):2258
- [9] Jian Z, Zeng L, Xu T, Sun S, Yan S, Yang L, et al. Antibiotic resistance genes in bacteria: Occurrence, spread, and control. *Journal of Basic Microbiology*. 2021;**61**(12):1049-1070
- [10] Manyi-Loh C, Mamphweli S, Meyer E, Okoh A. Antibiotic use in agriculture and its consequential resistance in environmental sources: Potential public health implications. *Molecules*. 2018;**23**(4):795
- [11] Ribeiro J, Silva V, Monteiro A, Vieira-Pinto M, Igrejas G, Reis FS, et al. Antibiotic resistance among gastrointestinal bacteria in broilers: A review focused on *Enterococcus* spp. and *Escherichia coli*. *Animals*. 2023;**13**(8):1362
- [12] CLSI. Performance Standards for Antimicrobial Susceptibility Testing, M100. 31st ed. Wayne, PA: Clinical and Laboratory Standards Institute; 2021
- [13] Ahmed OB, Dablood AS. Quality improvement of the DNA extracted by boiling method in gram negative bacteria. *International Journal of Bioassays*. 2017;**6**(4):5347-5349
- [14] Sunde M, Norström M. The prevalence of, associations between and conjugal transfer of antibiotic resistance genes in *Escherichia coli* isolated from Norwegian meat and meat products. *Journal of Antimicrobial Chemotherapy*. 2006;**58**(4):741-747

- [15] Kerrn MB, Klemmensen T, Frimodt-Møller N, Espersen F. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. *Journal of Antimicrobial Chemotherapy*. 2002;**50**(4):513-516
- [16] Perreten V, Boerlin P. A new sulfonamide resistance gene (sul3) in *Escherichia coli* is widespread in the pig population of Switzerland. *Antimicrobial Agents and Chemotherapy*. 2003;**47**(3):1169-1172
- [17] Guardabassi L, Dijkshoorn L, Collard JM, Olsen JE, Dalsgaard A. Distribution and *in-vitro* transfer of tetracycline resistance determinants in clinical and aquatic *Acinetobacter* strains. *Journal of Medical Microbiology*. 2000;**49**(10):929-936
- [18] Hartman AB, Essiet II, Isenbarger DW, Lindler LE. Epidemiology of tetracycline resistance determinants in *Shigella* spp. and enteroinvasive *Escherichia coli*: Characterization and dissemination of tet (a)-1. *Journal of Clinical Microbiology*. 2003;**41**(3):1023-1032
- [19] Brinas L, Zarazaga M, Sáenz Y, Ruiz-Larrea F, Torres C. B-Lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. *Antimicrobial Agents and Chemotherapy*. 2002;**46**(10):3156-3163
- [20] Sørum H, L'Abée-Lund TM, Solberg A, Wold A. Integron-containing IncU R plasmids pRAS1 and pAr-32 from the fish pathogen *Aeromonas salmonicida*. *Antimicrobial Agents and Chemotherapy*. 2003;**47**(4):1285-1290
- [21] Mashak Z. Prevalence and antibiotic resistance of *Escherichia coli* O157: H7 isolated from raw meat samples of ruminants and poultry. *Journal of Food and Nutrition Research*. 2018;**6**(2):96-102
- [22] Almasri M, Ahmed QA, Turkestani A, Memish ZA. Hajj abattoirs in Makkah: Risk of zoonotic infections among occupational workers. *Veterinary Medicine and Science*. 2019;**5**(3):428-434
- [23] Ashraf I. Studies on isolation and characterization of *Escherichia coli* from sheep, goats and their handlers (thesis). Chatha, Jammu, India: Division of Veterinary Public Health and Epidemiology Sher-e-Kashmir University of Agricultural Sciences & Technology of Jammu Main Campus; 2016
- [24] Abreham S, Teklu A, Cox E, Sisay Tessema T. *Escherichia coli* O157: H7: Distribution, molecular characterization, antimicrobial resistance patterns and source of contamination of sheep and goat carcasses at an export abattoir, Mojo, Ethiopia. *BMC Microbiology*. 2019;**19**:1-14
- [25] Chatzopoulos DC, Sarrou S, Vasileiou NGC, Ioannidi KS, Peteinaki E, Valiakos G, et al. Dissemination of intestinal pathogens between lambs and puppies in sheep farms. *Small Ruminant Research*. 2016;**141**:5-10
- [26] Boukli-Hacene F, Djouadi LN, Raddaoui A, Hachem Y, Boumerdassi H, Achour W, et al. Sheep and goats as reservoirs of colistin-resistant *E. coli*: First detection of ETEC ST10 and *E. coli* ST6396 mcr-1 positive strains in North Africa. *Journal of Applied Microbiology*. 2024;**135**:lxae227
- [27] Eltai N, Al-Thani A, Alhadidi S, Abdfarag A, Romaiha H, Mahmoud M, et al. Antibiotic resistance profile of commensal *Escherichia coli* isolated from healthy sheep in Qatar. *Journal*

of Infection in Developing Countries. 2020;**14**:138-145. DOI: 10.3855/jidc.11827

[28] Herawati O, Bejo SK, Zakaria Z, Ramanoon SZ. The global profile of antibiotic resistance in bacteria isolated from goats and sheep: A systematic review. *Veterinary World*. 2023;**16**(5):977

[29] Amador P, Fernandes R, Prudêncio C, Duarte I. Prevalence of antibiotic resistance genes in multidrug-resistant *Enterobacteriaceae* on Portuguese livestock manure. *Antibiotics*. 2019;**8**(1):23

[30] Jiang H, Cheng H, Liang Y, Yu S, Yu T, Fang J, et al. Diverse mobile genetic elements and conjugal transferability of sulfonamide resistance genes (sul1, sul2, and sul3) in *Escherichia coli* isolates from *Penaeus vannamei* and pork from large markets in Zhejiang, China. *Frontiers in Microbiology*. 2019;**10**:1787

[31] Van Duijkeren E, Schwarz C, Bouchard D, Catry B, Pomba C, Baptiste KE, et al. The use of aminoglycosides in animals within the EU: Development of resistance in animals and possible impact on human and animal health: A review. *Journal of Antimicrobial Chemotherapy*. 2019;**74**(9):2480-2496

[32] Crawford PE, Hamer K, Lovatt F, Behnke MC, Robinson PA. Antibiotic use in the Northern Irish sheep flock: What lessons can be learnt from medicine records and farmer attitudes to improve stewardship of these essential medicines? *Preventive Veterinary Medicine*. 2024;**226**:106169

[33] Serwecińska L. Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. *Water*. 2020;**12**(12):3313

[34] Hatem ME, Arab RH, Ata SN, El-Moez SIA, Khairy EA, Fouad EA.

Bacterial Abscessation in sheep and goat in Giza governorate with full antibiogram screening. *Global Veterinaria*. 2013;**10**(4):372-381

[35] Crawford PE, Hamer K, Lovatt F, Behnke MC, Robinson PA. Improving analgesia provision for sheep: An analysis of farm medicine records and attitudes towards pain relief on sheep farms in Northern Ireland. *Veterinary Record Open*. 2023;**10**(2):e75

Section 3

Advanced Therapeutics against *Escherichia coli*

Therapeutic Advances against Uropathogenic *E. coli*: From Beta-Lactamase Inhibition to Anti-Biofilm Agents

Rahimi Fateh and Qasemi Ali

Abstract

This chapter provides an in-depth analysis of the complex relationship between biofilm formation and antibiotic resistance in uropathogenic *Escherichia coli* (UPEC), which remains a predominant cause of recurrent urinary tract infections (UTIs). Biofilm development serves as a crucial survival mechanism for UPEC, facilitating robust adherence to surfaces, evasion of host immune responses and heightened tolerance to antimicrobial agents. The biofilm's extracellular matrix not only limits the penetration of antibiotics but also promotes the emergence of resistance mechanisms, most notably through the activity of beta-lactamase enzymes such as ESBLs, AmpC and carbapenemases. These enzymes effectively neutralize many frontline antibiotics, complicating treatment regimens and contributing to chronic, persistent infections. In response to these challenges, this chapter explores a range of innovative therapeutic strategies, with particular emphasis on anti-biofilm interventions aimed at dismantling biofilm architecture. Additionally, alternative approaches; including bioactive compounds derived from medicinal plants, the application of metallic nanoparticles and the use of bacteriophages specifically targeting UPEC; are critically assessed. When integrated with advances in drug delivery systems, these emerging therapies present promising prospects for overcoming antibiotic resistance and reducing the recurrence of UTIs. Ultimately, this overview aims to guide ongoing research and inform clinical practice, with the goal of improving outcomes in the management of infections caused by antibiotic-resistant UPEC.

Keywords: recurrent urinary tract infections (UTIs), uropathogenic *Escherichia coli* (UPEC), beta-lactamases, anti-biofilm strategies, liposome-based drug delivery systems

1. Introduction

Urinary tract infections (UTIs) constitute a significant global health burden, straining healthcare systems [1]. Defined by microbial presence and symptoms,

UTIs affect any urinary system part and are clinically categorized into lower (cystitis/urethritis) and more severe upper (pyelonephritis) infections [2]. Women are disproportionately affected due to anatomical factors (shorter urethra), with nearly half experiencing a UTI lifetime. Female-specific risk factors include sexual activity, certain contraceptives, pregnancy and postmenopausal changes. General risks encompass catheterization, diabetes mellitus, immunosuppression, urinary tract abnormalities and incomplete bladder emptying [2, 3].

Uropathogenic *Escherichia coli* (UPEC), originating from the host's gut microbiota, is the predominant pathogen (70–90% of uncomplicated UTIs) [4]. UPEC employs specialized virulence factors for colonization and immune evasion [5]. Pathogenesis involves periurethral colonization, urethral ascent, adhesion *via* fimbriae (e.g., Type 1 for bladder, P for kidney) [6] and invasion of bladder cells to form protective intracellular bacterial communities (IBCs) [7]. IBCs act as reservoirs, shielding UPEC from immunity and antibiotics, enabling replication and contributing to recurrence upon host cell exfoliation [8]. Pathogenicity is enhanced by toxins (e.g., alpha-hemolysin, CNF1), causing cell damage and modulating inflammation and iron acquisition systems essential for survival [9]. Virulence genes are frequently located on mobile genetic elements, facilitating adaptation and resistance acquisition [4, 10]. Biofilm formation is critical for UPEC persistence, particularly in chronic and catheter-associated UTIs (CAUTIs) [11]. UPEC forms biofilms on abiotic surfaces like catheters and biotic uroepithelium [12]. The self-produced extracellular polymeric substance (EPS) matrix provides a barrier against antimicrobials and host defenses, aids surface anchoring against urine flow and fosters physiologically distinct states, including antibiotic-tolerant persister cells [13]. The biofilm microenvironment facilitates horizontal gene transfer, potentially accelerating antibiotic resistance spread [14]. IBCs exhibit key biofilm-like characteristics, underscoring this community lifestyle's role in persistence [7].

The escalating antimicrobial resistance (AMR) crisis compounds these challenges [15]. Dissemination of resistance genes in UPEC, especially those encoding beta-lactamases that inactivate crucial antibiotics, severely compromises UTI treatment [16]. These genes, often on mobile elements, drive the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) strains [10, 17], increasing treatment failure, costs and complications like urosepsis [1, 16].

This chapter provides an in-depth analysis of the complex relationship between biofilm formation and antibiotic resistance in UPEC, which remains a predominant cause of recurrent UTIs (rUTIs). Biofilm development serves as a crucial survival mechanism for UPEC, facilitating robust adherence to surfaces, evasion of host immune responses and heightened tolerance to antimicrobial agents. The biofilm's EPS matrix not only limits the penetration of antibiotics but also promotes the emergence of resistance mechanisms, most notably through the activity of beta-lactamase enzymes such as ESBLs, AmpC and carbapenemases. These enzymes effectively neutralize many frontline antibiotics, complicating treatment regimens and contributing to chronic, persistent infections. In response to these challenges, this chapter explores a range of innovative therapeutic strategies, with particular emphasis on anti-biofilm interventions aimed at dismantling biofilm architecture. Additionally, alternative approaches, including bioactive compounds derived from medicinal plants, the application of metallic nanoparticles and the use of bacteriophages specifically targeting UPEC, are critically assessed. When integrated with advances in drug delivery systems, these emerging therapies present promising prospects for overcoming antibiotic resistance and reducing the rUTIs. Ultimately, this overview aims to guide

ongoing research and inform clinical practice, with the goal of improving outcomes in the management of infections caused by antibiotic-resistant UPEC.

2. Uropathogenic *Escherichia coli* and beta-lactamase production

2.1 The scope and impact of urinary tract infections

Urinary tract infections (UTIs) are common bacterial infections posing a significant global healthcare burden [1]. Affecting any urinary system part, they are clinically divided into lower UTIs (cystitis; localized symptoms) and more severe upper UTIs (pyelonephritis; systemic illness) [2]. Women are disproportionately affected (nearly half lifetime) due largely to anatomical factors (shorter urethra) [3]. Uropathogenic *E. coli* (UPEC) utilizes specialized virulence factors for colonization and immune evasion following ascent into the urinary tract [5].

2.2 Uropathogenic *E. coli* virulence: Mechanisms of colonization and invasion

UPEC pathogenesis relies on diverse virulence mechanisms [5]. Adhesion is critical, mediated by Type 1 fimbriae for bladder colonization (binding uroplakins) and P fimbriae for kidney infection (binding globoside) [6]. Invasion into bladder epithelial cells allows UPEC to form intracellular bacterial communities (IBCs), protected reservoirs shielding bacteria from immunity and antibiotics, facilitating replication, and contributing to recurrence upon host cell exfoliation [7, 8]. Secreted toxins like alpha-hemolysin (HlyA) and cytotoxic necrotizing factor 1 (CNF1) cause host cell damage and modulate inflammation [5]. High-affinity iron acquisition systems (e.g., siderophores) are essential for survival in the iron-limited urinary tract [9]. Key virulence factors of UPEC strains are summarized in **Table 1**. Virulence genes are often concentrated in specific phylogenetic groups (e.g., B2) and frequently located on mobile genetic elements, promoting their horizontal spread and co-acquisition of antibiotic resistance [10].

2.3 The biofilm lifestyle: A key strategy for UPEC persistence

Biofilm formation is a crucial strategy for UPEC persistence, particularly in chronic and catheter-associated UTIs (CAUTIs) [11, 12]. These structured microbial communities, encased in a self-produced extracellular polymeric substance (EPS) matrix, adhere to both abiotic (e.g., catheters) and biotic (uroepithelium) surfaces [12, 13]. The EPS matrix provides a physical barrier against antimicrobials and host defenses, aids surface anchoring and fosters inherent antibiotic tolerance through altered bacterial physiology and microenvironment heterogeneity [13, 18]. Biofilms also facilitate horizontal gene transfer [14] and harbor highly tolerant, dormant persister cells that survive antibiotic exposure and contribute to infection recurrence [19]. IBCs share key protective characteristics with surface biofilms [7].

2.4 Clinical challenges: Antibiotic resistance and recurrence

The clinical impact of UTIs is exacerbated by escalating antibiotic resistance and high rates of recurrence [8]. The global spread of multidrug-resistant (MDR) UPEC, particularly strains producing extended-spectrum beta-lactamases (ESBLs), AmpC

Factor category	Specific factor/ mechanism	Description/function	Role in pathogenesis
Adhesion	Type 1 Fimbriae	Bind mannosylated glycoproteins (e.g., uroplakins) on bladder cells.	Initial bladder colonization; facilitates invasion.
	P Fimbriae	Bind globosides (e.g., GbO3, GbO4) on kidney epithelial cells.	Ascent to kidneys; associated with pyelonephritis.
	Curli fibers	Amyloid fibers promoting cell aggregation and adhesion to surfaces/matrix proteins.	Biofilm formation; adhesion to catheters and host cells.
Invasion and intracellular survival	Invasion mechanisms	Trigger host cell uptake (e.g., via Type 1 fimbriae interaction).	Establishment of intracellular niche.
	Intracellular bacterial communities (IBCs)	Structured bacterial aggregates within host bladder cell cytoplasm.	Protected reservoir; immune evasion; source for recurrence.
Toxins	Hemolysin (HlyA)	Pore-forming toxin lysing host cells.	Tissue damage; nutrient release; immune modulation.
	Cytotoxic necrotizing factor 1 (CNF1)	Modifies host Rho GTPases.	Promotes invasion; cytoskeletal rearrangement; immune modulation.
Nutrient Acquisition	Siderophores (Enterobactin, Yersiniabactin, Salmochelin, Aerobactin)	High-affinity iron chelators.	Scavenge essential iron from host.
Persistence & protection	Biofilm formation (Extracellular)	EPS matrix-encased communities on surfaces.	Persistence; antibiotic tolerance; immune evasion; HGT facilitation.
	Persister cells	Metabolically dormant, highly tolerant bacterial subpopulation.	Survival of antibiotic exposure; relapse after treatment.

Table 1.
Key virulence factors of uropathogenic Escherichia coli (UPEC).

beta-lactamases and carbapenemases, severely limits therapeutic options and worsens patient outcomes [15–17]. Major classes of beta-lactamases found in UPEC and their characteristics are detailed in **Table 2**. Recurrent UTIs (rUTIs), common particularly in women, are strongly associated with UPEC persistence within protective niches like biofilms and IBCs, which allow bacteria to evade clearance and cause subsequent infections [3, 8]. The confluence of UPEC virulence, persistence strategies (biofilms/ IBCs) and antimicrobial resistance presents a major clinical challenge demanding and exploring novel therapies.

Beta-lactamase production by UPEC critically undermines treatment effectiveness [16]. Enzymes like ESBLs, AmpC and particularly carbapenemases confer resistance to essential antibiotics, including last-resort carbapenems [16, 20]. The genes encoding these enzymes often reside on mobile genetic elements, facilitating their spread and contributing to MDR phenotypes [10]. This resistance crisis necessitates novel therapeutic strategies [21, 22]. Key areas include developing new antimicrobials and

Class	Key enzyme types (examples)	Substrate spectrum	Inhibition by classical inhibitors (e.g., Clavulanate)	Key features/significance
Extended-spectrum beta-lactamases (ESBLs)	TEM variants, SHV variants, CTX-M (globally dominant)	Penicillins, Cephalosporins (generations 1–4), Monobactams	Yes	Major cause of resistance to essential cephalosporins; often plasmid-mediated and co-carry other resistance genes.
AmpC beta-lactamases	Plasmid-mediated (e.g., CMY, DHA, FOX) or Chromosomal (inducible/derepressed)	Penicillins, Cephalosporins (incl. Cephamycins), Monobactams	No	Resistance to cephamycins; Not inhibited by standard inhibitors; plasmid types spread rapidly; Chromosomal type can be induced.
Carbapenemases	Class A: KPC Class B (Metallo- β -Lactamases): NDM, VIM, IMP Class D: OXA-48-like	Penicillins, Cephalosporins, Monobactams, Carbapenems	Class A: Variable (inhibited by avibactam) Class B: No (inhibited by EDTA) Class D: Generally No	Hydrolyze carbapenems (“last-resort” drugs); lead to MDR/XDR/PDR strains; Diverse mechanisms; pose major therapeutic challenges.

Table 2.
Major beta-lactamase classes conferring resistance in uropathogenic Escherichia coli (UPEC).

resistance breakers (e.g., novel beta-lactamase inhibitor combinations, siderophore-antibiotic conjugates) [22, 23], alternative therapies (bacteriophages, anti-virulence agents, microbiome modulation) [24–26], specific anti-biofilm approaches [21, 27], immunotherapies [28] and advanced drug delivery systems using nanotechnology [29, 30]. A multifaceted approach involving surveillance, stewardship, research and clinical translation of innovations is essential [15, 31].

3. Uropathogenic *Escherichia coli* (UPEC) biofilms: Mechanisms of persistence and therapeutic targeting

UPEC’s ability to form biofilms is a critical virulence trait supporting its persistence, driving recurrent UTIs (rUTIs) [11] and presenting significant obstacles to effective treatment [8]. Biofilms are surface-associated microbial communities embedded within a self-secreted extracellular polymeric substance (EPS) matrix [12]. This protective structure shields UPEC from environmental stresses, including host immunity and antimicrobial drugs, often significantly limiting agent penetration into deeper layers [13, 18].

The protection offered by the biofilm lifestyle substantially contributes to infection chronicity and creates a microenvironment promoting the development and spread of antibiotic resistance [13]. This further complicates treatment outcomes, particularly given rising global resistance rates [1]. Therefore, understanding UPEC biofilm formation mechanisms and devising counteracting strategies are paramount for developing innovative therapies to tackle persistent and rUTIs.

3.1 Dissecting the mechanisms of UPEC biofilm formation

Developing effective anti-biofilm therapies requires a fundamental understanding of the complex, multi-stage process of biofilm development. Key stages generally include:

Initial attachment: Initial reversible, then irreversible, attachment of planktonic UPEC cells to a suitable biotic (e.g., uroepithelial cells) or abiotic (e.g., catheter) surface [12]. Various adhesins mediate this crucial first step, including Type 1 fimbriae (important for bladder colonization) and potentially P fimbriae (implicated in kidney colonization) [6]. Other surface structures like curli amyloid fibers and autotransporter proteins (e.g., Ag43) also contribute significantly to initial adhesion and cell-cell aggregation [32].

Microcolony development: Attached bacteria proliferate and aggregate, forming distinct microcolonies [12]. Cell-cell interactions mediated by adhesins and early EPS production are important during this stage [18].

Maturation and matrix production: Microcolonies expand and differentiate into complex, three-dimensional structures. The matrix, which is composed of polysaccharides (e.g., cellulose, colanic acid), proteins (e.g., curli), lipids, and extracellular DNA (eDNA), confers structural integrity, protection, and nutrient-trapping capabilities [13, 32].

Dispersion: From mature biofilms, individual planktonic cells or clumps detach and disperse, often triggered by environmental cues like nutrient limitation or waste accumulation [33]. This allows UPEC to colonize new sites, contributing to infection spread and potentially seeding recurrent episodes [8, 33].

Each stage is tightly regulated by intricate gene expression networks involving numerous regulators and signaling pathways [34]. Global regulators like CsgD (master regulator of curli and cellulose) [32] respond to environmental signals (e.g., nutrients, temperature, osmolarity) to coordinate biofilm development [34]. Quorum sensing (QS) systems, allowing bacteria to sense population density and coordinate gene expression, also play important roles. In *E. coli*, the AI-2-mediated QS system (regulated by *lsr*) influences biofilm maturation and virulence factor expression [35].

Furthermore, biofilms harbor specialized cell populations, including persister cells [13]. These phenotypic variants exist in metabolic quiescence or low activity, making them highly tolerant to conventional antibiotics targeting active cellular processes. Persisters survive intensive antibiotic therapy and can reactivate upon removal of antibiotic pressure, leading to relapse or recurrence [19]. Understanding the molecular mechanisms governing biofilm formation, maturation, dispersal and persister formation reveals potential therapeutic targets.

3.2 Developing anti-biofilm strategies against UPEC

Combating UPEC biofilms effectively requires multifaceted strategies targeting different lifecycle stages or structural components. The goals are generally to prevent initial formation or to disrupt and eradicate established communities [21]. This encompasses diverse approaches detailed in **Table 3**. Key approaches under investigation include:

Inhibiting initial adhesion: Preventing the critical first step.

- Targeting adhesins: Developing molecules (small molecules, peptides, carbohydrate analogs like mannosides) that block key adhesins (e.g., FimH) [6, 24].

Strategy category	Target/mechanism	Examples of approaches/agents
Inhibition of initial adhesion	Block bacterial attachment to surfaces (biotic/abiotic).	Fimbrial adhesin inhibitors (e.g., mannosides); anti-adhesion phytochemicals (e.g., cranberry PACs); anti-adhesive surface coatings.
EPS matrix disruption/inhibition	Degrade existing matrix components or prevent synthesis/assembly of the EPS.	Enzymes (DNases, proteases, glycoside hydrolases like dispersin B); chemical destabilizers (chelators, surfactants); inhibitors of matrix synthesis regulators (e.g., targeting CsgD).
Quorum sensing (QS) interference	Disrupt bacterial cell-to-cell communication essential for biofilm maturation and coordinated behaviors.	QS inhibitors (natural or synthetic) targeting signaling pathways (e.g., AI-2 system antagonists).
Targeting persister cells	Eradicate dormant, highly antibiotic-tolerant bacterial subpopulation or sensitize them to antibiotics.	Compounds specifically active against persisters; agents that “awaken” dormant cells.
Enhanced killing within biofilms	Improve the efficacy of bactericidal agents against bacteria protected within the biofilm.	Engineered bacteriophages (potentially expressing depolymerases); metallic Nanoparticles (e.g., AgNPs, ZnO-NPs); certain natural antimicrobials (e.g., essential oils); antimicrobial peptides (AMPs).
Advanced drug delivery systems	Overcome delivery barriers (stability, solubility, penetration, targeting) for anti-biofilm agents.	Liposomal encapsulation; nanoparticle carriers; other polymer-based or stimuli-responsive systems.

Table 3.
Key anti-biofilm strategies against uropathogenic Escherichia coli (UPEC).

- Leveraging natural compounds: Utilizing phytochemicals (e.g., cranberry PACs) known to interfere with fimbrial attachment [27, 36].
- Modifying surfaces: Developing anti-adhesive coatings or impregnating devices (e.g., catheters) with antimicrobials [12, 21].

Disrupting or degrading the EPS matrix: Compromising biofilm structural integrity and protection.

- Enzymatic approaches: Using enzymes (DNases, proteases, glycoside hydrolases like dispersin B) targeting key matrix components [18, 37].
- Chemical destabilization: Employing agents (small molecules, peptides, surfactants, chelators like EDTA) interfering with matrix structure [18, 21].
- Inhibiting matrix synthesis: Targeting biosynthetic pathways for major components (cellulose, curli) or key regulators (CsgD) [21, 32].

Interfering with QS: Disrupting cell-to-cell communication regulating biofilm development and virulence.

- Quorum quenching: Using inhibitors (natural or synthetic) blocking QS molecule (e.g., AI-2) production, detection or signaling [21, 35].

Targeting biofilm-specific physiology and persister cells: Addressing unique bacterial characteristics within biofilms, especially tolerance.

- Eradicating persisters: Developing agents/strategies to kill dormant persisters or induce susceptibility to conventional antibiotics [19, 21].

Enhancing killing of biofilm-embedded bacteria: Improving lethal agent delivery or efficacy within biofilms.

- Bacteriophage therapy: Utilizing phages (potentially engineered with matrix-degrading enzymes) to infect and lyse biofilm bacteria [25, 38].
- Nanoparticles: Employing metallic nanoparticles (e.g., AgNPs, ZnO-NPs) for biofilm penetration and antimicrobial effects (e.g., ROS generation, membrane disruption) [29, 39].
- Natural or synthetic antimicrobials: Exploring compounds (e.g., essential oil components, AMPs) disrupting the matrix or embedded bacteria [27, 40].

Utilizing advanced drug delivery systems: Improving anti-biofilm agent delivery.

- Nanotechnology carriers: Using nanoparticle platforms (liposomes, polymeric nanoparticles) for encapsulation and deep delivery [29, 30].
- Encapsulation strategies: Employing systems like liposomes to enhance penetration, stability and controlled release within biofilms [30].

In conclusion, a detailed understanding of UPEC biofilm formation and persistence mechanisms provides a roadmap for developing targeted therapies. Diverse approaches, from inhibiting adhesion to disrupting the matrix and targeting persisters, offer significant potential to augment current UTI treatments and overcome challenges posed by biofilm-mediated persistence and resistance. Combination therapies, potentially pairing matrix-disrupting or QS-interfering agents with antimicrobials, might achieve synergistic effects, improving clearance and reducing recurrence [21, 41]. Continued vigorous research and successful clinical translation of promising anti-biofilm strategies are essential for improving the management of biofilm-driven UTIs.

3.3 Beyond antibiotics: Emerging strategies targeting uropathogenic *E. coli* biofilms

UPEC's ability to form resilient biofilms is a primary factor in the persistence and recurrence of UTIs [8]. This complicates treatment, particularly amid escalating global antibiotic resistance [15]. The biofilm's protective EPS matrix shields bacteria from host immunity and limits conventional antibiotic efficacy, leading to chronic infections and treatment failures.

This clinical challenge has spurred research into alternative or adjunctive therapies beyond traditional antibiotics [21]. These strategies often focus on preventing biofilm formation, disrupting established biofilms or targeting tolerant bacteria within them. Promising avenues include medicinal plant compounds, metallic nanoparticles (MNPs) and bacteriophages.

3.3.1 Medicinal plants: A reservoir of anti-biofilm compounds

The traditional use of medicinal plants for infections is long-established, and scientific investigation increasingly validates this ethnobotanical knowledge [40]. Plants offer a vast, chemically diverse reservoir of bioactive compounds, many active against pathogens like UPEC [42]. Plant extracts, essential oils and purified phytochemicals show potential as alternatives or adjuncts to conventional antibiotics for UTIs [27].

Key plant-derived compounds (phenolics, alkaloids, terpenoids) show significant anti-biofilm effects against UPEC in preclinical studies. Their multifaceted mechanisms may reduce resistance development compared to single-target drugs [27, 42]. Reported activities span various biofilm lifecycle stages:

Inhibiting adhesion: Compounds like cranberry proanthocyanidins (PACs) hinder UPEC attachment to uroepithelial cells by interfering with P and Type 1 fimbriae [27, 36].

Disrupting biofilm development: Phytochemicals (e.g., from garlic, oregano, thyme, cinnamon) can interfere with maturation by inhibiting matrix synthesis, disrupting aggregation, or interfering with QS [27, 40].

Breaking down mature biofilms: Some agents may degrade matrix components or damage embedded bacteria in mature biofilms [18, 27].

Direct antimicrobial effects: Many exert bactericidal/bacteriostatic effects *via* membrane disruption, enzyme inhibition or metabolic interference [27, 42].

Advantages of plant-based therapies include potentially favorable toxicity profiles and enhanced biocompatibility. Furthermore, synergy with conventional antibiotics may allow reduced dosages and mitigate resistance development [41]. However, clinical translation faces hurdles. Standardization for consistent potency is crucial but challenging. Additionally, poor bioavailability or rapid metabolism limits *in vivo* effectiveness of many phytochemicals. Rigorous preclinical and clinical trials are essential to confirm efficacy, safety and dosage for UTI treatment or prevention [42, 43]. Advanced formulations (e.g., encapsulation in nanoparticles or liposomes) are explored to overcome stability, bioavailability and delivery issues [27, 30]. Addressing these challenges is critical to realizing the therapeutic potential of medicinal plants against UPEC biofilms.

3.3.2 Metallic nanoparticles (MNPs) in biofilm disruption

Advances in nanotechnology have introduced MNPs as innovative anti-biofilm agents. MNPs are engineered particles typically 1–100 nm in size. Various MNPs (AgNPs, ZnO-NPs, TiO₂-NPs, CuO-NPs, AuNPs) show potent antimicrobial and anti-biofilm activity against UPEC *in vitro*. Their efficacy stems from nanoscale properties like high surface area-to-volume ratio, enhancing reactivity and potentially matrix penetration [29, 39].

MNPs combat biofilms *via* multiple simultaneous mechanisms, potentially reducing resistance development compared to single-target antibiotics:

Oxidative stress induction: Many MNPs (e.g., AgNPs, ZnO-NPs) generate ROS, causing oxidative damage to DNA, proteins and lipids, leading to cell death [29, 44].

Membrane damage: Direct interaction compromises membrane integrity, causing leakage and lysis [29, 39].

Interference with cellular functions: Released ions (e.g., Ag⁺ from AgNPs) can interfere with essential processes like enzyme function, DNA replication and metabolism [29, 44].

Matrix destabilization: Interaction with EPS components can weaken biofilm structure (e.g., AgNPs) [29, 39].

Inhibition of biofilm formation: Some MNPs inhibit initial steps like adhesion or interfere with QS. Therapeutic potential is amplified through combination approaches; MNPs often show synergy with conventional antibiotics, enhancing efficacy against resistant or biofilm bacteria [41, 45]. Advanced strategies (integration into DDS, surface modification) aim to improve performance and safety (stability, targeting, penetration, biocompatibility) [29, 46].

However, ensuring safety is critical for MNP clinical translation. Potential cytotoxicity, tissue accumulation and environmental impact require thorough investigation. Extensive *in vitro* and *in vivo* toxicology studies are paramount to establish safety profiles, therapeutic windows, biodistribution and fate before widespread adoption [44, 46]. Addressing safety concerns is key to harnessing the anti-biofilm potential of MNPs for challenging UTIs [29].

3.3.3 Bacteriophage therapy: A resurging and targeted approach against UPEC biofilms

Bacteriophages, viruses that specifically infect and lyse bacteria, are re-emerging as a viable alternative or adjunct to antibiotics, particularly against biofilm-forming and antibiotic-resistant UPEC. Their high specificity minimizes disruption to the host microbiome. Phages can overcome biofilm defenses through enzymatic degradation of the EPS matrix or by infecting and replicating within biofilm-embedded bacteria [25, 47]. Key advancements include the characterization of novel phages like vB_EcoM-phiUZ and vB_EcoP_P64441, which demonstrate efficacy in reducing UPEC biofilm biomass and lysing MDR strains, respectively [48, 49]. Phage cocktails are increasingly utilized to broaden target range, mitigate resistance and have shown success in diminishing biofilm viability of XDR UPEC on surfaces mimicking urinary catheters [50, 51]. A promising avenue involves phages possessing inherent EPS-degrading capabilities, such as those encoding depolymerases (e.g., in phages phT4A and 590B), which actively breaks down biofilm matrix components, enhancing bacterial susceptibility to other antimicrobials [52, 53]. Furthermore, synergistic interactions between bacteriophages and conventional antibiotics (e.g., ciprofloxacin, trimethoprim) have demonstrated enhanced UPEC biofilm eradication compared to monotherapies [38]. Genetic engineering is also refining phage therapeutics by developing phages with enhanced biofilm-degrading enzymes or broader host ranges, alongside the development of purified phage-derived lytic proteins like endolysins, which offer rapid bactericidal action with potentially lower resistance propensity [54]. Collectively, these multifaceted approaches, from natural isolates and strategic cocktails to enzyme-equipped and engineered phages, underscore the evolution of phage therapy into an increasingly sophisticated and targeted modality for combating recalcitrant UPEC biofilm-associated infections. Genetic engineering creates designer phages with enhanced biofilm-tackling capabilities:

Expressing matrix-degrading enzymes: Engineering phages to express enzymes (e.g., depolymerases, DNases) degrades EPS components, clearing a path for deeper phage or antibiotic penetration [38].

Targeting specific bacterial processes: Engineering can modify phages to disrupt functions like QS or virulence factor production [55].

Optimizing phage biology: Modifications can broaden host range, increase killing efficiency or ensure a strictly lytic cycle [56].

Delivering therapeutic payloads: Engineering enables phages to deliver payloads like CRISPR-Cas systems (targeting virulence/resistance genes) or toxins [57].

The high specificity of phages, and the refinement of natural and engineered phages, offers significant promise against challenging, MDR UPEC-associated UTIs, including those involving biofilms. Despite this promise, widespread clinical implementation faces challenges. Bacterial resistance can develop, but may be countered by phage cocktails or combination therapies. Efficient delivery and potential host immune responses are considerations [25, 38]. Establishing clear regulatory pathways, especially for engineered phages, remains ongoing globally. Addressing these hurdles involves optimized cocktails, combination therapies and advanced formulations/delivery [25].

These diverse emerging strategies, medicinal plants, nanotechnology (MNPs) and engineered bacteriophages, represent vital frontiers against persistent, resistant UPEC infections [25, 27, 29]. They offer innovative approaches beyond traditional antibiotics [21]. Medicinal plants provide multi-targeting compounds potentially less prone to resistance [27]; MNPs offer broad-spectrum physicochemical disruption of biofilms [29]; and engineered phages promise highly specific, self-amplifying bacterial killing [56]. The future likely involves judicious combination therapies, potentially integrating novel modalities with each other or existing antibiotics (e.g., using a matrix-disrupting agent alongside an antibiotic or phage) [41]. Rigorous research addressing key challenges (standardization, safety, delivery, regulation) and successful clinical translation are imperative to provide effective new solutions for these difficult-to-treat infections [43].

4. Liposome-based drug delivery systems: Enhancing therapeutic efficacy

A critical bottleneck hindering the clinical translation of many promising discoveries for treating urinary tract infections (UTIs) of uropathogenic *Escherichia coli* (UPEC) is the biofilm barrier [12]. The dense biofilm extracellular polymeric substance (EPS) matrix significantly impedes therapeutic agent penetration, hindering access to bacteria within [18]. Moreover, biofilms harbor physiologically diverse bacteria, including highly tolerant persister cells that survive conventional antibiotics [13]. This combination contributes substantially to treatment failure, recurrence and potential resistance development, highlighting the limitations of traditional drug delivery [8, 21].

Addressing this requires sophisticated delivery strategies to overcome the biofilm barrier and target persistent bacteria. Advanced drug delivery systems (DDS), particularly liposome-based platforms, offer versatile means to enhance therapeutic efficacy against resilient UPEC biofilm infections [30, 58].

Liposomes are attractive DDS platforms due to their established biocompatibility, biodegradability (similar phospholipid composition to cell membranes), versatility in encapsulation and surface modification and clinical track record. Structurally,

liposomes are microscopic vesicles of one or more concentric phospholipid bilayers surrounding an aqueous core. This structure allows encapsulation of diverse payloads: hydrophilic agents (e.g., antibiotics, peptides) in the aqueous core and hydrophobic compounds (e.g., phytochemicals, lipophilic drugs) within the bilayer(s) [30, 58]. Consequently, liposomes can carry a wide spectrum of anti-UPEC agents, from conventional antibiotics and phytochemicals to metallic nanoparticles (MNPs) and bacteriophages enables controlled/sustained release, maintaining therapeutic concentrations longer at the target site [27, 30].

Crucially, liposomes can be engineered to address biofilm challenges. Lipid composition can be tailored to optimize interaction with/fusion to bacterial membranes or facilitate EPS matrix penetration, delivering cargo deep within the biofilm. Furthermore, targeted delivery is achievable *via* surface modification with specific ligands (e.g., antibodies, peptides, mannose recognizing UPEC structures). Targeting promotes liposome accumulation at the infection site, maximizing local drug concentration while minimizing systemic exposure and side effects [30, 59]. Controlled release is also relevant for biofilms, as sustained exposure may be needed to eliminate slow-growing/dormant persister cells responsible for relapse [19, 30].

Liposomal delivery holds promise for enhancing various emerging anti-biofilm agents facing delivery challenges:

Medicinal plant compounds: Many phytochemicals (e.g., PACs, carvacrol, curcumin) show potent *in vitro* anti-UPEC/anti-biofilm activity but often have poor water solubility, low bioavailability, rapid metabolism and thus inefficient biofilm delivery/penetration. Liposomal encapsulation enhances solubility, protects from degradation, improves stability and facilitates transport across barriers and deeper biofilm penetration. Preclinical studies show liposomal PACs or carvacrol exhibit improved anti-biofilm activity against UPEC compared to free compounds. Liposomes enable co-delivery of multiple phytochemicals or phytochemicals with antibiotics for synergistic combinations [30, 41, 58].

Metallic nanoparticles (MNPs): While MNPs (e.g., AgNPs, ZnO-NPs) show potent antimicrobial activity, clinical use can be hampered by aggregation, potential host toxicity and limited biofilm penetration. Encapsulating MNPs in liposomes helps prevent aggregation, potentially reduces cytotoxicity by shielding host tissues, enhances biocompatibility, facilitates biofilm delivery and allows controlled release within the biofilm. Studies show liposome-encapsulated AgNPs/ZnO-NPs exhibit enhanced anti-biofilm efficacy (e.g., vs. *Pseudomonas aeruginosa*) with reduced host toxicity compared to bare MNPs. Co-encapsulation of MNPs with other antimicrobials in liposomes could enable potent synergistic combinations [29, 41, 46].

Engineered bacteriophages: Phage therapy, especially using engineered phages expressing matrix-degrading enzymes, offers highly specific UPEC killing and biofilm disruption. However, challenges include potential inactivation (immune system, environment) and inefficient EPS matrix penetration. Liposomal encapsulation protects phages from neutralizing antibodies and harsh conditions, prolonging *in vivo* viability and infectivity. Furthermore, liposomes might act as ‘Trojan horses,’ facilitating phage delivery through the EPS matrix. Research indicates liposome-encapsulated phages show improved stability, enhanced biofilm disruption and better preclinical outcomes compared to free phages. Co-encapsulation of phages with synergistic antibiotics or enzymes in liposomes could enable sophisticated multi-pronged attacks [25, 38, 59].

In summary, advanced DDS, by enhancing stability, solubility, penetration and targeted delivery of diverse agents (conventional antibiotics, phytochemicals, MNPs,

phages), liposomal formulations hold significant potential to improve treatment efficacy for persistent/recurrent UTIs [21]. They also provide a platform for rational combination therapies, potentially reducing required dosages and aiding the fight against antimicrobial resistance [21, 41]. Continued innovation in liposome design (e.g., stimuli-responsive release, enhanced targeting) coupled with rigorous preclinical/clinical evaluation is essential to translate laboratory discoveries into effective patient care [30, 58].

5. Discussion and future directions

Uropathogenic *Escherichia coli* (UPEC) remains a significant clinical challenge, primarily due to its adeptness at causing persistent and recurrent urinary tract infections (UTIs) [1]. This challenge is amplified by two converging factors including UPEC's capacity to form resilient biofilms [11] and the escalating global crisis of antimicrobial resistance (AMR) [15]. Most UPEC isolates from recurrent UTIs and catheter-associated UTIs (CAUTIs) are robust biofilm producers [12]. Biofilm formation is a cornerstone of UPEC's survival and persistence strategy, providing a protected niche [12]. This niche involves initial adhesion (*via* Type 1 and P fimbriae) [6] and subsequent encasement within a self-produced extracellular polymeric substance (EPS) matrix [13]. This structure, coordinated by adhesins, regulatory networks and quorum sensing (QS), shields bacteria from host immunity and limits antimicrobial penetration [18, 32, 35].

Compounding this physical defense is the surge in prevalence and diversity of antibiotic resistance determinants in clinical UPEC isolates. Particularly concerning is the dissemination of genes encoding beta-lactamases, including extended-spectrum beta-lactamases (ESBLs, dominated by CTX-M types) [10], AmpC beta-lactamases (plasmid-mediated and chromosomal) [20], and increasingly, carbapenemases (KPC, NDM, OXA-48 types) [16]. Global surveillance reports alarming increases in MDR, XDR and carbapenemase-producing UPEC, with resistance rates sometimes exceeding 40% in certain regions or populations [15, 60]. These enzymes dismantle key beta-lactams, severely restricting therapeutic options, especially against biofilm-protected bacteria [16]. This dual challenge increases treatment failure, patient morbidity, healthcare costs and the emergence of difficult-to-treat infections potentially progressing to urosepsis, necessitating innovative therapeutic approaches [1, 21].

In response, diverse alternative and adjunctive strategies targeting UPEC pathogenicity, biofilms and resistance factors are under investigation [21]. Preclinical evidence supports strategies disrupting the biofilm lifestyle at various stages. These include enzymatic matrix degradation to expose bacteria, interfering with QS to disrupt coordinated behavior or blocking initial adhesion via fimbriae [35, 37]. Medicinal plants offer phytochemicals with multi-targeted anti-UPEC activities, including anti-biofilm effects, potentially circumventing rapid resistance development associated with single-target drugs [27, 42]. Nanotechnology offers distinct tools for combating biofilms. Metallic nanoparticles (MNPs) leverage unique nanoscale properties for enhanced biofilm penetration and multi-mechanism antimicrobial effects (e.g., ROS generation, membrane disruption, enzyme interference) [29, 44]. These multi-pronged attacks may be less prone to resistance and could re-sensitize resistant bacteria when used in combination [41]. Engineered bacteriophages offer another promising frontier, providing highly specific lysis of UPEC (including MDR strains) while minimizing microbiota disruption [25, 57]. Genetic engineering enhances phage potency against biofilms, for example, by adding matrix-degrading enzymes to improve access [38, 55].

Despite their promise, successful clinical translation of these innovative approaches faces limitations. Challenges include poor stability/solubility, limited bioavailability, potential toxicity and critically, inefficient delivery and penetration through the EPS matrix [21]. Advanced drug delivery systems (DDS), particularly liposomes, offer transformative potential here [51]. Liposomes encapsulate diverse cargo, from conventional antibiotics and small molecules to phytochemicals, MNPs and bacteriophages [25, 30].

Liposomal encapsulation provides multiple benefits crucial for tackling biofilm infections. Benefits include payload protection from degradation, enhanced solubility/stability, controlled/sustained release (vital for persisters), promoted matrix penetration and targeted delivery via surface functionalization, concentrating therapeutic effect locally while potentially minimizing systemic side effects [30, 58]. Liposomal DDS also provide an elegant means for synergistic combination therapies. Preclinical research increasingly shows that co-delivering agents with complementary mechanisms (e.g., antibiotic + matrix-disrupting enzyme, phytochemical + MNP, phage + synergistic antibiotic) in one liposome significantly enhances eradication of biofilm-embedded UPEC compared to individual agents [38, 41]. These DDS-delivered combination strategies may also slow resistance emergence. Orchestrating multi-pronged attacks against resistant UPEC biofilms is a promising frontier in UTI therapeutics [15, 30].

Looking ahead, successful clinical implementation requires navigating remaining hurdles. Rigorous validation *via* well-designed clinical trials is paramount [43]. Further DDS optimization for stability, predictable release and robust safety margins is critical [58]. Scalable, cost-effective manufacturing is necessary for accessibility. Clear, streamlined regulatory pathways for advanced products (e.g., phages, nanomedicines) are also essential [56]. Alongside translational efforts, continued fundamental research into UPEC biofilm formation, persistence and biofilm capacity is essential for guiding personalized treatment.

6. Conclusion

The fight against biofilm-forming, antibiotic-resistant UPEC necessitates a sustained, multifaceted and collaborative approach. Integrating innovative therapeutic modalities, such as natural products, nanotechnology and phage biology, with sophisticated drug delivery systems like liposomes, provides a powerful framework for developing more effective treatments for recurrent UTIs. These scientific advancements must proceed in tandem with essential public health measures, including robust antibiotic stewardship programs to preserve the efficacy of existing drugs and stringent infection prevention and control practices, particularly within healthcare settings. Vigorous interdisciplinary collaboration among clinicians, microbiologists, pharmacologists, materials scientists and regulatory agencies offers the best hope for improving clinical outcomes for these difficult-to-treat infections. Successfully addressing the challenges posed by UPEC will not only alleviate the burden of these specific infections but also offer valuable lessons for combating other biofilm-associated diseases and the broader global threat of antimicrobial resistance.

Conflict of interest

The authors declare no conflict of interest.

Author details

Rahimi Fateh^{1*} and Qasemi Ali²

1 University of Isfahan, Isfahan, Iran

2 University of Sistan and Baluchestan, Zahedan, Iran

*Address all correspondence to: fateh.rahimi@hotmail.com

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Zhou Y, Zhou Z, Zheng L, Gong Z, Li Y, Jin Y, et al. Urinary tract infections caused by uropathogenic *Escherichia coli*: Mechanisms of infection and treatment options. *International Journal of Molecular Sciences*. 2023;**24**(13):10537. DOI: 10.3390/ijms241310537
- [2] Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C. Urinary tract infections: The current scenario and future prospects. *Pathogens*. 2023;**12**(4):623. DOI: 10.3390/pathogens12040623
- [3] GBD 2021 Forecasting Collaborators. Burden of disease scenarios for 204 countries and territories, 2022–2050: A forecasting analysis for the global burden of disease study 2021. *Lancet*. 2024;**403**(10440):2204–2256. DOI: 10.1016/S0140-6736(24)00685-8
- [4] Whelan S, Lucey B, Finn K. Uropathogenic *Escherichia coli* (UPEC)-associated urinary tract infections: The molecular basis for challenges to effective treatment. *Microorganisms*. 2023;**11**(9):2169. DOI: 10.3390/microorganisms11092169
- [5] Sarowska J, Futoma-Koloch B, Jama-Kmiecik A, Frej-Madrzak M, Ksiazczyk M, Bugła-Ploskonska G, et al. Virulence factors, prevalence and potential transmission of extraintestinal pathogenic *Escherichia coli* isolated from different sources: Recent reports. *Gut Pathogens*. 2019;**11**:10. DOI: 10.1186/s13099-019-0290-0
- [6] García V, Stærk K, Alobaidallah MSA, Grønnemose RB, Guerra PR, Andersen TE, et al. Genome-wide analysis of fitness factors in uropathogenic *Escherichia coli* in a pig urinary tract infection model. *Microbiological Research*. 2022;**265**:127202. DOI: 10.1016/j.micres.2022.127202. Epub 2022 Sep 15
- [7] Sharma K, Dhar N, Thacker VV, Simonet TM, Signorino-Gelo F, Knott GW, et al. Dynamic persistence of UPEC intracellular bacterial communities in a human bladder-chip model of urinary tract infection. *eLife*. 2021;**10**:e66481. DOI: 10.7554/eLife.66481
- [8] Josephs-Spaulding J, Krogh TJ, Rettig HC, Lyng M, Chkonina M, Waschina S, et al. Recurrent urinary tract infections: Unraveling the complicated environment of uncomplicated rUTIs. *Frontiers in Cellular and Infection Microbiology*. 2021;**11**:562525. DOI: 10.3389/fcimb.2021.562525
- [9] Cavas L, Kirkiz I. Characterization of siderophores from *Escherichia coli* strains through genome mining tools: An antiSMASH study. *AMB Express*. 2022;**12**(1):74. DOI: 10.1186/s13568-022-01421-x
- [10] Pitout JD, DeVinney R. *Escherichia coli* ST131: A multidrug-resistant clone primed for global domination. *F1000Research*. 2017;**6**:195. DOI: 10.12688/f1000research.10609.1
- [11] Zhao A, Sun J, Liu Y. Understanding bacterial biofilms: From definition to treatment strategies. *Frontiers in Cellular and Infection Microbiology*. 2023;**13**:1137947. DOI: 10.3389/fcimb.2023.1137947
- [12] Mancuso G, Trinchera M, Midiri A, Zummo S, Vitale G, Biondo C. Novel antimicrobial approaches to combat bacterial biofilms associated with urinary tract infections. *Antibiotics*

(Basel). 2024;**13**(2):154. DOI: 10.3390/antibiotics13020154

[13] Lila ASA, Rajab AAH, Abdallah MH, Rizvi SMD, Moin A, Khafagy ES, et al. Biofilm lifestyle in recurrent urinary tract infections. *Life (Basel)*. 2023;**13**(1):148. DOI: 10.3390/life13010148

[14] Yang S, Li X, Cang W, Mu D, Ji S, An Y, et al. Biofilm tolerance, resistance and infections increasing threat of public health. *Microbial Cell*. 2023;**10**(11):233-247. DOI: 10.15698/mic2023.11.807

[15] Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *Lancet*. 2022;**399**(10325):629-655. DOI: 10.1016/S0140-6736(21)02724-0

[16] Schifano E, Marazzato M, Ammendolia MG, Zanni E, Ricci M, Comanducci A, et al. Virulence behavior of uropathogenic *Escherichia coli* strains in the host model *Caenorhabditis elegans*. *Microbiology*. 2019;**8**(6):e00756. DOI: 10.1002/mbo3.756

[17] Frickmann H, Hahn A, Berlec S, Ulrich J, Jansson M, Schwarz NG, et al. On the etiological relevance of *Escherichia coli* and *Staphylococcus aureus* in superficial and deep infections - A hypothesis-forming, retrospective assessment. *European Journal of Microbiology and Immunology (Bp)*. 2019;**9**(4):124-130. DOI: 10.1556/1886.2019.00021

[18] Donlan RM. Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases*. 2002;**8**(9):881-890. DOI: 10.3201/eid0809.020063

[19] Niu H, Gu J, Zhang Y. Bacterial persists: Molecular mechanisms and therapeutic development. *Signal Transduction and Targeted*

Therapy. 2024;**9**(1):174. DOI: 10.1038/s41392-024-01866-5

[20] De Angelis G, Del Giacomo P, Posteraro B, Sanguinetti M, Tumbarello M. Molecular mechanisms, epidemiology, and clinical importance of β -lactam resistance in *Enterobacteriaceae*. *International Journal of Molecular Sciences*. 2020;**21**(14):5090. DOI: 10.3390/ijms21145090

[21] Nasrollahian S, Graham JP, Halaji M. A review of the mechanisms that confer antibiotic resistance in pathotypes of *E. coli*. *Frontiers in Cellular and Infection Microbiology*. 2024;**14**:1387497. DOI: 10.3389/fcimb.2024.1387497

[22] Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, et al. β -Lactamases and β -lactamase inhibitors in the 21st century. *Journal of Molecular Biology*. 2019;**431**(18):3472-3500. DOI: 10.1016/j.jmb.2019.04.002

[23] Watkins RR, Bonomo RA. Overview: The ongoing threat of antimicrobial resistance. *Infectious Disease Clinics of North America*. 2020;**34**(4):649-658. DOI: 10.1016/j.idc.2020.04.002

[24] Dehbanipour R, Ghalavand Z. Anti-virulence therapeutic strategies against bacterial infections: Recent advances. *Germs*. 2022;**12**(2):262-275. DOI: 10.18683/germs.2022.1328

[25] Górski A, Międzybrodzki R, Węgrzyn G, Jończyk-Matysiak E, Borysowski J, Weber-Dąbrowska B. Phage therapy: Current status and perspectives. *Medicinal Research Reviews*. 2020;**40**(1):459-463. DOI: 10.1002/med.21593. Epub 2019 May 7

[26] Cusumano G, Flores GA, Venanzoni R, Angelini P. The impact of antibiotic therapy on intestinal

microbiota: Dysbiosis, antibiotic resistance, and restoration strategies. *Antibiotics (Basel)*. 2025;**14**(4):371. DOI: 10.3390/antibiotics14040371

[27] Wu SC, Liu F, Zhu K, Shen JZ. Natural products that target virulence factors in antibiotic-resistant *Staphylococcus aureus*. *Journal of Agricultural and Food Chemistry*. 2019;**67**(48):13195-13211. DOI: 10.1021/acs.jafc.9b05595. Epub 2019 Nov 21

[28] Mak Q, Greig J, Dasgupta P, Malde S, Raison N. Bacterial vaccines for the management of recurrent urinary tract infections: A systematic review and meta-analysis. *European Urology Focus*. 2024;**10**(5):761-769. DOI: 10.1016/j.euf.2024.04.002. Epub 2024 Apr 21

[29] Franco D, Calabrese G, Guglielmino SPP, Conoci S. Metal-based nanoparticles: Antibacterial mechanisms and biomedical application. *Microorganisms*. 2022;**10**(9):1778. DOI: 10.3390/microorganisms10091778

[30] Paliwal R, Paliwal SR, Kenwat R, Kurmi BD, Sahu MK. Solid lipid nanoparticles: A review on recent perspectives and patents. *Expert Opinion on Therapeutic Patents*. 2020;**30**(3):179-194. DOI: 10.1080/13543776.2020.1720649

[31] World Health Organization. Global Action Plan on Antimicrobial Resistance. Geneva, Switzerland: World Health Organization; 2022. ISBN: 978 92 4 004198 1

[32] Yan Z, Yin M, Chen J, Li X. Assembly and substrate recognition of curli biogenesis system. *Nature Communications*. 2020;**11**(1):241. DOI: 10.1038/s41467-019-14145-7

[33] Grooters KE, Ku JC, Richter DM, Krinock MJ, Minor A, Li P, et al.

Strategies for combating antibiotic resistance in bacterial biofilms. *Frontiers in Cellular and Infection Microbiology*. 2024;**14**:1352273. DOI: 10.3389/fcimb.2024.1352273

[34] Abdelhamid AG, Yousef AE. Combating bacterial biofilms: Current and emerging antibiofilm strategies for treating persistent infections. *Antibiotics (Basel)*. 2023;**12**(6):1005. DOI: 10.3390/antibiotics12061005

[35] Markowska K, Szymanek-Majchrzak K, Pituch H, Majewska A. Understanding quorum-sensing and biofilm forming in anaerobic bacterial communities. *International Journal of Molecular Sciences*. 2024;**25**(23):12808. DOI: 10.3390/ijms252312808

[36] Hirakawa H, Suzue K, Kurabayashi K, Tomita H. The Tol-Pal system of uropathogenic *Escherichia coli* is responsible for optimal internalization into and aggregation within bladder epithelial cells, colonization of the urinary tract of mice, and bacterial motility. *Frontiers in Microbiology*. 2019;**10**:1827. DOI: 10.3389/fmicb.2019.01827

[37] Thorn CR, Howell PL, Wozniak DJ, Prestidge CA, Thomas N. Enhancing the therapeutic use of biofilm-dispersing enzymes with smart drug delivery systems. *Advanced Drug Delivery Reviews*. 2021;**179**:113916. DOI: 10.1016/j.addr.2021.113916

[38] Faruk O, Jewel ZA, Bairagi S, Rasheduzzaman M, Bagchi H, Tuha ASM, et al. Phage treatment of multidrug-resistant bacterial infections in humans, animals, and plants: The current status and future prospects. *Infectious Medicine (Beijing)*. 2025;**4**(1):100168. DOI: 10.1016/j.imj.2025.100168

[39] Chakraborty N, Jha D, Roy I, Kumar P, Gaurav SS, Marimuthu K,

et al. Nanobiotics against antimicrobial resistance: Harnessing the power of nanoscale materials and technologies. *Journal of Nanobiotechnology*. 2022;**20**(1):375. DOI: 10.1186/s12951-022-01573-9

[40] Iseppi R, Mariani M, Condò C, Sabia C, Messi P. Essential oils: A natural weapon against antibiotic-resistant bacteria responsible for nosocomial infections. *Antibiotics (Basel)*. 2021;**10**(4):417. DOI: 10.3390/antibiotics10040417

[41] Brochado AR, Telzerow A, Bobonis J, Banzhaf M, Mateus A, Selkig J, et al. Species-specific activity of antibacterial drug combinations. *Nature*. 2018;**559**(7713):259-263. DOI: 10.1038/s41586-018-0278-9

[42] Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. A comprehensive review on medicinal plants as antimicrobial therapeutics: Potential avenues of biocompatible drug discovery. *Metabolites*. 2019;**9**(11):258. DOI: 10.3390/metabo9110258

[43] Choudhury A, Singh PA, Bajwa N, Dash S, Bisht P. Pharmacovigilance of herbal medicines: Concerns and future prospects. *Journal of Ethnopharmacology*. 2023;**309**:116383. DOI: 10.1016/j.jep.2023.116383

[44] Eker F, Duman H, Akdaşçi E, Witkowska AM, Bechelany M, Karav S. Silver nanoparticles in therapeutics and beyond: A review of mechanism insights and applications. *Nanomaterials (Basel)*. 2024;**14**(20):1618. DOI: 10.3390/nano14201618

[45] Mohanta YK, Biswas K, Jena SK, Hashem A, Abd Allah EF, Mohanta TK. Anti-biofilm and antibacterial activities of silver nanoparticles synthesized by the reducing activity

of phytoconstituents present in the Indian medicinal plants. *Frontiers in Microbiology*. 2020;**11**:1143. DOI: 10.3389/fmicb.2020.01143

[46] Li M, Liu Y, Gong Y, Yan X, Wang L, Zheng W, et al. Recent advances in nanoantibiotics against multidrug-resistant bacteria. *Nanoscale Advances*. 2023;**5**(23):6278-6317. DOI: 10.1039/d3na00530e

[47] Yang X, Ye W, Qi Y, Ying Y, Xia Z. Overcoming multidrug resistance in bacteria through antibiotics delivery in surface-engineered nano-cargos: Recent developments for future nano-antibiotics. *Frontiers in Bioengineering and Biotechnology*. 2021;**9**:696514. DOI: 10.3389/fbioe.2021.696514

[48] González-Villalobos E, Ribas-Aparicio RM, Montealegre GER, Belmont-Monroy L, Ortega-García Y, Aparicio-Ozores G, et al. Isolation and characterization of novel bacteriophages as a potential therapeutic option for *Escherichia coli* urinary tract infections. *Applied Microbiology and Biotechnology*. 2021;**105**(13):5617-5629. DOI: 10.1007/s00253-021-11432-6

[49] Wintachai P, Thaion F, Clokie MRJ, Thomrongsuwanakij T. Isolation and characterization of a novel *Escherichia* bacteriophage with potential to control multidrug-resistant avian pathogenic *Escherichia coli* and biofilms. *Antibiotics (Basel)*. 2024;**13**(11):1083. DOI: 10.3390/antibiotics13111083

[50] Sanchez BC, Heckmann ER, Green SI, Clark JR, Kaplan HB, Ramig RF, et al. Development of phage cocktails to treat *E. coli* catheter-associated urinary tract infection and associated biofilms. *Frontiers in Microbiology*. 2022;**13**:796132. DOI: 10.3389/fmicb.2022.796132

- [51] Abedon ST, Danis-Włodarczyk KM, Wozniak DJ. Phage cocktail development for bacteriophage therapy: Toward improving spectrum of activity breadth and depth. *Pharmaceuticals (Basel)*. 2021;**14**(10):1019. DOI: 10.3390/ph14101019
- [52] Islam MM, Mahbub NU, Shin WS, Oh MH. Phage-encoded depolymerases as a strategy for combating multidrug-resistant *Acinetobacter baumannii*. *Frontiers in Cellular and Infection Microbiology*. 2024;**14**:1462620. DOI: 10.3389/fcimb.2024.1462620
- [53] Chaudhary N, Maurya RK, Singh D, Mohan B, Taneja N. Genome analysis and antibiofilm activity of phage 590B against multidrug-resistant and extensively drug-resistant uropathogenic *Escherichia coli* isolates, India. *Pathogens*. 2022;**11**(12):1448. DOI: 10.3390/pathogens11121448
- [54] Wesołowski W, Łukasiak A, Bloch S, Kuligowska K, Neumann J, Lewandowska N, et al. Phage endolysins as promising and effective candidates for use against uropathogenic *Escherichia coli*. *Viruses*. 2025;**17**(4):560. DOI: 10.3390/v17040560
- [55] Fang K, Park OJ, Hong SH. Controlling biofilms using synthetic biology approaches. *Biotechnology Advances*. 2020;**40**:107518. DOI: 10.1016/j.biotechadv.2020.107518
- [56] Subramanian A. Emerging roles of bacteriophage-based therapeutics in combating antibiotic resistance. *Frontiers in Microbiology*. 2024;**15**:1384164. DOI: 10.3389/fmicb.2024.1384164
- [57] Ling H, Lou X, Luo Q, He Z, Sun M, Sun J. Recent advances in bacteriophage-based therapeutics: Insight into the post-antibiotic era. *Acta Pharmaceutica Sinica B*. 2022;**12**(12):4348-4364. DOI: 10.1016/j.apsb.2022.05.007
- [58] Nsairat H, Khater D, Sayed U, Odeh F, Al Bawab A, Alshaer W. Liposomes: Structure, composition, types, and clinical applications. *Heliyon*. 2022;**8**(5):e09394. DOI: 10.1016/j.heliyon.2022.e09394
- [59] Easwaran M, Raja N, Saravanan M, Belete MA. Liposome-loaded phage cocktail: A promising therapeutic option against postsurgical wound infections - A critical appraisal. *Annals of Medicine and Surgery (London)*. 2024;**86**(8):4319-4321. DOI: 10.1097/MS9.0000000000002275
- [60] Asokan GV, Ramadhan T, Ahmed E, Sanad H. WHO global priority pathogens list: A bibliometric analysis of Medline-PubMed for knowledge mobilization to infection prevention and control practices in Bahrain. *Oman Medical Journal*. 2019;**34**(3):184-193. DOI: 10.5001/omj.2019.37

Catabolite Repression of Dual-Species Biofilm Formation by Participation of *Escherichia coli* Strains

Ivo Ganchev

Abstract

The composition of the microbial community in the biofilms is determined by the nature and content of compounds of the nutrient medium. The structure of biofilms, formed by the participation of one or more bacterial species, is composed of a large number of populations, distinguished by their metabolic features and by the genetic mechanisms determining their formation. Changes in the conditions of their cultivation or the environment in which their formation takes place affect the metabolic activity of *Bacillus subtilis* 170 and 168 and *Escherichia coli* K-12 strains and their distribution in the structure of biofilms in the process of their co-development. The influence of the content of glucose in the medium on bacterial biofilm formation and the mechanisms were determined by the method, based on crystal violet staining, on the cultivated microbial analysis and confocal laser scanning microscopy. In the recent research, the glucose content at the concentration over of 0.5% in the cultural medium caused the inhibition of their growth and influenced negatively on the process of biofilm formation by the co-cultivation of *B. subtilis* 170 and *E.coli* K-121655, *B. subtilis* 168 and *E.coli* K-121655 strains. In regard to static methods of the biofilm formation at laboratory conditions is a highly relevant growing of the most of soil bacteria in the environment, the evaluation of the role of the catabolite regulation on the sessile growth of the soil bacteria is required to decrease of the information in regard to biofilm production during glucose excess.

Keywords: catabolite glucose repression, confocal laser scanning microscopy, *Escherichia coli* K-12, multispecies biofilms, relationships in biofilms

1. Introduction

In the most ecological niches, bacterial species are organized in communities and develop together in the form of heterogeneous structures called biofilms, which are formed on surfaces of different nature and as a result of the

participation and the interaction of cells of two or more bacterial species [1]. The microbial community in the biofilms is determined by the nature and medium in the environment. The structure of bacterial biofilms is composed of a large number of populations, distinguished by their metabolic features and the genetic mechanisms determining their formation [2].

Glucose induces biofilm formation by *Bacillus subtilis* and *Escherichia coli* strains [3]. The mechanism of catabolite repression and glucose metabolism plays an important role in the formation of biofilms by strains of *Escherichia coli*, which affects the synthesis of autoinducers 2 and indoles regulated by cyclic adenosine monophosphate and the receptor protein CRP. The presence of glucose is associated with an increase in the activity of adenylate cyclase, which leads to a decrease in the intracellular level of cAMP and to the repression of the synthesis of the receptor protein, and in its absence, they form a complex that ensures the activation of genes for the biosynthesis of exocellular polysaccharides from the composition of matrix [4]. The regulatory protein CsgD affects the formation of fringes, which represent amyloid fibers, and the glycan chains of the cellulose structure, which is part of the matrix of biofilms in *Escherichia coli* strains [5]. Its main function is reduced to suppressing the transcription of genes responsible for the biosynthesis of fringes and pili, taking part in their cell motility [6], and increasing its activity ensures the adhesion of *Escherichia coli* cells on different surfaces and their development into biofilms [5]. The increase in its activity appears to be the result of the formation of cyclic adenosine monophosphate [7] at low glucose content in the biofilm culture medium [8]. As a result of this regulatory process, conditions are created to inhibit the synthesis of type 1 pili in *Escherichia coli* strains, which prevents the implantation of their cells on the surface layer of the epithelial tissue of the host organism [9]. The catabolite repression protein binds to *kps* operon responsible for K1 capsule secretion [5]. Capsular polysaccharides secreted by uropathogenic *Escherichia coli* strains bind to the surface layer of the substrate with the help of covalent bonds [10], as a result of which a barrier is created for the colonization of the coexisting species during their development to the formation of biofilms [11]. The ability to form and release capsular polysaccharides 2 ensures the competitive advantage of *Escherichia coli* strains in the microbial community during their colonization on surfaces different in nature and structure [3].

The formation of biofilms by *Bacillus subtilis* strains occurs at a low concentration of glucose in the culture medium and is inhibited of glucose in the nutritional medium and is inhibited when it increases. The stimulating effect of low concentrations is due to its metabolism to acetoin, which activates the positive regulator SpoOA of the process of biofilm formation on different surfaces. The catabolite repression of the process carried out by *Bacillus subtilis* strains occurs with an increase in the concentration of glucose in the medium, which is determined by the increase in the activity of the regulatory protein CspA, as a result of which the proportion of plankton cells increases compared to their number in the structure of biofilms [12]. In some metabolic pathways for the synthesis of exocellular polysaccharides from the structure of the matrix of biofilms in *Bacillus subtilis* strains, uridine diphosphate-glucose and uridine diphosphate-galactose serve as starting compounds, which are intermediate metabolites in the catabolism of galactose in the Leloir pathway, the first step of which it results in the phosphorylation of galactose to galactose-1-phosphate by galactose-1-phosphate kinase. The phosphate form is converted to uridine diphosphate-galactose by galactose-1-phosphate uridylyltransferase. Under the influence of UDP-galactose-4-epimerase, UDP-galactose is converted to UDP-glucose, which serve as starting compounds in biosynthesis reactions of polysaccharides of the

matrix structure of the biofilms of *Bacillus subtilis* strains [13]. The minimal content of glucose in the medium stimulates the biosynthesis of surfactants by *B. subtilis* strains with a lipoprotein nature, the structure of which includes a hydrophobic and a hydrophilic part and is made up of β -hydroxyl fatty acid and β -amino fatty acid residues [14]. This group of compounds is distinguished by the property of changing the character of the surface layer of the substrate and hindering the colonization of the coexisting microbial species in the process of biofilm formation. Their impact is determined by the hydrophobicity of the bacterial surface, their structural features and their concentration in the intercellular space [15]. Most biofilms in the nature include species of different microbial genus in their composition, but the mechanism about glucose excess regulation on the structure of microbial communities and the biofilm matrix component and spore formation in them remains scarce in the modern scientific literature [16].

The aim of the present study is to evaluate the effect of the glucose concentration in the cultural medium on the biofilm formation and structure of biofilms, that are formed by *B. subtilis* strains during their relationships with *Escherichia coli* K-121655 strain. The influence of the content of glucose in the medium on bacterial biofilm formation and the mechanisms were determined by the method, based on the crystal violet staining, on the cultivated microbial analysis and confocal laser scanning microscopy. The results of this study leads to the conclusion, that there exists a narrow relationship between biofilm formation and glucose concentration during interaction of *B. subtilis* and *E. coli* strains, i.e., the glucose content at the concentration over of 0,5% in the cultural medium caused the inhibition of their growth and influenced negatively on the process of biofilm formation by the co-cultivation of *B. subtilis* 170 and *E. coli* K-121655, *B. subtilis* 168 and *E. coli* K-121655 strains.

2. Materials and methods

2.1 Bacterial strains and culture media

Bacillus subtilis and *Escherichia coli* strains in the present study are part of the microbial collection of The National Bank of Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria, and “Stefan Angelov” Institute of Microbiology in Sofia, Bulgaria. The cultures to be studied were stored at -80°C in the presence of 20 (v/v) % glycerol (NCZPB, Sofia), after being cultivated in Luria Bertani broth (LB, NCZPB, Sofia) until reaching a stationary phase of development. The total incubation time of *Bacillus subtilis* and *Escherichia coli* strains to the stationary phase of development was 24 hours at 37°C . The colony from the cultures stored on the agar plate was transferred to an Erlenmeyer flask containing 20 mL of LB medium (LB, NCZPB, Sofia). The preparation of *Bacillus subtilis* and *Escherichia coli* strains involved incubation for 24 hours at 37°C . After this liquid culture time, the total cell density was about $\sim 10^9$ CFU/mL.

2.2 Biofilm development conditions

The previously prepared stationary phase cultures were subjected to dilution by transferring 100 μl of it to 5 ml of LB broth until a cell density of $\sim 10^7$ CFU/mL was reached. The mixing ratios of the cultures of the two strains (*Bacillus subtilis* and *Escherichia coli*) were 1:1, and the growth medium was Luria Bertani Broth

(NCZPB, Sofia), which proved to be the optimal medium for the development of *Bacillus subtilis* and *Escherichia coli* biofilms. About 150 µL of the diluted culture was transferred to a 96-well plates, made of polystyrene (50 mm diameter, 9 mm height). After inoculation, the wells are capped and gently shaken to ensure that the inoculum covers the entire surface. The total duration of incubation of the biofilms was 24 h at 20°C, which is the optimal temperature for the formation of a single-species and multi-species biofilm of *Bacillus subtilis* and *Escherichia coli*, respectively.

2.3 Crystal violet assay

Staining of the biofilms after washing with 0.85% NaCl was performed for 15 min with 200 µL of Crystal Violet at concentration of 0.01% (w/v), after which the excess dye was washed three times with NaCl (36 g.l⁻¹). Biofilm quantification was performed by solubilizing the dye fixed on the biofilm structure with absolute ethanol for 10 min at 20°C, at 120 rpm, and measuring the absorbance of the Crystal Violet solution at 595 nm.

2.4 Quantification of colonies

In the second half of the wells, 150 µl of 0.85% NaCl was added, and the biofilms from each well were separated using of sterile knife, so that the total volume of the suspension was 9 mL. Then, aliquots of 25 µL of the suspension cultures were subjected to falling dilutions (10⁻¹, 10⁻², 10⁻³, and 10⁻⁴), and inoculations were carried out on Plate Count Agar (NCIPD, Sofia) and MacConkey Agar media (NCIPD, Sofia) for the identification of *Bacillus subtilis* and *Escherichia coli*, respectively. Red and pink colonies grown on MacConkey Agar (NCZPB, Sofia) are characteristic of *Escherichia coli* strains. After incubation at 37°C for 24 hours, colony forming units per milliliter (CFU/ml) were determined and converted to log (log₁₀).

2.5 Multispecies biofilm formation

For the cultivation of biofilm of the both microbial strains in this study, the cultures in stationary growth phase were placed in LB broth, and 150 µl of them is transferred in 96-well plates reaching optical density of the suspensions at 600 nm to 0.3 (0.15 per strain). The control samples included only diluted culture of one strain at the same concentrations in the comparison to the multispecies biofilm, at the same conditions for incubation.

To study the influence of the glucose content of the medium on biofilm growth, nutrient media were prepared with different concentration of glucose from 0.1 to 1.0%. For this purpose, 18-hour cultures of *B. subtilis* 170, *B. subtilis* 168 and *E. coli* K-121655 strains were prepared previously in Laura Bertani broth (BulBio Laboratory – Sofia).

2.6 Matrix components staining

The staining of the matrix of the biofilms in the microscopic studies was preceded by their static cultivation for 24 hours on glass coverslips. Each biofilm was stained with DAPI at the concentration of 5 µg/mL. Exopolysaccharides were stained with wheat germ agglutinin (WGA) conjugated to Alexa Fluor™ 555 at the concentration of 100 µg/mL to fix N-acetyl-glucosamine. After 30 min incubation of each sample, each coverslip was washed three times in PBS 1×. Coverslips were transferred onto

glass slides, and visualization of the formed structures and determination of their morphometric characteristics was performed with Leica TCS SPE confocal laser scanning microscopy at a wavelength of 540 nm.

2.7 Data extraction from images and statistics

The COMSTAT is an early biofilm image analysis software tool that has been used to evaluation of the biovolume, the average thickness and the evaluation of the maximum coverage based the CLSM pictures, that described the formed structures of the studied biofilms [7]. Analysis of variance (ANOVA) of the viable counts and the lag times corresponding to the different treatments, was carried out using the statistical program Statistix 8 Software. Differences were tested with paired sample t tests, and if $p < 0.05$, the difference was considered statistically significant.

3. Results and discussion

The change in the glucose content in the range of 0.1 to 0.5% in the biofilm culture medium resulted in the increase of the optical density value to 0.194 ± 0.001 for the co-culture of *B. subtilis* 170 and *E. coli* 1655 strains and 0.142 ± 0.002 in the co-culture of *B. subtilis* 168 and *E. coli* 1655 strains, which is statistically significantly higher than in the monospecies biofilms of *B. subtilis* 170 ($p < 0.05$) and *B. subtilis* 168 strains ($p < 0.05$) and is not significantly different in monospecies biofilms of *E. coli* K-121655 ($p > 0.05$) (Figure 1).

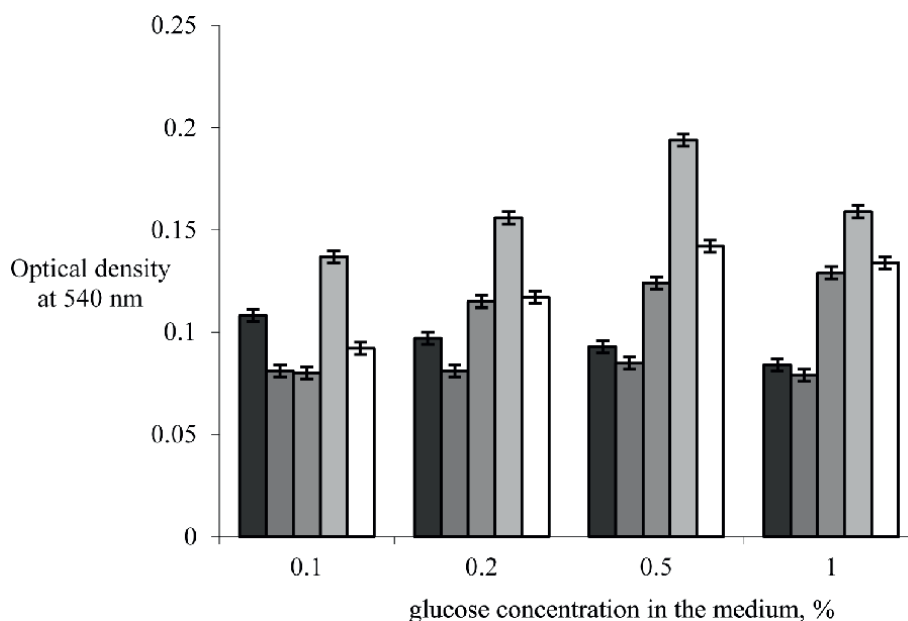


Figure 1.

The Influence of content of glucose in the culture medium on the biofilm growth: ■ *B. subtilis* 170; ■ *B. subtilis* 168; ■ *E. coli* 1655; ■ *B. subtilis* 170 + *E. coli* 1655; □ *B. subtilis* 168 + *E. coli* 1655. $y = -0.0076.x + 0.1142$, $r^2 = 0.972$, $p < 0.05$ - *B. subtilis* 170. $y = -0.0078.x + 0.2412$, $r^2 = 0.951$, $p < 0.05$ - *B. subtilis* 168. $y = 0.0016.x + 0.0730$, $r^2 = 0.930$, $p < 0.05$ - *E. coli* K-121655. $y = 0.1134.x + 0.1739$, $r^2 = 0.979$, $p < 0.05$ - *B. subtilis* 170-*E. coli* K-121655. $y = 0.0746.x + 0.2179$, $r^2 = 0.962$, $p < 0.05$ - *B. subtilis* 168-*E. coli* K-121655.

The increase in content in the range from 0.5 to 1.0% was accompanied in the present study by a statistically insignificant decrease in the biomass increment of the biofilms formed in the co-cultivation of *B. subtilis* 170 and *E. coli* K-121655 strains from 0.194 to 0.159 ($p > 0.05$), as well as for the co-culturer of *B. subtilis* 168 and *E. coli* K-121655 strains ($p > 0.05$). At the same time, the relationship between the concentration of glucose and the growth of the biomass of the biofilm in the studied pair of strains has a linear character ($r^2 = 0.96$), which is distinguished by its character in the monospecies biofilms of *B. subtilis* 170 and *B. subtilis* 168 strains, whose biomass of biofilms decreases directly proportional to the increase in the glucose concentration in the range from 0.1 to 1.0% ($r^2 = 0.97$); while under the same cultivation conditions, a statistically insignificant increase in the value of the optical density at 540 nm is found for the monospecies biofilms of *E. coli* K-121655 strain ($r^2 = 0.96$).

An increase in glucose and fructose content from 0.1 to 1.0% in the medium was accompanied by a twofold decrease in biofilm biomass of *B. subtilis* WT strain in the study by Stanley et al. [17]. Similar observations were made for *E. coli* strains in which the process of biofilm formation also undergoes catabolite repression [5]. The catabolite control protein CcpA in *B. subtilis* is a major regulator that represses genes for exopolysaccharide formation from matrix composition and cell development of strains to biofilm formation, when the content of carbohydrate sources in the medium changes [17]. Mutations in *ccpA* gene of *B. subtilis* WT strain provided an increase in biofilm biomass of about 2.5-fold compared to the parent or wild-type strain in the study of Stanley et al. [17], which confirmed the role of the regulatory protein CcpA in the formation of biofilms by *B. subtilis* strains. The stimulatory effect of glucose on biofilm formation appears to be the result of the effect of acetoin, which is an intermediate product of glucose metabolism [18]. Accumulation of acetoin activates the SpoOA regulator, resulting in a 2.9-fold increase in biofilm biomass of *B. subtilis* WT strain in 20 mM acetoin medium [17]. The derivation by Stanley et al. [17] regularity between medium glucose content and biofilm biomass growth is not in agreement with the nature of the relationships found in the present study, according to which the optical density value at 540 nm reached a maximum value in the co-cultivation of *B. subtilis* 170 and *E. coli* K-121655, *B. subtilis* 168 and *E. coli* K-121655 strains at a glucose content of 0.5%, immediately after which a decrease in its size was observed.

Similar observations were made in the study of biofilm formation in *E. coli* strains, which is also regulated by glucose through the mechanism of catabolite repression, which is initiated by cyclic adenosine monophosphate and the receptor protein for cyclic adenosine monophosphate [5]. Deletion of *crp* gene in *E. coli* MG1655 strain and in *csrA* mutant strain produced biofilm biomass reductions of 30 and 75%, when cultured in 0.2% glucose medium in the study by Jha et al. [14]. In the present study, the effect of catabolite repression on biofilm formation by the multispecies population of *B. subtilis* and *E. coli* strains was observed at a glucose content of 1.0%, which is in agreement with the findings of Jackson et al. [8], that catabolite repression is partly due to cyclic adenosine monophosphate and the receptor protein CRP. In other studies, the formation of a complex between the two molecules ensures the activation of biofilm formation genes and the *flhDC* operon in *E. coli* strains, responsible for the biosynthesis of the flagellar fringe protein [17].

In the course of the study, the predominant number of populations of *B. subtilis* 170 strains of $(12.93 \pm 0.40) \cdot 10^6$ cfu/cm³ and of *B. subtilis* 168 strains of $(9.73 \pm 0.40) \cdot 10^6$ cfu/cm³ in the structure of biofilms was established during their association with of *E. coli* K-121655 strains at a glucose content in the medium of 0.1%. The increase in its value to 1.0% was accompanied by a significant decrease in the

colonies of *B. subtilis* 170 strain from $(12.93 \pm 0.40) \cdot 10^6$ cfu/cm³ to $(5.8 \pm 0.3) \cdot 10^6$ cfu/cm³ in biofilms at the expense of the increase of the number of populations of *E. coli* K-121655 strain to a value of $(10.3 \pm 0.26) \cdot 10^6$ cfu/cm³. The interaction of *E. coli* K-121655 strain with *B. subtilis* 168 strain in the process of biofilm formation leads to an increase in the number of its population to a value of $(10.66 \pm 0.15) \cdot 10^6$ cfu/cm³ at a glucose content of 1.0%. Their values significantly exceeded the number of colonies in the monospecies biofilms of *E. coli* K-121655 strain, *B. subtilis* 170 and *B. subtilis* 168 strains ($p < 0.05$), which was associated with metabolizing glucose and reducing the effect of catabolite repression (**Tables 1 and 2**).

Uropathogenic strains of *E. coli* species are distinguished by the ability to prevent biofilm formation of a large number of gram-positive and gram-negative bacteria (*E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Enterococcus faecalis*) thanks to the formation of a group 2 capsule whose biosynthesis is encoded by *kps* locus [10, 11]. Group 2 capsule formation in the process of biofilm formation promotes an increase in surface hydrophobicity and a reduction in bacterial adhesion by inhibiting cell-to-surface contact and cell-to-cell contacts during biofilm maturation [11]. The study by Rendueles et al. [1] found that the formation of polymeric molecules with anti-adhesion activity with a molecular weight of 100 kDa and resistance to the effects of proteinase K in *E. coli* Ec300 and Ec111 strains was determined by the environmental conditions in which biofilm maturation took place, which corresponds to the regularity established in the present study related to the association of *E. coli* 1655 strains with *B. subtilis* 170 and *B. subtilis* 168 strains. The anti-adhesion polysaccharide formed by *E. coli* Ec300 strain, which is characterized by a lower number of galactosamine and glucosamine residues compared to Ec111 strain, took place in M63B1 medium with a glucose content of 0.4% and inhibited the surface colonization of *S. aureus* strains. The increase in its concentration from 5 to 30 µg/ml in extracts of *E. coli* Ec300 and Ec111 strains is accompanied by a reduction in the biomass of biofilms of *S. aureus* strains in the range of 20 to 80% [1]. Inhibition of the biofilm formation process, initiated by the biosynthesis of capsular polysaccharides by *E. coli* strains, ensures the competitive relationships between microbial species in the process of their colonization on the surface [11], which explains the higher numbers of the population of *E. coli* K-121655 strains in the structure of biofilms during their interaction with *B. subtilis* 170 and *B. subtilis* 168 strains at a glucose content of more than 0.5% in the medium.

The development of *B. subtilis* 170 and *B. subtilis* 168 strains at a glucose concentration in the medium in the range of 0.1 to 0.2% creates conditions for the appearance of spores in the structure of biofilms, with a corresponding maximum value of $0.6 \cdot 10^3$ cfu/cm³, and $0.3 \cdot 10^3$ cfu/cm³ was found at a low glucose content of 0.1%, while at the concentration of 0.5%, no presence of spores was observed in the formed structures (**Table 3**).

Mutations in *spo0A* gene ensure the formation of the phosphorylated form of the factor Spo0A, which stimulates the appearance of spores when *B. subtilis* strains are cultivated in a medium with a glucose content in the range of 0.1 to 0.2%, while increasing glucose content did not result in sporulation in the study of Quisel et al. [16]. At the same time, pyruvate, which is an intermediate product of glycolysis, has insignificant effects on the sporulation of the studied strains. A similar effect is observed in the presence of malate, which is an intermediate metabolite of the cycle of tricarboxylic acids in the process of metabolizing glucose. The results of the study, which approach the spore values in the structure of biofilms formed by *B. subtilis*

No.	Glucose, %	Colony forming units in biofilms of <i>B. subtilis</i> 170 cfu/cm ³	Colony forming units in biofilms of <i>E. coli</i> K-121655, cfu/cm ³	Colony forming units in dual-species biofilms, cfu/cm ³	
				<i>B. subtilis</i> 170	<i>E. coli</i> K-121655
1.	0.1	(1.6 ± 0.10).10 ⁶	(0.66 ± 0.05).10 ⁶	(12.93 ± 0.40).10 ⁶	(4.16 ± 0.15).10 ⁶
2.	0.2	(0.63 ± 0.11).10 ⁶	(1.36 ± 0.25).10 ⁶	(5.93 ± 0.40).10 ⁶	(1.3 ± 0.20).10 ⁶
3.	0.5	(0.66 ± 0.05).10 ⁶	(5.1 ± 0.10).10 ⁶	(15.3 ± 0.16).10 ⁶	(14.2 ± 0.10).10 ⁶
4.	1.0	(0.46 ± 0.06).10 ⁶	(4.0 ± 0.10).10 ⁶	(5.8 ± 0.3).10 ⁶	(10.3 ± 0.26).10 ⁶

Table 1.
The impact of glucose concentration in the cultural medium on colony forming units of B. subtilis 170 and E. coli 1655 strains in the structures of single-species and dual-species biofilms.

No.	Glucose, %	Colony forming units in biofilms of <i>B.subtilis</i> 168, cfu/cm ³	Colony forming units in biofilms of <i>E.coli</i> K-121655, cfu/cm ³	Colony forming units in dual-species biofilms, cfu/cm ³	
				<i>B.subtilis</i> 168	<i>E.coli</i> K-121655
1.	0.1	(1.2 ± 0.10).10 ⁶	(0.66 ± 0.05).10 ⁶	(9.73 ± 0.40).10 ⁶	(6.5 ± 0.20).10 ⁶
2.	0.2	(0.53 ± 0.06).10 ⁶	(1.36 ± 0.25).10 ⁶	(8.73 ± 0.29).10 ⁶	(1.4 ± 0.20).10 ⁶
3.	0.5	(0.53 ± 0.06).10 ⁶	(5.1 ± 0.10).10 ⁶	(10.7 ± 0.25).10 ⁶	(10.36 ± 0.32).10 ⁶
4.	1.0	(0.5 ± 0.0).10 ⁶	(4.0 ± 0.10).10 ⁶	(1.6 ± 0.26).10 ⁶	(10.66 ± 0.15).10 ⁶

Table 2.
The impact of glucose concentration in the cultural medium on colony forming units of *B. subtilis* 168 and *E. coli* 1655 strains in the structures of single-species and dual-species biofilms.

№	Glucose, % /	Spore forming units in biofilms of <i>B. subtilis</i> 170, cfu/cm ³	Spore forming units in biofilms of <i>B. subtilis</i> 168, cfu/cm ³	Spore forming units in dual-species biofilms, cfu/cm ³	
				<i>B. subtilis</i> 170 + <i>E. coli</i> 1655	<i>B. subtilis</i> 168 + <i>E. coli</i> 1655
1.	0.1	(6.66 ± 0.5).10 ³	(10 ± 0.1).10 ³	(2.54 ± 0.47).10 ³	(2.66 ± 0.5).10 ³
2.	0.2	(2 ± 0.05).10 ²	(4 ± 0.02).10 ²	(3.1 ± 0.57).10 ³	(3 ± 0.30).10 ³
3.	0.5	0	0	(5.6 ± 0.57).10 ³	(6.0 ± 0.07).10 ³
4.	1.0	0	0	0	0

$y = -3.3.x + 9.4467, r^2 = 0.9508, p < 0.05$ - *B. subtilis* 170.
 $y = -5.x + 14.667, r^2 = 0.9868, p < 0.05$ - *B. subtilis* 168.
 $y = -1.79.x^2 + 8.338.x - 4.36, r^2 = 0.9557, p < 0.05$ - *B. subtilis* 170-*E. coli* K-121655.
 $y = -2.085.x^2 + 9.727.x - 5.526, r^2 = 0.9252, p < 0.05$ - *B. subtilis* 168-*E. coli* K-121655.

Table 3.

The impact of glucose concentration in the cultural medium on spore forming units of *B. subtilis* 170, *B. subtilis* 168 and *E. coli* 1655 strains in the structures of single-species and dual-species biofilms.

170 and *B. subtilis* 168 strains, confirm the conclusion that a high level of glucose at a content above 0.5% does not lead to the appearance of spores in the structure of biofilms, while its low concentrations in the range of 0.1 to 0.2% activate the sporulation process [16].

Their co-development with *E. coli* K-121655 strains favors the spore-forming process in biofilms at a glucose content of 0.5%, and the dependence of the spore content on the glucose concentration in the range from 0.1 to 0.5% has an exponential characteristic of the studied pair of strains, increasing its value to 1.0% does not create conditions for sporulation both in the structure of dual-species biofilms of *B. subtilis* 170 and *E. coli* K-121655, and in the co-cultivation of *B. subtilis* 168 and *E. coli* K-121655 strains to form biofilms in the present study. The number of spores in the conducted research reached 2.7.10³ cfu/cm³ in the biofilm structures formed by the co-cultivation of *B. subtilis* 170 and *E. coli* K-121655 strains, and 2.9.10³ cfu/cm³ in the dual-species biofilms of *B. subtilis* 168 and *E. coli* K-121655 strains.

The obtained data testify to the fact that the sporulation of *B. subtilis* 170 and *B. subtilis* 168 strains in the structure of monospecies and biofilms and the biofilms formed in their association with *E. coli* K-121655 strains is stimulated by the formation of end products of glucose metabolism of both strains. The development of *B. subtilis* strain in DS medium with a glucose content of 0.1% ensures the formation and accumulation of pyruvate, acetate, acetoin, isobutyrate. Acetate and pyruvate had a weak effect on the spore formation of *spo0A* mutant strains, while the presence of isobutyrate had no effect on the process. Sporulation in *spo0A*, *spo0A* and *spo0A* mutant strains of *B. subtilis* was favored by the addition of acetoin in the medium, with their number increasing on average from 4.1.10³ to 6.5.10⁵ cfu/cm³ in the structure of the formed biofilms. Accumulation of acetoin promotes activation of the phosphorylated form of Spo0A, as confirmed by the appearance of spores in the biofilms of *spo0A* mutant strains with high content of the regulatory proteins KinC and Spo0A and the absence them in mutant strains with a low level of KinC kinase. The basis of the action of acetoin is its metabolism to acetyl phosphate, which serves as a donor of phosphate groups to the main regulators of biofilm formation and spore formation in *B. subtilis* strains. The intermediate products of acetoin metabolism – acetyl-CoA and acetaldehyde, are converted to acetylphosphates in the cells of *B. subtilis* and *E.*

coli strains [16], which explains the significant increase in the number of spores in the structure of biofilms of *B. subtilis* 170 and *E. coli* K-121655, *B. subtilis* 168 and *E. coli* K-121655 strains compared to their numbers in the monospecies biofilms of *B. subtilis* 170 and *B. subtilis* 168 strains in the present study (**Figures 2 and 3; Table 4**).

The formation of the exocellular matrix by *B. subtilis* and *E. coli* strains is regulated by transcription factors whose activity is determined by the level of environmental factors [5, 17]. The main regulatory protein CsgD in the uropathogenic *E. coli* UTI89 strains expresses the genes responsible for the biosynthesis of cellulose and curli, which are part of the exocellular matrix of biofilms [5]. Cyclic adenosine monophosphate also takes part in this mechanism of regulation of the process of biofilm formation, which positively affects the activity of *csgD* gene and the genes for the biosynthesis of cellulose and curli-structures [17], the formation and secretion of autoinducers 2 [5]. The established dependence is in agreement with the study

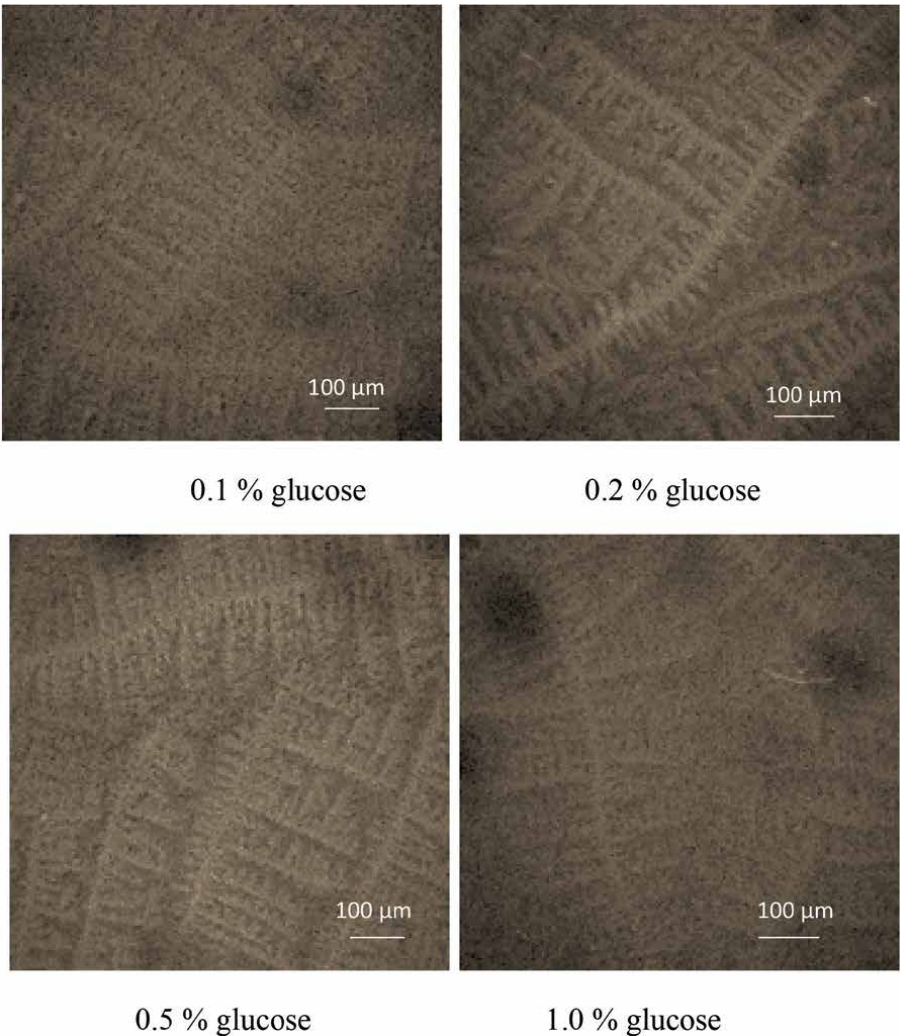


Figure 2.
 Microscopic study of biofilms of *Bacillus subtilis* 170 and *Escherichia coli* K-121655 strains at different concentrations of glucose in the cultural medium.

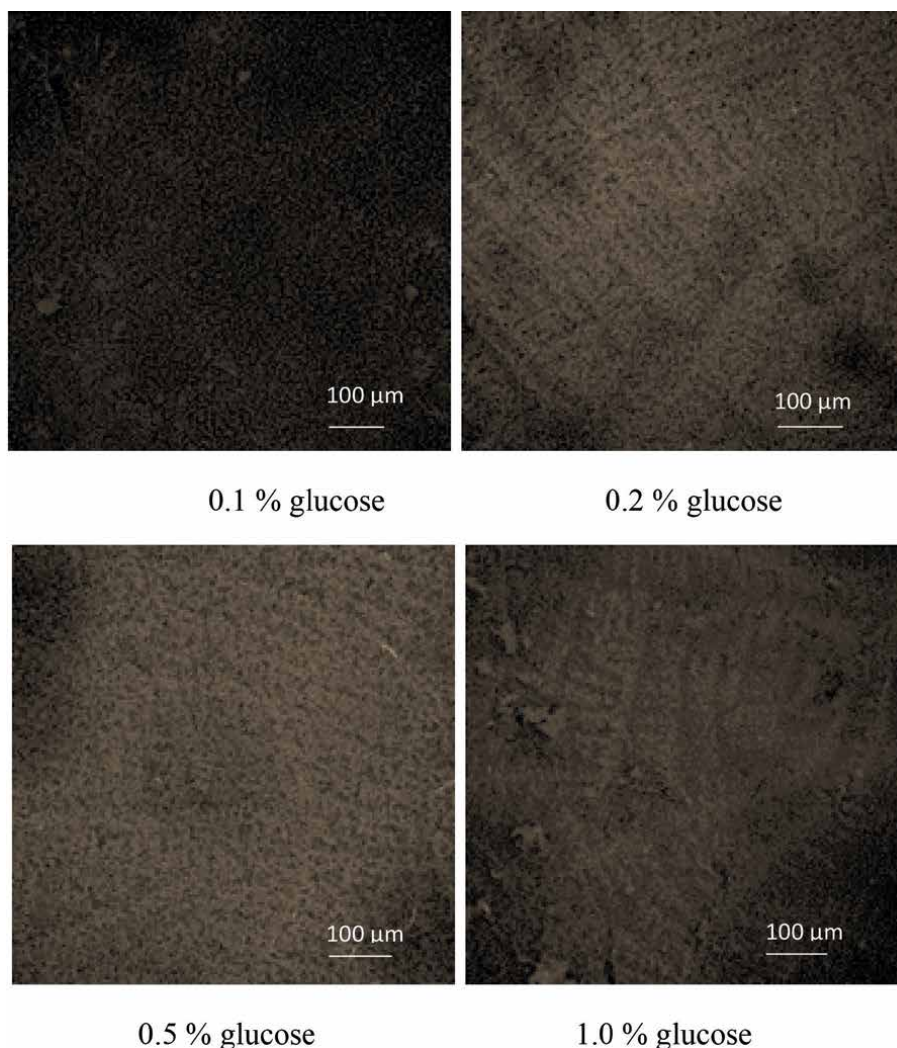


Figure 3.
Microscopic study of biofilms of Bacillus subtilis 168 and Escherichia coli K-121655 strains at different concentrations of glucose in the cultural medium.

of Domka et al. [4], according to which mutations in *yceP* and *yliH* genes lead to an increase in the biomass of the formed biofilms of *E. coli* K-12 strains, their average thickness and relative spreading area in medium with a glucose content of 0.1% as a result of the expression of the genes responsible for the biosynthesis and secretion of autoinducers 2 and indole, while in *B. subtilis* 170 strain it occurs at a glucose concentration in the medium of 1.0% as a result of the reduction of the activity of the genes, responsible for the biosynthesis of the biofilm matrix by the regulatory protein CcpA [17].

The average thickness of the formed structures in the process of static cultivation of the co-culture of strains was changed by *B. subtilis* 170 and *E. coli* K-121655 from $2.24 \pm 0.51 \mu\text{m}$ to $10.35 \pm 0.41 \mu\text{m}$ at a roughness coefficient of 0.19 ± 0.09 to 0.02 ± 0.06 as the glucose content in the medium increased from 0.1 to 0.5%,

Glucose, %	Mean thickness, μm	Coefficient of unevenness	Relative area, μm^2 **	Relationship area/volume, $\mu\text{m}^2 \cdot \mu\text{m}^{-3}$ ***
Dual-species biofilms of <i>Bacillus subtilis</i> 170 and <i>Escherichia coli</i> K-121655 strains				
0.1	2.24 ± 0.51	0.19 ± 0.09	0.76 ± 0.03	0.50 ± 0.02
0.2	2.50 ± 0.43	0.15 ± 0.05	0.93 ± 0.01	0.34 ± 0.07
0.5	10.35 ± 0.41	0.02 ± 0.06	0.99 ± 0.12	0.09 ± 0.01
1.0	2.69 ± 0.24	0.07 ± 0.04	0.96 ± 0.01	0.39 ± 0.05
Dual-species biofilms of <i>Bacillus subtilis</i> 168 and <i>Escherichia coli</i> K-121655 strains				
0.1	1.85 ± 0.53	0.19 ± 0.18	0.62 ± 0.02	0.58 ± 0.06
0.2	2.88 ± 0.86	0.22 ± 0.17	0.67 ± 0.03	0.28 ± 0.05
0.5	10.78 ± 0.37	0.03 ± 0.04	0.99 ± 0.21	0.09 ± 0.03
1.0	3.04 ± 0.89	0.24 ± 0.12	0.94 ± 0.05	0.35 ± 0.15

* $y = 4.005 \cdot x + 2.18$, $r^2 = 0.9763$, $p < 0.05$ – *B. subtilis*170-*E. coli* K-121655; $y = 4.465 \cdot x + 3.093$, $r^2 = 0.9667$, $p < 0.05$ – *B. subtilis*170-*E. coli* K-121655.
 ** $y = 0.115 \cdot x + 0.663$, $r^2 = 0.929$, $p < 0.05$ – *B. subtilis*170-*E. coli* K-121655; $y = 0.185 \cdot x + 0.423$, $r^2 = 0.9882$, $p < 0.05$ – *B. subtilis*170-*E. coli* K-121655.
 *** $y = -0.205 \cdot x + 0.21$, $r^2 = 0.984$, $p < 0.05$ – *B. subtilis*170-*E. coli* K-121655; $y = -0.245 \cdot x + 0.8067$, $r^2 = 0.983$, $p < 0.05$ – *B. subtilis*170-*E. coli* K-121655.

Table 4.
 Morphometric features of dual-species biofilms of *Bacillus subtilis* 170 and *Escherichia coli* K-121655, *Bacillus subtilis* 168 and *Escherichia coli* K-121655 strains, depending on the concentration of glucose in the cultural medium.

immediately after which a statistically significant decrease was observed to $2.69 \pm 0.24 \mu\text{m}$, and the unevenness coefficient acquires have a value of 0.07 ± 0.04 ($p < 0.05$). The ratio of the relative area to the volume of the biofilms decreased linearly until the glucose content of 0.5% was reached ($y = -0.205 \cdot x + 0.72$, $r^2 = 0.984$, $p < 0.05$), which was followed by a significant an increase in its value to $0.39 \pm 0.05 \mu\text{m}^2 \cdot \mu\text{m}^{-3}$ ($p < 0.05$). The dependence between the average thickness of the biofilms from the co-cultivation of *B. subtilis* 168 and *E. coli* K-121655 strains on the glucose content also has an exponential character ($y = 0.662 \cdot e^{0.881 \cdot x}$, $r^2 = 0.923$, $p < 0.05$) until reaching a value of 0.5%. Increasing the concentration of glucose in the culture medium was accompanied by the formation of structures with an average thickness of $3.04 \pm 0.89 \mu\text{m}$ at a roughness factor of 0.24 ± 0.12 . The ratio of the relative area to their volume decreases to a size of $0.09 \pm 0.03 \mu\text{m}^2 \cdot \mu\text{m}^{-3}$ as a result of the increase in the glucose content of 0.5%, and when its concentration reaches 1.0%, its value changes significantly to $0.35 \pm 0.15 \mu\text{m}^2 \cdot \mu\text{m}^{-3}$ ($p < 0.05$) (Figures 2 and 3; Table 4).

The relative spreading area of the biofilms reached a maximum value of $0.99 \pm 0.12 \mu\text{m}^2$ for the co-culture of *B. subtilis* 170 and *E. coli* K-121655 strains and $0.99 \pm 0.21 \mu\text{m}^2$ as a result of the co-cultivation of *B. subtilis* 168 and *E. coli* K-121655 strains at a concentration of glucose in the culture medium of 0.5%, which significantly exceeds their value when its value is reduced to 0.1% ($p < 0.05$) and statistically not changes when it increases to 1.0% ($p > 0.05$). The obtained regularities are in agreement with the study of Stanley et al. [17], according to which a repression of the genes for the biosynthesis of structures for flagellar motility and chemotactic movement was found in *B. subtilis* strains, when the glucose content reached 1.0%.

The decrease in the activity of the genes responsible for the formation of the surface cell structures participating in the appearance of flagellar motility was observed in the formation of biofilms of *P. aeruginosa* and *E. coli* strains under the conditions of catabolite repression from the increase in the concentration of glucose in the medium [16].

4. Conclusion

At cultivation at the concentration of glucose in the cultural medium in the range of 0.1 to 1.0%, the bacterial growth rates of single-species biofilms of *Bacillus subtilis* strains and biofilms, formed as a consequence of their interaction with *Escherichia coli* strains, have similar characteristics and determines the concurrence between two strains in the structure of biofilms, but the cultivating of the concentration of glucose in the cultural medium over of 0.5% lead to the impeded growth and affect negatively on the process of biofilm formation by the participation of *B. subtilis* 170 and *E. coli* K-121655, *B. subtilis* 168 and *E. coli* K-121655 strains, as a consequence of which there is a decrease in the value of the optical density, average thickness, and relative spreading area and an inversely proportional increase in the ratio of the spreading area of the structures to their volume, but inhibits spore formation. The recent research focused on the catabolite regulation by glucose excess by biofilm formation of soil -drawing bacteria of *Bacillus subtilis* under conditions of their sessile growth and relationship with other gram-negative bacterial species. The biosynthesis of extracellular polysaccharides, which constitute a significant proportion of the composition of the biofilm matrix, is also affected by the glucose content of the medium and determined the morphometric characteristics of the structures during the association of the two strains in the study. In regard to static methods of the biofilm formation at laboratory conditions is a highly relevant growing of the most of soil bacteria in the environment, the evaluation of the role of the catabolite regulation on the sessile growth of the soil bacteria is required to decrease of the information in the regard to biofilm production at the glucose excess.

Acknowledgements

This research was supported by the Bulgarian Ministry of Education and Science under National Science Foundation, Contract No. KP-06-PM67/6 - SUNY BG-175467353-2022-2103-0019.

Conflict of interest

The authors declare no conflict of interest.

Author details

Ivo Ganchev
Trakia University of Stara Zagora, Stara Zagora, Bulgaria

*Address all correspondence to: ivotg@abv.bg

IntechOpen

© 2025 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Rendueles O, Ghigo J. Multi-species biofilms: How to avoid unfriendly neighbors. *FEMS Microbiology Reviews*. 2012;**36**:972-989
- [2] Simoes LC, Simoes M, Vieira MJ. Biofilm interactions between distinct bacterial genera isolated from drinking water. *Applied and Environmental Microbiology*. 2007;**73**(19):6192-6200
- [3] Karatan E, Watnick P. Signals, regulatory networks, and materials that build and break bacterial biofilms. *Microbiology and Molecular Biology Reviews*. 2009;**73**(2):310-347
- [4] Domka J, Lee J, Wood T. YliH (BssR) and YceP (BssS) regulate *Escherichia coli* K-12 biofilm formation by influencing cell signaling. *Applied and Environmental Microbiology*. 2006;**72**(4):2449-2459
- [5] Hufnagel D, Evans M, Greene S, Pinkner J, Hultgren S, Chapman M. The catabolite repressor protein-cyclic AMP complex regulates csgD and biofilm formation in uropathogenic *Escherichia coli*. *Journal of Bacteriology*. 2016;**198**(24):3329-3334
- [6] Römling U, Galperin MY. Bacterial cellulose biosynthesis: Diversity of operons, subunits, products and functions. *Trends in Microbiology*. 2015;**23**(9):545-557
- [7] Heydorn A, Nielsen A, Hentzer M, Sternberg C, Givskov M, Ersbøll B, et al. Quantification of biofilm structures by the novel computer program COMSTAT. *Microbiology*. 2000;**146**:2395-2407
- [8] Jackson DW, Simecka JW, Romeo T. Catabolite repression of *Escherichia coli* biofilm formation. *Journal of Bacteriology*. 2002;**184**:3406-3410
- [9] Murray EJ, Stanley-Wall NR. The sensitivity of *Bacillus subtilis* to diverse antimicrobial compounds is influenced by Abh. *Archives of Microbiology*. 2010;**192**:1059-1067
- [10] Whitfield C. Biosynthesis and assembly of capsular polysaccharides in *Escherichia coli*. *Annual Review of Biochemistry*. 2006;**75**:39-68
- [11] Valle J, Da Re S, Henry N, Fontaine T, Balestrino D, Latour-Lambert P, et al. Broad-spectrum biofilm inhibition by a secreted bacterial polysaccharide. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;**103**:12558-12563
- [12] Sawada K, Araki H, Takimura Y, Masuda K, Kageyama Y, Ozaki K, et al. Poly-l-gamma glutamic acid production by recombinant *Bacillus subtilis* without *pgsA* gene. *AMB Express*. 2018;**8**:110
- [13] Chai Y, Norman T, Kolter R, Losick R. Evidence that metabolism and chromosome copy number control mutually exclusive cell fates in *Bacillus subtilis*. *The EMBO Journal*. 2011;**30**(7):1402-1141
- [14] Jha S, Joshi S, Geetha SJ. Lipopeptide production by *Bacillus subtilis* R1 and its possible applications. *Brazilian Journal of Microbiology*. 2016;**47**:955-964
- [15] Rivardo F, Turner RJ, Allegrone G, Ceri H, Martinotti MG. Anti-adhesion activity of two biosurfactants produced by *Bacillus* spp. prevents biofilm formation of human bacterial pathogens. *Applied Microbiology and Biotechnology*. 2009;**83**:541-553

[16] Quisel J, Burkholder W, Grossman A.
In vivo effects of sporulation kinases
on mutant Spo0A proteins in *Bacillus*
subtilis. Journal of Bacteriology.
2001;**183**(22):6573-6578

[17] Stanley N, Britton R, Grossman A,
Lazazzera B. Identification of catabolite
repression as a physiological regulator
of biofilm formation by *Bacillus subtilis*
by use of DNA microarrays. Journal of
Bacteriology. 2003;**185**(6):1951-1957

[18] Blanton LV, Wang LT, Hofmann J,
DuBow J, Lafrance A, Kwak S, et al.
Aggregative adherence and intestinal
colonization by enteroaggregative
Escherichia coli are produced by
interactions among multiple surface
factors. mSphere. 2018;**3**(2):78-88

Edited by Ping Zeng

Escherichia coli is a mirror which could reflect grand themes of biology. By investigating this important microorganism, we find lessons about our relationship with microbes. As we stand on the brink of a new era of biology, understanding *Escherichia coli* becomes imperative. This book invites you to reconsider a microbe which co-exists with us from birth to death. Here, you will encounter *Escherichia coli* as a guardian, ally or enemy. In any case, the knowledge and perspectives mentioned in the book will further deepen your understanding of *Escherichia coli*. All the authors who have contributed to this book believe that readers can benefit from reading it.

*Alfonso J. Rodriguez-Morales,
Infectious Diseases Series Editor*

Published in London, UK

© 2025 IntechOpen
© Tess_Trunk / iStock

IntechOpen

ISSN 2631-6188

ISBN 978-1-83635-299-0

