



Ihtisham Ul Haq, M.Sc.

Doctoral dissertation

**Biofunctionalized urinary catheter surfaces with antimicrobial peptides to prevent
urinary tract infections**

Under a cotutelle agreement between

Silesian University of Technology, Gliwice, Poland.

And

Universidade Federal de Minas Gerais. Belo Horizonte, Brazil.

Advisors: Maria Elena De Lima

Katarzyna Krukiewicz

Co-Advisors: Rafael Pinto Vieira

William Gustavo de Lima

PREFACE

This thesis is submitted as the partial fulfillment of the requirements for the degrees of Doctor of Philosophy in Chemical Sciences and in Technological Innovation, area of concentration in Biotechnology and Biopharmaceutical. This doctoral dissertation was conducted jointly at the Department of Physical Chemistry and Technology of Polymers, Silesian University of Technology (SUT), Poland (host university), and the Universidade Federal de Minas Gerais Federal University of Minas Gerais (UFMG), Brazil (home university), under a cotutelle agreement.

Acknowledgment

First and foremost, I give all glory and thanks to **God Almighty**, who has granted me the grace and strength to see this day and complete this journey. Without His guidance and mercy, none of this would have been possible.

I would like to express my gratitude to my supervisors, Prof. **Katarzyna Krukiewicz** (Silesian University of Technology, Poland), Prof. **Maria Elena De Lima** (Federal University of Minas Gerais, Brazil), and co supervisors, Prof. **Rafael Pinto Vieira** (UFMG), and **Dr. William Gustavo Lima** (Santa Casa), for their continuous guidance, and invaluable scientific support throughout my doctoral studies.

I am sincerely thankful to Prof. **Vera Lúcia** (UFMG) and her team, Dr. **Julio Cesar** (FUNED, Brazil), Prof. **Janusz Szewczenko** and his team, Prof. **Ewa Zablocka-Godlewska**, Dr. **Krzysztof Karoń**, and Dr. **Roman Turczyn** (SUT) for their assistance during experimental work. I would also like to thank my colleague, **Maryam Sajida** (SUT) for her valuable assistance during the experimental work. Her support and willingness to help were greatly appreciated. I would like to express my sincere gratitude to **Santa Casa Faculdade** for its institutional support and for providing access to the facilities.

I would also like to thank all members of the Faculdade de Saúde **Santa Casa** de Belo Horizonte, Brazil, **Bioelectrochemistry group** (SUT), and **Plus group** (UFMG) for providing a supportive and collaborative research environment.

I would like to sincerely thank **Eni** and **Letícia** (PPGIT, UFMG) for their kind assistance with course-related matters and administrative procedures throughout my doctoral studies.

I am deeply grateful to my dear friends **Pedro, Dr. Felipe, Dr. Kashif, Dr. Humayun, Abdullah, Mujahid, Shakir, Kamran, and Dr. Hamza** for their encouragement, support, and companionship throughout this journey. My parents and siblings have been my lifelong source of inspiration, teaching me the value of education, perseverance, and integrity.

Finally, I would like to express my sincere gratitude to my supervisor, Prof. Dr. **Katarzyna Krukiewicz**, for introducing me to the opportunity of pursuing a double PhD degree and for her valuable support throughout this journey. I am particularly grateful for her initiative in establishing this opportunity and for her guidance during the academic and administrative processes involved in the cotutelle programme.

LIST OF CONTENT

Acknowledgment.....	Iii
Abstract.....	Ix
Abstrakt.....	Xi
Resumo.....	Xiii
List of acronyms, abbreviations, and symbols.....	Xv
List of publications from thesis.....	Xviii
Chapter I: Theoretical part.....	1
1. Introduction.....	1
2. Literature Review.....	3
2.1. Urinary catheters and CAUTI.....	3
2.2. Risk factors and chances of developing CAUTI.....	4
2.3. Pathogens in involved in CAUTIs.....	5
2.4. Uropathogenic <i>E. coli</i>.....	6
2.5. Polymicrobial communities in CAUTI.....	7
2.6. Establishment of CAUTI.....	8
2.7. CAUTI-associated secondary complications.....	10
2.8. Healthcare and economic impact of CAUTI.....	11
2.9. Antimicrobial coatings on catheters to prevent CAUTI.....	12
2.10. Antimicrobial peptides (AMPs).....	15

2.11.	Advantages and challenges of AMPs.....	16
2.12.	The conjugation of AMPs with polymers.....	18
2.13.	The breadth of polymers used in antimicrobial catheter coatings.....	20
2.14.	AMPs-polymers coatings on urinary catheters.....	23
2.15.	Justification.....	29
2.16.	Hypothesis and objectives.....	31
Chapter II: Experimental part		35
3.	Materials and Methods.....	32
3.1.	AMPs.....	32
3.2.	Antimicrobial activity of AMPs.....	32
3.3.	MIC and MBC.....	33
3.4.	LyeTx I mnΔK Effects on biomass and viability of UPEC biofilm....	34
3.5.	Effects LyeTx I mnΔK on UPECs adhesion.....	35
3.6.	Evaluation of <i>in vitro</i> and <i>in vivo</i> toxicity of LyeTx I mnΔK.....	36
3.7.	LyeTx I mnΔK stability.....	37
3.8.	Biopolymers selection for the incorporation of LyeTx I mnΔK.....	37
3.9.	Stability of polymers/PLL films.....	38
3.10.	PVA/PLL deposition on urinary catheters through.....	38
3.11.	SA/LyeTx I mnΔK deposition on flat silicon sheets through.....	39
3.12.	Surface characterization of coatings.....	39
3.13.	<i>In vitro</i> biocompatibility of coatings.....	49

3.14.	Antimicrobial effects of coatings.....	40
3.15.	SEM analysis of microbial adhesion on coatings.....	41
3.16.	Antimicrobial effect of SA/LyeTx I mnΔK against CAUTI.....	42
3.17.	Stability of SA/LyeTx I mnΔK coating.....	42
3.18.	In silico/docking analysis of PLL and LyeTx I mnΔK.....	42
3.19.	Statistical analysis.....	43

Chapter III: Result part

4.	Results.....	44
4.1.	UPEC isolates identification.....	44
4.2.	Antimicrobial activity of LyeTx I and its analogue.....	45
4.3.	Structure prediction of the LyeTx I mnΔK and validation.....	47
4.4.	LyeTx I mnΔK docking with FimH.....	49
4.5.	ADMET profile of LyeTx I mnΔK.....	49
4.6.	Anti-biofilm activities of LyeTx I mnΔK against UPEC	49
4.7.	Anti-adhesive effects of LyeTx I mnΔK of UPECs on catheter.....	51
4.8.	Stability of LyeTx I mnΔK.....	53
4.9.	Toxicity of LyeTx I mnΔK <i>in vitro</i> and <i>in vivo</i>	54
4.10.	Deposition and surface characterization of coatings of PVA.....	60
4.11.	FTIR analysis of coatings of PVA and PVA/PLL.....	63
4.12.	Wettability analysis of coatings of PVA and PVA/PLL.....	63
4.13.	Biocompatibility of coatings of PVA and PVA/PLL.....	64

4.14.	Polylysine docking with FimH and PapG.....	65
4.15.	SEM analysis for microbial adhesion on PVA and	66
4.16.	Viability of microorganisms on PVA and PVA/PLL coated.....	68
4.17.	Deposition and surface characterization of SA and.....	70
4.18.	FTIR analysis and wettability of SA and SA/LyeTx I mnΔK.....	72
4.19.	Biocompatibility of SA and SA/LyeTx I mnΔK coating.....	74
4.20.	Antimicrobial activity of SA/LyeTx I mnΔK coated silicon.....	75
4.21.	SEM analysis for microbial adhesion on SA and.....	76
4.22.	Antimicrobial effect of SA/LyeTx I mnΔK against CAUTI.....	78
4.23.	Antimicrobial stability of the SA/LyeTx I mnΔK coating in urine.....	80
5.	Chapter IV: Discussion.....	82
6.	Chapter V: Conclusion and future directions.....	94
7.	Institutional review board statement.....	95
8.	Financial support.....	95
9.	Chapter VI: Research output.....	96
9.1.	Internships.....	96
9.2.	List of publications with UFMG, Brazil (Home University).....	96
9.3.	Conferences attended.....	98
9.4.	Participation in research projects.....	100
9.5.	Research projects proposed for funding (not approved)	100
9.6.	Awards and other achievements	100

10.	References.....	101
11.	List of tables.....	134
12.	List of figures.....	135
13.	Appendices.....	139

Abstract

Urinary tract infections (UTIs) have placed tremendous stress on public health sector. UTIs reported as the fourth most common type of healthcare-associated infection, making up 12.9% of such infections among which more than half are associated with catheter. In this work antimicrobial peptides (AMPs) derived from *Lycosa erythrognatha* spider venom, particularly LyeTx I mnΔK was assessed against UTIs.

Bacteriostatic and bactericidal properties of AMP were assessed, and its docking was performed with the adhesin of UPEC. The antibiofilm effects of the AMP were evaluated using the microplate-based model and catheter-based model. The toxicity of AMP was tested *in vitro* and in animals as well. Subsequently, AMP was coated on medical grade silicon using the ultrasonic spray method in the presence of polymer. The coatings were characterized through scanning electron microscopy (SEM), energy-dispersive spectroscopy integrated with SEM, Fourier-transform infrared spectroscopy (FTIR), contact-angle analysis and tested for antimicrobial effects.

The results showed that LyeTx I mnΔK stands out as a highly active AMP against UPECs, hypothetically due to its predicted binding to the FimH protein. LyeTx I mnΔK reduced the biomass of few isolates but significantly reduced viability of UPEC cells within biofilms. AMP exhibited negligible cytotoxicity *in vitro* and less toxicity *in vivo*. However, the highest dose of AMP increases the serum creatinine levels and promotes mild tubular degeneration of animal kidney.

Coating of the medical-grade silicon and silicon catheters with AMP-loaded polymers was performed with the use of an ultrasonic deposition method. To assess whether this method can affect AMP activity, preliminary optimization studies were performed with poly (vinyl alcohol) (PVA) as a polymer matrix and poly-L-lysine (PLL) as a model AMP. The results proved that the ultrasonic deposition can be used to form uniform coatings on the surface of silicon without affecting their antimicrobial activity.

Polymer screening tests indicated sodium alginate (SA) as the polymer matrix able to stabilize the activity of AMP. Consequently, an ultrasonic deposition method was used to coat the silicon with LyeTx I mnΔK. Antimicrobial tests revealed that SA/LyeTx I mnΔK coating reduced the bacterial growth and SEM images showed very less bacterial adhesion on the SA/LyeTx I mnΔK. The SA/LyeTx I mnΔK coating showed antimicrobial activity against

catheter-associated pathogens. Additionally, SA/LyeTx I mn Δ K coating also showed excellent biocompatibility. The SA/LyeTx I mn Δ K was therefore proven as an efficient way to provide antibacterial effects to urinary catheters.

Keywords: Catheters associated infections; Quinolone-resistant uropathogenic *Escherichia coli*; Antimicrobial peptides; Polymers; Antimicrobial coatings.

Abstrakt

Zakażenia układu moczowego (ZUM) stanowią ogromne wyzwanie dla sektora zdrowia publicznego. ZUM są czwartym pod względem częstości występowania rodzajem zakażeń związanych z opieką zdrowotną (stanowiąc 12,9% takich przypadków), przy czym ponad połowa z nich jest związana z cewnikowaniem. W niniejszej pracy oceniono skuteczność peptydów przeciwdrobnoustrojowych (AMP) pochodzących z jadu pająka *Lycosa erythrogna* -a w szczególności wariantu LyeTx I mnΔK – w zwalczaniu ZUM.

Zbadano właściwości bakteriostatyczne i bakteriobójcze tego peptydu oraz przeprowadzono analizę dokowania molekularnego z udziałem adhezyny szczepów UPEC (uropatogennych szczepów *Escherichia coli*). Aktywność antybiofilmową AMP oceniono przy użyciu modeli płytkowych oraz modeli wykorzystujących cewniki. Toksyczność peptydu zbadano zarówno *in vitro*, jak i *in vivo* (na modelach zwierzęcych). Następnie naniesiono AMP na silikon klasy medycznej metodą natrysku ultradźwiękowego w obecności polimeru. Uzyskane powłoki poddano charakterystyce z wykorzystaniem skaningowej mikroskopii elektronowej (SEM), spektroskopii dyspersji energii promieniowania rentgenowskiego (EDS) zintegrowanej z SEM, spektroskopii w podczerwieni z transformacją Fouriera (FTIR) oraz analizy kąta zwilżania, a także zbadano ich aktywność przeciwdrobnoustrojową.

Wyniki wykazały, że LyeTx I mnΔK jest peptydem wykazującym wysoką aktywność wobec szczepów UPEC, co prawdopodobnie wynika z przewidywanego wiązania się z białkiem FimH. LyeTx I mnΔK ograniczył biomasę niektórych izolatów, a przede wszystkim istotnie zmniejszył żywotność komórek UPEC w obrębie biofilmów. Peptyd wykazywał znikomą cytotoksyczność *in vitro* oraz niską toksyczność *in vivo*. Jednakże najwyższa zastosowana dawka powodowała wzrost poziomu kreatyniny w surowicy oraz łagodne zwyrodnienie kanalików nerkowych u zwierząt.

Pokrywanie silikonu klasy medycznej oraz cewników silikonowych polimerami zawierającymi AMP przeprowadzono metodą osadzania ultradźwiękowego. Aby sprawdzić, czy metoda ta wpływa na aktywność peptydu, przeprowadzono wstępne badania optymalizacyjne z wykorzystaniem poli(alkoholu winylowego) (PVA) jako matrycy polimerowej oraz poli-L-lizyny (PLL) jako modelowego AMP. Wyniki potwierdziły, że osadzanie ultradźwiękowe pozwala na tworzenie jednorodnych powłok na powierzchni silikonu bez utraty aktywności przeciwdrobnoustrojowej.

Badania przesiewowe polimerów wskazały alginian sodu (SA) jako matrycę zdolną do stabilizacji aktywności AMP. W konsekwencji, do naniesienia peptydu LyeTx I mnΔK na silikon wykorzystano metodę osadzania ultradźwiękowego. Badania właściwości przeciwdrobnoustrojowych wykazały, że powłoka SA/LyeTx I mnΔK ograniczała wzrost bakterii, a obrazy uzyskane za pomocą mikroskopii elektronowej (SEM) ukazały znacznie mniejszą adhezję bakterii do powierzchni pokrytej tym materiałem. Powłoka SA/LyeTx I mnΔK wykazała aktywność przeciwdrobnoustrojową wobec patogenów związanych z cewnikowaniem. Ponadto charakteryzowała się ona doskonałą biokompatybilnością. Tym samym potwierdzono, że zastosowanie SA/LyeTx I mnΔK stanowi skuteczną metodę nadawania właściwości przeciwbakteryjnych cewnikom urologicznym.

Słowa kluczowe: zakażenia związane z cewnikowaniem; szczepy *Escherichia coli* uropatogenne i odporne na chinolony; peptydy przeciwdrobnoustrojowe; polimery; powłoki przeciwdrobnoustrojowe.

Resumo

As infecções do trato urinário (ITUs) têm exercido uma enorme pressão sobre o setor de saúde pública. As ITUs são relatadas como o quarto tipo mais comum de infecção associada à assistência à saúde, representando 12,9% dessas infecções, das quais mais da metade estão associadas a cateteres. Neste trabalho, peptídeos antimicrobianos (AMPs) derivados do veneno da aranha *Lycosa erythrogatha*, particularmente LyeTx I mnΔK, foram avaliados contra ITUs.

As propriedades bacteriostáticas e bactericidas do AMP foram avaliadas e seu acoplamento foi realizado com a adesina da UPEC. Os efeitos antibiofilme do AMP foram avaliados usando o modelo baseado em microplaca e o modelo baseado em cateter. A toxicidade do AMP foi testada *in vitro* e *em animais*. Posteriormente, o AMP foi revestido em silício de grau médico usando o método de pulverização ultrassônica na presença de polímero. Os revestimentos foram caracterizados por microscopia eletrônica de varredura (MEV), espectroscopia de energia dispersiva integrada à MEV, espectroscopia de infravermelho com transformada de Fourier (FTIR), análise de ângulo de contato e testados quanto aos efeitos antimicrobianos.

Os resultados mostraram que LyeTx I mnΔK se destaca como um AMP altamente ativo contra UPECs, hipoteticamente devido à sua ligação prevista à proteína FimH. LyeTx I mnΔK reduziu a biomassa de alguns isolados, mas reduziu significativamente a viabilidade das células UPEC dentro dos biofilmes. O AMP exibiu citotoxicidade insignificante *in vitro* e menor toxicidade *in vivo*. No entanto, a dose mais alta de AMP aumenta os níveis de creatinina sérica e promove degeneração tubular leve nos rins dos animais.

O revestimento de silicone de grau médico e cateteres de silicone com polímeros carregados com AMP foi realizado utilizando um método de deposição ultrassônica. Para avaliar se esse método pode afetar a atividade do AMP, estudos preliminares de otimização foram realizados com poli(álcool vinílico) (PVA) como matriz polimérica e poli-L-lisina (PLL) como um AMP modelo. Os resultados comprovaram que a deposição ultrassônica pode ser usada para formar revestimentos uniformes na superfície do silício sem afetar sua atividade antimicrobiana.

Testes de triagem de polímeros indicaram o alginato de sódio (SA) como a matriz polimérica capaz de estabilizar a atividade do AMP. Consequentemente, um método de deposição ultrassônica foi usado para revestir o silício com LyeTx I mnΔK. Testes antimicrobianos

revelaram que o revestimento SA/LyeTx I ΔK reduziu o crescimento bacteriano e imagens de MEV mostraram muito pouca adesão bacteriana no SA/LyeTx I ΔK . O revestimento SA/LyeTx I ΔK mostrou atividade antimicrobiana contra patógenos associados a cateteres. Além disso, o revestimento SA/LyeTx I ΔK também mostrou excelente biocompatibilidade. O SA/LyeTx I ΔK foi, portanto, comprovado como uma maneira eficiente de fornecer efeitos antibacterianos a cateteres urinários

Palavras-chave: Infecções associadas a cateteres; *Escherichia coli* uropatogênica resistente a quinolonas; Peptídeos antimicrobianos; Polímeros; Revestimentos antimicrobianos.

List of acronyms, abbreviations, and symbols

3D	Three-dimensional
3MCY	Protein Data Bank structure ID
Ag QDs	Silver quantum dots
AgPLig-NPs	Silver-phenolated lignin nanoparticles
Afa	Fimbrial adhesins
AMP	Antimicrobial peptide
AMR	Antimicrobial resistance
ANOVA	One-way analysis of variance
Arg	Arginine
AS	Aggregation substance
Asn	Asparagine
AST	Antimicrobial susceptibility testing
β -CD	β -cyclodextrin
BrCAST	Brazilian Committee on Antimicrobial Susceptibility Testing
CAGR	Compound annual growth rate
CAUTIs	Catheter-associated urinary tract infections
C-di-GMP	Cyclic-diguanylic acid
CecA	Cecropin A
CFU	Colony-forming units
CI	Confidence interval
CLSI	Clinical and Laboratory Standards Institute
CS	Chitosan
dfrA	Dihydrofolate reductase genes
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
EC	Ethyl cellulose
EDC	1-ethyl-3-(3-dimethylaminopropyl) carbodiimide
EDS	Energy-dispersive spectroscopy
ELA	Ethyl lauroyl arginate
ESBL	Extended-spectrum β -lactamases
FDA	Food and Drug Administration
FTIR	Fourier-transform infrared spectroscopy
Glu	Glutamine
GNP	Graphene nanoplatelet
HC	Hemorrhagic colitis
HDPs	Host defence peptides
HEK	Human Embryonic Kidney
HLC	Human-like collagen

HNPs	Human neutrophil peptides
HUS	Hemolytic uremic syndrome
IBD	Inflammatory bowel disease
ICUs	Intensive care units
IL	Interleukin
Ile	Isoleucine
ISO	International Organization for Standardization
LB	Luria-Bertani
L929	Mouse fibroblast cell line
LCN2	Lipid-carrying protein-2
LUHMES	Lund Human Mesencephalic cells
MedPAR	Medicare Provider Analysis and Review
MG63	Human osteosarcoma cell line
MOE	Molecular Operating Environment
mPep	Mixed-charge polypeptide
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSSA	Methicillin-sensitive <i>Staphylococcus aureus</i>
NCA	N-carboxyanhydrides
NHDF	Normal human dermal fibroblasts
NIH3T3	Mouse embryonic fibroblast cell line
NK2	Natural Killer cell-derived peptide 2
OD	Optical density
PAAm	Polyacrylamide
PAM	Pressure-assisted microsyringe
PapG	P fimbrial adhesin G
PCL	Polycaprolactone
PDA	Polydopamine
PDADMAC	Poly(diallyldimethylammonium chloride)
PDB	Protein Data Bank
PEG	Poly(ethylene glycol)
PepSMI	Peptide–Small Molecule Interaction
PHMB	Poly(hexamethylene biguanide) hydrochloride
PLA	Poly(lactic acid)
PLGA	Poly(lactic-co-glycolic acid)
PLL	Poly-L-lysine
PolySBMA-TA	Poly(sulfobetaine methacrylate)-tannic acid
PVA	Poly(vinyl alcohol)
PVDF-HFP	Polyvinylidene difluoride-hexafluoropropylene
PVP	Polyvinylpyrrolidone
PyMOL	Python Molecular Graphics System
QMDEAs	Quaternized ammonium-modified methyldiethanolamines

QS	Quorum sensing
ROP	Ring-opening polymerization
SA	Sodium alginate
SI-ATRP	Surface-Initiated Atom Transfer Radical Polymerization
SMILES	Simplified Molecular Input Line Entry System
SPU	Segmented polyurethane
TcpF	Toxin-coregulated pilus factor F
TfaLyse	Trifluoroacetyl-L-lysine
uhPDMA	Poly(N,N-dimethylacrylamide)
WCA	Water contact angle
ZnO	Zinc oxide
γ -PGA	Poly(γ -glutamic acid)

List of publications related to this thesis

#shared first authorship (Equal distribution)

Haq, I.U#., Lima, W.G#, de Souza, Silva., F.H., Brito, J.C.M., Dos Santos, V.L., Verly, R.M., Vieira, R.P., Krukiewicz, K. and de Lima, M.E.

Anti-adhesive activity of LyeTx I mnΔK, a short peptide derived from the Brazilian spider *Lycosa erythrognatha* venom, against quinolone-resistant uropathogenic *Escherichia coli*

Microbial Pathogenesis, 2026, 108404.

IF₂₀₂₆=3.9, Ministry of Science and Higher Education (MNiSW) =70

Maryam, S#, **Haq, I.U#.**, Wilk, K., Shakibania, S., Shyntum, D.Y., Student, S., Szewczenko, J. and Krukiewicz, K.

Antimicrobial efficacy of ultrasonically deposited poly (vinyl alcohol)/poly (l-lysine)-based coatings for urinary catheters.

Surface and Coatings Technology, 2025, 521, 133094.

IF₂₀₂₆=6.9, MNiSW=100

Haq, I. U., & Krukiewicz, K.

Antimicrobial approaches for medical implants coating to prevent implants associated infections: Insights to develop durable antimicrobial implants.

Applied Surface Science Advances, 2023, 18, 100532.

IF₂₀₂₆=10.00, MNiSW=20

Haq, I. U., Khan, T. A., & Krukiewicz, K.

Etiology, pathology, and host-impaired immunity in medical implant associated infections.

Journal of Infection and Public Health, 2024, 17 (2), 189-203.

IF₂₀₂₆=4.8, MNiSW=70

Haq I. U., Pinto Vieira, R., Lima, W.G., de Lima, ME., Krukiewicz, K.

Antimicrobial polymers: elucidating the role of functional groups on antimicrobial activity.

Arab Journal of Basic and Applied Sciences. 2024, 31, 325–344.

IF₂₀₂₆=N/A, MNiSW=20

Chapter I: Theoretical part

1. Introduction

Urinary catheters are flexible tubes used for patients presenting impaired bladder function to allow bladder emptying (Skok *et al.*, 2025). Catheters have benefitted the patients by restoring organ function, though microbial adhesion to the catheter surface during insertion may lead to development of catheter-associated urinary tract infections (CAUTI) (Milo *et al.*, 2019). Each other day of catheterization increase in the risk of CAUTI. Urinary tract infections (UTIs) are reported as the one of the most prevalent healthcare-associated infection, making up 12.9% of such infections among which more than half are CAUTI (Magill *et al.*, 2014; Butement *et al.*, 2022). Roughly, 1 million CAUTIs annually contribute to 80% of hospital-acquired UTIs (Lo *et al.*, 2014). The daily risk of bacteriuria due to catheterization ranges between 3% and 10%, and the risk of symptomatic UTI is 20% in patients with bacteriuria (Hashmi *et al.*, 2003).

The catheter insertion procedures disturb the mechanical and antimicrobial defences of the bladder, increases the patient vulnerability of CAUTI (Guiton *et al.*, 2010). Individuals with immunocompromised status, advanced age, nephrolithiasis or vesical calculi, prior urological interventions, and structural abnormalities of the urinary tract are at increased risk for the development of CAUTIs. Several pathogens potentially cause infections such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Morganella morganii* (Matsukawa *et al.*, 2005; Jacobsen *et al.*, 2008; Macleod *et al.*, 2007; Saint *et al.*, 2011; Dudeck *et al.*, 2011; Tambyah *et al.*, 2001; Armbruster *et al.*, 2021). In catheterized patients, bacteriuria may progress to polymicrobial communities (Learman *et al.*, 2020). Microbial infections on catheter surfaces shaped by various factors, such as prophylaxis measures, host immune responses and treatment strategies. Interactions of pathogens, which can be either synergistic or antagonistic, may influence the pathogen's diversity (Azevedo *et al.*, 2017). The adhesion and colonization of uropathogens on catheters favoured by the surface of the catheter and host cell binding receptors with the involvement of adhesins proteins of pathogens (Jacobsen *et al.*, 2008; Werneburg, 2022). Initially, adhesion molecules and physical forces used by CAUTI pathogens to adhere to the catheter surface (Gupta *et al.*, 2016).

The major adhesion factors of CAUTI pathogens include flagella and pili, which allow them to attach to the catheter (Nicolle, 2014; Mireles *et al.*, 2015). The adhesins, such as

fimbriae, reported to initiate bacterial adherence to catheters (Stahlhut *et al.*, 2012). Therefore, preventing microbial adhesion and colonization on catheter surfaces through antimicrobial and anti-adhesive coatings is crucial for improving catheter longevity and performance (Gambrill *et al.*, 2025). The coating using of metal nanoparticles (Saikawa *et al.*, 2024), polymers (Dave *et al.*, 2011; Massoumi *et al.*, 2024) antibiotics, (Kranjec *et al.*, 2024), quorum sensing (QS) disrupting enzymes (Ivanova *et al.*, 2015), bacteriophages (Curtin *et al.*, 2006), and antimicrobial peptides (AMPs) (Lim 2015; Marie *et al.*, 2021) on catheter has been reported to reduce microbial attachment and infections. Particularly AMPs are excellent antimicrobial agents to create antimicrobial coatings for urinary catheters (Singha *et al.*, 2017). AMPs offer several advantages such as excellent antimicrobial activity, proteolytic stability, biocompatibility, and a reduced propensity to induce antimicrobial resistance (Li *et al.*, 2023). AMPs disrupts the membrane integrity of microorganisms allowing the exposition of intracellular targets (Zhang *et al.*, 2021). In recent times, the interest in using AMPs in medical implants has significantly increased, which leverages the natural antimicrobial properties of AMPs to address common challenges in medical implantology, such as infection-associated scenarios (Ul Haq *et al.*, 2023). Several studies of AMP-based medical implants showed osteoconductive properties even *in vivo* studies (Narbat *et al.*, 2010, Narbat *et al.*, 2012; Pihl *et al.*, 2021).

Despite being exceptional as antimicrobial agents, AMPs suffer from poor *in vivo* stability, salt sensitivity, and toxicity; however, their conjugation with other compounds, such as functional polymers, proved fruitful in reducing such limitations (Alkarri *et al.*, 2024). The antimicrobial activity of AMPs and stability could be enhanced through conjugation with polymers (Cui *et al.*, 2021). Several AMPs accommodated in AMP-polymer conjugates often retain their potent antimicrobial potential. Polymer based coatings can present antifouling and bactericidal properties, as well as they can create bifunctional antibacterial polymer coatings, depending on the surface functionality. Polymers explored as potential agents in controlled-release drug platforms to overcome the limitations of conventional controlled-release antimicrobial coatings on implants (Jacobsen *et al.*, 2013; Kumari *et al.*, 2021; Temtem *et al.*, 2009). The addition of non-fouling polymers also increases the antibiofilm properties of AMP coatings. The polymers provide regenerating surface for AMPs to inhibit the growth of microorganisms (Yu *et al.*, 2021a).

2. Literature Review

2.1. Urinary catheters and CAUTI

The usage of urethral catheters in medical practice dates back more than 3500 years. The word "catheter" originates from the Greek word "kathienai," which translates to 'to insert' or 'to let down' (Pelling *et al.*, 2019). A urinary catheter implanted for either short-term or long-term use, depending on the patient's clinical condition and postoperative care requirements. Following specific surgical procedures, catheterization provides a safe and effective means of urine drainage, collection, and monitoring (Corrado *et al.*, 2025). The first report of catheter usage published in 1960 (Kaitz, 1960). The United States of America (USA) is the leading country in terms of usage of catheter, and its percentages lie in the ranges of 7.5% to 31% (Dudeck *et al.*, 2013). In general hospitals, 15-25% of individuals require implantation of a urinary catheter during their hospital stay, and more than 100,000 individuals in nursing homes are living with catheters in place (Laan *et al.*, 2020). The nurses or health professionals, which are responsible for catheters insertion must follow standard protocols for procedures to avoid the risk of infections (Corrado *et al.*, 2025). The usage of catheters positively affects patients' quality of life, although surgical procedures required for catheterization may cause infections (Milo *et al.*, 2019). Fourcade et al. studied the incidence of UTIs among 289 patients, among which 122 patients (40.8 %) had indwelling catheters (Fourcade *et al.*, 2015). Wang et al. studied 234 patients with catheter, among which 30.5% of patients displayed CAUTI (Wang *et al.*, 2017). The CAUTIs in adult Intensive Care Units (ICUs) of National Healthcare Safety Network-affiliated hospitals experienced a decline ranging from 18.5% to 67% from 1990 to 2007 (Burton *et al.*, 2011). A 66% decrease in CAUTI occurred in France from 1995- 2004 (Vanhems *et al.*, 2008). According to the study of Magill et al. CAUTI and Ventilator-associated pneumonia account for 25.6% of healthcare-associated infections (Magill *et al.*, 2014). They also reported that among 504 healthcare-associated infections, 44 were UTIs, among which 67.7% were associated with catheter (Werneburg, 2022; Nicolle, 2014). Furthermore, a British study comparing various catheter types found that 10.6% to 12.6% of patients with catheters experienced CAUTIs. Duszyńska et al. reported a 7% incidence of UTIs, among which 36% attributed to CAUTI, in a single-centre study in Poland (Duszyńska *et al.*, 2016). Walaszek and colleagues reported marginally lower figures, noting a UTI incidence rate of 4% (with an incidence density of 5), and found that 99% of these infections were CAUTI in Poland (Walaszek *et al.*, 2018). A study conducted between 01.01.2012 and 31.12.2014

reported 6.9%, 7.3%, and 7.6% in 2012, 2013, and 2014 respectively in Poland (Duszyńska *et al.*, 2016).

2.2. Risk factors and chances of developing CAUTI

The chances of CAUTI are associated with the catheterization procedures, catheter surface features, and patient underlying diseases and gut microbiota (Figure 1). The vulnerability of CAUTI significantly increases with the disturbing mechanical and antimicrobial defences of the bladder upon catheter insertion, which allows pathogens adhesion, colonization, establishment of infections, and dispersal (Guiton *et al.*, 2010) while using the catheter surface as a substrate (Macleod *et al.*, 2007). 15% to 25% of patients have a urethral catheter inserted in general hospitals (Hooton *et al.*, 2010), and 22.2% undergo long-term catheterization (Shackley *et al.*, 2017). This scenario poses a risk of cross-contamination to other patients or different sites on the same patient (Knoll *et al.*, 2011). After the third day of the indwelling of the catheter, the pathogens start colonizing the catheter surface; however, the infection chances depend on underlying diseases in patients such as diabetes, hypertension, and cardiovascular disease (Niveditha *et al.*, 2012). The daily risk of bacteriuria due to catheterization ranges between 3% and 10%, and the risk of symptomatic UTI is 20% in patients with bacteriuria (Hashmi *et al.*, 2003).

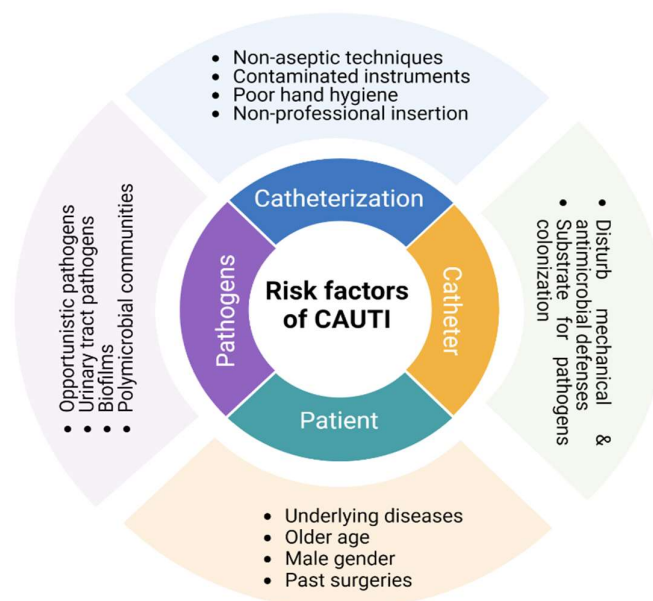


Figure 1. The risk factors for CAUTI related to procedures of catheterization, catheter surface, and patient. Created in BioRender.com

Gender is also a significant determinant of the risk of CAUTI (Chenoweth *et al.*, 2014; Mireles *et al.*, 2015). Adegun *et al.* reported CAUTI incidence as 90.2% and 80.6% among middle-aged and older men, respectively, indicating old age as a CAUTI risk factor (Adegun *et al.*, 2019). Niveditha *et al.* found a 36% prevalence of CAUTI in the age group 61-75 years (Adegun *et al.*, 2019). On gender-based comparison, CAUTI has a female-to-male ratio of about 2:1. About 30% to 43% of CAUTI reported in males (Kakaria *et al.*, 2018; Daniels *et al.* 2014; Mireles *et al.*, 2019). Berendsen *et al.* studied the indwelling catheter users and observed that the percentage of male users was 71.56% and females was 28.44% (Berendsen *et al.*, 2021).

2.3. Pathogens in involved in CAUTIs

Pathogens associated with CAUTI generally exhibit lower virulence than those commonly implicated in non-catheter-associated UTIs. However, the process of catheter insertion enables them to establish infection quickly with a less virulence profile. Gram-positive and Gram-negative bacteria reported to colonize, form biofilms on catheters, and cause CAUTIs (Guiton *et al.*, 2012). Somewhere, in CAUTI, the incidence of Gram-negative bacteria (57%) reported higher compared to Gram-positive bacteria (43%) among 1092 bacterial isolates of CAUTI

(Armbruster *et al.*, 2021). The older catheterized patients are mostly infected with *M. morganii*, which is a rare opportunistic pathogen (Liu *et al.*, 2016) and less prevalent than other common CAUTI pathogens such as *P. mirabilis* or *E. coli*. Usually, it causes co-infections with other CAUTI pathogens. The presence of co-colonizing microorganisms can promote the colonization, persistence, and pathogenicity of *M. morganii* in the urinary tract (Learman *et al.*, 2020).

A study comprising 97 patients found that polymicrobial CAUTIs were more common in older individuals, which may be associated with immunocompromised status and the presence of multiple underlying comorbidities (Galván *et al.*, 2016). Wang *et al.* studied 234 patients with CAUTI (Wang *et al.*, 2017), and highly prevalent pathogens were *Enterococcus*, *E. coli*, and MRSA. A considerable change observed in the microbial pathology on the catheter surface inside the biofilms (White *et al.*, 2021). MRSA causes complicated CAUTI due to limited treatment options (Paudel *et al.*, 2023). The United States of America (USA) tertiary care academic centre reported the incidence of *Enterococcus* spp 28.4% and *Candida* spp (19.7%) in CAUTI (Chang *et al.*, 2011). Antibiotic resistant pathogens in CAUTI are also a major problem (Köves *et al.*, 2017; Mancuso *et al.*, 2023). Sievert *et al.* reported extended spectrum cephalosporins resistant pathogens from patients with CAUTIs (Sievert *et al.*, 2013).

2.4. Uropathogenic *E. coli*

Uropathogenic *E. coli* (UPEC)-induced UTI considered as a common nosocomial infection. In healthcare settings, in 50-65% of healthcare-associated UTIs, UPEC is the causative agent. The UPEC prevalence is even higher in community-acquired UTIs than in healthcare-associated UTIs (Mireles *et al.*, 2015; Tarlton *et al.*, 2019), highly contributing to global mortality and morbidity (Halaji *et al.* 2022). Compared to non-uropathogenic *E. coli*, UPEC isolates can adjust and colonize rapidly within the urinary tract (Forsyth *et al.*, 2018). The main virulence factor of UPEC involved in CAUTIs is its ability to form biofilms on inanimate surfaces such as vesico-urethral catheters. Biofilms increase antibiotic resistance in UPEC isolates. (Abo *et al.*, 2024) and infection severity (Rastegar *et al.*, 2023). UPEC uses several virulence factors to adhere to the host cell surface, enter the cell, produce toxins, and establish infections (Mireles *et al.*, 2015). The highly reported virulence factors used by UPEC are F1C pili, and Dr adhesins. In contrast, the factors that help UPEC in immune invasion are HlyA, Capsular antigens, CNF1, and Yersiniabactin (Mireles *et al.*, 2015). In UPEC, the adhesins

from the Afa/Dr family (fimbrial adhesins with Dr fimbriae and Afa) are important virulence factors that facilitate critical infections (Lehmann *et al.*, 2013). FimH has also suggested as a candidate for vaccine development (Dadi *et al.*, 2020). There is an association between biofilm formation and antibiotic resistance; cephalosporin, gentamicin, and ceftazidime-resistant UPEC are low biofilm producers (Gajdács *et al.*, 2021; Rolo *et al.*, 2019). Compared to planktonic UPEC cells, the antimicrobial resistance is 1000-fold higher in biofilms, which complicates the treatment and eradication of biofilm-related infections (Alshaikh *et al.*, 2024). The increased resistance in UPEC, especially to quinolones, such as nalidixic acid (first generation); ciprofloxacin, norfloxacin, ofloxacin, levofloxacin, pefloxacin (second generation); sparfloxacin, gatifloxacin, gemifloxacin (third generation); moxifloxacin, and trovafloxacin (fourth generation) reported (Haslund *et al.*, 2013). The treatment of quinolone-resistant UPEC-induced infections become particularly challenging due to its widespread incidences all over the world (Rashki *et al.*, 2019; Bunduki *et al.*, 2021; Białek *et al.*, 2017; Fasugba *et al.*, 2015). The resistance of low-level-quinolone-resistant UPEC increased due to the urinary tract physiological parameters (Gutiérrez *et al.*, 2016). UPECs also reported as multi-drug resistant (MDR) pathogens (Fenner, 2020; Kalsy *et al.*, 2020; Chen *et al.*, 2022). The average prevalence of UPEC MDR strains was 50% in Iran (Hadifar *et al.*, 2017), 80% in India, and 65% in Pakistan (Reza *et al.*, 2019). Furthermore, the prevalence of MDR UPEC is also high in developed countries such as the USA (Sanchez *et al.*, 2014). Europe and the USA have reported 22% and 31% fluoroquinolone-resistant UPEC.

2.5. Polymicrobial communities in CAUTI

Pathogenic microbes usually exist or prefer to form polymicrobial communities rather than live as a single entity. In catheterized patients, bacteriuria quickly turns into a polymicrobial condition involving a mix of various bacteria such as *P. mirabilis*, *Enterococcus* spp, *P. aeruginosa*, *K. pneumoniae*, *Citrobacter*, *Staphylococcus* species, and *Streptococcus* species (Learman *et al.*, 2020). The incidence of polymicrobial CAUTI varies, ranging between 31-97% (Dedeić *et al.*, 2009; Hollenbeak *et al.*, 2018; Armbruster *et al.*, 2021). CAUTI has a higher incidence of polymicrobial infections compared to UTIs without catheters (Fourcade *et al.*, 2015). About 86% of CAUTI are polymicrobial infections (Govindarajan *et al.*, 2022). Fourcade *et al.* studied polymicrobial communities in UTIs and reported a 53% incidence of polymicrobial infections in the presence of a catheter (Fourcade *et al.*, 2015; Fouts *et al.*, 2012;

Azevedo *et al.*, 2017). Interactions of pathogens, which can be either synergistic or antagonistic (Azevedo *et al.*, 2017). Typically, the initial episode of bacteriuria is caused by a single species in the initial days of catheterization; however, the absence of antimicrobial therapies and long-term stay of catheters leads to polymicrobial bacteriuria comprised of 3 to 5 organisms (Pelling *et al.*, 2019).

In polymicrobial CAUTI, *E. faecalis* and *P. mirabilis* are involve (Gaston *et al.*, 2020). Several bacterial species inhabiting the urinary tract of catheterized individuals can either increase or reduce the severity of the disease. This influence on *P. mirabilis* occurs without the necessity for physical contact or proximity between the bacteria (Learman *et al.*, 2020). *K. pneumoniae*, *E. coli*, *E. faecalis*, and *P. mirabilis* have been reported to form biofilms on silicon surfaces *in vitro* study. In another study, 106 patients with urinary catheters were examined, and 76 catheters were found with the biofilms of numerous bacteria (Macleod *et al.*, 2007). *P. mirabilis* can form crystalline biofilms on all types of catheters (Holling *et al.*, 2014; Wilks *et al.*, 2015). The possible outcomes of interactions between multiple microbes in the urinary system is shown in Figure 2.

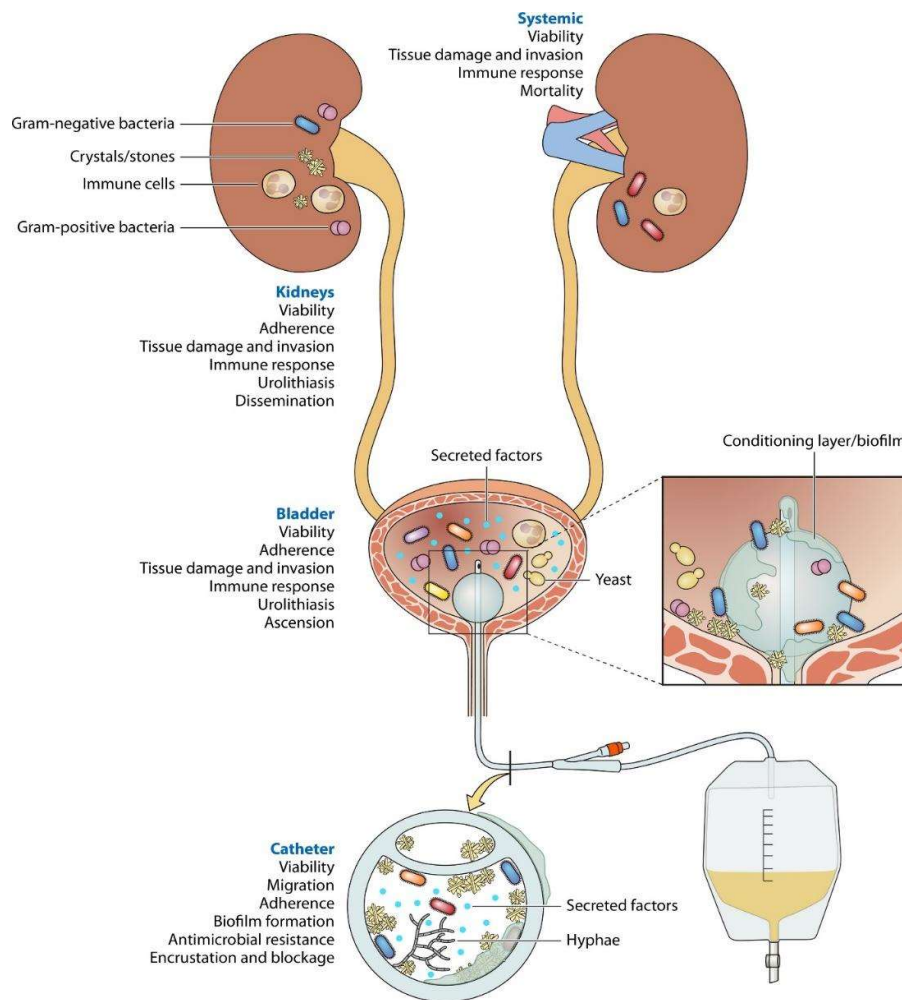


Figure 2. The possible outcomes of interactions between multiple microbes in the urinary system. Reprinted from (Gaston *et al.*, 2021) under a CC by license. Copyrights (2021) ASM.

2.6. Establishment of CAUTI

The pathogens can attach to a catheter during the insertion and reach the bladder (Mobley, 2017). The process begins with inserting a urinary catheter, which may disrupt the natural defences of the urinary tract and increase the shedding or harm to the urothelial lining (Guiton *et al.*, 2010). More binding sites are created to attach adhesins due to damage to protective uroepithelial mucosa by inserting catheter (Adegun *et al.*, 2019). The host mechanical and immune defences in the vicinity of the urinary tract are also disrupted by catheter insertion, which causes over distension of the bladder and incomplete voiding (Mireles *et al.*, 2015). Catheterization procedures cause histological and immunological changes, leading to robust inflammatory response, exfoliation, edema, and mucosal lesions (Mireles *et al.*, 2015). The

catheter-induced inflammatory responses also influence the functions of host immune responses (Leendertse *et al.*, 2009). Usually, the flushing of urine flow and the protective mucosal layer of the bladder help prevent infection. However, the presence of a catheter can impair these defences and provide a surface for bacteria to adhere to in two ways: extraluminally and intraluminally (Tenke *et al.*, 2006). Most extraluminal pathogens originate internally, primarily from the gastrointestinal tract that colonizes the patient's perineum and ascends the urethra after catheter insertion (Warren, 2001). Extraluminal entry usually takes place in catheterized women in approximately 70% of cases and 34% of the pathogens are enter by intraluminal contamination (Saint *et al.*, 2003). CAUTI pathogens also infiltrate the catheter through an intraluminal pathway, typically when they enter an internal passage due to the failure of the closed drainage system or contamination of the collection bag (Tenke *et al.*, 2006).

2.7. CAUTI-associated secondary complications

The CAUTIs-related complications, such as the risk of hospitalization, length of hospitalization, and length of antibiotic therapy, are significantly higher than regular UTI (Werneburg, 2022). CAUTI leads to further health complications such as pyelonephritis, bacteremia, enterococcal cystitis, urosepsis, and in some cases, death (Maki *et al.*, 2001). The crystalline biofilms on the catheter surface led to encrustation, resulting in the development of urinary tract stones (Jacobsen *et al.*, 2011). Struvite and apatite are the two significant crystals in catheters due to crystalline biofilms made of magnesium ammonium phosphate and hydroxylated calcium phosphate (Stickler *et al.*, 2010). These crystals are generated by urease enzymes while hydrolyzing urea in the residual bladder urine (Stickler *et al.*, 2010). The high urease activity increases the urinary pH, crystalline biofilms, and urinary stones, which causes catheter blockage (Learman *et al.*, 2020). The probability of developing struvite crystals and urolithiasis during pathogenesis is likely associated with certain urease-enhancing organisms. The crystalline biofilms formed by *P. mirabilis* cause the blockage of urine, which causes leakage of urine around the catheter (Macleod *et al.*, 2007). MRSA is reported to cause other medical complications, such as urosepsis and shock (Paudel *et al.*, 2023). The immune responses towards catheters and CAUTI are complicated; the regular responders are neutrophils, macrophages, basophils, and some eosinophils (Guiton *et al.*, 2013). *In vivo*, the implanted materials colonized by *E. faecalis* increase neutrophil levels approximately 5-fold

due to the activation of the neurogenic inflammatory pathway (Dale *et al.*, 2011). It is speculated that neurogenic inflammatory responses are also triggered by mast cells, bradykinins, and NK2 receptors (Birder *et al.*, 2018). *E. faecalis* has an immune evasion mechanism (Tien *et al.*, 2017). CAUTI polymicrobial communities, particularly *E. faecalis* and *P. aeruginosa*, result in aggravated pyelonephritis (Tien *et al.*, 2017). Some pathogens, such as *P. mirabilis*, a highly prevalent and intense biofilm producer of catheters, can spread to the bloodstream and other organs, such as the spleen and heart (Gaston *et al.*, 2020).

2.8. Healthcare and economic impact of CAUTI

The financial costs of catheter-associated bacteriuria and infections significantly impact public health economy (Knoll *et al.*, 2011). Healthcare institutions invest significant time and resources to decrease the incidence of CAUTI (Patel *et al.*, 2023). The high occurrence of CAUTI leads to substantial medical costs in the USA, with an estimated annual expenditure of \$424 million to \$451 million for treating these infections (Jacobsen *et al.*, 2008). The estimated expenses spent on CAUTI in surgical patients are around \$558 to \$676 per case in USA (Saint 2000) (Givens *et al.*, 1980). At the same time, the cost of treating an uncomplicated case of UTI is estimated to be \$40, which includes diagnosis and antibiotic treatment. In the USA, an estimated 62,700 CAUTI were reported in 2015, which cost around \$864 million (Dickerson *et al.*, 2022). The annual medical expenditures of CAUTI reached \$600 million in 2010 (Guiton *et al.*, 2010), while Werneburg reported \$115 million to \$1.82 billion in costs (Werneburg, 2022). Asymptomatic CAUTI increases the direct expenses of acute-care hospitalization by between \$500 and \$1,000 in USA (Maki *et al.*, 2001). In 1999, in Scotland, the annual cost spent on the treatment of CAUTI was estimated at £ 125 million (Feneley *et al.*, 2015). It was speculated that a total of £2.5 billion was spent annually on treating CAUTI in the UK during 1999-2015. Therefore, it is estimated that the total yearly additional cost related to CAUTI in the UK likely ranges between £1.0 billion and £2.5 billion (Feneley *et al.*, 2015). In the USA, the annual cost of healthcare-acquired infections is \$ 45 billion (Kennedy *et al.*, 2013), among which 80% (\$ 36 billion) spent on CAUTI. USA hospitals used to compensate for the additional cost spent on CAUTI by recovering the extra payment before October 2008. However, it was stopped due to Medicare rule (Saint *et al.*, 2009). A report on estimating the cost associated with hospital-acquired conditions analysed the cost data from six studies. Approximately \$13,793 was the additional cost in these six studies on CAUTI, with a 95% confidence interval

(CI) of \$5,019 to \$22,568 (Bysshe *et al.*, 2017). Paul *et al.* studied 123 CAUTI patients and found that approximately \$56,534 spent on diagnosis and additional medication costs, with a mean cost of \$589 and a median cost of \$356 (Paul *et al.*, 2022).

Additionally, each episode of catheter-associated bacteremia is estimated to cost around \$2,900 (Saint, 2000). However, in 2011, Burton *et al.* (Burton *et al.*, 2011) reported that \$600 is the total cost of one episode of CAUTI, which significantly increases to \$2800 if CAUTI is associated with a bloodstream infection. Although the estimated cost of medical care is \$131 million, thus a significant amount is spent on CAUTI (Burton *et al.*, 2011). The cost associated with CAUTI depends on causative pathogens; for example, fungal infections costs (\$821.2) are higher than gram-negative bacteria infections (\$690.4), and among gram-negative bacteria, the cost is lower in the case of *E. coli* (\$363.3). In 2016, these costs were \$540 for *E. coli*, \$1,027 for other gram-negative bacteria, and \$1,222 for yeasts, according to (Hollenbeak *et al.*, 2018). However, the cost spent on one episode of CAUTI increases to approximately \$3790 (Azevedo *et al.*, 2017). In 2007, the cost associated with catheter reached \$ 380 million (Feneley *et al.*, 2015). In 2015, the market size amounted to \$ 3.4 billion, considering all urinary catheters' economic burden and a gradual increase in future growth prospects (Berendsen *et al.*, 2021). In the 1980s, \$204,000 was spent on hospital-acquired UTIs in one hospital every year. The terms bacteriuria and UTI are often interchangeably used because the precise definition of catheter UTI used in published reports is different (Saint, 2003).

2.9. Antimicrobial coatings on catheters to prevent CAUTI

In recent times, the research on antimicrobial catheters has accelerated significantly (Yu *et al.*, 2015b; Anjum *et al.*, 2018). The most frequent antimicrobial agents used in catheter coatings are metal nanoparticles (Saikawa *et al.*, 2024), polymers (Massoumi *et al.*, 2024; Dave *et al.*, 2011), antibiotics, bacteriocin (Kranjec *et al.*, 2024), QS disrupting enzymes (Ivanova *et al.*, 2015), antimicrobial peptides (Lim, 2015; Marie *et al.*, 2021), and bacteriophages (Curtin *et al.*, 2006). For instance, latex silicone-coated Foley catheter coated with bio-AgNPs showed antimicrobial activity against multidrug-resistant *P. mirabilis* infection (Saikawa *et al.* 2024). The silicon catheters modified with polydopamine (PDA) and poly (2-hydroxyethyl methacrylate) (PHEMA) hydrogel showed antifouling characteristics (Massoumi *et al.*, 2024). Modified catheters prevented the adhesion of *P. aeruginosa* and was non-cytotoxic with L929 fibroblast cells. Kranjec *et al.* fabricated antimicrobial coatings for indwelling medical devices, including catheters, using bacteriocin (Kranjec *et al.*, 2024). The coating showed antibiofilm

effects and no cytotoxic effects were observed up to 80 µg/ml towards MG63 cells. Duan *et al.* fabricated a membrane using the piezoelectric zinc oxide nanoparticles (ZnO NPs)-incorporated polyvinylidene difluoride-hexafluoropropylene (ZnO-PVDF-HFP) for urinary catheter (Duan *et al.*, 2024). ZnO-PVDF-HFP functionalised urinary catheter showed significant antimicrobial effects, stability and biocompatibility. Puertas *et al.* fabricated antimicrobial coatings on silicone surfaces via ultrasound and nanoparticle-assisted self-assembly (Segura *et al.*, 2024). The coatings showed antimicrobial activities and inhibited biofilms and no adverse effects on fibroblasts and keratinocytes were observed. Lim *et al.* developed a strategy using a 2-step polydopamine-based surface immobilization for conjugation of an antimicrobial peptide (AMP) onto commercially available Foley catheter (Lim, 2015). The AMP-based catheter inhibited the growth of bacteria. Dave *et al.* fabricated a novel biocatalytic polymer-based antimicrobial coatings for urinary catheter (Dave *et al.*, 2011), which showed antimicrobial effects against highly active catheter associated pathogens. Cai *et al.* fabricated a PDA-carboxymethylcellulose-Ag nanoparticle coating on the latex catheter (Cai *et al.*, 2023), which showed antimicrobial and anti-adhesive effects.

Miao *et al.* used hydrogel coating containing poly (sulfobetaine methacrylate)-tannic acid (polySBMA-TA) to fabricate antimicrobial responsive platforms for urinary catheters (Miao *et al.*, 2023). The modified surfaces showed antibacterial and anti-encrustation effects in *in vivo* studies and released antimicrobials in the response to change in pH. Yong *et al.* fabricated a thin conformal lubricating hydrogel coating comprised of an antifouling, zwitterionic moiety sulfobetaine and biocidal N-halamine monomer, on catheters (Yong *et al.*, 2018). The coating did not allow the microbial growth and biofilms combined with excellent mechanical properties like lubricity and stability. *In vivo* studies showed that the foreign body response was greatly reduced by sulfobetaine group addition in the hydrogel coating. Gayani *et al.* employed trifluoropropyl spray-coated polydimethylsiloxane (TFP-PDMS) (Gayani *et al.*, 2021) which reduced *P. mirabilis* attachment till two weeks. The properties of PDMS, such as nontoxicity, flexibility, biocompatibility, and autoclavability made it an ideal tool for fabrication of medical devices (Neoh *et al.*, 2017).

Bai *et al.* fabricated a photocurable zwitterionic copolymer poly (2-methacryloyloxyethyl phosphatidylcholine-co-hydroxyethyl methacrylate-co-(benzoyl) phenyl methacrylate (poly(MPC-co-HEMA-co-BP) coatings for urinary catheters with potential antifouling and lubrication properties (Bai *et al.*, 2024). The coatings showed antimicrobial behaviour against

several clinically important pathogens. Roy et al. coated the urinary catheter with cobalt containing antimicrobial mesoporous bioactive glass (Roy *et al.*, 2024). The samples showed antimicrobial behaviour towards *Candida albicans*, *E. coli* and *P. aeruginosa* and were non-toxic towards NIH3T3 cell line. Puertas-segura et al. built an enzymatically nano-enabled antimicrobial coating on urinary catheters using the PDMS surface loaded with silver-phenolated ligninnanoparticles-poly(carboxybetaine) zwitterions (AgPLig-NPs) (Segura *et al.*, 2024). The AgPLig-NPs coated PDMS surfaces showed antimicrobial effects against *P. aeruginosa* and *S. aureus in vivo* and fibroblasts exhibited over 90% viability after 1 and 7 days of direct contact with the coated samples. Santos et al. fabricated a graphene nanoplatelet (GNP)-based composites as coatings for catheter (Santos *et al.*, 2023). The coatings showed antimicrobial effects against several bacteria. Pulido et al. coated catheters with carboxymethylcellulose and ciprofloxacin loaded chitosan-silver complexes through a layer-by-layer method. The coatings showed a contact-killing effect and controlled antimicrobial release. However, the reducing of silver to its elemental form which may affect the biological functionality of the coating (Pulido *et al.*, 2025).

Polyurethane-based coatings incorporating a positively charged antibacterial agent quaternized ammonium-modified methyldiethanolamines (QMDEAs) were developed and applied to the catheter. The coatings showed excellent and long-term antibacterial effects and also was biocompatible (Jiang *et al.*, 2024b). Ring open polymerization of ϵ -trifluoroacetyl-L-lysine (Tfa-Lys)-N-carboxyanhydride allowed the immobilization of poly-L-lysine (PLL) brush on the catheter, which exhibited inhibitory effects *in vitro* and *in vivo* as well (Liu *et al.*, 2023). Catheter retained antimicrobial effects after incubation at 60 °C for two months approximately. Soyhan et al. immobilized thiolated AMPs on the hydroxylated silicone catheters activated with allyltrimethoxysilane solution. The modified catheters showed antibiofilm effects *E. coli* 25922 (Soyhan *et al.*, 2024).

The polymers such as sodium alginate (SA), methylcellulose, and poly (ethylene glycol) (PEG) impregnated with antimicrobial agent secnidazole were used for 3D printing of catheter to suppress QS mediated virulence factors (Archana *et al.*, 2023). Cross-linking between SA and calcium chloride is required to produce a suitable polymer ink for pressure-assisted microsyringe (PAM) 3D printing of catheters. Initial burst release of antibiotic was observed from drug-loaded catheter followed by sustained release which disrupted biofilms by

suppressing all the QS mediated virulence factors. Liu *et al.* prepared nanoparticles of chitosan-SA followed by loading with PLL which was three times more bacteriostatic than that of free PLL (Liu *et al.*, 2018). Similarly, composite films of sodium carboxymethyl cellulose/SA/CS were prepared by dissolving SA, CS, and glycerol in an acetic acid solution followed by addition of sodium carboxymethyl cellulose solution which was then casted onto a glass plate to obtain films. The composite films inhibited the microbial growth and therefore suggested as promising for antibacterial food packaging applications (Lan *et al.*, 2018).

2.10. Antimicrobial peptides (AMPs)

Antimicrobial peptides (AMPs) have attracted the attention of worldwide researchers for their excellent broad spectrum antimicrobial activity, biocompatibility, and fewer chances of resistance (Li *et al.*, 2023). AMPs are excellent antimicrobial agents to create antimicrobial coatings for urinary catheters (Singha *et al.*, 2017). Nature has several sources of AMPs, including living organisms where AMPs serve as an innate immune system (Yang *et al.*, 2025). The global AMP market in 2015 was valued at \$ 1.06 billion and is anticipated to have a compound annual growth rate (CAGR) of 7.5% through 2017–2030 (Kazemzadeh-Narbat *et al.* 2020) (Cresti *et al.*, 2024). AMPs are ubiquitous; the first AMP, i.e. gramicidin, was discovered in 1939 and as of 18 March 2026, the AMP database version 6 platform housed records for 5188 peptides (Wang *et al.*, 2026). The last decade observed a significantly higher increase in articles published on AMPs (Cresti *et al.*, 2024) (Figure 3).

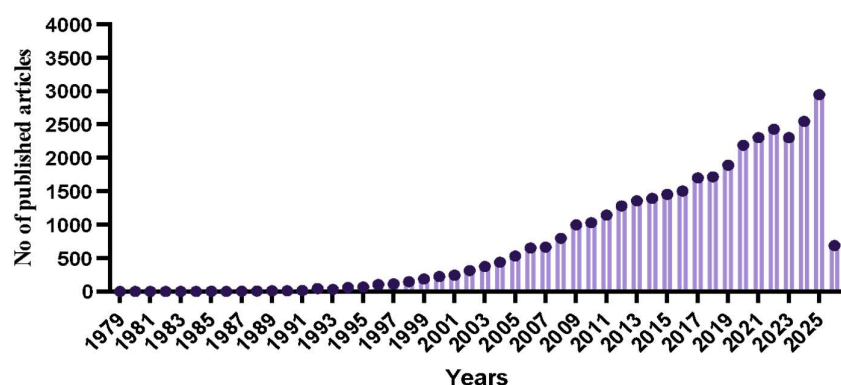


Figure 3. Number of scientific articles published in academic journals since 1979. The antimicrobial peptides” OR “AMPs” OR “host defence peptides” OR “HDPs” were used as keywords to search the PubMed databases in March 2026.

Most of the AMPs comprise 10-50 amino acids, a proportion of hydrophobic residues. AMPs kill microorganisms by disrupting their membrane integrity and reaching intracellular targets (Zhang *et al.*, 2021). Few AMPs are being used in medical practice. Most of the AMPs are membrane-active peptides (Chen *et al.*, 2020). The interaction of AMPs with bacterial surfaces is mediated by electrostatic forces (Ebenhan *et al.*, 2014). AMPs also form complexes with small organic anions to permeabilize the bacterial membrane and easily reach intracellular targets. Comparatively, AMPs interact less with mammalian cells due to cholesterol and phosphatidylcholine in their cell membrane (Lin *et al.*, 2021). AMPs have also exhibited substantial antimicrobial efficacy in the context of implant coatings to improve their performance and biocompatibility (Zhou *et al.*, 2017). Several studies of AMP-based medical implants showed osteoconductive properties (Narbat *et al.* 2010; Narbat *et al.*, 2012; Pihl *et al.*, 2021). Number of synthetic AMPs is steadily increasing; however, most of the AMPs are derived from natural sources.

2.11. Advantages and challenges of AMPs

AMPs have multi-antimicrobial mechanisms on the plasma membrane and intracellular targets, making them less prone to antimicrobial resistance (Xuan *et al.*, 2023). The less tendency to antimicrobial resistance in AMPs, particularly in α -helical AMPs, is due to its rapid antimicrobial action (Zhang *et al.*, 2022). The multi-antimicrobial mechanism is also associated with their hydrophobicity, amphipathicity, secondary structure such as α -helix or β -sheet, and aromatic amino acid residues (Sun *et al.*, 2018; Narbat *et al.*, 2020). AMPs are less toxic for the mammalian cell membranes due to bearing positive charge and preferentially interacts with negatively charged membranes (Singha *et al.*, 2017). Besides antimicrobial potential, AMPs are immunomodulatory and have angiogenesis properties, wound healing, and antitumor activity (Ebenhan *et al.*, 2014; Chen *et al.*, 2020). AMPs enhance the chemotaxis of immune cells, activate immune cell differentiation, initiate adaptive immunity, and repress the cytokine and TLR-mediated release of proinflammatory cytokines (Mahlapuu *et al.*, 2020).

Additionally, versatility is the key advantage of AMPs, which allows them to be modified for therapeutic purposes (Sierra, 2021). A group of modified AMPs with selectivity for different membranes was tested by Hicks *et al.* against ESKAPE pathogens (Hicks *et al.*, 2013). They found that the AMP potency increased with modifications, which can potentially help in

designing new peptide-based drugs with a narrow but selective antimicrobial spectrum. Such modifications allow the synthesis of novel peptide-based antimicrobials with less toxicity. Other structural changes in AMPs, such as increased hydrophobicity, increase the bacterial killing rate (Zhang *et al.*, 2022). Such modifications allow the AMPs to bind rapidly to bacterial membranes, which is also very fruitful for *in vivo* applications as, typically, AMPs degrade due to low bioavailability. Most AMPs are receptor-binding peptides that bind to the microbial membrane, while some inhibit the biological pathways of the targeted organisms (Chen *et al.*, 2020). Some AMPs inhibit the synthesis of proteins and nucleic acids, induce apoptosis, and block enzyme activities (Luong *et al.*, 2020). Receptor-binding peptides can be both agonists and antagonists.

Several challenges, including stability and toxicity issues, limit the clinical applications of AMPs. The serum's proteolytic enzymes, such as trypsin and chymotrypsin, degrade the AMPs (Yang *et al.*, 2025), where multiple doses are needed, which reduces patient adherence and increases treatment costs. Enfuvirtide is a peptide-based structure that necessitates twice-daily injections due to rapid degradation that inflicts a burden on the human body (Lai, 2021). The hepatic and renal clearance of AMPs is enhanced due to rapid proteolytic degradation (Vlieghe *et al.*, 2010). The AMP synthesis is very complex, making large-scale production challenging, increasing the high production cost, and potentially imposing the cost barrier (Zenin *et al.*, 2022). Low oral bioavailability is the problem that limits the clinical application of AMPs because digestive enzymes break the AMPs in the gut. The high polarity and molecular weight of AMPs limit their permeability in the intestine. *In vitro*, AMPs are highly active; however, *in vivo* due to the complexity of the microenvironment, the antimicrobial potential is reduced (Kumar *et al.*, 2020b). Some AMPs exert inhibit the growth of microorganisms by modulating the immune system rather than directly killing bacteria that may cause additional chronic inflammatory diseases in case of high concentrations of AMPs (Takahashi *et al.*, 2018). Alamethicin, melittin, and other membrane-active peptides are reported to cause hemolysis and cytotoxicity by interaction with eukaryotic cells. The membrane affinity of AMPs may also result in interaction with eukaryotic cells and can potentially cause hemolysis. It is a major hurdle in designing AMPs with high antimicrobial activity and minimum cytotoxicity by maintaining balance in charge, hydrophobicity, and amphipathicity (Maturana *et al.*, 2017). Additionally, dermcidin resistance is induced by a particular protease from *S. aureus*. The antibiotic resistance in pathogens can be overcome by treating them with AMPs, which

permeabilize the membrane. The lengthy time required for experimental work or clinical trials is the major obstacle to developing peptide-based antimicrobials.

In recent times, the interest in using AMPs coatings on medical implants has increased, which leverages the natural antimicrobial properties of AMPs to address common challenges in medical implantology, such as infection, inflammation, and implant failure. Despite being exceptional antimicrobials, AMPs suffer from poor *in vivo* stability, salt sensitivity, and toxicity; however, their conjugation with other compounds, such as functional polymers, proved effective in reducing these limitations (Alkarri *et al.*, 2024). The therapeutic efficiency of AMPs can be improved through conjugation with polymers which also increases their stability (Cui *et al.*, 2021). Several AMPs accommodated in AMP-polymer conjugates often retain their potent antimicrobial potential. Polymers are also being explored as potential agents in controlled-release drug platforms to overcome the limitations of conventional controlled-release antimicrobial coatings on implants (Jacobsen *et al.*, 2013; Kumari *et al.* 2021; Temtem *et al.*, 2009).

2.12. The conjugation of AMPs with polymers

The trends of AMPs coatings for medical implants to avoid implant associated infections and biofilms continue growing (Min *et al.*, 2024). The challenges with AMPs such as stability, toxicity, and biocompatibility issues can be mitigated by conjugation with polymers (Zhang *et al.*, 2025). Polymers are optimal agents for medical implants coatings and offer several advantages such as antimicrobial properties (Palomero *et al.*, 2020; Armugam *et al.*, 2021) immune modulation (Kalirajan *et al.*, 2021), immune adjuvant (Allen *et al.* 2018), increase microhardness (Hay *et al.*, 2018; Kumar *et al.*, 2020a; Kumari *et al.*, 2021; Smith *et al.*, 2022), reconstruction, bone, cartilage, and muscle repair (Kang *et al.*, 2016) and increase dendritic cell levels (Kalirajan *et al.*, 2021). The AMP's biocompatibility, *in vivo* stability, and antimicrobial efficacy can be increased by conjugation with polymers (Cheng *et al.*, 2022; Dizaj *et al.*, 2022; Ali *et al.*, 2023), a biodegradable and biocompatible polymer (Zhang *et al.*, 2020). PLGA-AMP nanoparticles showed excellent wound healing potential and upregulated the expression of IL-6 and Vascular Endothelial Growth Factor-A (Kumar *et al.*, 2014). The AMP toxicity in mammalian cells is reduced by conjugation with neutral hydrophilic polymers (Cui *et al.*, 2021). Polymers have high mechanical and chemical robustness, which assure the

long-term and consistent release of AMPs, paving the way to develop state-of-the-art multifunctional implant coatings. Figure 4 shows the strategies for polymer-AMP conjugates.

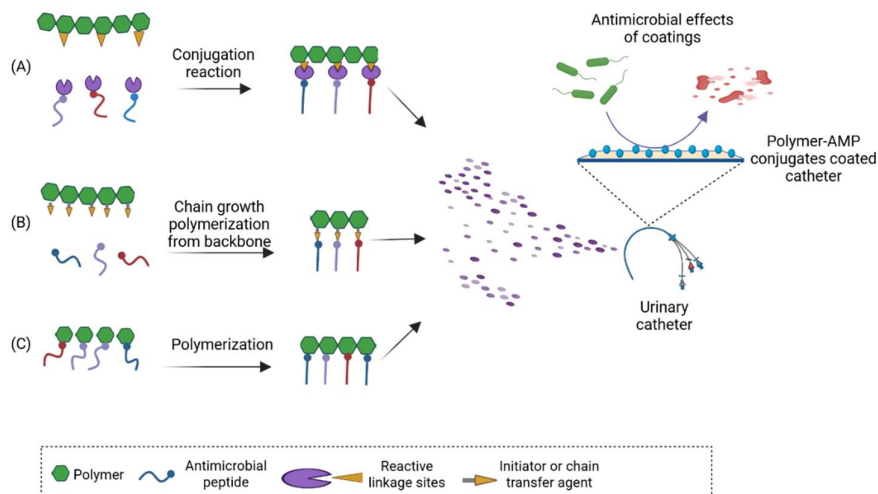


Figure 4. Illustration of strategies for polymer-AMP conjugation. (a) grafting-to; (b) grafting-from, and (c) grafting-through methods and coatings of conjugates on urinary catheters to produce antimicrobial effects. The figure was created in BioRender.com and modified from (Cui *et al.*, 2021).

The polymer-AMP conjugates can be prepared using several approaches. The connection of AMP to a polymer when the AMP reactive group reacts with a complementary reactive group of the polymer is called grafting-to conjugation. Heterobifunctional linkers can be used when the polymer and AMP lack complementary functional groups. In grafting-to conjugation, the N-terminal primary amines and the amine groups on lysine side chains of AMP act as reactive sites for polymers (Hartlieb *et al.*, 2019; Song *et al.*, 2012; Lin *et al.*, 2019). In grafting-from conjugation, conjugates are prepared by growing AMPs from polymers or polymers from AMPs, mostly through ring-opening polymerization (ROP) of amino acid N-carboxyanhydrides (NCA). Polypeptide-based AMPs can be synthesized through NCA ROP, having precise control over composition and molecular weight (Kricheldorf, 2006; Hadjichristidis *et al.*, 2009; Deming, 2016). This strategy is useful for AMPs whose antimicrobial effects are not dependent on amino acid sequences (Lam *et al.*, 2016; Chen *et al.*, 2019). The AMPs functionalised with an initiator allow the growth of polymers from them using several polymerization methods (Perrier, 2017; Corrigan *et al.*, 2020; Messina *et al.*,

2020). Trithiocarbonate-based chain-transfer agents are aminolysis resistant, which makes them ideal candidates for attachment to the N-terminal amine of AMPs (Messina *et al.*, 2020). The AMP-polymer conjugates prepared by grafting-through approaches use a polymerizable group to modify the AMP and then its polymerization into a structure with pendant AMPs (Sun *et al.*, 2019a; Messina *et al.*, 2020). The linear AMP and sugar-containing polymer chains are linked together to prepare star-shaped conjugates through polymerizing a difunctional monomer from the CTA at the chain ends (Wong *et al.*, 2016). The bond between polymers and AMPs through their functional groups form by amide bond formation and click chemistry allows covalent conjugation (Song *et al.*, 2012). By employing engineered spacer sequences, conjugating the peptide creates a new opportunity to incorporate several different AMP sequences with controlled spatial distribution and conformational stability in the composite material. Additionally, by expanding the above approach, a multifunctional/multi-biomolecular matrix can be fabricated with potential antimicrobial with anti-inflammatory, and/or antihistaminic properties in these polymeric materials (Xie *et al.*, 2020).

2.13. The breadth of polymers used in antimicrobial catheter coatings

Polymer based coatings can present antifouling and antimicrobial properties. Antifouling polymer coatings which are commonly fabricated with hydrophilic polymers, which resist bacterial adhesion. Many ether bonds, hydroxyl, and ionic groups are present in hydrophilic polymers, which enables them to be used as antifouling coatings (Chu *et al.*, 2022). Lin *et al.* loaded rifampicin antibiotic on urinary catheters pre-coated with polylactic acid (PLA) (Lin *et al.*, 2025). PLA is used to achieve porous coatings with multi-layered arrays of micro-pores for controlled release of rifampicin. The coating showed long term antimicrobial effects against *P. aeruginosa* through controlled releasing of rifampicin. A hydrogel coating was developed by using the poly (vinylpyrrolidone)-iodine, copper ions, and nitrofurazone-loaded poly(sulfobetaine methacrylate)-tannic acid (polySBMA-TA) to fabricated controlled release antimicrobial coatings for catheter. The coating showed excellent antimicrobial effects as antimicrobial release platform. Additionally, the coating showed good biocompatibility (Miao *et al.* 2023). Wang *et al.* coated lumen of slender catheters with cationic ethyl lauroyl arginate (ELA) functionalized poly(γ -glutamic acid) (γ -PGA-ELA). Coating showed potent antimicrobial effects and good hemocompatibility (Wang *et al.*, 2020). Gao *et al.* deposited the mixed-charge polypeptide (mPep) and other bioactive agents on silicone catheter (Gao *et al.*, 2020). Catheters coated with a thin and stable layer comprised of poly(N,N-

dimethylacrylamide) (uhPDMA) and dopamine showed antimicrobial and antibiofilm activities (Yu *et al.*, 2021b).

A double-network hydrogel matrix composed of polyvinyl alcohol (PVA) and chitosan (CS), incorporated with silver quantum dots (Ag QDs), was developed as an antibacterial coating for latex urinary catheters (Li *et al.*, 2024). Double network hydrogels improved the mechanical properties, such as strength and toughness. The modified catheters showed controlled release of Ag QDs which prevented the microbial attachment, and also demonstrated long-term antibacterial activity, excellent biocompatibility and hemocompatibility. Similarly, Yang *et al.* created a thin lubricious layer of CS/PVA on the surface of segmented polyurethane (SPU) using a multiple surface modification method (Yang *et al.*, 2007). This method allows high hydrophilicity, reduces protein adsorption, promotes surface homogeneity, and gradual elution of chitosan. The inflammation could be reduced due to the low friction on the catheter surface (Yang *et al.*, 2007). The modified catheters showed antimicrobial effects. In another study, PVA membrane was deposited on silicone catheter surface using the electrospinning method, which showed anti-adhesive effects and excellent biocompatibility and hemocompatibility (Sun *et al.*, 2026). Similarly, PDA and low-alcoholysis PVA were co-deposited on the surface of microtube catheters (Chen *et al.*, 2026). The PDA and low-alcoholysis PVA deposited microtube showed excellent lubricity, *in vitro* biocompatibility and antibacterial effects. Ran *et al.* used hydrogen comprised of PVA with β -cyclodextrin (β -CD) grafts for coating of silicon rubber catheters (Ran *et al.*, 2024). The modified catheters exhibited robust stabilities, twisting, knotting, and compression. Additionally, modified catheters showed persistent antibacterial efficacy *in vivo* and lower levels of inflammation. Additionally, PVA/polyvinylpyrrolidone based thermoplastic polyurethane exhibited excellent hydrophilicity and stability. Such coating may decrease coating-related adverse events (Qian *et al.*, 2025). PVA-based membranes incorporated with lipopeptides, and zinc oxide nanoparticles developed by solvent casting method showed antimicrobial effects. Additionally, PVA-lipopeptides based membranes showed hemocompatibility, enhanced optical, thermal, mechanical, and water barrier properties.

A hydrogel-based layer of PVA loaded with antimicrobial agents such as acetohydroxamic acid or ciprofloxacin hydrochloride was coated on catheter surface and then covered with an upper pH-responsive layer to release antimicrobial agents in response to elevation of urinary pH (Slate *et al.*, 2023). Anti-blockage activity was tested against *P. mirabilis* associated crystalline biofilms that cause the blockage of catheters. It was observed that compared to

empty coatings, blockage time for catheters coated antimicrobial agents was high. Ciprofloxacin coated catheters significantly increased blockage time and tripled catheter life span (Slate *et al.*, 2023).

SA being anionic and hydrophilic in nature, form a crosslinked polymeric structure with elasticity and strength which make it ideal for tissue regeneration (Kurowiak *et al.*, 2020). The α -L-guluronic acid (G-blocks) having a snake structure and β -D-mannuronic acids (M-blocks) having stretched structure are main residues of SA connected by glycosidic bonds (Kurowiak *et al.*, 2020). SA is easily dissolved in water at room temperature and due to its high biocompatibility, its also used in tissue engineering scaffolds combining with other agents (Zhou *et al.*, 2014; Wang *et al.*, 2018). Other properties of SA are high oxygen permeability, haemostasis ability, good biodegradability, and prevention of new granulation tissue from sticking to the dressing and preventing “secondary damage” during removal. Other biomedical applications of SA include patches, scar healing, and treatment of bone injuries (Klekiel *et al.*, 2020).

PDA modified latex urinary catheters pretreated with dopamine was coated with nanoparticles and polymers including SA using the dip coating method, showed excellent good biocompatibility, anti-adhesive and antibacterial effects (Yang *et al.*, 2024). SA in combination with antimicrobial agents can be used as dual-functional composite coating system for thermoplastic polyurethane (TPU) catheters (Sun *et al.*, 2025). Prior to coating, glycidoxypyrrolidone (KH-560) and polyvinylpyrrolidone (PVP) were used to form a hydrophilic coating. It was followed by formation of a long-lasting antibacterial functional layer using the anionic properties of SA combined with cationic antimicrobial agents. The coating showed antimicrobial effects, cytocompatibility and hemocompatibility (Sun *et al.*, 2025). A compact, and crack-free polyelectrolyte film created with SA and poly (diallyldimethylammonium chloride) (PDADMAC) showed antiviral and antibacterial properties.

The antimicrobial effects of the antifouling polymer coatings can be enhanced by incorporation of AMPs (Chu *et al.*, 2022). Bactericidal polymer coatings use cationic polymers to kill bacteria by disrupting their membrane and to mimic the amphiphilicity and charge composition of AMPs. The local positive charge density is increased by adding the AMPs that efficiently kill bacteria by constructing star-shaped architectures due to the self-assembly (Chu *et al.*, 2022). The films of SA are supplemented with probiotic microorganisms, showed

antimicrobial effects against pathogenic bacteria. The physicochemical and barrier properties of films and their shelf life were enhanced with the use of probiotics (Kalkan *et al.*, 2025). Similarly, SA/carboxymethylcellulose/glycerol-based films enriched with *Lactococcus lactis* presented as edible films showed bacteriostatic effects against *S. aureus* (Ye *et al.*, 2018). Similarly, the composite films created with SA, glycerol, and gallnut extract showed antibacterial and antifungal effects (Aloui *et al.*, 2021). Nesic *et al.* prepared antimicrobial films using the pectin, SA and polyglycerol by simple casting method and cross-linked with zinc chloride, which showed antibacterial and antifungal effects (Nešić *et al.*, 2017). SA was cross-linked with calcium chloride to produce suitable polymer ink for PAM 3D printing to prepare the catheter. Initial burst release of antibiotic was observed from drug-loaded/coated catheter followed by sustained release which disrupted biofilms by suppressing all the QS mediated virulence factors. Additionally, the coated catheter showed biocompatibility towards L929 mouse fibroblasts. CS, carboxymethyl chitosan, and hyaluronic acid in combination with a lysine-based surfactant coated silicone sheets and silicone tubes prevented microbial growth and biofilms (Bračić *et al.*, 2019).

2.14. AMPs-polymers coatings on urinary catheters

Several AMPs accommodated in AMP-polymer conjugates often retain their potent antimicrobial potential. The peptides immobilize on surface through C-terminus have high antimicrobial potential, therefore, to achieve high antimicrobial activity, it is important to immobilize the peptides on the surface through C-terminus that allow N-terminal hydrophobic domain freely to interact with microorganisms (Yu *et al.*, 2021a). The Cysteine labelled AMP E6 (RRWRIVVIRVRRC) was coated on polyurethane surfaces through using an anti-adhesive hydrophilic polymer coating (Yu *et al.*, 2017). The aqueous surface-initiated ATRP (SI-ATRP) was used to graft the catheter surfaces with a copolymer brush followed by modification with linkers. Next, the modified catheters were immersed in cysteine labelled peptide E6 solution to immobilize the peptides on polymer brush polyurethane PU catheter. The modified PU catheter surface showed long term antibacterial effects *in vitro* against *P. aeruginosa*, *S. aureus* and *S. saprophyticus*. Additionally, it also showed biocompatible properties. Lim *et al.* developed a strategy using a 2-step PDA-based surface immobilization to inject an AMP onto a commercially available Foley catheter (Lim, 2015). PDA-based platform enhanced peptide grafting efficacy and antimicrobial potential retained after exposure to a different type of

solvent conditions, which made it an attractive choice for subsequent translational studies. However, being in contact with active catheters, the planktonic bacteria were continuing to proliferate in the surrounding environment. Therefore, controlled release antimicrobial coating was created by dual-layered assembly (poly- ϵ -caprolactone(P)-1-palmitoyl-2-oleoyl-sn-glycero3-phosphocholine(P)) (POPC) consisting of an AMP-impregnated basal polycaprolactone (PCL) matrix layered with a thin POPC film (Lim, 2015). The coating showed antimicrobial and antibiofilm potential against several bacteria.

Marie et al. fabricated controlled release antimicrobial coatings on silicone urinary catheters using the AMP-impregnated PEG-PCL anhydrous polymer (Marie *et al.*, 2021). The coated catheter showed antimicrobial activity for 4 days. Additionally, catheters presented superior antimicrobial activity and durability compared to commercial silver-based catheters. Low et al. designed an AMP-based antimicrobial coating for catheters (Low *et al.*, 2021). The modification did not affect the physical characteristics of catheter. The antimicrobial coating formulation was efficiently designed for achieving desirable AMP release kinetics. Syringe coating was used for catheters (Figure 5). Additionally, the modified catheters did not allow the biofilm formation up to one week.

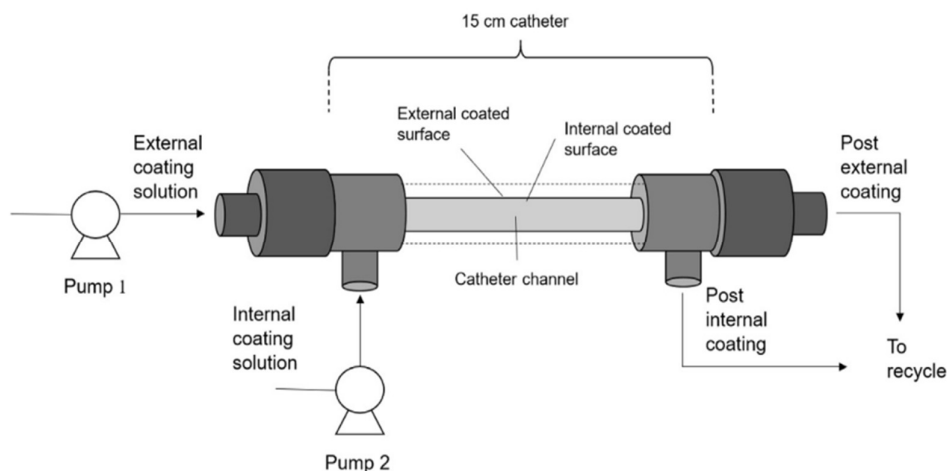


Figure 5. Catheter modifications by syringe coating. The coating solution was pumped through intra and extraluminal surfaces and allowed to dry and AMPs were impregnated through dip and syringe coating methods into a PCL coating layer. Reproduced with permission of (Low *et al.*, 2021). Copyrights (2021), Elsevier.

Anhydrous PCL and AMP HHC36-based coating on silicone urinary catheters showed controlled release of AMP in *in vivo* studies with high biocompatibility (Lim *et al.*, 2018). A polymer brush comprised of poly(3-[dimethyl-[2-(2-methylprop-2-enoyloxy)ethyl]azaniumyl]propane-1-sulfonate) and AMP HHC36 was created with hierarchical architecture on polyurethane catheters. Modified catheter showed broad spectrum antibacterial effects by inhibiting the growth of *E. coli* and *S. aureus*, high hemocompatibility and low cytotoxicity (Zhang *et al.*, 2019). Polymyxin B was nanohybridized with a zwitterionic antifouling polymer to fabricate antimicrobial catheter through sonochemical coating using the ultrasonic transducer Ti-horn (Ivanova *et al.*, 2021). The catheters showed antimicrobial and antibiofilm effects against *P. aeruginosa*, a one of the most common CAUTI pathogens. Additionally, catheters showed negligible toxicity against two mammalian cell lines, namely fibroblasts and keratinocytes. Ring open polymerization of ϵ -trifluoroacetyl-L-lysine (Tfa-Lys)-TFA-Lys N-carboxyanhydride allowed the immobilization of antimicrobial PLL brush on the surface of catheter, which showed potent antibacterial effects against several bacteria *in vitro* (Liu *et al.*, 2023). Additionally, the catheters also showed antimicrobial effects and was biocompatible *in vivo*.

Yu *et al.* dissolved copolymer N,N-Dimethylacrylamide and N-(3-Aminopropyl) Methacrylamide (PDMA-co-APMA) in anhydrous acetonitrile following by addition of iodoacetic acid N-hydroxysuccinimide ester into the solution (Yu *et al.*, 2021a). The polyurethane catheters were dipped into a 24-well plate containing dopamine and uhPDMA, for one day. PDA/PDMA-co-APMA-I/polydopamine coated catheters were immersed overnight in solution of several peptides including E6, Tet20C, DJK5C, 3002C with cysteine at the C-terminus, IDR-1018, and Tet20LC (Yu *et al.*, 2021a). The catheters showed antimicrobial and antibiofilm effects against several bacteria *in vivo*, as well as biocompatibility. The addition of non-fouling polymer such as PDMA enhances the antibiofilm properties of AMP coatings. PDMA prevents the accumulation of bacterial debris thus provide regenerating surface for killing of bacteria through thus antimicrobial potential of AMPs retained (Yu *et al.*, 2021a). Additionally, presenting AMP within the antifouling layer without direct attachment to the polymer chains is highly efficient, substrate-independent and do not need the any further modification which make it superior to other AMP conjugation methods (Gallardo *et al.*, 2014; Salwiczek *et al.*, 2014). It was suggested that interaction with coating components causes alteration of secondary structure of the conjugated peptides (Gao *et al.*, 2012; Yu *et al.*, 2015a) which may reduce antimicrobial effects of AMP-conjugated surfaces.

The testing and identification of AMPs for relevant conditions is very important. Mishra *et al.* immobilize AMP onto silicone catheters by chemical coupling method employing allyl glycidyl ether (AGE) brush (Mishra *et al.*, 2014). Before AMP immobilization, the catheters were incubated with allyl glycidyl ether. Next, catheters were treated with peptide CysLasio-III in phosphate-buffered saline. The AMP functionalised catheter showed antimicrobial effects against (Mishra *et al.*, 2014).

Liu *et al.* developed a durable antibacterial polyurethane-based coating incorporating crosslinked PLL on catheter using the drop casting method (Liu *et al.*, 2026). The modified catheter showed excellent antimicrobial activity and maintain nearly 100% bactericidal activity for more than 3 weeks and have good low-friction properties which allow easy insertion of the catheter. The polymers such as SA, methylcellulose, and PEG impregnated with antimicrobial agent secnidazole used for 3D printing of catheter to suppress QS mediated virulence factors (Archana *et al.*, 2023). The AMPs and polymers used in urinary catheters to present antimicrobial properties as shown in Table 1.

Table 1. Efficacy of antimicrobial peptides-polymers coatings on urinary catheters.

AMPs	Polymers	Outcomes	Reference
Cysteine-modified AMP (synthetic AMP)	Poly(N,N-dimethylacrylamide-co-N-(3) aminopropyl)methacrylamide)	Coated catheter showed biocompatibility and antimicrobial effects.	(Yu <i>et al.</i> , 2017).
CWR11 (synthetic AMP)	Allyl glycidyl ether	Contact killing surface exhibited biocompatibility and antimicrobial potential against	(Lim, 2015).
	Poly(ϵ -caprolactone) & 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine	Controlled-release antimicrobial coating inhibited the growth of bacteria.	
HHC36 (synthetic AMP)	Poly(ethylene glycol) & polycaprolactone	Coating showed controlled-release antimicrobial effects.	(Marie <i>et al.</i> , 2021)

Synthetic AMPs	Polycaprolactone, Ethyl Cellulose, and 1-Palmitoyl-2-oleoylphosphatidylcholine	Coating showed controlled-release antimicrobial effects.	(Low <i>et al.</i> , 2021).
HHC36 (synthetic AMP)	Polycaprolactone & 1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine	Coating showed controlled-release antimicrobial effects	(Lim <i>et al.</i> , 2018).
HHC36 (synthetic AMP)	Poly(3-[dimethyl-[2-(2-methylprop-2-enoyloxy)ethyl]azaniumyl]propane-1-sulfonate)	The coated catheter showed broad spectrum antimicrobial effects, good hemocompatibility and low cytotoxicity.	(Zhang <i>et al.</i> , 2019).
Polymyxin B <i>Paenibacillus polymyxa</i> -derived AMP	Poly(sulfobetaine) methacrylate	Coated catheters showed antimicrobial effects and negligible toxicity.	(Ivanova <i>et al.</i> , 2021).
E6, Tet20C, DJK5C, 3002C, IDR-1018, and Tet20LC (synthetic AMPs)	N,N-Dimethylacrylamide and N-(3-Aminopropyl) Methacrylamide	The catheters showed antimicrobial and antibiofilm effects against several bacteria <i>in vitro</i> and in a mouse infection model, as well as biocompatibility.	(Yu <i>et al.</i> , 2021a).
CysLasio-III (synthetic cysteine-modified AMP)	Poly(allyl glycidyl ether)	The functionalised catheter inhibited the growth of <i>E. coli</i> and <i>E. faecalis</i> .	(Mishra <i>et al.</i> , 2014).
Cationic epsilon-poly-l-lysine (Derived	Polyurethane	The coated catheters effectively inhibited the growth of bacteria	(Liu <i>et al.</i> , 2026).

from
Streptomyces
albulus)

under static and dynamic flow
conditions.

Polylysine ϵ -trifluoroacetyl-L-lysine
(Synthetic carboxyanhydride
polypeptide)

N- The modified catheter showed (Liu *et al.*, 2023)
potent antibacterial effects *in*
vitro and *in vivo*. Additionally,
the catheters also showed
biocompatibility *in vivo*.

Poly-l-lysine PVA
(Synthetic
polypeptide)

The coated catheter showed (Maryam *et al.*,
biocompatibility and 2026)
antimicrobial effects against
bacteria and fungi.

2.15. Justification

To curb CAUTI, a wide variety of antimicrobial agents have been coated on the surface of catheters, among which AMPs being potent antimicrobial agents are one of the most appealing candidates. Their multi-antimicrobial mechanisms on the plasma membrane and rapid antimicrobial action (Zhang *et al.*, 2022) make them less prone to antimicrobial resistance (Xuan *et al.*, 2023). AMPs are generally less toxic for the mammalian cells due to bearing positive charge because of selective affinity for negatively charged membranes (Singha *et al.*, 2017). Besides antimicrobial potential, AMPs are immunomodulatory and have angiogenesis properties, wound healing, and antitumor activity (Ebenhan *et al.*, 2014; Chen *et al.*, 2020). A wide variety of AMPs showed antimicrobial effects as coatings on catheter (Mishra *et al.*, 2014; Soyhan *et al.*, 2024; Marie *et al.*, 2021; Low *et al.*, 2021; Lim *et al.*, 2018; Wang *et al.*, 2020). AMPs derived from the toxin of the Brazilian spider *Lycosa erythrognatha* including LyeTx I and its analogues including LyeTx I mnΔK emerge potent antimicrobial agents and can be potentially coated on catheter to provide antimicrobial effects. These AMPs have already shown antimicrobial potential against several drug-resistant pathogens, including *S. aureus*, *E. coli*, *Candida krusei*, *Cryptococcus neoformans*, *A. baumannii*, quinolone-resistant uropathogenic *E. coli* (Santos *et al.*, 2010, Nunes *et al.*, 2019; Brito *et al.*, 2021; Lima *et al.*, 2021; Souza *et al.*, 2025; Ul Haq *et al.*, 2026) and are less toxic to mammalian cells than microbial cells (Reis *et al.*, 2018; Fuscaldi *et al.*, 2021; Lima *et al.*, 2021).

Considering the broad-spectrum antimicrobial potential of LyeTx I mnΔK (Fuscaldi *et al.*, 2021; Vieira *et al.*, 2024a; Lima *et al.*, 2021; Ul Haq *et al.*, 2026), it was decided to coat it on the catheter to functionalize it with antimicrobial effects.

This is the first time investigating LyeTx I mnΔK for the coating of medical implants coating such as catheters. LyeTx I mnΔK was incorporated in a biopolymer sodium alginate (SA) and deposited on medical grade silicon through ultrasonic deposition method. SA is easily dissolved in water, has high biocompatibility (Wang *et al.*, 2018), oxygen permeability, haemostasis ability, and good biodegradability (Klekiel *et al.*, 2020). SA form a stable system with antimicrobial agents (Thakur *et al.*, 2018). In the previous study, the SA-based urethral stent showed mechanical strength, biocompatibility, maintained satisfactory urine flow and met strength requirements (Klekiel *et al.*, 2020). The ultrasonic deposition system forms ultrathin and uniform coatings and offers good control over thickness (Maryam *et al.*, 2026).

Additionally, coating properties can be precisely tuned through programmable parameters including number of coating layers and flexible spray speed.

2.16. Hypothesis and objectives

The present study hypothesized that coating urinary catheters with polymers and AMPs produces antimicrobial and anti-biofilm effects with minimal toxicity towards mammalian cells, thereby preventing CAUTIs and extending catheter lifespan.

Consequently, the following objectives have been indicated:

1. To identify UPECs pathogens based on the biochemical-physiological and morphological features.
2. To assess the antimicrobial effects of spider *Lycosa erythrognatha* derived AMPs.
3. To assess anti-adhesive and antibiofilm effects of LyeTx I mnΔK peptide.
4. To evaluate the mechanism of action of LyeTx I mnΔK peptide by performing molecular docking of LyeTx I mnΔK with bacterial adhesin *in silico*.
5. To assess pharmacokinetic properties of LyeTx I mnΔK through ADMET analysis *in silico*.
6. To assess LyeTx I mnΔK toxicity *in vitro* and *in vivo*.
7. To assess whether the incorporation of LyeTx I mnΔK into the polymers enhances its stability.
8. To optimize the ultrasonic spray deposition method to produce a continuous polyvinyl (PVA) coating loaded with the model antimicrobial peptide poly-L-lysine (PLL) on the urinary catheter.
9. To deposit sodium alginate/LyeTx I mnΔK on medical-grade silicon sheets and verify its applicability as antibacterial and antibiofilm coating.

Chapter II: Experimental part

3. Materials and methods

3.1. AMPs

The *Lycosa erythrognatha* spider venom derived AMPs such as LyeTx I and its analogues (Table 2), were supplied by (SynTM; Shanghai, China).

Table 2: Sequences and chemical properties of LyeTx I and its analogues. Reproduced from (Ul Haq et al. 2026).

Name	ϵ [M ⁻¹ cm ⁻¹]*	Mon.M [Da]	Amino acid residues sequence	Number of residues
LyeTx I	5690	2832.48	H-IWLTALKFLGKNLGKHLAKQQLAKL-NH ₂	25
LyeTx Ib	5690	2695.34	H-IWLTALKFLGKNLGKLAKQQLAKL-NH ₂	24
LyeTx I mn	5690	1701.11	H-IWLTALKFLGKNLGK-NH ₂	15
LyeTx I mnΔK	5690	1829.28	H-IWLTALKFLGKNLGK-NH ₂	16
LyeTx I mnΔKAc	5690	1871.32	Ac-IWLTALKFLGKNLGK-NH ₂	16
LyeTx I mnΔKL	5690	1942.44	H-IWLTALKFLGKNLGKL-NH ₂	17
LyeTx I mnΔKwN	5690	1716.16	H-IWLTALKFLGKL GK-NH ₂	15

* ϵ : Molar extinction coefficient at 280 nm; Mon.M: Monoisotopic mass. Molecular mass and molar extinction coefficient were determined empirically from the primary peptide sequence using the free and open-source software PepCalc (Innovagen ABTM; Lund, Sweden; <https://pepcalc.com>).

3.2. Antimicrobial activity of AMPs

Antimicrobial assays of all AMPs were performed against 10 clinical quinolone-resistant UPECs isolates through Minimum Inhibitory Concentration (MIC) and minimum bactericidal concentrations (MBC) according to CLSI M07 (2018). The mechanism of quinolones resistance of UPECs isolates were identified by Paiva et al. (Paiva *et al.*, 2012) (Table 3). Prior to antimicrobial assays, quinolone resistance and resistance to other antimicrobials were determined for UPECs by disk diffusion method following the recommendations of Brazilian

Committee on Antimicrobial Susceptibility Testing (BrCAST, 2024). *E. coli* ATCC 25922 was included as a reference strain for experimental control purposes.

Table 3: Antimicrobial resistance profile of UPECs used in this study.

Code	MIC to quinolones (µg/mL)*					Inhibition holes in mm (Interpretation)			Resistance mechanisms to quinolones*
	NAL	OFL	NOR	CIP	LVF	SUT	FOS	NIT	
EC11	>1024	64	256	64	16	31 (S)	25 (S)	0 (R)	GyrA mutation: S38L; D87N
EC31	>1024	64	256	64	32	32 (S)	27 (S)	0 (R)	GyrA mutation: S38L; D87N
EC49	>1024	256	512	512	128	28 (S)	24 (S)	0 (R)	GyrA mutation: S38L; D87N
EC58	>1024	64	256	32	32	30 (S)	27 (S)	0 (R)	GyrA mutation: S38L; D87N
EC71	>1024	8	16	8	8	32 (S)	21 (R)	0 (R)	GyrA mutation: S38L; D87N; K162N
EC74	>1024	64	64	16	32	0 (R)	12 (R)	0 (R)	GyrA mutation: S38L; D87N
EC81	>1024	32	512	16	32	16 (S)	0 (R)	0 (R)	GyrA mutation: S38L; D87N
EC90	>1024	16	32	8	16	0 (R)	0 (R)	26 (S)	●GyrA mutation: S38L; D87N ●ParC mutation: S80L ●PMQR: <i>qnrS1</i>
EC97	>1024	64	512	16	32	0 (R)	27 (S)	17 (S)	GyrA mutation: S38L; D87N; E153A; K154T; P156L
EC99	>1024	64	512	16	32	34 (S)	29 (S)	0 (R)	GyrA mutation: S38L; D87N

*Previously characterized by PAIVA *et al.*, 2020. PMQR: Plasmid-mediated quinolone resistance; NAL: Nalidixic acid; OFL: Ofloxacin; NOR: Norfloxacin; CIP: Ciprofloxacin; LVF: Levofloxacin; SUT: Sulfamethoxazole-Trimethoprim; FOS: Fosfomycin; NIT: Nitrofurantoin. S: Susceptible; R: Resistant. MIC: Minimum inhibitory concentrations.

3.3. MIC and MBC of AMPs

To perform MIC, bacterial inoculum was prepared using the 24-hour culture from nutrient agar (Vieira *et al.*, 2024b). Few bacterial colonies were transferred to sterile saline (NaCl 0.85%) from bacterial culture. The suspension turbidity was adjusted to 0.5 McFarland

standard (10^8 colonies-forming units (CFU)/mL) using a spectrophotometer (optical density, OD) at 630 nm between 0.08 to 0.13; Shimadzu UVmini-1240TM, Kyoto, KY, Japan). To prepare an inoculum of 10^6 CFU/mL, 50 μ L of saline suspension was transferred to 10 mL of Mueller–Hinton broth (MHB). A 100 μ L of inoculum added to 96 well plates containing the AMPs previously two-fold diluted in a concentration range of 0.5 to 128 μ M as previously described (Lima *et al.*, 2021). Tween-80TM (0.002%) was added to wells to prevent the adherence of AMPs to plates (Ling *et al.*, 2015). For the positive control, colistin and meropenem were added in wells, and the untreated wells were included as negative controls. The plates were incubated for one day at 37 °C. MIC was defined as the lowest concentration of AMP that completely inhibited visible growth of bacteria. There were three replicates for this experiment. To determine MBC, a 100 μ L of the culture medium was aspirated from optically free growth wells of MIC plates and transferred to Muller-Hinton agar plates (Lima *et al.*, 2025). The medium was spread with a Drigalski loop on the surface of the agar plates which were incubated for 24 h at 37 °C.

3.4. LyeTx I Δ mnAK effects on biomass and viability of UPECs biofilms

Anti-biofilm activity of the AMP was assessed in a microplate-based model against ten isolates of UPECs. MHB supplemented with 1% of glucose suspension was prepared for each UPEC isolate. The suspension was added to microplate previously containing LyeTx I Δ mnAK at sub-inhibitory concentrations (1/2, 1/4, and 1/8 of MIC) and incubated at 37 °C for one day. After incubation, the medium was removed and NaCl 0.85% was used to wash the plates. After washing, methanol was used to fix the biofilms for 5 min at 37 °C and then stained with 0.1% (w/v) crystal violet for 30 minutes. Again, plates were washed with saline (three times; NaCl 0.85%), air-dried, and solubilized with 30% glacial acetic acid. In the last stage, absorbance at 595 nm was measured using a spectrophotometer (Bio-Tek InstrumentsTM, Winooski, VT, USA). In relation to the negative control (considered 100% biomass) the biomass percentage was calculated (Herrera *et al.*, 2022). On the other hand, the plates used in MTT assay were washed three times with sterile saline after removing the medium. MTT solution (0.25 mg/L; 125 μ L) was introduced in the well of microplates and incubated at 37 °C for 4 h while covered with aluminium foil to protect them from light. The supernatant was removed from plates after 4 h, the formazan crystals were solubilized with 125 μ L of DMSO, and plates were incubated after 30 min of incubation at room temperature. After incubation, the OD of wells was

determined photometrically at 595nm (Bio-Tek Instruments™; Winooski, VT, US). The cell viability percentage was calculated relative to the untreated control (considered 100% viability) and was graphically presented (Trafny *et al.*, 2013). Both assays were conducted in sextuplicate with at least two independent experiments.

3.5. Effect of LyeTx I mnAK on UPECs adhesion on urinary catheters

Anti-adhesive effect of AMP against UPECs was evaluated on catheter segments (Sousa *et al.*, 2019). 1 cm sterilized PVC-urethral catheter segments were introduced 10 ml MHB tubes supplemented with 1% glucose. 1 µg/mL AMP was added to the tube and then 100 µL of a suspension containing 10⁸ CFU/mL of UPECs. The tubes were incubated in shaking incubator at 37 °C for 24 h. The experiment was conducted in triplicate. After incubation, the medium was removed and catheter segments were transferred to sterile saline (NaCl 0.85%) (5mL) containing vials and incubated in a shaking incubator for half an hour. Next, the catheters were removed in an aseptic way and placed in 5 mL of sterile saline tube and sonicated at 40 kHz for 5 min (Unique™; Indaiatuba, SP, Brazil). Samples were homogenized and serially diluted in sterile saline (10⁻¹ to 10⁻⁵). A 100 µL of sample from each dilution was spread on MacConkey agar surface plates and incubated at 37 °C for 24 h. After incubation, the morphotypic colonies of UPEC *E. coli* were counted to determine the bacterial load (CFU/cm catheter). The samples which were proceeded without peptide were considered as negative control. Additionally for Scanning Electron Microscopy (SEM) analysis, AMP treated catheters segments incubated in UPEC-inoculated MHB. Next day, before SEM analysis, the non-adherent cells were removed by washing (three times) the catheters with sterile saline (NaCl 0.85%) and catheters fixed with 2.5% glutaraldehyde for 3 h at 4 °C. Different concentrations of ethanol (30%, 50%, 70%, 90%, and 100%) were used to dehydrate them by 10 min soaking. The catheters were placed on aluminium stubs and sputter-coated 10 nm thin layer of gold (Denton Vacuum/US) after drying. To see adherent bacteria on catheters, they were examined under SEM (Zeiss™; Oberkochen, WG, Germany) under high vacuum at an accelerating voltage of 10 kV. The images of adherent bacteria captured at different magnifications. Sample which was proceeded without peptide were considered as negative control.

3.6. Evaluation of *in vitro* and *in vivo* toxicity of LyeTx I mnΔK

To assess the cytotoxic effect of LyeTx I mnΔK *in vitro*, human embryonic kidney cell (HEK-293) was used using the MTT assay (Zheng *et al.*, 2016) as suggested by ISO 10993:2009 (Standardization, 2009). Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) was used for culturing of cells and incubated at 37 °C in a 5% CO₂ incubator. After cells reaching 70-80% confluency, were detached by using the 0.25% trypsin-EDTA and counted through a hemocytometer after neutralizing. Next, 100 µL of cell suspension (5×10^4 cells/mL) was seeded into each well of a 96-well plate for 24 h. Cells were washed and incubated in a fresh medium, followed by addition of AMP the peptide. Next, PBS was used for washing of cells after incubation. MTT solution (5 mg/mL) was added to wells and incubated again for 4 h followed by adding 100 µL of DMSO and mixed for 30 minutes. The microplate reader (Bio-Tek Instruments™; Winooski, VT, USA) at 600 nm was used to read the plates. The cell viability in relation to peptide concentration was analysed to determine the 50% cytotoxic concentration (CC₅₀), identifying the concentration that caused a 50% reduction in viability.

Acute toxicity of AMP was assessed using the Six-week-old female BALB/c mice, according to the Organization for Economic Co-operation and Development (OECD) protocol 423 (OECD, 2002) with modifications. All the international protocols for laboratory animal management were strictly followed. Animals were kept in Bioterio of *Faculdade de Saúde Santa Casa de Belo Horizonte*, Brazil under controlled temperature range of 25±2 °C, 40% humidity, water and food *ad libitum* and a 12/12 h light/dark cycle. Three different concentrations of LyeTx I mnΔK were administered in animals (3 animals for each animal through the intraperitoneal route. Animals were monitored for one hour after administration to notice any behavioural alterations. The food, water, and animal's weight were examined every day for 14 days. After 14 days, anesthesia was given to given to animals to euthanize them. After euthanization, blood was collected from all the animals to measure creatinine levels using commercial kits (Quibasa-Bioclin™; Belo Horizonte, MG, Brazil). Additionally, the kidneys were removed from all the animals. The left kidney was weighed before and after drying in an oven at 37 °C for 24 h to determine renal edema. On the other hand, histological slides stained with hematoxylin and eosin were prepared using the right kidney. The slides were analysed under a light microscope (Zeiss™; Oberkochen, WG, Germany).

3.7. LyeTx I Δ mn stability

The stability AMP was assessed by incubating in filter sterilized mammalian urine at 37 °C for 1, 3, 6, 9, 12, 24, and 48 hours. Aliquotes were removed during specific incubation times to perform MIC against a clinical isolate of UPEC (Isolate EC 90). The AMP incubated in water (sterilized) was included as a control.

3.8. Biopolymers selection for the incorporation of LyeTx I Δ mn

Different biopolymers, including PVA, chitosan, pectin, and sodium alginate, were screened with the model antimicrobial peptide poly-L-lysine (PLL) to determine the most suitable polymer for LyeTx I Δ mn coatings. Briefly, a 20 ml 2.8% pectin solution was prepared by adding the pectin in deionized water at 70°C under continuous magnetic stirring for 2 h. Subsequently, temperature of solution was reduced after achieving homogeneity to add the 0.15 ml of 0.1% (w/v) PLL (MW: 150,000–300,000 g/mol). The pectin/PLL solution was stirred to dissolve the PLL, and solution was cast on the Petri dish to obtain dry films. The following day, the pectin/PLL films peeled off and cross-linked with 0.8-0.33% CaCl_2 . A 1.3% SA solution was prepared by slowly sprinkling the SA into deionized water heated to 60–65°C while stirring continuously with a magnetic stirrer for 1 hour. After SA fully dissolved it was then allowed to cool to room temperature and 0.15 ml PLL was mixed with 20 ml SA solution by stirring. The SA/PLL solution was cast on the Petri dish and next day peeled off and cross-linked with 5% CaCl_2 . The 20 ml 2.8% chitosan solution was prepared in 2% (v/v) acetic acid under continuous stirring for 3 hours. Next, 0.15 ml PLL was added to the 20 ml chitosan solution, and chitosan/PLL solution was cast on the Petri dish. The following day, the films were peeled off and cross-linked with 5% glutaraldehyde. Similarly, a 20 ml 9.3% PVA solution was prepared by slowly adding the PVA into deionized water to approximately 80-90°C while stirring continuously with a magnetic stirrer overnight. The following day, the solution was allowed to cool to room temperature, 0.15 ml PLL was added and the resulting PVA/PLL solution was stirred to properly dissolve the PLL. The PVA/PLL solution was cast on Petri dish and next day the films were peeled off and cross-linked with 5-9% glutaraldehyde.

3.9. Stability of polymers/PLL films

All the polymers' films were evaluated for stability in the phosphate-buffered saline (PBS; pH 7.4) for 7 days and were observed daily to evaluate their stability. The polymer films that did not disintegrate during incubation were selected for further evaluation.

3.10. PVA/PLL deposition on urinary catheters through ultrasonic spray system

To achieve uniform, stable, and reproducible coatings, preliminary experiments were performed using PVA with PLL to optimize ultrasonication-based coating parameters including polymer concentration and number of coating layers. For preliminary experiments PLL as an antimicrobial agent was used in PVA coating on catheters. 0.5% (w/v) PVA (molecular weight of 85,000 g/mol) solution was prepared by dissolving PVA in deionized water by heating at 70 °C with continuous stirring. The temperature of the solution was reduced to add the PLL with volume ratio between 400:1 and 100:1 (Table 4) as reported in previous studies on PVA/PLL systems (Wahid *et al.*, 2019). Ultrasonic coating system (UCS) (ExactaCoat from Sono-Tek Corporation) was employed to deposit the PVA/PLL solution on commercially available two-way Foley silicone catheter, and silicon flat sheets. The catheters coated with PVA only were included as control. The nozzle speed of the UCS was 5 mm/sec, a flow rate of 0.25 cm³/min, nozzle rotational speed of 90 revolutions per minute, ultrasonic frequency of 60 kHz and an ultrasonic power of 3 W. Air at a pressure of 2 Pa was applied to direct the polymer solution onto the applied surface. Multiple layers were deposited to attain uniform and compact coatings.

Table 4. Chemical composition of PVA/PLL and SA/LyeTx I mnΔK.

PVA/PLL			
Samples	PVA (0.5%)	PLL (0.1%)	PVA: PLL Volume Ratio
PVA/PLL-1	20 mL	0.05 mL	400:1
PVA/PLL-2	20 mL	0.10 mL	200:1
PVA/PLL-3	20 mL	0.20 mL	100:1
PVA (control)	20 mL	0 mL	1:0
SA/LyeTx I mnΔK			
Samples	SA (0.17%)	SA/LyeTx I mnΔK	Mass ratio of LyeTx I mnΔK to SA

SA/PLL	20 mL	2 mg	1 mg: 10 ml
SA (control)	20 mL	0 mg	1:0

3.11. SA/LyeTx I mnΔK coating on flat silicon sheets

A 0.17% (w/v) SA solution was prepared by dissolving SA in 20 mL deionized water with continuous stirring. Once solution was homogenized 2 mg LyeTx I mnΔK was added. Prior to coating, the silicone sheets were plasma-treated using a low-pressure plasma cleaner (femto-ARS-200, Diener Electronic, Germany) at 120 W for 15 min with a 13.56 MHz RF generator. SA/LyeTx I mnΔK solution was deposited on plasma treated silicon using the UCS using the same parameters which were employed for PVA/PLL except increasing ultrasonic power to 7.5 W from 3 W. Multiple layers were deposited to attain uniform and compact coatings.

3.12. Surface characterization of coatings

The surface features of PVA, PVA/PLL, SA, and SA/LyeTx I mnΔK and controls were analysed using the scanning electron microscopy (SEM) (Phenom ProX, Thermo Fisher Scientific, Waltham, MA, USA) with voltage 15 keV at different magnifications. The energy-dispersive spectroscopy (EDS) integrated with SEM was used to evaluate the elemental composition of the coatings. The specific chemical groups on coatings were identified by Fourier-transform infrared spectroscopy (FTIR) using an IR PerkinElmer Spectrum Two equipped with a UATR diamond accessory (Waltham, MA, USA). Spectra were obtained in the range of 4000-400 cm^{-1} , with 16 scans collected for background and coated catheters and compared with pristine silicon catheter. Additionally, surface wettability was evaluated using the sessile drop method with a contact-angle goniometer (OCA 15EC, Data Physics, Filderstadt, Germany) by dropping a 4 μL of water on coatings and left to spread for 60 seconds. Water contact angles (WCAS) were recorded and analysed by SCA20_U software. Average WCA values were calculated from triplicate measurement of each sample to ensure accuracy.

3.13. *In vitro* biocompatibility of coatings

Normal human dermal fibroblasts (NHDFs) were used to check the biocompatibility of coating. The segments of PVA/PLL coated catheter, SA/LyeTx I mnΔK coated silicon and controls were sterilized with UVP CL-1000 ultraviolet crosslinker at UV 254 nm for 3 times, 1 min each side. The segments of coated catheters along with controls (pristine catheters) were added to the cell culture plates previously contained NHDFs seeded in DMEM) supplemented with 10% FBS and 1% penicillin–streptomycin, at a density of 2×10^4 cells per well in 500 μ L. The plates were incubated for 48 h at 37 °C in 5% CO₂. Each sample consisted in replicate (n = 3), standardized to dimensions of 1 $\times$ 1 cm. After incubation, catheter segments were aseptically removed and 50 μ L (10%) of Alamar blue reagent was added to plates and incubated for 4 h. A microplate reader was used to measure the fluorescence values (excitation: 530-560 nm, emission: 590 nm). The readings of the PVA/PLL coated catheter, SA/LyeTx I mnΔK coated silicon, and controls were compared to cells without any treated samples (viability of 100%) to determine cell viability. Cell viability was assessed using the following formula:

$$\text{Cell viability (\%)} = \frac{\text{Fluorescence of treated cells}}{\text{Fluorescence of control cells}} \times 100\%$$

3.14. Antimicrobial effects of coatings

Antimicrobial effects of PVA/PLL coatings were evaluated against *E. coli* ATCC25922, *S. epidermidis* ATCC12228, and *C. albicans* ATCC18804. Briefly, the inoculum of each microorganism was prepared by adding a single colony in 10 mL of Luria-Bertani (LB) broth and incubated at 37 °C overnight in a shaking incubator (250 rpm). Next day, cultures were diluted by a factor of 1:100 in fresh LB broth and grown to reach the OD₆₀₀ of 0.7 and then standardised to 0.5 McFarland standard (approximately 1.5×10^8 CFU/ml) using a densitometer. LIVE/DEAD™ BacLight™ Bacterial Viability Kit (ThermoFisher, USA) was used to assess the antimicrobial effects of all coated catheters. This kit identifies dead and live cells of microorganisms based on the compromised membrane. Dead or dying cells are stained red by propidium iodide while live cells are stained green by SYTO™ 9. Prior to LIVE/DEAD assay, all the catheters' samples were sterilized by UV irradiation for 30 min. After sterilization the samples were transferred to cell culture plates consisting of 1 mL LB broth and 10 μ L of microbial inoculum in each well and incubated at 37 °C overnight. Next day, samples were removed and washed twice with sterile 1 x phosphate-buffered saline (PBS, pH 7.0) (Merck, USA). A 3 μ L of Propidium Iodide and SYTO™ 9 was added into 1 mL of PBS and vortexes

for 5 s to prepare staining solution. A 50 μ L of the staining solution was dispensed on all the catheters' surfaces and incubated in the dark for half an hour. The mounting medium was spread on the stained catheter surfaces already affixed to a microscope slide and covered with a cover slip. Catheters were incubated for 15 minutes and analyzed using fluorescence confocal microscope (Model: Fluoview FV 3000, Lense: UPLSAPO 60XS, Mag: 60x) to visualize live cells (488 nm) and dead cells (561 nm). In case of SA/LyeTx I Δ mnK-coatings, antimicrobial effects were determined by OD measurements. The standard reference strains, *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) were used in antimicrobial experiments. The inoculum of each bacterium was prepared by adding a single colony in 10 mL of Luria-Bertani (LB) broth and incubated at 37 °C overnight in a shaking incubator (250 rpm). Next day, cultures were diluted by a factor of 1:100 in fresh LB broth and grown to reach the OD₆₀₀ of 0.7 and then standardised to 0.5 McFarland standard (approximately 1.5×10^8 CFU/ml) using a densitometer. Samples were immersed in 48 well plates consisting of 900 μ L LB broth and 100 μ L diluted culture and plates were incubated at 37 °C for 24 h. Next day samples were removed and culture medium was used to measure the OD and determine bacterial inhibition percentages. Samples coated with SA were used as control. The experiment was performed in triplicate.

3.15. SEM analysis of microbial adhesion on coatings

To perform SEM analysis, the coated catheters and silicon were added to 12 well plates consisting of 1 mL LB broth inoculated with 10 μ L microbial inoculums. Microbial inoculum was prepared as discussed in the earlier section. The coated catheters and silicon substrates with microorganisms were incubated at 37 °C for 24 h. Pristine catheters were included as a control. The next day, the catheters were washed with PBS for 10 minutes. Then catheters were dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 90%, 95%, and 99.8%), with each concentration applied for 10 minutes. Catheters were kept at room temperature for one day to air dry and then sputter-coated with a 2 nm layer of gold to achieve a conductivity. The images of catheters were taken at 600x, 1000x, and 5000x magnification using a scanning electron microscope (Phenom ProX, Thermo Fisher Scientific, Waltham, MA, USA) at 10 kV.

3.16. Antimicrobial effects of SA/LyeTx I mnΔK coating against CAUTI pathogens

Antimicrobial activity of SA/LyeTx I mnΔK coating was evaluated against 4 different CAUTI pathogens such as *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, and UPEC, according to de Sousa et al. (Sousa *et al.*, 2019). Uncoated silicon, SA, and SA/LyeTx I mnΔK coatings were introduced into sterile tubes containing 10 ml MHB supplemented with 1% glucose. Each tube contained approximately 10^8 CFU/mL of the respective pathogen. The tubes samples and pathogens were incubated in shaking incubator at 37 °C for 24 h. The experiment was conducted in triplicate. Next day, the coatings were transferred to sterile saline (NaCl 0.85%) (5mL) containing vials and placed in a shaking incubator for 30 minutes. Afterwards, the samples were removed in an aseptic way and placed in another tube containing 5 mL of sterile saline and sonicated at 40 kHz for 5 min (Unique™; Indaiatuba, SP, Brazil). Samples were homogenized and serially diluted in sterile saline (10^{-1} to 10^{-5}). A 100 µL of sample from each dilution was spread on Tryptic Soy Agar surface plates and incubated at 37 °C for 24 h. Next day, the morphotypic colonies of pathogens were counted to determine the bacterial load.

3.17. Stability of SA/LyeTx I mnΔK coating

To test antimicrobial stability of SA/LyeTx I mnΔK coating, its activity was tested after incubation in freshly collected sterile mammalian urine for 1, 3, 5, 7, 9, 12, and 14 days. After specific incubation times coatings were removed and evaluated for their antimicrobial activity against a clinical UPEC isolate (EC90) by measuring bacterial growth using OD. The SA coating was included as a control.

3.18. *In silico*/docking analysis of PLL and LyeTx I mnΔK

Docking analysis was performed to check the interactions of PLL (ID:162282) and LyeTx I mnΔK with adhesion proteins of *E. coli*. The SMILES (Simplified Molecular Input Line Entry System) structure of PLL was obtained from PubChem. PLL was docked with adhesin proteins such as FimH and PapG through the ClusPro webserver. The 3D crystal structures of PapG and FimH were obtained from the Protein Data Bank (PDB) and prepared for interaction studies using Molecular Operating Environment (MOE) to evaluate potential receptor–ligand binding geometries (Maryam et al. 2026). Regarding LyeTx I mnΔK, ITASSER web server was used

to predict the three-dimensional (3D) structure (Roy et al. 2010) visualized via PyMOL (Zhang *et al.*, 2012) and validated through a Ramachandran plot (Sobolev *et al.*, 2021). AMP was docked with adhesin of *E. coli* such as FimH through software ClusPro web server (Kozakov *et al.*, 2017). The 3D crystal structure of FimH (PDB ID: 3MCY) was downloaded from the PDB and prepared for interaction studies with the MOE (Agu *et al.*, 2023). The Simplified Molecular Input Line Entry System structural format of the LyeTx I Δ K sequence obtained using a web-based tool, PepSMI (<https://www.novoprolabs.com/tools/convert-peptide-to-smiles-string>). ADMET profiles involving key parameters were assessed using the latest version (July 2021 Release) of ADMETlab 2.0 platform (<https://admetmesh.scbdd.com/>).

3.19. Statistical analysis

Experiments were conducted in triplicate or sextuplicate depending on the assay. Shapiro-Wilk test was used to assess the data normality. The groups were compared by one-way analysis of variance (ANOVA), using Dunnet's post hoc test for comparison with the control group. Tukey's post hoc test was used to compare the different doses/concentrations of AMP. In both cases, statistical differences were considered when the p-value was less than 0.05. The GraphPad PrismTM version 5.03 software was used to generate the graphs. The results were expressed as mean $\pm$ standard deviation. Data analysis was carried out using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA). Quantitative data obtained from confocal microscopy images were analyzed using ImageJ software (version 1.54g; National Institutes of Health, Bethesda, MD, USA).

Chapter III: Results part

4. Results

4.1. UPEC isolates identification

The isolates were identified using biochemical-physiological and morphological techniques. As shown in Table 5, all included isolates presented a biochemical profile like *E. coli*. All were glucose fermenters and indole producers, relevant chemical markers in the identification of *E. coli*. In addition, all isolates presented a gram-negative rod morphology.

Table 5: Biochemical-physiological profile, morphology, and growth characteristics of UPEC isolates included in the study.

Code	Biochemical test									MacConkey agar			Gram
	Suc	Glu	H ₂ S	Ind	Ure	DL	Gas	Mot	Lac	Growth	Visc.	BS	
<i>E. coli</i> ATCC*	+	+	-	+	-	+	+	+	+	+	Low	+	GNB
EC11	+	+	-	+	-	-	+	+	+	+	High	+	GNB
EC31	+	+	-	+	-	+	+	+	+	+	Low	+	GNB
EC49	-	+	-	+	-	+	+	+	+	+	High	+	GNB
EC58	-	+	-	+	-	+	+	+	+	+	Low	+	GNB
EC71	-	+	-	+	-	+	+	+	+	+	Low	+	GNB
EC74	-	+	-	+	-	+	+	+	+	+	Low	+	GNB
EC81	-	+	-	+	-	+	+	+	+	+	Low	+	GNB
EC90	-	+	-	+	-	+	+	+	+	+	High	+	GNB
EC97	-	+	-	+	-	+	+	+	+	+	Low	+	GNB
EC99	-	+	-	+	-	-	+	+	+	+	High	+	GNB

Suc: Sucrose; Glu: Glucose; H₂S: Hydrogen sulfide; Ind: Indole; Ure: Urea; DL: Lysine decarboxylation; Mot: Motility; Visc: Mucosity; BS: Biliary salt precipitation; GNB: Gram-negative bacilli. **E. coli* ATCC 39218.

4.2. Antimicrobial activity of LyeTx I and its analogues

All the tested peptides showed antimicrobial effects against UPEC isolates as shown in Table 6. The peptides showed MICs ranging from 2 μ M to 64 μ M as shown in Table 6. The inhibitory concentration for 50% of the tested isolates (MIC_{50}) of the prototype LyeTx I was 4 μ M, LyeTx I-b had 16 μ M MIC, LyeTx I mn 64 μ M, and LyeTx I mn Δ K showed 2 μ M MIC. Additionally, another analogue of LyeTx I named LyeTx I mn showed an MIC_{50} of 64 μ M. On the other hand, LyeTx I mn Δ K, another analogue synthesized by addition of a lysine (K) residue to the LyeTx I mn peptide, was also effective against UPEC isolates showed MIC_{50} of 2 μ M. Other analogues such as Ac-LyeTx I mn Δ K showed MIC_{50} 64 μ M and LyeTx I mn Δ KL showed MIC of 4 μ M and 2 μ M respectively. The antibiotics such as meropenem and colistin showed MIC against all UPEC isolates within expected range i-e MIC_{50} of 375 nM and MIC_{50} of 92.5 nM respectively. The MBC results showed that all the peptides have bactericidal potential revealed by their MBC value ranging from 4 μ M to 128 μ M (Table 6). For highly antimicrobial efficacy against UPEC isolates, LyeTx I mn Δ K was selected for further experiments.

Table 6: MIC and MBC of LyeTx I and its derivatives against quinolone-resistant UPECs. Reproduced from (Haq *et al.*, 2026).

Code	Peptides (μM)														Controls (nM)	
	LyeTx I		L. Ib		L. mn		L. mnΔK		L. mnΔKAc		L. mnΔKL		L. mnΔKwN		Colistin	Meropenem
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MIC
EC11	4	8	32	32	64	64	2	8	64	128	4	8	2	8	125	60
EC31	4	8	16	16	64	64	2	8	64	64	4	8	4	8	250	60
EC49	4	8	16	16	64	64	2	8	64	64	4	8	2	4	500	60
EC58	4	8	32	32	64	64	2	8	64	128	8	16	2	8	250	250
EC71	4	8	16	16	64	64	2	8	64	128	4	8	2	8	250	60
EC74	8	64	16	16	64	64	2	8	64	128	4	8	2	8	250	60
EC81	8	16	16	32	64	64	2	8	64	128	4	8	4	8	500	125
EC90	4	8	16	32	64	64	2	8	64	64	4	8	4	8	500	125
EC97	16	16	16	32	64	64	2	8	64	64	4	8	2	4	500	125
EC99	8	64	16	16	64	64	2	8	64	64	4	8	2	8	500	125
MIC ₅₀	4		16		64		2		64		4		2		375	92.5
MBC ₅₀	8		24		64		8		96		8		8		-	-

L=LyeTx I

4.3. Structure prediction of the LyeTx I mnΔK and validation

The 3D structure of the AMP is formed mainly by an alpha-helical fold as shown in Figure 6. A predominant helical region (red) observed in by peptide structure profile. Additionally, a coil region is also observed (blue), and some residues of peptide acting as extended regions (green). The quality factor was 87.5% of the peptide as shown in Ramachandran plot Figure 7 which also shows that 91.7% of residues lie in favoured regions.

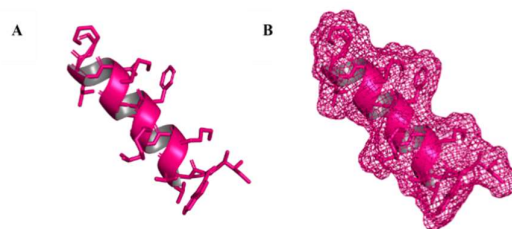


Figure 6. The predicted 3D structure of LyeTx I mnΔK in cartoon and stick form (A) and in mesh form (B). Reproduced from (Haq *et al.*, 2026).

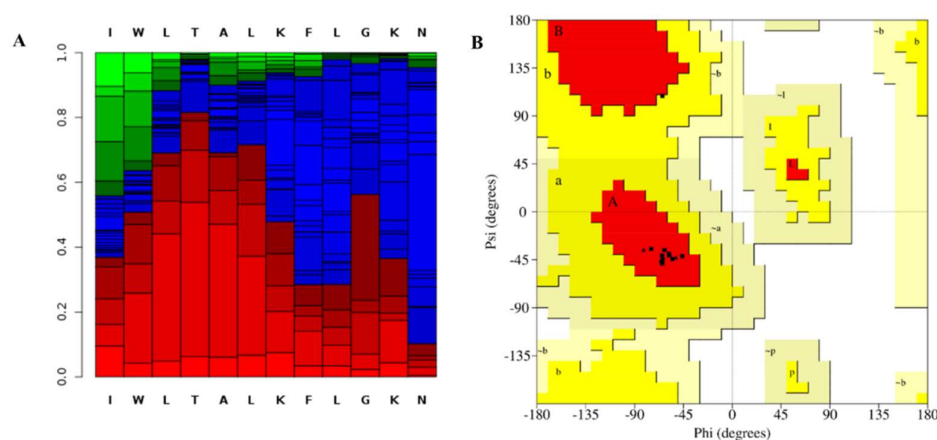


Figure 7. Validation of LyeTx I mnΔK (A) and its structure profile. (B) The Ramachandran plot depicts 91.7% residues in favoured regions and 8.3% in the additional allowed regions. (Haq *et al.*, 2026).

4.4. Membrane docking of LyeTx I mnΔK docking with FimH

Membrane docking studies showed that LyeTx I mnΔK binds with the adhesion protein of UPEC called FimH (Figure 8) through several key amino acids. Hydrogen bond was observed by Ile1 from the N-terminal portion of the peptide with Ser17 residue of FimH and Phe9 of LyeTx I mnΔK and the Arg60 residue of FimH as shown in Figure 9. The C-terminal region of the peptide also binds with FimH protein through hydrogen bonds by its Asn13 and Leu14 with Gln59/Gln143 and Arg132 in FimH, respectively.

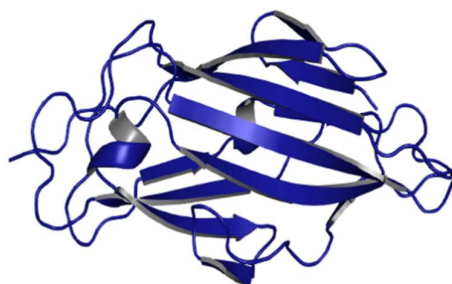


Figure 8. Bacterial protein FimH 3D structure in cartoon form. Reproduced from (Haq *et al.*, 2026).

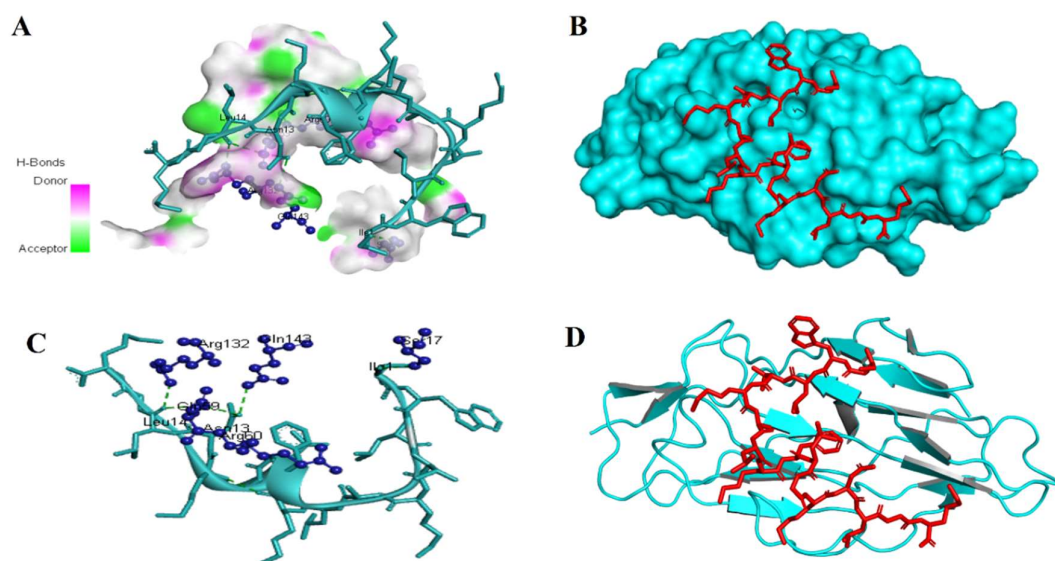


Figure 9. LyeTx I mnΔK docking with FimH in different representation modes (A). Display receptor surface as H-bond donor and acceptor (B). LyeTx I mnΔK-FimH interaction along labeled residues (C). LyeTx I mnΔK in cartoon form while protein in surface form is depicted

in cyan color (D). LyeTx I mnΔK-FimH is in cartoon form in red and cyan, respectively. Reproduced from (Haq *et al.*, 2026).

4.5. ADMET profile of LyeTx I mnΔK

The *in silico* ADMET analysis of LyeTx I mnΔK indicated a low probability of adverse toxicological effects. The predicted AMES mutagenicity score was 0.008, while the carcinogenicity probability was 0.036. The probability of drug-induced liver injury was negligible (0.000), and the predicted skin sensitization probability was 0.117. In terms of distribution, plasma protein binding was estimated at 23%. The predicted blood–brain barrier penetration was low, with a value of 0.018. Metabolism predictions indicated that LyeTx I mnΔK is neither an inhibitor nor a substrate of CYP1A2. For CYP3A4, the peptide showed a low probability of inhibition (0.093. Regarding absorption, LyeTx I mnΔK demonstrated high predicted human intestinal absorption with a value of 0.99.

4.6. Anti-biofilm activities of LyeTx I mnΔK

Sub inhibitory concentrations of LyeTx I mnΔK such as 0.25 μM, 0.50 μM, and 1 μM for its anti-biofilm potential using the crystal violet and MTT for biomass and viability of UPECs biofilms respectively. The biomass reduction results varied based on the tested isolate as shown in Figure 10. The highest 20% of the biomass was reduced by all LyeTx I mnΔK concentrations of the EC 81 and EC 90 isolates. Subsequently, the average biomass of EC 58 and EC 74 was reduced by 10% at 1 μM peptide concentration. The biomass of other isolates was not affected by any concentrations of the peptide.

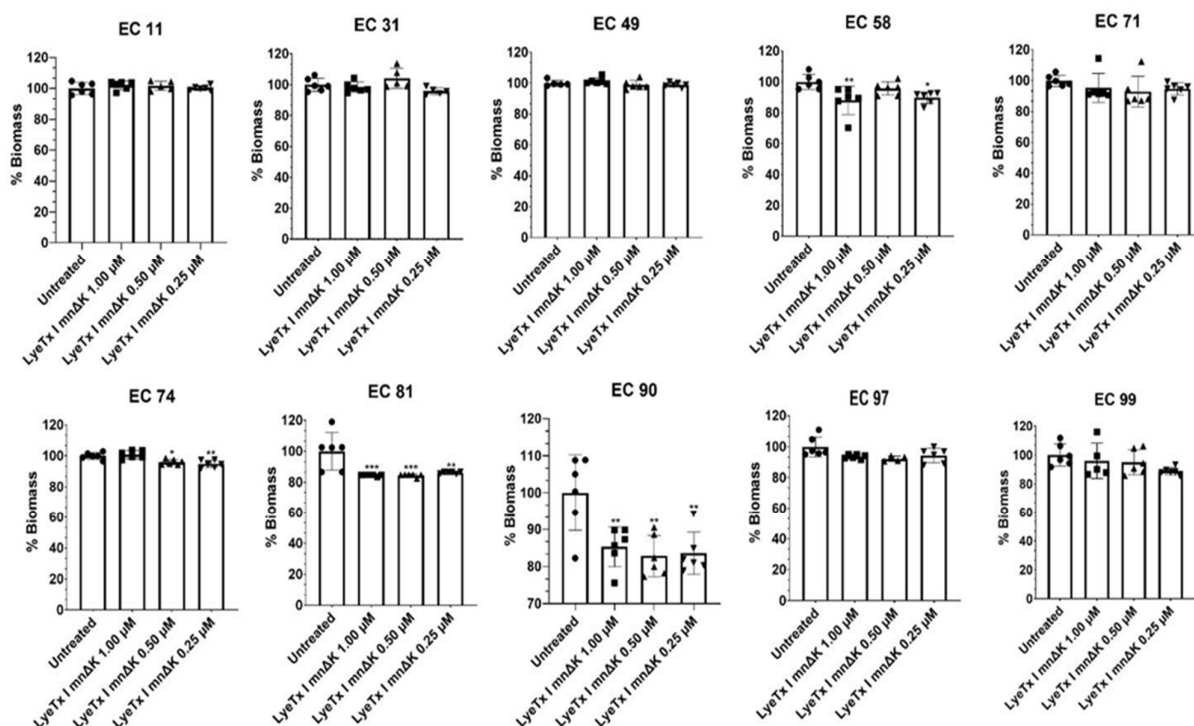


Figure 10. The inhibition of biofilm biomass of UPEC isolates by LyeTx I mnΔK at various concentrations. (values are presented as mean $\pm$ standard deviation). One, two, three, and four asterisks (*, **, ***, and ****) indicate statistically significant differences compared with the control at $0.01 < p < 0.05$, $0.001 < p < 0.01$, $0.0001 < p < 0.001$, and $p < 0.0001$, respectively. Reproduced from (Haq *et al.*, 2026).

However, MTT assay shows that the peptide effectively reduced the viability of UPECs inside biofilms in dose dependent manner but varied based on the isolate number. The highly susceptible isolate was EC 11 which 80% of viability was reduced by peptide all concentrations. The viability of EC 90 only decreased at high concentration of the peptide (approximately 80% at 1 μM). The viability of other isolates was found decreased from 20% to 40% at concentration of 1 μM (Figure 11).

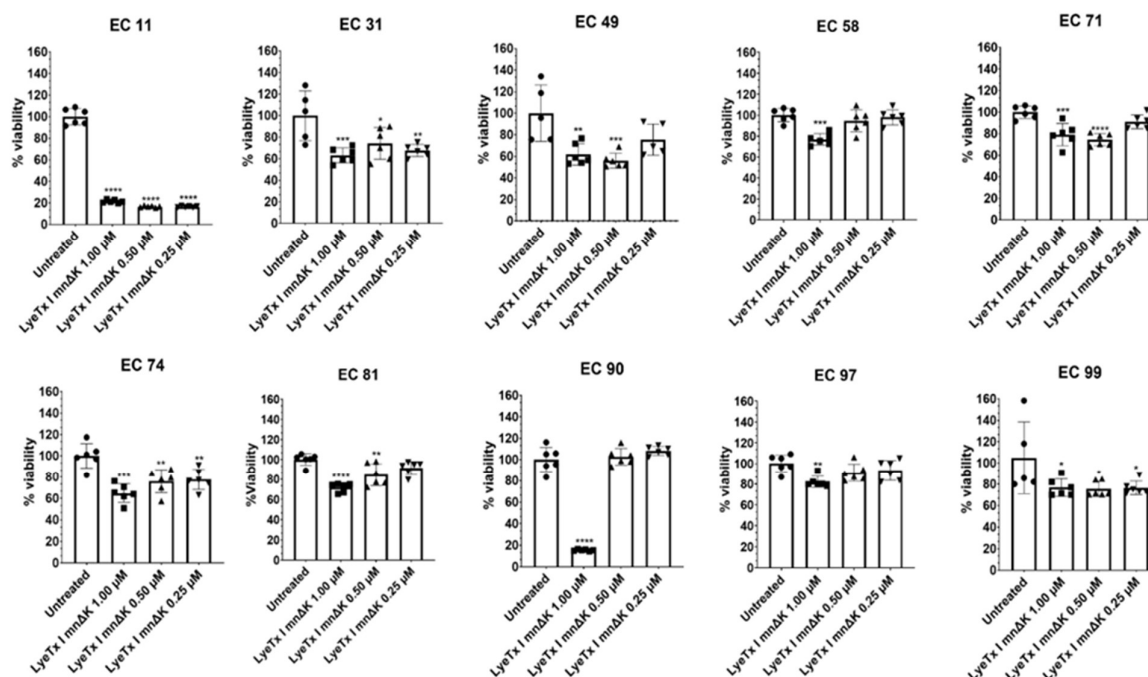


Figure 11. The inhibition of biofilm viability of UPEC isolates by LyeTx I mnΔK at various concentrations. Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks. Reproduced from (Haq *et al.*, 2026).

4.7. Anti-adhesive effects of LyeTx I mnΔK of UPECs on catheter

AMP showed anti-adhesive effects against UPECs on the urinary catheter at concentration of 1 μg/ml. Compared to control, peptide reduces 1-Log reduction of UPECs biofilm on catheter surface (Figure 12).

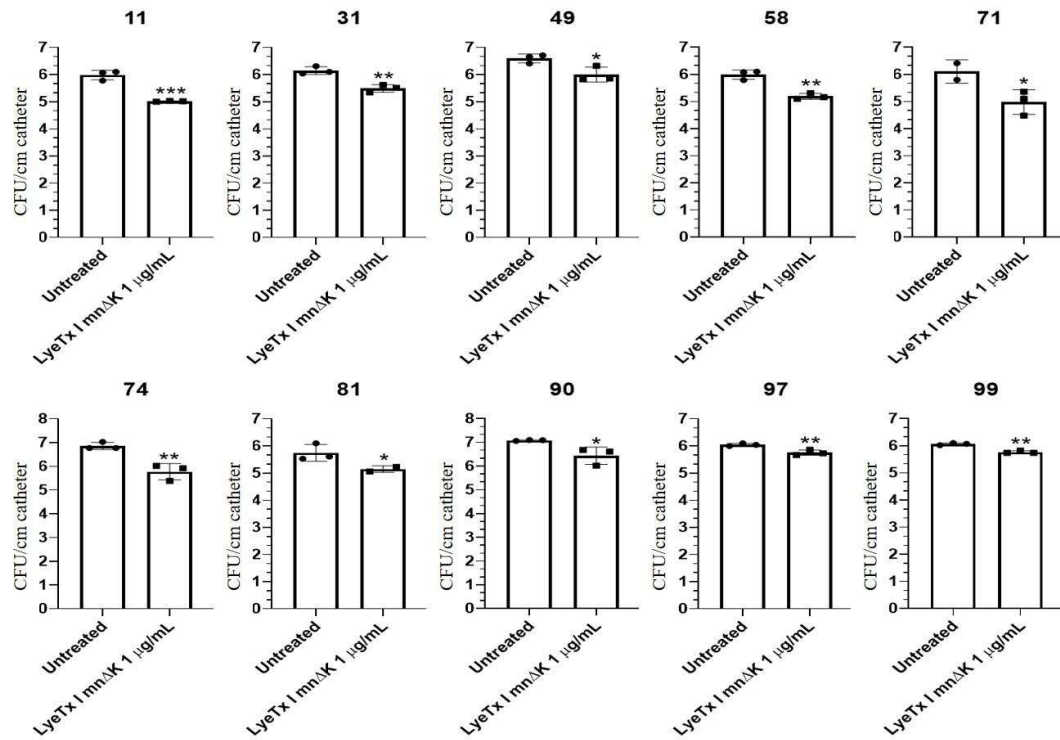


Figure 12. Reduction of bacterial biofilm formation of UPEC isolates on catheter surfaces after treatment with LyeTx I mnΔK (1 μg/mL). Bacterial (CFU counts per cm of catheter surface for different UPEC isolates. Untreated: without LyeTx I mnΔK. Data are presented as mean ± SD. Statistical significance is indicated by asterisks. Reproduced from (Haq *et al.*, 2026).

SEM analysis showed that LyeTx I mnΔK prevented the adherence of UPECs cells on the surface of the catheter while the control group catheter (without peptide treatment) showed a higher number of UPEC cells (Figure 13).

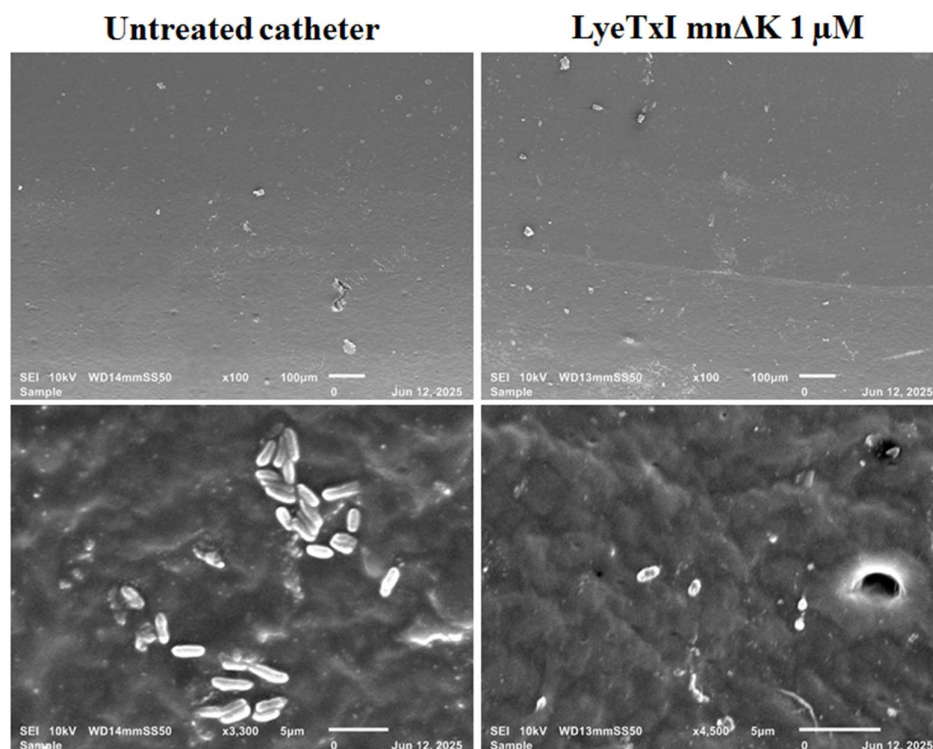


Figure 13. SEM analysis of untreated and AMP-treated catheters at low ($\times 100$) and high ($\times 3,300$ – $\times 4,500$) magnifications. AMP treatment markedly reduced UPEC colonization on the catheter surface. Reproduced from (Haq *et al.*, 2026).

4.8. Stability of LyeTx I mnΔK

Stability tests showed that antimicrobial potential of peptide against UPEC was greatly affected by incubation in urine. Since the peptide which was incubated in urine was used in MIC at different intervals. Initially, at 0 h the MIC was typically $2 \mu\text{M}$ but reach to $32 \mu\text{M}$ after 1-3 h of incubation. After 6 h of incubation, the MIC reached to $64 \mu\text{M}$ and was same till 9 h then reach to $128 \mu\text{M}$ after 24-48 h of incubation. In turn, the LyeTx I mnΔK incubated in water consistently showed $2 \mu\text{M}$ (Table 7).

Table 7: MIC of the AMP after incubation in urine at different time intervals. Reproduced from (Haq *et al.*, 2026).

Times	MIC of LyeTx I mnΔK (μM)	
	Urine	Water
0 h	2	2
1-6 h	32	2
9-12 h	64	2
24-48 h	128	2

MIC: Minimum Inhibitory Concentration.

Cytotoxicity results showed that LyeTx I mnΔK exhibited a CC₅₀ value of 32.72 μM in HEK-293 cells.

4.9. Toxicity evaluation of LyeTx I mnΔK *in vitro* and *in vivo*

Cytotoxicity results showed that AMP at 32.72 μM concentration became toxic to 50% of HEK-293 cells (CC₅₀) (Figure 14).

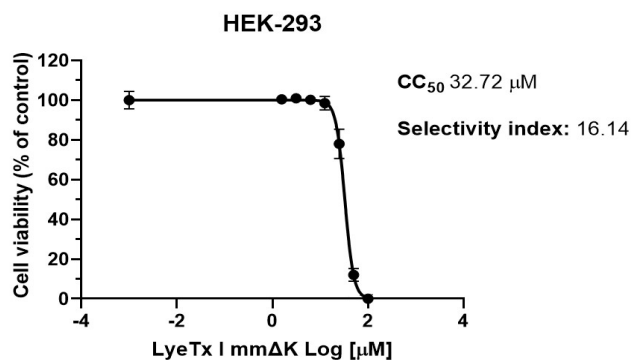


Figure 14. Cytotoxicity of AMP against HEK-293 cells. Reproduced from (Ul Haq *et al.*, 2026).

In vivo toxicity studies showed that no animal died from AMP treatment (Figure 15). Compared to the control, LyeTx I mnΔK slightly affected the food intake, including caloric, protein, carbohydrates, and lipids, but highly affected the food efficiency, as shown in Table 8.

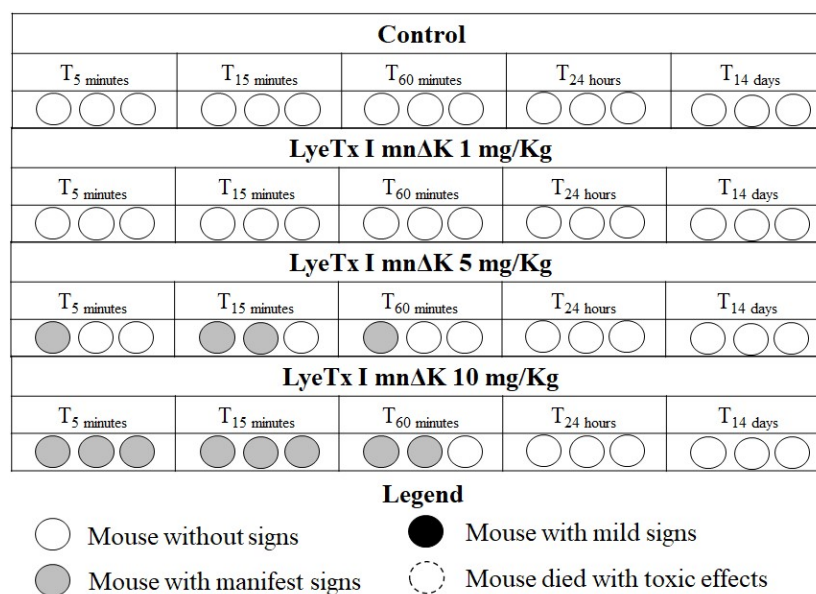


Figure 15. Dose-dependent assessment of clinical signs in mice treated with different concentrations of LyeTx I mnΔK. Reproduced from (Ul Haq *et al.*, 2026).

Table 8. LyeTx I Δ mn Δ K effect on mice food intake and metabolic parameters. The food intake (caloric, carbohydrate, protein, and lipid intake) slightly reduced in control animals, whereas food efficiency increased somewhat in all experimental animals. (values are presented as mean $\pm$ standard deviation). Reproduced from (Ul Haq *et al.*, 2026).

Food intake (g/g of animal)			
Control	AMP 1 μg/Kg	AMP 5 μg/Kg	AMP 10 μg/Kg
6.77 $\pm$ 0.20	5.10 $\pm$ 0.52	5.33 $\pm$ 0.24	5.20 $\pm$ 0.13
Carbohydrates intake (g/g of animal)			
3.11 $\pm$ 0.09	2.34 $\pm$ 0.24	2.45 $\pm$ 0.11	2.39 $\pm$ 0.06
Proteins intake (g/g of animal)			
1.56 $\pm$ 0.05	1.17 $\pm$ 0.12	1.22 $\pm$ 0.06	1.20 $\pm$ 0.03
Lipid intake (g/g of animal)			
0.27 $\pm$ 0.01	0.20 $\pm$ 0.21	0.21 $\pm$ 0.001	0.21 $\pm$ 0.005
Calorie intake Kcal/g of animal)			
21.11 $\pm$ 0.63	15.90 $\pm$ 1.62	15.34 $\pm$ 0.70	15.09 $\pm$ 0.40
Feed Efficiency (mg/Kcal)			
3.2 $\pm$ 1.40	5.62 $\pm$ 3.69	2.41 $\pm$ 0	4.02 $\pm$ 1.40

The weight gain of all the animals with all tested concentrations and control was same ($p > 0.05$) (Figure 16).

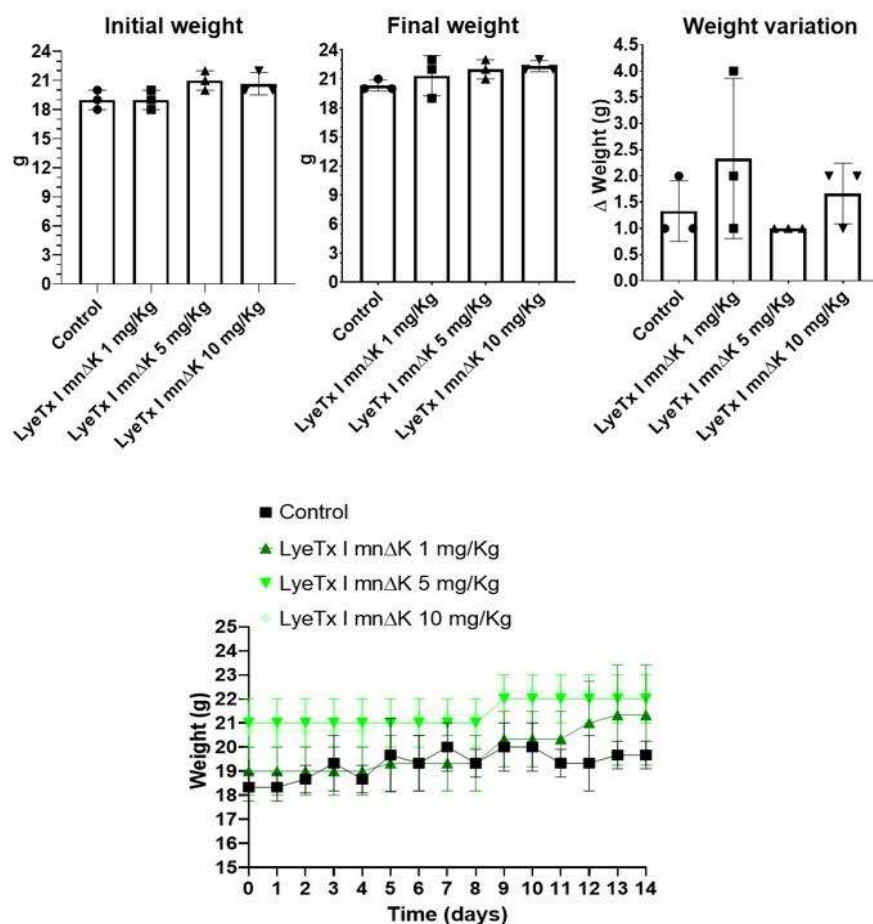


Figure 16. LyeTx I mnΔK effect on body weight of mice. Weight variation was observed in animals administered with different concentrations of peptides. The animals administered with 1 $\mu\text{g/kg}$ peptide showed high growth; the animals with 5 $\mu\text{g/kg}$ peptide showed less growth compared to control animals and 1 $\mu\text{g/kg}$ peptide animals. Whereas the animals administered with 10 $\mu\text{g/kg}$ peptide showed higher growth than 5 $\mu\text{g/kg}$ peptide animals and control. (values are presented as mean $\pm$ standard deviation). Reproduced from (Haq *et al.*, 2026).

The toxicity study focused on the urinary system revealed that no change was observed in renal masses nor any signal of edema (Figure 17). The serum creatinine level was high in the animal administered with the 10 $\mu\text{g/kg}$ AMP which indicates that renal function could be impaired.

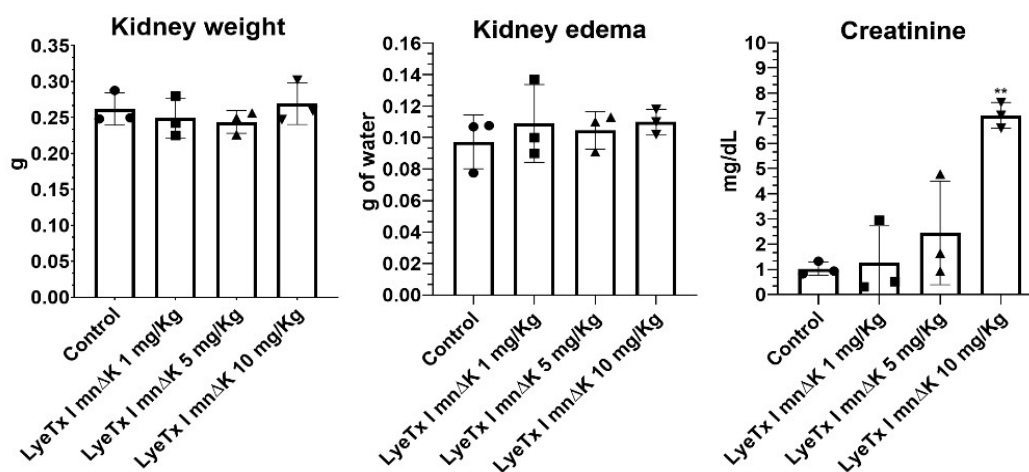


Figure 17. Renal toxicity of AMP in mice. All the tested concentrations do not significantly alter kidney weight or induce edema; however, the increase in creatinine levels at the highest dose (10 μ g/kg) indicates a reduced glomerular filtration rate and possible nephrotoxicity. Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks. Reproduced from (Haq *et al.*, 2026).

Moreover, mild tubular degeneration was observed at 10 μ g/kg of AMP as shown in renal histological analyses (Figure 18).

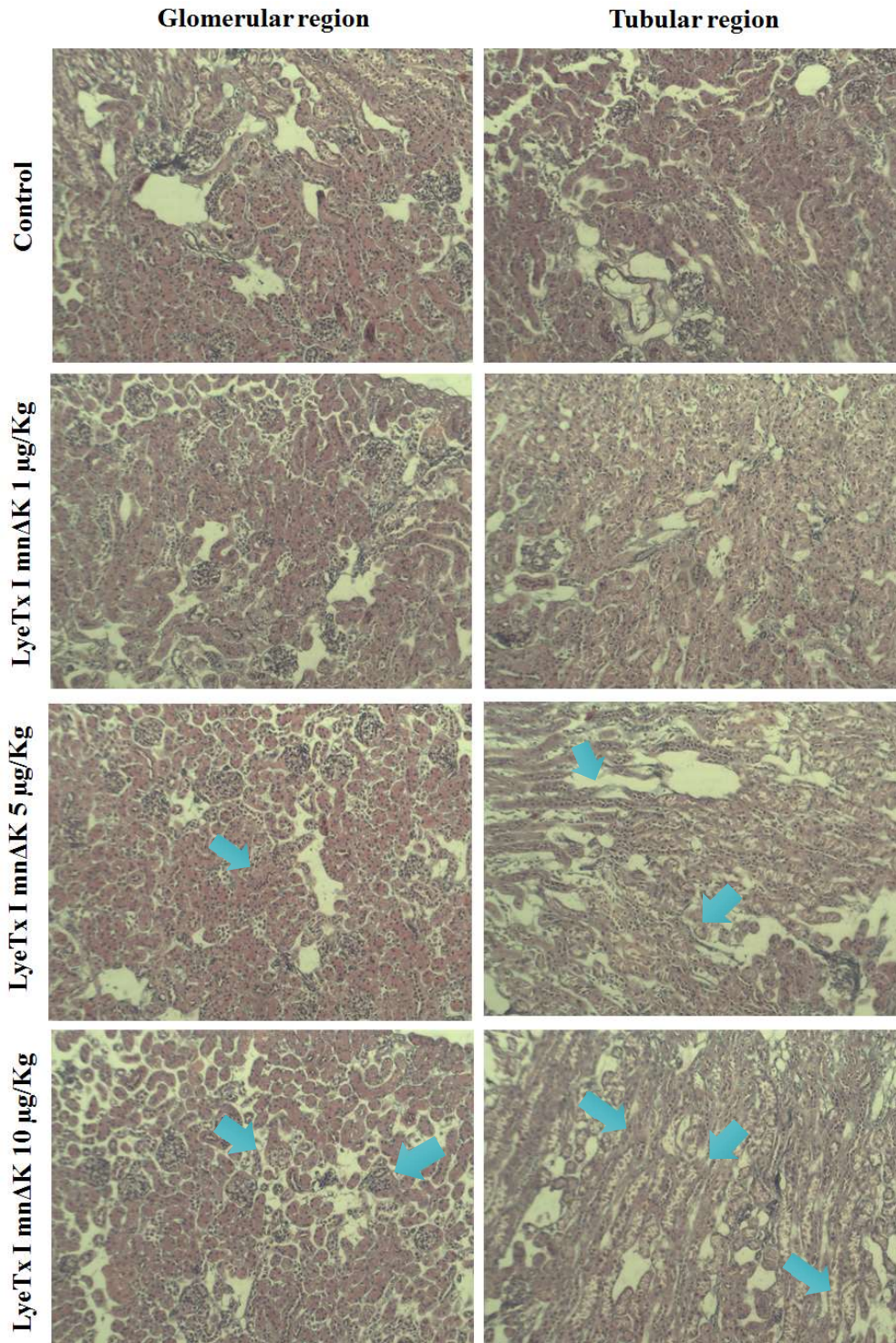


Figure 18. Histological analysis of kidney tissues from mice treated with different concentrations of peptide. Glomerular and tubular regions of kidneys. The control group exhibits normal histological architecture in both glomerular and tubular regions. Mice treated with LyeTx I mnΔK at doses of 1, 5, and 10 $\mu\text{g/kg}$ show dose-dependent morphological

changes. At higher doses (5 and 10 $\mu\text{g/kg}$), notable alterations are observed, including tubular dilation, interstitial inflammation, and glomerular congestion, particularly prominent in the tubular region. Reproduced from (Haq *et al.*, 2026).

4.10. Deposition and surface characterization of coatings of PVA and PVA/PLL

PVA exhibited good film-forming properties and was therefore selected for further optimization of coating studies using the model AMP PLL. Several polymer layers were applied to achieve a uniform and compact coating on catheters. SEM images collected at 1000x magnification showed that 5- and 9-layers coatings of PVA are not even (Figure 19A) while 11 layers coatings revealed uniform and even coatings on catheters. Additionally, surface roughness values of each layer were calculated in Average maximum peak-to-valley height (Rz) and Arithmetic average roughness (Ra) (Figure 19B-C). The surface roughness values for the 5-layer sample were measured as Rz of $6.4 \pm 0.1 \mu\text{m}$ and Ra of $1.7 \pm 0.2 \mu\text{m}$ 9-layer as Rz of $2.4 \pm 0.5 \mu\text{m}$ and Ra of $0.96 \pm 0.05 \mu\text{m}$, while 11 layer as Rz of $1.3 \pm 0.2 \mu\text{m}$ and Ra of $0.52 \pm 0.03 \mu\text{m}$ which indicate uniformity and smoothness. EDS was used to determine the chemical compositions of each layer of samples (Figure 19D). It was observed that increasing the layers' numbers gradually cover the catheter's surface therefore from a smooth and uniform polymer layer.

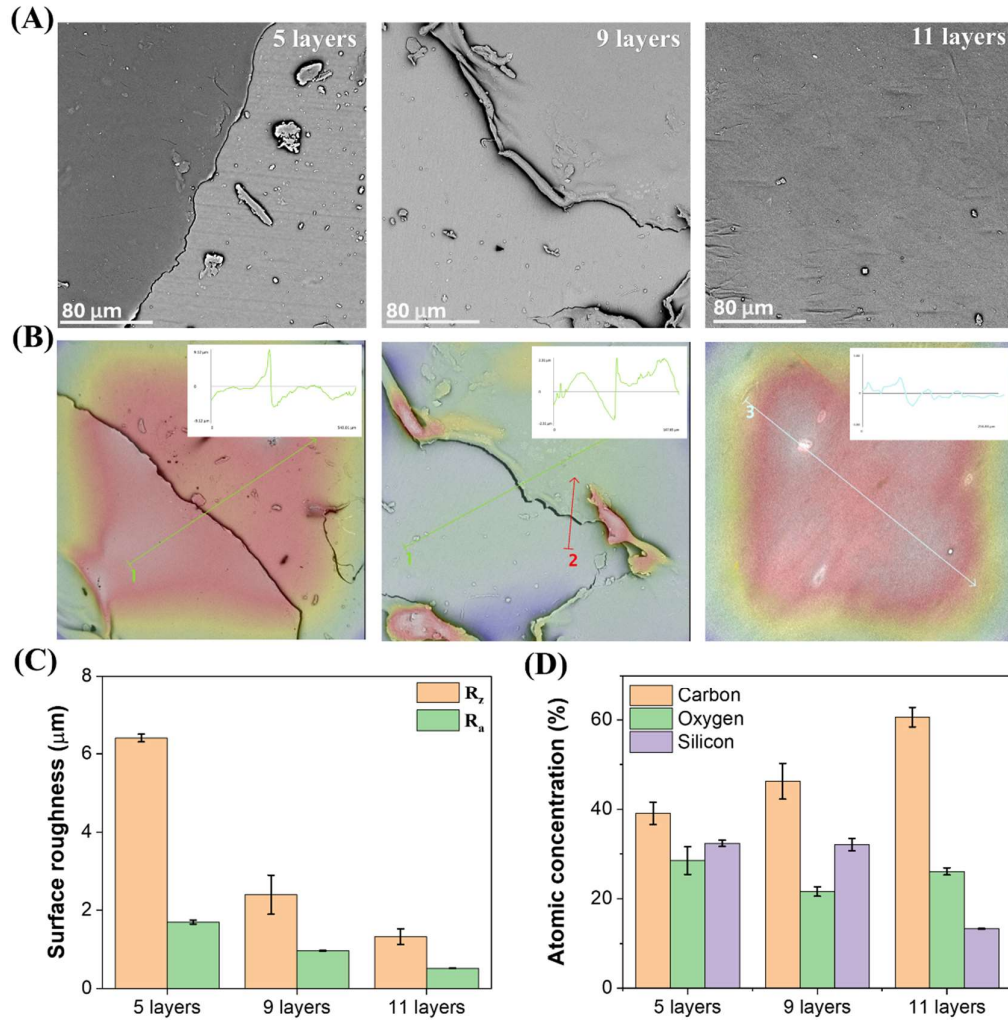


Figure 19. SEM images at 1000x magnification of catheters coated with different layers (A). 11-layered surface revealed a uniform coating. Surface roughness values in R_z and R_a were calculated for each layer (B) to provide an analysis of surface textures (C). Chemical composition of each layer was analysed through EDS (D). (values are presented as mean $\pm$ standard deviation). Reproduced from (Maryam *et al.*, 2026).

After optimizing the PVA coating layers catheters, PVA/PLL solution was coated on catheters. SEM images showed uniformity of the PVA to PLL coated catheters regardless of their ratio (Figure 20A). Carbon and oxygen were high in highest ratio PVA/PLL coating while silicon (48.9 ± 5.2 at.%) and oxygen (51.1 ± 2.0 at.%) as primary chemical components observed in untreated samples (Figure 20B). Catheter coatings with different concentrations of PVA/PLL showed the same level of silicon content (9-10 at.%) without statistically significant differences, confirming the uniformity of coating regardless of PLL content. Carbon content of 66-67 at.% was observed in PVA and PVA/PLL coated catheters while no carbon detected in

uncoated catheters. However, regarding roughness profiles, PLL appeared to affect the roughness, as PVA/PLL 400:1 showed Rz value of $1.6 \pm 0.2 \mu\text{m}$ and an Ra value of $0.29 \pm 0.03 \mu\text{m}$ while roughness was high for uncoated and PVA samples which exhibited Rz values of $2.6 \pm 0.3 \mu\text{m}$ and $2.5 \pm 0.5 \mu\text{m}$, and Ra values of $0.49 \pm 0.02 \mu\text{m}$ and $0.6 \pm 0.1 \mu\text{m}$, respectively (Figure 20C).

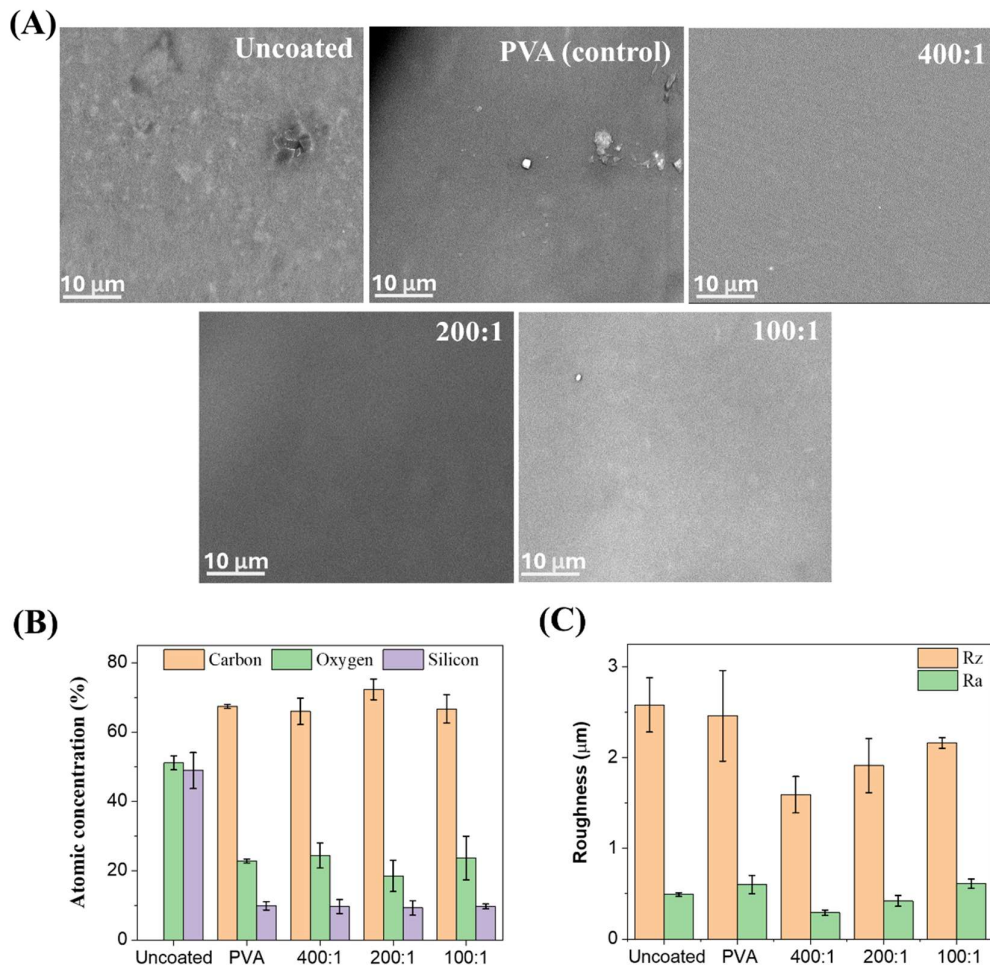


Figure 20. SEM images of all the samples at 1000x magnification (A). Chemical composition (B), roughness values (C) provide a comparative analysis of surface texture of pristine and coated catheters. (values are presented as mean $\pm$ standard deviation). Reproduced from (Maryam *et al.*, 2026).

4.11. FTIR analysis of coatings of PVA and PVA/PLL

In FTIR analysis, the Si-O-Si asymmetric and symmetric stretching vibration bands at 1010 cm^{-1} and 780 cm^{-1} , respectively was observed in pristine silicon catheter (Figure 21A). The band at 1260 cm^{-1} is represent the vibrations of the Si-O-Si network. Additionally, transmittance bands associated with silanols were identified within the range of 2850 cm^{-1} to 3300 cm^{-1} . Regarding the PVA spectrum, the bands within the $1480\text{--}1380\text{ cm}^{-1}$ range was observed that corresponds to the bending vibrations modes of O-H and C-H bonds of PVA. Another prominent band at approximately at 1715 cm^{-1} , was identified, corresponding to the stretching vibration modes of carbonyl (C=O) groups and additional peaks at 1090 cm^{-1} and 843 cm^{-1} attributed to C-O and C-C stretching vibrations, respectively. Whereas the PLL peaks in FTIR spectrum of all PVA/PLL coatings displayed at $2848\text{--}2915\text{ cm}^{-1}$, 1462 cm^{-1} , and 720 cm^{-1} , which correspond to N-H stretching, CH_2 bending, and C-H bending vibrations, respectively. Furthermore, the coating uniformity was also confirmed by absence of characteristic stretching and vibrational bands for silicon (Si-O-Si) in PVA and PVA/PLL samples.

4.12. Wettability analysis of coatings of PVA and PVA/PLL

Wettability tests were performed for all the catheters to assess a surface's hydrophilicity as presented in Figure 21B. The contact angle of the pristine catheter was $176.4 \pm 2.0^\circ$ that is characteristic of hydrophobic silicon. The contact angle of PVA coated catheter $67.6 \pm 0.9^\circ$, was low compared to pristine catheter. It was suggested that presence of hydroxyl groups in the structure of PVA coatings improve the hydrophilicity of the surface. Moreover, surface wettability did not influence significantly with addition of PLL, maintaining contact angles around $72\text{--}73^\circ$ for all PLL ratios.

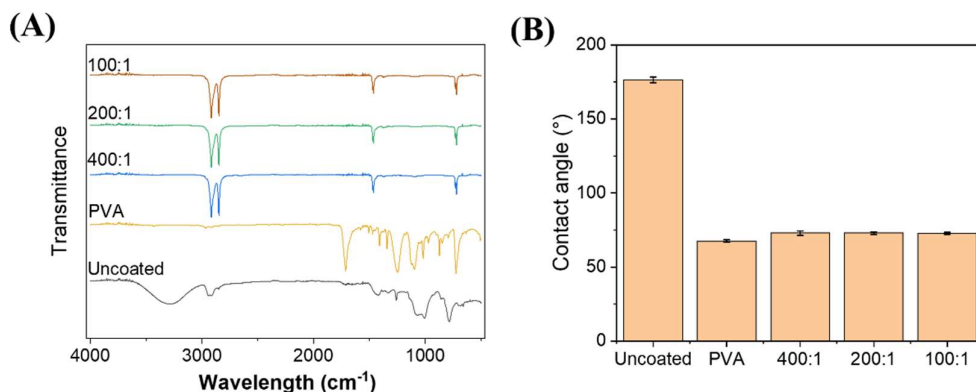


Figure 21. FTIR (A) and contact angle analysis of uncoated catheter, PVA and all the samples (B). (values are presented as mean $\pm$ standard deviation) Reproduced from (Maryam *et al.*, 2026).

4.13. Biocompatibility of coatings of PVA and PVA/PLL

Cytotoxicity assay revealed the high cell viability of the PVA/PLL towards Normal Human Dermal Fibroblast *or* Normal Human Dermal Fibroblasts (NHDFs), namely 90 ± 2 % in the presence of PVA/PLL (400:1) coated catheter, 85 ± 2 % for 200:1 coating and 83 ± 1 % for 100:1 while 91 ± 1 % for PVA coating and 94 ± 1 % for pristine catheter (Figure 22).

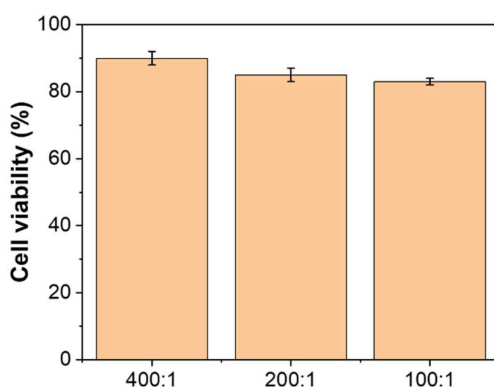
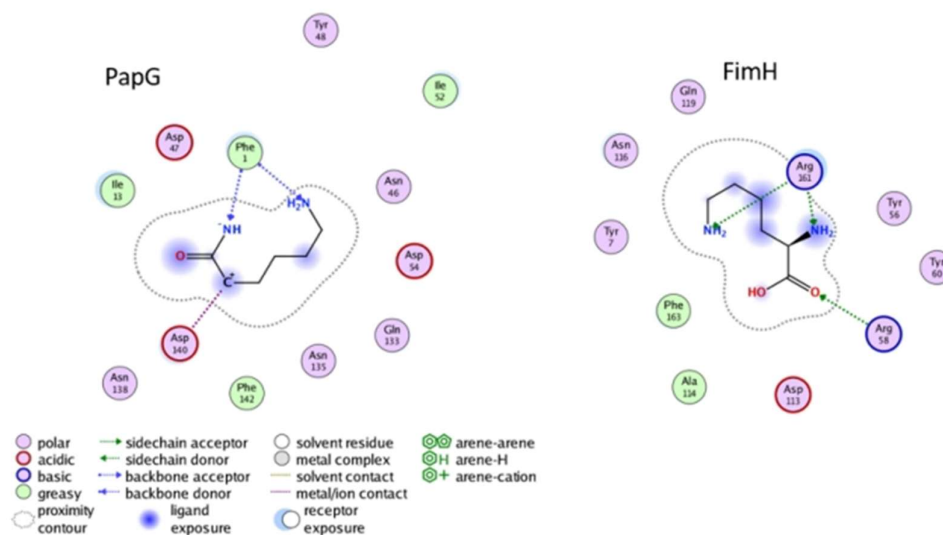


Figure 22. NHDFs cell viability percentages for PLL coated catheters (n = 3). As a control, cells without any treatment were used (viability 100 %). (values are presented as mean $\pm$ standard deviation). Reproduced from (Maryam *et al.* 2026).

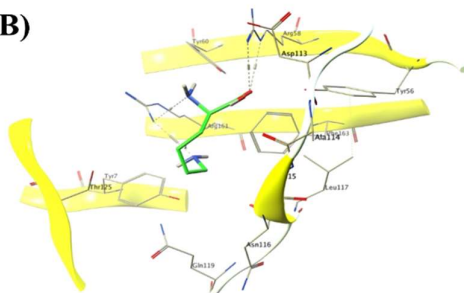
4.14. Polylysine docking with FimH and PapG

A single ϵ -PL showed binding with FimH and PapG proteins of *E. coli* as shown in Figure 23A. Based on the three-dimensional box binding configurations between the protein and ligand polymer was revealed by the Molecular operating environment (MOE) that produced a docking score using the molecular mechanics forcefields. The binding energy with PapG was -8.3608 and FimH -7.8268. Figure 23A shows the 2D interactions and FimH, and Figure 23B and C show the 3D interactions.

(A)



(B)



(C)

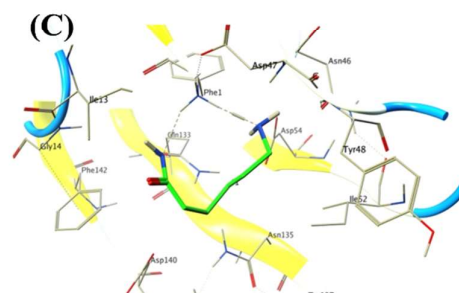


Figure 23. 2D interactions of ϵ -PL with PapG and FimH (A); 3D interactions of ϵ -PL with PapG (B) and 3D interactions of ϵ -PL with FimH (C). Reproduced from (Maryam *et al.*, 2026).

4.15. SEM analysis for microbial adhesion on PVA and PVA/PLL coated catheters

SEM images showed that microbial attachment was significantly decreased on the surface of catheters coated with PVA/PLL (Figure 24-26). The coating with high PLL (100:1) concentrations lead to highest microbial reduction on surface while 400:1 coating reduces less microbial attachment compared to 100:1 coating. The coating with highest PLL concentrations, 100:1 significantly reduces the clusters of *E. coli* and is observed to be more dispersed. Coating with a 200:1 ratio also reduces *E. coli* adhesion however less compared to 100:1 but higher reduction compared to 400:1. On a PVA/PLL coated surface, *E. coli* cells isolated and distorted compared to PVA coating that observed with uniform cells distribution exhibiting a rod-shaped morphology which show higher microbial density which suggest less anti-adhesive activity (Figure 24).

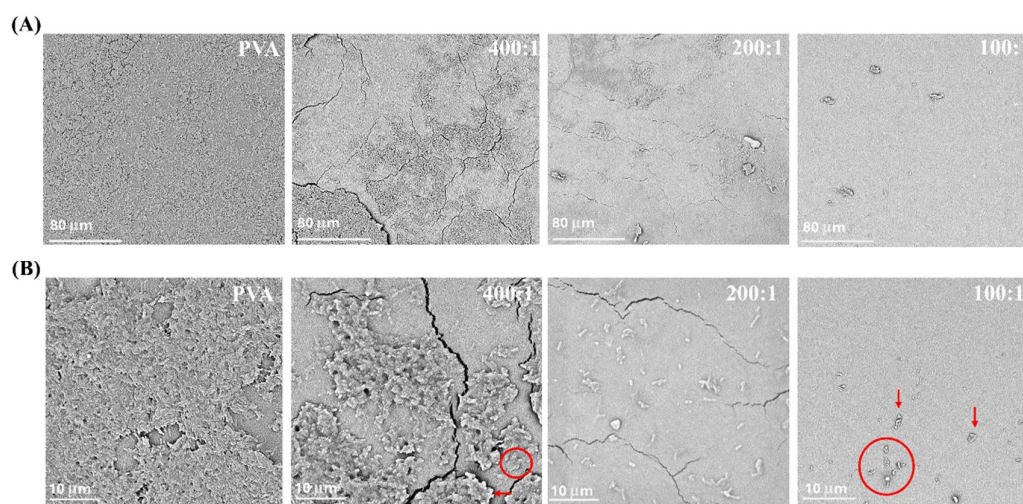


Figure 24. The SEM images obtained at magnifications of 1000x (A) and 5000x (B) shows reduced *E. coli* adhesion on PLL coated catheters compared to the PVA. Reproduced from (Maryam *et al.*, 2026).

Similarly, anti-adhesive effects of PVA/PLL coatings against *S. epidermidis* were also found to be dependent on PLL concentration (Figure 25) as in higher PLL concentration coatings few cells observed on the surface. While on PVA coatings showed typical spherical morphology of *S. epidermidis* and appear mostly dispersed, with some small clusters which are different from PVA/PLL coatings that showed altered bacterial arrangement due to presence of PLL.

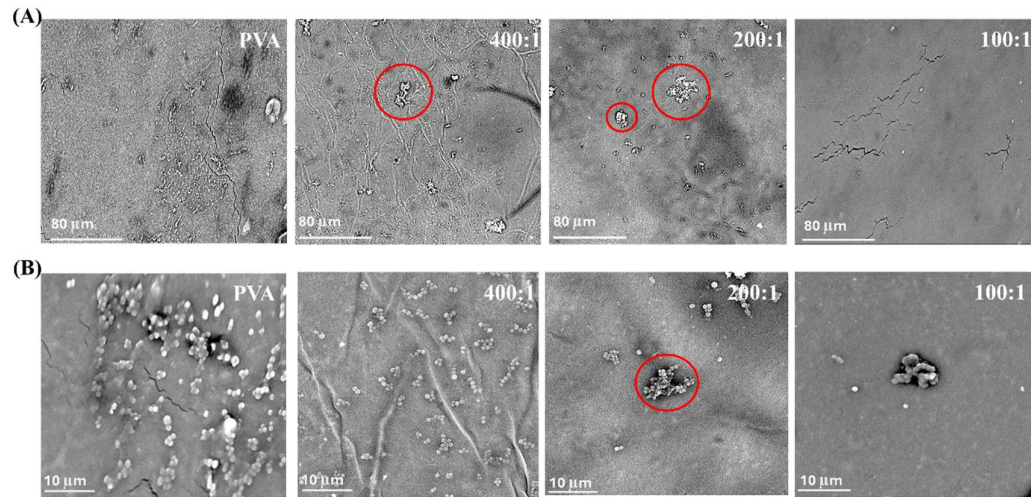


Figure 25. The SEM images obtained at magnifications of SEM 1000x (A) and 5000x (B) shows reduced *S. epidermidis* adhesion on PLL coated catheters compared to the PVA. Reproduced from (Maryam *et al.*, 2026).

Regarding *C. albicans*, 100:1 coating revealed pronounced filamentous growth (hyphae/pseudohyphae) with reduced clustering while the 200:1 based coatings showed a mixed morphology including yeast cells and emerging pseudohyphal structures. The 400:1 sample showed abundant yeast cells, although slightly less aggregated while control coating, based on pure PVA showed a typical dense network of yeast cells and small number of hyphae indicating biofilm development (Figure 26). Overall, increasing PLL content reduced microbial attachment and influenced *C. albicans* morphology, indicating a PLL concentration-dependent anti-adhesive and antifungal effect.

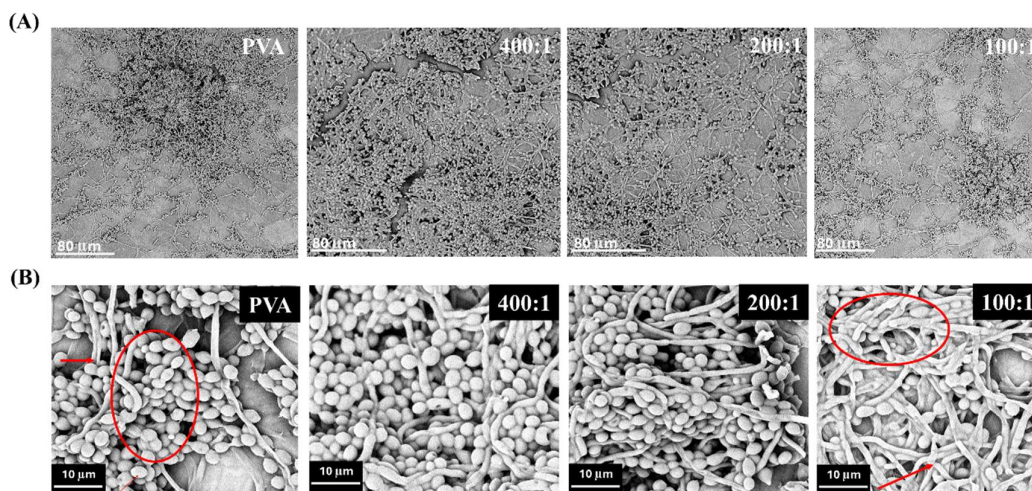


Figure 26. The SEM images obtained at magnifications of 1000x (A) and 5000x (B) shows reduced *Candida albicans* adhesion on PLL coated catheters and more sparse areas. Reproduced from (Maryam *et al.*, 2026).

4.16. Viability of microorganisms on PVA and PVA/PLL coated catheters

LIVE/DEAD assay was performed to distinguish viable and non-viable cells on coated catheters to assess antimicrobial effect. It was found that viability of *E. coli*, *S. epidermidis*, and *C. albicans* on coated catheter decreased with increasing PLL content (Figure 27A) while control PVA based coating showed highest viability of microbial cells. Increasing PLL content in the coatings (from PVA: PLL 400:1 to 200:1 and 100:1) resulted in decreasing microbial viability on the catheter surface. The coating with highest PLL ratio 100:1, significantly reduced the viability of all microbial cells that was revealed by reduction in fluorescence due to impaired microbial membrane. The quantitative analysis of LIVE/DEAD assay found 65 ± 4 % *E. coli* live cells on PVA coating (Figure 27C), the coating 400:1 found with 42 ± 2 %), and 100:1 found with very low number of *E. coli* live cells percentage declines to below 30 ± 1 % which showed that highest antimicrobial potential of coating with higher PLL concentrations.

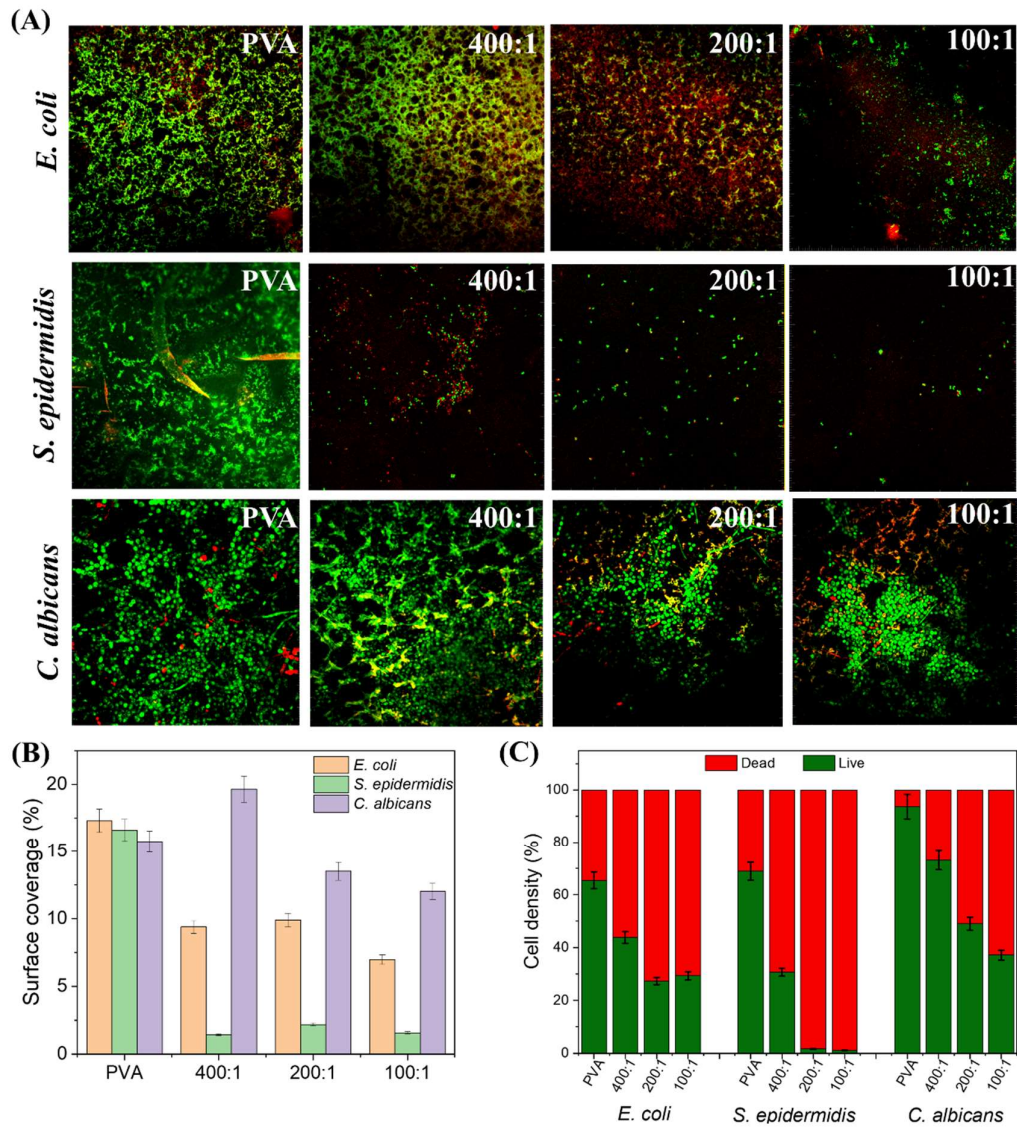


Figure 27. Confocal microscopy images of live (green) and dead/dying (red) cells of all tested microorganisms (A); coverage of live and dead microbial cells (B); cell density of live/dead cells tested microorganisms (C). (values are presented as mean $\pm$ standard deviation). Reproduced from (Maryam *et al.* 2026).

Compared to *E. coli*, PVA/PLL coated catheters cause high reduction of the *S. epidermidis* at all concentrations of PLL viability (Figure 27A), with the most pronounced effect observed at the 100:1 ratio (Figure 27A). Overall, *S. epidermidis* cells coverage was approximately 16.6 ± 0.8 % on PVA coated catheters, however, reduce to 1.6 ± 0.1 % on the PVA/PLL (100:1) indicating the PLL mediated antimicrobial effects (Figure 27B). The living cells percentage of *S. epidermidis* was 70 ± 3 % PVA coated catheters which is quite high compared to the 100:1 sample that was found with 1.1 ± 0.1 % (Figure 27C). Regarding *C. albicans*, increased hyphal

formation was observed with dispersed green fluorescence and localized red signal on 400:1 catheter due to low PLL concentration (Figure 27A). 200:1 catheter showed clumping of the green fluorescence signal due to clustering of cell aggregation. The percentage of living cells of *C. albicans* was 90 ± 4 % on PVA coated catheters while reduced to around 72 ± 3 % on 400:1 indicating antifungal effects due to PLL presence. Interestingly, 100:1 coated catheter, with highest PLL concentrations showed approximately 38 ± 1 % living cells of *C. albicans* (Figure 27C) indicating a high antimicrobial potential.

4.17. Deposition and surface characterization of SA and SA/LyeTx I mnΔK coatings

During the screening of biopolymers, SA exhibited the greatest structural stability and was therefore selected for deposition of LyeTx I mnΔK. Several SA layers were deposited to achieve a uniform and compact coating on silicon sheets. SEM images showed that 15, 25, and 30 layers coatings of SA are not even (Figure 28A) while 35 layers coatings revealed uniform and even coatings on silicon. The plasma treatment of samples greatly supports the SA coating. The uniform coating of SA/LyeTx I mnΔK was optimized in 30 layers (Figure 28B).

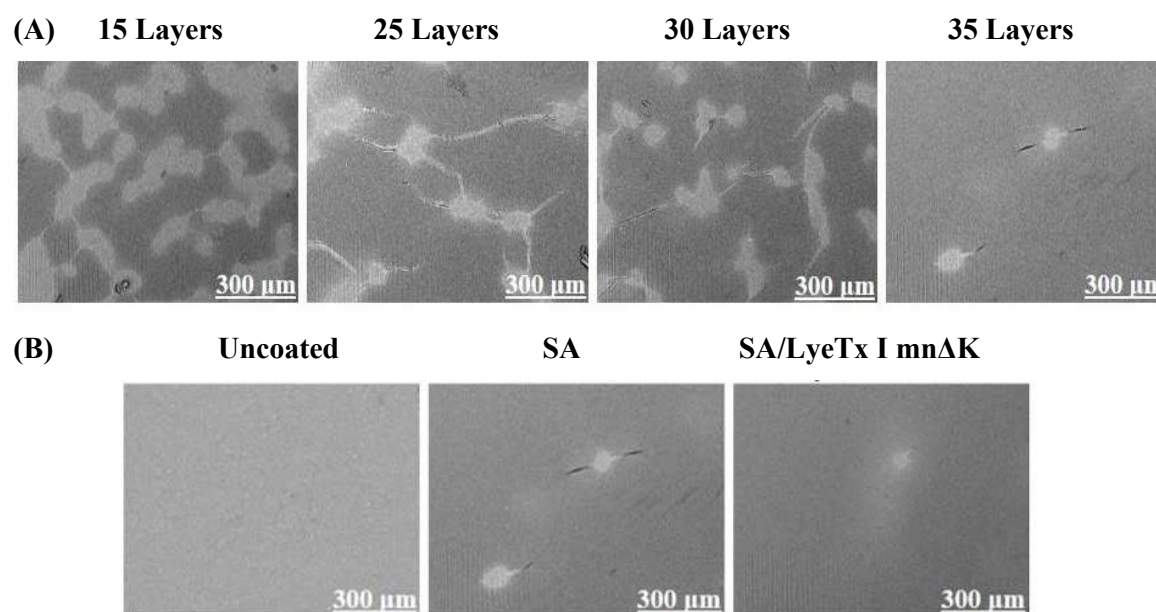


Figure 28. SEM images at magnification of 270x of silicon coated with several layers of SA, 35-layered surface reveals a uniform coating (A). Uncoated, SA coated and SA/LyeTx I mnΔK coated samples (B).

Elemental analysis was performed for all the coatings through SEM-EDS. SA coating and SA/LyeTx I mnΔK coating revealed higher contents of carbon 44.96 ± 3.91 at.% and 42.28 ± 1.32 at.% respectively (Figure 29). The oxygen content was 33.80 ± 3.49 at.% in SA coating, 29.85 ± 0.75 at.% in SA/LyeTx I mnΔK coating, and 52.46 ± 1.17 at.% in the uncoated silicon. The silicon content was high in uncoated silicon, 47.54 ± 1.18 at.%, which reduced to 19.33 ± 0.79 at.% and 25.03 ± 1.57 at.% for the SA and SA/LyeTx I mnΔK coatings, respectively, indicating successful surface coverage. On the other hand, sodium content increased from 1.91 ± 0.47 at.% in the SA coating to 2.90 ± 0.31 at.% in the SA/LyeTx I mnΔK coating. No significant difference in carbon atomic percentage was observed between the SA and SA/LyeTx I mnΔK coatings ($p = 0.351$). In contrast, the oxygen and silicon atomic percentages differed significantly among the groups ($p < 0.001$). Both coatings significantly reduced the oxygen and silicon contents compared with the uncoated surface, while the SA/LyeTx I mnΔK coating exhibited a significantly higher silicon content than the SA coating ($p = 0.0029$). Additionally, the SA/LyeTx I mnΔK coating showed a significantly higher sodium content than the SA coating ($p = 0.045$).

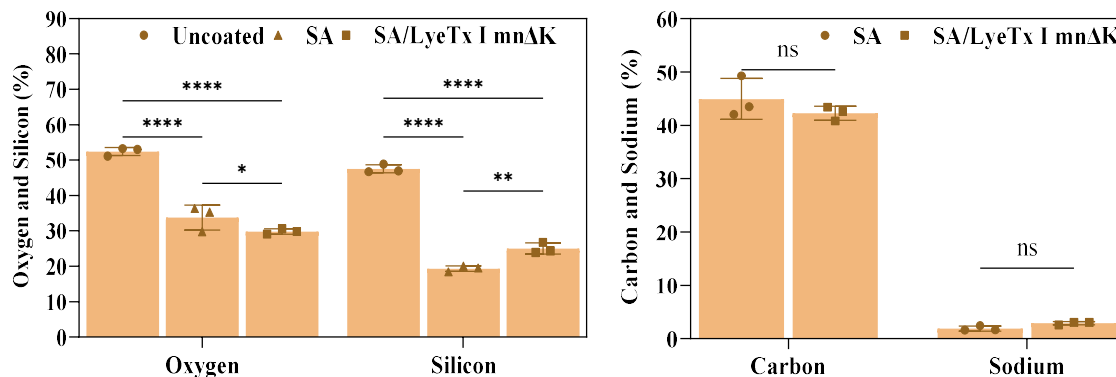


Figure 29. Atomic concentrations of uncoated, SA, and SA/LyeTx I mnΔK coating determined using EDS. Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks. ns, not significant ($p > 0.05$).

The uncoated surface exhibited an aerial surface roughness (S_a) of 213.3 ± 2.5 nm (0.213 ± 0.003 μ m) (Figure 30A). Deposition of the SA coating reduced the roughness to 174.3 ± 52.1 nm (0.174 ± 0.052 μ m). The incorporation of LyeTx I mnΔK AMP increased the roughness to 317.0 ± 39.1 nm (0.317 ± 0.039 μ m) which may be due to its interactions with anionic alginate matrix, therefore, altering the coating microstructure and promoted the formation of nanoscale

protrusions. The SA/AMP coating significantly increased the Sa compared with both the uncoated ($p < 0.05$) and SA-coated ($p < 0.01$) surfaces. The three-dimensional surface topography images obtained by optical profilometry are presented in Figure 30B, illustrating the morphological differences among the surfaces.

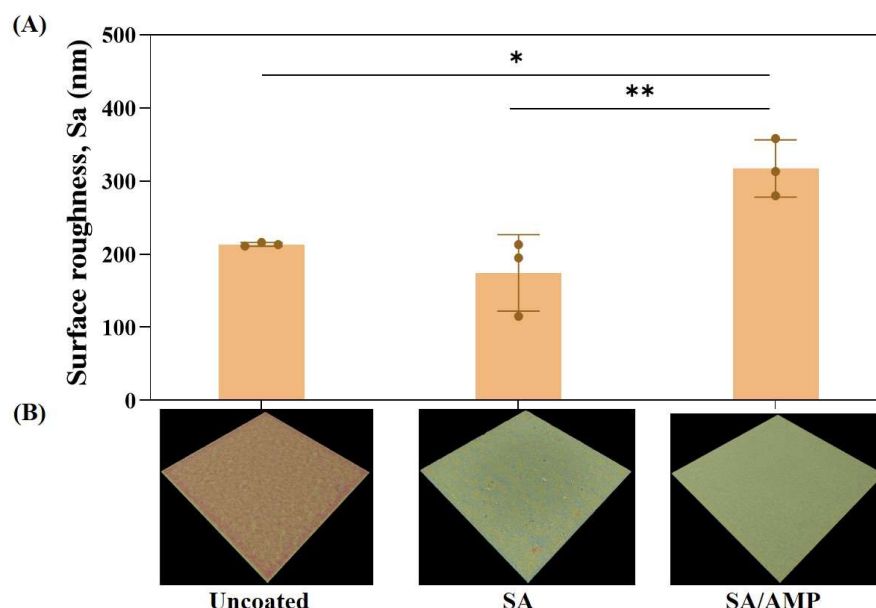


Figure 30. Roughness values of uncoated, SA, and SA/LyeTx I mnΔK coated surfaces provide a comparative analysis of their surface texture (A). Three-dimensional surface topography of the uncoated surface, SA coating and SA/LyeTx I mnΔK coating obtained by optical profilometry (B). Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks.

4.18. FTIR analysis and wettability and of SA and SA/LyeTx I mnΔK coatings

A broad band observed in the 3200–3500 cm^{-1} region in FTIR spectrum of the SA corresponds to O-H stretching vibrations associated with hydroxyl groups and hydrogen-bonded water molecules (Figure 31A). The peaks in the 1600–1400 cm^{-1} region are characteristic of the asymmetric and symmetric stretching modes of carboxylate (COO^-) groups present in the alginate structure while bands within the 1200–800 cm^{-1} region assigned to C–O–C and C–O stretching vibrations confirms the presence of SA (Figure 31A). The presence of the peptide was indicated by the Amide I band (1600–1690 cm^{-1}), arising primarily from C=O stretching vibrations of the peptide backbone, as previously reported by (Aboul-

enein *et al.*, 2014). Changes in the intensity and shape of this band were observed in the SA/LyeTx I mnΔK spectra (Figure 31A).

Water contact angles of the uncoated surface were $127.5 \pm 2.7^\circ$, while SA and SA/LyeTx I mnΔK coated silicon exhibited contact angle values of $51.3 \pm 1.6^\circ$ and $42.4 \pm 3.3^\circ$, respectively, which indicates a moderately hydrophilic surface (Figure 30B). With the coating of the SA and SA/ LyeTx I mnΔK, the surface wettability of the silicon is greatly increased. The water contact angle of the SA/LyeTx I mnΔK coating was noticeably reduced compared with those of the PVA-coated catheter ($67.6 \pm 0.9^\circ$) and the PVA/PLL-coated catheter ($72\text{--}73^\circ$), indicating that incorporation of LyeTx I mnΔK increased the surface hydrophilicity. Both coatings significantly reduced the contact angle compared with the uncoated surface ($p < 0.0001$), with the SA/LyeTx I mnΔK coating showing a lower contact angle than the SA coating ($p < 0.05$).

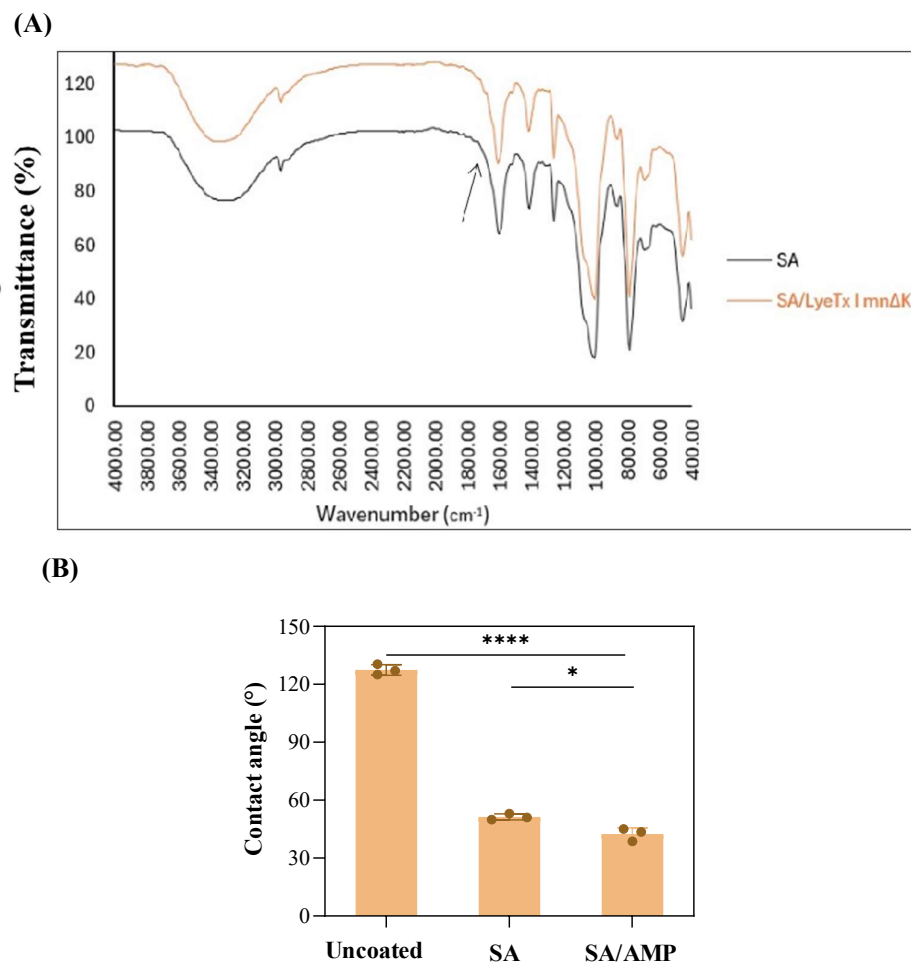


Figure 31. FTIR (A) and contact angle analysis of uncoated silicon, SA and SA/AMP (B). Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks.

4.19. Biocompatibility of SA and SA/LyeTx I mnΔK coatings

The cytotoxicity assay revealed that NHDFs cultured in the presence of the uncoated silicon maintained high cell viability ($96.8 \pm 7.5\%$) as shown in figure 32. The viability of NHDFs slightly reduced to $92.2 \pm 5.0\%$ in the presence of SA coating, which was comparable to that of the uncoated control. While the viability was $84.9 \pm 8.1\%$ in the presence of SA/LyeTx I mnΔK coatings. Although viability of cells exposed to SA and SA/LyeTx I mnΔK was slightly reduced ($p > 0.05$).

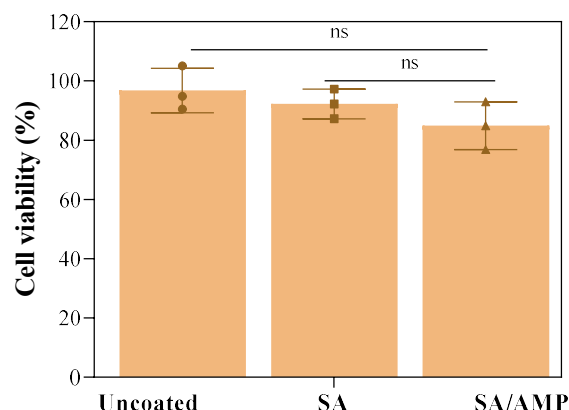


Figure 32. NHDFs cell viability percentages for uncoated silicon surface, SA coated surface, and SA/LyeTx I mnΔK coated surface. As a control, cells without any treatment were used (viability 100 %). Data are presented as mean ± SD. ns, not significant ($p > 0.05$).

4.20. Antimicrobial activity of SA and SA/LyeTx I mnΔK coatings

The OD₆₀₀ measurements revealed that *S. aureus* growth was lowest on the SA/LyeTx I mnΔK-coated samples (OD₆₀₀ = 0.975 ± 0.302), representing a significant reduction relative to growth control ($p < 0.001$), uncoated silicon ($p < 0.05$), and SA-coated samples ($p < 0.01$) (Figure 33A). The highest OD₆₀₀ value was observed for the SA-coated samples (OD₆₀₀ = 1.837 ± 0.064), while cultures incubated on uncoated silicon exhibited an intermediate OD₆₀₀ value (1.536 ± 0.032) (Figure 33A). The SA/LyeTx I mnΔK coating inhibited *S. aureus* growth by $36.43 \pm 20.31\%$ compared to the uncoated surface ($p < 0.05$) and by $47.27 \pm 14.84\%$ relative to the SA coating alone ($p < 0.01$) (Figure 33B). The OD₆₀₀ value of the *E. coli* culture incubated with SA/LyeTx I mnΔK-coated samples was 0.675 ± 0.095 , representing a significant reduction relative to the growth control ($p < 0.0001$), uncoated samples ($p < 0.01$), and SA-coated samples ($p < 0.05$) (Figure 33A). In contrast, the OD₆₀₀ values for the SA-coated and uncoated samples were 1.030 ± 0.064 and 1.543 ± 0.011 , respectively. The SA/LyeTx I mnΔK-coated samples exhibited *E. coli* inhibition of $56.29 \pm 5.86\%$ relative to the uncoated samples ($p < 0.001$), and $34.63 \pm 4.99\%$ relative to the SA-coated samples ($p < 0.01$) (Figure 33B).

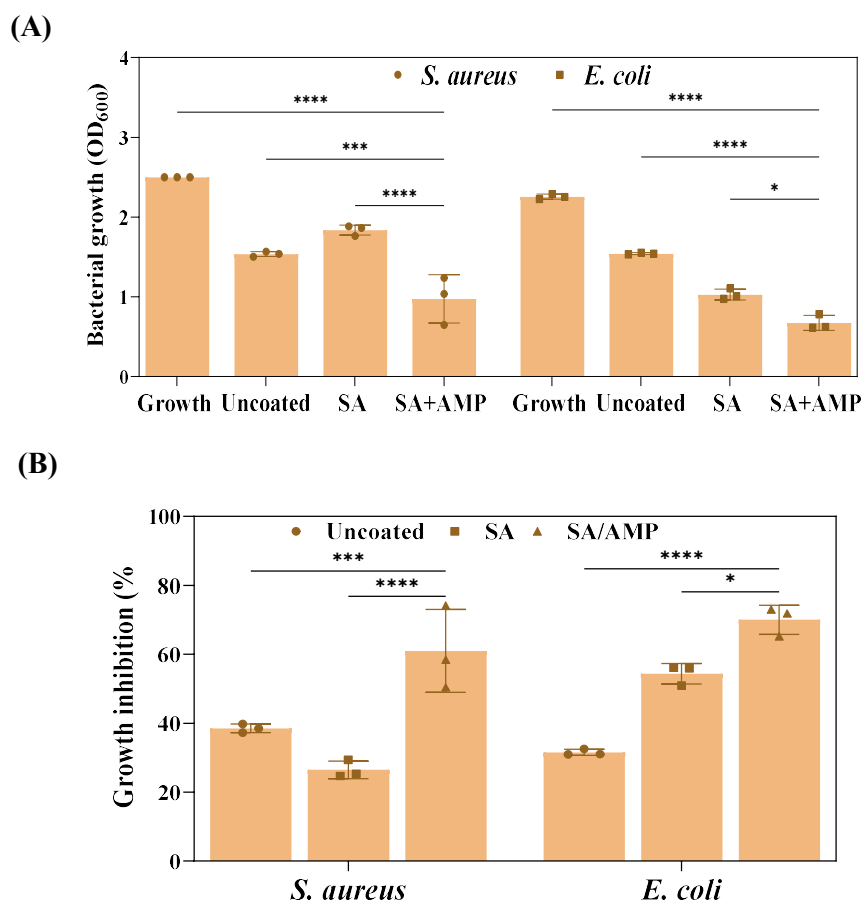


Figure 33. OD₆₀₀ of *S. aureus* and *E. coli* cultures incubated with SA/LyeTx I mnΔK-coated, SA-coated, and uncoated samples (A). Bacterial growth inhibition percentages (B). Data are presented as mean ± SD. Statistical significance is indicated by asterisks.

4.21. SEM analysis for microbial adhesion on SA and SA/LyeTx I mnΔK coatings

SEM analysis showed that microbial attachment was low on the surface of samples coated with SA/LyeTx I mnΔK when compared with uncoated surface and SA-coating (Figure 34, 35). SA/LyeTx I mnΔK coated sample showed only few isolated *S. aureus* cells without any cell clusters or biofilms (Figure 34). While the uncoated surface showed several *S. aureus* cells and extensive colonization on the surface. The SA-coating that was without peptide also showed localized aggregates of *S. aureus* cells which surrounded the surface indicating the microcolony formation.

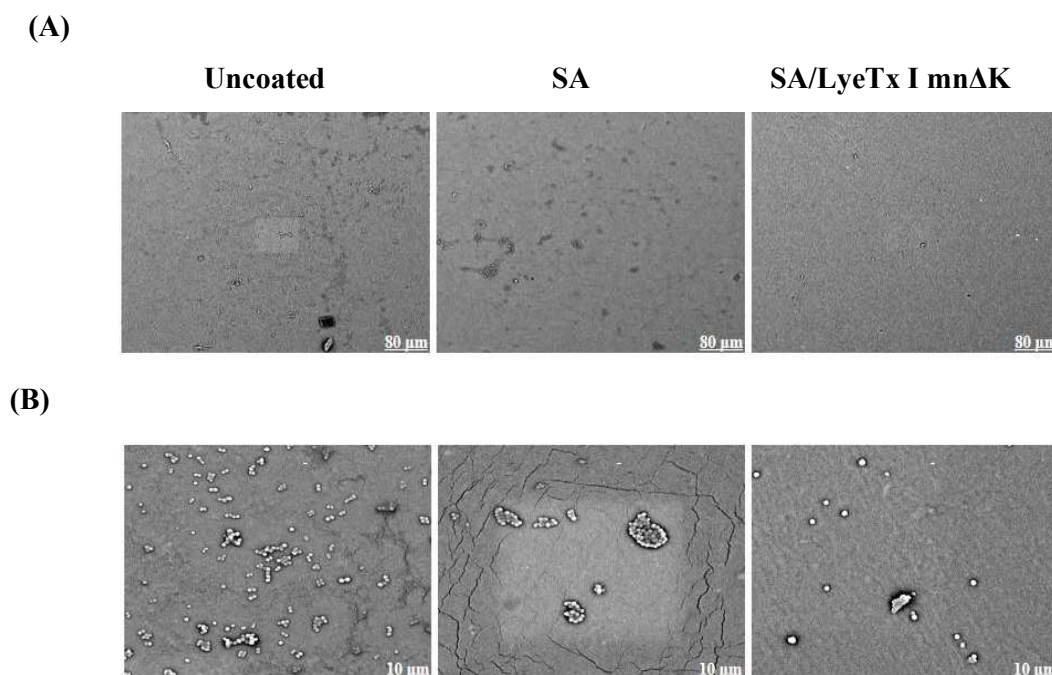
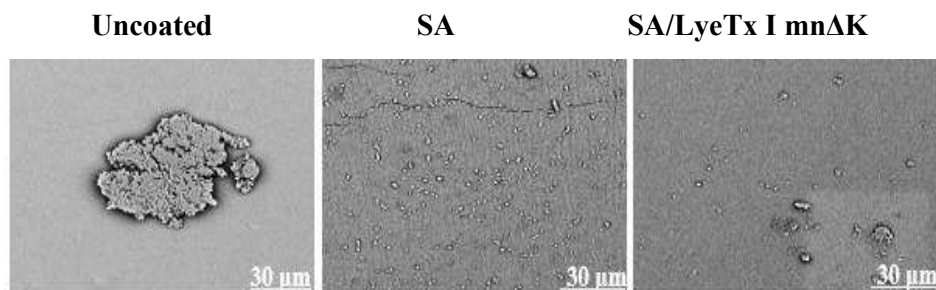


Figure 34. SEM images of samples exposed to *S. aureus* at magnifications of 1000x (A) and 5000x (B) showed differences in bacterial distribution. SA/LyeTx I mnΔK coatings showed less adhesion of *S. aureus*.

Additionally, SEM micrographs of SA/LyeTx I mnΔK coating showed only a few *E. coli* cells and no dense adhesion on the surface. There are no extensive aggregation or mature biofilms observed on the surface (Figure 35). A SA coating sample was found with smaller clusters and scattered adherent cells of *E. coli* showing high microbial adhesion. The uncoated surface showed a large and dense aggregate of *E. coli* suggesting a high biofilms growth covering a significant portion of the surface. The addition of peptide in the coating greatly reduces the microbial adhesion on the surface.

(A)



(B)

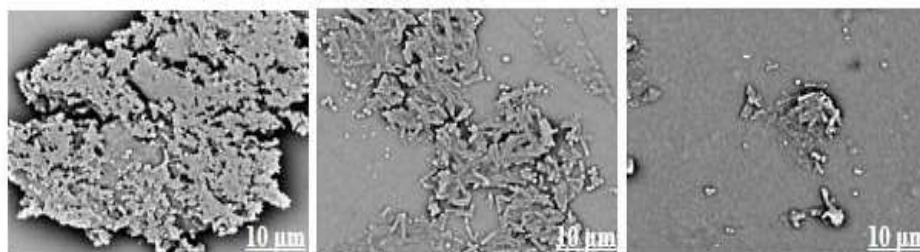
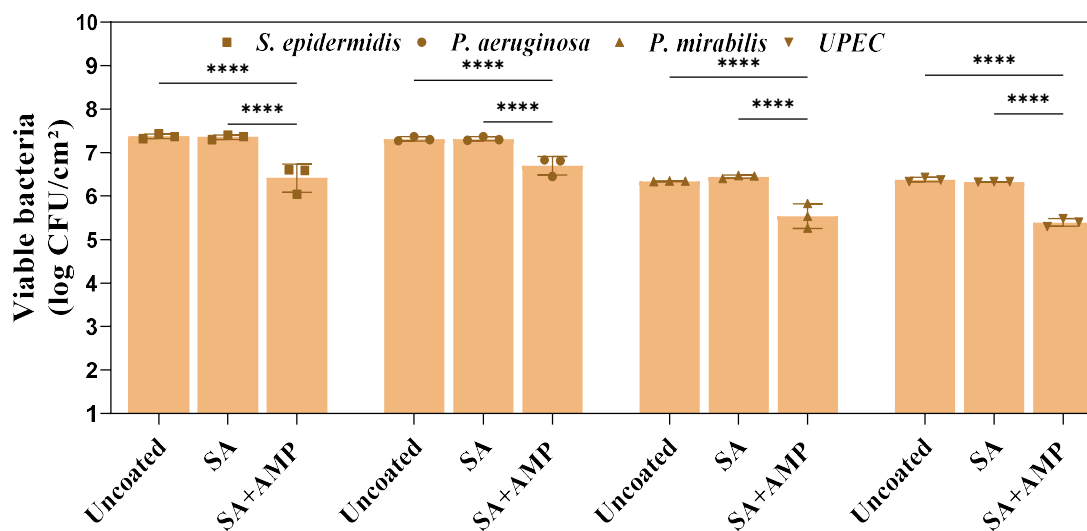


Figure 35. SEM images of samples exposed to *E. coli* at magnifications of 2000x (A) and 5000x (B) showed differences in bacterial distribution. SA/LyeTx I mnΔK coatings showed less adhesion of *E. coli*.

4.22. Antimicrobial activity of SA and SA/LyeTx I mnΔK coating against CAUTI pathogens

SA/LyeTx I mnΔK coating showed anti-microbial activity against CAUTI pathogens such as *S. epidermidis*, *P. aeruginosa*, *P. mirabilis*, and UPEC (Figure 36A) by reducing their viability by 0.96 ± 0.32 , 0.61 ± 0.22 , 0.80 ± 0.29 , and 0.99 ± 0.08 log, respectively, compared to uncoated samples (all $p < 0.01$). While the reductions compared to SA-coating were 0.95 ± 0.34 , 0.62 ± 0.22 , 0.90 ± 0.28 , and 0.94 ± 0.09 log, respectively (all $p < 0.01$). The inhibition of SA/LyeTx I mnΔK coating against *S. epidermidis*, *P. aeruginosa*, *P. mirabilis*, and UPEC, was 89.0%, 75.5%, 84.2%, and 89.8%, respectively (Figure 36B), compared with the uncoated samples. Compared with the SA-coating, the SA/LyeTx I mnΔK coating exhibited approximately 88.8%, 76.0%, 87.4%, and 88.5% inhibition of *S. epidermidis*, *P. aeruginosa*, *P. mirabilis*, and UPEC (Figure 36B), respectively. However, no significant differences were observed between inhibition percentages calculated relative to the uncoated and SA-coated surfaces for any bacterial strain ($p > 0.05$).

(A)



(B)

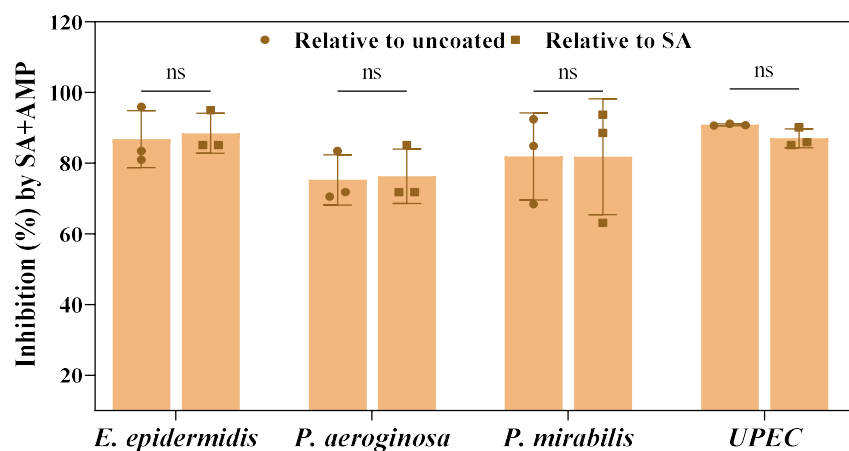
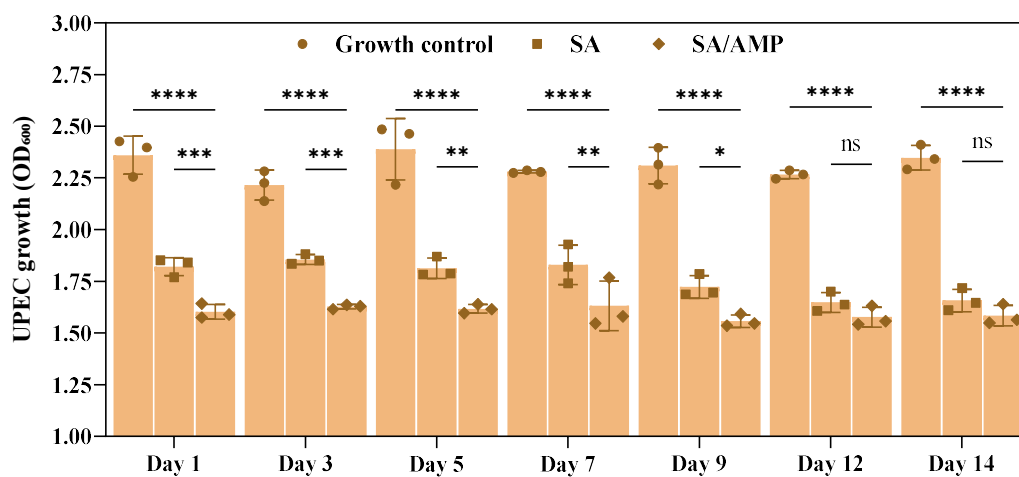


Figure 36. Viable bacterial counts (log CFU/cm²) recovered from uncoated, SA-coated, and SA/LyeTx I mnΔK-coated silicon surfaces (A). Inhibition (%) by SA/LyeTx I mnΔK relative to uncoated silicon and SA-coating (B). Data are presented as mean ± SD. Statistical significance is indicated by asterisks.

4.23. Antimicrobial stability of SA/LyeTx I Δ mnK coating in urine

The antimicrobial activity of the SA/LyeTx I Δ mnK coating after incubation in urine for up to 14 days was evaluated against a clinical UPEC isolate (EC90) by measuring bacterial growth (OD_{600}) (Figure 37A). The cultures incubated with the SA/LyeTx I Δ mnK-coated samples consistently exhibited lower OD_{600} values relative to growth control and SA-coated samples. However, the OD_{600} values gradually increased after 5 days of incubation. The growth control consistently exhibited the highest OD_{600} values (2.22 ± 0.07 – 2.39 ± 0.15), followed by the SA coating (1.63 ± 0.06 – 1.86 ± 0.02), whereas the SA/LyeTx I Δ mnK coating showed the lowest OD_{600} values throughout the 14-day study (1.21 ± 0.12 – 1.62 ± 0.03) (Figure 37A). During the first 5 days, the OD_{600} values of the SA/LyeTx I Δ mnK coating remained significantly lower than those of both the growth control ($p < 0.0001$) and the SA coating (day 1, $p < 0.001$; day 3, $p < 0.001$; day 5, $p < 0.01$). On day 7, the coating continued to exhibit significantly lower OD_{600} values than the growth control ($p < 0.0001$) and SA coating ($p < 0.01$). By day 9, significant differences were maintained relative to both the growth control ($p < 0.0001$) and SA coating ($p < 0.05$). After 12 and 14 days of incubation, the SA/LyeTx I Δ mnK coating remained significantly different from the growth control ($p < 0.0001$), whereas no significant difference was observed compared with the SA coating ($p > 0.05$). The 1-day incubated SA/LyeTx I Δ mnK coating exhibited $48.60 \pm 3.99\%$ inhibition relative to the growth control and $33.39 \pm 7.88\%$ relative to the SA coating ($p < 0.01$). After 3 and 5 days of incubation, the coating maintained high antibacterial activity, with inhibition percentages of $43.61 \pm 1.00\%$ and $47.82 \pm 4.78\%$ relative to the growth control, and $32.67 \pm 2.21\%$ ($p > 0.05$) and $31.30 \pm 3.05\%$ ($p < 0.01$) relative to the SA coating, respectively. Thereafter, the inhibition relative to the SA coating progressively decreased to $20.23 \pm 3.49\%$ after 7 days ($p < 0.01$), $10.82 \pm 3.27\%$ after 9 days ($p < 0.001$), $7.28 \pm 3.44\%$ after 12 days ($p < 0.0001$), and $2.79 \pm 0.67\%$ after 14 days of incubation ($p < 0.0001$) (Figure 37B). It is suggested that prolonged exposure of the SA/LyeTx I Δ mnK coating to urine gradually reduced its antimicrobial effects.

(A)



(B)

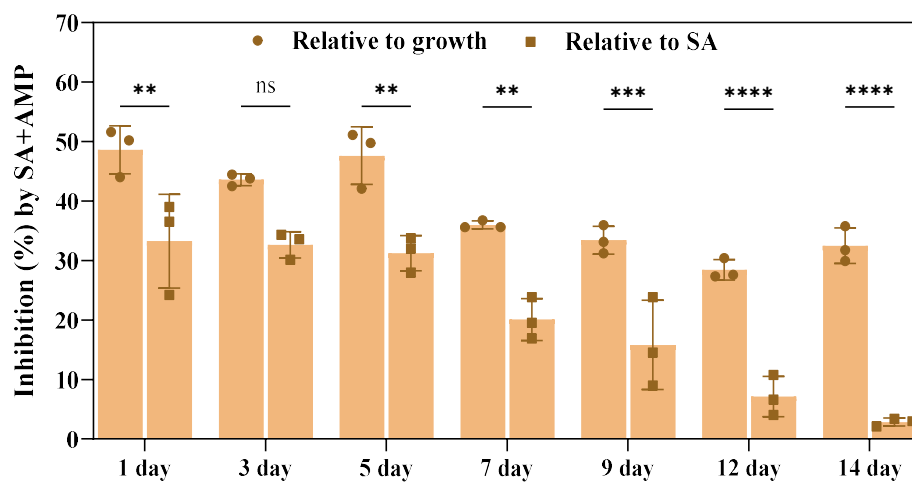


Figure 37. OD₆₀₀ of UPEC culture exposed to SA/LyeTx I mnΔK coating and SA coating after incubation in mammalian urine for 1, 3, 5, 7, 9, 12, and 14 days to assess coating stability (A). Bacterial growth inhibition by SA/LyeTx I mnΔK-coating relative to control and SA-coating (B). Data are presented as mean ± SD. Statistical significance is indicated by asterisks.

5. Discussion

The AMPs have emerged as potent antimicrobial agents derived from several nature sources (Yang *et al.*, 2025). AMPs have multi-antimicrobial mechanisms on the plasma membrane and intracellular targets, making them less prone to antimicrobial resistance (Xuan *et al.*, 2023) (Zhang *et al.*, 2022). AMPs derived from arthropods sources such as spiders have shown promising antimicrobial effects. Several AMPs have been isolated from venom of spiders including LyeTx I (Santos *et al.*, 2010) and its analogues such as LyeTx I-b (Reis *et al.*, 2018), LyeTx I-bPEG, (Brito *et al.*, 2021), LyeTx I mn, LyeTx I mn Δ K, and LyeTx I mn Δ KAc, (Fuscaldi *et al.*, 2021) showed broad spectrum antimicrobial effects against several drug-resistant pathogens (Fuscaldi *et al.*, 2021). Previously, LyeTx I mn Δ K showed antimicrobial effects against several pathogens including carbapenem-resistant *A. baumannii* (Lima *et al.*, 2021), MRSA (Vieira *et al.*, 2024a), and *C. albicans* (Cardoso *et al.*, 2022).

Additionally, interest in using AMPs coatings on medical implants such as urinary catheters also has been increased, which uses the natural antimicrobial properties of AMPs. The therapeutic efficacy of AMPs can be enhanced through conjugation with polymers which also increases their stability and regulates interactions with eukaryotic cells (Cui *et al.*, 2021). Several AMPs accommodated in AMP-polymer conjugates often retain their potent antimicrobial potential. Polymer based coatings can present antifouling properties, bactericidal, and can create bifunctional antibacterial polymer coatings, depending on the surface functionality. While considering the high incidence of CAUTI, significant research efforts are focused to produce infection free catheters and biocompatible catheters (Liu *et al.*, 2025).

Herein, in this study, it was decided to coat AMP LyeTx I mn Δ K on urinary catheters using the ultrasonic coating system to prevent microbial biofilms and catheters associated infections. Prior to coating, LyeTx I mn Δ K was assessed for MIC/MBC, antibiofilm effects in microplate model and catheters against quinolone resistant UPECs isolates and its interaction with membrane proteins of UPECs *in silico*. Additionally, LyeTx I mn Δ K was assessed for toxicity *in vitro* and *in vivo*. Furthermore, preliminary studies employed PLL in combination with PVA polymer to refine ultrasonication-based coating parameters and number of coating layers.

LyeTx I AMP showed MIC₅₀ of 4 μ M against ten isolates while the most highly active AMP was LyeTx I mn Δ K showed MIC₅₀ of 2 μ M. MIC less than 10 μ M of any natural

compound is considered good (Mbaveng *et al.*, 2015). The analogue, LyeTx I-b showed MIC₅₀ of 16 µM. Compared to prototype, the antimicrobial effects of LyeTx I-b were found to be decreased. LyeTx I mnΔK, a rationally designed derivative of LyeTx I obtained by truncating nine amino acid residues from the C-terminal region and introducing a lysine (K) residue at position 5, exhibited potent activity against UPEC, with a MIC₅₀ of 2 µM. Further, the acetylation of the *N*-terminal region of LyeTx I mnΔK generated Ac-LyeTx I mnΔK showed MIC₅₀ of 64 µM which is lower than LyeTx I mnΔK. Other modifications to LyeTx I mnΔK such as addition of a leucine residue to the *C*-terminal and the removal of the asparagine generated two analogues such as LyeTx I mnΔKL and LyeTx I mnΔKwN respectively, showed MIC₅₀ of 4 µM and 2 µM. The antimicrobials meropenem and colistin have MIC₅₀ of 375 nM and 92.5 nM respectively against all isolates tested, thus validating the experimental conditions.

Additionally, MBC studies were done for all the peptides to assess its bactericidal activity and all the peptides showed bactericidal activity in this case MBC ranging from 4 µM to 128 µM while the lowest MBC presented by LyeTx I mnΔK. The addition of lysine residue contributes to antimicrobial activity of LyeTx I mnΔK, which tends to be protonated at physiological pH. Therefore, it increases the peptide's overall positive charge (Cutrona *et al.*, 2015; Huan *et al.*, 2020; Yuan *et al.*, 2022; Ma *et al.*, 2024). Moreover, the antimicrobial effect of the peptides against quinolone-resistant UPECs increases by the presence of the leucine residue in the *C*-terminal region. Leucine is mostly present at the end of their primary sequence of the arthropods derived from natural AMPs (Li *et al.*, 1988; Park *et al.*, 1995). The biological activities of jelleine-I AMP were completely lost after removing leucine from its primary sequence (Ma *et al.*, 2024). Bactericidal compounds have the potential to treat infections effectively and are strong candidates to be used as alternative antimicrobials agents (Oliveira *et al.*, 2025; Sadeeq *et al.*, 2025). AMPs are bacteriolytic which kills the bacteria by acting on bacterial membranes (Sadeeq *et al.*, 2025). In past, LyeTx I mnΔK have shown potent antimicrobial effects against multi-drug-resistant bacteria including carbapenem-resistant *A. baumannii* against which this peptide showed MIC and MBC 1 to 16 µM and 2 to 32 µM, respectively (Lima *et al.*, 2021). A hydrogel formulated with LyeTx I mnΔK showed anti-MRSA effects *in vivo* studies and the MIC was 8 to 32 µM (Vieira *et al.*, 2024a). LyeTx I mnΔK also showed antifungal effects against fluconazole-resistant *C. albicans* through MIC between 4 and 32 µM (Cardoso *et al.*, 2022). Additionally, several studies reported that LyeTx I mnΔK is less prone to microbial resistance (Lima *et al.*, 2021; Vieira *et al.*, 2024; Cardoso *et al.*, 2022).

Pharmacokinetic parameters of the LyeTx I mn Δ K were evaluated through ADMET studies *in silico* (Okella *et al.*, 2022). ADMET scores are important for virtual screening of AMPs libraries (Okella *et al.*, 2022). The *in silico* ADMET analysis of LyeTx I mn Δ K indicated a low probability of adverse toxicological effects. The predicted AMES mutagenicity score was 0.008, while the carcinogenicity probability was 0.036. These results are like myxinidin peptide which showed predicted AMES mutagenicity values as 0.003 and carcinogenicity values as 0.062 (Okella *et al.*, 2022). The probability of drug-induced liver injury was negligible (0.000), and the predicted skin sensitization probability was 0.117. In terms of distribution, plasma protein binding was estimated at 23%. The predicted blood–brain barrier penetration was low, with a value of 0.018. Metabolism predictions indicated that LyeTx I mn Δ K is neither an inhibitor nor a substrate of CYP1A2. For CYP3A4, the peptide showed a low probability of inhibition (0.093). Regarding absorption, LyeTx I mn Δ K demonstrated high predicted human intestinal absorption with a value of 0.99. Overall, the *in silico* ADMET analysis suggests that LyeTx I mn Δ K predicted favorable pharmacokinetic characteristics with low predicted toxicity. Distribution analysis revealed a volume of distribution of 0.601, plasma protein binding of 22.25%, and minimal blood–brain barrier penetration (0.036). Metabolic predictions suggested negligible interaction with major cytochrome P450 enzymes, with CYP1A2 inhibitor and substrate values of 0.000 and 0.002.

Additionally, *in silico* analyses were performed to assess docking of LyeTx I mn Δ K with membrane protein, FimH of *E. coli*, which is one of the most important virulence factors in UPEC. FimH protein helps UPEC to adhere to bladder epithelium, colonize and form biofilms (Terlizzi *et al.*, 2017) also on catheters surface (Sarshar *et al.*, 2020; Werneburg, 2022). Novel antimicrobials or compounds with anti-UPEC potential are mostly FimH inhibitors FimH inhibitors (Han *et al.*, 2010; Sarshar *et al.*, 2020). The peptide showed interaction with FimH through hydrogen bonds with several amino acids such as Ser17, Gln59, Arg60, Arg132, and Gln143. Han *et al.* optimised a weak compound called (R-D-phenylmannoside) to design small molecules which shows binding with FimH by interaction with amino acids such as Tyr-48 and Arg-98 (Han *et al.*, 2010).

A lectin domain (FimHLD) present at the N-terminal region of FimH adhesion, which includes the carbohydrate-binding site responsible for its functional activity, basically its mannose-binding pocket consists of a hydrophobic region, a stretch of seven polar amino acids, the tyrosine gate, and the Thr51 amino acid residue. The FimH protein's affinity state is

modulated by conformational switching due to Gln59 residue found within the β -bulge (Rodriguez *et al.*, 2013). The high-affinity conformation of the binding pocket is stabilized by Gln143 (Rodriguez *et al.*, 2013). The FimH function is abrogated by mutation within the amino acids of the mannose-binding pocket (Sarshar *et al.*, 2020). The interactions of LyeTx I Δ mnAK with the residues Gln59 and Gln143 suggest that it may destabilize the FimH. With computational studies it was revealed that the C-terminal region has antibiofilm and anti-adhesive effects as it appears to be involved in binding to FimH protein. Two different approaches used to assess the affinity of FimH antagonists include competitive assays and inhibition of biological function assay (Sarshar *et al.*, 2020). Therefore, LyeTx I Δ mnAK was tested for its anti-biofilm activity against quinolone-resistant UPECs to determine its possible mechanism of action. LyeTx I Δ mnAK reduces the biomass and viability of UPEC biofilms in microplates and on catheters as well. Previously, anti-biofilm effects have been reported against the biofilms of *A. baumannii*. The biomasses were reduced upto 46.68% and 63.22% after 24 h and 48 h of incubation, respectively, at 32 μ M concentration (Lima *et al.* 2021). LyeTx I Δ mnAK at 16 μ M concentrations reduced the mature biofilms of MRSA (Vieira *et al.*, 2024a).

Some AMPs also have some affinity towards zwitterionic mammalian cells which make them toxic thus poses a major clinical challenge in clinical use (Greco *et al.*, 2020). The toxicity results of LyeTx I Δ mnAK showed that it is less toxic to kidney cells as its selectivity index was much lower compared to UPECs. In the past LyeTx I Δ mnAK showed less toxicity towards erythrocytes and Lund Human Mesencephalic (LUHMES) cells compared to prototype LyeTx I (Fuscaldi *et al.*, 2021). Additionally, the *in vivo* study showed that LyeTx I Δ mnAK does not appear toxic, does not cause macroscopic changes in the kidneys nor cause any death of animals. However, tubular degeneration was observed in histological observations of kidneys of animals which were treated with high dose (10 μ g/kg). It was suggested that peptides may interact with podocyte cells which form the epithelial lining of Bowman's capsule. Additionally, the animals treated with peptides showed reduced food intake and caloric intake compared with the control group. The animals treated with LyeTx I Δ mnAK showed reduced food intake ranging from 5.0 to 5.3 g/g of animal, while the control group exhibited the highest food intake (approximately 6.7 g/g of animal). The caloric consumption for the LyeTx I Δ mnAK treated animals ranges from approximately 15–16 kcal/g of animal while the control group consumed approximately 21 kcal/g of animal. Regarding the intake of macronutrients, carbohydrate intake was approximately 2.2 to 2.4 g/g of animal in peptide treated animals while in the control group it was $\sim$ 3.1 g/g of animal on the other hand, protein intake was 1.1–1.2 g/g of animal in the peptide

treated animals and 1.5 g/g of animal in the control group. Similarly, lipid intake in peptide treated animals was approximately 0.20–0.21 g/g of animal and around 0.27 g/g of animal in the control group. Overall, LyeTx I mnAK resulted in a reduction in total food intake, caloric consumption, and macronutrient intake. It may be speculated that the peptide could interfere with receptors, enzymes, or signalling pathways, which may lead to decreased cellular activity, protein expression, or assay signal. However, these proposed mechanisms require further experimental investigation. Lima et al. reported that LyeTx I treated animals has less water and food intake (Lima *et al.*, 2025).

The antimicrobial effects of LyeTx I mnAK reduced after exposure to urine showing the low stability of peptide which has still been a great concern in AMPs research. AMPs are degraded in presence of proteases. Previously, LyeTx I mnAK already showed low stability in presence of plasma peptidases and no stability in presence of trypsin (Lima et al. 2021). The structural modifications of peptides may solve this problem such as PEGylation, cyclization, amidation or acetylation or introduction of D-amino acids (Dijksteel *et al.*, 2021; Xuan *et al.*, 2023). Brito et al. and Fuscaldi et al. have worked on modifications of LyeTx I AMP family to enhance its stability by PEGylation and acetylation (Brito *et al.*, 2021; Fuscaldi *et al.*, 2021).

After the MIC, MBC, antibiofilm activity, anti-adhesive properties, and toxicity profile of LyeTx I mnAK, it was coated onto medical-grade silicone surfaces through SonoTek ExactaCoat Ultrasonic Coating System. Prior to LyeTx I mnAK coating, the equipment's parameters were optimized through coating the PVA/PLL solution on urinary catheter and flat silicon employing ultrasonic spraying technology that is known to deposit thin, homogeneous layers. A smooth and uniform coating of PVA/PLL and SA/LyeTx I mnAK without any defect was obtained, as shown in SEM images. Controlled droplet deposition and rapid evaporation allowed the uniform and streak-free coatings with consistent thickness. Ultrasonic spraying technology is efficiency in forming homogeneous layers, which made it an ideal technique for biomedical applications even for coating tubular surfaces such as catheters, where other surface modification techniques such as dip coating (Yu *et al.*, 2018), drop cast coating (Zhao *et al.*, 2020), and immersion processing (Maharubin *et al.*, 2019) suffers from poor thickness control, non-uniform coverage, and limited reproducibility (Chen *et al.*, 2006; Ourabi *et al.*, 2023). In addition to that, medical implants such as catheters required larger area modification, which is not possible through a dip coating method (Eslamian *et al.*, 2018). In ultrasonic spraying technology, flow rates and nozzle motion can be adjusted which enable the accurate tuning and

preservation of the desired polymer: peptide ratios, thus improving experimental reproducibility. This technique is also useful to form multi-layered coating (Goldsztajn *et al.*, 2024).

A consistent chemical composition was observed on PVA/PLL and SA/LyeTx I mnΔK coatings on all the surface as demonstrated by FTIR and contact angle measurements which ensure surface behaviour across the coating. Additionally, coating showed integrity and no delamination, cracking during handling or extended storage which indicate strong adhesion and no particulate entrapment and pinholes was found by surface analysis techniques such as SEM, EDS, and FTIR. Uniformity of antimicrobial coating plays an essential role in antimicrobial effects as microorganisms easily colonize on non-uniform surfaces using microenvironments (Yoda *et al.*, 2014). The wettability of the PVA and PVA/PLL coatings was high which is another factor contributing to anti-adhesive effects of the coated surface. PVA is responsible for hydrophilic properties of the coating (Jiang *et al.*, 2024a). Here, the contact angle of the pristine catheter was $176.4 \pm 2.0^\circ$ that reduces to approx. 70° on PVA coated catheter, and to $51.3 \pm 1.6^\circ$ on the surface of SA.

In this study hydrophilicity of SA based coatings was slightly decreased by addition of AMP LyeTx I mnΔK, but the final SA/LyeTx I mnΔK coating, with a water contact angle of $42.4 \pm 3.3^\circ$, should still be considered moderately hydrophilic. *E. coli* and *S. epidermidis* cannot grow efficiently on the surface with high wettability (Lorenzetti *et al.*, 2015; Verhorstert *et al.*, 2020). *S. epidermidis* easily adhere to hydrophobic surfaces compared to hydrophilic surfaces (Wassmann *et al.*, 2017), also hydrophilic surfaces resist proteins and contaminants (Rana *et al.*, 2010).

It has been reported that surfaces with an R_a between 0.1 and 0.35 μm exhibit minimal microbial adhesion, with no significant additional reduction below the proposed threshold of approximately 0.2 μm (Bollen *et al.*, 1996). Surface with roughness of $0.17 \pm 0.01 \mu\text{m}$ and $1.14 \pm 0.15 \mu\text{m}$ allow low and moderate microbial growth respectively, while the surface with roughness of $3.17 \pm 0.23 \mu\text{m}$ was found with highest microbial growth (Silva *et al.*, 2005). In this study, 400:1 coating ($R_a = 0.29 \pm 0.03 \mu\text{m}$) exhibited the lowest roughness and was the closest to the proposed threshold, whereas the 200:1 and 100:1 coating showed slightly higher R_a values of $0.42 \pm 0.05 \mu\text{m}$ and $0.60 \pm 0.05 \mu\text{m}$, respectively. The SA/LyeTx I mnΔK coating exhibited an aerial surface roughness (S_a) of $317.0 \pm 39.1 \text{ nm}$ ($0.317 \pm 0.039 \mu\text{m}$), which falls

within the range associated with reduced microbial adhesion. Nevertheless, antimicrobial performance of a coating does not only depend on the surface roughness but is also influenced by surface chemistry, functionalization, and the presence of antimicrobial agents. Bacterial adhesion may be reduced by specific micro/nanostructures on rough surfaces, as they can trap air pockets and decrease the bacteria-surface contact area (Fujii *et al.*, 2024). A hydration layer in PVA/PLL coatings induces antifouling characteristics. Interestingly, it has been reported that microbial adhesion can be low on hydrophilic substrates (Oh *et al.*, 2018) but also can be higher (Pranzetti *et al.*, 2012) therefore, it's suggested that single parameter of surface is not responsible for antimicrobial effects. The catheter with highest PLL content (100:1) was observed with lowest *E. coli* cells coverage (7.0 ± 0.3 %) among which most of them were dead cells; however, on PVA coated catheter cell coverage was 17.3 ± 0.8 %. The study showed that PLL targets microbial membranes as disruption of the cytoplasmic membrane of bacteria.

Previous studies already reported the antimicrobial effects of PLL coatings (Guo *et al.*, 2023; Wahid *et al.*, 2019). PLL brushes on silicone catheters showed anti-adhesive and bactericidal effects against *E. coli*, a most common pathogen of CAUTIs (Liu *et al.*, 2023). PLL is a membrane active antimicrobial agent (Hyldgaard *et al.*, 2014), however, also reported to induce oxidative stress (Ye *et al.*, 2013a). PLL electrostatically interferes with membranes bearing negatively charge resulting in destabilization of the membrane (Lebaudy *et al.*, 2023). It is proposed that a carpet-like mechanism is used by PLL to destabilize the membranes interacting with negatively charged membrane, which ultimately leads to partial removal of the outer membrane (Hyldgaard *et al.*, 2014). Zhang *et al.* (Zhang *et al.*, 2018) reported that ϵ -PL damages *E. coli* cell membrane that causes leakage of cytoplasmic contents. The ϵ -PLL coated catheter also showed broad spectrum potent antimicrobial effects against *S. aureus* and *E. coli* and low toxicity *in vivo* study (Yu *et al.*, 2019).

A larger and denser clump of *S. epidermidis* was observed on catheter with highest PLL concentrations that indicate it may promote cell-to-cell adhesion through interactions with surface proteins (Hyldgaard *et al.*, 2014), therefore, promoting bacterial aggregation and adhesion through this attraction, thereby influencing its overall antimicrobial activity (Morsin *et al.*, 2021). Additionally, Tan *et al.* (Tan *et al.*, 2019) showed that antimicrobial mechanism of PLL altered the structure of peptidoglycan layer. The suppression of metabolic pathways has been observed under ϵ -PL stress which impairs bacterial cellular function therefore, it is indicated that under ϵ -PL also interferes with metabolic pathway. PLL are less prone to

antimicrobial resistance and can be potentially used as alternative antimicrobial (Lebaudy *et al.*, 2023).

Bacterial cellulose functionalized with PLL created as wound dressing showed antimicrobial effects against *S. epidermidis* (Fürsatz *et al.*, 2018). In this study, a catheter with 100:1 coating (highest PLL concentrations, 0.2 mg/mL) inhibited the growth of *S. epidermidis* by 98.8 ± 0.5 %. A study reported complete growth inhibition of *S. epidermidis* and *E. coli* by PLL (Naghadeh *et al.*, 2017). Here, PLL coated catheters also showed antifungal effects against viable *C. albicans* as confirmed by their less viability and increased dispersion of hyphal branches that indicate stimulation of hyphal growth by PLL. Stress responses in *C. albicans* increases hyphal growth in response to antifungal agents or environmental stressors to avoid stress under antifungal pressure (Nobile *et al.*, 2015; Tosiano *et al.*, 2025). It has been observed that increasing hyphal growth in fungi is used as adaptation to antifungal stress that happens in the response to antifungal drugs such as azole drugs (Ha *et al.*, 1999). The antifungal mechanism of PLL is associated with interaction with fungal membranes through electrostatic interactions with their negatively charged fungal surface as reported by Padilla *et al.* (Padilla *et al.*, 2022). Additionally, membrane permeability decreased even more with high PLL concentration resulting in leakage of cations (Ca^{2+} and K^{+}) from the cytoplasm. The growth of *C. albicans* decreases the pH of the cultured medium while they observed pH decreased upon adding PLL, which disrupts fermentation and cellular respiration (Zhang *et al.*, 2023; Shu *et al.*, 2021). PLL also forms pores in fungal membranes (Tan *et al.*, 2019) and damages fungal morphology of fungi *Trichothecium roseum* (Jiao *et al.*, 2020; Liu *et al.*, 2020). Interestingly, it has been observed that PLL influences the genes which are important for cell wall proteins of fungi (Ye *et al.*, 2013b). ϵ -PL also produces intracellular reactive oxygen species as revealed by fluorometric analysis, which causes peroxidation of lipid and ultimately leads to membrane damage (Shu *et al.*, 2021).

The SA/LyeTx I $\text{mn}\Delta\text{K}$ -coated samples showed *E. coli* inhibition by $70.04 \pm 4.20\%$ relative to growth control, $56.29 \pm 5.86\%$ relative to uncoated sample, and $34.63 \pm 4.99\%$ relative to SA coating. The inhibition of *S. aureus* by SA/LyeTx I $\text{mn}\Delta\text{K}$ coating was $60.97 \pm 11.95\%$ relative to the positive control, $36.43 \pm 20.31\%$ relative to the uncoated surface, and $47.27 \pm 14.84\%$ relative to the SA coating. Additionally, SA/LyeTx I $\text{mn}\Delta\text{K}$ exhibited antibiofilm activity against common CAUTI-associated pathogens, including *S. epidermidis*, *P. aeruginosa*, *P. mirabilis*, and UPEC, reducing bacterial viability by 0.96 ± 0.32 , 0.61 ± 0.22 ,

0.80 $\pm$ 0.29, and 0.99 $\pm$ 0.08 log, respectively, compared with uncoated sample while the reductions compared to SA-coated samples were 0.95 $\pm$ 0.34, 0.62 $\pm$ 0.22, 0.90 $\pm$ 0.28, and 0.94 $\pm$ 0.09 log, respectively.

Janik et al. reported that SA-based films containing chestnut extract showed 7 to 8-fold reduction of *E. coli* and *S. epidermidis* growth in the recovered CFU/ml, respectively (Janik *et al.*, 2023). Additionally, SA films based incorporated with quaternary ammonium lignin–cinnamaldehyde composite particles showed inhibition rates of 96% against *S. aureus* and 65% against *E. coli* (Miao *et al.*, 2025). In this work, *in silico* study predicted that the C-terminal region of the LyeTx I mn Δ K has anti-adhesive effects and prevented UPEC growth on catheter. LyeTx I mn Δ K has shown broad spectrum antimicrobial results against several drug resistant clinically important pathogens. LyeTx I mn Δ K based formulations effectively treated the wounds. The biomedical applicability of LyeTx I mn Δ K was extended by investigating the peptide as a surface-bound antimicrobial agent, thereby exploring its application in antimicrobial coatings for medical implants.

Moreover, antimicrobial activity of the SA/LyeTx I mn Δ K coating was maintained till 5 days of incubation in urine and slight decrease was observed after 5 days. The 1-day incubated SA/LyeTx I mn Δ K coating exhibited significant inhibition relative to the growth control and 33.39 $\pm$ 7.88% relative to the SA coating. After 3 and 5 days of incubation, the coating maintained high antibacterial activity, after 7-14 days of incubation. It is suggested that prolonged exposure of the SA/LyeTx I mn Δ K coating to urine gradually reduced its antimicrobial efficacy.

This study showed that PVA/PLL based coatings are biocompatible with the tested cell lines as they showed viability more than 70 % in the presence of all coatings. Compared to PVA coating, cell viability was slightly higher in case of PVA/PLL based coatings. Similar biocompatibility results also have been reported in past studies of PLL or PVA (Alexandre *et al.*, 2014; Amariei *et al.*, 2018; Sun *et al.*, 2019b). Additionally, PLL-tethered catheters also showed biocompatibility *in vivo* studies as reported by Liu et al (Liu *et al.*, 2023). Patel et al. (Patel *et al.*, 2019) showed that Foley catheters coated with lysine showed biocompatibility along with antimicrobial effects. This study showed a slight drop in cell viability by increasing PLL concentrations. The number of amine groups of PLL influences its behaviour towards cells, is linked to its molecular weight and concentration (Cai *et al.*, 2012). PLL also reduced

the viability of L929 mouse fibroblasts, possibly due to the interaction of its polycationic chains with membrane (Fischer *et al.*, 2003). The cationic polymers with high molecular weights, cause cytotoxicity and necrosis due to their accumulation on the outer cell membrane. PLL, being positively charged, have more tendency to bind with negatively charged biological molecules. The interaction of polycations with membranes is determined by their molecular weight and charge density that govern the extent of cellular damage (Alinejad *et al.*, 2019). The molecular weight of PLL I used in this study was 150,000–300,000 g/mol. PLL with high molecular weight was selected for providing a high density of positively charged amine groups per polymer chain (Flynn *et al.*, 2026) that promote stronger electrostatic interactions and improve coating stability.

On the other hand, this study showed that SA/LyeTx I mnΔK based coatings are biocompatible with the tested lines as the cell viability was more than 70 %. Compared to uncoated samples and SA coated samples the viability of NHDFs was decreased in case of SA/LyeTx I mnΔK due to presence of antimicrobial peptide. The SA/LyeTx I mnΔK coating showed similar biocompatibility to the PVA/PLL coating. Cell viability was $84.9 \pm 8.1\%$ for the SA/LyeTx I mnΔK coating and $83 \pm 1\%$ for the PVA/PLL coating (100:1) containing the highest PLL concentration. The antimicrobial behaviour of AMPs is associated with the membrane, which may pose a risk of interacting with the mammalian membrane as well (Zhang *et al.*, 2021). Due to the presence and neutrally charge, mammalian cells may require a higher concentration of AMPs to be affected compared to the bacterial membrane. In its free form, LyeTx I mnΔK exhibited low toxicity toward kidney cells while maintaining potent activity against UPEC bacteria, resulting in a high selectivity index. In another study, LyeTx I mnΔK showed less toxicity towards erythrocytes and LUHMES cells compared to prototype LyeTx I (Fuscaldi *et al.* 2021). Additionally, the *in vivo* study of this work showed that LyeTx I mnΔK does not appear toxic and does not cause macroscopic changes in the kidneys. However, tubular degeneration was observed in histological observations of kidneys of animals which were treated with high dose.

In silico membrane binding studies, lysine was used to assess its interaction with membrane proteins of bacteria. The functional groups of lysine including terminal amine ($-\text{NH}_2$), the terminal carboxylic group ($-\text{COOH}$), and the side-chain amine group ($-\text{NH}_2$). The terminals $-\text{NH}_2$ and $-\text{COOH}$ groups are used during polymerization of polylysine and form peptide bonds. After polymerization, in PLL, for interaction only the side-chain amines remain

free and "inert" due to distinct functional group distribution. Also, there is an increase in positively charged groups in PLL due to presence of multiple repeating lysine units, which may improve the docking interactions. The terminal -NH₂ and -COOH groups are involved in docking interactions in case of lysine, which are absent in PLL, so docking interactions are driven by side-chain amines. Additionally, due to abundance of side-chain amines, which may increase the chances for interaction with negatively charged surfaces. The extended structure may cause steric hindrance, which may affect the docking interactions of PLL. But on another hand, the presence of numerous side-chain amines in PLL may result in multivalent binding capacity which allows simultaneous interaction with bacterial membranes. There are substantial challenges associated while work with complex molecules like PLL may require high computational costs and accurate structural models.

This work suggested that the coatings based on PLL do not allow microbial adhesion and could be potentially used to fabricate infection free catheters to avoid microbial biofilms CAUTI. The catheter with PVA/PLL in 100:1 significantly inhibited the growth of all tested microorganisms. The PLL based coatings could be translated to commercial applications by the biomedical industry to fabricate antimicrobial medical implants. Additionally, to increase antimicrobial breadth PLL based coatings; they could be combined with other antimicrobials including antifungal drugs, which may enhance the efficacy of both PLL and combined drugs (Lv *et al.*, 2022). For controlled release antimicrobial coatings PLL can be incorporated into micro- or nano scaffolds and advanced drug delivery systems can be developed by encapsulation in nanoparticles and films (Niaz *et al.*, 2019; Mousavi *et al.*, 2021).

This work has several limitations including lack of antimicrobial assessment of the peptide *in vivo* but *in vitro* thus, peptide's efficacy may influence by several urine composition, host immune responses and microbial biofilms. In mammalian urine, peptides exhibited lower stability due to proteolytic degradation, which required chemical optimization for translational potential. *In vivo* toxicity studies showed that at higher concentrations peptide showed moderate effects on kidney, suggesting long-term *in vivo* toxicity studies. *In vivo* toxicity of peptide was evaluated with a focus on the urinary tract and lacking direct evaluation of other organ systems. *In silico* studies, there were no controls in docking of the peptide with FimH as it was performed to provide only predictive evidence of interaction. Thus, *in vitro* mechanistic studies are required to validate this mechanism experimentally.

The limitations of PVA/PLL study include lacking antimicrobial tests *in vivo* as they were performed *in vitro*, which do not reflect therefore assessment of long-term antimicrobial testing and biocompatibility are important. The aqueous immersion, protein adsorption, and enzymes *in vivo* environment may affect the efficacy and stability of PLL based catheters therefore, it is necessary to validate the *in vitro* antimicrobial and biocompatibility results. Further investigations are important to assess the antibiofilm properties of PLL against the strains tested in this study. Moreover, SA/ LyeTx I Δ mnK was coated on medical-grade silicon substrates rather than a clinically relevant urinary catheter that may not reflect the actual antimicrobial and biological behaviour of the urinary catheter. The antimicrobial tests and biocompatibility tests were performed *in vitro*; therefore, *in vivo* experiments would be important to validate the antimicrobial effects and cytotoxicity of the coating materials. SA/LyeTx I Δ mnK was tested against only bacteria not fungi therefore, broad spectrum antimicrobial potential also needs to be assessed.

6. Chapter V: Conclusion and future directions

This work showed efficacy of LyeTx I mnΔK against UPECs. LyeTx I mnΔK did not show significant results against UPECs biofilm mass, however, was able to significantly reduce the viability of UPECs biofilms and prevented biofilm formation on urinary catheter. Since the computational studies showed that LyeTx I mnΔK bind with FimH protein, it may be playing a key role in preventing the UPEC to adhere and form biofilms on the surface of urinary catheters. The antimicrobial activity of LyeTx I mnΔK was reduces after incubation in urine which indicates the low stability in presence of proteases. *In vitro*, the peptide shows safety towards HEK-293 cells, while *in vivo* acute toxicity assay, it shows safety at concentrations of 1 μg/kg and 5 μg/kg but at higher concentration (10 μg/kg) it increases the serum creatinine levels and promotes mild tubular degeneration. However, none of the animals died during the *in vivo* toxicity assay. We suggest that the stability of the peptide could be improved and its renal toxicity at high concentrations of peptide can be decreased by structural modifications of the molecule in future studies. Overall, LyeTx I mnΔK stands out as a highly active antimicrobial against UPECs and anti-adhesive agent and can be potentially used as a prototype.

In the preliminary experiments for LyeTx I mnΔK coatings, PVA/PLL was coated on urinary catheters to optimize the coating process and equipment parameters. The PVA-PLL coated catheters presented excellent antimicrobial effects and biocompatibility. The coating process of PVA/PLL enables the successful coating of SA/LyeTx I mnΔK on silicon surfaces using the same technique. The areal surface roughness (S_a) of the SA/LyeTx I mnΔK coating falls within the surface roughness range reported in the literature to be associated with reduced microbial adhesion. SA/LyeTx I mnΔK coating inhibited the growth of *S. aureus* and *E. coli*, and CAUTI associated pathogens such as *S. epidermidis*, *P. aeruginosa*, *P. mirabilis*, and UPEC. SA/LyeTx I mnΔK coating also showed excellent biocompatibility. SA/LyeTx I mnΔK coating maintained high antimicrobial activity for up to 5 days of urine incubation. Although its efficacy gradually declined thereafter, the coating retained measurable antibacterial activity throughout the 14-day study. The bacterial biofilms reduction by LyeTx I mnΔK on catheters when co-incubated with bacteria and anti-adhesive and antibiofilm effects of LyeTx I mnΔK coated silicon sheets suggest that novel and infection free urinary catheters can be fabricated using this peptide. The overall coating process and number of LyeTx I mnΔK layers used in silicon coating provide a foundation for the subsequent translation of the coating on the urinary catheter. Long-term stability under physiological flow and *in vivo* evaluation of the LyeTx I

mnΔK coated catheters would need to be assessed against microbial infections and biofilms. Considering the *in silico* prediction of LyeTx I mnΔK–FimH binding obtained in the present study, together with the antibiofilm activity observed in both the microplate and catheter-based biofilm models and the anti-adhesive activity of the LyeTx I mnΔK-coated silicon surfaces, LyeTx I mnΔK–FimH interactions may contribute to the observed effects. However, this mechanism requires further validation. The successful clinical translation of the catheter would require emphasis on process optimization and scalable manufacturing to develop robust, reproducible, and cost-effective fabrication strategies to ensure consistent coating performance at an industrial scale. Further translational research could benefit from the current work to apply such coatings on the medical devices and implants.

7. Institutional review board statement

The animal study protocol was approved by the Ethics Committee of Faculdade Santa Casa de Belo Horizonte (protocol code 001/2023; date of approval: March 23, 2023).

8. Financial support

I would also like to thank the Doctoral School, Silesian University of Technology (SUT), Poland and the NAWA STER Programme for their financial support during my study abroad. I gratefully acknowledge the financial support provided by NAWA, including the A38/2022 – Programa de Desenvolvimento da Pós-Graduação – Parcerias Estratégicas nos Estados III (CAPES/FAPEMIG), project APQ-04900-22, National Council for Scientific and Technological Development (CNPq), Brazil, through the research project “Peptides Derived from Animal Venoms as Potent (Model) Drugs” (Project No. CNPq-310638/2023-2). I also gratefully acknowledge the financial support provided by the Minas Gerais State Agency for Research and Development (FAPEMIG), Brazil, through the research project “Synthetic Peptides Derived from Arthropod Secretions as Potent Anticancer and Antimicrobial Agents” (Project No. APQ-00754-24).

9. Chapter V: Research outputs

9.1. Internships

One year internship at Federal University of Minas Gerais, Belo Horizonte Brazil

Duration: 25 August 2023 to 13 July 2024

Funding source: Grant for at least 3-months internships in leading foreign research centers

Title: Antimicrobial activity of peptides derived from *Lycosa erythrognatha* spider venom against quinolone-resistant uropathogenic *Escherichia coli* and ADMET profiling in silico

Six weeks internship at Faculdade de Saúde Santa Casa de Belo Horizonte, Brazil

Duration: 22 August 2024 to 29 September 2024

Funding source: Best PhD scholarship's mobility grant

Title: Hands-on experience in conducting *in vivo* antimicrobial experiments

9.2. List of publications with Home University (UFMG)

1. Moraes, I. M. C#, **Haq, I. U#**., de Souza Monteiro, A., Araujo, D. P., da Silva, C. M., de Fátima, Â., Dos Santos, V. L. (2026). Synergistic antimicrobial and antiadhesive effects of benzazole derivatives and lactobacillus biosurfactants *in vitro*. Journal of Biotechnology. IF₂₀₂₆=4.00, MNiSW=70.
2. Oliveira, A.G.G#, **Haq, I.U#**., de Oliveira, P.L. et al (2025). Isolation and partial characterization of antimicrobial peptide-enriched fractions from Parabacteroides distasonis. *Scientific Reports*, 15, 43043. IF₂₀₂₆=4.90, MNiSW 140.
3. Dias-Souza, M.V., **Haq, I.U.**, Pagnin, S., Veiga, A.A., Dos Santos, V.L. (2025). Liposome-encapsulated antibiotics and biosurfactants: an effective strategy to boost biofilm eradication in cooling towers. *Microbial Cell Factories*, 24(1):135. IF₂₀₂₆=5.80, MNiSW=100.
4. Dias-Souza, M.V., Alves, A.L., Pagnin, S. **Haq, I.U.** et al. (2024). The activity of hydrolytic enzymes and antibiotics against biofilms of bacteria isolated from industrial-scale cooling towers. *Microbial Cell Factories*, 23, 282. IF₂₀₂₆=5.80, MNiSW=100.

5. Silva, C.P., Silva, C.L., **Haq, I.U.** et al. (2026). Phenotypic and genotypic antimicrobial resistance profiles of gram-positive cocci and non-fermenting gram-negative rods. *Scientific Reports*. IF₂₀₂₆=4.90, MNiSW=140.
6. Oliveira, A. G. G., Dias, M. F., **Ul Haq, I.**, Ferreira, J. F. G., Silva, C. P., Moreira, M., da Silva Lanna, M. C., Santos Rodrigues, L. d., de Mâcedo Farias, L., & Magalhães, P. P. (2026). Seasonal and source-associated microbiome dynamics in Brazilian drinking water. *Microbial Ecology*, 89. IF₂₀₂₆=4.30, MNiSW=100.

9.3. Conferences attended

1. **Poster Presentation:** Antimicrobial Effects of Sodium Alginate/Poly-L-lysine-Based Films and Their Deposition onto Urinary Catheters Using Ultrasonic Spray Coating **Haq, I.U.**, Abdullah, A., Ayaz, K., Szewczenko, J., and Krukiewicz. 2nd International Online Conference on Functional Biomaterials, (online), 8-10 July 2026.
2. **Invited lecture:** Presentations by doctoral students pursuing their doctorates in international cooperation. International Doctoral Week, Education and Congress Centre, Silesian University of Technology in Gliwice, Poland, 11-15 May, 2026.
3. **Poster presentation:** Antibiofilm Activity of LyeTx I mn Δ K, a Peptide Derived from the Venom of the Spider *Lycosa erythrognatha*, on Urinary Catheters and Its Toxicity Evaluation *In Vitro* and *In Vivo*. **Haq, I.U.**, Lima, W. G., Vieira, R. P., Krukiewicz, K., and de Lima, M.E. Gliwice Scientific Meetings, Gliwice, Poland, 20-21 November 2025.
4. **Oral presentation:** Poly(vinyl alcohol)/poly(L-lysine)-functionalised urinary catheters with potential antibacterial and antifungal effects. Maryam, S., **Haq, I.U.**, Wilk, K., Abdullah, A., Shakibania, S., Shyntum, D. Y., Student, S., Szewczenko, J., and Krukiewicz, K. Young Science Beyond Borders Conference (online), 23-24 October 2025.
5. **Invited lecture:** “Antibiofilm effects of LyeTx I mn Δ K, a synthetic peptide derived from *Lycosa erythrognatha* spider venom, against quinolone-resistant uropathogenic *Escherichia coli* (UPEC). International Meeting on Animal Venom Peptides With Therapeutic Properties Brasilia, Brazil, 11-14 December 2024.
6. **Poster presentation:** Antibiofilm effects of LyeTx I mn Δ K, a synthetic peptide derived from *Lycosa erythrognatha* spider venom, against quinolone-resistant uropathogenic *Escherichia coli* (UPEC). **Haq, I.U.**, Lima, W. G., Vieira, R. P., Krukiewicz, K., and de Lima, M.E. International Meeting on Animal Venom Peptides With Therapeutic Properties Brasilia, Brazil, 11-14 December 2024.

7. **Oral presentation:** Antimicrobial activity of polymers-functionalized urinary catheters against *Staphylococcus aureus*. **Haq, I. U.**, Shyntum, D. Y., Abdullah, A., Maryam, S., & Krukiewicz, K. 1st International Online Conference on Functional Biomaterials (online), 10-12 July 2024.
8. **Oral presentation:** Investigating the antimicrobial efficacy of polymer-coated urinary catheters: an integrated *in vitro* and in silico approach with ADMET profiling. Haq, I.U., Shyntum, D. Y., Abdullah, Maryam, S., Alam, Z., Krukiewicz, K. Computational Oncology and Personalized Medicine (online), 22nd May 2024. (Abstract awarded Third Place in the conference competition).
9. **Oral presentation:** Antimicrobial activity of peptides derived from *Lycosa erythrognatha* spider venom against quinolone-resistant uropathogenic *Escherichia coli* and ADMET profiling in silico. Haq, I.U, Lima, W. G., Krukiewicz, K., Vieira, R. P., and de Lima, M. E. Computational Oncology and Personalized Medicine (online) 22nd May 2024. (Abstract awarded **Second Place** in the conference competition).
10. **Poster presentation:** Antimicrobial Activity of Peptides Derived from *Lycosa erythrognatha* Spider Venom Against Quinolone-Resistant Uropathogenic *Escherichia coli* (UPECs). **Haq, I.U**, Lima, W. G., de Lima, M. E., Krukiewicz, K., and Vieira, R. P. 6th Edition of the World Congress on Infectious Diseases, Paris, France (Hybrid) 24-26 June 2024.
11. **Oral presentation:** Antimicrobial Effects of Polymers-Functionalized Urinary Catheters Against *Staphylococcus aureus*. **Haq, I.U.**, Shyntum, D. Y., Abdullah, A., Krukiewicz, K. 6th Annual International Conference on Advances in Materials Science (Hybrid) 29-30, November 2023.
12. **Poster presentation.** A Peptide Isolated from the Venom of *Lycosa erythrognatha* Spider Shows Potential to Treat Pneumonia Induced by KPC-Producing *Klebsiella pneumoniae* Without Detectable Resistance. **Haq, I. U.**, Lima, W. G., and de Lima, M. E. at the XVIII Congress of the Brazilian Society of Toxinology (SBTx). XIII Congress of the Panamerican section of the International Society of Toxinology, Brasilia, Brazil, 9-13 November 2023.

9.4. Participation in research projects

1. Research project titled Peptides Derived from Animal Venoms as Potent (Model) Drugs [01/08/2024 -10/04/2025] Funded by National Council for Scientific and Technological Development (CNPq), Brazil. Project Number: CNPq-310638/2023-2.
2. Research project titled Synthetic Peptides Derived from Arthropod Secretions as Potent Anticancer and Antimicrobial Agents [20/11/2023 -20/10/2024] Funded by the Minas Gerais State Agency for Research and Development (FAPEMIG), Brazil. Project Number: APQ-00754-24.

9.5. Research projects proposed for funding (not approved)

1. NCN Preludium: Development of antimicrobial peptides-polymer conjugates as medical implant coatings with potential, antimicrobial, anti-biofilm, and immunomodulatory effects. Project registration number: 2024/53/N/ST5/03176
2. IDUB proposal-Ground-breaking research: Optimization of immune responses on urinary catheters through polymer coatings: A way forward to control microbial infections and biofilms by reducing immune cells-mediated inflammatory reactions.

9.6. Awards and other achievements

1. Best PhD student award for the years of 2024 and 2025 (SUT).
2. Pro-quality award for highly-scored JCR publication in journal from TOP1 and TOP10 ranking lists (SUT) (2024).
3. Awarded a competitive scholarship recognizing outstanding PhD candidates pursuing their doctoral thesis under a co-tutelle (joint doctoral) agreement.
4. First Place in the Ph.D. Admission Selection, Postgraduate Program in Technological Innovations, Federal University of Minas Gerais (UFMG), Belo Horizonte, Brazil (2023).
5. Achieved a CAPES PDPG scholarship for Doctoral Research in Biological Sciences (Physiology and Pharmacology, Brazil (2023–2027)

10. References

- (BrCAST) BC on AST. Teste de Sensibilidade aos Antimicrobianos-Método de Difusão BrCAST-EUCAST. 2024.
- Abdal-hay A, Agour M, Kim Y, Lee M, Hassan MK, El-ainin HA, et al. Magnesium-particle/Polyurethane Composite Layer Coating on Titanium Surfaces for Orthopedic Applications. *Eur Polym J*. 2018; <https://doi.org/10.1016/j.eurpolymj.2018.10.012>
- Abo D, Enas A, Ahmed T. Biofilm - producing and carbapenems - resistant *Escherichia coli* nosocomial uropathogens : a cross - sectional study. *Int Microbiol*. 2024;27:1633–40. <https://doi.org/10.1007/s10123-024-00495-w>
- Aboul-enem, Y, Bunaciu, A, & Fleschin, S. Evaluation of the Protein Secondary Structures Using Fourier Transform Infrared Spectroscopy. *Gazi University Journal of Science*, 2014; 27(1), 637-644
- Adamus-Białek W, Lechowicz Ł, Kubiak-Szeligowska AB, Wawszczak M, Kamińska E, Chrapek M. A new look at the drug-resistance investigation of uropathogenic *E. coli* strains. *Mol Biol Rep*. 2017;44:191–202.
- Adegun PT, Odimayo MS, Olaogun JG, Emmanuel EE. Comparison of uropathogens and antibiotic susceptibility patterns in catheterized ambulant middle-aged and elderly nigerian patients with bladder outlet obstruction. *Turkish J Urol*. 2019;45:48–55.
- Agu PC, Afiukwa CA, Orji OU, Ezech EM, Ofoke IH, Ogbu CO, et al. Molecular docking as a tool for the discovery of molecular targets of nutraceuticals in diseases management. *Sci Rep*. 2023;1–18. <https://doi.org/10.1038/s41598-023-40160-2>
- Alan L. Kaitz and EJW. Bacteriuria and Urinary-Tract Infections in Hospitalized Patients List of authors. *N Engl J Med*. 1960;212:425–30.
- Alexandre N, Ribeiro J, Gärtner A, Pereira T, Amorim I, Fragoso J, et al. Biocompatibility and hemocompatibility of polyvinyl alcohol hydrogel used for vascular grafting - In vitro and in vivo studies. *J Biomed Mater Res - Part A*. 2014;102:4262–75.
- Ali M, van Gent ME, de Waal AM, van Doodewaerd BR, Bos E, Koning RI, et al. Physical and Functional Characterization of PLGA Nanoparticles Containing the Antimicrobial Peptide SAAP-148. *Int J Mol Sci*. 2023;24.

- Alinejad-Mofrad E, Malaekheh-Nikouei B, Gholami L, Mousavi SH, Sadeghnia HR, Mohajeri M, et al. Evaluation and comparison of cytotoxicity, genotoxicity, and apoptotic effects of poly-l-lysine/plasmid DNA micro- and nanoparticles. *Hum Exp Toxicol*. 2019;38:983–91.
- Alkarri S, Bin Saad H, Soliman M. On Antimicrobial Polymers: Development, Mechanism of Action, International Testing Procedures, and Applications. Vol. 16, *Polymers*. 2024.
- Allen RP, Bolandparvaz A, Manickam VA, Jamal S, States U. Latent, Immunosuppressive Nature of Poly(lactic-co-glycolic acid) Microparticles. *ACS Biomater Sci Eng*. 2018;4:900–18.
- Aloui H, Deshmukh AR, Khomlaem C, Kim BS. Novel composite films based on sodium alginate and gallnut extract with enhanced antioxidant , antimicrobial , barrier and mechanical properties. *Food Hydrocoll*. 2021;113:106508.
<https://doi.org/10.1016/j.foodhyd.2020.106508>
- Alshaikh SA, El T, Sonbol F, Farghali MH. Correlation between antimicrobial resistance , biofilm formation , and virulence determinants in uropathogenic *Escherichia coli* from Egyptian hospital. *Ann Clin Microbiol Antimicrob*. 2024;2:1–22.
<https://doi.org/10.1186/s12941-024-00679-2>
- Amariei G, Kokol V, Vivod VB, Letón P, Rosal R. Biocompatible antimicrobial electrospun nanofibers functionalized with ϵ -poly-l-lysine. *Int J Pharm*. 2018;553:141–8.
- Anjum S, Singh S, Benedicte L, Roger P, Panigrahi M. Biomodification Strategies for the Development of Antimicrobial Urinary Catheters : Overview and Advances. *Glob challenges*. 2018;2:1700068.
- Archana M, Rubini D, Priya K, Narayanan B, Hari V, Jayasankari S, et al. Development of an anti-infective urinary catheter composed of polyvinyl alcohol / sodium alginate / methylcellulose / polyethylene glycol by using a pressure-assisted 3D-printing technique. *Int J Biol Macromol*. 2023;249:1–12.
- Armbruster CE, Brauer AL, Humby MS, Shao J, Chakraborty S. Prospective assessment of catheter-associated bacteriuria clinical presentation, epidemiology, and colonization dynamics in nursing home residents. *JCI Insight*. 2021;6.
- Armugam A, Teong SP, Lim DSW, Chan SP, Yi G, Yew DS, et al. Broad spectrum

- antimicrobial PDMS-based biomaterial for catheter fabrication. 2021;1–13.
- Azevedo AS, Almeida C, Melo LF, Azevedo NF. Impact of polymicrobial biofilms in catheter-associated urinary tract infections. *Crit Rev Microbiol*. 2017;43:423–39. <http://dx.doi.org/10.1080/1040841X.2016.1240656>
- Bai G, Zhu Y, Cao H, Zhuo R, Li Y, Wang C, et al. Progress in Organic Coatings Fabrication of photocurable zwitterionic polymer coatings with enhanced antifouling and lubrication properties for urinary catheter applications. *Prog Org Coatings*. 2024;197:108748. <https://doi.org/10.1016/j.porgcoat.2024.108748>
- Benjamin Jung GJH. MacConkey Medium. 2024.
- Berendsen SA, van Doorn T, Blok BFM. Urinary catheterization from 1997 to 2018: a Dutch population-based cohort. *Ther Adv Urol*. 2021;13:1–9.
- Birder LA, Kullmann FA. Role of neurogenic inflammation in local communication in the visceral mucosa. *Semin Immunopathol*. 2018;40:261–79.
- Bollen, C. M., Papaioanno, W., Van Eldere, J., Schepers, E., Quirynen, M., & Van Steenberghe D. The influence of abutment surface roughness on plaque accumulation and peri-implant mucositis. *Clin Oral Implants Res*. 1996;7:201-211.
- Bračič M, Šauperl O, Strnad S, Kosalec I, Plohl O, Zemljič LF. Surface modification of silicone with colloidal polysaccharides formulations for the development of antimicrobial urethral catheters. *Appl Surf Sci*. 2019;463:889–99.
- Brito. JCM, L. WG, R. JM, Assis. DCS, B. D, E.d.Li M. Pegylated LyeTx I-b peptide is effective against carbapenem-resistant *Acinetobacter baumannii* in an in vivo model of pneumonia and shows reduced toxicity. *Int J Pharm*. 2021;609:121156.
- Bunduki GK, Heinz E, Phiri VS, Noah P, Feasey N, Musaya J. Virulence factors and antimicrobial resistance of uropathogenic *Escherichia coli* (UPEC) isolated from urinary tract infections: a systematic review and meta-analysis. *BMC Infect Dis*. 2021;21:1–13.
- Burton DC, Edwards JR, Srinivasan A, Fridkin SK, Gould C V. Trends in Catheter-Associated Urinary Tract Infections in Adult Intensive Care Units—United States, 1990–2007. *Infect Control Hosp Epidemiol*. 2011;32:748–56.
- Butement JT, Noel DJ, Bryant CA, Wilks SA, Eason RW. A light-guiding urinary catheter

- for the inhibition of *Proteus mirabilis* biofilm formation. *Front Microbiol.* 2022;13:1–13.
- Buxadera-Palomero J, Albó K, Gil FJ, Mas-Moruno C, Rodríguez D. Polyethylene glycol pulsed electrodeposition for the development of antifouling coatings on titanium. *Coatings.* 2020;10.
- Bysshe T, Gao Y, Al. H-HK et. Estimating the Additional Hospital Inpatient Cost and Mortality Associated With Selected Hospital-Acquired Conditions | Agency for Healthcare Research and Quality. Vol. 602, National Score Card Reports. 2017. <https://www.ahrq.gov/hai/pfp/haccost2017.html%0Ahttps://www.ahrq.gov/hai/pfp/haccost2017-results.html>
- Cai L, Lu J, Sheen V, Wang S. Optimal poly (L-lysine) grafting density in hydrogels for promoting neural progenitor cell functions. *Biomacromolecules.* 2012;13:1663-1674.
- Cai Y, Gu R, Dong Y, Zhao Q, Zhang K, Cheng C. Fabrication of antibacterial nanoparticle hydrogel coating for urinary catheters. *J Biomater Appl.* 2023;38:73–84.
- Cardoso BG, de Lima WG, Fernandes SOA, de Lima ME, Cardoso VN. Antifungal activity of a shortened analogue of the natural peptide LyeTx I isolated from the venom of the spider *Lycosa erythrognatha*. *Nat Prod Res.* 2022;37:759–763.
- Chang R, Greene MT, Chenoweth CE, Kuhn L, Shuman E, Rogers MAM, et al. Epidemiology of Hospital-Acquired Urinary Tract–Related Bloodstream Infection at a University Hospital. *Infect Control Hosp Epidemiol.* 2011;32:1127–9.
- Chen, C. H, Lu, T. K. Development and Challenges of Antimicrobial Peptides for Therapeutic Applications. *Antibiotics.* 2020;9:24.
- Chen MM, Zhang XM, Ma JP, Chen SC, Chen WX, Feng LF. Experimental study on film thickness and the problem of free surface film flow in dip coating. *Asia-Pacific J Chem Eng* 11(5), 695-704. 2006;11:695-704.
- Chen PC, Ho CH, Fan CK, Liu SP, Cheng PC. Antimicrobial Peptide LCN2 Inhibited Uropathogenic *Escherichia coli* Infection in Bladder Cells in a High-Glucose Environment through JAK/STAT Signaling Pathway. *Int J Mol Sci.* 2022;23:15763.
- Chen Y, Lai Y, Chang C, Tsai Y, Tang C, Jan J. Star-shaped polypeptides exhibit potent antibacterial activities. *Nanoscale.* 2019;11:11696–708.

- Chen Z, Wang X, Li L. Dual-functional PVA based microtubes: Leveraging natural lubrication and surface modification for antibacterial interventions. *Polymer (Guildf)*. 2026;344:129508.
- Cheng Y, Qin J, Huang Y, Wang T. The antimicrobial effects of PLGA microspheres containing the antimicrobial peptide OP-145 on clinically isolated pathogens in bone infections. *Sci Rep*. 2022;12:1–9. <https://doi.org/10.1038/s41598-022-18690-y>
- Chenoweth CE, Gould C V., Saint S. Diagnosis, management, and prevention of catheter-associated urinary tract infections. *Infect Dis Clin North Am*. 2014;28:105–19.
- Chenoweth CE, Saint S. Urinary Tract Infections. *Infect Dis Clin North Am*. 2011;25:103–15. <http://dx.doi.org/10.1016/j.idc.2010.11.005>
- Chu X, Yang F, Tang H. Recent Advance in Polymer Coatings Combating Bacterial Adhesion and Biofilm Formation†. *Chinese J Chem*. 2022;40:2988–3000.
- Corrado B, Cammarano A, Iacono S Dello, Renzi E, Moretta R, Mercurio ME, et al. A Comprehensive Review of Progress in Preventing Urinary Infections Associated with the Use of Urinary Catheters : A Dual Analysis of Publications and Patents. *Infect Dis Rep*. 2025;17:64.
- Corrigan N, Jung K, Moad G, Hawker CJ, ... Reversible-deactivation radical polymerization (Controlled/living radical polymerization): From discovery to materials design and applications. *Prog Polym* 2020; <https://www.sciencedirect.com/science/article/pii/S0079670020301040>
- Cresti L, Cappello G, Pini A. Antimicrobial Peptides towards Clinical Application — A Long History to Be Concluded. *Int J Mol Sci*. 2024;25:4870.
- Cui Z, Luo Q, Bannon MS, Gray VP, Bloom TG, Clore MF, et al. Molecular engineering of antimicrobial peptide (AMP)-polymer conjugates. *Biomater Sci*. 2021;9:5069–91.
- Curtin JJ, Donlan RM. Using Bacteriophages To Reduce Formation of Catheter-Associated Biofilms by *Staphylococcus epidermidis*. *Antimicrob Agents Chemother*. 2006;50:1268–75.
- Cutrona KJ, Kaufman BA, Figueroa DM, Elmore DE. Role of arginine and lysine in the antimicrobial mechanism of histone-derived antimicrobial peptides. *FEBS Lett*. 2015;589:3915–20.

- Dadi BR, Abebe T, Zhang L, Mihret A, Abebe W, Amogne W. Distribution of virulence genes and phylogenetics of uropathogenic *Escherichia coli* among urinary tract infection patients in Addis Ababa , Ethiopia. *BMC Infect Dis*. 2020;20:108.
- Dale E. Bjorling, Zun-Yi Wang WB. Models of Inflammation of the Lower Urinary Tract. *Neurourol Urodyn*. 2011;30:673–682.
- Daniels KR, Lee GC, Frei CR. Trends in catheter-associated urinary tract infections among a national cohort of hospitalized adults, 2001-2010. *Am J Infect Control*. 2014;42:17–22. <http://dx.doi.org/10.1016/j.ajic.2013.06.026>
- Dave RN, Joshi HM, Venugopalan VP. Novel Biocatalytic Polymer-Based Antimicrobial Coatings as Potential Ureteral Biomaterial: Preparation and In Vitro Performance Evaluation. *Antimicrob Agents Chemother*. 2011;55:845–53. <http://dx.doi.org/10.1128/aac.00477-10>
- Dedeić-Ljubović A, Hukić M. Catheter-related urinary tract infection in patients suffering from spinal cord injuries. *Bosn J Basic Med Sci*. 2009;9:2–9.
- Deming TJ. Synthesis of Side-Chain Modified Polypeptides. *Chem Rev*. 2016;16:786–808.
- Dickerson Mayes K, Schuur JD CS. Trends in Urinary Catheter Use by Indication in US Emergency Departments, 2002-2019. *JAMA*. 2022;328:1557–9.
- Dijksteel GS, Ulrich MMW, Middelkoop E, Boekema BKHL. Review : Lessons Learned From Clinical Trials Using Antimicrobial Peptides (AMPs). *Front Microbiol*. 2021;12:12:616979.
- Duan X, Xu Y, Zhang Z, Ma X, Wang C, Ma W, et al. Piezoelectrically-activated antibacterial catheter for prevention of urinary tract infections in an on-demand manner. *Mater Today Bio*. 2024;26:101089. <https://doi.org/10.1016/j.mtbio.2024.101089>
- Dudeck MA, Horan TC, Peterson KD, Allen-Bridson K, Morrell G, Pollock DA, et al. National Healthcare Safety Network (NHSN) Report, data summary for 2010, device-associated module. *Am J Infect Control*. 2011;39:798–816. <http://dx.doi.org/10.1016/j.ajic.2011.10.001>
- Duque-Sanchez L, Qu Y, Voelcker NH, Thissen H. Tackling catheter-associated urinary tract infections with next-generation antimicrobial technologies. *J Biomed Mater Res - Part A*. 2023;312–35.

- Duszyńska W, Rosenthal VD, Szczêsny A, Woznica E, Ulfik K, Ostrowska E, et al. Urinary tract infections in intensive care unit patients-a single-centre, 3-year observational study according to the INICC project. *Anaesthesiol Intensive Ther.* 2016;48:1–6.
- Ebenhan T, Gheysens O, Kruger HG, Zeevaart JR, Sathekge MM. Antimicrobial Peptides : Their Role as Infection-Selective Tracers for Molecular Imaging. *Biomed Res Int.* 2014;2014.
- Eslamian M, Soltani-Kordshuli F. Development of multiple-droplet drop-casting method for the fabrication of coatings and thin solid films. *J Coatings Technol Res.* 2018;15:271–80.
- Fasugba O, Gardner A, Mitchell BG, Mnatzaganian G. Ciprofloxacin resistance in community- and hospital-acquired *Escherichia coli* urinary tract infections: A systematic review and meta-analysis of observational studies. *BMC Infect Dis.* 2015;15. <http://dx.doi.org/10.1186/s12879-015-1282-4>
- Feneley RCL, Hopley IB, Wells PNT. Urinary catheters: History, current status, adverse events and research agenda. *J Med Eng Technol.* 2015;39:459–70.
- Fenner A. Antimicrobial peptide derived from moths can eradicate UPEC biofilms and could offer a novel therapeutic option. *Nat Rev Urol.* 2020;17:191.
- Fischer D, Li Y, Ahlemeyer B, Krieglstein J, Kissel T. In vitro cytotoxicity testing of polycations: Influence of polymer structure on cell viability and hemolysis. *Biomaterials.* 2003;24:1121–31.
- Flores-Mireles, A. L., Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: Epidemiology, mechanisms of infection and treatment options. *Nat Rev Microbiol.* 2015;13:269–84.
- Flores-Mireles A, Hreha TN, Hunstad DA. Pathophysiology, treatment, and prevention of catheter-associated urinary tract infection. *Top Spinal Cord Inj Rehabil.* 2019;25:228–40.
- Forsyth VS, Armbruster CE, Smith SN, Pirani A, Springman AC, Walters MS, et al. Rapid Growth of Uropathogenic *Escherichia coli* during Human Urinary Tract Infection. *MBio.* 2018;9:e00186-18.
- Fourcade C, Canini L, Lavigne JP, Sotto A. A comparison of monomicrobial versus

- polymicrobial *Enterococcus faecalis* bacteriuria in a French University Hospital. *Eur J Clin Microbiol Infect Dis*. 2015;34:1667–73.
- Fouts DE, Pieper R, Szpakowski S, Pohl H, Knobloch S, Suh MJ, et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. *J Transl Med*. 2012;10:1–17.
- Fürsatz M, Skog M, Sivilér P, Palm E, Aronsson C, Skallberg A, et al. Functionalization of bacterial cellulose wound dressings with the antimicrobial peptide ϵ -poly-L-Lysine. *Biomed Mater*. 2018;13.
- Fuscaldi LL, de Avelar Júnior JT, Dos Santos DM, Boff D, de Oliveira, V. L. S., Gomes K, de Lima ME. Shortened derivatives from native antimicrobial peptide LyeTx I: In vitro and in vivo biological activity assessment. *Exp Biol Med*. 2021;246:414–25.
- Gajdács M, Kárpáti K, Nagy ÁL, Gugolya M, Stájer A, Burián and K. Association between biofilm-production and antibiotic resistance in *Escherichia coli* isolates : A laboratory-based case study and a literature review. *Acta Microbiol Immunol Hung*. 2021;68:217–26.
- Galván EM, Mateyca C, Ielpi L. Role of interspecies interactions in dual-species biofilms developed in vitro by uropathogens isolated from polymicrobial urinary catheter-associated bacteriuria. *Biofouling*. 2016;32:1067–77.
<http://dx.doi.org/10.1080/08927014.2016.1231300>
- Gambrill B, Pertusati F, Shergill I, Hughes S, Prokopovich P. A novel multilayer antimicrobial urinary catheter material with antimicrobial properties. *Mater Adv*. 2025;6:1020–30.
- Gao. Q, Li X, Yu W, Jia F, Yao T, Jin Q, et al. Fabrication of Mixed-Charge Polypeptide Coating for Enhanced Hemocompatibility and Anti-Infective Effect. *ACS Appl Mater Interfaces*. 2020;12:2999–3010.
- Gao G, Cheng JTJ, Kindrachuk J, Hancock REW, Straus SK. Biomembrane Interactions Reveal the Mechanism of Action of Surface-Immobilized Host Defense. *Chem Biol*. 2012;19:199–209. <http://dx.doi.org/10.1016/j.chembiol.2011.12.015>
- Garcia EC, Brumbaugh AR, Mobley HL. Redundancy and Specificity of *Escherichia coli*

- Iron Acquisition Systems during Urinary Tract Infection □. *Infect Immun.* 2011;79:1225–35.
- Gaston JR, Andersen MJ, Johnson AO, Bair KL, Sullivan CM, Guterman LB, et al. Enterococcus faecalis polymicrobial interactions facilitate biofilm formation, antibiotic recalcitrance, and persistent colonization of the catheterized urinary tract. *Pathogens.* 2020;9:1–20.
- Gaston JR, Johnson AO, Bair KL, White AN, Armbruster CE. Polymicrobial interactions in the urinary tract: Is the enemy of my enemy my friend? *Infect Immun.* 2021;89.
- Gayani B, Dilhari A, Kottegoda N, Ratnaweera DR, Weerasekera MM. Reduced Crystalline Biofilm Formation on Superhydrophobic Silicone Urinary Catheter Materials. *ACS Omega.* 2021;6:11488–96.
- Givens CD, Wenzel RP. Clinical and economic consequences of nosocomial catheter-related bacteriuria. *J Urol.* 1980;124:646–8.
- Godoy-gallardo M, Mas-moruno C, Yu K, Manero JM, Gil FJ, Kizhakkedathu JN, et al. Antibacterial Properties of hLfl – 11 Peptide onto Titanium Surfaces : A Comparison Study Between Silanization and Surface Initiated Polymerization. *Bioacromoleculesmacromol.* 2014;16:483–96.
- Goldberg S, Doyle RJ, Rosenberg M. Mechanism of enhancement of microbial cell hydrophobicity by cationic polymers. *J Bacteriol.* 1990;172:5650–4.
- Goldsztajn K, Godzierz M, Hercog A, Włodowski M, Jaworska J, Jelonek K, et al. Properties of biodegradable polymer coatings with hydroxyapatite on a titanium alloy substrate. *Acta Bioeng Biomech.* 2024;26:121–32.
- Gottenbos B, Grijpma DW, van der Mei HC, Feijen J, Busscher HJ. Antimicrobial effects of positively charged surfaces on adhering Gram-positive and Gram-negative bacteria. *J Antimicrob Chemother.* 2001;48:7–13.
- Govindarajan DK, Kandaswamy K. Virulence factors of uropathogens and their role in host pathogen interactions. *Cell Surf.* 2022;8:100075.
<https://doi.org/10.1016/j.tcs.2022.100075>
- Greco I, Molchanova N, Holmedal E, Jenssen H, Hummel BD, Watts JL, et al. Correlation between hemolytic activity, cytotoxicity and systemic in vivo toxicity of synthetic

- antimicrobial peptides. *Sci Rep.* 2020;10:1–13. <https://doi.org/10.1038/s41598-020-69995-9>
- Guiton PS, Cusumano CK, Kline KA, Dodson KW, Han Z, Janetka JW, et al. Combinatorial small-molecule therapy prevents uropathogenic *Escherichia coli* catheter-associated urinary tract infections in mice. *Antimicrob Agents Chemother.* 2012;56:4738–45.
- Guiton PS, Hannan TJ, Ford B, Caparon MG, Hultgren SJ. *Enterococcus faecalis* overcomes foreign body-mediated inflammation to establish urinary tract infections. *Infect Immun.* 2013;81:329–39.
- Guiton PS, Hung CS, Hancock LE, Caparon MG, Hultgren SJ. Enterococcal biofilm formation and virulence in an optimized murine model of foreign body-associated urinary tract infections. *Infect Immun.* 2010;78:4166–75.
- Guo S, Zhang X, Ma R, Ge X, Shen H, Liang W, et al. Preparation and characterization of polyvinyl alcohol/glutaraldehyde cross-linked chitosan/ε-Polylysine degradable composite film and its antibacterial effect. *J Food Eng.* 2023;359:111698.
- Gupta P, Sarkar S, Das B, Bhattacharjee S, Tribedi P. Biofilm, pathogenesis and prevention—a journey to break the wall: a review. *Arch Microbiol.* 2016;198:1–15.
- Lima, G.W, Júlio L, Moreira C, Maria B, Lima E De, Cristina A, et al. A short synthetic peptide , based on LyeTx I from *Lycosa erythrognatha* venom , shows potential to treat pneumonia caused by carbapenem-resistant *Acinetobacter baumannii* without detectable resistance. *J Antibiot (Tokyo).* 2021;425–34. <http://dx.doi.org/10.1038/s41429-021-00421-6>
- Ha KC, White TC. Effects of azole antifungal drugs on the transition from yeast cells to hyphae in susceptible and resistant isolates of the pathogenic yeast *Candida albicans*. *Antimicrob Agents Chemother.* 1999;43:763–8.
- Ha Min K, Hee Kim K, Mi-Ran Ki, Seung PP. Antimicrobial Peptides and Their Biomedical Applications: A Review. *Antibiotics.* 2024;13:394.
- Hadifar S, Moghoofei M, Nematollahi S, Ramazanzadeh R. Epidemiology of Multidrug Resistant Uropathogenic *Escherichia coli* in Iran : a Systematic Review and Meta-Analysis. *Japanese J Infect Dis.* 2017;70:19–25.
- Hadjichristidis N, Iatrou H, Pitsikalis M, Sakellariou G. Synthesis of Well-Defined

- Polypeptide-Based Materials via the Ring-Opening Polymerization of α -Amino Acid N-Carboxyanhydrides. *Chem Rev.* 2009;109:5528–78.
- Halaji M, Fayyazi A, Rajabnia M, Zare D. Phylogenetic Group Distribution of Uropathogenic *Escherichia coli* and Related Antimicrobial Resistance Pattern : A Meta-Analysis and Systematic Review. *Front Cell Infect Microbiol.* 2022;12:790184.
- Han Z, Pinkner JS, Ford B, Obermann R, Nolan W, Wildman SA, et al. Structure-based drug design and optimization of mannoside bacterial fimH antagonists. *J Med Chem.* 2010;53:4779–92.
- Hartlieb M, Peltier R, Catrouillet S, Kuroki A, Sanchez-Cano C, Perrier S. Stimuli-responsive membrane activity of cyclicpeptide–polymer conjugates. *Chem Sci.* 2019;10:5476–83.
- Hashmi S, Kelly E, Rogers SO, Gates J. Urinary tract infection in surgical patients. *Am J Surg.* 2003;186:53–6.
- Herrera KMS, Lopes GFM, Oliveira ME, Sousa JF, Lima WG, Silva FK, et al. A 3-alkylpyridine-bearing alkaloid exhibits potent antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) with no detectable resistance. *Microbiol Res.* 2022;261:127073. <https://doi.org/10.1016/j.micres.2022.127073>
- Hicks RP, Abercrombie JJ, Wong RK, Leung KP. Bioorganic & Medicinal Chemistry Antimicrobial peptides containing unnatural amino acid exhibit potent bactericidal activity against ESKAPE pathogens. *Bioorg Med Chem.* 2013;21:205–14. <http://dx.doi.org/10.1016/j.bmc.2012.10.039>
- Hollenbeak CS, Schilling AL. The attributable cost of catheter-associated urinary tract infections in the United States: A systematic review. *Am J Infect Control.* 2018;46:751–7. <https://doi.org/10.1016/j.ajic.2018.01.015>
- Holling N, Lednor D, Tsang S, Bissell A, Campbell L, Nzakizwanayo J, et al. Elucidating the genetic basis of crystalline biofilm formation in *Proteus mirabilis*. *Infect Immun.* 2014;82:1616–26.
- Hooton TM, Bradley SF, Cardenas DD, Colgan R, Geerlings SE, Rice JC, et al. Diagnosis, prevention, and treatment of catheter-associated urinary tract infection in adults: 2009 international clinical practice guidelines from the infectious diseases society of America. *Clin Infect Dis.* 2010;50:625–63.

- Huan Y, Kong Q, Mou H, Yi H. Antimicrobial Peptides: Classification, Design, Application and Research Progress in Multiple Fields. *Front Microbiol.* 2020;11:1–21.
- Hyldgaard M, Mygind T, Vad BS, Stenvang M, Otzen DE, Meyer RL. The antimicrobial mechanism of action of epsilon-poly-L-lysine. *Appl Environ Microbiol.* 2014;80:7758–70.
- Ivanova A, Ivanova K, Tzanov T. Simultaneous ultrasound-assisted hybrid polyelectrolyte/antimicrobial peptide nanoparticles synthesis and deposition on silicone urinary catheters for prevention of biofilm-associated infections. *Nanomaterials.* 2021;11:3143.
- Ivanova K, Fernandes MM, Mendoza E, Tzanov T. Enzyme multilayer coatings inhibit *Pseudomonas aeruginosa* biofilm formation on urinary catheters. *Appl Microbiol Biotechnol.* 2015;99:4373–85.
- Jacobsen PAL, Rafaelsen J, Nielsen JL, Juhl M V, Theilgaard N, Larsen KL. Microporous and Mesoporous Materials Distribution of grafted β -cyclodextrin in porous particles for bone tissue engineering. *Microporous Mesoporous Mater.* 2013;168:132–41.
<http://dx.doi.org/10.1016/j.micromeso.2012.10.001>
- Jacobsen SM, Shirtliff ME. *Proteus mirabilis* biofilms and catheter-associated urinary tract infections. *Virulence.* 2011;2:460–5.
- Jacobsen SM, Stickler DJ, Mobley HLT, Shirtliff ME. Complicated catheter-associated urinary tract infections due to *Escherichia coli* and *Proteus mirabilis*. *Clin Microbiol Rev.* 2008;21:26–59.
- Janik W, Nowotarski M, Ledniowska K, Shyntum DY, Krukiewicz K, Turczyn R, et al. Modulation of physicochemical properties and antimicrobial activity of sodium alginate films through the use of chestnut extract and plasticizers. *Sci Rep.* 2023;13:1–14.
<https://doi.org/10.1038/s41598-023-38794-3>
- Jiang J, Zhang W, Yi X, Lei Q, Liao Y, Tan Y, et al. Recent progress in properties and application of antibacterial food packaging materials based on polyvinyl alcohol. *E-Polymers.* 2024a;24.
- Jiang R, Liu XY, Gao S, Kang K, Ding X, Wu D, et al. A scalable and universal strategy for constructing long-term antibacterial coatings with lubricant property on medical

- catheters. *Prog Org Coatings*. 2024b;196:108738.
<https://doi.org/10.1016/j.porgcoat.2024.108738>
- Jiao W, Liu X, Chen Q, Du Y, Li Y, Yue F, et al. Epsilon-poly-L-lysine (ϵ -PL) exhibits antifungal activity in vivo and in vitro against *Botrytis cinerea* and mechanism involved. *Postharvest Biol Technol*. 2020;168:111270.
<https://doi.org/10.1016/j.postharvbio.2020.111270>
- Kakaria BA, K. A, Tushar R. Study of incidence and risk factors of urinary tract infection in catheterised patients admitted at tertiary care. *Int J Res Med Sci*. 2018;6:1730.
- Kalirajan C, Dukle A, Nathanael AJ, Oh T. A Critical Review on Polymeric Biomaterials for Biomedical Applications. 2021;1–27.
- Kalkan S, Yilmaz Ş, Selahaddin Ç, Akben B, Remzi M. Effect of Incorporation of Mix Probiotic Culture in Sodium Alginate Film Characteristics : Antimicrobial , Physiochemical , Mechanical , and Barrier Properties. *Probiotics Antimicrob Proteins*. 2025;17:3774–89. <https://doi.org/10.1007/s12602-025-10651-x>
- Kalsy M, Tonk M, Hardt M, Dobrindt U, Zdybicka-Barabas A, Cytrynska M, et al. The insect antimicrobial peptide cecropin A disrupts uropathogenic *Escherichia coli* biofilms. *npj Biofilms Microbiomes*. 2020;6. <http://dx.doi.org/10.1038/s41522-020-0116-3>
- Kang H, Lee SJ, Ko IK, Kengla C, Yoo JJ, Atala A. A 3D bioprinting system to produce human-scale tissue constructs with structural integrity. *Nat Biotechnol*. 2016;34:312–9. <http://dx.doi.org/10.1038/nbt.3413>
- Kazemzadeh-Narbat M, Cheng H, Chabok R, Alvarez MM, de la Fuente-Nunez C, Phillips KS, et al. Strategies for antimicrobial peptide coatings on medical devices: a review and regulatory science perspective. *Crit Rev Biotechnol*. 2020;41:94–120.
<https://doi.org/10.1080/07388551.2020.1828810>
- Kazemzadeh-Narbat M, Kindrachuk J, Duan K, Jenssen H, Hancock REW, Wang R. Antimicrobial peptides on calcium phosphate-coated titanium for the prevention of implant-associated infections. *Biomaterials*. 2010;31:9519–26.
<http://dx.doi.org/10.1016/j.biomaterials.2010.08.035>
- Kazemzadeh-narbat M, Noordin S, Masri BA, Garbuz DS, Duncan CP, Hancock REW, et al. Drug release and bone growth studies of antimicrobial peptide-loaded calcium

- phosphate coating on titanium. *J Biomed Mater Res Part B Appl Biomater.* 2012;100:1344–1352.
- Kennedy EH, Greene MT, Saint S. Estimating hospital costs of catheter-associated urinary tract infection. *J Hosp Med.* 2013;8:519–22.
- Klekiel T, Mackiewicz A, Kaczmarek-Pawelska A, Skonieczna J, Kurowiak J, Piasecki T, et al. Novel design of sodium alginate based absorbable stent for the use in urethral stricture disease. *J Mater Res Technol.* 2020;9:9004–15.
- Knoll BM, Wright D, Ellingson L, Kraemer L, Patire R, Kuskowski MA, et al. Reduction of inappropriate urinary catheter use at a veterans affairs hospital through a multifaceted quality improvement project. *Clin Infect Dis.* 2011;52:1283–90.
- Köves B, Magyar A, Tenke P. Spectrum and antibiotic resistance of catheter-associated urinary tract infections. *GMS Infect Dis.* 2017;5:Doc06.
<http://www.ncbi.nlm.nih.gov/pubmed/30671328>
<http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC6301742>
- Kozakov D, Hall DR, Xia B, Porter KA, Padhorny D, Yueh C, et al. The ClusPro web server for protein – protein docking. *Nat Protoc.* 2017;12:255–78.
- Kranjec C, Puthiarambil J, Ovchinnikov K, Fadayomi I, Yang Y, Kjos M, et al. Biofilm A bacteriocin-based coating strategy to prevent vancomycin-resistant *Enterococcus faecium* biofilm formation on materials of interest for indwelling medical devices. *Biofilm.* 2024;8:100211. <https://doi.org/10.1016/j.biofilm.2024.100211>
- Kricheldorf HR. Polypeptides and 100 Years of Chemistry of a -Amino Acid N - Carboxyanhydrides *Angewandte. Angew Chemie Int Ed.* 2006;45:5752–84.
- Kumar AM, Yusuf A, Hussein MA, Umoren SA, Ramakrishna S, Saravanan S. Preparation and characterization of Pectin / Polypyrrole based multifunctional coatings on TiNbZr alloy for orthopaedic applications. *Carbohydr Polym.* 2020a;242:116285.
<https://doi.org/10.1016/j.carbpol.2020.116285>
- Kumar K, Her C, Comune M, Moia C, Lopes A, Porporato PE, et al. PLGA nanoparticles loaded with host defense peptide LL37 promote wound healing. *J Control Release.* 2014;194:138–47. <http://dx.doi.org/10.1016/j.jconrel.2014.08.016>
- Kumar R, Diep DB, Hjorth H. Topical antimicrobial peptide formulations for wound

- healing : Current developments and future prospects. *Acta Biomater.* 2020b;103:52–67.
- Kumari S, Reddy H, Beeran Y, Kumar K, Ponnammam D, Douglas TEL, et al. Surface functionalization of chitosan as a coating material for orthopaedic applications : A comprehensive review. *Carbohydr Polym.* 2021;255:117487.
<https://doi.org/10.1016/j.carbpol.2020.117487>
- Kurowiak J, Kaczmarek-Pawelska A, Mackiewicz AG, Bedzinski R. Analysis of the degradation process of alginate-based hydrogels in artificial urine for use as a bioresorbable material in the treatment of urethral injuries. *Processes.* 2020;8:304.
- Laan BJ, Maaskant JM, Spijkerman IJB, Borgert MJ, Godfried MH, Pasmooij BC, et al. De-implementation strategy to reduce inappropriate use of intravenous and urinary catheters (RICAT): a multicentre, prospective, interrupted time-series and before and after study. *Lancet Infect Dis.* 2020;20:864–72. [http://dx.doi.org/10.1016/S1473-3099\(19\)30709-1](http://dx.doi.org/10.1016/S1473-3099(19)30709-1)
- Lai Y. Small Molecule HIV-1 Attachment Inhibitors: Discovery, Mode of Action and Structural Basis of Inhibition. *Viruses.* 2021;13:843.
- Lam SJ, Brien-simpson NMO, Pantarat N, Sulistio A, Wong EHH, Chen Y, et al. Combating multidrug-resistant Gram-negative bacteria with structurally nanoengineered antimicrobial peptide polymers. *Nat Microbiol.* 2016;1:16162.
- Learman BS, Brauer AL, Eaton KA, Armbruster CE. A rare opportunist, *Morganella morganii*, decreases severity of polymicrobial catheter-associated urinary tract infection. *Infect Immun.* 2020;88.
- Lebaudy E, Guilbaud-chéreau C, Frisch B, Vrana NE, Laval P. The High Potential of ϵ -Poly-L-Lysine for the Development of Antimicrobial Biomaterials. *Adv NanoBiomed Res.* 2023;3:2300080.
- Leendertse M, Willems RJL, Giebelen IAJ, Van Den Pangaart PS, Bonten MJM, Van Der Poll T. The acute-phase response impairs host defence against *Enterococcus faecium* peritonitis. *Immunology.* 2009;128:335–42.
- Lehmann W, Roggenbuck D, Zinke O, Ansorge H, Vogel S, Klemm P, et al. Adhesion of Human and Animal *Escherichia coli* Strains in Association with Their Virulence-Associated Genes and Phylogenetic. *Appl Environ Microbiol.* 2013;79.
- Li G, Lai Z, Shan A. Advances of Antimicrobial Peptide-Based Biomaterials for the

- Treatment of Bacterial Infections. *Adv Sci*. 2023;10:1–23.
- Li J, Yang H, Cai Y, Gu R, Chen Y, Wang Y, et al. Ag quantum dots-doped poly (vinyl alcohol)/chitosan hydrogel coatings to prevent catheter-associated urinary tract infections. *Int J Biol Macromol*. 2024;282:136405.
<https://doi.org/10.1016/j.ijbiomac.2024.136405>
- Li P, Zhou C, Rayatpisheh S, Ye K, Poon Y, Hammond P, et al. Cationic Peptidopolysaccharides Show Excellent Broad-Spectrum Antimicrobial Activities and High Selectivity. *Adv Mater*. 2012;24:4130–47.
- Li Z qu, Merrifield RB, Boman IA, Boman HG. Effects on electrophoretic mobility and antibacterial spectrum of removal of two residues from synthetic sarcotoxin IA and addition of the same residues to cecropin B. *FEBS Lett*. 1988;231:299–302.
- Lim K. Antimicrobial peptides engineering and their immobilization studies for catheter coating development. Doctoral thesis, Nanyang Technological University, Singapore. 2015.
- Lim K, Saravanan R, Chong KKL, Goh SHM, Chua RRY, Tambyah PA, et al. Anhydrous polymer-based coating with sustainable controlled release functionality for facile, efficacious impregnation, and delivery of antimicrobial peptides. *Biotechnol Bioeng*. 2018;115:2000–12.
- Lima, WG, AS F, FR da SS, F de LT, AN de S, et al. Highlighting the Potential of LyeTx I, a Peptide Derived from the Venom of the Spider *Lycosa erythrognatha*, as a Potential Prototype for the Development of a New Antimicrobial Against Carbapenem-Resistant *Klebsiella pneumoniae*. *Pharmaceuticals*. 2025;18:679.
- Lima WG, Brito JCM, de Lima ME, Pizarro A, Vianna, M. A. M. D. M., de Paiva MC. A short synthetic peptide, based on LyeTx I from *Lycosa erythrognatha* venom, shows potential to treat pneumonia caused by carbapenem-resistant *Acinetobacter baumannii* without detectable resistance. *J Antibiot (Tokyo)*. 2021;74:425-434.
- Lin L, Chi J, Yan Y, Luo R, Feng X, Zheng Y, et al. Membrane-disruptive peptides/peptidomimetics-based therapeutics: Promising systems to combat bacteria and cancer in the drug-resistant era. *Acta Pharm Sin B*. 2021;11:2609–44.
<https://doi.org/10.1016/j.apsb.2021.07.014>

- Lin P, Ni H, Wang W, Sun C, Xing S, Xi J, et al. Fabrication of highly porous polymeric coatings loaded with rifampicin for preventing catheter-associated urinary tract infections. *Prog Org Coatings*. 2025;203:109190.
<https://doi.org/10.1016/j.porgcoat.2025.109190>
- Lin Z, Wu T, Wang W, Li B, Wang M, Chen L, et al. International Journal of Biological Macromolecules Biofunctions of antimicrobial peptide-conjugated alginate / hyaluronic acid / collagen wound dressings promote wound healing of a mixed-bacteria-infected wound. *Int J Biol Macromol*. 2019;140:330–42.
<https://doi.org/10.1016/j.ijbiomac.2019.08.087>
- Ling LL, Schneider T, Peoples AJ, Spoering AL, Engels I, Conlon BP, et al. A new antibiotic kills pathogens without detectable resistance. *Nature*. 2015;517:455–459.
- Liu H, Chen J, Xia Z, An M, Wu Y. Effects of ϵ -poly-L-lysine on vegetative growth, pathogenicity and gene expression of *Alternaria alternata* infecting *Nicotiana tabacum*. *Pestic Biochem Physiol*. 2020;163:147–53. <https://doi.org/10.1016/j.pestbp.2019.11.005>
- Liu H, Zhu J, Hu Q, Rao X. *Morganella morganii*, a non-negligent opportunistic pathogen. *Int J Infect Dis*. 2016;50:10–7. <http://dx.doi.org/10.1016/j.ijid.2016.07.006>
- Liu J, Xiao J, Li F, Shi Y, Li D, Huang Q. Chitosan-sodium alginate nanoparticle as a delivery system for ϵ -polylysine: Preparation, characterization and antimicrobial activity. *Food Control*. 2018;91:302–10.
- Liu J, Xu Y, Lin X, Ma N, Zhu Q, Yang K, et al. Immobilization of poly-L-lysine brush via surface initiated polymerization for the development of long-term antibacterial coating for silicone catheter. *Colloids Surfaces B Biointerfaces*. 2023;221.
- Liu L, Wang K, Yan Q, Yuan S. Durable antibacterial polyurethane based on crosslinked epsilon-poly-l-lysine for urinary catheter coating. *Prog Org Coatings*. 2026;213:109921.
- Liu X, Kamperman M. Smart bacteria-responsive coatings for combating catheter-associated urinary tract infections. *Mater Today Bio*. 2025;34:102191.
<https://doi.org/10.1016/j.mtbio.2025.102191>
- Lo E, Nicolle LE, Coffin SE, Gould C, Maragakis LL, Meddings J, et al. Strategies to Prevent Catheter-Associated Urinary Tract Infections in Acute Care Hospitals: 2014 Update. *Infect Control Hosp Epidemiol*. 2014;35:464–79.

- Lorenzetti M, Dogša I, Stošicki T, Stopar D, Kalin M, Kobe S, et al. The influence of surface modification on bacterial adhesion to titanium-based substrates. *ACS Appl Mater Interfaces*. 2015;7:1644–51.
- Low J le, Kao PH, Tambyah PA, Liang G, Koh E, Ling H, et al. Development of a polymer-based antimicrobial coating for efficacious urinary catheter protection. *Biotechnol Notes*. 2021;2:1–10. <https://doi.org/10.1016/j.biotno.2020.12.001>
- Luong HX, Thanh TT, Tran TH. Antimicrobial peptides—Advances in development of therapeutic applications. *Life Sci*. 2020;260:118407.
- Lv Z, Lu Y, Li B, Shi L, Zhang K, Ge B. Effects of ϵ -Poly-L-Lysine Combined with Wuyiencin as a Bio-Fungicide against *Botrytis cinerea*. *Microorganisms*. 2022;10:1–17. f
- Ma X, Wang Q, Ren K, Xu T, Zhang Z, Xu M, et al. A Review of Antimicrobial Peptides : Structure , Mechanism of Action , and Molecular Optimization Strategies. *Fermentation*. 2024;10:540.
- Macleod SM, Stickler DJ. Species interactions in mixed-community crystalline biofilms on urinary catheters. *J Med Microbiol*. 2007;56:1549–57.
- Magill SS, Edwards JR, Bamberg W, Beldavs ZG, Dumyati G, Kainer MA, et al. Multistate Point-Prevalence Survey of Health Care–Associated Infections. *N Engl J Med*. 2014;370:1198–208.
- Maharubin S, Nayak C, Phatak O, Kurhade A, Singh M, Zhou Y, et al. Polyvinylchloride coated with silver nanoparticles and zinc oxide nanowires for antimicrobial applications. *Mater Lett*. 2019;249:108–11. <https://doi.org/10.1016/j.matlet.2019.04.058>
- Mahlapuu M, Björn C, Ekblom J. Critical Reviews in Biotechnology Antimicrobial peptides as therapeutic agents : opportunities and challenges. *Crit Rev Biotechnol*. 2020;40:978–92. <https://doi.org/10.1080/07388551.2020.1796576>
- Maki DG, Tambyah PA. Engineering out the risk for infection with urinary catheters. *Emerg Infect Dis*. 2001;7:342–7.
- Maleki Dizaj S, Salatin S, Khezri K, Lee JY, Lotfipour F. Targeting Multidrug Resistance With Antimicrobial Peptide-Decorated Nanoparticles and Polymers. *Front Microbiol*. 2022;13.

- Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C. Urinary Tract Infections: The Current Scenario and Future Prospects. *Pathogens*. 2023;12.
- Margaret A. Dudeck TCH, Kelly D. Peterson KA-B, Gloria Morrell, Angela Anttila Daniel A. Pollock and JRE. National Healthcare Safety Network (NHSN) Report, Data Summary for 2011, Device-associated Module. Atlanta; 2013.
- Marie E, Lin J, Lay CL, Subramanian GS, Tan WS, Su S, et al. Control Release Coating for Urinary Catheters with Enhanced Released Profile for Sustained Antimicrobial Protection. *ACS Appl Mater Interfaces*. 2021;
- Martín-gutiérrez G, Rodríguez-beltrán J, Rodríguez-martínez, José.m Manuel Costas C, Aznar J. Resistance in Low-Level-Quinolone-Resistant *Escherichia coli*. *Antimicrob Agents Chemother*. 2016;60:4252–8. <http://www.ncbi.nlm.nih.gov/pubmed/27139482>
- Maryam S, Haq I,U, Wilk K, Abdullah, Shakibania S, Shyntum DY, et al. Antimicrobial efficacy of ultrasonically deposited poly(vinyl alcohol)/poly(L-lysine)-based coatings for urinary catheters. *Surf Coatings Technol*. 2026;521:133094. <https://doi.org/10.1016/j.surfcoat.2025.133094>
- Massoumi B, Sarvari R, Fakhri E. Silicon surfaces coated with polydopamine and poly (2-hydroxyethyl methacrylate) for medical device applications. *Polym Bull*. 2024; <https://doi.org/10.1007/s00289-024-05353-3>
- Matsukawa M, Kunishima Y, Takahashi S, Takeyama K, Tsukamoto T. Bacterial colonization on intraluminal surface of urethral catheter. *Urology*. 2005;65:440–4.
- Maturana P, Martinez M, Noguera ME, Santos NC, Disalvo EA, Semorile L, et al. Colloids and Surfaces B : Biointerfaces Lipid selectivity in novel antimicrobial peptides : Implication on antimicrobial and hemolytic activity. *Colloids Surfaces B Biointerfaces*. 2017;153:152–9. <http://dx.doi.org/10.1016/j.colsurfb.2017.02.003>
- Mbaveng AT, Sandjo LP, Tankeo SB, Ndifor AR, Pantaleon A, Nagdju BT, et al. Antibacterial activity of nineteen selected natural products against multi-drug resistant Gram-negative phenotypes. *Springerplus*. 2015;4:1–9.
- Mcquiston Haslund J, Rosborg Dinesen, M., Sternhagen Nielsen AB, Llor C, Bjerrum L. Different recommendations for empiric first-choice antibiotic treatment of uncomplicated urinary tract infections in Europe. *Scand J Prim Health Care*.

2013;31:235–40.

- Messina MS, Messina KMM, Bhattacharya A, Montgomery HR, Maynard HD. Preparation of biomolecule-polymer conjugates by grafting-from using ATRP , RAFT , or ROMP. *Prog Polym Sci.* 2020;100:101186. <https://doi.org/10.1016/j.progpolymsci.2019.101186>
- Miao J, Lai Y, Zhang Y, Wei J, Fan K, Sun N, et al. Sodium Alginate-Based Antibacterial Coatings Reinforced with Quaternized Lignin–Cinnamaldehyde Composite Particles for Fruit Preservation. *Foods.* 2025;14:1–21.
- Miao J, Wu X, Fang Y, Zeng M, Huang Z, Ouyang M, et al. Multifunctional hydrogel coatings with high antimicrobial loading efficiency and pH-responsive properties for urinary catheter applications. *J Mater Chem B.* 2023;11:3373–86.
- Milo S, Nzakizwanayo J, Hathaway HJ, Jones B V., Jenkins ATA. Emerging medical and engineering strategies for the prevention of long-term indwelling catheter blockage. *Proc Inst Mech Eng Part H J Eng Med.* 2019;233:68–83.
- Mishra B, Basu A, Chua R, Saravanan R, Tambyah PA, Ho B, et al. Site specific immobilization of a potent antimicrobial peptide onto silicone catheters: evaluation against urinary tract infection pathogens. *J Mater Chem B.* 2014;2:1706–16.
- Mobley CEA. SNS. AOJ. VD. KAE. AY. LM. WW. HLT. The Pathogenic Potential of *Proteus mirabilis* Is Enhanced by Other Urinary Tract Infection. *Infect Immun.* 2017;85:85:e00808-16.
- Morsin M, Nafisah S, Sanudin R, Razali NL, Mahmud F, Soon CF. The role of positively charge poly-L-lysine in the formation of high yield gold nanoplates on the surface for plasmonic sensing application. *PLoS One.* 2021;16:1–15.
<http://dx.doi.org/10.1371/journal.pone.0259730>
- Mousavi Z, Naseri M, Babaei S, Hosseini SMH, Shekarforoush SS. The effect of cross-linker type on structural, antimicrobial and controlled release properties of fish gelatin-chitosan composite films incorporated with ϵ -poly-L-lysine. *Int J Biol Macromol.* 2021;183:1743–52. <https://doi.org/10.1016/j.ijbiomac.2021.05.159>
- Naghadeh HT, Sharifi Z, Soleimani S, Jamaat ZPM, Ferdowsi S. Efficacy of ϵ -poly-l-lysine as an antibacterial additive for platelets stored at room temperature. *Iran J Med Sci.* 2017;42:509–11.

- Neoh KG, Li M, Kang E-T, Chiong E, Tambyah Anantharajah P. Surface modification strategies for combating catheter-related complications : recent advances and challenges. *J Mater Chem B*. 2017;5:2045.
- Nešić A, Onjia A, Davidović S, Dimitrijević S, Errico ME, Santagata G, et al. Design of pectin-sodium alginate based films for potential healthcare application: Study of chemico-physical interactions between the components of films and assessment of their antimicrobial activity. *Carbohydr Polym*. 2017;157:981–90.
- Niaz T, Ihsan A, Abbasi R, Shabbir S, Noor T, Imran M. Chitosan-albumin based core shell-corona nano-antimicrobials to eradicate resistant gastric pathogen. *Int J Biol Macromol*. 2019;138:1006–18. <https://doi.org/10.1016/j.ijbiomac.2019.07.165>
- Nicolle LE. Catheter associated urinary tract infections. *Antimicrob Resist Infect Control*. 2014;3:1–8.
- Niveditha S, Pramodhini S, Umadevi S, Kumar S, Stephen S. The isolation and the biofilm formation of uropathogens in the patients with catheter associated urinary tract infections (UTIs). *J Clin Diagnostic Res*. 2012;6:1478–82.
- Nobile CJ, Johnson AD. *Candida albicans* Biofilms and Human Disease. *Annu Rev Microbiol*. 2015;69:71–92.
- Nunes C, Rodrigues F, Fernanda L, Dourado N, Victor P, Silva RO, et al. A New Topical Eye Drop Containing LyeTxI-b , A Synthetic Peptide Designed from A *Lycosa erithrognata* Venom Toxin , Was Effective to Treat Resistant Bacterial Keratitis. *Toxins (Basel)*. 2019;11:203.
- OECD. Test No. 423: Acute Oral Toxicity-Acute Toxic Class Method. 2002.
- Oh JK, Yegin Y, Yang F, Zhang M, Li J, Huang S, et al. The influence of surface chemistry on the kinetics and thermodynamics of bacterial adhesion. *Sci Rep*. 2018;8:17247. <http://dx.doi.org/10.1038/s41598-018-35343-1>
- Okella H, Okello E, Mtewa AG, Ikiriza H, Kaggwa B, Aber J, et al. ADMET profiling and molecular docking of potential antimicrobial peptides previously isolated from African cat fish , *Clarias gariepinus*. *Front Mol Biosci*. 2022;9:1039286.
- Oliveira Júnior NG, Souza CM, Buccini DF, Cardoso MH, Franco OL. Antimicrobial peptides: structure, functions and translational applications. *Nat Rev Microbiol*. 2025;

- Ourabi M, Garg S, Mirka B, Ewenike R, Tousignant MN, Ranne M, et al. Networks of Conjugated Polymer-Wrapped Single-Walled Carbon Nanotubes through Controlled Drop-Dispensing for Thin-Film Transistors. *ACS Appl Nano Mater.* 2023;6:11242–51.
- Padilla-Garfias F, Ríos-Cifuentes L, Sánchez NS, Calahorra M, Peña A. Study of the mechanism of ϵ -poly-L-lysine as an antifungal on *Candida albicans* and *Saccharomyces cerevisiae*. *Biochim Biophys Acta - Gen Subj.* 2022;1866.
- Paiva MC, Maria A, Nascimento A, Lopes I, Cunha B, Lima-bittencourt CI, et al. The first report of the *qnrB19*, *qnrS1* and *aac (6')-Ib-cr* genes in urinary isolates of ciprofloxacin-resistant *Escherichia coli* in Brazil. *Mem Inst Oswaldo Cruz.* 2012;107:687–9.
- Park NG, Yamato Y, Lee S, Sugihara G. Interaction of mastoparan-B from venom of a hornet in taiwan with phospholipid bilayers and its antimicrobial activity. *Biopolymers.* 1995;36:793–801.
- Patel K, Kushwaha P, Kumar S, Kumar R. Lysine and α -Aminoisobutyric Acid Conjugated Bioinspired Polydopamine Surfaces for the Enhanced Antibacterial Performance of the Foley Catheter. *ACS Appl Bio Mater.* 2019;2:5799–809.
- Patel PK, Advani SD, Kofman AD, Lo E, Maragakis LL, Pegues DA, et al. Strategies to prevent catheter-associated urinary tract infections in acute-care hospitals: 2022 Update. *Infect Control Hosp Epidemiol.* 2023;44:1209–31.
- Paudel S, Guedry S, Obernuefemann CLP, Hultgren SJ, Walker JN, Kulkarni R. Defining the Roles of Pyruvate Oxidation, TCA Cycle, and Mannitol Metabolism in Methicillin-Resistant *Staphylococcus aureus* Catheter-Associated Urinary Tract Infection. *Microbiol Spectr.* 2023;11.
- Paul A. Tambyah, Valerie Knasinski DGM. THE DIRECT COSTS OF NOSOCOMIAL CATHETER-ASSOCIATED URINARY TRACT INFECTION IN THE ERA OF MANAGED CARE. *Infect Control Hosp Epidemiol.* 2022;23:27–31.
- Pelling H, Nzakizwanayo J, Milo S, Denham EL, MacFarlane WM, Bock LJ, et al. Bacterial biofilm formation on indwelling urethral catheters. *Lett Appl Microbiol.* 2019;68:277–93.
- Perrier S. 50th Anniversary Perspective: RAFT Polymerization—A User Guide.

- Macromolecules. 2017;50:7433–47.
- Pihl M, Galli S, Jimbo R, Andersson M. Osseointegration and antibacterial effect of an antimicrobial peptide releasing mesoporous titania implant. *J Biomed Mater Res - Part B Appl Biomater*. 2021;109:1787–95.
- Pranzetti A, Salaün S, Mieszkina S, Callow ME, Callow JA, Preece JA, et al. Model organic surfaces to probe marine bacterial adhesion kinetics by surface plasmon resonance. *Adv Funct Mater*. 2012;22:3672–81.
- Puertas-segura A, Morena AG, Silvia P, Ivanova K, Ivanov I, Todorova K, et al. Enzymatically Built Nanoenabled Antimicrobial Coating on Urinary Catheters. *ACS Appl Mater Interfaces*. 2024;16:39139.
- Pulido R, Naveas N, Fernández-Alonso FJ, Manso-Silván M, Soriano L, Torres-Ulloa C, et al. Drug delivery system based on an antibacterial layer-by-layer coating on urinary catheters: an experimental and simulation approach. *Front Bioeng Biotechnol*. 2025;13:1–14.
- Qian L, Xu H, Lu Y, Chen Z. Preparation and performance optimization of PVP/PVA coatings with high adhesion and high hydrophilicity for coronary guidewires. *Prog Org Coatings*. 2025;209:109600. <https://doi.org/10.1016/j.porgcoat.2025.109600>
- Ran P, Qiu B, Zheng H, Xie S, Zhang G, Cao W, et al. On-demand bactericidal and self-adaptive antifouling hydrogels for self-healing and lubricant coatings of catheters. *Acta Biomater*. 2024;186:215–28.
- Rana D, Matsuura T. Surface modifications for antifouling membranes. *Chem Rev*. 2010;110:2448–71.
- Rashki, A., Rahdar, M., Ghalehnoo ZR. Characterization of uropathogenic *Escherichia coli*: Distribution of adhesin-encoding genes and O-serotypes among ciprofloxacin susceptible and resistant isolates. *Jundishapur J Microbiol*. 2019;12.
- Rastegar E, Malekzadegan Y, Khashei R, Hadi N. Quinolone resistance and biofilm formation capability of uropathogenic *Escherichia coli* isolates from an Iranian inpatients' population. *Mol Biol Rep*. 2023;50:8073–9. <https://doi.org/10.1007/s11033-023-08704-x>
- Reis P V., Boff D, Verly RM, Melo-Braga MN, Cortés ME, Santos DM, et al. LyeTxI-b, a

- synthetic peptide derived from *Lycosa erythrognatha* spider venom, shows potent antibiotic activity in vitro and in vivo. *Front Microbiol.* 2018;9:1–12.
- Reza M, Karam A, Habibi M, Bouzari S. Urinary tract infection : Pathogenicity , antibiotic resistance and development of effective vaccines against Uropathogenic *Escherichia coli*. *Mol Immunol.* 2019;108:56–67. <https://doi.org/10.1016/j.molimm.2019.02.007>
- Rodriguez VB, Kidd BA, Interlandi G, Tchesnokova V, Sokurenko E V, Thomas WE. Allosteric Coupling in the Bacterial Adhesive Protein FimH *. *J Biol Chem.* 2013;288:24128–39.
- Rolo D, Ardanuy C, Xercavins M, Horcajada JP, Bosch J, Soto SM. Relationship Between Biofilm Formation and Antimicrobial Resistance in Gram-Negative Bacteria. *Microb Drug Resist.* 2019;25:72–9.
- Roy A, Kucukural, A., Zhang, Y. I-TASSER : a unified platform for automated protein structure and function prediction. *Nat Protoc.* 2010;5:725–38.
- Roy P, Saha R, Pawlik J, Samol Z, Dziadek M, Cholewa-Kowalska K, et al. Cobalt containing antimicrobial bioactive glass coated urinary catheter towards management of catheter associated urinary tract infection (CAUTI): Significant in vitro characterizations. *Ceram International.* 2024;50:11625-11638.
- Sadeeq M, Li Y, Wang C, Hou F, Zuo J, Xiong P. Unlocking the power of antimicrobial peptides: advances in production, optimization, and therapeutics. *Front Cell Infect Microbiol.* 2025;15.
- Saikawa GIA, Guidone GHM, Noriler SA, Reis GF, de Oliveira AG, Nakazato G, et al. Green-Synthesized Silver Nanoparticles in the Prevention of Multidrug-Resistant *Proteus mirabilis* Infection and Incrustation of Urinary Catheters BioAgNPs Against *P. mirabilis* Infection. *Curr Microbiol.* 2024;81:1–10. <https://doi.org/10.1007/s00284-024-03616-w>
- Saint S. Clinical and economic consequences of nosocomial catheter-related bacteriuria. *Am J Infect Control.* 2000;28:68–75.
- Saint S, Chenoweth CE. Biofilms and catheter-associated urinary tract infections. *Infect Dis Clin North Am.* 2003;17:411–32.
- Saint S, Meddings J, Calfee D, Kowalski C, Krein S. *Annals of Internal Medicine Perspective*

- Catheter-Associated Urinary Tract Infection and the Medicare. *Ann Intern Med*. 2009;150:877–84.
- Salwiczek M, Qu Y, Gardiner J, Strugnell RA, ... Emerging rules for effective antimicrobial coatings. *Trends Biotechnol*. 2014;32:82–90.
[https://www.cell.com/trends/biotechnology/fulltext/S0167-7799\(13\)00207-2](https://www.cell.com/trends/biotechnology/fulltext/S0167-7799(13)00207-2)
- Sanchez G V, Baird AMG, Karlowsky JA, Master RN, Bordon JM. Nitrofurantoin retains antimicrobial activity against multidrug-resistant urinary *Escherichia coli* from US outpatients. *Antimicrob Agents Chemother*. 2014;69:3259–62.
- Santos DM, Verly RM, Piló-Veloso D, De Maria M, De Carvalho MAR, Cisalpino PS, et al. LyeTx I, a potent antimicrobial peptide from the venom of the spider *Lycosa erythrognatha*. *Amino Acids*. 2010;39:135–44.
- Sarshar M, Behzadi P, Ambrosi C, Zagaglia C. FimH and Anti-Adhesive Therapeutics : A Disarming Strategy Against Uropathogens. *Antibiotics*. 2020;9:397.
- Shackley DC, Whytock C, Parry G, Clarke L, Vincent C, Harrison A, et al. Variation in the prevalence of urinary catheters: A profile of National Health Service patients in England. *BMJ Open*. 2017;7:1–8.
- Shih IL, Shen MH, Van YT. Microbial synthesis of poly(ϵ -lysine) and its various applications. *Bioresour Technol*. 2006;97:1148–59.
- Shu C, Cui K, Li Q, Cao J, Jiang W. Epsilon-poly-L-lysine (ϵ -PL) exhibits multifaceted antifungal mechanisms of action that control postharvest *Alternaria* rot. *Int J Food Microbiol*. 2021;348:109224. <https://doi.org/10.1016/j.ijfoodmicro.2021.109224>
- Sierra JM, Viñas M. Expert Opinion on Drug Discovery Future prospects for Antimicrobial peptide development : peptidomimetics and antimicrobial combinations antimicrobial combinations. *Expert Opin Drug Discov*. 2021;16:601–4.
<https://doi.org/10.1080/17460441.2021.1892072>
- Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. Antimicrobial-Resistant Pathogens Associated with Healthcare-Associated Infections Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009–2010. *Infect Control Hosp Epidemiol*. 2013;34:1–14.
- Da Silva CHFP, Vidigal GM, De Uzeda M, De Almeida Soares G. Influence of titanium

- surface roughness on attachment of streptococcus sanguis: An in vitro study. *Implant Dent.* 2005;14:88–93.
- Singha P, Locklin J, Handa H, Author AB. A Review of the Recent Advances in Antimicrobial Coatings for Urinary Catheters. *Acta Biomater.* 2017;50:20–40. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5316300/pdf/nihms837082.pdf>
- Skok K, Bele U, Pintar Š, Peršin Z, Kuzmič K, Bračič M, et al. Urinary catheters: state of the art and future perspectives - a narrative review. *Mater Today Bio.* 2025;34:102225.
- Slate AJ, Clarke OE, Kerio M, Nzakizwanayo J, Patel BA, Jones B V. Infection responsive coatings to reduce biofilm formation and encrustation of urinary catheters. *J Appl Microbiol.* 2023;134:1–9.
- Smith JR, Lamprou DA, Larson C, Upson SJ, Lamprou DA, Larson C, et al. Transactions of the IMF The International Journal of Surface Engineering and Coatings Biomedical applications of polymer and ceramic coatings : a review of recent developments developments. 2022; <https://doi.org/10.1080/00202967.2021.2004744>
- Sobolev O V, Afonine P V, Moriarty NW, Hekkelman ML, Joosten RP, Perrakis A, et al. A global Ramachandran score identifies protein structures with unlikely stereochemistry. *Structure.* 2021;28:1249–58.
- Song A, Rane AA, Christman KL. Antibacterial and cell-adhesive polypeptide and poly(ethylene glycol) hydrogel as a potential scaffold for wound healing. *Acta Biomater.* 2012;8:41–50. <http://dx.doi.org/10.1016/j.actbio.2011.10.004>
- de Sousa JKT, Haddad JPA, de Oliveira AC, Vieira CD, dos Santos SG. In vitro activity of antimicrobial-impregnated catheters against biofilms formed by KPC-producing *Klebsiella pneumoniae*. *J Appl Microbiol.* 2019;127:1018–27.
- Souza AN De, Cardoso GDA, Nunes LO, Aisenbrey C, Salnikov E, Souza KR De, et al. Comparative Structural and Biophysical Investigation of *Lycosa erythrognatha* Toxin I (LyeTx I) and Its Analog LyeTx I-b. *Antibiotics.* 2025;14:66.
- Soyhan I, Polat T, Mozioglu E, Ozal Ildeniz TA, Acikel Elmas M, Cebeci S, et al. Effective Immobilization of Novel Antimicrobial Peptides via Conjugation onto Activated Silicon Catheter Surfaces. *Pharmaceutics.* 2024;16.
- Stahlhut SG, Struve C, Krogfelt KA, Reisner A. Biofilm formation of *Klebsiella pneumoniae*

- on urethral catheters requires either type 1 or type 3 fimbriae. *FEMS Immunol Med Microbiol.* 2012;65:350–9.
- Standardization IO for. ISO 10993-5:2009 — Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity. 2009.
- Stickler DJ, Feneley RCL. The encrustation and blockage of long-term indwelling bladder catheters: A way forward in prevention and control. *Spinal Cord.* 2010;48:784–90.
<http://dx.doi.org/10.1038/sc.2010.32>
- Sun H, Hong Y, Xi Y, Zou Y, Gao J, Du J. Synthesis, Self-Assembly, and Biomedical Applications of Antimicrobial Peptide-Polymer Conjugates. *Biomacromolecules.* 2018;19:1701–20.
- Sun H, Yang L, Thompson MP, Schara S, Cao W, Choi W, et al. Recent Advances in Amphiphilic Polymer – Oligonucleotide Nanomaterials via Living / Controlled Polymerization Technologies. *Bioconj Chem.* 2019a;30:1889–1904.
- Sun T, Xue B, Shao Z, Niu M, Yang Y, Zhang L. Effect on the bacterial adhesion of PVA electrospinning membrane deposited on silicone catheter surface Effect on the bacterial adhesion of PVA electrospinning membrane deposited on silicone catheter surface. *Biomed Phys Eng Express.* 2026;12:025003.
- Sun T, Zheng Z, Cheng L, Hou J, Hou C, Feng Z, et al. Hydrophilic-antimicrobial dual-function modification of TPU catheter based on sodium alginate and cationic composite coating. *React Funct Polym.* 2025;216:106410.
<https://doi.org/10.1016/j.reactfunctpolym.2025.106410>
- Sun X, Dai H, Guo P, Sha X. Biocompatibility of a New Kind of Polyvinyl Alcohol Embolic Microspheres: In Vitro and In Vivo Evaluation. *Mol Biotechnol.* 2019b;61:610–21.
<https://doi.org/10.1007/s12033-019-00166-6>
- Takahashi T, Nitin N, Lee EY, Zhang L, Wong GCL, Gallo RL. Cathelicidin promotes inflammation by enabling binding of self-RNA to cell surface scavenger receptors. *Sci Rep.* 2018;4032.
- Tan Z, Shi Y, Xing B, Hou Y, Cui J, Jia S. The antimicrobial effects and mechanism of ϵ -poly-lysine against *Staphylococcus aureus*. *Bioresour Bioprocess.* 2019;6.
<https://doi.org/10.1186/s40643-019-0246-8>

- Tarlton NJ, Moritz C, Adams-sapper S, Riley LW. Genotypic analysis of uropathogenic *Escherichia coli* to understand factors that impact the prevalence of β -lactam-resistant urinary tract infections in a community. *J Glob Antimicrob Resist*. 2019;19:173–80. <https://doi.org/10.1016/j.jgar.2019.03.002>
- Teixeira-santos R, Gomes LC, Vieira R, Sousa-cardoso F, Soares SGP, Mergulh FJ. Exploring Nitrogen-Functionalized Graphene Composites for Urinary Catheter Applications. *Nanomaterials*. 2023;13:2604.
- Temtem M, Pompeu D, Jaraquemada G, Cabrita EJ, Casimiro T, Aguiar-Ricardo A. Development of PMMA membranes functionalized with hydroxypropyl- β -cyclodextrins for controlled drug delivery using a supercritical CO₂-assisted technology. *Int J Pharm*. 2009;376:110–5.
- Tenke P, Kovacs B, Jäckel M, Nagy E. The role of biofilm infection in urology. *World J Urol*. 2006;24:13–20.
- Terlizzi ME, Gribaudo G, Maffei ME. UroPathogenic *Escherichia coli* (UPEC) Infections : Virulence Factors , Bladder Responses , Antibiotic , and Non-antibiotic Antimicrobial Strategies. *Front Microbiol*. 2017;8:1566.
- Thakur S, Sharma B, Verma A, Chaudhary J, Tamulevicius S, Thakur VK. Recent progress in sodium alginate based sustainable hydrogels for environmental applications. *J Clean Prod*. 2018;198:143–59.
- Tien BYQ, Goh HMS, Chong KKL, Bhaduri-Tagore S, Holec S, Dress R, et al. *Enterococcus faecalis* Promotes Innate Immune Suppression and Polymicrobial. *Infect Immun*. 2017;85:1–14.
- Tosiano MA, Lanni F, Mitchell AP, McManus CJ. Roles of P-body factors in *Candida albicans* filamentation and stress response. *PLoS Genet*. 2025;21:1–31. <https://doi.org/10.1371/journal.pgen.1011632>
- Trafny EA, Lewandowski R, Zawistowska-Marciniak I, Stępińska M. Use of MTT assay for determination of the biofilm formation capacity of microorganisms in metalworking fluids. *World J Microbiol Biotechnol*. 2013;29:1635–43.
- Haq IU, Lima, G.W, Henrique F, Silva DS, Cesar J, Brito M, et al. Anti-adhesive activity of LyeTx I mn Δ K , a short peptide derived from the Brazilian spider *Lycosa*

- erythrognatha venom , against quinolone-resistant uropathogenic *Escherichia coli*. *Microb Pathog*. 2026;214.
- Haq I.U, Krukiewicz K. Antimicrobial approaches for medical implants coating to prevent implants associated infections: Insights to develop durable antimicrobial implants. *Appl Surf Sci Adv*. 2023;18:100532. <https://doi.org/10.1016/j.apsadv.2023.100532>
- Vanhems P, Baratin D, Voirin N, Savey A, Caillat-Vallet E, Metzger MH, et al. Reduction of urinary tract infections acquired in an intensive care unit during a 10-year surveillance program. *Eur J Epidemiol*. 2008;23:641–5.
- Verhorstert KWJ, Guler Z, De Boer L, Riool M, Roovers JPWR, Zaat SAJ. In Vitro Bacterial Adhesion and Biofilm Formation on Fully Absorbable Poly-4-hydroxybutyrate and Nonabsorbable Polypropylene Pelvic Floor Implants. *ACS Appl Mater Interfaces*. 2020;12:53646–53.
- Vieira APGC, Bechinger B, Verly RM, de Lima ME. The Synthetic Peptide LyeTx I mnΔK, Derived from *Lycosa erythrognatha* Spider Toxin, Is Active against Methicillin-Resistant *Staphylococcus aureus* (MRSA) In Vitro and In Vivo. *Antibiotics*. 2024a;13:248.
- Vieira APGC, de Souza AN, Lima WG, Brito JCM, Simião DC, Gonçalves LVR, et al. The Synthetic Peptide LyeTx I mnΔK, Derived from *Lycosa erythrognatha* Spider Toxin, Is Active against Methicillin-Resistant *Staphylococcus aureus* (MRSA) In Vitro and In Vivo. *Antibiotics*. 2024b;13:248.
- Vlieghe P, Lisowski V, Martinez J, Khrestchatisky M. Synthetic therapeutic peptides : science and market. *Drug Discov Updat*. 2010;15:40–56.
- Wahid F, Wang F, Xie Y, Chu L, Jia S, Duan Y. Reusable ternary PVA films containing bacterial cellulose fibers and ϵ - polylysine with improved mechanical and antibacterial properties. *Colloids Surfaces B Biointerfaces*. 2019;183:110486. <https://doi.org/10.1016/j.colsurfb.2019.110486>
- Walaszek M, Rozanska A, Bulanda M, Wojkowska-Mach J. Epidemiology of healthcare-associated infections in Polish intensive care. A multicenter study based on active surveillance. *Biomed Pap*. 2018;162:190–7.
- Wang G, Schmidt C, Li X, Wang Z. APD6: the antimicrobial peptide database is expanded to

- promote research and development by deploying an unprecedented information pipeline. *Nucleic Acids Res.* 2026;54:D363–D374.
- Wang J, Foxman B, Mody L, Snitkin ES. Network of microbial and antibiotic interactions drive colonization and infection with multidrug-resistant organisms. *Proc Natl Acad Sci U S A.* 2017;114:10467–72.
- Wang L, Zhou W, Wang Q, Xu C, Tang Q, Yang H. An injectable, dual responsive, and self-healing hydrogel based on oxidized sodium alginate and hydrazide-modified poly (ethyleneglycol). *Molecules.* 2018;23.
- Wang X, Shi H, Tang H, Yu H, Yan Q, Yang H, et al. Electrostatic assembly functionalization of poly (γ -glutamic acid) for biomedical antibacterial applications. *J Mater Sci Technol.* 2020;59:14–25. <https://doi.org/10.1016/j.jmst.2020.05.017>
- Warren JW. Catheter-associated urinary tract infections. *Int J Antimicrob Agents.* 2001;17:299–303.
- Wassmann T, Kreis S, Behr M, Buergers R. The influence of surface texture and wettability on initial bacterial adhesion on titanium and zirconium oxide dental implants. *Int J Implant Dent.* 2017;3.
- Werneburg GT. Catheter-Associated Urinary Tract Infections : Current Challenges and Future Prospects. *Res Reports Urol.* 2022;14:109–33.
- White AN, Learman BS, Brauer AL, Armbruster CE. Catalase Activity is Critical for *Proteus mirabilis* Biofilm Development , Extracellular Polymeric Substance Composition , Infection. 2021;1–20.
- Wilks SA, Fader MJ, Keevil CW. Novel insights into the *proteus mirabilis* crystalline biofilm using real-time imaging. *PLoS One.* 2015;10:1–13.
- Wong EHH, Khin MM, Ravikumar V, Si Z, ... Modulating antimicrobial activity and mammalian cell biocompatibility with glucosamine-functionalized star polymers. 2016; <https://pubs.acs.org/doi/abs/10.1021/acs.biomac.5b01766>
- Xie SX, Song L, Yuca E, Boone K, ... Antimicrobial peptide–polymer conjugates for dentistry. *ACS Appl Polym* 2020; <https://pubs.acs.org/doi/abs/10.1021/acsapm.9b00921>

- Xuan J, Feng W, Wang J, Wang R, Zhang B, Bo L, et al. Antimicrobial peptides for combating drug-resistant bacterial infections. *Drug Resist Updat*. 2023;68:100954. <https://doi.org/10.1016/j.drup.2023.100954>
- Yang H, Chen Y, Cai Y, Li J, Qiu L, Wang Y, et al. A feasible strategy of Ag nanoparticles-sodium alginate-polyacrylamide-polyvinyl alcohol hydrogel coatings for preventing catheter-associated urinary tract infections. *Prog Org Coatings*. 2024;196:108706. <https://doi.org/10.1016/j.porgcoat.2024.108706>
- Yang R, Ma X, Peng F, Wen J, Allahou LW, Williams GR, et al. Advances in antimicrobial peptides : From mechanistic insights to chemical modifications. *Biotechnol Adv*. 2025;81:108570. <https://doi.org/10.1016/j.biotechadv.2025.108570>
- Yang SH, Lee YSJ, Lin FH, Yang JM, Chen KS. Chitosan/poly(vinyl alcohol) blending hydrogel coating improves the surface characteristics of segmented polyurethane urethral catheters. *J Biomed Mater Res - Part B Appl Biomater*. 2007;83:304–13.
- Ye J, Ma D, Qin W, Liu Y. Physical and Antibacterial Properties of Sodium Alginate-Sodium Carboxymethylcellulose Films. *Molecules*. 2018;23:2645.
- Ye R, Xu H, Wan C, Peng S, Wang L, Xu H, et al. Antibacterial activity and mechanism of action of ϵ -poly-l-lysine. *Biochem Biophys Res Commun*. 2013a;439:148–53. <http://dx.doi.org/10.1016/j.bbrc.2013.08.001>
- Ye RS, Zhang ZH, Xu HY, Xu F, Aguilar ZP, Xiong YH, et al. Differentially-expressed genes in *Candida albicans* exposed to ϵ -poly-l-lysine. *Biotechnol Lett*. 2013b;35:2147–53.
- Yoda I, Koseki H, Tomita M, Shida T, Horiuchi H, Sakoda H, et al. Effect of surface roughness of biomaterials on *Staphylococcus epidermidis* adhesion. *BMC Microbiol*. 2014;14:1–7.
- Yong Y, Qiao M, Chiu A, Fuchs S, Pardo Y, Worobo R, et al. Biological and Environmental Phenomena at the Interface Conformal hydrogel coatings on catheters to reduce biofouling. *Langmuir*. 2018;35.
- Yu H, Liu L, Li X, Zhou R, Yan S, Li C, et al. Fabrication of polylysine based antibacterial coating for catheters by facile electrostatic interaction. *Chem Eng J*. 2019;360:1030–41. <https://doi.org/10.1016/j.cej.2018.10.160>

- Yu H, Liu L, Yang H, Zhou R, Che C, Li X, et al. Water-Insoluble Polymeric Guanidine Derivative and Application in the Preparation of Antibacterial Coating of Catheter. *ACS Appl Mater Interfaces*. 2018;10:39257–67.
- Yu K, Alzahrani A, Khoddami S, Cheng JTJ, Mei Y, Gill A, et al. Rapid Assembly of Infection-Resistant Coatings: Screening and Identification of Antimicrobial Peptides Works in Cooperation with an Antifouling Background. *ACS Appl Mater Interfaces*. 2021a;13:36784–99.
- Yu K, Alzahrani A, Khoddami S, Ferreira D, Scotland KB, Cheng JTJ, et al. Self-Limiting Mussel Inspired Thin Antifouling Coating with Broad-Spectrum Resistance to Biofilm Formation to Prevent Catheter-Associated Infection in Mouse and Porcine Models. *Adv Healthc Mater*. 2021b;10:1–18.
- Yu K, Lo J, Mei Y, Haney EF, Siren EMJ, Kalathottukaren MT, et al. Toward Infection-Resistant Surfaces: Achieving High Antimicrobial Peptide Potency by Modulating the Functionality of Polymer Brush and Peptide. *ACS Appl Mater Interfaces*. 2015a;7:28591–28605.
- Yu K, Lo JCY, Yan M, Yang X, Brooks DE, ... Anti-adhesive antimicrobial peptide coating prevents catheter associated infection in a mouse urinary infection model. *Biomaterials*. 2017; <https://www.sciencedirect.com/science/article/pii/S0142961216306718>
- Yu Q, Wu Z, Chen H. Acta Biomaterialia Dual-function antibacterial surfaces for biomedical applications. *ACTA Biomater*. 2015b;. <http://dx.doi.org/10.1016/j.actbio.2015.01.018>
- Yuan Y, Li S, Zhao Z, Deng B, Li Z, Jing X, et al. In vitro and in vivo antibacterial activity of a lysine-rich scorpion peptide derivative. *Toxicon*. 2022;209:1–9.
- Zenin V, Yurkova M, Tsedilin A, Fedorov A. Enfuvirtide biosynthesis in thermostable chaperone-based fusion. *Biotechnol Reports*. 2022;35:e00734. <https://doi.org/10.1016/j.btre.2022.e00734>
- Zhang C, Yang L, Wan F, Bera H, Cun D, Rantanen J. Quality by design thinking in the development of long-acting injectable PLGA / PLA-based microspheres for peptide and protein drug delivery. *Int J Pharm*. 2020;585:119441. <https://doi.org/10.1016/j.ijpharm.2020.119441>
- Zhang, H., Lv, J., Ma, Z., et al. Advances in Antimicrobial Peptides : Mechanisms , Design

- Innovations , and Biomedical Potential. *Molecules*. 2025;30:1529.
- Zhang L, Zhao J, Ding G, Li X, Liu H. Screening of Potent Inhibitor of H1N1 Influenza NS1 CPSF30 Binding Pocket by Molecular Docking. *Adv Infect Dis*. 2012;2.
- Zhang, M., Ouyang, J., Fu, L., et al. Hydrophobicity Determines the Bacterial Killing Rate of α - Helical Antimicrobial Peptides and Influences the Bacterial Resistance Development. *J Med Chem*. 2022;65:14261–970.
- Zhang QY, Yan Z Bin, Meng YM, Hong XY, Shao G, Ma JJ, et al. Antimicrobial peptides: mechanism of action, activity and clinical potential. *Mil Med Res*. 2021;8:48.
<https://doi.org/10.1186/s40779-021-00343-2>
- Zhang S, Chen H, Shi Z, Liu Y, Liu L, Yu J, et al. Preparation of amino cellulose nanofiber via ϵ -poly-L-lysine grafting with enhanced mechanical, anti-microbial and food preservation performance. *Ind Crops Prod*. 2023;194:116288.
- Zhang X, Shi C, Liu Z, Pan F, Meng R, Bu X, et al. Antibacterial activity and mode of action of ϵ -polylysine against Escherichia coli O157: H7. *J Med Microbiol*. 2018;67:838–45.
- Zhang XY, Zhao YQ, Zhang Y, Wang A, Ding X, Li, Y. ., et al. Antimicrobial Peptide-Conjugated Hierarchical Antifouling Polymer Brushes for Functionalized Catheter Surfaces. *Biomacromolecules*. 2019;20:4171–9.
- Zhao Z, Ma X, Chen R, Xue H, Lei J, Du H, et al. Universal antibacterial surfaces fabricated from quaternary ammonium salt-based pnipam microgels. *ACS Appl Mater Interfaces*. 2020;12:19268–76.
- Zheng Y, Chen Y, Jin LW, Ye HY, Liu G. Cytotoxicity and Genotoxicity in Human Embryonic Kidney Cells Exposed to Surface Modify Chitosan Nanoparticles Loaded with Curcumin. *AAPS PharmSciTech*. 2016;17:1347–52.
<http://dx.doi.org/10.1208/s12249-015-0471-1>
- Zhou XCXC, Wu SLRF, Wu CAJY. In vivo osseointegration of dental implants with an antimicrobial peptide coating. *J Mater Sci Mater Med*. 2017;
- Zhou Z, Chen J, Peng C, Huang T, Zhou H, Ou B, et al. Fabrication and physical properties of gelatin/sodium alginate/hyaluronic acid composite wound dressing hydrogel. *J Macromol Sci Part A Pure Appl Chem*. 2014;51:318–25.

11. List of tables

- Table 1: Efficacy of antimicrobial peptides-polymers coatings on urinary catheters
- Table 2: Sequences and chemical properties of LyeTx I and its analogues. Reproduced from (Ul Haq et al. 2026).
- Table 3: Antimicrobial resistance profile of UPECs used in this study.
- Table 4: Chemical composition of PVA/PLL and SA/LyeTx I Δ mnK.
- Table 5: Biochemical-physiological profile, morphology, and growth characteristics of UPEC isolates included in the study
- Table 6: MIC and MBC of LyeTx I and its derivatives against quinolone-resistant UPECs. Reproduced from (Haq *et al.*, 2026).
- Table 7: MIC of the AMP after incubation in urine at different time intervals. Reproduced from (Haq *et al.*, 2026).
- Table 8: LyeTx I Δ mnK effect on mice food intake and metabolic parameters. The food intake (caloric, carbohydrate, protein, and lipid intake) slightly reduced in control animals, whereas food efficiency increased somewhat in all experimental animals. (values are presented as mean $\pm$ standard deviation). Reproduced from (Ul Haq *et al.*, 2026).

12. List of figures

- Figure 1: The risk factors for CAUTI related to procedures of catheterization, catheter surface, and patient. Created in BioRender.com
- Figure 2: The possible outcomes of interactions between multiple microbes in the urinary system. Reprinted from (Gaston et al., 2021) under a CC by license. Copyrights (2021) ASM.
- Figure 3: Number of scientific articles published in academic journals since 1979. The antimicrobial peptides” OR “AMPs” OR “host defence peptides” OR “HDPs” were used as keywords to search the PubMed databases in March 2026.
- Figure 4: Illustration of strategies for polymer-AMP conjugation. (a) grafting-to; (b) grafting-from, and (c) grafting-through methods and coatings of conjugates on urinary catheters to produce antimicrobial effects. The figure was created in BioRender.com and modified from (Cui et al., 2021).
- Figure 5: Catheter modifications by syringe coating. The coating solution was pumped through intra and extraluminal surfaces and allowed to dry and AMPs were impregnated through dip and syringe coating methods into a PCL coating layer. Reproduced with permission of (Low *et al.*, 2021). Copyrights (2021), Elsevier.
- Figure 6: The predicted 3D structure of LyeTx I mnΔK in cartoon and stick form (A) and in mesh form (B). Reproduced from (Haq et al., 2026).
- Figure 7: Validation of LyeTx I mnΔK (A) and its structure profile. (B) The Ramachandran plot depicts 91.7% residues in favoured regions and 8.3% in the additional allowed regions. (Haq *et al.*, 2026).
- Figure 8: Bacterial protein FimH 3D structure in cartoon form. Reproduced from (Haq *et al.*, 2026).
- Figure 9: LyeTx I mnΔK docking with FimH in different representation modes (A). Display receptor surface as H-bond donor and acceptor (B). LyeTx I mnΔK-FimH interaction along labeled residues (C). LyeTx I mnΔK in cartoon form while protein in surface form is depicted in cyan color (D). LyeTx I mnΔK-FimH is in cartoon form in red and cyan, respectively. Reproduced from (Haq *et al.*, 2026).
- Figure 10: The inhibition of biofilm biomass of UPEC isolates by LyeTx I mnΔK at various concentrations. (values are presented as mean ± standard deviation). One, two, three, and four asterisks (*, **, ***, and ****) indicate statistically significant differences compared with the control at $0.01 < p < 0.05$, $0.001 < p < 0.01$, $0.0001 < p < 0.001$, and $p < 0.0001$, respectively. Reproduced from (Haq *et al.*, 2026).

- Figure 11: The inhibition of biofilm viability of UPEC isolates by LyeTx I mnΔK at various concentrations. Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks. Reproduced from (Haq *et al.*, 2026).
- Figure 12: Reduction of bacterial biofilm formation of UPEC isolates on catheter surfaces after treatment with LyeTx I mnΔK (1 μ g/mL). Bacterial (CFU counts per cm of catheter surface for different UPEC isolates. Untreated: without LyeTx I mnΔK. Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks. Reproduced from (Haq *et al.*, 2026).
- Figure 13: SEM analysis of untreated and AMP-treated catheters at low ($\times 100$) and high ($\times 3,300$ – $\times 4,500$) magnifications. AMP treatment markedly reduced UPEC colonization on the catheter surface. Reproduced from (Haq *et al.*, 2026).
- Figure 14: Cytotoxicity of AMP against HEK-293 cells. Reproduced from (Ul Haq *et al.*, 2026).
- Figure 15: Dose-dependent assessment of clinical signs in mice treated with different concentrations of LyeTx I mnΔK. Reproduced from (Ul Haq *et al.*, 2026).
- Figure 16: LyeTx I mnΔK effect on body weight of mice. Weight variation was observed in animals administered with different concentrations of peptides. The animals administered with 1 μ g/kg peptide showed high growth; the animals with 5 μ g/kg peptide showed less growth compared to control animals and 1 μ g/kg peptide animals. Whereas the animals administered with 10 μ g/kg peptide showed higher growth than 5 μ g/kg peptide animals and control. (values are presented as mean $\pm$ standard deviation). Reproduced from (Haq *et al.*, 2026).
- Figure 17: Renal toxicity of AMP in mice. All the tested concentrations do not significantly alter kidney weight or induce edema; however, the increase in creatinine levels at the highest dose (10 μ g/kg) indicates a reduced glomerular filtration rate and possible nephrotoxicity. Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks. Reproduced from (Haq *et al.*, 2026).
- Figure 18: Histological analysis of kidney tissues from mice treated with different concentrations of peptide. Glomerular and tubular regions of kidneys. The control group exhibits normal histological architecture in both glomerular and tubular regions. Mice treated with LyeTx I mnΔK at doses of 1, 5, and 10 μ g/kg show dose-dependent morphological changes. At higher doses (5 and 10 μ g/kg), notable alterations are observed, including tubular dilation, interstitial inflammation, and glomerular congestion, particularly prominent in the tubular region. Reproduced from (Haq *et al.*, 2026).
- Figure 19: SEM images at 1000x magnification of catheters coated with different layers (A). 11-layered surface revealed a uniform coating. Surface roughness values in R_z and R_a were calculated for each layer (B) to provide an analysis of surface textures (C). Chemical composition of each layer was analysed through EDS

(D). (values are presented as mean $\pm$ standard deviation). Reproduced from (Maryam *et al.*, 2026).

Figure 20: SEM images of all the samples at 1000x magnification (A). Chemical composition (B), roughness values (C) provide a comparative analysis of surface texture of pristine and coated catheters. (values are presented as mean $\pm$ standard deviation). Reproduced from (Maryam *et al.*, 2026).

Figure 21: FTIR (A) and contact angle analysis of uncoated catheter, PVA and all the samples (B). (values are presented as mean $\pm$ standard deviation) Reproduced from (Maryam *et al.*, 2026).

Figure 22: NHDFs cell viability percentages for PLL coated catheters (n = 3). As a control, cells without any treatment were used (viability 100 %). (values are presented as mean $\pm$ standard deviation). Reproduced from (Maryam *et al.* 2026).

Figure 23: 2D interactions of ϵ -PL with PapG and FimH (A); 3D interactions of ϵ -PL with PapG (B) and 3D interactions of ϵ -PL with FimH (C). Reproduced from (Maryam *et al.*, 2026).

Figure 24: The SEM images obtained at magnifications of 1000x (A) and 5000x (B) shows reduced *E. coli* adhesion on PLL coated catheters compared to the PVA. Reproduced from (Maryam *et al.*, 2026).

Figure 25: The SEM images obtained at magnifications of SEM 1000x (A) and 5000x (B) shows reduced *S. epidermidis* adhesion on PLL coated catheters compared to the PVA. Reproduced from (Maryam *et al.*, 2026).

Figure 26: The SEM images obtained at magnifications of 1000x (A) and 5000x (B) shows reduced *Candida albicans* adhesion on PLL coated catheters and more sparse areas. Reproduced from (Maryam *et al.*, 2026).

Figure 27: Confocal microscopy images of live (green) and dead/dying (red) cells of all tested microorganisms (A); coverage of live and dead microbial cells (B); cell density of live/dead cells tested microorganisms (C). (values are presented as mean $\pm$ standard deviation). Reproduced from (Maryam *et al.* 2026).

Figure 28: SEM images at magnification of 270x of silicon coated with several layers of SA, 35-layered surface reveals a uniform coating (A). Uncoated, SA coated and SA/LyeTx I mn Δ K coated samples (B).

- Figure 29: Atomic concentrations of uncoated, SA, and SA/LyeTx I Δ K coating determined using EDS. Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks. ns, not significant ($p > 0.05$).
- Figure 30: Roughness values of uncoated, SA, and SA/LyeTx I Δ K coated surfaces provide a comparative analysis of their surface texture (A). Three-dimensional surface topography of the uncoated surface, SA coating and SA/LyeTx I Δ K coating obtained by optical profilometry (B). Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks.
- Figure 31: FTIR (A) and contact angle analysis of uncoated silicon, SA and SA/AMP (B). Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks.
- Figure 32: NHDFs cell viability percentages for uncoated silicon surface, SA coated surface, and SA/LyeTx I Δ K coated surface. As a control, cells without any treatment were used (viability 100 %). Data are presented as mean $\pm$ SD. ns, not significant ($p > 0.05$).
- Figure 33: OD₆₀₀ of *S. aureus* and *E. coli* cultures incubated with SA/LyeTx I Δ K-coated, SA-coated, and uncoated samples (A). Bacterial growth inhibition percentages (B). Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks.
- Figure 34: SEM images of samples exposed to *S. aureus* at magnifications of 1000x (A) and 5000x (B) showed differences in bacterial distribution. SA/LyeTx I Δ K coatings showed less adhesion of *S. aureus*.
- Figure 35: SEM images of samples exposed to *E. coli* at magnifications of 2000x (A) and 5000x (B) showed differences in bacterial distribution. SA/LyeTx I Δ K coatings showed less adhesion of *E. coli*.
- Figure 36: Viable bacterial counts (log CFU/cm²) recovered from uncoated, SA-coated, and SA/LyeTx I Δ K-coated silicon surfaces (A). Inhibition (%) by SA/LyeTx I Δ K relative to uncoated silicon and SA-coating (B). Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks.
- Figure 37: OD₆₀₀ of UPEC culture exposed to SA/LyeTx I Δ K coating and SA coating after incubation in mammalian urine for 1, 3, 5, 7, 9, 12, and 14 days to assess coating stability (A). Bacterial growth inhibition by SA/LyeTx I Δ K-coating relative to control and SA-coating (B). Data are presented as mean $\pm$ SD. Statistical significance is indicated by asterisks.

13. Appendices

13.1. Appendix A: Animal research ethics approval certificate



COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO EXPERIMENTAÇÃO ANIMAL (CEPEEA)

CERTIFICADO

Certificamos que o projeto intitulado “Peptídeos antimicrobianos sintéticos inspirados em análogos isolados de secreções de artrópodes contra bactérias multirresistentes do grupo ESKAPE: Eficácia *in vitro* e determinação do potencial terapêutico em modelos de infecção murina”, protocolo nº001-2023, sob a responsabilidade de Maria Elena de Lima Perez Garcia e William Gustavo de Lima - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovado, após solicitações de diligência, pelo Comitê de Ética em Pesquisa Envolvendo Experimentação Animal (CEPEEA) da Santa Casa BH Ensino e Pesquisa, em reunião de 23/03/2023.

Vigência do Projeto	23/03/2023 a 23/03/2028
Espécie/linhagem	Camundongo/Balb-c
Nº de animais	125
Peso/Idade	~25g/6 semanas
Sexo	Machos e fêmeas
Origem	Cebio-ICB/UFG

Prof. Marcus Vinicius Gomez

Docente Pesquisador, Mestrado/Doutorado acadêmico em Medicina/Biomedicina

Coordenador do CEPEEA – Santa Casa BH

Rua Domingos Vieira, 590, Santa Efigênia, Belo Horizonte-MG. CEP:30150-240. Tel. (31)3238-8525. email: cepeea@santacasabh.org.br

13.2. Appendix B: Copyright Permission for Figure 2.



Copyrights
Permission Figure 2.p

13.3. Appendix C: Copyright Permission for Figure 4.



Copyrights
Permission Figure 4.p