Development of Ceftriaxone Containing Chitosan-Coated Catheters to Target Common Uropathogens for Preventing Catheter Associated Urinary Tract Infections

Afraz Saeed

Reg No. 00000318677

Master of Science in Industrial Biotechnology

Supervisor

Dr. Shah Rukh Abbas

Department of Industrial Biotechnology

Atta-Ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST)

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By

Afraz Saeed

Reg No. 00000318677

Master of Science in Industrial Biotechnology

Supervisor

Dr. Shah Rukh Abbas

Department of Industrial Biotechnology

Atta-Ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

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Examination Committee Members

1. Name: Dr. Shehzad Abid Signature:

2. Name: Dr. Abdur Rahman Signature:

3. Name: Dr. Mudassir Iqbal Signature:

Supervisor's name: Dr. Shah Rukh Abbas Signature:
Date:

Dr. Shah Rukh Abbas
Tenured Associate Professor
Deptt of Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Date: 21/8/23

Dr. Amjad Ali, PhD
Head of Department (HoD)
Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Head of Department

COUNTERSINGED

Date: 21/8/23

Prof. Dr. Muhammad Asghar
Principal
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Dean/Principal

THESIS ACCEPTANCE CERTIFICATE

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Signature: _____

Dr. Shah Rukh Abbas
Tenured Associate Professor
Deptt of Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Name of Supervisor: Dr. Shah Rukh Abbas

Date: 24.8.2023

Signature (HOD): _____

Dr. Amjad Ali, PhD
Head of Department (HoD)
Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Date: 4/9/23

Signature (Dean/Principal) _____

Principal
Atta ur Rahman School of Applied Biosciences (ASAB)
NUST Islamabad

Date: 5/9/23

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Supervisor
Dr. Shah Rukh Abbas
Industrial Biotechnology Biotechnology
ASAB, NUST

Dr. Shah Rukh Abbas
Tenured Associate Professor
Deptt of Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

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DEDICATED TO

My Beloved Parents & Siblings

For their support throughout this journey

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Praise be to **Allah Almighty**, the most beneficent, the most merciful, the ultimate source of all knowledge. The One who has been with me at every step of the way and never left me even at my lowest; The Creator, The Exalted, The Bestower who has blessed me immensely and kept me firm on this path of knowledge that I chose.

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"My success is only by ALLAH."

Qur'an [11:88]

Afraz Saeed

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List of Abbreviations

ACRONYM	GENERIC NAME
CAUTIs	Catheter Associated Urinary Tract Infections
UTI	Urinary tract infection
HAI	Healthcare-associated infections
AMR	Antimicrobial resistance
UPEC	Uropathogenic *Escherichia coli*
UPs	Uropathogens
MIC	Minimum Inhibitory Concentration

Abstract

Catheter Associated Urinary Tract Infections (CAUTIs) are a major healthcare concern and are linked to the use of indwelling urinary catheters. In this study, we present an approach for the prevention and treatment of CAUTIs by developing ceftriaxone-containing chitosan-coated catheters. Chitosan, a biocompatible and antimicrobial polymer, was utilized as a coating material for urinary catheters. Additionally, Ceftriaxone, a potent broad-spectrum antibiotic, was incorporated into the chitosan coating to establish a dual-action mechanism against UTI-causing pathogens. The uropathogens accumulate around the catheter surface and form biofilms which are sometimes very difficult to remove. The chitosan coating acts as a physical barrier, inhibiting bacterial adhesion and subsequent biofilm formation on the catheter surface. In vitro studies were conducted to assess the antimicrobial efficacy of ceftriaxone-containing chitosan-coated catheters against common uropathogens i.e., Staphylococcus aureus and Escherichia coli. Results of ceftriaxone containing chitosan-coated catheters in the form of Zone of Inhibition demonstrated better results as compared to uncoated catheters. Furthering up the research by taking in some other uropathogens and with further degradation, toxicity as well as surface morphological analyses effectivity of catheters can be enhanced.

Chapter 1

INTRODUCTION

The condition known as urinary tract infection (UTI) is characterized by the detection of a specific quantity of bacteria in the urine, typically equal to or greater than 108 colony forming units (CFU) per liter (1). This presence may occur with or without pyuria, which is characterized by a urinary white cell count (WCC) over 100 per microliter. Additionally, UTIs are commonly accompanied with symptoms that affect the bladder, ureters, and kidneys. UTIs can be categorized into two main types: those that solely affect the bladder, known as cystitis or urethritis, and those that additionally include the kidneys, referred to as pyelonephritis. The majority of UTIs mostly affect the lower urinary tract, including acute cystitis (2). These conditions can manifest in both females and males, although they are more prevalent in females. Approximately 60% of females aged 18 years and older are susceptible to experiencing one or many UTIs. The clinical presentation encompasses dysuria, urgency, frequency, and sporadically, hematuria. Numerous women exhibit symptoms that indicate the presence of UTIs yet exhibit either an absence of bacterial growth or bacterial counts that are less than 108 colony-forming units per liter (CFU/L) upon conducting multiple urine cultures.

The current consensus in the field admits that a colony count of $\geq$ 106 CFU/L is the established microbiological threshold for diagnosing urinary tract infections (UTIs) in symptomatic women who do not exhibit any other underlying abnormalities. Pyelonephritis is characterized by several clinical manifestations, such as discomfort in the flank or back region, elevated body temperature, shivering accompanied by chills, a sense of overall malaise, in addition to the symptoms often associated with a lower urinary tract infection. While the majority of individuals seeking medical attention exhibit symptoms of UTIs, it is worth noting that certain individuals may be asymptomatic. Specifically, only those individuals who are asymptomatic and at a heightened risk of getting subsequent infections, such as pregnant women and the elderly, are deemed to require treatment. Approximately 30% of women are estimated to experience recurrent UTIs, typically presenting with an average frequency of anywhere from two to three episodes annually (3,4). The illness can be acquired through community transmission, surgical transmission, or self-infection.

The majority of community-acquired diseases are attributed to inadequate personal hygiene practices, limited adherence to sanitary precautions, or engagement in multiple sexual partnerships. Self-infection typically occurs in patients with weakened immune systems or in high-risk situations, often resulting from the presence of commensal microorganisms found in the periurethral, vaginal, or rectal microbiota (5,6).

The utilization of medical instruments has significantly altered the dynamics of present-day healthcare, leading to enhanced quality of life and improved illness outcomes for those afflicted with chronic and lifestyle-related ailments. The aforementioned medical interventions encompass invasive devices such as intravenous catheters, artificial heart valves, stents, urine catheters, and intrauterine devices, all of which are utilized to enhance medical well-being. Indwelling catheters are widely recognized as one of the most prevalent invasive medical devices employed in clinical settings. Urinary catheters, which can be classified as either urethral or suprapubic, are essential medical instruments employed for the purpose of quantifying urine flow, collecting urine in surgical procedures, and facilitating its drainage (7,8). In general, the administration of urinary catheters is commonly performed by introducing them via the urethral route in patients. This procedure is commonly referred to as transurethral catheterization. Nevertheless, in situations where traditional approaches are not viable from a therapeutic standpoint or when persons are unable to void their bladder via the urethra, suprapubic catheterization presents an alternative way (9). The latest statistics published by the Centres for Disease Control and Prevention (CDC) indicates that approximately 12-16% of adult patients admitted to hospitals are administered an indwelling urinary catheter during the course of their hospitalization. Nevertheless, it is crucial to acknowledge that the data pertaining to catheterization rates in patients admitted to hospitals exhibit a range of 12% to 26% owing to disparities arising from diverse clinical environments, facilities, patient demographics (such as age, gender, and severity of illness), and clinical specialties (10).

In addition to the advantageous impact of these medical instruments, they also render patients vulnerable to infections. The introduction of a foreign object has been observed to compromise the local host's defense mechanisms in individuals, hence enabling the spread of microbial pathogens. This, in turn, leads to the occurrence of healthcare-associated infections (HAI) such as respiratory infections, infections in the UTIs, bloodstream infections. These infections contribute

to higher rates of illness and death and result in increased expenses for hospitalization of affected individuals. UTIs exhibit a notably high incidence within the majority of hospitals and healthcare settings, with CAUTIs accounting for around 80% of these cases (11). Within the context of a hospital environment, it has been observed that roughly 75% of UTI cases can be solely attributable to CAUTI. This particular type of infection imposes a significant financial burden, resulting in an estimated yearly spending of roughly 451 million U.S. dollars in the United States (12). According to estimates, over 100% of patients undergoing catheterization will experience bacteriuria within a period of 7-10 days. It is noteworthy that the likelihood of acquiring a CAUTI is closely linked to the duration of catheterization, with an increase of around 3-5% (in short-term care settings) and 3-10% (in long-term care settings) for each consecutive day of catheter use. Additionally, cohort research conducted in India revealed that CAUTIs ranked as the second most prevalent illness within healthcare institutions (13,14).

Numerous alternative approaches have been investigated in the realm of managing CAUTI. One effective strategy is minimizing the possibility of improper urinary catheter placement and restricting its duration. The most recent advancements encompass the utilization of external catheters. Previous research has investigated the efficacy of several interventions, such as bladder cleaning with antimicrobial drugs, introduction of antimicrobial fluids into the bag used for drainage, and pre-insertion catheter soaked in antiseptic solutions, with the aim of mitigating the incidence of CAUTIs (15). Regrettably, these methodologies have demonstrated ineffectiveness, as they possess a poor therapeutic index and hence pose a substantial likelihood of promoting the emergence of antibiotic resistance. Hence, a potential alternative approach to address CAUTI involves the adaptation of the catheter through the application of a surface coating containing antimicrobial agents with wide-ranging effectiveness, anti-virulent substances, or anti-fouling medications, aiming to impede the establishment of bacterial colonies.

The interest towards solutions for biomodification of catheters has increased due to the understanding that bacterial colonization of catheter surfaces and the subsequent production of biofilms are the main causes of CAUTIs. In this methodology, the composition of the catheter material is deliberately manipulated by chemical engineering techniques to possess antibacterial, anti-virulent, and anti-fouling characteristics. These features are designed to effectively impede

the attachment, colonization, and formation of biofilms by bacteria on the surface of the catheter (16).

This work aimed to construct catheters coated with chitosan containing ceftriaxone in order to address the issue of biofilm formation. Chitosan, an antibacterial polymer, has demonstrated efficacy in targeting a wide range of bacterial species. The combination of ceftriaxone, a broad-spectrum antibiotic, with chitosan has the potential to serve as a promising approach for inducing a synergistic effect against uropathogens commonly found in UTIs.

Aims and Objectives

The aims and objectives of this study are:

1. Optimization of chitosan and drug concentrations
2. Physically and chemically ensuring the attachment of coating
3. Antibacterial analysis of the prepared catheters against common uropathogens like *E.coli* and *Staphylococcus aureus.*

Chapter 2

LITERATURE REVIEW

2.1 Urinary tract Infections

Urinary tract infections (UTIs) are a prevalent form of bacterial infection on a global scale, ranking as the second most frequently encountered type. Annually, approximately 120-150 million cases are diagnosed. Distinguishing UTIs can be achieved by considering the anatomical location of the bacterial infections. When the infection reaches the upper portion of the urinary system, it is categorized as pyelonephritis or a kidney infection. This condition is characterized by the presence of severe symptoms including abdominal pain, chills, a high temperature, flank pain, nausea, and vomit. If left untreated, it can result in permanent harm to the kidneys and sepsis (17). Furthermore, urinary tract infections (UTIs) can be clinically categorized into two categories: uncomplicated UTIs (uUTIs) and complicated UTIs (cUTIs) in order to differentiate between infections of benign nature and those with an increased likelihood of recurring or progressing to severe pathology (18). Currently, the precise description and categorization of UTIs lack uniformity and universal consensus, instead undergoing ongoing development. In the past, it was commonly understood that uncomplicated UTIs referred to infections in nonpregnant, medically fit women that often cured with antibiotic therapy. Conversely, all other UTIs, including cystitis in men, were categorized as difficult. An additional categorization of UTI is recurring UTI (rUTI). This pertains to a subsequent symptomatic infection that occurs following the completion of adequate treatment and symptomatic remission of a prior infection. The prevailing definition commonly entails a minimum occurrence of two UTIs within a span of six months, or at least three UTIs throughout the course of a year. The prevailing consensus in the scientific community is that the primary causative agent of UTIs is uropathogenic Escherichia coli (UPEC). UPEC is responsible for around 85% of documented cases of cystitis on a global scale during a given year (19).

E. coli bacteria exhibit a migratory behavior, transitioning from their usual habitat in the gastrointestinal tract to the urethra, and then infiltrating the bladder. The potential migration of Escherichia coli (E. coli) germs from the perianal region to the urethra can be attributed to

inadequate hygiene practices following defecation, engaging in sexual intercourse, or delaying urination (20). The process of urination aids in the elimination of bacteria from the body. In addition to Escherichia coli, various Gram-negative bacterial strains, such as Klebsiella pneumoniae, Pseudomonas aeruginosa, and Proteus mirabilis, as well as certain Gram-positive bacteria, including select species of Staphylococcus and Enterococcus, have been identified as additional causative agents of UTIs. These pathogens are particularly prevalent among immunocompromised individuals and those who are frail (21). In general, females have a higher vulnerability to bacterial infections compared to males. This susceptibility can be attributed primarily to factors such as the absence of prostatic secretion, the somewhat shorter length of the female urethra, the physiological changes during pregnancy, and the increased likelihood of urinary tract contamination by fecal flora (22). The heightened vulnerability of women to UTIs can be attributed to various physiological factors. Although UTIs can be caused by multiple strains of bacteria, the current traditional treatment approach has not been successful in providing targeted antimicrobial medication. Instead, it mostly focuses on alleviating symptoms (23). In recent times, there has been a notable restriction in the range of antimicrobial drugs acceptable for effective treatment of UTIs because of the substantial global emergence of antimicrobial resistance (AMR). In certain instances of UTIs, strains of AMR bacteria have been identified. These strains include Pseudomonas spp. that are resistant to carbapenems and fluoroquinolones, Enterococcus spp. that are resistant to vancomycin, and Enterobacterales that are resistant to β-lactamase (24).

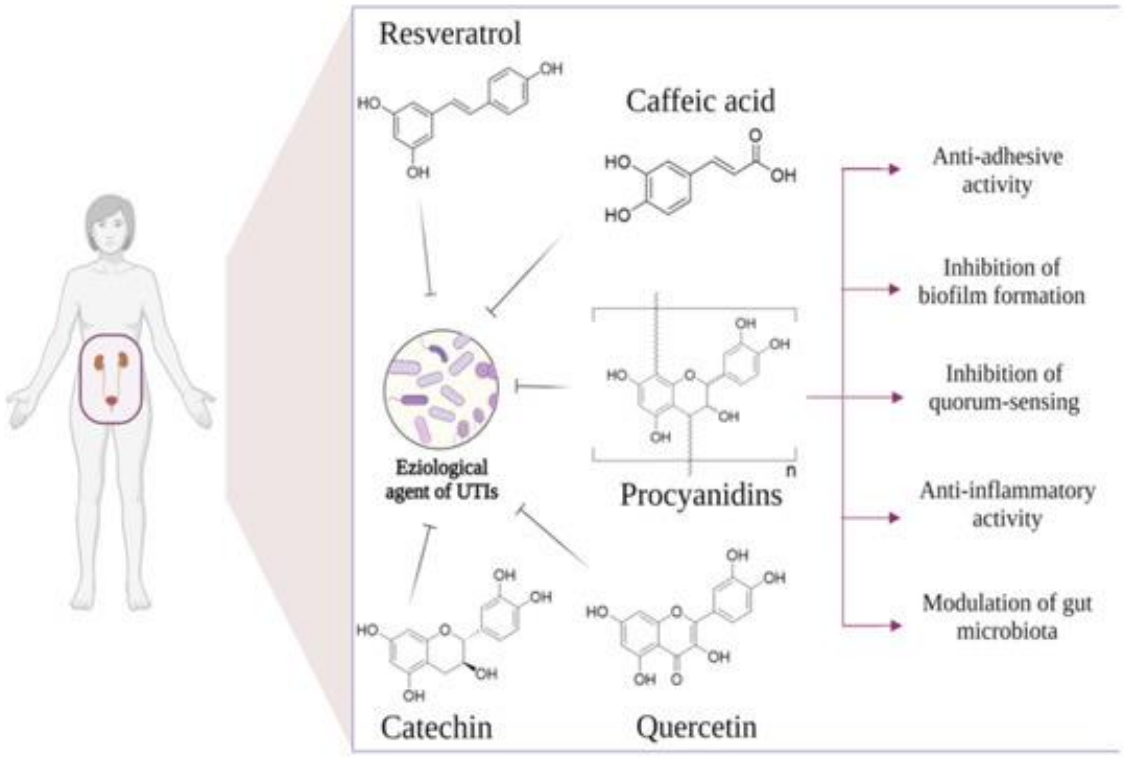


Figure 2. 1:Illustration of the primary polyphenols that have been investigated in relation to their molecular targets for the prevention and treatment of UTIs.

2.2 Catheter Associated Urinary Tract Infection (CAUTI)

CAUTIs encompass instances of urinary tract infections occurring in individuals who currently possess a urinary bladder catheter or have done so within the preceding 48 hours. These infections represent a prominent category of nosocomial infections, contributing significantly to the landscape of healthcare-associated infections. Within the United States alone, the occurrence of CAUTIs translates to over one million reported cases annually (25). Notably, these infections are often accompanied by secondary bloodstream infections. A substantial subset, spanning from 3% to 10% of individuals residing in long-term care facilities, receive care that necessitates the use of persistent indwelling catheters. The anticipated annual expenses associated with avoidable CAUTI vary between $115 million and $1.82 billion (26).

The projected yearly financial outlay associated with preventable CAUTI spans from $115 million to $1.82 billion. Multiple risk factors have been identified in relation to CAUTI, encompassing advanced age, female gender, diabetes, and extended catheterization duration. The duration for

which a catheter is retained plays a significant role in the onset of bacteriuria, with a daily risk ranging from 3% to 7%. An investigation conducted within long-term care facilities in the United States unveiled an average occurrence of 3.2 urinary tract infections per 1000 catheter days. Within the confines of the intensive care unit (ICU), where infection rates surpass those noted in other segments of patient care within the hospital by a ratio of 3 to 5, the prevalence of catheter-associated urinary tract infection (CAUTI) is documented to be 7.78 cases per 1000 catheter days (27,28).

Urinary tract infections (UTIs) can emerge due to the presence of both gram-negative and gram-positive bacteria, along with fungal pathogens. UPEC stands as the predominant causal agent for both uncomplicated and complex UTIs, contributing to approximately 75% and 65% of cases, respectively. In the context of complex UTIs, catheter associated UTIs (CAUTIs) represent the predominant instances. Following UPEC, the most prevailing causative organisms encompass Enterococcus spp. (11%), Klebsiella pneumoniae (8%), Candida spp. (7%), Staphylococcus aureus (3%), Proteus mirabilis (2%), Pseudomonas aeruginosa (2%), and Group B Streptococcus (2%). Antibiotics stand as the foundational cornerstone for the management of CAUTIs (29). Nevertheless, the non-living surface of the catheter is prone to the development of biofilms, resulting in increased resistance to the penetration of antibiotics. Moreover, the use of antibiotics is associated with adverse effects, as it promotes the emergence of antibiotic-resistant bacterial strains and disrupts the composition of the vaginal and intestinal microbiota. Consequently, this disturbance may create opportunities for colonization by resistant organisms in previously unoccupied ecological niches. Pili, which are sticky virulence-associated factors, have been found to play a dual role in both antibiotic escape and facilitating bacterial colonization of the intracellular environment. The prevalence of antibiotic resistance is on the rise, as evidenced by the declaration made by the CDC in 2013, stating that humanity has entered a phase known as the "post-antibiotic era." Furthermore, the World Health Organization issued a warning in 2014, highlighting the increasingly catastrophic nature of the antibiotic resistance phenomena. Therefore, there is a crucial need for methods aimed at preventing CAUTIs as well as exploring alternative treatments to antibiotics (30,31).

2.2.1 The mechanisms underlying catheter-associated urinary tract infection (CAUTI)

UTIs typically arise from the introduction of rectal bacteria into the urethra, subsequently leading to the migration, adhesion, and colonization of microorganisms in the bladder. The colonization of the bladder is facilitated by pili and adhesins, leading to the initiation of neutrophil infiltration. Bacterial replication subsequently occurs, leading to the formation of biofilms, while the presence of bacterial proteases and chemicals induces epithelial harm. The core steps of infection remain same regardless of the presence or absence of a urinary catheter. Urinary catheters serve as a direct pathway from the external environment to the urinary bladder. The conduit serves as an essential means of urine drainage in certain patients, but it also functions as a pathway by which microbes from the rectum and periurethral region can ascend to the bladder, potentially leading to the establishment of an infection. Catheters have the ability to circumvent the urethral sphincters, mitigate the turbulence often associated with voluntary urination, and function as a potential site for infection, therefore elevating the susceptibility to UTIs. Furthermore, catheters have the potential to cause irritation and stress to the uroepithelium, leading to the disruption of the natural mucopolysaccharide covering. This disruption increases the vulnerability of the uroepithelium to bacterial adhesion and subsequent invasion (32). The robust immunological response elicited by catheterization results in the deposition of fibrinogen on the catheter, creating an ideal setting for adherence by uropathogens that possess fibrinogen-binding proteins. As an illustration, Enterococcus faecalis exhibits an inability to proliferate in pee or adhere to catheter material under laboratory conditions. However, it demonstrates the capacity to thrive in urine supplemented with fibrinogen and stick to catheters coated with fibrinogen (33).

Adherence is a crucial primary stage in the development of urinary tract infections. In cases of uncomplicated UTI, bacteria have the ability to cling directly to the uroepithelium of the bladder, so establishing a favorable environment for infection to occur. Nevertheless, within the framework of a urinary catheter, whether it is a urethral catheter or a suprapubic tube, UTIs can occur when bacteria attach to the catheter and create biofilms (34). Biofilms consist of populations of microorganisms and their associated metabolic byproducts that are attached to both each other and a solid surface, such as a catheter. Biofilms are composed of several components such as extracellular DNA, ex-polys and bacterial surface structures like pili and flagella, which form a

framework. Biofilms facilitate the ability of bacteria to evade antibiotics and host immune responses, hence promoting the duration of infections. Biofilms play a crucial role in the pathogenesis of CAUTIs by serving as reservoirs for the colonization and subsequent seeding of microorganisms in the urinary tract. The process of biofilm production is beginning shortly after the catheterization procedure is performed. Biofilms subsequently develop in relation to the duration of indwelling (35,36).

Microbial species employ diverse processes to facilitate the production of biofilms. One example is UPEC, which is the predominant pathogen responsible for causing CAUTI. UPEC employs many mechanisms, such as pili, antigen 43, and curli, to facilitate interbacterial interaction, adhesion to surfaces, and subsequent production of biofilms (37). The production of UPEC catheter biofilm is contingent upon the presence of type 1 pili UPEC biofilm formation, and is governed by mechanisms involving oxidative stress, iron sensing, and quorum sensing. Pseudomonas aeruginosa demonstrates the ability to build biofilms on urinary catheters by many methods, such as the creation of alginate, quorum sensing, and modification of surface hydrophobicity (38).

Biofilms have a significant role in the pathogenicity of Pseudomonas aeruginosa, resulting in the development of persistent or recurrent infections. Proteus mirabilis is a species known for its ability to produce urease, an enzyme that catalyzes the breakdown of urea. This enzymatic activity leads to an elevation in urine pH, resulting in the formation of calcium crystals and precipitates of magnesium ammonium phosphate. Consequently, these factors collectively contribute to the development of catheter-associated biofilms with a crystalline structure. A comprehension of these pathways and additional ones has established a conceptual structure for the advancement of innovative inhibitors and antibiotics aimed at the prevention or treatment of CAUTI.

2.3 Prevention and Treatment of Catheter-Associated Urinary Tract Infections (CAUTI)

Urine catheters are widely utilized medical instruments within hospital settings, with an approximate prevalence of catheterization reaching 25% among hospitalized individuals. This technique is performed for various purposes, including addressing urine retention, facilitating surgical interventions, and managing lengthy periods of immobility (39). With the progression of our society's demographic shift towards an aging population, there has been a notable rise in the

utilization of urinary catheters. This can be attributed to the heightened prevalence of chronic ailments and lifestyle-related diseases. Upon admission to nursing homes, it has been shown that approximately 13% of men and 12% of women require an indwelling urine catheter. The incidence of hospitalizations attributed to problems associated with catheter usage is on the rise. In 2008, the Centers for Medicare and Medicaid Services discontinued the provision of reimbursement for the additional expenses incurred due to the occurrence of hospital acquired CAUTI. Hospitals have implemented several ways to mitigate expenses and enhance patient outcomes in response to this issue (40,41). The following measures can be taken to prevent CAUTI.

- Minimizing the implementation of Urine Catheters and Reducing Dwell Time
- Antibiotic Preventative Measures
- Utilization of Urinary Catheter with antimicrobial coating

2.4 Utilization of Chitosan in Catheter coating

Chitosan, a natural polysaccharide substance, exhibits considerable potential for uses in the medical and packaging of food industries due to its notable antimicrobial, anti-inflammatory, and antioxidants characteristics. Currently, the primary commercial production of chitosan, ranging from 109 to 1010 tonnes annually, relies on the utilization of crustacean shells. These shells are plentiful and easily accessible as they are a byproduct of the seafood industry. Therefore, the synthesis of chitosan can be considered as a sustainable and cost-effective procedure. The market size of Chitosan industry was estimated to be more than $1.2 billion in 2015 and is projected to reach $4.2 billion by 2021, with a compound annual growth rate of 15.4%.

Numerous experiments were conducted to demonstrate the antibacterial capabilities and mechanism of action of the subject under investigation (42). The aforementioned applications frequently necessitate the application of a thin chitosan coating onto diverse polymer substrates. Nevertheless, the majority of polymers exhibit hydrophobic properties and possess a relatively low surface energy, hence impeding the effective adhesion of coatings. Insufficient wettability results in suboptimal contact and limited spreading of the liquid containing chitosan that is deposited onto the surface of the polymer. Consequently, the adhesion between the two materials is compromised. To modify the surface properties of the polymer, it is necessary to activate the surface. The desired outcome can be achieved by the implementation of diverse chemical treatments. Nevertheless, to mitigate the adverse environmental impacts associated with chemical treatments, the utilization of

non-thermal plasma treatment presents a viable alternative technique. The functionalization of polymer material surfaces through plasma treatment allows for the adjustment of their wettability and adhesive properties (43).

Chitosan is a linear polysaccharide consisting of β-(1-4)-linked 2-amino-2-deoxy-d-glucopyranose (deacetylated units) and 2-acetamido-2-deoxy-d-glucopyranose (acetylated units) that are randomly distributed. Chitosan is obtained from chitin (depicted in Figure 2), which is the second most prevalent natural biopolymer following cellulose. Chitosan possesses a significant cationic potential (44). Chitin constitutes the external skeletal structure of several creatures, such as crustaceans, arthropods, insects, and the cellular walls of fungi. Chitosan has advantageous physicochemical and biological characteristics, including biocompatibility, biodegradability, biological activity, a high degree of permeability, and low toxicity. The aforementioned attributes render chitosan a multifaceted biomaterial that exhibits utility in various technical domains, including cosmetics, pharmaceuticals, and biomedicine (45).

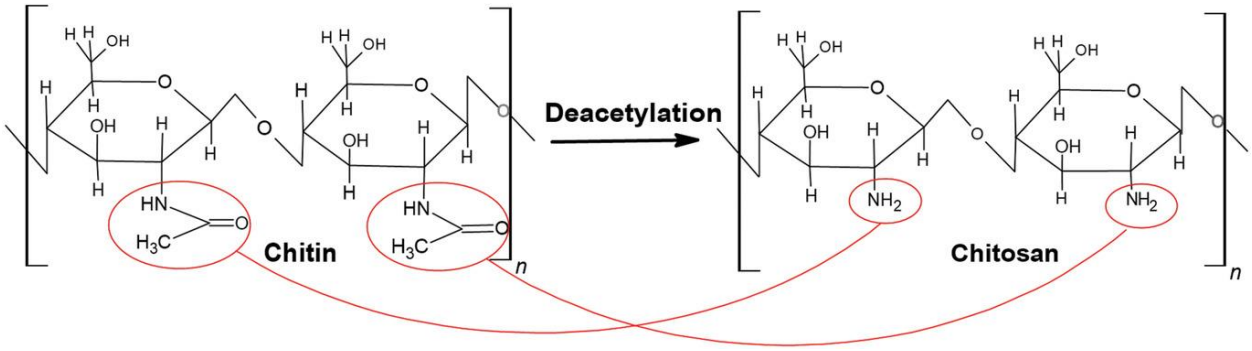


Figure 2. 2: Chemical structures of chitin and chitosan.

Chitosan and its derivatives have demonstrated activity against both gram-negative and gram-positive bacteria, filamentous fungus, and yeasts. Additionally, they exhibit reduced toxicity towards mammalian cells, making them highly desirable materials for the development of surface coatings in medical applications. The precise method by which CS operates remains incompletely understood. However, three primary processes have been proposed to explain its ability to limit bacterial growth; (i) disruption of cell wall charge, (ii) chelation of metal ions, and (iii) formation of complexes with DNA. Initially, the CS molecules, which carry a positive charge, engage in a reaction with the anionic phosphate groups present on the phospholipids located on the bacterial cell wall (46). This interaction occurs through the utilisation of the NH3+ amino group possessed

by the CS molecules. Consequently, this process results in alterations in the permeability of the cell and ultimately leads to the discharge of the cellular content. The uptake of metal cations (such as Ca2+ or Mg2+) by chelation, which leads to the destruction of the bacterial cell wall, is attributed to the amino groups present in the CS molecules. Another method is characterised by the interaction between dispersed hydrolysis products and microbial DNA, resulting in the suppression of messenger RNA (mRNA) and protein synthesis. The potential antibacterial effects of chitosan may arise from a variety of pathways. Diverse factors contribute to the antimicrobial attributes of chitosan and its derivatives. These encompass environmental conditions like pH, microbial type, and adjacent components. Moreover, the antimicrobial effectiveness is substantially shaped by the inherent structural attributes of chitosan, such as molecular weight, degree of deacetylation, derivative formulation, concentration, and origin (47,48).

Currently, the primary commercial production of chitosan, ranging from 109 to 1010 tonnes annually, relies on the utilization of crustacean shells. These shells are plentiful and easily accessible as they are a byproduct of the seafood industry. Therefore, the synthesis of chitosan can be considered as a sustainable and cost-effective procedure. The market size of chitosan industry was estimated to be more than $1.2 billion in 2015 and is projected to reach $4.2 billion by 2021, with a compound annual growth rate of 15.4% (49).

2.5 Ceftriaxone

Ceftriaxone, a member of the 3rd generation cephalosporin antibiotic class. Ceftriaxone is widely utilized as the predominant drug in the context of outpatient parenteral antibiotic therapy. The compound possesses a half-life that enables its administration on a regular basis, rendering it a favored substance for utilization in outpatient settings. Ceftriaxone is usually considered to be a safe and well-tolerated medication. Cephalosporin antibiotics are generated from the fungus Acremonium and belong to the class of beta-lactam antibiotics, characterized by the presence of a beta-lactam ring. Beta-lactam antibiotics are commonly employed in the therapeutic management of many strains of gram-positive and gram-negative bacteria. Ceftriaxone, a pharmaceutical compound, was formulated by Hoffmann-La Roche. The initial scientific publication in 1980 highlights its enhanced in vivo efficacy and extended half-life in comparison to alternative cephalosporin antibiotics. The plasma half-life of ceftriaxone in human subjects is estimated to be around 6 to 8 hours, which is notably 2 to 10 times longer than the half-life of other cephalosporins.

This extended half-life enables the administration of ceftriaxone once daily. In the context of rats, it has been observed that the plasma half-life is around 35 minutes. Ceftriaxone exhibits the ability to traverse the blood-brain barrier, rendering it a suitable therapeutic agent for the treatment of meningitis (50).

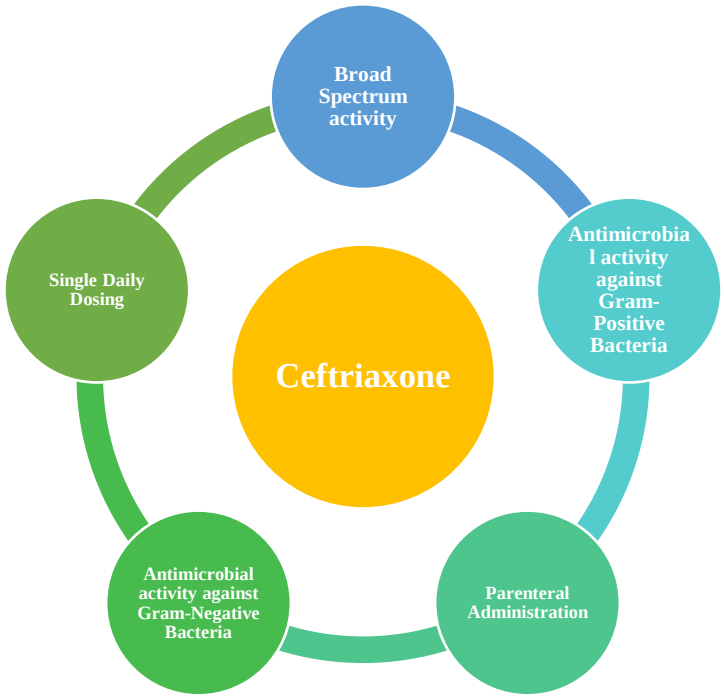


Figure 2. 3Advantages of using ceftriaxone for treatment of UTI

The recommended daily dosage of ceftriaxone as an antimicrobial agent in human patients is 2 grams per day, to be administered for a duration of 4 to 6 weeks. The administration of ceftriaxone is limited to intravenous (IV) or intraperitoneal (IP) routes, as there is currently no orally accessible formulation available for ceftriaxone. The authorization of the utilization of Body Surface Area (m2) by the United States Food and Drug Administration for the purpose of converting dosages from various species to the corresponding human dose is accompanied by the provision of a standardized formula (51). According to the formula employed, the rat's daily dosage of ceftriaxone would amount to 200 mg/kg, a dosage frequently observed in the reviewed literature. The dose equivalent in rats is 50% of that in mice. Therefore, the administration of 200 mg/kg of ceftriaxone to rats can be considered similar to the administration of 400 mg/kg to mice. The administration of ceftriaxone at these levels for an extended period of time, ranging from weeks to months, has been determined to be safe and well-tolerated in both human and rodent subjects (52). Nevertheless, it is imperative to acknowledge that the prolonged utilization of antibiotics has

a significant role in promoting the emergence of antibiotic-resistant bacteria and modifying the composition of the gastrointestinal microbiota.

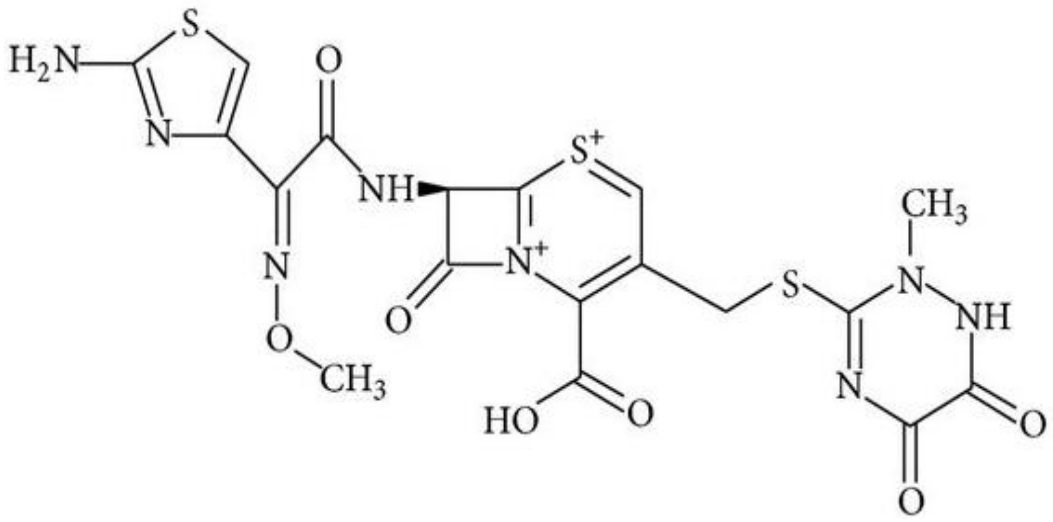


Figure 2. 4: Chemical structure of ceftriaxone

Chapter 3

MATERIALS AND METHODS

3.1 Reagents

The chitosan utilized in the investigation was obtained from Sigma-Aldrich. Ceftriaxone was procured from a nearby medical provider. The Foley catheters utilized in the investigation were procured from Chrome. Glacial acetic acid was obtained from Sigma Aldrich. All solutions were made using distilled water.

3.2 Optimization of Ceftriaxone

During the first stage of the experiment, the Minimum Inhibitory Concentration (MIC) levels of *E. coli* and *Staphylococcus aureus* were analyzed in order to determine how different concentrations of the antibiotic ceftriaxone affected these two strains of bacteria. This study comprised the examination of four different dosages of the medication: 125 milligrams (mg), 259 milligrams (mg), 375 milligrams (mg), and 500 milligrams (mg).

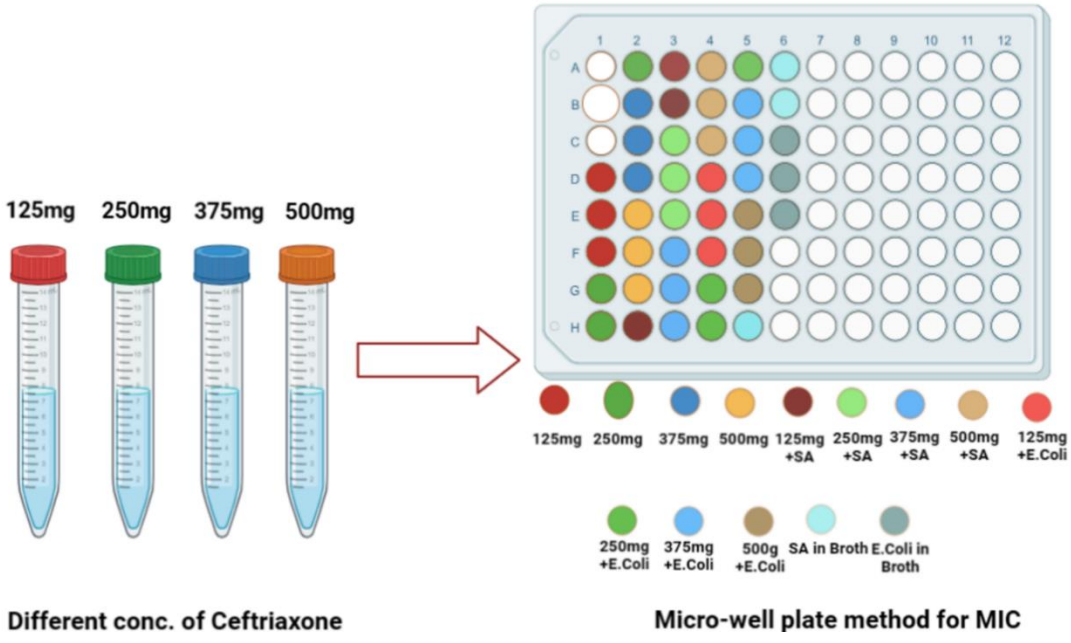


Figure 3. 1:displays 96 well-plate method for the optimization of different concentrations of Ceftriaxone and checking their Minimum Inhibitory concentrations against E.Coli and Staphylococcus aureus.

3.3 Optimization of Chitosan

During the subsequent stage of the experiment, different concentrations of chitosan (2%, 1.5%, 1%, and 0.5%) were prepared through the dissolution of chitosan in an acetic acid solution. The solution that was produced underwent testing against *E. coli* and *Staphylococcus aureus* in order to ascertain the minimum inhibitory concentration (MIC) of chitosan.

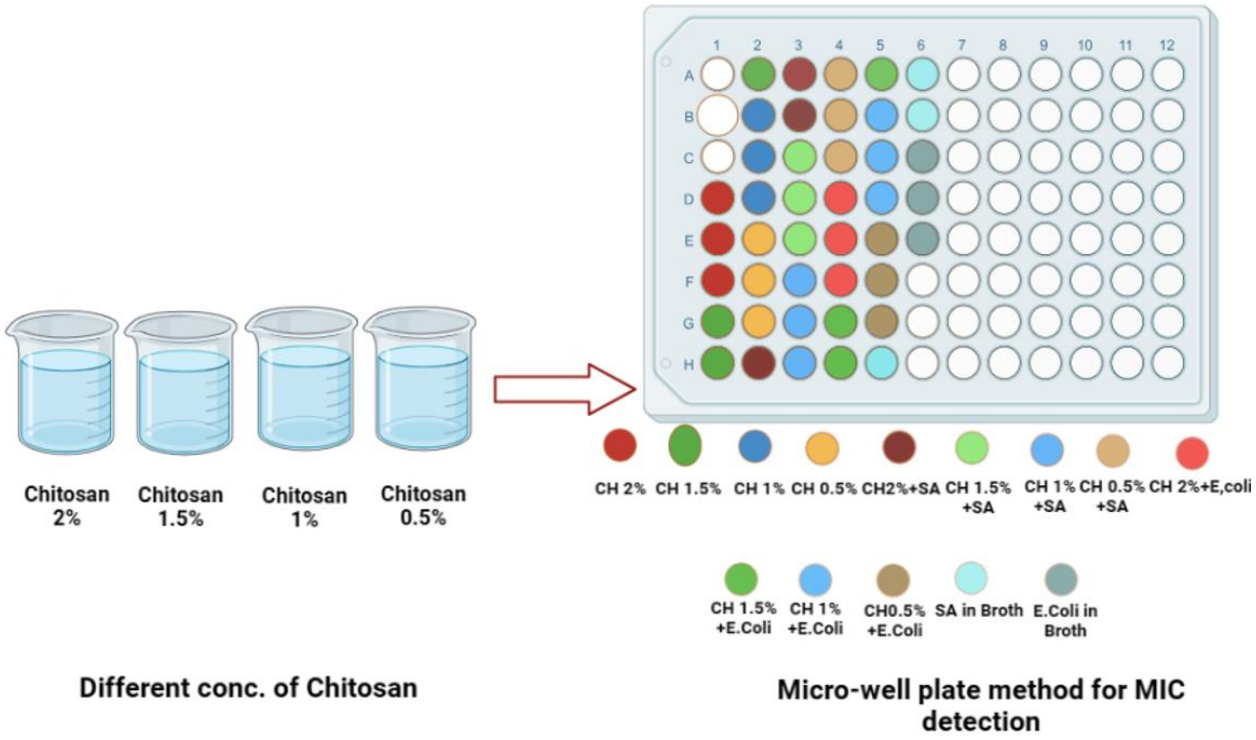


Figure 3. 2: displays 96 well-plate method for the optimization of different concentrations of Chitosan and check their Minimum Inhibitory concentrations against E.Coli and Staphylococcus aureus.

3.4 Chitosan coating of Catheters

A solution of chitosan with a concentration of 1.5% weight/volume (w/v) was chosen for the purpose of coating foley catheters. The process of degassing the distilled water involved immersing it in an ultrasonic bath for a duration of 10 minutes. Subsequently, the catheters were immersed in degassed distilled water to undergo photopolymerization under ultraviolet (UV) radiation for a duration of one hour. During the photopolymerization process, the formation of polar groups occurred on the surface of catheters. The catheters were subsequently immersed in a

1.5% chitosan solution for an extended period. Subsequently, the catheter coated with chitosan was stored at a temperature of 4°C for subsequent utilization.

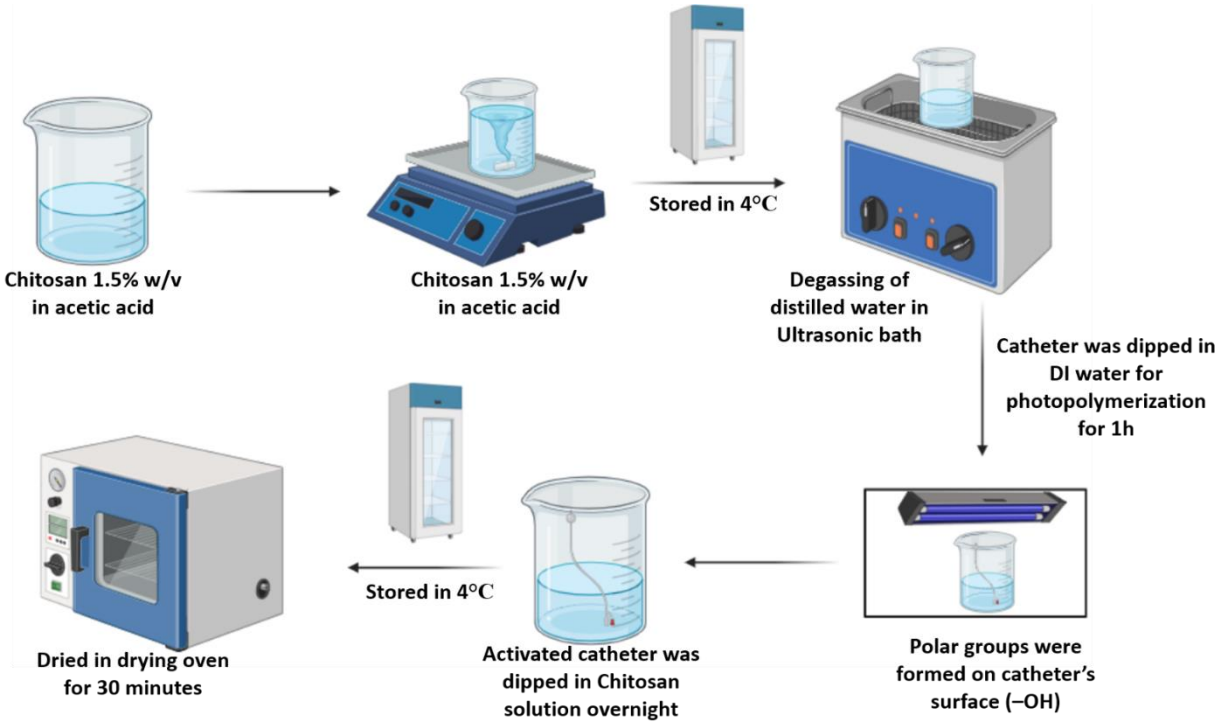


Figure 3. 3: Schematics of Preparation of Chitosan Coated Catheter

3.5 Preparation of Ceftriaxone-Chitosan Catheters

A solution was prepared by dissolving 100mg of ceftriaxone in 5ml of the provided solvent. The medication was added dropwise to a solution of chitosan with a concentration of 1.5% w/v while maintaining continuous stirring, resulting in the formation of spindle-like structures. The degassing of the distilled water was achieved by soaking it in an ultrasonic bath filled with distilled water for a period of 10 minutes. Following that, the catheters were placed in deoxygenated distilled water in order to undergo photopolymerization when exposed to ultraviolet (UV) radiation for a period of one hour. Following activation, the catheters were immersed in a chitosan solution containing

ceftriaxone for an extended period of time, specifically overnight. Subsequently, the catheters were stored at a temperature of 4°C for future utilization.

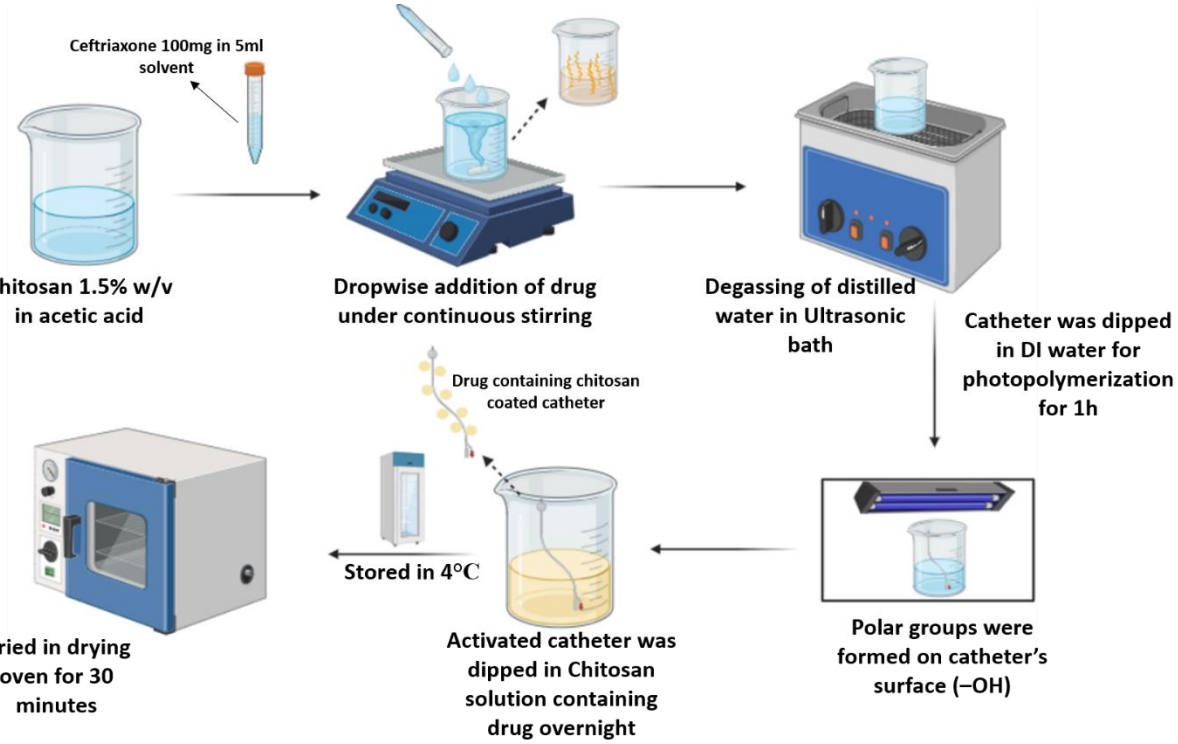


Figure 3. 4: Schematics of Preparation of Ceftriaxone Containing Chitosan Coated Catheter.

3.6 Revival of the Bacterial Cultures

The nutrient agar was subjected to autoclaving at a temperature of 121°C for a duration of 15 minutes, following which it was carefully poured into petri plates. In the subsequent stage, bacterial strains were streaked onto petri plates and underwent overnight incubation at a temperature of 37°C. The nutrient broth was prepared in a similar fashion to the nutrient agar. A 50 mL flask was filled with nutrient broth, and subsequently, the bacterial inoculum was mixed with the nutrient broth. The mixture was then subjected to overnight shaking incubation at a temperature of 37°C. The recovered strains were stored at a temperature of 4°C.

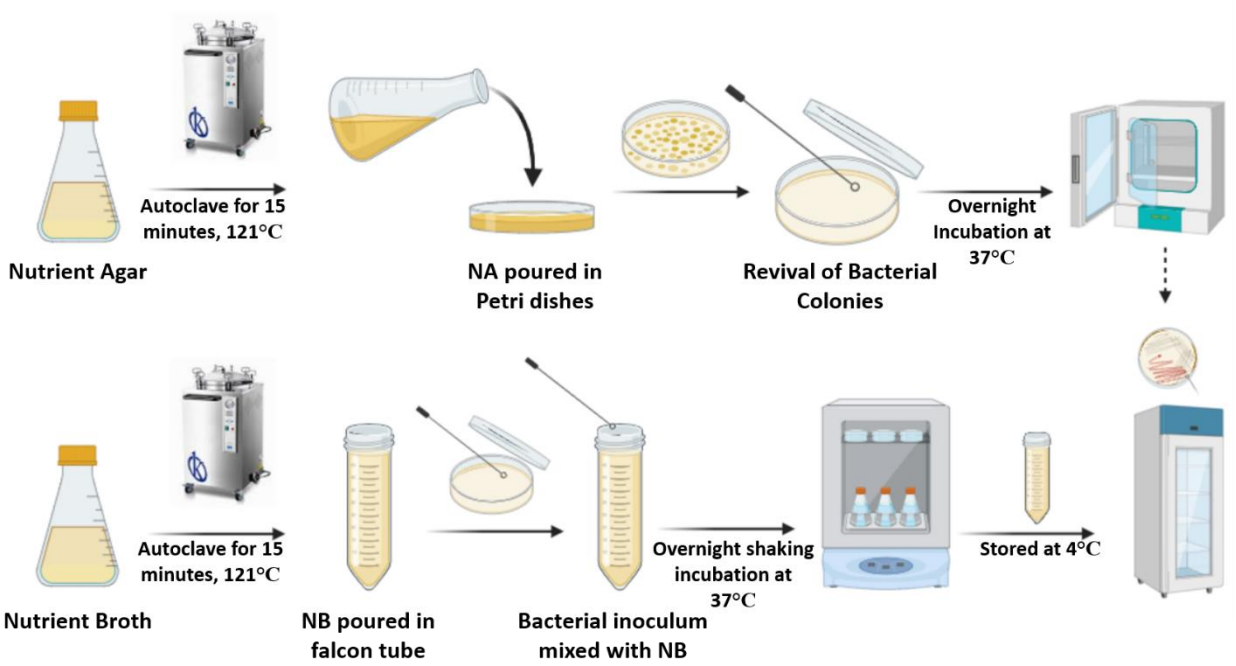


Figure 3. 5: Preparation of Nutrient Agar and Broth and Revival of Bacterial Colonies.

3.7 Antimicrobial Activity

After this nutrient agar was poured in petri plate and nutrient broth containing bacterial inoculum was spread on petri plates. Subsequently, a bacterial inoculum was dispersed onto the petri plates containing nutrient broth. Afterwards, a bare catheter, a chitosan-coated catheter, a drug-coated catheter, and a drug-chitosan coated catheter were positioned onto petri plates that contained bacterial cultures. These plates were then incubated for the duration of one night. The analysis of inhibitory zones was conducted the next day.

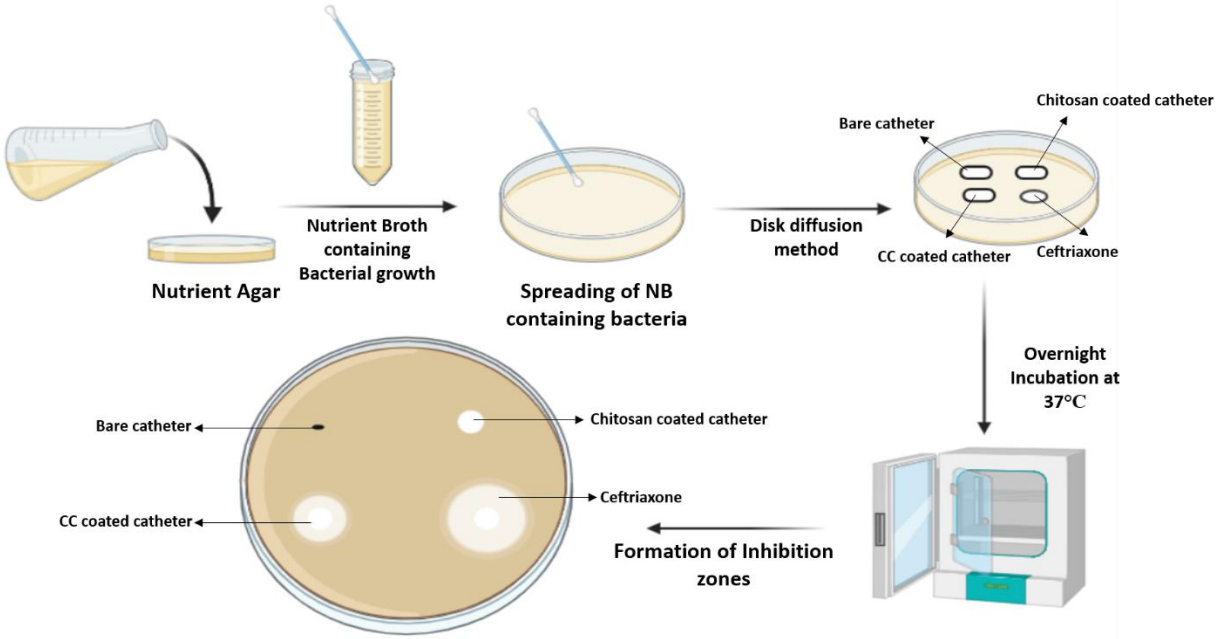


Figure 3. 6: Schematic illustration of the study of Antimicrobial activity.

Chapter 4

RESULTS

4.1 Minimum Inhibitory Concentration for Ceftriaxone

The minimum inhibitory concentration of Ceftriaxone was calculated through 96-well plate method through which it was estimated which concentration of Ceftriaxone would be best suitable to be used in future experiments. The spectrophotometric analysis revealed that C1 concentration of the drug was giving optimum inhibition against both *E.coli* and *S. aureus*. It is also evident from the graphs obtained that concentration of 125mg ceftriaxone (C1) prepared in 5ml of distilled water was giving effective inhibition against both *E.coli* and *Staphyloccous aureus*, therefore it was selected for future experiments.

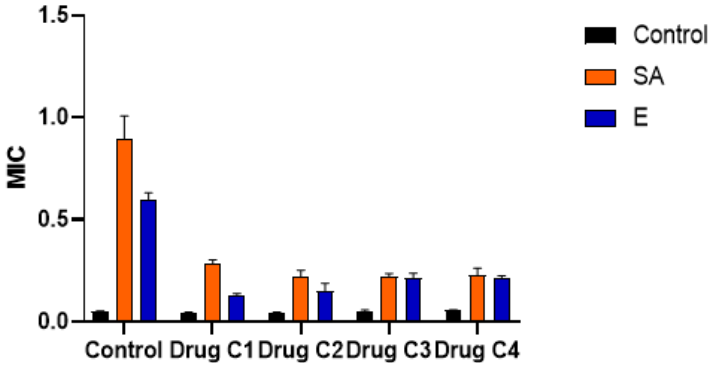


Figure 4. 1: Minimum inhibitory concentrations. Ss

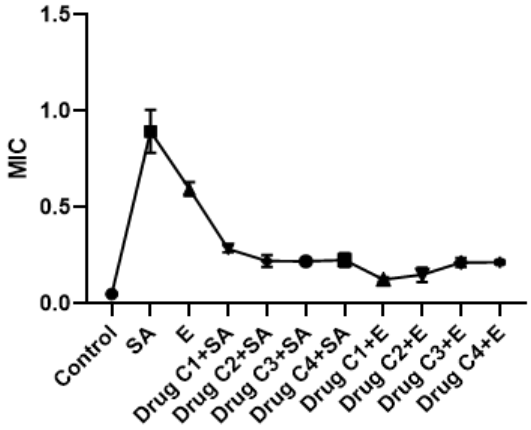


Figure 4. 2Graphical representation for minimum inhibitory concentration for ceftriaxone

4.2 Minimum Inhibitory Concentration for Chitosan

In order to choose the optimum chitosan concentration to target the Uropathogens, 96-well plate method was carried out with different concentrations of chitosan (2%, 1.5% , 1%, 0.5%) targeting E.coli and S. aureus. It was found after spectrophotometric analysis that C2 (1.5%) represented the best candidate in terms of microbial inhibition against the uropathogens as well as viscosity. The viscosity factor was kept in mind to get a better adsorption of chitosan on the catheter surface.

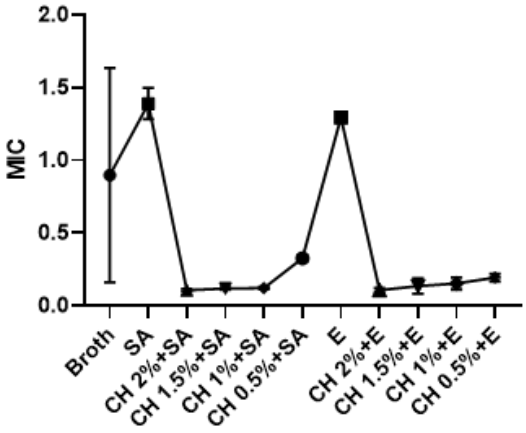


Figure 4. 3: Minimum inhibitory concentration for chitosan

4.3 Antimicrobial Analysis

Antibicrobial analysis was carried out for bare catheter, chitosan-coated, ceftriaxone containing chitosan-coated catheter and the antibiotic ceftriaxone which served as a positive control. The prepared media along with the samples was kept overnight in incubation at 37ºC for both *E.coli* and *S. aureus.*

4.3.1 Antimicrobial Analysis of *Staphylococcus aureus*

Bare and Chitosan coated catheters showed little to no antimicrobial activity while the zone of inhibition (ZOI) of ceftriaxone was found to be 20.67±3.86 similarly zone of inhibition (ZOI) of Chitosan-ceftriaxone catheter was found to be 15±0.816. Therefore, Ceftriaxone containing chitosan-coated catheters gave distinguishable results, against *Staphylococcus aureus,* when compared to their uncoated counterparts.

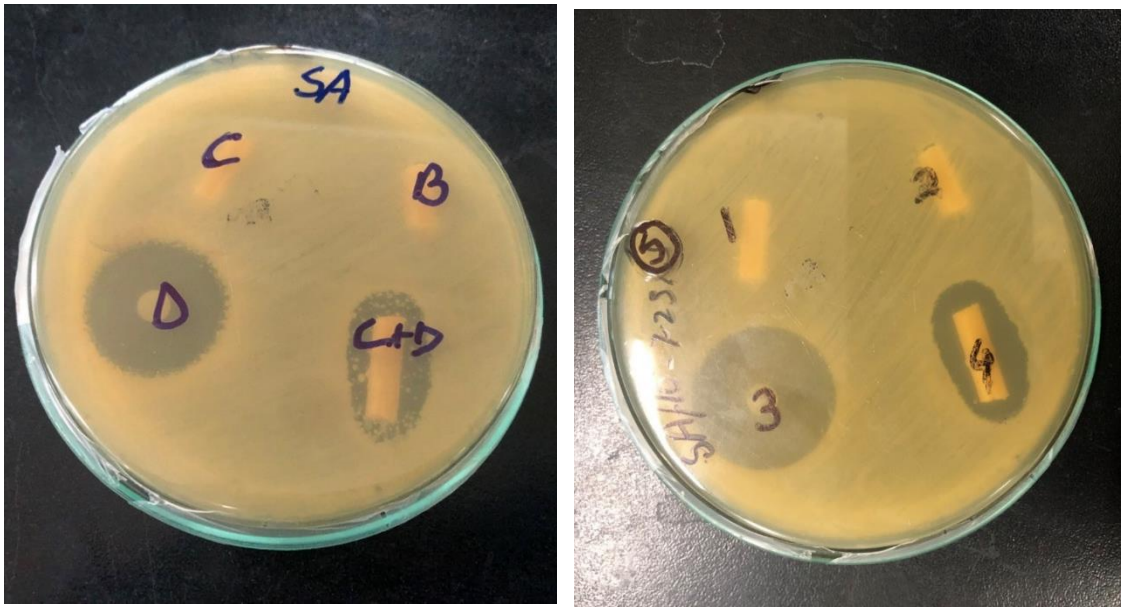
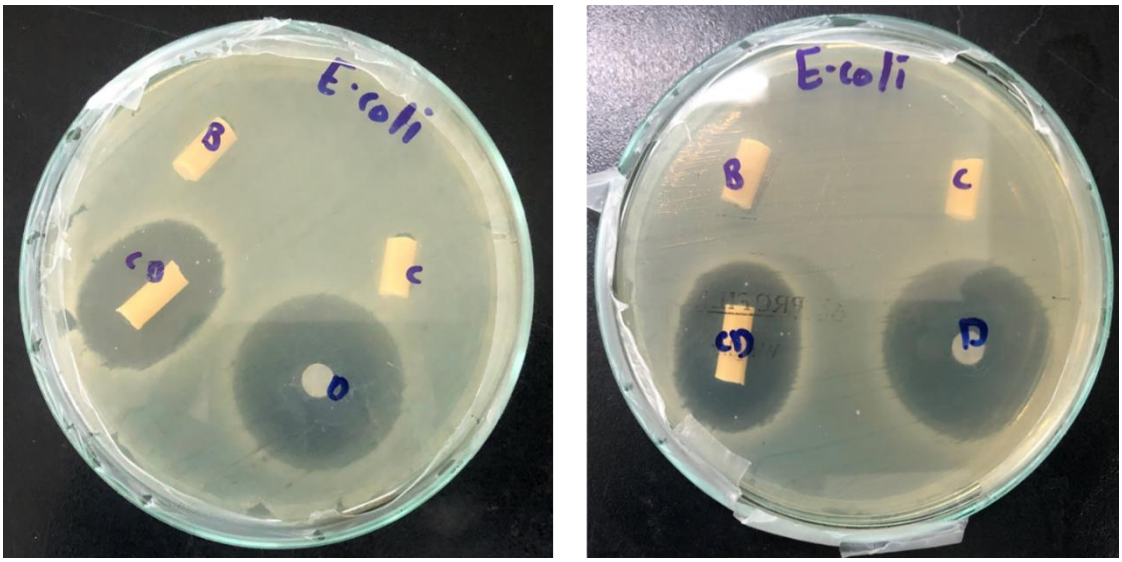


Figure 4. 4: Antimicrobial Analysis of S. aureus



Bare and

Figure 4. 5:Antimicrobial analysis of the catheters against E. coli

Chitosan coated catheters showed little to no antimicrobial activity while the zone of inhibition (ZOI) of ceftriaxone was found to be 33.83±0.76. Similarly zone of inhibition (ZOI) of Chitosan-ceftriaxone catheter was found to be 28.42±1.13. Therefore, ceftriaxone containing chitosan-coated catheters showed distinguishable activity, against *E.coli*, when compared to their uncoated counterparts.

4.4 Morphological Characterization

4.4.1 Scanning Electron Microscopy

The Scanning electron microscope images of bare catheters, chitosan-catheters as well as ceftriaxone coated chitosan-coated catheters reveal that there is no extra layer on the catheter surface when bare catheters are observed. While in case of chitosan and ceftriaxone-containing chitosan coated catheters, a visible layer of chitosan can be seen on the siliconized latex catheters. This means that chitosan has been effectively coated on the catheter surface.

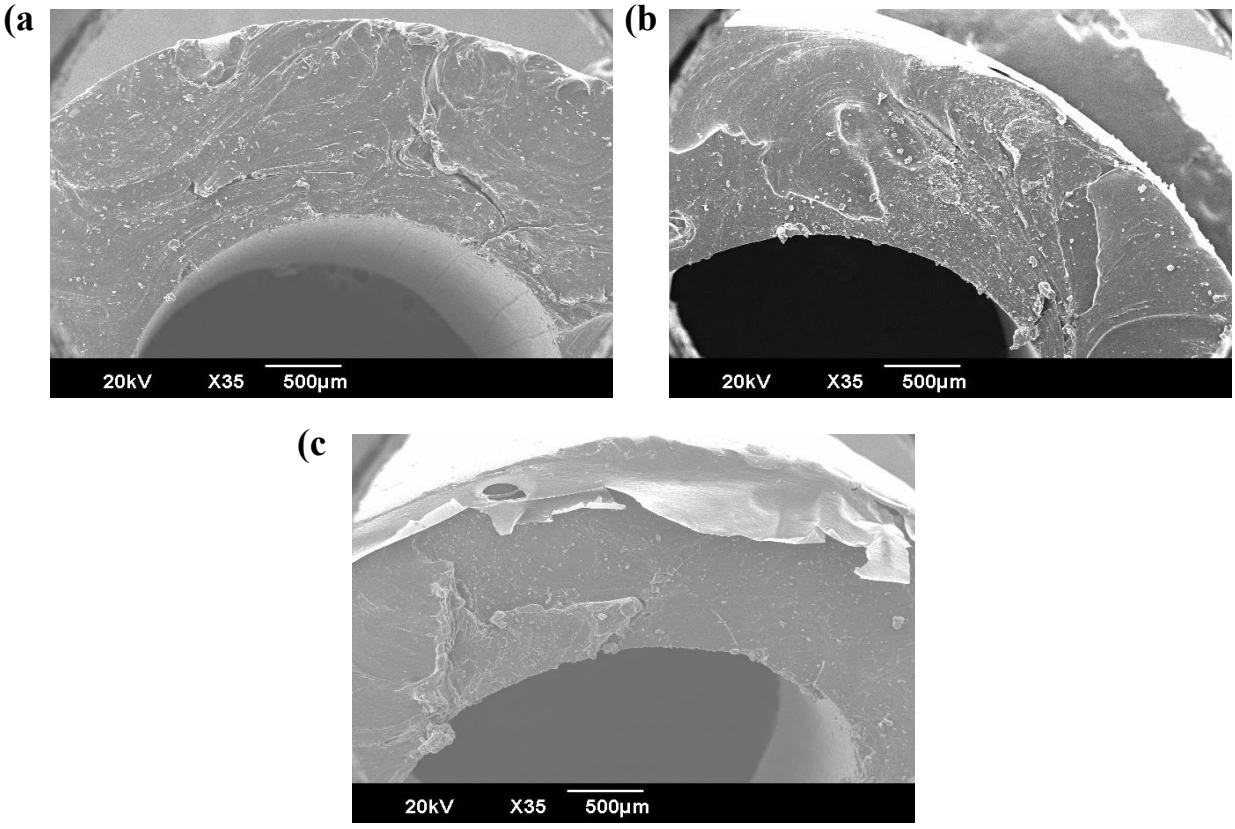


Figure 4. 6: The Scanning electron microscope images of bare catheters, chitosan-catheters as well as ceftriaxone coated chitosan-coated catheters.

4.4.2 Fourier Transfer Infrared Microscopy

The FTIR spectrum of the siliconized latex catheter (Figure 1A) depicts distinct absorption bands characteristic of both the silicone and latex constituents. Peaks falling within the spectrum of 1020 to 1260 cm−1 are indicative of the presence of Si–O–Si bonds inherent in the silicone material. A specific peak situated around 1100 cm−1 corresponds to the intrinsic C–O–C bonds of the latex,

likely originating from the polyacrylate latex. Particularly noteworthy is the appearance of absorption peaks related to the polyacrylate latex at approximately 3300 cm−1, as well as within the 3000–2850 cm−1 range and at 1735 cm−1. These peaks signify the stretching vibrations of O–H, C–H, and C=O bonds, respectively. Additionally, the absorption bands observed in the vicinity of 1480–1380 cm−1 are attributed to the bending vibrations of O–H and C–H bonds.

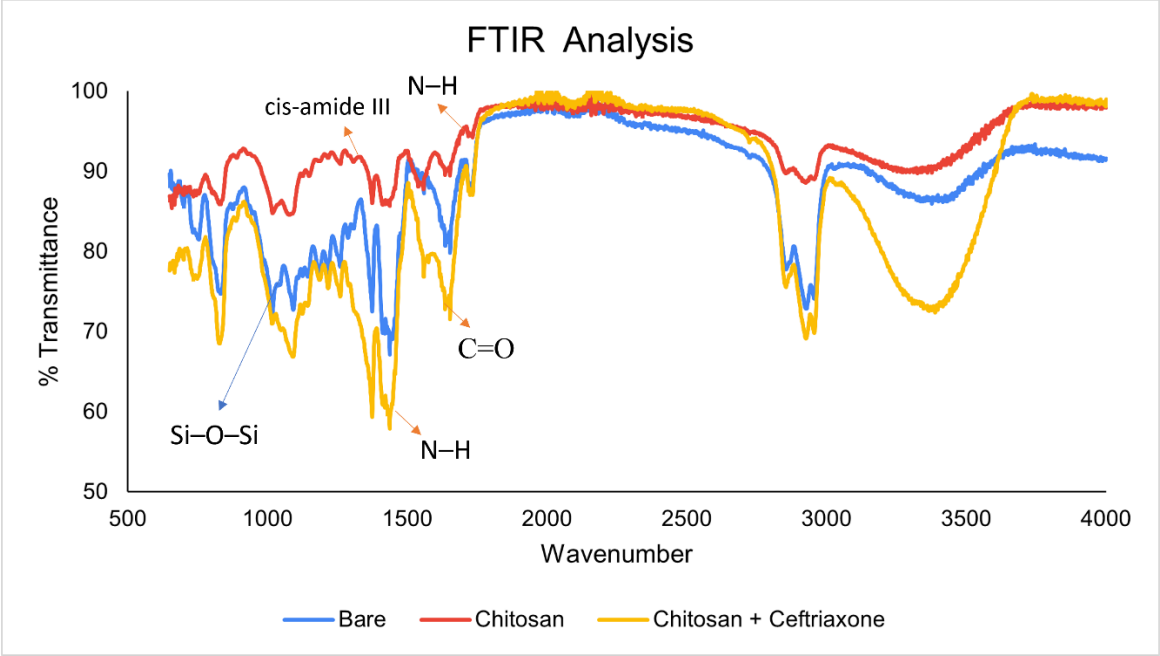


Figure 4. 7: Fourier transform infrared spectroscopy analysis of bare latex siliconized catheter, chitosan-coated catheters, and ceftriaxone chitosan-coated catheter reveled bonds comparable to the ones reported in literature.

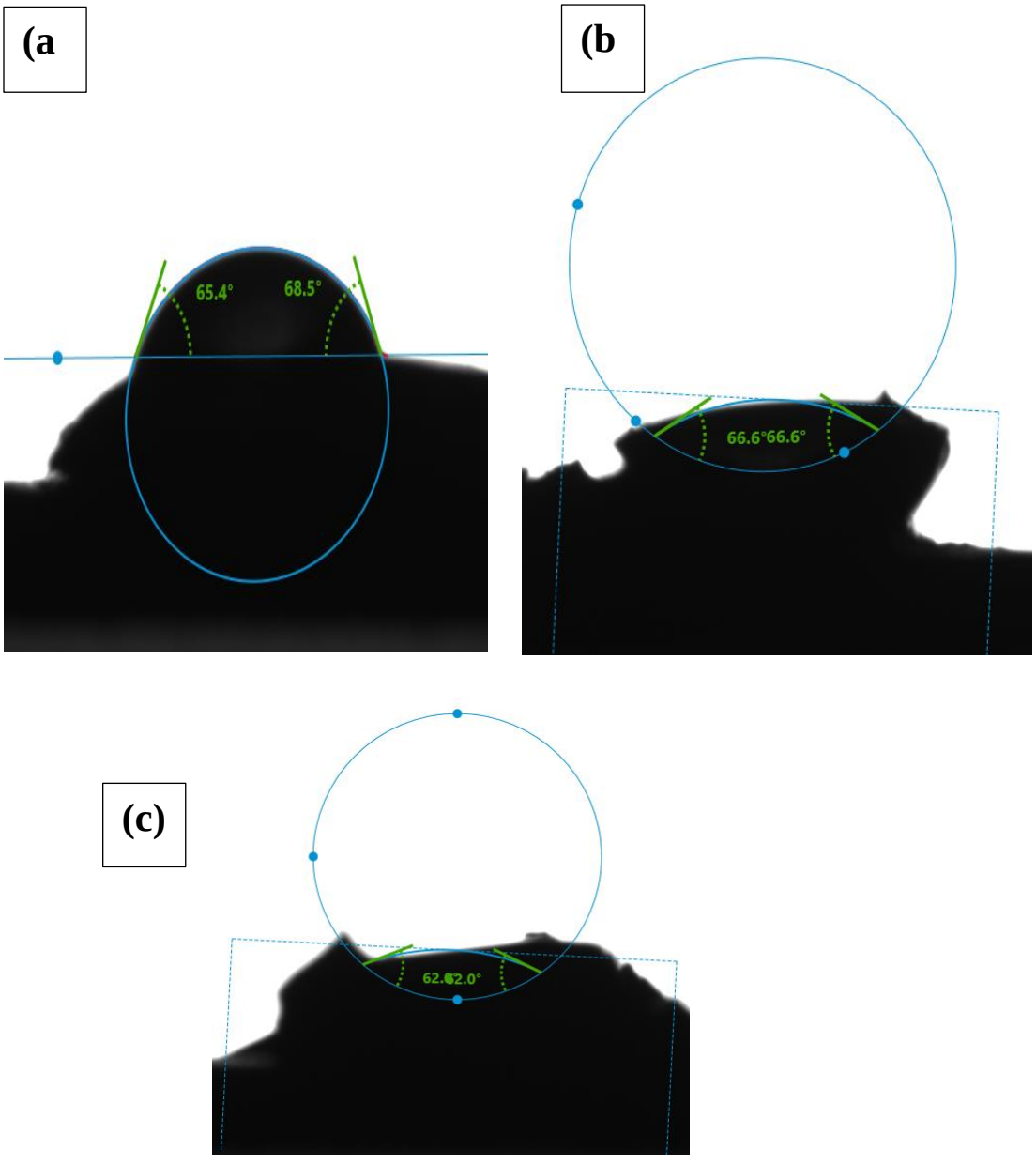
Within the chitosan spectra, distinct bands representing amide-I (C=O) and amide-II (N–H) were identified approximately at 1640 cm−1 and 1538 cm−1, correspondingly. The cis-amide III band exhibited a presence at 1248 cm−1 [62,67]. Furthermore, a discernible peak at 1078 cm−1 substantiated the occurrence of C–O–C stretching vibrations.

The IR spectra analysis of the ceftriaxone (CFT) ligand reveals several significant vibrational bands associated with its molecular structure. Amidic N-H stretching vibrations are prominently represented by strong intensity bands at 3440 and 3261 cm−1, corresponding to the asymmetric and symmetric stretching of N-H groups, respectively [38]. Notably, a robust intensity band observed at 1649 cm−1 corresponds to amidic C=O stretching vibrations [38]. Amidic N-H deformation and C-N stretching are evident through strong bands at 1608, 1537, and 1500 cm−1, reflecting the amide N-H deformation vibrations [38, 39]. The vibrational modes of C-H stretching

are manifested by weak bands at 2891 cm−1, attributed to CH3 symmetric stretching, and bands at 2934 cm−1 in the IR spectra, which are indicative of CH3 asymmetric stretching vibrations.

4.4.3 Contact Angle Measurement

Contact Angle Measurement of the bare catheters, chitosan-coated catheters, and ceftriaxone containing chitosan-coated catheters done from both inside and outside of the catheters. The decrease in contact angle from bare to ceftriaxone containing chitosan-coated catheters when viewed from outside reveals that hydrophilicity of the catheters has been increased due to incorporation of the ceftriaxone drug. The catheters have become somewhat in between purely hydrophilic (Contact angle: <90º) and hydrophilic (Contact angle: >90º) which can be further adjusted according to the patient requirements.



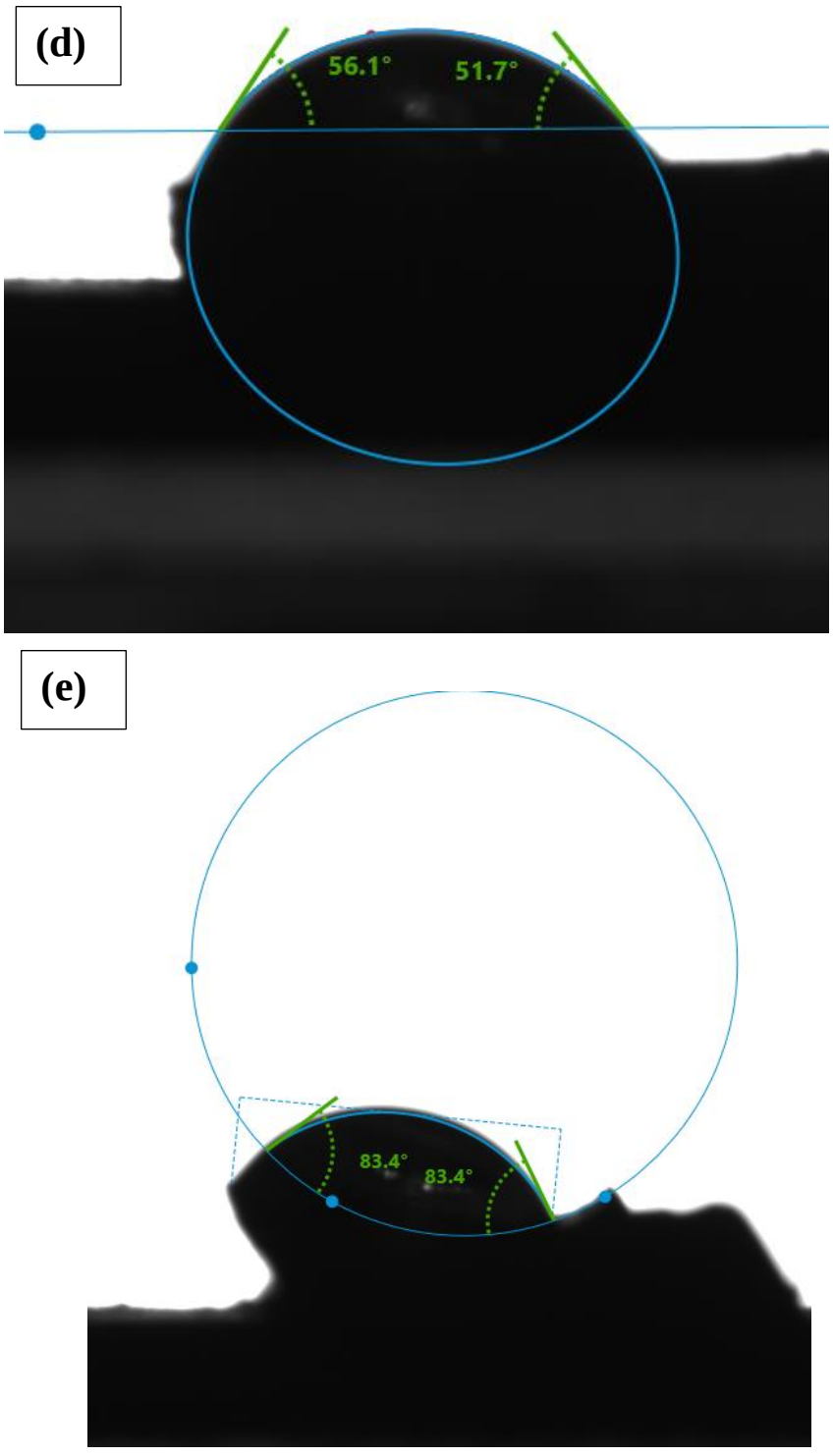


Figure 4. 8: Contact angle measurement of (a) Bare catheter outside (b) Bare catheter inside (c) Chitosan coated catheter inside (d) Chitosan-Ceftriaxone catheter outside (e) Chitosan-Ceftriaxone catheter inside

Chapter 5

DISCUSSION

Catheter-associated urinary tract infections (CAUTIs) represent a significant challenge in healthcare settings, necessitating innovative approaches to prevent their occurrence. The results of this study contribute valuable insights into the development of effective antimicrobial strategies using ceftriaxone and chitosan-coated catheters. By evaluating their minimum inhibitory concentrations (MICs), antimicrobial activities, molecular characterization, and surface properties, this research advances our understanding of potential interventions to mitigate CAUTIs.

Minimum Inhibitory Concentration (MIC) Determination:

The selection of optimal MIC concentrations for ceftriaxone and chitosan was pivotal in ensuring their effectiveness while avoiding potential cytotoxicity. The careful consideration of MICs, represented by C1 and C2 concentrations respectively, aligns with established principles of antimicrobial efficacy (53) These concentrations were identified through a meticulous spectrophotometric analysis, highlighting the precision required in establishing appropriate dosages. By doing so, this study forms a solid foundation for subsequent experiments, ensuring that antimicrobial concentrations are optimized for therapeutic outcomes.

Antimicrobial Analysis:

The observed enhancement in the zone of inhibition for ceftriaxone-containing chitosan-coated catheters against both E. coli and Staphylococcus aureus underscores the potential of synergistic antimicrobial interventions. The combination of ceftriaxone's antibiotic action with chitosan's antimicrobial properties has been suggested in the literature as a promising approach to combat infections (54, 55). This synergy aligns with the contemporary paradigm that emphasizes combination therapies to address microbial resistance. The utilization of scanning electron microscopy (SEM) to visualize the successful application of chitosan on catheter surfaces validates its role as an effective antimicrobial coating. Such visual evidence is crucial for translating laboratory findings into practical clinical applications.

Fourier Transform Infrared Microscopy (FTIR) Analysis:

The molecular characterization using FTIR provides an intricate understanding of the composition of catheters and their coatings. The distinctive peaks detected for silicone, latex, chitosan, and ceftriaxone spectra validate the successful integration of these components. This analysis supports the premise that catheters have been effectively modified to incorporate antimicrobial agents. Moreover, it exemplifies the importance of material analysis in confirming the intended modifications at the molecular level (56).

Contact Angle Analysis:

The alteration in contact angles on ceftriaxone-containing chitosan-coated catheters indicates significant changes in surface properties. The increased hydrophilicity observed implies reduced bacterial adhesion potential, a key factor in preventing CAUTIs (57). This alteration resonates with the current emphasis on surface modification to curtail bacterial attachment and subsequent biofilm formation. By successfully modifying the catheter's surface properties, this study contributes to the broader goal of enhancing medical device biocompatibility.

The outcomes of this study are consistent with existing literature, highlighting the necessity of multifaceted approaches to address microbial infections associated with medical devices (58). The combination of antibiotic agents with antimicrobial coatings, as demonstrated here, holds promise for improving device biocompatibility and reducing infection risks. This approach aligns with recommendations to combat the growing concern of antimicrobial resistance.

The present study demonstrates the potential of ceftriaxone and chitosan-coated catheters as effective antimicrobial interventions against uropathogenic bacteria. The precise determination of MIC concentrations, successful catheter coating, molecular characterization, and surface property alteration collectively contribute to the advancement of our knowledge regarding antimicrobial strategies for medical devices. However, it is important to acknowledge the limitations of this study, including the need for further in vivo investigations to validate the efficacy of these interventions under real-world conditions.

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Development of Ceftriaxone Containing Chitosan-Coated Catheters to Target Common Uropathogens for Preventing Catheter Associated Urinary Tract Infections

Afraz Saeed

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Master of Science in Industrial Biotechnology

Supervisor

Dr. Shah Rukh Abbas

Department of Industrial Biotechnology

Atta-Ur-Rahman School of Applied Biosciences (ASAB)

National University of Sciences and Technology (NUST)

Islamabad, Pakistan

2023

Dr. Shah Rukh Abbas
Tenured Associate Professor
Deptt of Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

Dr. Shah Rukh Abbas
Tenured Associate Professor
Deptt of Industrial Biotechnology
Atta-ur-Rahman School of Applied Biosciences (ASAB), NUST Islamabad

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