The Design, Development, and Testing of Novel Next Generation *In Vitro* Agar-based Urethral Models for Medical Device Innovation

A thesis submitted for the degree of

Doctor of Philosophy

to the

Technological University of the Shannon

By

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Based on the research carried out under the supervision of

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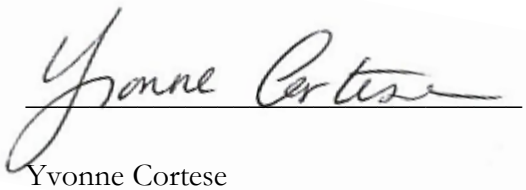
Declaration

I hereby declare that this thesis submitted to the Technological University of the Shannon for the degree of Doctor of Philosophy, is a result of my own work and has not in the same or altered form, been presented to this institute or any other institute in support for any degree other than for which I am now a candidate.



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Yvonne Cortese Date

"The only difference between screwing around and science, is writing it down."

-Adam Savage

Acknowledgements

My journey to PhD started a long time ago (sometimes longer than I'd like to admit), as an undergraduate student in Athlone Institute of technology. I have had the privilege of watching my small college evolve and expand over the years. It is now with great pride I get to watch my little home institute transform into the Technological University of the Shannon (TUS). With all that said, I'd first and foremost like to thank TUS Athlone for all the wonderful years and all of the incredible staff that have supported me to this point.

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List of Abbreviations

AgNPs	Silver Nanoparticles
AIT	Athlone Institute of Technology
ANOVA	Analysis of Variance
ASM	American Society for Microbiology
ATCC	American Type Culture Collection
ATLA	Alternatives to Laboratory Animals
BINI	Bioengineering in Ireland
BJU	British Journal of Urology
CAUTI	Catheter Associated Urinary Tract Infection
CFU	Colony Forming Unit
CFU ml^{-1}	Colony Forming Units per Millilitre
Ch	French Gauge
CHX	Chlorhexidine
CPZ	Chlorpromazine
DNA	Deoxyribonucleic Acid
ENIUS	European Network of Multidisciplinary research to Improve Urinary Stents
FEMS	Federation of Microbiological Societies
GIT	Gastrointestinal Tract
H_2O_2	Hydrogen Peroxide
HAIs	Hospital-Acquired Infections
IC	Intermittent Catheter
ID	Indwelling Catheter
IP	Intellectual Property
LB	Luria Broth
LPS	Lipopolysaccharide
MHA	Mueller Hinton Agar
MHB	Mueller Hinton Broth
MRSA	Methicillin-resistant *Staphylococcus aureus*
NCTC	The National Collection of Type Cultures
Ø	Diameter
OD	Optical Density
pHn	pH of Nucleation
PP	Polypropylene
PVC	Polyvinyl chloride
R^2	Coefficient of Determination
RPM	Revolutions per Minute
rRNA	Ribosomal Ribonucleic Acid
SEM	Standard Error of the Mean
SURE	Science Undergraduate Research Experience
TSA	Tryptone Soy Agar
TSB	Tryptone Soy Broth
TUS	Technological University of the Shannon
UPEC	Uropathogenic *E. coli*
UTI	Urinary Tract Infection

Abstract

Catheters are one of the most utilised medical devices in modern medicine with catheter-associated urinary tract infections (CAUTI) being ubiquitous in worldwide healthcare settings. These infections negatively affect patient mortality and morbidity as well as providing reservoirs for the proliferation of antimicrobial resistant genes in culpable strains. The aim of this study was to develop a novel, robust and reproducible, *in vitro* urethra model that can aid in the development and testing of next generation urological devices; with a specific interest in testing the efficacy of intermittent catheters to prevent friction-mediated bacterial displacement and assessing indwelling catheters for the prevention of extraluminal bacterial migration.

The base *in vitro* urethra model comprised of multiple preformed channels within a chromogenic agar-based matrix. The base model was then modified. A **novel *in vitro* urethra friction model** was designed to study friction-mediated bacterial displacement during intermittent catheter insertion. A **novel *in vitro* urethra diffusion model** was developed to assess the diffusion of antimicrobial compounds from a novel polymer coating in the three dimensional space of the urethra. A **novel *in vitro* urethra extraluminal migration model** was devised to visualise bacterial migration on the outer surfaces of a catheter during long-term placement.

The design and development process for each model, and associated methodology, was long and at times arduous. The *in vitro* urethra friction and extraluminal migration models were both validated with *E. coli* and *S. aureus* through twelve independent tests each and were found to be highly reproducible ($P \geq 0.265$ and $P \geq 0.851$, respectively). The development and findings of these models have been disseminated at international conferences and submitted/published in peer review journals. The **novel *in vitro* urethra friction model** directly influenced IP protected changes in the design of a novel friction-eliminating intermittent catheter, developed by the industry partner of this study. The **novel *in vitro* urethra extraluminal migration model** guided the IP protected redesign of an antimicrobial coated catheter, developed by the industry partner of this study, to reduce the antimicrobial coating by ≥50% without reducing its antimicrobial efficacy. Unfortunately, the **novel *in vitro* urethra diffusion model** development did not reach fruition as the needs and the overall aims of the project evolved, it still presents a novel idea that could prove useful in other future studies.

Whilst the knowledge of this thesis could have been IP protected, it was openly published in the interest of public health to allow uninhibited innovation in urological medicine.

Innovation in the field of urological medical devices has been long overdue with little changes in the basic function and design of catheters since the early 20th century. Healthcare-associated infections and in particular CAUTIs are contributing to the global overuse of antibiotics and the looming problem of antibiotic resistance. The novel *in vitro* urethra models described herein, have the potential to inspire change in the medical device industry's design of urinary catheters and strategies for CAUTI prevention.

Publication and Dissemination Record

Relevant Journal Publications

Cortese, Y. J., Wagner, V. E., Tierney, M., Devine, D., & Fogarty, A. (**2018**). Review of Catheter-Associated Urinary Tract Infections and *In vitro* Urinary Tract Models. *Journal of Healthcare Engineering*, 2018, 1–16. DOI: 10.1155/2018/2986742 **Appendix A**

Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D. M., & Fogarty, A. (**2020**). Pathogen displacement during intermittent catheter insertion: a novel *in vitro* urethra model. *Journal of Applied Microbiology*, *128*(4), 1191–1200. DOI: 10.1111/jam.14533 **Appendix A**

Cortese, Y.J., Fayne, J., Devine, D., Fogarty, A., (**2022**). Prevention of extraluminal migration on indwelling urinary catheters: a novel *in vitro* model and method. [Under Review] **Appendix A**

Co-Authored Journal Publications

de Lima, G., Chee, B. S., Moritz, V. F., **Cortese, Y. J**., Magalhães, W. L. E., Devine, D. M., Nugent, M.J.D., (**2019**). The production of a novel poly (vinyl alcohol) hydrogel cryogenic spheres for immediate release using a droplet system. *Biomedical Physics & Engineering Express*. Express 5, 1–25. DOI: 10.1088/2057-1976/ab2547

Aliabadi, M., Chee, B. S., Matos, M., **Cortese, Y. J.**, Nugent, M. J. D., de Lima, T. A. M., Magalhães, W. L. E., & de Lima, G. G. (**2020**). Yerba mate extract in microfibrillated cellulose and corn starch films as a potential wound healing bandage. *Polymers*, *12*(12), 1–18. DOI: 10.3390/polym12122807

Aliabadi, M., Chee, B. S., Matos, M., **Cortese, Y. J.**, Nugent, M. J. D., de Lima, T. A. M., Magalhães, W. L. E., de Lima, G. G., & Firouzabadi, M. D. (**2021**). Microfibrillated cellulose films containing chitosan and tannic acid for wound healing applications. *Journal of Materials Science: Materials in Medicine*, *32*(6), 1–12. DOI: 10.1007/s10856-021-06536-4

Araujo, J. A., **Cortese, Y. J.**, Mojicevic, M., Fournet, M. B., & Chen, Y. (**2021**). Composite Films of Thermoplastic Starch and CaCl2 Extracted from Eggshells for Extending Food Shelf-Life. *Polysaccharides*, *2*(3), 677–690. DOI: 10.3390/polysaccharides2030041

de Lima, T. A., de Lima, G., Chee, B. S., Henn, J. G., **Cortese, Y. J.**, Mattos, M. M., Helm, C. V., Magalhães, W. L. E., Nugent M. J. D. (**2022**). In vitro, in silico, studies and characterisation of pinhão seed coat nanocellulose as a potential anticancer alternative material. [Under Review]

Oral Presentations

Cortese, Y. J., Wagner, V. E., Spence, G., Scully, D., Devine, D., Fogarty, A., (**2018**). Development of an *in vitro* agar based urethral model for testing novel medical devices. In: *Bioengineering in Ireland*. Ennis, Ireland. Abstract DO1-1. **Appendix B**

Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D., Fogarty, A., (**2018**). Development of an agar based *in vitro* urinary tract model to test novel urological medical devices. In: *European network of multidisciplinary research to improve urinary stents (ENIUS)*: *Update in Urinary Stents. Indications-Complications-Technology. Theory & Practice*. Cáceres, Spain. **Appendix D**

Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D., Fogarty, A., (**2019**). *In Vitro* Model of Bacterial Migration During Urinary Catheter Insertion. In: *Bioengineering in Ireland*. Limerick, Ireland. Abstract OB4-01. **Appendix B**

Cortese, Y. J., Fayne, J., Devine, D., Fogarty, A., (**2020**). A Novel *In vitro* Urethra Model to Visualise Bacterial Migration During Long-Term Urinary Catheter Insertion. In: *FEMS Online Conference on Microbiology*. Online. **Appendix B**

Cortese, Y. J., Fayne, J., Devine, D., Fogarty, A., (**2021**). Extraluminal Migration of Pathogens on Urological Medical Devices: A Novel *In vitro* Model. In: *European network of multidisciplinary research to improve urinary stents (ENIUS)*: *Latest advances in Urinary Stents. Biomaterials, Technology and Coatings*. Online. **Appendix D**

Poster Presentations

Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D., Fogarty, A., (**2018**). Development of an *in vitro* agar based urethral model for testing novel medical devices. In: *Science Undergraduate Research Experience (SURE)* and *Athlone Institute of Technology Research Day*. Athlone, Ireland. **Appendix C**

Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D., Fogarty, A., (**2019**). Demonstration of Bacterial Migration due to Medical Device Insertion in a Novel *In vitro* Urethral Model. In: *Federation of European Microbiological Societies (FEMS) 8th Congress of European Microbiologists*. Glasgow, Scotland. Abstract 2198. **Appendices B and C**

Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D., Fogarty, A., (**2019**). Catheter Type Affects Bacterial Migration During Insertion into A Novel *In Vitro* Urethral Model. In: *Athlone Institute of Technology Research Day*. Athlone, Ireland. Poster 60. **Appendix C**

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Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D., Fogarty, A., (**2019**). *In vitro* urethra model to demonstrate bacterial migration during medical device insertion. In: *European network of multidisciplinary research to improve urinary stents (ENIUS)*: *Urethral stents: from modelling to commercialism*. Bern, Switzerland. **Appendix D**

Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D., Fogarty, A., (**2020**). Development of a novel *in vitro* urethra model for improved medical use and research innovation. In: *Athlone Institute of Technology Research Day*. Athlone, Ireland. **Appendix C**

Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D. M., & Fogarty, A. (**2020**). A novel *in vitro* urethra model to demonstrate bacterial displacement during urinary catheter insertion. Access Microbiology, 2(7A), 610. DOI: 10.1099/acmi.ac2020.po0509 **Appendix B**

Cortese, Y. J., Fayne, J., Devine, D., Fogarty, A., (**2021**). Visualisation of Extraluminal Bacterial Migration on Indwelling Urinary Catheters: Development of a Novel *In vitro* Urethral Migration Model In: *The Microbiology Society's Annual Conference*. Online. **Appendices B and C**

Cortese, Y. J., Fayne, J., Devine, D., Fogarty, A., (**2021**). Prevention of Catheter-associated Urinary Tract Infections: A Novel *In vitro* Urethra Model to Observe Extraluminal Bacterial Motility In: *The American Society for Microbiology (ASM) and Federation of European Microbiological Societies (FEMS) World Microbe Forum*. Online. **Appendices B, C, and D.**

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Chapter 1 Literature Review

1.1 Introduction

The urinary system is one of the main routes through which the human body excretes liquid waste. The urinary tract is divided into two sections: the upper tract, consisting of the kidneys and ureters, where liquid wastes from the body are converted into urine and other products; and the lower tract that includes the bladder and urethra, where urine is stored in the bladder before being expelled from the body through the urethra (Chapple, 2011). The outermost section of the urethra and the tissue surrounding the urethral opening is known as the urethral meatus (Gaonkar *et al.,* 2003).

The filling and emptying of the bladder is controlled by the nervous system, specifically the sympathetic, parasympathetic, and somatic nervous system (Murphy, 2018). When the bladder is filling, the bladder wall muscles are relaxed whilst leakage is prevented by the tensed urethral and pelvic floor muscles as well as the sphincter at the neck of the bladder. Once bladder capacity is reached, signals are sent to the brain, via the nervous system, that the person will need to urinate soon. When emptying, the urethral and pelvic floor muscles in conjunction with the internal sphincter relax as the detrusor muscle in the bladder walls contracts until the bladder empties fully (Murphy, 2018).

When functioning normally, the lower urinary tract flushes out the urethra as the bladder empties, preventing the movement of uropathogens up from the periurethral skin or meatus, into the urethra and then into the bladder (Summers & Goeres, 2019; Feneley *et al.,* 2015). If uropathogens manage to enter the bladder of a healthy individual, they will usually be expelled during micturition (Summers & Goeres, 2019). However if they remain, the bladder's internal surface is resistant to pathogenic attachment due to its lining of urothelial cells that are covered in a glycosaminoglycan mucin that prevents uropathogens adhering to the internal bladder surface (Summers & Goeres, 2019; Feneley *et al.,* 2015). If a pathogen bypasses this first line of defence, the immune system should be able to eliminate it if the patient is healthy. If the immune system fails, a urinary tract infection (UTI) can occur and possibly lead to serious illness (Feneley *et al.,* 2015). When problems arise in the lower urinary tract such as nerve damage or muscle atrophy leading to incontinence, or by prostate enlargement or urethral stricture resulting in urinary retention, the use of a urinary catheter can become a necessity (Feneley *et al.,* 2015; Chapple, 2011).

1.2 Urinary Catheters

A urinary catheter is a long tube that is used for the drainage or instillation of fluid into the bladder. Constructed from any number of different polymers, with silicone, latex, and polyvinyl chloride (PVC) most commonly used (Murphy, 2018; Donlan, 2001). When required, the urinary catheter is inserted into the urethra as far as needed until the urine begins to flow. This is known as transurethral catheterisation. A catheter may also be inserted by a medical professional through the creation of an artificial track between the bladder and the abdominal wall, this is known as suprapubic catheterisation (Feneley *et al.,* 2015). A urinary catheter can be a temporary or long-term solution depending on the patient's personal mobility and their prognosis (Murphy, 2018).

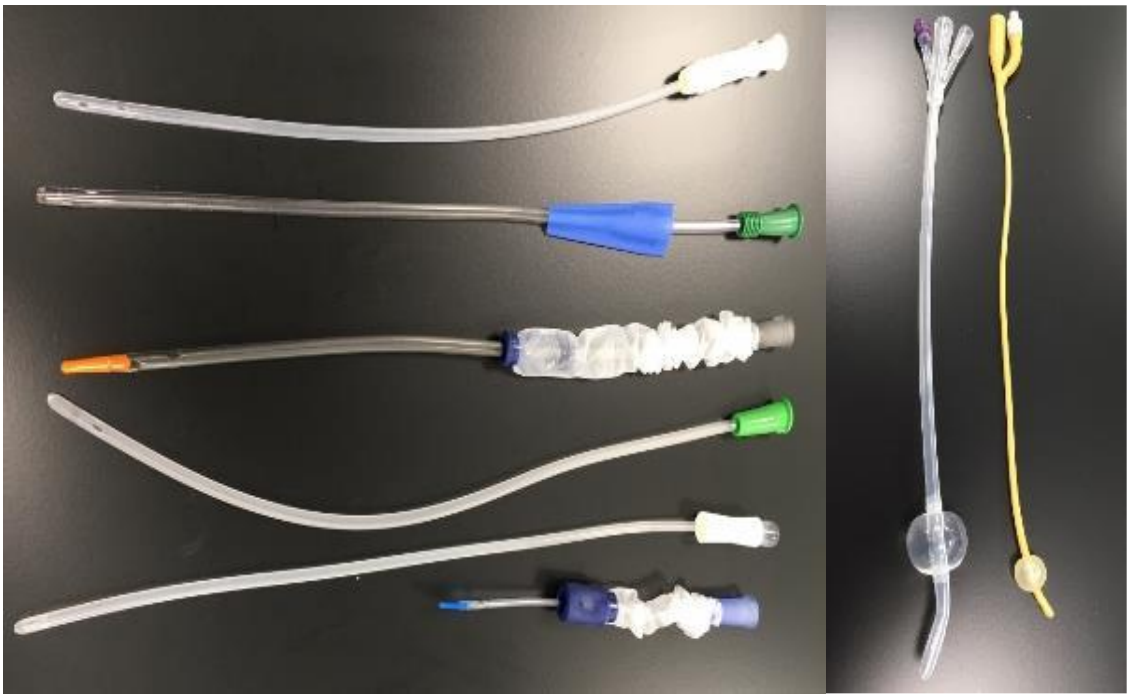


Figure 1.1 Various Intermittent Catheters (left). The upper five are male catheters, the lowest one is a female catheter. Two indwelling catheters with retention balloons inflated (right).

If a patient has the ability to take care of their own medical needs, temporary self-catheterisation can be the best option and performed easily (Feneley *et al.,* 2015). For patients in which self-catheterisation is not an option, indwelling catheters become a necessity to maintain proper function of the urinary system. The most commonly used urinary catheter in the world is the Foley Catheter that was invented by an American urologist named Frederic Foley (Feneley *et al.,* 2015). The Foley catheter consists of a tube containing two channels or lumen; the larger lumen allows the flow of urine from the bladder, and the smaller lumen allows inflation of a balloon just below the tip and eyelets of the catheter that, once inflated,

holds the catheter in place until deflated and removed (Figure 1.1). Under optimal conditions, a urinary catheter can stay in place for approximately 4-12 weeks (Nzakizwanayo *et al.,* 2019). However, this is often not the case as encrustation and infection can block the catheter or lead to medical complications (Feneley *et al.,* 2015).

1.3 Urinary Tract Infections & Catheter Associated Urinary Tract Infections

While catheters are one of the most commonly used medical devices. These devices are notoriously prone to infection. Infection is the largest concern with catheter use, whether long term or short term, with more than 75% of UTIs being associated with indwelling catheter use (Fasugba *et al.,* 2019; Schmudde *et al.,* 2019). Catheter Associated-Urinary Tract Infections (CAUTIs), are the most commonly faced hospital-acquired infections (HAIs) or nosocomial infections, accounting for 40% of all HAIs and the second most common cause of septicaemia (Raja *et al.,* 2019; Sidrim *et al.,* 2019; Jordan *et al.,* 2015; Jacobsen *et al.,* 2008). CAUTIs can lead to numerous medical complications such as: catheter encrustation, bladder stones, septicaemia, endotoxic shock, vesicoureteral reflux, and pyelonephritis (Jordan *et al.,* 2015). CAUTIs are typically caused by fungi or bacteria, including both Gram-positive and Gram-negative bacteria (Gharanfoli *et al.,* 2019; Donlan, 2001). The Center for Disease Control in the U.S.A. defines a CAUTI as a bacterial level $\geq 10^5$ Colony Forming Units (CFUs) ml^{-1} in conjunction with one other symptom *e.g.* fever, in a recently catheterised patient (Summers & Goeres, 2019).

A study by Chatterjee *et al.,* (2014) sampled 150 catheters from patients with no history of UTIs and found that 130 of the catheters had pathogens present both on the catheter and in accompanying urine samples. Some of the most common microorganisms found during the study by Chatterjee *et al.,* (2014) included "*Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Escherichia coli, Citrobacter freundii, Providentia rettgeri,* and *Candida albicans*". These uropathogens can cause asymptomatic bacteriuria or UTIs which can be devastating to at risk patients.

All catheter types and brands are vulnerable to CAUTIs, biofilm formation, or encrustation, and current methods to prevent these complications may just delay the process without treating the problem (Stickler, 2014; Hola & Ruzicka, 2011). Other approaches, such as prophylactic antibiotic courses, raise concerns about antimicrobial resistance and the evolution of numerous new resistant bacterial strains. This is especially relevant to resistance in the

treatment of biofilm linked infections (Jordan *et al.,* 2015). Additionally, the over use of antimicrobials could disturb the balance in the bladders naturally present microflora and further contribute to pathogenesis (Whiteside *et al.,* 2015).

The long held idea that the bladder and urine itself are sterile is a misconception postulated by early bacteriologists in the 1800's (Thomas-White *et al.,* 2016). This idea led a lot of doctors to believe that any UTI or CAUTI was from external contamination only. As the field of microbiology evolved, a better understanding of body's intricate microbiota has developed; with *Corynebacterium* species in males and *Lactobacillus* species in females being the predominant species observed in the respective urinary tracts (Thomas-White *et al.,* 2016; Stickler, 2014; Fouts *et al.,* 2012). Today, it is understood that the prevalence of CAUTIs appears to be caused by a combination of both internal microflora and external introduced contamination (Whiteside *et al.,* 2015).

Patients who practice intermittent catheterisation are most at risk from the microflora of the meatus being pushed up and into the bladder during catheter insertion, with *E. coli* and Staphylococcal species being responsible for the majority of CAUTIs in intermittent catheter users (Nicolle, 2014). With indwelling catheters, the main concern is bacterial biofilms or crystalline biofilms, with *P. mirabilis* infection being of lead concern to patients (Norsworthy & Pearson, 2017). These biofilms are most often initiated by bacterial migration along intra- and extra-luminal catheter surfaces. In roughly a third of cases, intraluminal bacterial migration is observed and is usually due to contamination in the collection bag or a break in the closed sterile system used (Summers & Goeres, 2019). Extraluminal bacterial migration, present the other ~60% of the time, is caused by contamination of the catheter tip during insertion or bacterial migration along an *in situ* catheter from the meatus and periurethral space over time (Summers & Goeres, 2019).

1.4 Gram Negative Uropathogens

The majority of uncomplicated and complicated UTIs are caused by Gram negative uropathogens form the *Enterobacteriaceae* and *Morganellaceae* Families (Grygorcewicz *et al.,* 2021; Gajdács & Urbán, 2019). For both UTIs and CAUTIs, the main Gram negative species include Uropathogenic *E. coli* (UPEC), *K. pneumoniae*, *P. mirabilis*, and *P. aeruginosa* with the main species implicated in both instances being UPEC strains (Flores-Mireles *et al.,* 2015). In regards to prevention of CAUTIs, the two most studied Gram negative uropathogens are UPEC strains

and *P. mirabilis* due to the high isolation rates for UPEC strains and the serious complications associated with crystalline biofilms formed by *P. mirabilis* (Jacobsen *et al.,* 2008).

Escherichia coli

E. coli is a member of the *Enterobacteriaceae* family of bacteria that includes some of the best-known pathogens that affect human health. *E. coli* is the most documented and studied bacterial species in the world, yet it still poses a consistent threat to human health particularly in a medical setting (Jacobsen *et al.,* 2008). Within human anatomy, *E. coli*, as a non-fastidious facultative anaerobe, can live anywhere however it is primarily found in its natural habitat, the gastrointestinal tract or GIT (Finn *et al.,* 2017). With the proximity of the urethra to the anus, especially in female patients, *E. coli* is a major contributor or initiator in the majority of CAUTIs for catheter users (Ayyash *et al.,* 2019; Jacobsen *et al.,* 2008).

UPEC strains that are associated with UTIs are part of a subset of strains referred to as extraintestinal pathogenic *E. coli* strains, and they can cause sepsis and meningitis in addition to UTIs (Jacobsen *et al.,* 2008). UPEC strains are amongst the most common isolates of nosocomial UTIs, and the most common cause of UTIs in the general public. *E. coli* accounts for 70 – 90% of UTIs in the general public and 50% of all nosocomial UTIs (Holroyd, 2019). *E. coli* is a motile bacterial species utilising flagellar-mediated motility to invade the urinary tract. When *E. coli* is introduced into the urinary tract on the surface of a catheter it can move out into the bladder and can eventually move into the upper urinary tract, potentially causing kidney infections (Jacobsen *et al.,* 2008).

Once inside the body, UPEC strains exhibit a number of virulence factors that contribute to the formation and recurrence of UTIs and CAUTIs (Jacobsen *et al.,* 2008). One such virulence factor is the expression of type 1 fimbriae which are found in ≥80% of UPEC strains (Summers & Goeres, 2019; Jacobsen *et al.,* 2008). This adhesin allows UPEC strains and other uropathogens to adhere to the uroepithelial cells lining the urinary tract as well as the surface of a catheter (Summers & Goeres, 2019). This ability to adhere to a catheter allows for the establishment of UPEC infections which can in turn support complex biofilm formation for UPEC and other strains (Jacobsen *et al.,* 2008).

Attachment to uroepithelial cells can lead to invasion and deterioration of the uroepithelial layer and perpetuation of CAUTIs after removal of the catheter (Ulett *et al.,* 2013). UPEC strains are also capable of avoiding the host immune system through capsule and lipopolysaccharide (LPS) production. The capsules produced by UPEC play an important role

in UTIs and CAUTIs as the capsules aid in host immune avoidance, masking the bacterial cells with surface structure similarities to human cells and providing resistance to phagocytosis by immune cells (Jacobsen *et al.,* 2008; Campos *et al.,* 2004). Capsules and LPS expression by UPEC strains has also been shown to aid in their resistance to complement-mediated lysis and endogenous antimicrobial peptides (Jacobsen *et al.,* 2008; Farnaud *et al.,* 2004).

Proteus mirabilis

P. mirabilis, formerly classified as a member of the *Enterobacteriaceae* family, is a member of the *Morganellaceae* under the order of *Enterobacterales* (Gajdács & Urbán, 2019; Adeolu *et al.,* 2016). Species within the *Proteus* genus are widely distributed in the environment and opportunistic, having been linked to numerous nosocomial infections throughout the body (Gajdács & Urbán, 2019). *P. mirabilis* is normally not associated with UTIs in healthy persons with unobstructed urinary tracts (Norsworthy & Pearson, 2017). *P. mirabilis* can, however, colonise the urinary tract of individuals who have structural or functional abnormalities. Catheterised patients are most at risk, as *P. mirabilis* can move along catheter surfaces from the outside in, most likely due to existing colonisation of GIT (M. J. González *et al.,* 2019).

P. mirabilis is the second most common bacterial strain isolated from up to 40% of CAUTIs in patients with long term indwelling catheters, and of all Gram-negative bacteria, it tends to display the greatest propensity to bind the surface of catheters and urological devices in general (Durgadevi *et al.,* 2020; Nzakizwanayo *et al.,* 2019). The greater adherence abilities seen in *P. mirabilis* are due to its production of multiple adherence factors such as hemagglutinins and fimbriae, which allow *P. mirabilis* to attach to devices and host urothelial cells with or without the presence of a conditioning film (M. J. González *et al.,* 2019; Stickler, 2012). The adherence ability of *P. mirabilis* plays a large role in CAUTIs, particularly in catheter encrustation/crystalline biofilm formation (Jacobsen *et al.,* 2008). *P. mirabilis* distinguishes itself from other uropathogens by its high motility and flagella-mediated swarming (Durgadevi *et al.,* 2020; Jones *et al.,* 2004). A study by Jones, *et al.,* (2004) demonstrated the conditions under which *P. mirabilis* swarms, which genes were involved, and visualised cells working together forming swarmer "cell rafts". This study not only discussed the high motility of *P. mirabilis* but also demonstrated how the cells move together across a catheter surface by interweaving their flagella together into helical connections to move rapidly across a surface as one mass (Jones *et al.,* 2004). Jones *et al.,* (2004) proposed that this movement not only contributes to the virulence of *P. mirabilis* but is also conducive to the movement of the bacterium from the skin

to the catheter, to the bladder. *P. mirabilis* has also been observed to travel from the bladder to the kidneys and contribute kidney stones formation (Norsworthy & Pearson, 2017).

P. mirabilis produces urease, which hydrolyses urea, and is essential for crystalline biofilm formation in the urinary tract (M. J. González *et al.,* 2019; Flores-Mireles *et al.,* 2015). *P. mirabilis* has the highest production of urease out of all uropathogens, and the urease it produces is extremely reactive, hydrolysing urea faster than any other species, leading to rapid crystal formation that can encrust catheters and form bladder or kidney stones (Holroyd, 2019; Summers & Goeres, 2019; Flores-Mireles *et al.,* 2015).

P. mirabilis infection can be especially dangerous in healthcare settings as the species is intrinsically drug resistant with clinical isolates often found to be resistant to flouroquinolones and aminoglycosides while having a high tolerance of polymyxin and tetracycline (Learman *et al.,* 2020). Most alarming, recent studies have shown increased resistance to β-lactamases and carbapenemases in clinical isolates, which could ultimately lead to developing resistance to "last-resort" antibiotics (Learman *et al.,* 2020). To further add to the dangers of antibiotic-resistant *P. mirabilis* strains, it has been hypothesised that these strains may act as a "hub" for other uropathogens to thrive whilst in a biofilm where resistance genes can be spread to other species via quorum sensing (Learman *et al.,* 2020; Trautner & Darouiche, 2004).

Many Patients who experience recurrent CAUTIs and in particular recurrent catheter encrustation and blockage have been found to be carriers of *P. mirabilis*. *Sabbuba, et al.,* (2003) whilst investigating indwelling catheter patients in a nursing home found that *P. mirabilis* was continually isolated from the same patient even after catheter removal and antibiotic treatment. They found through genetic analysis that the same strain of *P. mirabilis* was found both within the crystalline biofilm of their encrusted catheters and in the patient's urine without the presence of a catheter (Sabbuba *et al.,* 2003). These finding were later substantiated by another study by *Sabbuba, et al.,* (2004) where they genotyped *P. mirabilis* strains isolated from bladder stones and compared them to *P. mirabilis* strains isolated from the same patients encrusted catheters and found them to be identical, thus complications caused by *P. mirabilis* could be due to residual crystalline fragments in the urinary tract after catheter removal.

Another study by Mathur *et al.,* (2005) also isolated and genotyped *P. mirabilis* strains from patient urine and faecal samples. The patients tested were catheterised for at least 9 months prior to the study and they found that out of the 18 patients included in the study, 10 tested positive for *P. mirabilis* in both urine and faecal samples. Mathur *et al.,* (2005) also determined

that strains of *P. mirabilis* if isolated from both a faecal and urine sample of the same patient, were genetically identical and as such it was proposed that faecal contamination from the patient themselves could be the cause of recurrent CAUTIs and catheter encrustation. Alternatively this study also speculated that patients who don't experience catheter encrustation may not be faecal carriers of *P. mirabilis* (Mathur *et al.,* 2005).

1.5 Gram Positive Uropathogens

Gram positive uropathogens are a common cause of UTIs with Staphylococcal and Enterococcal species being responsible for the majority of Gram positive UTIs and CAUTIs (Kline & Lewis, 2016). Of the Staphylococcal genus, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, and *Staphylococcus aureus* are the main strains implicated in UTI/CAUTI formation. In regards to the Enterococcal genus, *Enterococcus faecalis* and *Enterococcus faecium* are the most often isolated from UTI/CAUTI sufferers (Kline & Lewis, 2016; Arias & Murray, 2012).

Staphylococcal Uropathogens

Staphylococci are a Gram-positive genus of bacteria that currently includes 49 species and 27 sub species. Staphylococci are able to grow in a variety of environmental conditions such as temperatures between 18 – 40°C, high salt concentrations, and aerobic or anaerobic atmospheres (Murray *et al.,* 2013). Staphylococcal species can be found throughout the environment, in particular they colonise the skin and mucus membranes of humans and animals with several species adapted to thrive in niche locations *e.g. S. aureus* within the anterior nares of humans and *S. saprophyticus* colonisation of the human urinary tract. Staphylococci are opportunistic pathogens that pose a significant risk to human health and act as a major contributor to uncomplicated UTI and a minor contributor to CAUTI (Kline & Lewis, 2016; Murray *et al.,* 2013).

Staphylococcus saprophyticus, originally misclassified as a Micrococci, is part of the commensal microflora for the urogenital mucosa and the GIT (Raz *et al*., 2005; Ishihara *et al*., 2001). *S. saprophyticus* is unique from other uropathogenic Staphylococci in that it is specifically adapted to grow in the urinary tract. It is the only coagulase negative Staphylococci that can adhere to the uroepithelial cells and as such cause a UTI without the presence of a catheter or any other functional abnormality (de Paiva-Santos *et al.,* 2018; Kline & Lewis, 2016; Kuroda *et al.,* 2005). *S. saprophyticus* is the second most common aetiological agent in community acquired UTI after *E. coli*. As the predominant cause of uncomplicated UTIs in young and sexually

active females, it is uncommonly isolated from males or patients experiencing CAUTIs (Kline & Lewis, 2016). The immune response to *S. saprophyticus* infection usually results in extensive inflammation throughout the urinary tract causing pyuria, haematuria, and flank pain, however serious complications such as septicaemia are rare (Kline & Lewis, 2016). The main virulence factors of *S. saprophyticus* are the adhesins it expresses allowing it to adhere to uroepithelial cells; a capsule that allows it to resist compliment-mediated opsonophagocytic death by human neutrophils; and urease production that allows the organism to utilise ammonia produced from the hydrolysis of urea as a nitrogen source for persistent growth (Kline & Lewis, 2016; Kuroda *et al.,* 2005). Whether contracted from environmental or endogenous sources, *S. saprophyticus* in the number one cause of Gram positive UTIs and is a prominent uropathogens that warrants further study (de Paiva-Santos *et al.,* 2018; Widerström *et al.,* 2012).

Staphylococcus epidermidis is a Gram positive, coagulase negative cocci that is ubiquitous on human epithelium, primarily colonising the axillae, head, and nares with 10-24 different strains found on healthy adults (Kline & Lewis, 2016; Otto, 2009; Rogers *et al.,* 2009). *S. epidermidis* is usually a commensal organism in healthy adults and is rarely implicated in infections of natural tissues and in some cases it has been theorised that it can have a probiotic affect in preventing *S. aureus* colonisation (Otto, 2009; Rogers *et al.,* 2009). *S. epidermidis* can also be an opportunistic pathogen; and is the coagulase negative Staphylococci that is most often implicated in community acquired and nosocomial infections, particularly those that include indwelling medical devices such as CAUTIs (Kline & Lewis, 2016; Otto, 2009). The pathogenesis of *S. epidermidis* medical device infections is a three stage process: (1) Contamination of medical device during insertion, (2) Rapid expression of adhesins and adherence to the biomaterial, (3) Accumulation, proliferation, and biofilm formation (Fey & Handke, 2006). *S. epidermidis* evades the host immune system and medical intervention in a number of ways including: biofilm formations to protect against antibiotic treatment; excretion of polysaccharide exopolymers to prevent immunoglobulin compliment deposition and neutrophil killing; and upregulation of antimicrobial peptide defences that lower the attraction efficiency of cationic antimicrobial peptides by decreasing the anionic charge of the bacterial membrane (Otto, 2009). With *S. epidermidis* being the most common cause of coagulase negative Staphylococci CAUTIs, further research needs to be done in the future to prevent these infections, particularly with recent increases in antibiotic resistance found in the strain, specifically emerging methicillin resistance (Kline & Lewis, 2016).

Staphylococcus aureus is a major human pathogen that is implicated in a wide range of human infections (Tong *et al.,* 2015). *S. aureus* is a Gram positive, coagulase positive Staphylococci that is both a human commensal and pathogenic species (Walker *et al.,* 2017). Colonising ~30% of the population, it can be found on the skin, perineum, and pharynx but is most often isolated from the anterior nares of healthy adults (Tong *et al.,* 2015; Wertheim *et al.,* 2005). *S. aureus* is not normally involved in UTIs but Methicillin Resistant *Staphylococcus aureus* (MRSA) is increasingly being isolated from patients with complicated UTIs and the leading cause for an MRSA UTI is urinary catheterisation (Looney *et al.,* 2017; Walker *et al.,* 2017; Kline & Lewis, 2016; Tong *et al.,* 2015; Routh *et al.,* 2009; Araki *et al.,* 2002). CAUTIs are the leading cause of secondary healthcare acquired bloodstream infections and those caused by MRSA often result in metastatic infections that greatly increase both morbidity and mortality rates (Walker *et al.,* 2017; Tong *et al.,* 2015). In Ireland 40-50% of *S. aureus* recovered from bloodstream infections is MRSA (Looney *et al.,* 2017). A UTI or CAUTI caused by *S. aureus* can cause fever, haematuria, altered mental states, suprapubic pain, flank pain, and dysuria (Tong *et al.,* 2015). *S. aureus* has evolved several attributes that protect the cell and increase its virulence including: a capsule to prevent phagocytosis (≥90% of clinical strains); adhesin expression to allow attachment to biomaterials; excretion of polysaccharides to aid in biofilm formation; and urease production to allow utilisation of ammonia as a nitrogen source for propagation (Kline & Lewis, 2016; Harris *et al.,* 2002). A particularly insidious route to infection used by MRSA is to use inflammation induced fibrinogen release to aid adherence to urinary catheters. Inflammation caused by the catheter insertion itself leads to the fibrinogen release which then in turn coats the catheter surface making an optimal colonisation environment for MRSA (Walker *et al.,* 2017). Penicillin was first used to treat infections in the 1940s and by the 1970s ≥80% of hospital and community *S. aureus* strains were resistant. *S. aureus* is the most pathogenic of all Staphylococci making infection prevention and control especially important as further antibiotic resistance in the species would be problematic (Deurenberg & Stobberingh, 2008; Chambers, 2001).

Enterococcal Uropathogens

Enterococci are Gram positive cocci that are morphologically indistinguishable from *Streptococcus pneumoniae*, as such they were not given their own distinctive genus until DNA hybridisation and 16S rRNA sequencing determined the formerly know *Streptococcus faecalis and Streptococcus faecium* were distinctly different from other Streptococci; designation into a new

genus Enterococci was then recommended (Murray *et al.,* 2013; K. Fisher & Phillips, 2009; Amyes, 2007).

Regarding UTI and CAUTI, ***Enterococcus faecalis*** and ***Enterococcus faecium*** are two of the most common causes of nosocomial infections with UTIs being the most common site of infection. Enterococcal species are the second highest contributors to CAUTIs after UPEC (Flores-Mireles *et al.,* 2015; Murray *et al.,* 2013; Arias & Murray, 2012; Hammerum, 2012; K. Fisher & Phillips, 2009). As their name implies, Enterococci are enteric bacteria *i.e.* they are natural colonisers of the GIT in humans and animals in what is thought to be a commensal relationship, however when infections do occur, the GIT can act as a reservoir in perpetuating the infection (Murray *et al.,* 2013; Arias & Murray, 2012; Arias *et al.,* 2010; Amyes, 2007; Kau *et al.,* 2005). Other infections that can be caused by Enterococcal species include: endocarditis, neonatal sepsis, surgical wound infections, bacteraemia, and hepatobiliary sepsis (K. Fisher & Phillips, 2009). Enterococci can cause infection in almost any part of their body due to their ability to survive in a variety of environments with tolerances of pH 4.6 – 9.9, temperatures of 10-45°C, and high sodium chloride or bile salts concentrations (Murray *et al.,* 2013; Arias & Murray, 2012).

Most infections caused by Enterococci are due to endogenous flora, often spread from one mucosal surface to another, usually occurring after the use of broad spectrum antibiotics, particularly those that are not specifically effective for Enterococcal infections (Murray *et al.,* 2013; K. Fisher & Phillips, 2009). UTIs and CAUTIs caused by Enterococcal species can be asymptomatic or lead to complications such as cystitis and pyelonephritis (Murray *et al.,* 2013). Enterococci have evolved a number or virulence factors, including: colonisation of the GIT, acting as a reservoir for infection; expression of adhesins that allow attachment to both biomaterials and uroepithelial; biofilm formation and quorum sensing; and inherent antibiotic resistance (Murray *et al.,* 2013; Arias *et al.,* 2010; K. Fisher & Phillips, 2009). Enterococcal species are characteristically resistant to several first line antibiotic and antimicrobial agents showing resistance to cephalosporins and low-level resistance to aminoglycosides and β-lactams (Murray *et al.,* 2013; Hammerum, 2012). Vancomycin has in the past often been viewed as a last line of defence against multi-drug resistant gram positive bacteria, in hospitalised patients with bloodstream infections, the majority of *E. faecium* strains isolated are now demonstrating vancomycin resistance (K. Fisher & Phillips, 2009; Boneca & Chiosis, 2003). Enterococcal vancomycin resistance is a major threat both directly to patients infected with such strains and an indirect threat in that there is potential that vancomycin resistance

capabilities could be transferred from Enterococci to other species. This has already been observed with some MRSA species now showing vancomycin resistance that occurred due to horizontal acquisition of a plasmid from *E. faecalis* (Lerminiaux & Cameron, 2018). While Enterococci may not possess a broad spectrum of virulence factors when compared to Staphylococcal species, the increase in life threatening antibiotic resistance seen in strains of *E. faecalis* and *E. faecium* pose a serious threat to patients and the community at large (Murray *et al.,* 2013).

1.6 Biofilms

The cellular structure of the bladder and the regular emptying of its contents usually prevent bacteria/fungi from multiplying to dangerous levels or adhering to the surrounding mucosa. When a foreign object like a catheter is introduced, bacterial or fungal contamination may occur (Feneley *et al.,* 2015). Normally in the bladder, microorganisms are present in a planktonic state where they are freely suspended in the urine. In this state, they are unlikely to cause a UTI unless present in large numbers that may overwhelm the bladder's innate immune defences (Donlan, 2001). When an indwelling urinary catheter is in place, or any medical device within in the body, microorganisms can attach to the medical device, forming large colonies bound together and usually enclosed in a polymer matrix known as a biofilm (Sayal *et al.,* 2014; Donlan, 2001).

A biofilm is defined as microorganisms bound to a surface or each other with the presence of an extracellular matrix composed of secreted products of the organisms and/or of components of the microorganisms themselves (Sayal *et al.,* 2014). The cells within the biofilm may be irreversibly bound to the surface and to each other via secreted, adhesive substances (Sayal *et al.,* 2014). The organisms contained within the biofilm usually demonstrate changes in gene expression differing from their planktonic state (Trautner & Darouiche, 2004). A biofilm can contain just one or multiple species, and the organisms involved can be gram-negative or gram-positive bacteria and fungi (Sayal *et al.,* 2014). The longer a urinary catheter is in place, the more likely it is for a biofilm to form on its surface and cause a CAUTI. Patients who are catheterised short term (≤7 days) experience biofilm formation 10-50% of the time; however, practically all patients who are catheterised long term (>28 days) are found to present with biofilm formation (Donlan, 2001).

Being a part of a biofilm is highly advantageous to a microorganism, as the group together is more resilient and resistant than any singular planktonic organism (Coenye & Nelis, 2010;

Donlan, 2001). The advantages for an organism being within a biofilm community include antimicrobial resistance, protection from physical forces, and safety from phagocytosis by immune cells (Trautner & Darouiche, 2004). The ability of biofilms to resist antimicrobial agents is particularly worrying, as mechanisms of resistance, such as genes encoding for antimicrobial resistance, can be transferred throughout the community and even further afield as microorganisms leave the biofilm to spread and multiply (Homeyer *et al.,* 2019; Sayal *et al.,* 2014). Within a biofilm, cell to cell communication can occur in a process known as quorum-sensing to choreograph changes in gene expression across the community (Jacobsen *et al.,* 2008). A biofilm can also influence and change aspects of the surrounding environment, and is of particular interest when examining the factors that lead to encrustation and blocking of urinary catheters (Broomfield *et al.,* 2009).

1.7 Crystalline Biofilms

Urinary catheter encrustation is an ongoing problem with no simple solution. Catheter encrustation can cause numerous unfavourable outcomes for patients beyond the previously mentioned health risks biofilms alone present. Encrusted catheters become blocked, leading to urine retention that is not only painful for the patient but also constitutes a medical emergency (Frant *et al.,* 2018; Feneley *et al.,* 2015). Once blocked by encrustation, the catheter must be removed to avoid damaging the bladder, urethra, ureters, and kidneys; if the pressure builds to a high enough level in the bladder, ureteric reflux can occur where urine is forced backwards up into the ureters and into the kidneys (Feneley *et al.,* 2015). In some patients, catheter crystallisation can be so extreme that removal of the catheter can require emergency surgery (Stickler, 2008).

Encrustation can occur due to metabolic dysfunction, but generally urinary catheter encrustation occurs due to bacterial influence, particularly urease-producing bacteria that form crystalline biofilms as shown in Figure 1.2 (Durgadevi *et al.,* 2020; Hola & Ruzicka, 2011). Although many various bacterial and yeast strains can and do lead to UTIs or CAUTIs, when it comes to urinary crystalline catheter encrustation, the urease-producing species *Proteus mirabilis, Proteus vulgaris,* and *Providentia rettgeri* are of interest in most studies (Jordan *et al.,* 2015; Broomfield *et al.,* 2009; Stickler, 2008; Morris *et al.,* 1997). Of these three bacteria, *P. mirabilis* is isolated most frequently from patients and produces the most urease, an enzyme that hydrolyses urea, breaking it down into ammonia and carbonate ions (Stickler, 2008). Urease-producing bacteria use ammonia as a source of nitrogen and carbon to support further colony growth (Jacobsen *et al.,* 2008). Increasing ammonia levels lead to an increase in the overall pH

of urine in the bladder, and the bacterially-produced alkaline environment causes calcium and magnesium to come out of solution and precipitate into crystals (Morris *et al.,* 1997). The crystallised mineral forms of magnesium and calcium are known as struvite, which is magnesium ammonium phosphate, and apatite, a poorly crystallised form of hydroxylated calcium phosphate (Stickler, 2008). This process of catheter encrustation via crystallisation is directly connected to the formation of biofilms and the products produced by the organisms within (Morris *et al.,* 1997).

As with the advantages for the bacteria to form into a biofilm, there are also specific advantages to forming crystallised biofilms. Infections can often persist in patients when a catheter is removed, several studies believe this could be due to the crystalline nature of these biofilms (Sabbuba *et al.,* 2003; Feneley *et al.,* 2002). As the previously encrusted catheter is removed, crystals can break off, containing the bacterium that they formed upon (Feneley *et al.,* 2002). These crystal fragments act as a nuclei on which newly forming minerals can grow and ultimately form bladder stones (Feneley *et al.,* 2002). These bladder stones can store pathogens, allowing the re-infection and crystallisation of a new catheter, thus perpetuating the cycle (Feneley *et al.,* 2002). Morris, *et al.,* (1997) and more recently Barros *et al.,* (2017) describe the development of a crystallised biofilm on the surface of urinary devices as follows:

1. The urinary tract is infected by a urease-producing bacterial species
2. The surface of the urinary device is prepared for bacteria adhesion by the production of an organic conditioning film by the deposition of urine components, ions, and minerals
3. The urease-producing bacteria adhere to the conditioning film
4. The biofilm community begins to form as they excrete an exopolysaccharide matrix
5. As bacterial numbers rise in the biofilm, so does the release of urease that goes on to hydrolyse urea into ammonia, increasing the pH of both the urine and the biofilm
6. Calcium and magnesium ions are attracted to the biofilm's gel matrix
7. The calcium and magnesium phosphate crystallise, forming struvite and apatite crystals on the device's surface bound with the biofilm

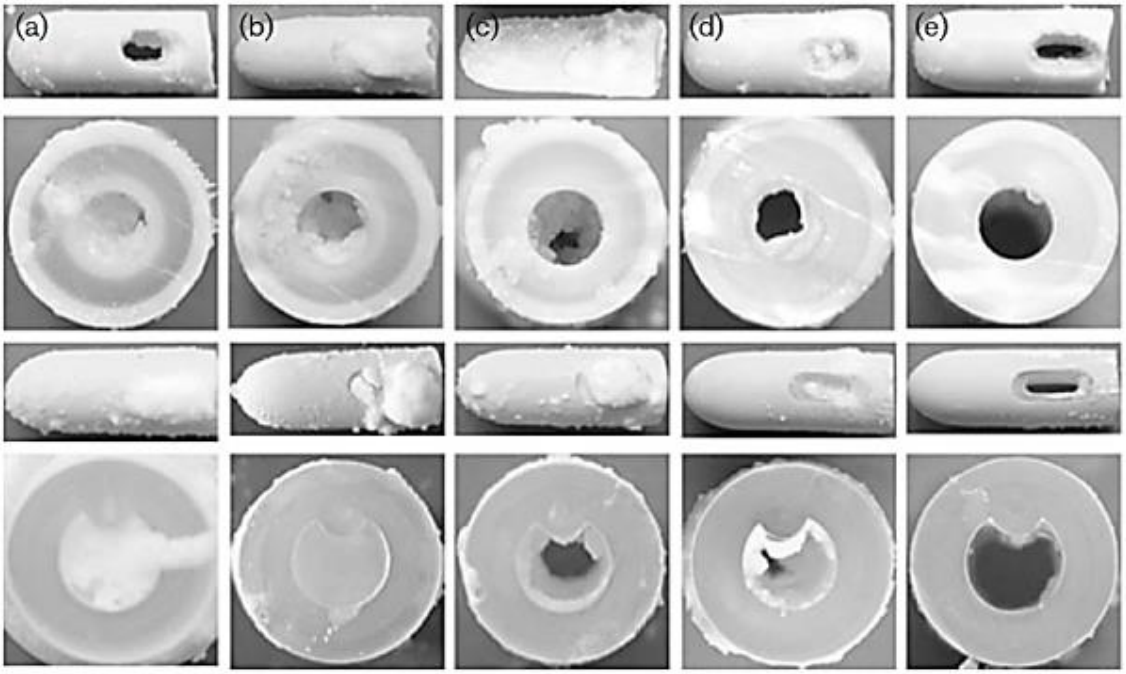


Figure 1.2 Examples of catheters encrusted by crystalline biofilms created by various bacteria, (a) *Proteus mirabilis,* (b) *Proteus vulgaris,* (c) *Providentia rettgeri,* (d) *Morganella morganii,* and (e) *Staphylococcus aureus*. The top two rows are are silver/latex catheters and the bottom two rows are nitrofurazone/silicone catheters (Broomfield *et al.,* 2009).

1.8 Non-Crystallised Biofilms

Some urease-producing bacterial species do not form crystallised biofilms as their urease production levels are too low. These include: *Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Morganella morganii,* and *Providencia stuarii,* to name a few (Stickler, 2014). While these microbes do have the ability to form a biofilm, it will not be crystalline in structure without help from other species as their lower urease output, although able to hydrolyse urea into ammonia, is not high enough to raise the urine to a pH of >8.0 which is needed for apatite and struvite to form (Broomfield *et al.,* 2009).

Interestingly *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* although they cannot produce crystals, can still block catheters and cause the same problems associated with reduced or halted bladder drainage (Stickler, 2008). Broomfield, *et al.,* (2009) investigated different approaches to controlling crystalline biofilms on catheters and during their testing they observed that both *Klebsiella pneumoniae* and *Pseudomonas aeruginosa,* while not able to produce a crystalline biofilm, produced large amounts of a mucoid material that did not block the catheter but did greatly reduce urine flow.

1.9 Control and Prevention of CAUTIs

Due to the ongoing problems caused by crystalline biofilm encrustation with indwelling catheters, and CAUTIs in general with intermittent catheters, numerous studies have been carried out proposing novel solutions. Many manufacturers and researchers have come out with new products, all with varying levels of success. This section will discuss previously implemented CAUTI mitigation strategies and their efficacy.

1.9.1 Indwelling Catheters

Indwelling catheters are primarily at risk of CAUTIs due to biofilm formation. A large body of research to date has been carried out to develop and test novel indwelling catheters to prevent CAUTIs or more specifically prevent bacterial adherence, biofilm formation, and catheter encrustation. These are generally characterised by the antimicrobial agent, material, design, or practice involved.

Antimicrobial Agents

Over the decades, many studies and manufacturers have tried to produce a urinary catheter that resists biofilm formation by impregnation or coating the catheter in an antiseptic, antimycotic, antimicrobial, or bactericidal compound. A study by Ghanwate *et al.,* (2014) investigated the efficacy of several antimicrobial agents effective against the urinary pathogen *P. aeruginosa* to determine if they could prevent or remove biofilm formations on coated catheters. **Cisplatin** is a medication used commonly in chemotherapy treatment but can also be effectively used as an antimicrobial. In the study by Ghanwate *et al.,* (2014), Cisplatin prevented biofilm growth for 8 days. Ghanwate *et al.,* (2014) also investigated the antimicrobial ability of **Heparin**, normally used as an anti-coagulant, and the antibiofilm enzyme **DNase** which, through endonucleic cleavage, degrades DNA by breaking the phosphodiester linkages in the DNA backbone. They found Heparin prevented biofilm formation for only 6 days and DNase only for 5 days, making them the least effective long term. The compounds identified by the work of Ghanwate *et al.,* (2014) could be useful for short-term or intermittent catheterisation but are not effective for long-term catheterisation or >28days.

Several studies investigated the antimicrobial **Nitrofurazone**, an antimicrobial organic compound, that prevents bacterial growth and biofilm formation by inhibiting DNA replication (Andersen & Flores-Mireles, 2020). While it has been proposed that it might be a suitable antimicrobial agent to coat catheters, its effects against *P. mirabilis* are negligible at best

and it does not prevent crystalline biofilm formation (Stickler, 2014; Johnson *et al.,* 1999). In a recent randomized clinical trial by Menezes *et al.,* (2019) Nitrofurazone coated catheters were tested to prevent bacteriuria and CAUTI in 214 kidney transplant patients. They found no significant difference in bacteriuria or CAUTI incidence between the test and control groups whilst also observing an increase in catheter-related side effects in the test group. Their findings further support the work of Stickler, (2014), demonstrating the limited clinical benefit of Nitrofurazone for CAUTI control/prevention (Menezes *et al.,* 2019).

Triclosan is a biocide with antimicrobial capabilities and can act as both an antibacterial and an antimycotic agent (Singha *et al.,* 2017; Dhillon *et al.,* 2015). Triclosan, like the other antimicrobials discussed, has also been used in several studies to control catheter encrustation and biofilm formation (Jordan *et al.,* 2015; Broomfield *et al.,* 2009; Williams & Stickler, 2008a; Gaonkar *et al.,* 2003; Stickler *et al.,* 2003). It has been well established that *P. mirabilis* is highly susceptible to Triclosan and it can be used as an alternative to antibiotic treatments (Williams & Stickler, 2008a). One method of Triclosan administration comprised of filling the retention balloon of a Foley catheter with a Triclosan/polyethylene glycol solution instead of water, allowing the Triclosan solution to permeate out of the catheter balloon slowly (Stickler *et al.,* 2003). With *P. mirabilis's* susceptibility to Triclosan so well documented, a study by Broomfield, *et al.,* (2009) aimed to also test Triclosan's efficacy against the other two main urease-producing strains; *P. vulgaris,* and *P. rettgeri.* Their study found that *P. vulgaris,* like *P. mirabilis,* is fully sensitive to Triclosan and catheter blockage was prevented, while *P. rettgeri* was resistant to the Triclosan levels achievable in the bladder, and catheter blockage occurred (Broomfield *et al.,* 2009). A recent study by Ayyash *et al.,* (2019) investigated the efficacy of a Triclosan/EDTA solution, as a balloon lock solution, to prevent catheter colonisation by several *E. coli* strains. They found that the test solution fully eradicated biofilm formation in the *E. coli* strains tested. Even with these positive results, Triclosan is not the final solution in encrustation defence. As with all antimicrobial use, microbial resistance is a never ending race that Triclosan may already be losing (Dhillon *et al.,* 2015). Triclosan's mechanism of antimicrobial action is concerning as it targets the enzyme enoyl reductase which is required by most bacteria for fatty acid biosynthesis. Numerous antibiotics also work by targeting enoyl reductase, and if microbes develop resistance to Triclosan they may develop cross-resistance to a variety of antibiotics (Singha *et al.,* 2017; Dhillon *et al.,* 2015).

Chlorpromazine (CPZ) is an antimicrobial that is traditionally used as a phenothiazine antipsychotic drug for the treatment of schizophrenia (Sidrim *et al.,* 2019). Mechanistically,

CPZ inhibits bacterial growth by altering cell membrane permeability and inhibiting efflux pumps which are transport pumps on the cells surface that are directly involved in the extrusion of toxic substances out of the cell (Sidrim *et al.,* 2019; Webber & Piddock, 2003). When considering bacteria, these toxic substances would include antibiotics and some multi-drug resistant bacterial strains have altered or increased expression of efflux pumps allowing for improved clearance of these drugs (Webber & Piddock, 2003). With this in mind a recent study by Sidrim *et al.,* (2019) created and tested a CPZ coated indwelling catheter to assess if CPZ alone or CPZ in conjunction with the antibiotics ciprofloxacin and meropenem could inhibit biofilm growth. In tests with antibiotic-susceptible and antibiotic-resistant uropathogenic strains of *E. coli*, *P. mirabilis*, and *K. pneumoniae*, they found that CPZ not only significantly reduced biofilm formation on its own, but that it also potentiated the effects of ciprofloxacin and meropenem. These promising results propose the CPZ coated urinary catheters could be become an important tool in fighting multi-drug resistant CAUTIs with CPZ acting as an adjuvant in the fight against infection (Sidrim *et al.,* 2019).

Chlorhexidine (CHX) was first discovered in the 1950's and ever since has been used extensively in the healthcare industry due to its broad spectrum antiseptic abilities and relatively low toxicity (Andersen & Flores-Mireles, 2020; Huang, 2019). CHX is a cationic bisbiguanide and acts by interaction between the cell membrane of bacteria and fungi which is negatively changed, and the positively changed functional group in the CHX structure which ruptures the cell membrane of the microbe (Srisang & Nasongkla, 2018; Singha *et al.,* 2017). At low concentrations CHX is bacteriostatic with higher concentrations required to act as an irreversible bactericide (Majeed *et al.,* 2019; Singha *et al.,* 2017). CHX has been traditionally used in medicine as a cleaning agent, often used to cleanse wounds, surgical site preparation, or in general to wash patients who are at increased risk of infection (Abdel-Sayed *et al.,* 2020; Fasugba *et al.,* 2019; Schmudde *et al.,* 2019). In regards to urinary catheters, CHX has customarily be used as a cleanser for the meatal area or in some instances to cleanse the patient from the navel down to prevent contamination of a catheter during insertion and the duration of its placement (Fasugba *et al.,* 2019; B. G. Mitchell *et al.,* 2019). In this use case two studies found that cleansing a patient with CHX versus normal saline resulted in a significant drop in CAUTI and as an added benefit, a reduction in patient care costs (Fasugba *et al.,* 2019; B. G. Mitchell *et al.,* 2019). In recent years CHX has been trialled as a catheter coating additive with one study by Gefter *et al.,* (2018) testing the efficacy of an indwelling catheter coated in a CHX varnish. They found that the CHX varnish decreased the growth and biomass of *P. aeruginosa* biofilms both *in vitro* and *in vivo* indicating some potential for the coating to prevent CAUTI

with future improvements. Another series of studies by one research group, sprayed or dip coated CHX micelles, nanospheres, or nanoparticles onto indwelling catheters which they found to prevent bacterial proliferation for 6, 14, and 28 days respectively (Srisang *et al.,* 2020; Srisang & Nasongkla, 2018, 2019). While these findings are promising, it should be noted that although CHX exhibits low mammalian toxicity, there has been a link associated between CHX and allergic reactions in patients (Fernandes *et al.,* 2019). These allergic reactions can cause mild symptoms such as rash or urticaria however in a minority of cases CHX exposure can cause anaphylaxis which can result in cardiac arrest and possible death (Fernandes *et al.,* 2019). Further investigation into using CHX as a catheter coating is warranted based on previous promising studies however, due to its potential for inducing allergic responses in patients, its use in clinical settings should be monitored to avoid allergic sensitisation.

Silver

A non-pharmaceutical antimicrobial that has been used in catheters is silver, either impregnated into the catheter's materials itself or as part of a polymer coating on the catheters surfaces (Broomfield *et al.,* 2009; Donlan, 2001). The antimicrobial effects of silver have long been utilised in medical dressings for burns and pressure ulcers as well as in consumer goods (Estores *et al.,* 2008). Silver exhibits broad spectrum antimicrobial activity that is effective against both anaerobic and aerobic bacteria as well as both gram-positive and gram-negative bacteria (Estores *et al.,* 2008). Silver's mechanism of action involves the release of ions that cause oxidative damage to a bacterium's cellular DNA and disruption of the cell's membrane (Thokala *et al.,* 2018; Durán *et al.,* 2016). This oxidative damage occurs as a result of strong binding between the silver ions and critical biological molecules, such as DNA, RNA, and proteins, damaging them and disrupting their functions (Thokala *et al.,* 2018). Some studies have found that silver can be effective in controlling bacterial levels during short term catheterisation; however, silver was found to only delay the onset of bacteriuria and has not been proven effective in prevention of CAUTIs (Thokala *et al.,* 2018; Hooton *et al.,* 2010; Brosnahan *et al.,* 2004).

When discussing catheter research, different forms of silver coatings have been trialled. Silver oxide, silver alloys, and silver nanoparticles (AgNPs) have all be assessed to determine their efficacy in preventing bacteriuria, CAUTIs, and biofilm formation. Silver oxide catheters are no longer on the market as any evidence they were effective in prevention of CAUTIs was deemed statistically insignificant (Hooton *et al.,* 2010). A more promising silver catheter design

contains a silver alloy where the silver has been stabilised by other metals such as gold and palladium which allows for the slow release of silver ions (Davenport & Keeley, 2005).

A notable catheter with a silver alloy coating is the Bardex® IC catheter which is coated in a silver alloy embedded in a hydrogel and palladium layer on both the internal and external surface (Verleyen *et al.,* 1999). Some studies in the past have demonstrated that silver alloy catheters postpone development and reduce instances of asymptomatic bacteriuria, and while this may make these catheters seem like they could prevent CAUTIs, they have been categorically dismissed by several largescale reviews (Holroyd, 2019; Chua *et al.,* 2017; Pickard *et al.,* 2012; Hooton *et al.,* 2010; Brosnahan *et al.,* 2004; Verleyen *et al.,* 1999). More recently a couple of studies have coated catheters in AgNPs to assess their antibiofilm or antifouling ability. A laboratory study by Wang *et al.,* (2015) coated catheter segments with layers of AgNPs immobilised on polydopamine and an outer "antifouling layer" of Poly(sulfobetaine methacrylate-co-acrylamide). Their belief was that the outermost layer would prevent catheter encrustation and the silver would prevent CAUTIs over time. Wang *et al.,* (2015) found that their AgNPs coating reduced overall bacterial concentrations when compared to a control by two orders of magnitude and that their coating resisted encrustations for up to 45 days depending on how many AgNP layers were present. Another laboratory study by Thomas *et al.,* (2015) also coated catheter segments in AgNPs and found the coating inhibited the adhesion of coagulase negative staphylococci to the catheter with between an 80-90% reduction in biofilm formation dependant on the test species.

While the efficacy of silver in the prevention of CAUTIs is still debated, it should be noted that bacterial resistance to silver is now being observed in members of the Enterobacteriaceae and Morganellaceae families including: *Proteus mirabilis, Enterobacter cloacae, Citrobacter freundii, and Klebsiella pneumoniae*; with *Proteus mirabilis'* resistance to silver of particular interest in preventing CAUTIs (Raja *et al.,* 2019; Estores *et al.,* 2008). To this end, different antimicrobial metals are now being investigated *e.g.* zinc, however their use to date is limited and under continuing scrutiny to determine their therapeutic use whilst balancing their potential for cytotoxic effects (Raja *et al.,* 2019).

pH Control and Citrate

As discussed previously, many uropathogenic bacteria produce urease which hydrolyses urea, increasing the pH of the urine, and ultimately leading to precipitation of calcium and magnesium in the urine which directly contributes to encrustation of catheters (Suller *et al.,*

2005). The pH at which calcium and magnesium precipitate in the urine is classified as the nucleation pH or pH_n (Mathur *et al.,* 2006b). Mathur *et al.,* (2006b) determined that the pH_n varies in each individual and can account for why some patients block catheters very quickly while others don't. They found that the mean pH_n of participant's urine ranged from 6.67 to 8.96. In addition, the fact that the pH_n of a patient's urine could vary from week to week suggested that the manipulation of pH_n could be a strategy for controlling encrustation (Mathur *et al.,* 2006b). Mathur *et al.,* (2006a) went onto undertake another study where they assessed if lowering calcium and magnesium concentrations in the urine would affect the pH_n and thus reduce/eliminate catheter encrustation. They found that by increasing a patients fluid intake there would be a decrease in magnesium and calcium concentration in the urine and a resulting increase in the patients pH_n (Mathur *et al.,* 2006a). They also found calcium concentration to have a larger impact on pH_n than magnesium (Mathur *et al.,* 2006a).

A study by Suller *et al.,* (2005) also investigated the link between pH_n and catheter encrustation with the aim to control a patients pH_n with citrate. Citrate acts as a chelating agent for divalent metal ions and as such it can keep calcium and magnesium in solution (A. Khan *et al.,* 2010; Stickler, 2008). Suller *et al.,* (2005) found that intake of citrate can increase a patients pH_n, decreasing the likely hood of their catheter blocking. Intake of fresh orange juice, daily, would cover the recommendations of both increased citrate and fluid intake which Suller *et al.,* (2005) found could raise a urine pH_n from 7.24 to 8.2 reducing the risk of catheter encrustation. Another later study by A. Khan *et al.,* (2010) found similar results when assessing the impact on patient pH_n when administered citrate in the forms of lemon juice and potassium citrate. They came to the same conclusion as the work carried out by Suller *et al.,* (2005) that citrate and increase fluid intake is effective in modulating a patient's pH_n and could be an inexpensive, simple, and effective way to reduce and control catheter encrustation (A. Khan *et al.,* 2010).

A study by Broomfield, *et al.,* (2009) specifically looked at the urease production levels of uropathogens in relation to catheter blockage time. They found that the thirteen bacterial species tested fell into three distinct groups based on levels of urease production which also correlated to the largest changes in urine pH recorded (Broomfield *et al.,* 2009). The highest urease producing species were *Proteus mirabilis, Proteus vulgaris and Providencia rettgeri* and all blocked catheters quickly and completely.

A recent study by Torrecilla *et al.,* (2020) carried out a double blind study where 52 patients were given a placebo and 53 patients were given a novel oral treatment, consisting of a urine acidifier and crystallisation inhibitor compound. The aim of this study was to determine if

increased pH in the urinary system could prevent crystallisation of double J stents. They found that after an 8 week period, patients in the experimental group experienced an 8-fold decrease in global stent encrustation when compared to the control group (Torrecilla *et al.,* 2020).

The use of citrate and increased fluid intake to increase pH_n and reduce encrustation has been part of the advice given to urinary catheterised and stented patients for over 20 years (Stickler, 2014). Although pH modulation can successfully reduce catheter encrustation *in vitro* and *in vivo*, it only addresses the crystallisation but not the underlying infection that possess the greatest risk to patient mortality and morbidity.

Furanones

Quorum sensing communication contributes to the development of biofilms, and some studies have looked towards furanones as a solution to disrupting a bacteria's ability to communicate and prevent or inhibit biofilm formation (Trautner & Darouiche, 2004). Furanones are naturally occurring compounds that are secreted by the red algae species *Delisea pulchra* that possess the ability to disrupt cell-to-cell signalling (Proctor *et al.,* 2020). Some studies have shown that *E. coli* exposed to furanones demonstrated a clear reduction in biofilm thickness, and when *P. aeruginosa* was exposed to furanones, quorum sensing-controlled gene expression was reduced in a manner that impacted *P. aeruginosa*'s biofilm architecture and total biomass (Hentzer *et al.,* 2002; Ren *et al.,* 2001). Furanones also inhibited the biofilm growth of *S. epidermidis, B. subtilis,* and *E. coli;* however, more research is needed to determine if the cause of inhibition is in fact quorum sensing disruption (Hentzer *et al.,* 2002; Ren *et al.,* 2001). Unfortunately, the potential toxicity of furanones has resulted in their limited clinical use (Jacobsen *et al.,* 2008; Trautner & Darouiche, 2004).

Polymers and Biomaterials

Throughout the modern history of catheter use, numerous materials have been tested to assess their ability to prevent UTIs or CAUTIs, including polyurethane, hydrogels, silicone, latex, and composite biomaterials (Holroyd, 2019; Donlan, 2001). No material alone has presented a universal solution; however, new innovations in material science and polymer science may hold the key. One such biomaterial catheter was studied by Vapnek, *et al.,* (2003), LoFric® catheters covered in a hydrophilic coating was found to have a decreased incidence of UTIs or CAUTIs in clinical settings. A study by Stickler, (2002) investigated another hydrophilic polymer catheter that was coated in phosphorycholine, which is the main polar head group found on erythrocytes. This phosphorycholine coated catheter was not only biocompatible with the

body but it was also stable. Although it has been shown to inhibit bacterial colonisation on medical devices like contact lenses; in urological tests it did not inhibit the growth or formation of crystalline biofilms by *P. mirabilis* (Stickler, 2014; Andrews *et al.,* 2002; Hentzer *et al.,* 2001; Ren *et al.,* 2001).

Probiotics

The use of non-pathogenic bacterial species as probiotics to displace pathogenic bacteria has had some successes in preventing UTIs. One study found that 21 patients who had been inoculated with *E. coli* 83972 experienced no bacteriuria when compared to the previous year where an average of 3.1 patients suffered a UTI (Hull *et al.,* 2000). Another study by Darouiche *et al.,* (2001), also discusses the success of non-pathogenic *E.coli* strains to prevent UTIs in patients with neurogenic bladders caused by spinal cord injuries. They found that direct insemination of *E.coli* into the bladder was safe and did not produce symptoms of a UTI but it did lower the overall instances of UTIs when compared to the patients' history (Darouiche *et al.,* 2001).

In a recent study by Learman *et al.,* (2020), it was found that *Morganella morganii* can inhibit urease induced catheter crystallisation and blockage by *P. mirabilis*. They found that while some species such as *E. coli*, *E. faecalis*, and *Providencia stuartii* can enhance *P. mirabilis* urease production, leading to increased infection severity (Learman *et al.,* 2020). *M. morganii* actively inhibits *P. mirabilis* and while it may be appealing, based on this study, to think that *M. morganii* could be used as a probiotic it should be noted that *M. morganii* is an opportunistic human pathogen in its own right. While its use as a probiotic organism may not be appropriate, Learman *et al.,* (2020) recommends future investigation into secretions produced by *M. morganii* that may lead to novel therapeutic treatments for CAUTIs, particularly those involving *P. mirabilis*.

Alternative Designs

The disruption of CAUTI biofilms by mechanical catheter-pathogen displacement has been investigated as a potential CAUTI solution. Chakravarti *et al.,* (2005) investigated the use of an electrical current to aid in the dispersion of silver ions from a silver impregnated catheter. The method works by passing an electric current through silver electrodes imbedded in the catheter which generates silver ions in the bladder (Chakravarti *et al.,* 2005). This method was effective in inhibiting biofilm growth on the catheter; however, the effect was temporary as

the electrical current caused the silver electrode to disintegrate after 150 hours, making this method only suitable for short-term catheterisation (Chakravarti *et al.,* 2005).

An alternative mechanical approach was reported in a study by Hazan *et al.,* (2006) where an elastic wave-generating actuator was attached to a Foley catheter before insertion into the urethra of a male rabbit. The actuator produced low-energy acoustic waves along the surface of the catheter, which was found to prevent biofilm production for up to 9 days in contrast to the control animals' catheters, that had biofilm formation by the second day (Hazan *et al.,* 2006).

Another study by Levering *et al.,* (2016) developed a novel ID design that aimed to disrupt and dislodge biofilms from the catheters inner drainage lumen. Their catheter was designed to have inflation lumens which run the length of the catheter between in urine drainage lumen and the outer catheter wall (Figure 1.3). When inflated they found that the pressure exerted by the inflation lumens was sufficient to dislodge crystalline biofilms from the inner luminal surface, breaking them apart, so the remnants could then be flushed out by the flow of the patients urine (Levering *et al.,* 2016).

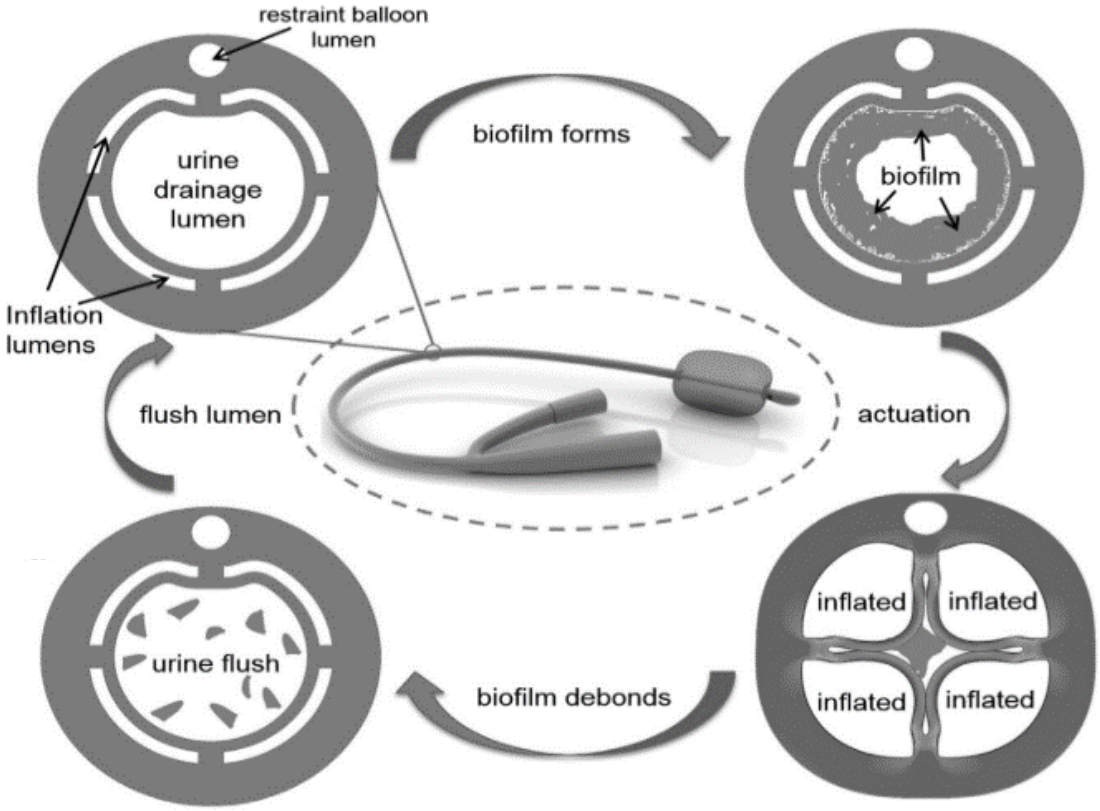


Figure 1.3 Schematic of Levering *et al.,* (2016) novel indwelling urinary catheter with internal inflation lumens to aid in the disruption and removal of crystalline biofilms.

Sun *et al.,* (2011) developed a new indwelling catheter, but unlike the last study discussed they focused on the catheter's outer surface structure. They refer to their novel catheter design as "trefoil shaped" with three longitudinal grooves that run the length of the catheter (Sun *et al.,* 2011). According to Sun *et al.,* this trefoil shape would allow bladder drainage like any other catheter but it would cause less tissue damage as a lower surface area is in contact with the urethral mucosa which in turn preserves normal tissue function and lowers risks of infection (Sun *et al.,* 2011). The trefoil design also enables normal secretions from the urethra that are not possible with the complete surface area contact of traditional indwelling catheters (Sun *et al.,* 2011).

Another alternative design is the Poiesis Duette™ shown in Figure 1.4, which is an indwelling catheter with two balloons, one for retention like other indwelling catheters and one above the drainage eyelet to protect the bladder urothelium from irritation caused by the catheter tip and drainage eye, preventing the urothelium being sucked into the drainage eyelet (Murphy, 2018; Newman *et al.,* 2017;). Maintaining the integrity of the bladder urothelium is integral to prevention of CAUTIs as once compromised it can allow invasive pathogens to colonise the urinary tract and potentially cause irreversible damage (Jeffery & Mundy, 2020; Newman *et al.,* 2017; Feneley *et al.,* 2012; Jacobsen *et al.,* 2008; Kunin, 1988). It should be noted that while this design may cause less tissue damage, it does prevent complete bladder drainage which can allow uropathogens to flourish in the stagnant urine remaining (Murphy, 2018).

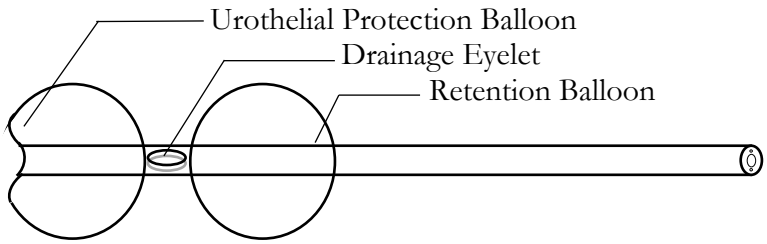


Figure 1.4 Illustration of the Poiesis Duette™ indwelling urinary catheter tip with distal and proximal balloons inflated.

Finally, a recent study by Vargas-Cruz *et al.,* (2019), described a novel catheter which was constructed with two balloons, one to act as a traditional retention balloon on the distal end just below the catheter eyelets at the tip, and a second balloon on the proximal end to act as an "irrigation cuff". The irrigation cuff was fed by its own lumen separate from the drainage and retention lumens; the irrigation cuff was smaller than the retention balloon to allow it to sit within the urethra without causing distention. Lastly, the irrigation cuff featured small slits at the bottom aimed down the catheter shaft towards the urethral meatus (Figure 1.5). Vargas-

Cruz *et al.,* (2019) used their double-cuffed Foley catheter to assess if urethral irrigation with an antimicrobial solution of 1% polygalacturonic acid, 0.4% caprylic acid, and 1.5% hydroxyethyl cellulose (thickener) could prevent uropathogen migration along the outer lumen of their novel catheter. They assessed this by placing their catheter in a modified version of the Gaonkar *et al.,* (2003) *in vitro* urinary tract model with the major change being that the agar used in the original model to mimic the urethra was replaced with a soft silicone tube. They first placed in a portion of a test catheter in Mueller Hinton Broth (MHB) with a mixture of uropathogens overnight to allow a biofilm to form, the discrete segment colonised was halfway down the catheter length. The catheter was then placed in their "*in vitro* Foley catheter colonisation" model where they then irrigated the urethra with MHB to entice the uropathogens of the biofilm to migrate up the urethra. After 4 hours incubation they then irrigated the urethra with the antimicrobial solution. Vargas-Cruz *et al.,* (2019) found that after a single irrigation with the antimicrobial solution that uropathogens colonising the upper half of the catheter nearest the bladder were eradicated and with a second irrigation the majority of uropathogens present in the periurethral space were eliminated. They found this method was successful in inhibition of *E. faecalis*, *P. mirabilis*, *P. aeruginosa*, and carbapenem-resistant strains of *K. pneumoniae* and *E. coli* (Vargas-Cruz *et al.,* 2019).

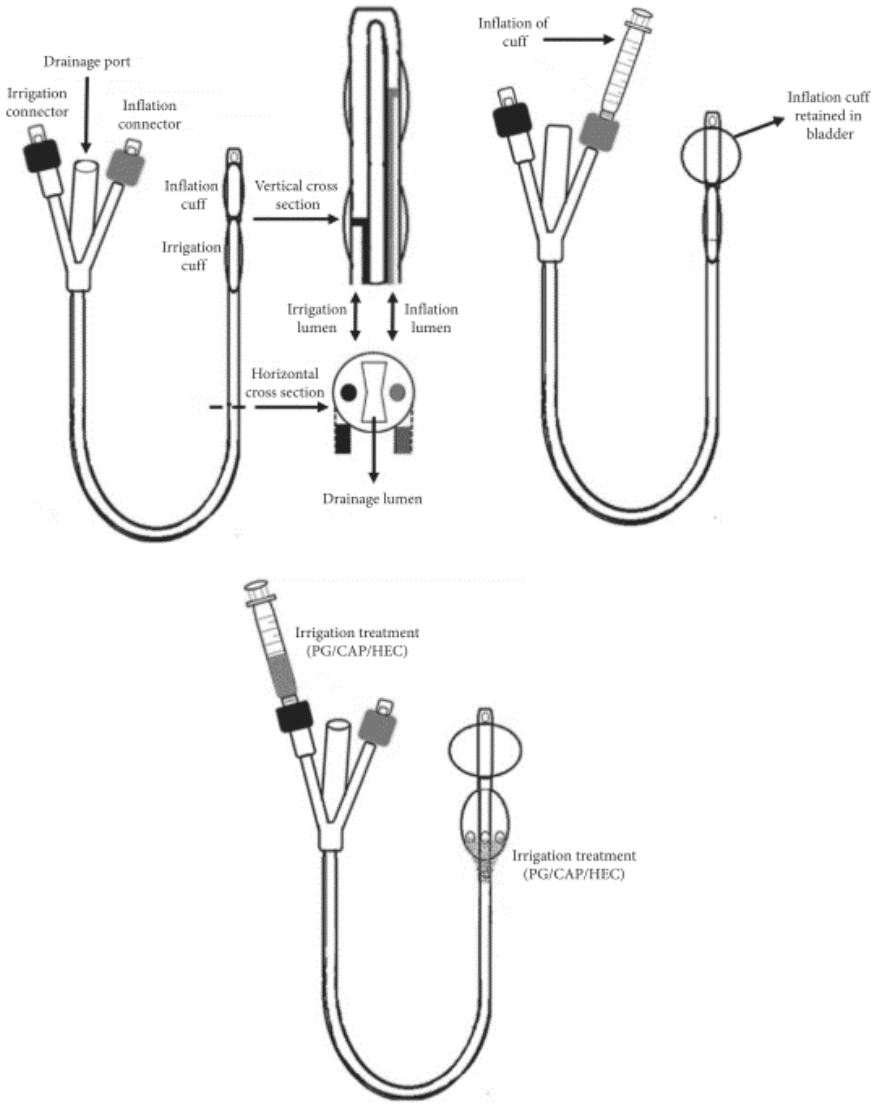


Figure 1.5 Illustration of the double cuffed irrigation Foley catheter developed by Vargas-Cruz *et al.,* (2019). PG-polygalacturonic acid, CAP-caprylic acid, and HEC-hydroxyethyl cellulose.

1.9.2 Intermittent Catheters

Intermittent catheters are often seen as a solution to CAUTI complications caused by indwelling catheters, as intermittent catheters pose no risk of biofilm formation due to their short time in the body and are a lower risk of bladder stone formation (Goetz *et al.,* 2018). Although there is a lower incidence of CAUTIs with intermittent catheter users compared to indwelling catheter users, there is still a high risk of CAUTI with infection rates as high as 60%. Recurrent CAUTIs are quite common, and often associated with the inappropriate over use of antibiotics (Goetz *et al.,* 2018). While indwelling catheter research is extensive with a diverse array of possible solutions to CAUTIs, the research in respect to intermittent catheters is lacking in comparison. Indwelling catheter research and development, as previously discussed,

is primarily focused on the use of antimicrobial and antibiofilm compounds to prevent catheter colonisation or extend the time before colonisation occurs. Intermittent catheter research and development, traditionally and currently, is focused on preventing bacteria entering the urinary tract or coming into contact with the catheter, rather than the direct treatment CAUTIs (Goetz *et al.,* 2018).

No Touch Closed Systems

Some intermittent catheters are encased in closed systems, consisting of what is known as a "no touch" sleeve that allow insertion of the catheter, by the patient or medical professional, without needing to ever touch the catheter itself, therefore maintaining sterility of the catheter (Goetz *et al.,* 2018). A study by Hudson and Murahata (2005), compared intermittent catheters that had physical barriers to hydrophilic coated intermittent catheters. One such catheter, included a self-contained lubricant and a "no touch" sleeve that covered the entirety of the catheter to prevent bacterial transfer from the user's hands to the device. They found a significant reduction in pathogen contamination with this no touch catheter, recovering on average <5.4 CFUs, whereas an average of $3.3x10^2$ CFUs was recovered from the hydrophilic coated catheters (Hudson & Murahata, 2005). A hybrid catheter, that contained a hydrogel coating and a small plastic sleeve that the patient could use to avoid touching the catheter, performed better than those with the hydrogel alone, however this was not as effective as the fully enclosed "no touch" catheters, with an average of $2.5x10^1$ CFUs recovered. The results Hudson and Murahata (2005) obtained, demonstrated the effectiveness of complete physical barriers to prevent pathogenic catheter contamination.

Insertion Tip

Another study reported in a monograph by Holland and Fish, (2012), compared two catheters produced by the same manufacturer. One catheter was the Coloplast Self-Cath®, a generic PVC catheter with no added lubricants or sleeve. The second one, was the Bard® Touchless® Plus intermittent catheter, that was enclosed in a no touch sleeve and included an insertion tip (Holland & Fish, 2012). Insertion tips, first in developed in 1982, are often used in intermittent catheter design as the tip allows the catheter to avoid the urethral meatus during insertion (Goetz *et al.,* 2018). The urethral meatus includes not only the area directly around the urethral opening, but also the first 15 mm inside the urethra, where high levels of bacterial colonisation is found, Figure 1.6 (Goetz *et al.,* 2018).

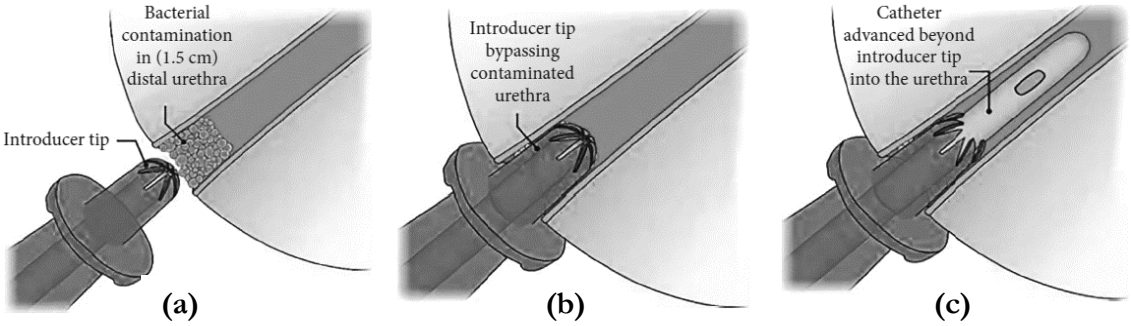


Figure 1.6 Insertion tip found on some intermittent catheters. (a) large bacterial numbers at urethral meatus, (b) insertion tip bypasses meatus, (c) catheter passes through the insertion tip up the urethra towards the bladder (Goetz *et al.,* 2018).

The study by Holland and Fish, (2012), assessed the effectiveness of both the insertion tip and the no touch system, with the aim, to prevent pathogen transfer from the meatus or hands during insertion, much like the study by Hudson and Murahata (2005). From their work, Holland and Fish, (2012) determined that the no touch catheter with the insertion tip performed significantly better than the no frills PVC catheter. In an insertion tip test, with the Bard® Touchless® Plus intermittent catheter, as low as 1.8 CFUs were recovered from the catheter after insertion through an agar plate inoculated with $1x10^7$ CFUs. They found that the generic catheter yielded an average of 22 CFUs. In regards to the contaminated handling test, which was performed in a similar fashion to that of Hudson and Murahata (2005), the no touch catheter outperformed the generic catheter with 0 CFUs and 144 CFUs recovered respectively (Holland & Fish, 2012).

Hydrophilic Coatings

Hydrophilic coated intermittent catheters have been around since the early 1990s, they have since become a standard in the industry (Goetz *et al.,* 2018; Woodward & Rew, 2003). Several studies have demonstrated that hydrophilic coated intermittent catheters reduce incidences of CAUTIs or delay their onset. One such study by De Ridder, *et al.,* (2005), found that 64% of patients using a hydrophilic coated intermittent catheter experienced one or more CAUTIs in a year compared to 82% of patients using un-coated PVC catheters. Similar results were found in a study by Cardenas, *et al.,* (2011), which reported a 22% reduction of symptomatic CAUTIs by using hydrophilic coated catheters. Finally, a meta-analysis by Li, *et al.,* (2013), reported a correlation between lower incidences of CAUTI when using hydrophilic catheters from their analysis of five clinical studies. The lower incidences of CAUTIs found when using hydrophilic catheters could be a product of high patient satisfaction, as the hydrophilic coating can reduce

pain, making the process easier and increasing a patient's quality of life resulting in adherence to clean intermittent catheterisation practices (Durán *et al.,* 2016; Ghanwate *et al.,* 2014; Van Achterberg *et al.,* 2008; Woodward & Rew, 2003). The lower incidence of CAUTIs may also be due to the hydrophilic coated catheters not requiring additional external lubrication, and so there is no additional need to touch the catheter before insertion (Goetz *et al.,* 2018).

Antimicrobial Coatings

Antimicrobial coatings are primarily utilised for indwelling catheterisation, with few available for intermittent catheters as the market shifts towards physical barriers and no touch engineering (Goetz *et al.,* 2018). There was at one point a hydrophilic intermittent catheter coated with the antimicrobial nitrofurazone, but this was later taken off the market as it efficacy in CAUTI prevention was negligible at best (Goetz *et al.,* 2018). Silver has been incorporated into or used to coat intermittent catheters for many years due to its broad spectrum antimicrobial abilities, however as previously discussed, the efficacy of silver in the prevention of CAUTIs is questionable and with the emergence of silver-resistant bacterial strains, its use in the industry has waned. (Mandakhalikar *et al.,* 2016). With all historic and recent studies in mind, there is a significant gap in the research where antimicrobial intermittent catheters are concerned (Goetz *et al.,* 2018; Mandakhalikar *et al.,* 2016; Stickler, 2012).

Reuse of Intermittent Catheters

Although the studies previously discussed show the advantage of enclosed and disposable catheters, due to financial issues, health insurers, environmental concerns, or just personal choice, some people use reusable intermittent catheters (Goetz *et al.,* 2018). While single use sterile catheters are becoming the norm in many developed countries, they are not readily available in most developing and underdeveloped countries depending on the health care system (Hakansson, 2014). While some studies report higher incidences of CAUTIs with reusable catheters, others report no statistical difference in sterile single use and reusable catheters, though this may be due to lack of evidence in the area with instances of catheter reuse under reported (Goetz *et al.,* 2018; Hakansson, 2014; Li *et al.,* 2013). The primary cause for concern with catheter reuse is that lack of standard procedure for the patient to clean, sanitize, or re-sterilise the catheter after use (Goetz *et al.,* 2018). Numerous techniques for sterilising reusable catheters are thought to be effective such as microwaving, boiling, and soaking in antiseptics such as; alcohol, hydrogen peroxide, and bleach (Hooton *et al.,* 2010). However there has been little research published to prove the efficacy of any available

technique (Goetz *et al.,* 2018; Goetz & Klausner, 2014). Sherbondy, *et al.,* (2002), investigated the variations in technique of patients who practised clean intermittent catheterisation with reusable catheters and utilised microwave sterilisation. They noted a large variation in both time and microwave wattage levels between patients, reinforcing the lack of a standard methodology or instructions given to patients. A study by Bogaert, *et al.,* (2004), performed several experiments to determine not only the antimicrobial efficacy of microwave heating and alcohol immersion sterilisation, but they also investigated the effects on the structure of the catheter, as often patients will reuse catheters that were never meant for reuse. Their work determined that microwave heating was adequate at eliminating *E.coli* but was not effective for *P. aeruginosa* or *S. aureus*, while having minimum effect on the catheter's physical properties (Bogaert *et al.,* 2004). In respect to alcohol immersion, they found that immersion in 70% alcohol was effective against all bacterial strains used and would be an appropriate sterilisation technique; however, immersion times should be kept short as the alcohol was found to create significant changes to the catheter's physical qualities (Bogaert *et al.,* 2004).

1.9.3 Prophylaxis

Prophylactic Antibiotic Use

Prophylactic systematic antibiotic use is common in both indwelling and intermittent catheter users (Hooton *et al.,* 2010). Though more common in indwelling catheter patients, with highly resistant or recurrent biofilms, intermittent catheter users are often given prophylactic treatments without any symptoms of UTI (Goetz *et al.,* 2018; Goetz & Klausner, 2014). Prophylactic antibiotics can be useful in some cases, and some studies have shown that their use can reduce CAUTI cases or off set the time before a problematic infection sets in (Hooton *et al.,* 2010). That said, prophylactic antibiotic use is no longer recommended as it can contribute to the development of antibiotic-resistant bacterial strains, as well as adverse outcomes for the patient, such as recurrent and resistant infections as treatment often does not completely eradicate the offending organisms (Goetz *et al.,* 2018; Hooton *et al.,* 2010).

Prophylactic Antimicrobial Use

Prophylactic use of non-antibiotic antimicrobials is common for catheter users as well as community UTI sufferers. Cranberry products have long been a home remedy for the treatment and prevention of UTIs, and many catheterised patients utilise them in the hopes of preventing CAUTIs (Ayyash *et al.,* 2019; Hooton *et al.,* 2010). Proanthocyanidins are the active ingredient in cranberries that acts as an anti-adherent for bacteria within the urinary tract due

to their tannin molecules containing irregular A-type linkages, which prevents adhesion of bacteria to the inner walls of the bladder (Goetz *et al.,* 2018). A study by Ayyash *et al.,* (2019), tested a novel lock solution for indwelling catheters consisting of Triclosan and cranberry. They found that this solution was able to diffuse out of the ID retention balloon into the bladder and was highly effective in eradicating *E. coli* biofilms but there was little to no affect observed on planktonic *E. coli*. While this study is promising and warrants further investigation, is should be noted that the majority of previous studies into the effectiveness of cranberry, and in particular proanthocyanidins, are inconclusive or insufficient at best, and are well covered by both Goetz, *et al.,* (2018) and Hooton, *et al.,* (2010).

Another prophylactic treatment used is d-mannose, a sugar that plays an important role in human metabolism, and like proanthocyanidins, it can prevent bacterial adhesion to uroepithelial cells by binding to the type 1 pili of enteric bacteria (Kranjčec *et al.,* 2014). A study by Kranjec, *et al,* (2014) found a correlation with d-mannose use and a reduction in recurrent UTIs in their trial. A more recent study found that d-mannose in combination with other plant extracts, including arbutin, forskolin, berberine, and birch, were effective in reducing recurrent UTIs and could be an alternative to prophylactic antibiotic use or in fighting resistant bacterial infections (Genovese *et al.,* 2017).

Methenamine salts, either in the form of methenamine mandelate or methenamine hippurate, are another group of antimicrobials used in the past for control or prevention of UTIs and CAUTIs (Hooton *et al.,* 2010). The antimicrobial mechanism of methenamine is due to its hydrolysis in the body to form ammonia and formaldehyde (Hooton *et al.,* 2010). The formaldehyde formed is bactericidal and broad spectrum. It is also unlikely to lead to the development of resistant strains, as formaldehyde is an antiseptic rather than an antibiotic (Lo *et al.,* 2014). Many studies have looked at the efficacy of prophylaxis with methenamine and the results are inconclusive, with some studies finding that methenamine can lower the risk of, or prolong time before a UTI/CAUTI develops, while other studies found it no more effective than placebos (Hooton *et al.,* 2010). Due to these inconclusive results, methenamine use was discouraged in the past; however, relatively recently there has been renewed interest in non-antibiotic treatments due to increasing antibiotic resistance and stagnating development in discovery of new antibiotics (Lo *et al.,* 2014).

1.10 Microbial *In vitro* Catheter Testing and Urinary Tract *In vitro* Models

In vitro testing is a key component of catheter research and development as it allows for potentially high throughput testing and initial screening for proving novel concepts, while avoiding the ethical issues and higher costs associated with *in vivo* testing (Roberts *et al.,* 2015; Coenye & Nelis, 2010). While current *in vitro* testing is not capable of truly representing the clinical realities of catheter use, it can provide valuable information in the early stages of novel catheter development (Roberts *et al.,* 2015). *In vitro* testing in the area of urological devices and more specifically CAUTIs and catheters includes a wide variety of general microbiological assays that are not necessarily specific to the field. According to available data, four *in vitro* models currently exist with the aim of more closely mimicking the anatomy of the urinary tract and/or the behaviours of bacteria within.

1.10.1 General Microbiology *In vitro* Testing

General microbiology *in vitro* assays are often used or adapted to test novel urological medical devices and to assess novel antimicrobials or coatings to prevent CAUTIs.

Kirby Bauer Disc/Well Diffusion

The Kirby Bauer disc diffusion assay, first developed in the 1960's by Bauer *et al.,* (1966), is based on the principle of diffusion, where either a proprietary antibiotic disc or a paper disc impregnated with a suspected antimicrobial, is placed on an inoculated solid agar surface. This allows the antimicrobial to diffuse out into the surrounding agar (Ramstedt *et al.,* 2019). Alternatively, a well can be created in the agar surface to allow for insertion of a sample *e.g.* catheter segment (Ramstedt *et al.,* 2019; Balouiri *et al.,* 2016). If the microorganism of interest is susceptible to the test solution, sample, or antibiotic a zone of inhibition will form around the disc/well where no bacteria have grown. The radius of the zone of inhibition is then correlated with the concentration of the antimicrobial agent. Advantages of the disc diffusion assay include high reproducibility, the simplicity of the assay with no requirements for specific equipment, the rapidity of results, and it is the lowest cost version of a bacterial susceptibility assay (Summers & Goeres, 2019). The main disadvantages of the technique are technician variability in results as they are assessed visually and are therefore susceptible to bias, the method may also underestimate the antimicrobial ability of compounds that are insoluble or poorly soluble in aqueous solutions, finally the method is only usable for antimicrobials that

can diffuse through the agar making it unsuitable for non-eluting antimicrobials (Summers & Goeres, 2019).

Serial Plate Transfer Test

The serial plate transfer test relies on producing zones of inhibition on a series of plates over time to establish both the duration of an antimicrobial's activity and the time taken to develop resistance (Saini *et al.,* 2016). The method is used in several studies, and the protocol starts with a segment of a device that is either imbedded or coated with an antimicrobial, and the segment is placed on the surface of an inoculated agar plate or into a well cut into the agar surface and incubated overnight (Rajamani *et al.,* 2018; Saini *et al.,* 2016; L. E. Fisher *et al.,* 2015). After incubation the zone of inhibition, is measured and recorded before the segment is transferred to a new inoculated plate and incubated again (Rajamani *et al.,* 2018; Saini *et al.,* 2016; L. E. Fisher *et al.,* 2015). This process is repeated with new plates each day, while making sure the same side of the segment is in contact with the agar each time it is transferred if the agar surface method is used, until a zone of bacterial inhibition no longer forms (Rajamani *et al.,* 2018; Saini *et al.,* 2016; L. E. Fisher *et al.,* 2015).

Minimum Inhibitory/Bactericidal Concentration

Determining the Minimum Inhibitory Concentration (MIC) or Minimum Bactericidal Concentration (MBC) of a novel antimicrobial is usually the next natural step after performing a Kirby Bauer disc diffusion. These tests are carried out to fine tune minimum concentrations to produce the desired effect whether that be mere inhibition of microbial growth or total eradication. The MIC of an antibiotic, antimycotic, antimicrobial, antiseptic, or bactericidal agent is the lowest concentration that can inhibit microbial growth, the MBC is the concertation at which total eradication of live microbes occurs (Balouiri *et al.,* 2016). There are several versions of a MIC/MBC assay including broth macrodilution and broth micro dilution methods. MIC/MBC tests provide a fast and inexpensive method to determine antimicrobial resistance or bacterial susceptibility to antimicrobials, and determine potential dosing thresholds for novel antimicrobials (Balouiri *et al.,* 2016). Broth dilution MIC/MBC testing is traditionally done using test tubes and is one of the earliest developed antimicrobial susceptibility testing methods. The method can be miniaturised and scaled down to utilise a 96-well multiwell plate. The broth microdilution method is high throughput allowing multiple antimicrobial agents to be tested simultaneously (Summers & Goeres, 2019; Wiegand *et al.,*

2008). The principle of the broth macro- and microdilution MIC assays is the assessment of turbidity, if the broth in a given test tube or well is turbid then microbial growth was not inhibited. The MIC is determined by the lowest concentration that entirely inhibited growth. When the proposed antimicrobial itself causes turbidity in the media, turbidity alone is no longer an appropriate indicator of microbial growth. In such cases, colorimetric or fluorescent dyes can be used to identify bacterial growth/inhibition. Commonly used dyes include Alamar blue dye also referred to as resazurin, and the Tetrazolium salts XTT and MTT (Balouiri *et al.,* 2016). To determine the MBC media is recovered from the well/tube at the MIC concentration as well as the concertation above and below the MIC, the total live CFU ml^{-1} is then quantified, the concentration at which no growth is observed is determined to be the MBC (Balouiri *et al.,* 2016; Wiegand *et al.,* 2008). The main advantages of MIC/MBC testing are that they are reproducible whilst also being rapid, cheap, and simplistic to run as they require no specialised equipment to run. The chief disadvantage is that while they may establish that a substance is in fact antimicrobial, the assay does not determine if the antimicrobial will be effective in preventing colonisation of a device or biofilm formation so the results would need to be supported by further testing in regards to CAUTI prevention research (Summers & Goeres, 2019).

Static Biofilms: Multiwell Plate Biofilm Formation/Bacterial Adhesion

The multiwell plate biofilm formation assay is a simple technique to form biofilms within the wells of a multiwell plate (Roberts *et al.,* 2015; Coenye & Nelis, 2010). The assay consists of a multiwell plate of 96, 48, 24, 12, or 6 wells, and bacteria are grown within the wells and bind either to the walls/base of the wells, or introduced items such as a catheter segments contained in plates with 24 wells or less (Coenye & Nelis, 2010). This assay can be used to assess two different endpoints: total bacterial adhesion/biomass, wherein the adherent bacteria and biofilm matrix are stained and then the total retained stain is quantified; an alternative end point is to quantify the live adherent bacteria through sonic recovery and subsequent quantification of live organisms either through traditional agar plate counting methods or analytical methods *e.g.* flow cytometry (Merritt *et al.,* 2005). The assay is high-throughput whilst also being relatively cheap and straight forward when compared to other biofilm models; however, the assay can be vulnerable to large variations from well to well (Roberts *et al.,* 2015; Coenye & Nelis, 2010). A macro version of this assay also exists where test tubes are used in place of multiwell plates (Sayal *et al.,* 2014).

Dynamic Biofilms: Bioreactor and Flow Cell Models

To grow dynamic biofilms, *i.e.,* biofilms grown in moving or flowing media that exhibits shearing forces and fluctuating nutrient levels mimicking the *in situ* environment, two general techniques are used. In the traditional method, a dynamic biofilm is grown in a bioreactor where whole samples, stabilised on a frame, or segments of samples, suspended in a receptacle, are placed in a nutrient rich media, agitated by a rotating impeller (Figure 1.7 a; Coenye & Nelis, 2010). This system is often referred to as a "continuous flow stirred tank reactor" and there are standardised versions of this reactor setup and methods to grow biofilms within such as the CDC biofilm reactor (Ramstedt *et al.,* 2019; Coenye & Nelis, 2010). While this method may be the most cost effective way to study dynamic biofilms, as the apparatus can be created with standard lab equipment if proprietary models are outside of a technician's budget, they are limited by the relatively simple environment they create. The major limitation of bioreactors, in consideration of urinary catheters, is that the movement of liquid inside the bioreactor is continuous and does not reflect the stop and start flow of urine within a catheter (Ramstedt *et al.,* 2019). Another limitation is the possible depletion of nutrients from the surrounding media depending of the duration of the experiment; finally the disparity in shearing forces *in situ* does not accurately mimic the *in situ* environment of the urethra (Ramstedt *et al.,* 2019).

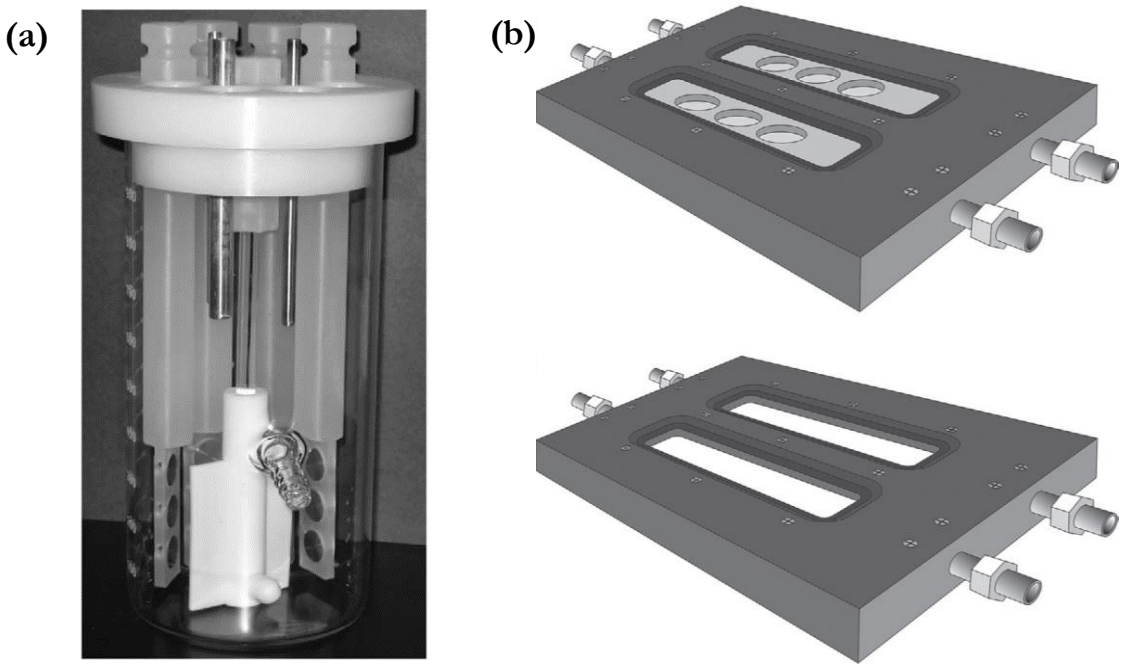


Figure 1.7 Examples of dynamic Biofilm growing apparatuses, (a) image of a CDC bioreactor, (b) illustration of a flow cell chamber (Ramstedt *et al.,* 2019; Stewart *et al.,* 2012).

As an alternative to bioreactors, several flow cell apparatuses have been developed. These models primarily consist of a flow chamber where a sample or segment of a sample is placed and then media containing the microorganisms of interest is passed though in either a continuous or peristaltic flow (Peterson *et al.,* 2011). Flowing media allows for greater control of shearing forces and the overall microfluidics of the model, allowing for conditions closely resembling the fluid dynamics of the urethra and eliminating the problems of nutrient depletion sometimes experienced in a bioreactor set up (Ramstedt *et al.,* 2019). Flows cells also offer the advantage of being able to observe the biofilm as it grows either micro- or macroscopically as the chamber is usually glass walled, this allows for observation of all stages of biofilm development (Summers & Goeres, 2019). The main disadvantages are the cost of the specialised equipment and experience needed to run said equipment, the morphological differences in the flow cell grown biofilms and naturally occurring biofilms, and the difficulty in recovering the biomass at the end of the experiment depending on the design of the flow chamber (Ramstedt *et al.,* 2019; Summers & Goeres, 2019; Peterson *et al.,* 2011).

Time to Kill Attached Bacteria Assay (tK100)

The tK100 assay establishes the time it takes for an antimicrobial to kill 100% of bacteria attached to a urological device (L. E. Fisher *et al.,* 2015; Bayston *et al.,* 2009). The assay protocol starts with segments of the test device, either uncoated or coated with a protein conditioning film, being exposed to a bacterial inoculum and allowing the bacteria to adhere to the device for a set period of time (L. E. Fisher *et al.,* 2015; Bayston *et al.,* 2009). Once bacteria are attached to the surface, the segments are washed to remove planktonic bacteria, then the segments are place in growth media and incubated for a set period of time, usually over several days (Saini *et al.,* 2016; L. E. Fisher *et al.,* 2015; Bayston *et al.,* 2009). Replicates are removed at set time intervals over the extended incubation time, then sonicated and the remaining live bacteria enumerated. These bacterial counts are then plotted over time to establish the time intervals to reach 100% bacterial death (Saini *et al.,* 2016; L. E. Fisher *et al.,* 2015; Bayston *et al.,* 2009).

1.10.2 *In vitro* CAUTI Models

Essentially five CAUTI models have been developed in an attempt to represent the anatomy more closely and/or infection conditions *in situ.* These models are the catheter bridge model by Sabbuba *et al.,* (2002), the bladder model by Stickler *et al.* (1999), the urinary tract model by Gaonkar *et al.* (2003), the CAUTI model by Rosenblatt, *et al.,* (2017a), and the meatus model by Holland and Fish, (2012).

Catheter Bridge Model

The catheter bridge model was first described by Sabbuba *et al.,* (2002) and was used to investigate the swarming of uropathogens across the outer lumen of urinary catheters. A rather simplistic model, it consisted of an agar plate with channels cut out of the agar ~10 mm wide where catheter segments could be placed (Figure 1.8). Once in place, the agar on one side of the catheter was inoculated and the plate was incubated. After incubation, if bacterial growth was observed on the adjacent side of the catheter, the species tested was successful in swarming over the outer catheter surface (Sabbuba *et al.,* 2002). This method has been used in other studies, primarily to study the swarming behaviours of *P. mirabilis* and to assess the efficacy of hydrogel coatings and antibiofilm impregnated catheters to prevent said swarming (Durgadevi *et al.,* 2020; Kazmierska *et al.,* 2010). The main advantage of this model is speed and low cost, with the ability to test multiple catheter types/bacterial species with little materials and media. This method also allows for the testing of non-eluting antimicrobials (Summers & Goeres, 2019). The primary disadvantage of this method is its simplicity, while it can possibly predict novel compounds that could prevent bacterial swarming on a urinary catheter, it lacks the complexity of the *in situ* infection environment, specifically the urethral membrane catheter interface. For these reasons caution should be taken when extrapolating results to *in vivo* and clinical settings (Summers & Goeres, 2019).

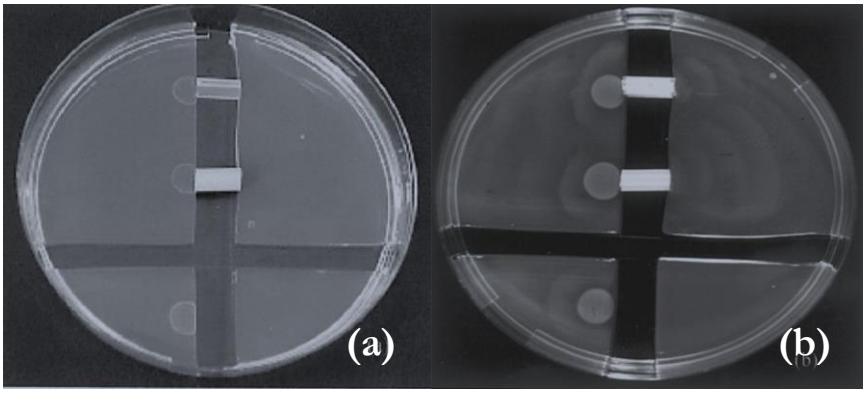


Figure 1.8 Example of catheter bridge model with a non-swarming bacterial species (a) and a swarming species (b) and their movement over hydrogel coated catheter segments (Sabbuba *et al.,* 2002).

In vitro Bladder Model

Stickler *et al.,* (1999), first developed a bladder model comprised of a glass vessel surrounded by a water jacket maintained at 37 °C. The entire model was sterilised before an indwelling catheter is inserted into a glass outlet tube, and the retention balloon was inflated to keep the catheter in place before it was attached to a drainage bag, Figure 1.9 (Stickler *et al.,* 1999). Sterile

urine was then pumped into the model via a peristaltic pump which then drained through the catheter into the attached drainage bag (Stickler *et al.,* 1999). This model can and has been used to produce bacterial biofilms on outer and inner catheter surfaces, and in particular crystalline biofilms where blockage of the catheter is the usual end point (Stickler *et al.,* 1999).

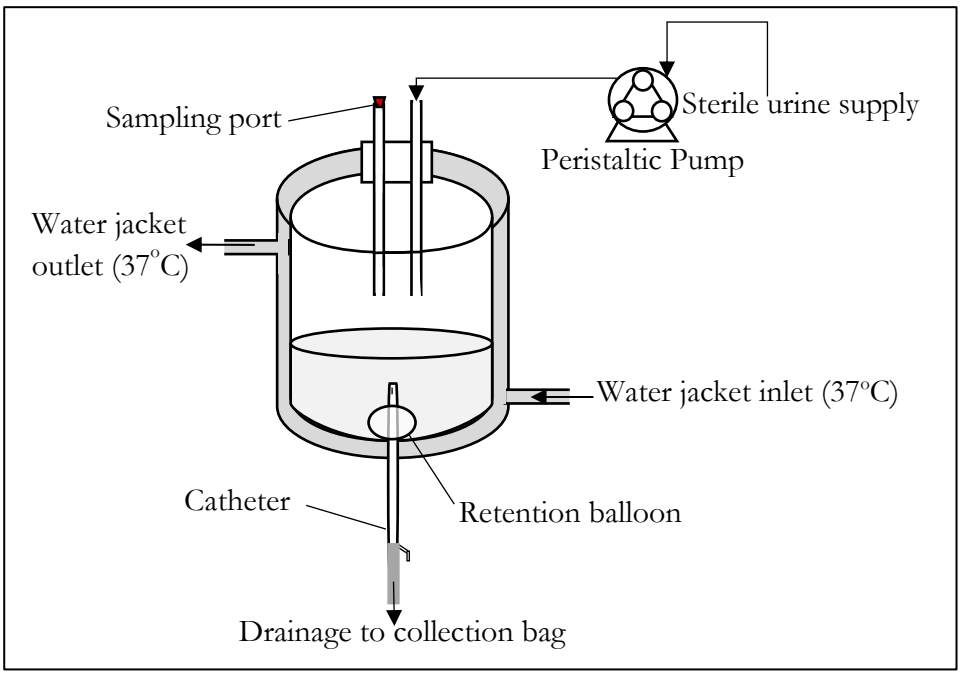


Figure 1.9 Illustration of an *in vitro* bladder model adapted from a paper by Stickler *et al.,* (1999).

The Stickler *et al.* (1999) model has been used and modified by several research studies for CAUTI development, and to test novel indwelling catheters during development containing or coated with novel antimicrobials (Nzakizwanayo *et al.,* 2019; Norsworthy & Pearson, 2017; Mandakhalikar *et al.,* 2016; Jordan *et al.,* 2015; Barford *et al.,* 2008; Davenport & Keeley, 2005). The advantages of this model are that it can simulate many aspects of the *in vivo* infection environment and allows for continuous flow with relatively realistic fluid dynamics. The complexity of the model is its greatest weakness as this can affect the reproducibility of results. This method can also be costly with large volumes of media needed (Summers & Goeres, 2019).

In vitro Urinary Tract Model

Gaonkar *et al.* (2003) developed a small scale model of the urinary tract to investigate the migration of pathogens along the surface of an indwelling catheter from the meatus to the bladder. The model consists of two tubes: tube 1 was an open narrower tube with a cap at one

end and a rubber cork with a hole in it at the other end, tube 2 was a larger vessel that was open at one end to connect to tube 1 and closed at the other end to collect urine; all parts were sterilised with ethylene dioxide (Gaonkar *et al.,* 2003). The agar urethra portion was formed by placing a catheter segment aseptically into the top of tube one and secured by pushing through the hole in the rubber cork before molten agar was poured down the sides of the catheter, and once solidified the rubber cork was removed (Gaonkar *et al.,* 2003). Tube 1 was then secured in the top of tube 2 for testing (Figure 1.10).

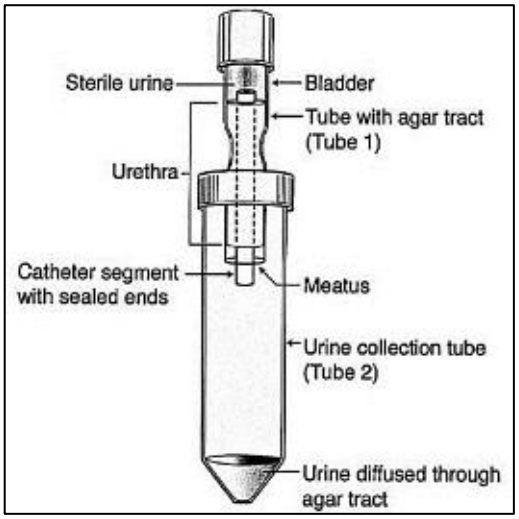


Figure 1.10 Gaonkar *et al.* (2003)*, in vitro* urinary tract model schematic.

The Gaonkar *et al.* (2003) model methodology involved inoculating the "meatus" daily with bacteria, then filling the top of tube 1 with sterile urine daily. Samples were taken from the urine at the top of tube 1 periodically to assess if bacteria have migrated up the catheter and at what concentrations. This model was designed to test indwelling catheters over a number of days (Gaonkar *et al.,* 2003). A modification of this model was used in a study by Williams & Stickler, (2008b) to assess the migratory abilities of specific bacterial strains over a catheter surface. In their work they left the bladder section of the model empty and replaced the trypticase soy agar used by Gaonkar *et al.,* (2003) with chromogenic UTI agar facilitating the visualisation of bacterial movement (Williams & Stickler, 2008b). The advantages of this design are its simplicity with little media required and no specialist equipment. The main disadvantage is that the agar channel does not control the direction of bacterial movement, allowing bacteria to migrate on all sides of the agar not just those in contact with the catheter which can interfere with the rate of bacterial migration and reproducibility (Summers & Goeres, 2019; Williams & Stickler, 2008b).

In vitro CAUTI model

Recently the Gaonkar *et al.* (2003) model has been adapted by Rosenblatt, *et al.,* (2017b). Communicated on a poster presented in October 2017 at the IDweek conference in San Diego, California, Rosenblatt, *et al.,* (2017b) revealed their modified version of the urinary tract model or CAUTI model (Figure 1.11). This model consisted of only one tube with an upper bulbous end to allow inflation of the retention balloon or cuff and a cap at the other end. The agar channel was wider than seen in the Gaonkar *et al.* (2003) model, which allowed room for the second catheter cuff or "proximal irrigation cuff" which they modified the model to specifically test (Rosenblatt, *et al.,* 2017a; Rosenblatt, *et al.,* 2017b). The Rosenblatt *et al.,* (2017a, 2017b) model methodology is similar to Gaonkar *et al.,* (2003), with the "meatus" being inoculated, and the periurethral space then rinsed via the proximal irrigation cuff. The model was incubated to promote any growth, after which the catheter was removed, segmented, and the bioburden of the catheter assessed. Like the Gaonkar *et al.* (2003) model, the Rosenblatt *et al.* (2017b) model is designed specifically for testing indwelling catheters, in particular those with an irrigation cuff.

In a later study by the same research group, Vargas-Cruz *et al.,* (2019) further modified the Gaonkar *et al.,* (2003) model by replacing the agar with a soft silicone tube to act as the urethra. This further allowed control of the migration of uropathogens within the model as nutrients, introduced by the irrigation cuff only, prevented pathogen movement outside of the periurethral space. Vargas-Cruz *et al.,* (2019) called this iteration for the CAUTI model the "*In vitro* Foley Catheter Colonisation Model". As with the previous version, they used this model to assess the efficacy of their novel double-cuff periurethral irrigation indwelling catheter to prevent uropathogenic migration along the outer lumen from meatus to bladder (Vargas-Cruz *et al.,* 2019).

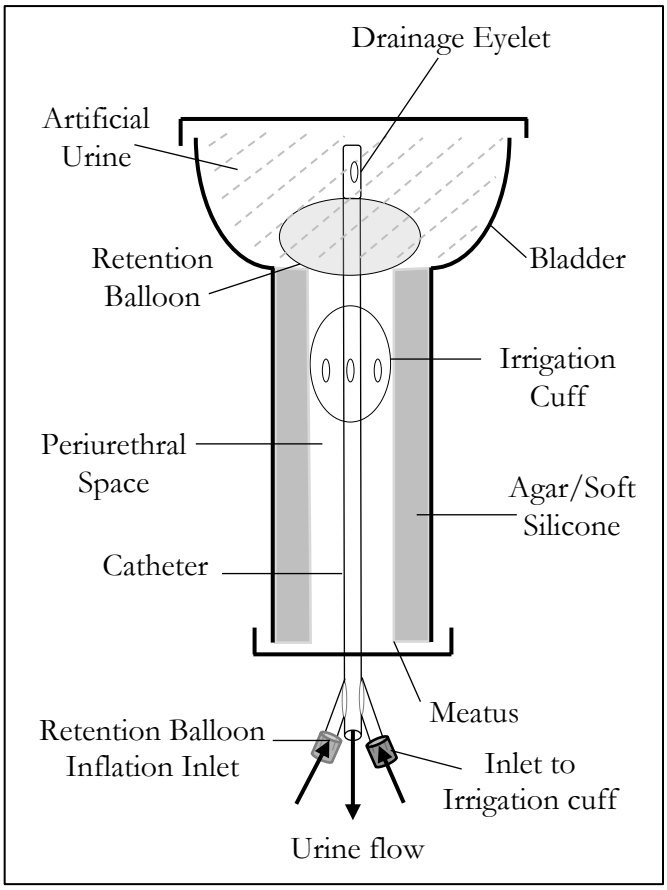


Figure 1.11 Illustration of the *in vitro* CAUTI model adapted from a study by Rosenblatt *et al.,* (Rosenblatt, *et al.,* 2017a).

Meatus Model

A monograph study by Holland & Fish, (2012) developed a simple meatus model for the testing of intermittent catheters, specifically those with an introducer tip. The model consisted of an agar plate with one or more bore-holes through both the agar and the plastic petri plate (Holland & Fish, 2012). The top surface of the agar acted as the outer tissue surrounding the urethra or meatus. The entire agar surface was inoculated with bacteria and then a catheter was then passed through the agar then the boreholes. The portion of the catheter that has passed through the agar was cut off aseptically and the bacteria was recovered from the surface of the catheter by sonication, bacterial enumeration was then carried out, Figure 1.12 (Holland & Fish, 2012). The advantage of this model is its speed and simplicity. This simplicity is also its greatest disadvantage as it only examines one aspect of CAUTI, that being initiation by contamination of the catheter tip.

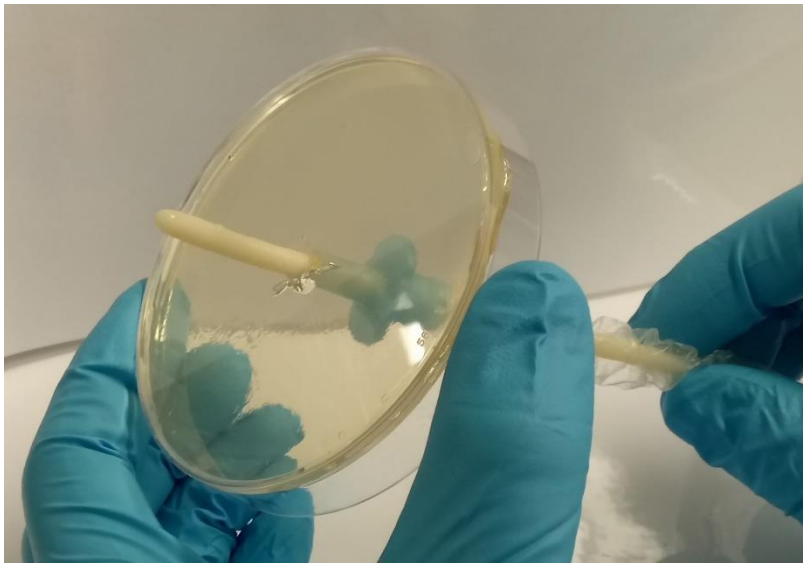


Figure 1.12 Laboratory reproduction of the Holland and Fish, (2012) *in vitro* meatus model. Catheter being inserted though the agar and through the bottom of the petri plate.

1.11 Concluding Remarks and Aims of Current Study

With the historic and ongoing problems associated with catheter use, research and innovation is vital if CAUTIs are to be controlled or eliminated. ID development has largely stagnated since the development of the Foley catheter. Research has focused primarily on antimicrobials and new materials while there are still fundamental flaws with the basic design of the original Foley catheter. This contributes to CAUTI development by damaging the uroepithelial lining of the bladder, disrupting the bladder's immune defences, and allowing stagnation of urine with incomplete drainage of the bladder thus not allowing full flushing of pathogens (Feneley *et al.,* 2012; Stickler & Feneley, 2010; Kunin, 1988). Intermittent catheters on the other hand are often recommended to catheterised patients as a safer alternative to indwelling catheters but they also have remained largely unchanged is modern medical history with a few noted exceptions (Goetz *et al.,* 2018). Medical device research can be a costly and time consuming venture that doesn't appeal to manufacturers, *in vitro* tests could bridge this gap (Feneley *et al.,* 2012; Kunin, 1988). Although *in vitro* tests do not truly represent the anatomy and complex environment *in vivo,* they do offer many advantages including high throughput, relative low cost, low/no inter sample (patient) variability, and no ethical concerns associated with *in vivo* tests (Roberts *et al.,* 2015). Flexibility and timeframes for testing are also an important advantage, and developing novel *in vitro tests* or models that can be quickly adapted to suit the product being tested are of great interest (Roberts *et al.,* 2015).

The *in vitro* models discussed in section 1.10.2 are examples of innovative and convenient *in vitro* tests that incorporate the basic structure of the urinary tract. They allow "proof of

concept" testing before resources are potentially wasted on more intensive and invasive *in vivo* or clinical testing (Coenye & Nelis, 2010). During the literature review process, it became evident that with the exception of the model described by Holland and Fish, (2012), **there are no specific *in vitro* urethral models that can be used to test intermittent catheters.** Intermittent catheter design is focused on preventing bacteria entering the urinary tract or coming into contact with the catheter, and **there is no standardised *in vitro* model to aid in the design of novel intermittent catheters or test the efficacy of manufacturer CAUTI prevention claims** (Goetz *et al.,* 2018). In regards to indwelling catheters, with the exception of the model described by Williams & Stickler, (2008b), **there is no *in vitro* model purposely designed to visualise the extraluminal migration of bacteria within the urethra during long term catheterisation. As such, it was the aim of this project to develop *in vitro* urethra models and associated methodology to visualise the initiation of CAUTI and test the efficacy of prototype intermittent and indwelling catheters to prevent bacterial movement and CAUTI initiation.**

Research Problem

There is currently no *in vitro* urethral model to test the displacement of bacteria due to intermittent catheter insertion or to visualise and quantify extraluminal bacterial migration along indwelling catheters over long-term insertion.

Aim and Objectives

The overall aim of this thesis was to design, develop, test, and validate novel *in vitro* urethral models and associated methodologies to test next-generation urological medical devices with a focus on intermittent and indwelling urinary catheters

The objectives were:

(i) To design and develop a novel *in vitro* urethra model to quantitatively assess and visualise the friction-mediated movement of bacteria as a result of **intermittent** urinary catheter insertion.

(ii) To design and develop a novel *in vitro* urethra model to quantitatively assess and visualise extraluminal bacterial migration during long-term **indwelling** urinary catheterisation.

(iii) Dissemination of novel research by submission of at least two peer reviewed research papers and four posters or presentations at national/international conferences.

(iv) To advance the field of urinary catheterisation and submit a dissertation of PhD level for external examination and obtain a PhD in Microbiology.

Chapter 2 Experimental Design

2.1 Materials

The materials and catheters used throughout the research project are outlined in Table 2.1 and Table 2.2.

Table 2.1 List of materials**.**

Equipment/Material/Software	Manufacturer	Identifier
Polypropylene Containers	Satco	5060123317879
Digital Callipers	Work Zone	ANS-18-027
18v Cordless Drill	ROK	1114544
48 MP Digital Camera	Samsung	A51
Visible Spectrophotometer	Jenway	6300
UV-Visible Spectrophotometer	Shimadzu	UV-1280
Ultrasonic Bath	Guyson	Pulsatron KC2
Vortex Mixer	Fisher Scientific	ZX3
Microplate Reader	Biotek	Synergy HTX
Oscillating Incubator	New Brunswick Scientific	Innova 4000
Medical Applicator (Paediatric Swab)	Heinz Herenz	1032749
Luria Broth	Sigma Life Science	L3022
Agar Bacteriological	Sigma Life Science	A6686
Harlequin E. coli/Coliform Agar	Neogen	NCM1005A
CHROMagar Staph aureus Agar	CHROMagar	TA672
CHROMagar MH Orientation Agar	CHROMagar	MH482(B & S)
Tryptone Soy Agar	Lab M	LAB011
Tryptone Soy Broth	Lab M	LAB014
Mueller Hinton Agar	Lab M	LAB039
Mueller Hinton Broth	Lab M	LAB114
Chlorhexidine diacetate	Sigma Aldrich	282227
Resazurin Sodium Salt	Sigma Aldrich	199303
Cryobeads	Hardy Diagnostics	CS100B
Graphpad Prism	Graphpad Software	Ver. 8.0.2
Minitab Statistical Software	Minitab LLC	Ver. 19
Escherichia coli	American Type Culture Collection	25922
Staphylococcus aureus	National Collection of Type Cultures	12981

Table 2.2 List of urinary catheters.

Abbreviation	Catheter Description	Model	Chapter
IC UnC	Uncoated PVC Intermittent urinary catheter	*In vitro* Urethra Friction Model	3
IC Hydro	Hydrophilic coated PVC Intermittent urinary catheter	*In vitro* Urethra Friction Model	3
IC Proto	Prototype Intermittent urinary catheter	*In vitro* Urethra Friction Model	3
ID -CHX3B	Silicone Indwelling urinary catheter banded with polymer coated without chlorhexidine	*In vitro* Urethra Diffusion Model	4,5
ID +CHX3B	Silicone Indwelling urinary catheter banded with polymer coated with chlorhexidine	*In vitro* Urethra Diffusion Model	4,5
ID UnC	Uncoated silicone Indwelling urinary catheter	*In vitro* Urethra Extraluminal Migration Model	5
ID -CHX	Silicone Indwelling urinary catheter fully polymer coated without chlorhexidine	*In vitro* Urethra Extraluminal Migration Model	5
ID +CHX	Silicone Indwelling urinary catheter fully polymer coated with chlorhexidine	*In vitro* Urethra Extraluminal Migration Model	5
ID +CHX50	Silicone Indwelling urinary catheter 50% polymer coated with chlorhexidine	*In vitro* Urethra Extraluminal Migration Model	5

2.2 Bacterial Strains

The bacterial strains used for all *in vitro* model tests and validation were *E. coli* ATCC 25922 and *S. aureus* NCTC 12981, lyophilised cultures were obtained from their respective culture collection institutes. Primary bacterial strains were maintained at -80°C on Cryobeads™. For each independent test, a secondary culture was grown from the primary culture stock. Growth standard curves were prepared for each strain and later used to quantify bacterial density by spectrophotometry to ensure reproducible inoculation for testing purposes.

Table 2.3 Bacterial strain characteristics.

Bacterial Species	**Strain**	**Storage**	**Biosafety**	**Growth Conditions**
Escherichia coli	ATCC 25922	-80°C	Level 1	Aerobic, Mesophilic
Staphylococcus aureus	NCTC 12981	-80°C	Level 2	Aerobic, Mesophilic

2.3 Urethra Model Development

The development process involved the initial formation of the urethra model and associated methodology whilst also tackling the problems of temporal contamination, condensation, bacterial swarming, reducing variability, and later modifying the methodology to diversify the uses of the model.

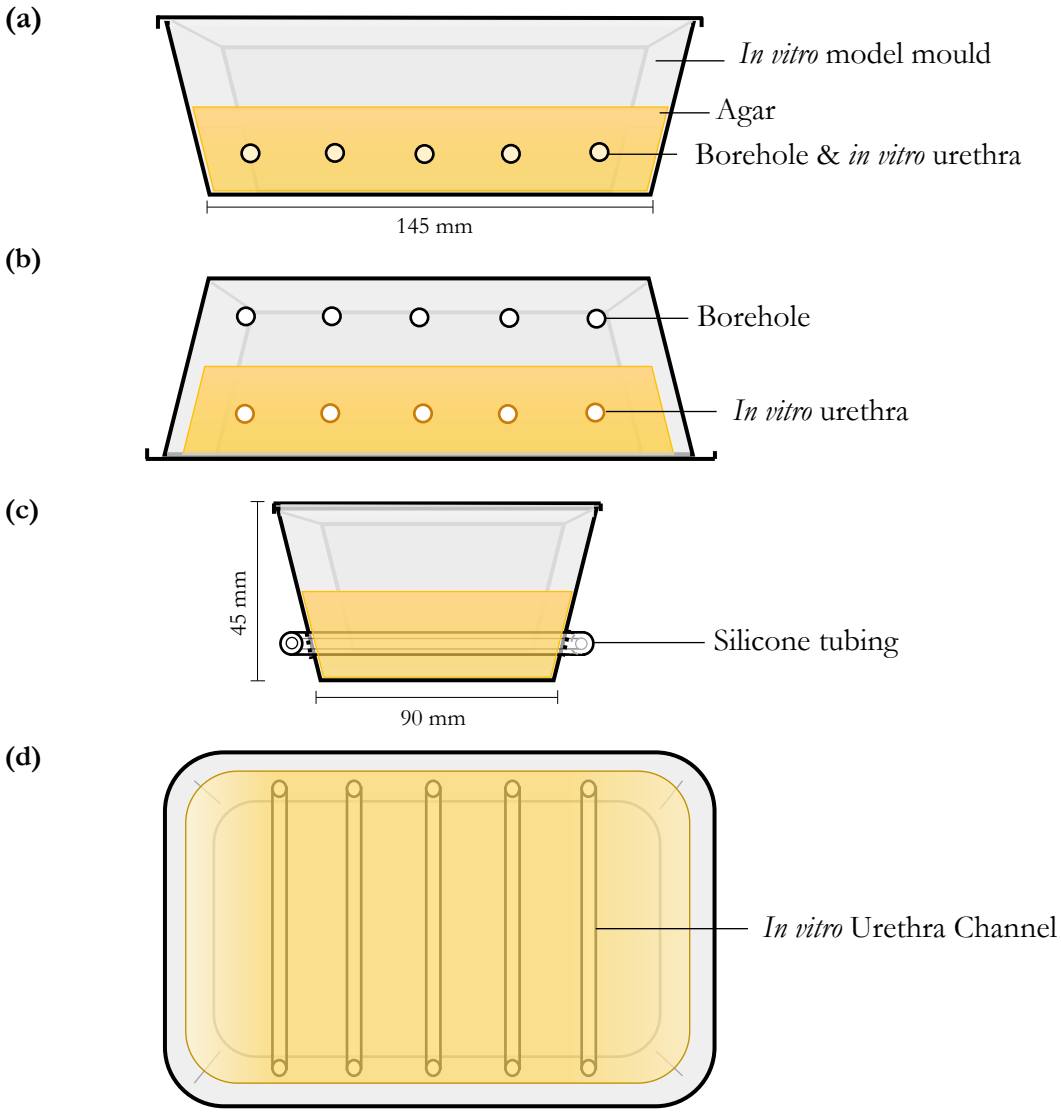


Figure 2.1 *In vitro* Urethra Model mould apparatus in friction model configuration; (a) Transection of the urethra model in mould (b) Transection of the urethra model mould inverted, (c) End elevation of the urethra model mould with silicone tubing in place, (d) Planar view of *in vitro* urethra model in mould.

2.4 *In vitro* Urethra Models

The structure of the urethra model and mould was similar for all three *in vitro* models and concomitant methodologies developed. Deviations were made in channel length/diameter, media used, and test methodologies to suit the specific diagnostic needs of the different medical devices tested and the experimental outcome desired.

2.3.1 *In vitro* Urethra Model Mould and Agar preparation

Preparation of *in vitro* Friction/Extraluminal Migration Models and Model Moulds

Polypropylene (PP) containers were prepared to form urethral moulds. Parallel holes were drilled in the sides of the containers just smaller than the diameter of the silicone tubing. Silicone tubing was pushed through one hole, then through the corresponding parallel hole on the other side of the container.

For the friction model, this was done five times for each mould on the long side of the container to form an 80 mm channel, alternatively this was done in duplicate on the short sides of the mould to form a 140 mm channel for the migration model (Figure 2.1). The moulds were then placed in individual air-permeable sterilisation pouches and autoclaved. After autoclaving, the moulds were places in a 60°C oven to dry fully. After drying, in a laminar flow hood, either Harlequin™ E. coli/Coliform agar or CHROMagar™ Staph aureus agar was then poured over top of the silicone tubing creating a straight channel in the agar. The agar was allowed to set with the container open in the laminar flow hood to avoid condensation. Once the agar was set, the silicone tubing was carefully removed leaving a formed *in vitro* urethra channel. After removal of the silicone tubing, each urethra model was inverted, and the model released from the container base. The model remained on the lid of the container for the remainder of testing with no contact between the sides of the container and the agar (Figure 2.1).

Preparation of *in vitro* Urethra Diffusion Models and Model Moulds

Parallel holes were drilled in the sides of PP containers just smaller than the diameter of the silicone tubing. Silicone tubing was pushed through one hole, then through the corresponding hole on the other side of the container (Figure 2.1). The moulds were then placed in individual air-permeable sterilisation pouches and autoclaved. After autoclaving, the moulds were places in a 60°C oven to dry fully. After drying, in a laminar flow hood, the prepared CHROMagar™ MH Orientation Agar (CHROMagar™) was then poured over top of the silicone tubing

creating a straight channel in the agar. The agar was allowed to set with the container open in the laminar flow hood to avoid condensation. Once set, the silicone tubing was carefully removed forming an *in vitro* urethra. After removal of the silicone tubing, each urethral mould was inverted, and the agar model released from the container base. The model remained on the lid of the container for the remainder of testing with no contact between the sides of the container base (Figure 2.1).

2.4.1 *In vitro* Urethra Friction Testing Methodology

This method quantitatively measures the pathogen movement along the urethra due to introduction of a urinary catheter. The protocol was designed to simulate a scenario of an intermittent catheter being inserted in a clinical setting through a contaminated meatus, and the subsequent movement of bacteria from the meatus to the bladder due to friction investigated. This protocol was informed by previous studies reported by Holland and Fish, (2012); Hudson and Murahata, (2005).

Urethra Friction Model Testing Method

A microbial suspension was prepared and diluted to ~10^5 – 10^6 CFU ml^{-1}. A sterile cotton swab was inserted into the test inoculum for 10 seconds, the excess was removed by running the sterile swab along the rim of the container, (Figure 2.2 a). The swab was then inserted ~10 mm into one end of the urethra channel and moved along the inside of the channel clockwise one turn, removed and discarded. A fresh swab was prepared for each replicate. A sterile swab with no bacterial inoculum was inserted similarly into the sterility control channel, (Figure 2.2 b). The inoculum was allowed to absorb into the agar for 5 minutes. A catheter was then inserted into the agar channel, left in place for 30 seconds, then removed through the same entry hole (Figure 2.2 c). All urethra models were then incubated for 24 hours at 37°C.

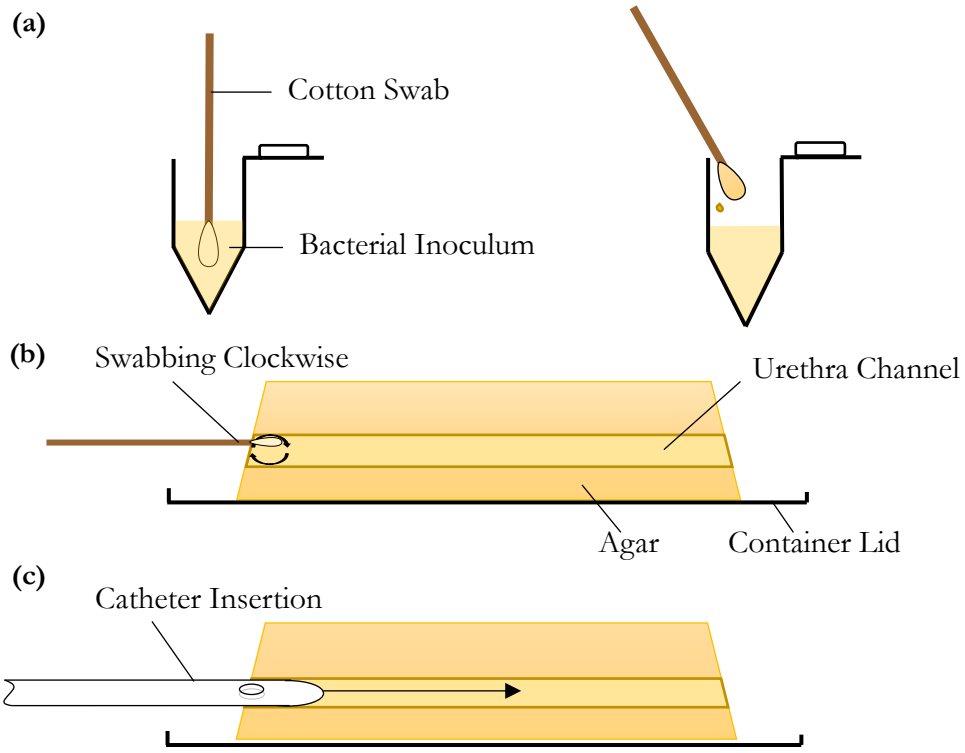


Figure 2.2 Illustration of the urethra friction model testing methodology; (a) inoculation of sterile swab, (b) inoculation of urethra channel with swab, (c) insertion of catheter into urethra channel.

Quantification of the *In vitro* Urethra Friction Model

After incubation of the urethra friction model, excess agar was dissected from around each channel and discarded. Each channel was assessed visually then bisected with a scalpel to expose the inner channel walls and colonies within (Figure 2.3 a). After photography, each channel was dissected into 8 equal segments or octants (Figure 2.3 b). Each pair of octants was place in a separate 15 ml centrifuge tube with 10 ml of TSB (Figure 2.3 c). All octants were sonicated for 10 minutes to dislodge the bacteria from the agar surface. All octants were then vortexed for 30 seconds. The media containing each octant pair was serial diluted in a 96 well plate containing TSB down to a 10^{-6} dilution. Five 20 µl drops of each concentration in the dilution range of 10^{-3} – 10^{-6} were pipetted onto TSA plates and the media was allowed time to absorb into the agar (Reed & Reed, 1948). The TSA plates were inverted and incubated for 24 hours at 37°C. After incubation, the colonies in each drop on each plate were counted and recorded (Figure 2.3 d). The CFU ml^{-1} for each segment was calculated using the formula below:

$$CFU\ ml^{-1} = Total\ Colonies\ in\ 5\ Drops \times \frac{1}{Dilution\ Factor} \times 10$$

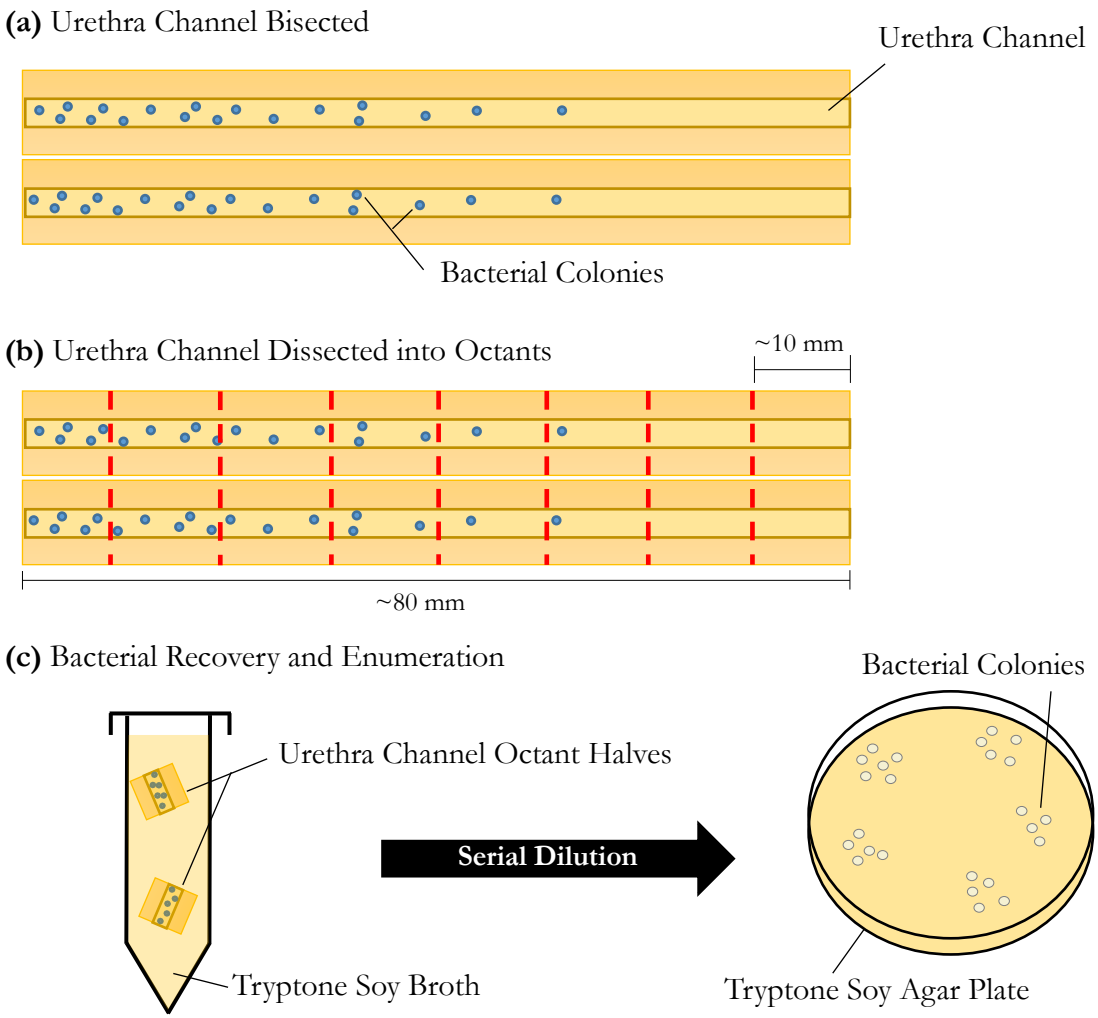


Figure 2.3 Illustration of the recovery and quantification of the bacteria within the urethra friction model.

2.4.2 *In vitro* Urethra Diffusion Model

This method quantitatively measures the zone of bacterial growth inhibition around antimicrobial coatings on a urinary catheter extraluminal surface. The protocol was designed to simulate a scenario wherein antimicrobials diffuse from an antimicrobial coating to inhibit bacterial growth in a fully contaminated urethra. It was envisaged that this model would provide a method to determine the length and location of antimicrobial coatings or bands on a catheter surface to inhibit catheter colonisation. This model was informed by the historical works of Bauer *et al.,* (1966).

Urethra Diffusion Model Method

A microbial suspension was prepared to a density of ~10^8 CFU ml^{-1}. A sterile cotton swab was inserted into the test inoculum for 10 seconds, the excess was removed by running the sterile swab along the rim of the container (Figure 2.4 a). The swab was then inserted into the *in vitro* urethra channel and run along the full length of the channel ensuring continuous surface contact before being withdrawn. This was repeated approximately every 2 mm working in a clockwise pattern until the entire internal surface of the *in vitro* urethra was inoculated (Figure 2.4 b). A prepared catheter sample was then swabbed with a sterile swab dipped in sterile water based lubricant and then inserted into the *in vitro* urethra (Figure 2.4 c). The model was then incubated at 37°C for 24 hours. After incubation, the model was assessed by eye and the zone of inhibition around coated areas or bands was recorded in millimetres with the use of a digital callipers (Figure 2.4 d).

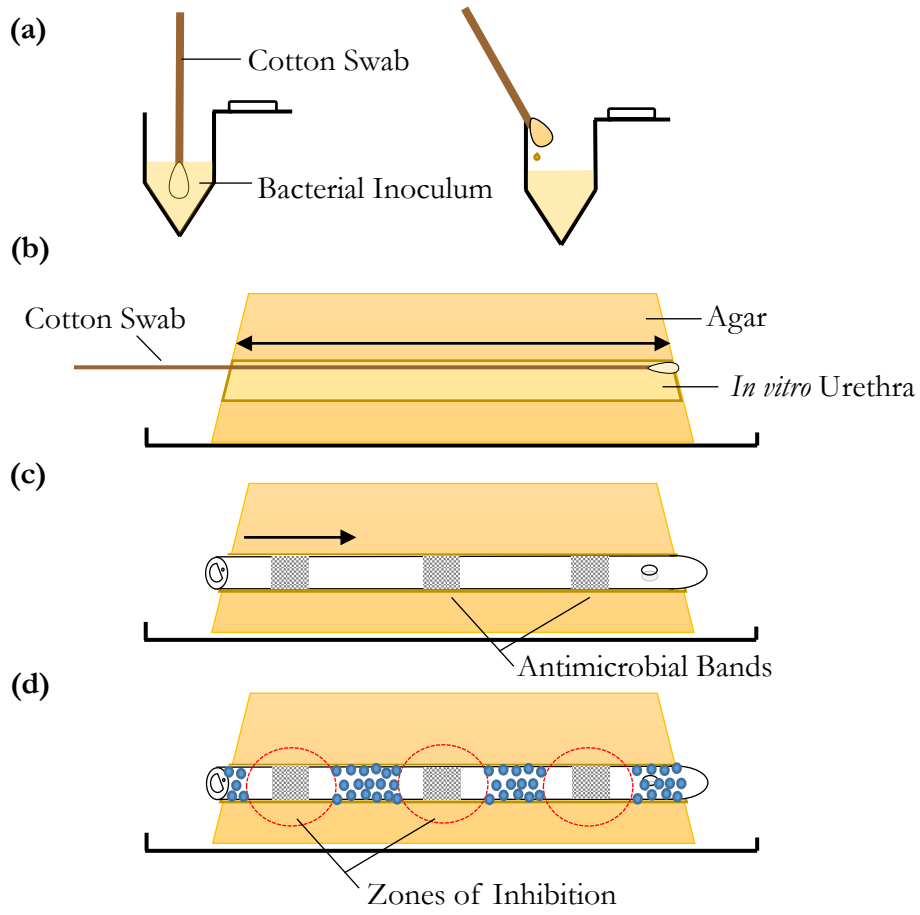


Figure 2.4 Illustration of the *in vitro* urethra diffusion model methodology; (a) inoculation of swab with bacteria, (b) inoculation of the *in vitro* urethra with arrow indicating in and out motion of swab, (c) insertion of catheter with antimicrobial bands, (d) model after 24-hour incubation with bacterial colonies represented as blue dots and zones of inhibition encircled in red.

2.4.3 *In vitro* Urethra Extraluminal Migration Model

This model and method quantitatively measures and visualises the migration of bacteria along the extraluminal surface of an indwelling urinary catheter. The protocol was developed to simulate the migration of bacteria from the urethral meatus to the bladder within the urethra during long-term catheterisation. This protocol was informed by a publication by Gaonkar *et al.,* (2003).

Extraluminal Migration Model Testing Method

A previously prepared catheter was aseptically inserted into the *in vitro* urethra channel until the proximal end was flush with the meatal end of the channel (Figure 2.5 a). A microbial suspension was prepared to a density of ~10^5 – 10^6 CFU ml^{-1}. A sterile paediatric swab was inserted into the test inoculum for 10 seconds, the excess was removed by running the swab along the rim of the container (Figure 2.5 b). The swab was then inserted ~10 mm into one end of the urethra channel between the catheter and the agar. The swab was moved along the inside of the channel clockwise one turn, removed and discarded. A fresh swab was prepared for each replicate (Figure 2.5 c). All urethra models were then incubated for 30 days at 37°C.

Quantification of the *In vitro* Urethra Extraluminal Migration Model

To quantify the migration of bacteria along the extraluminal catheter surface, the models were taken from the incubator at the same time each day and the distance of the bacterial migration from the meatal end was measured with the use of a digital callipers and, photographs were taken (Figure 2.6). This was repeated for 30 days to represent the average time that an indwelling catheter remains within a patient. After the completion of the 30 day study, the final migration distance was compared between control and test samples.

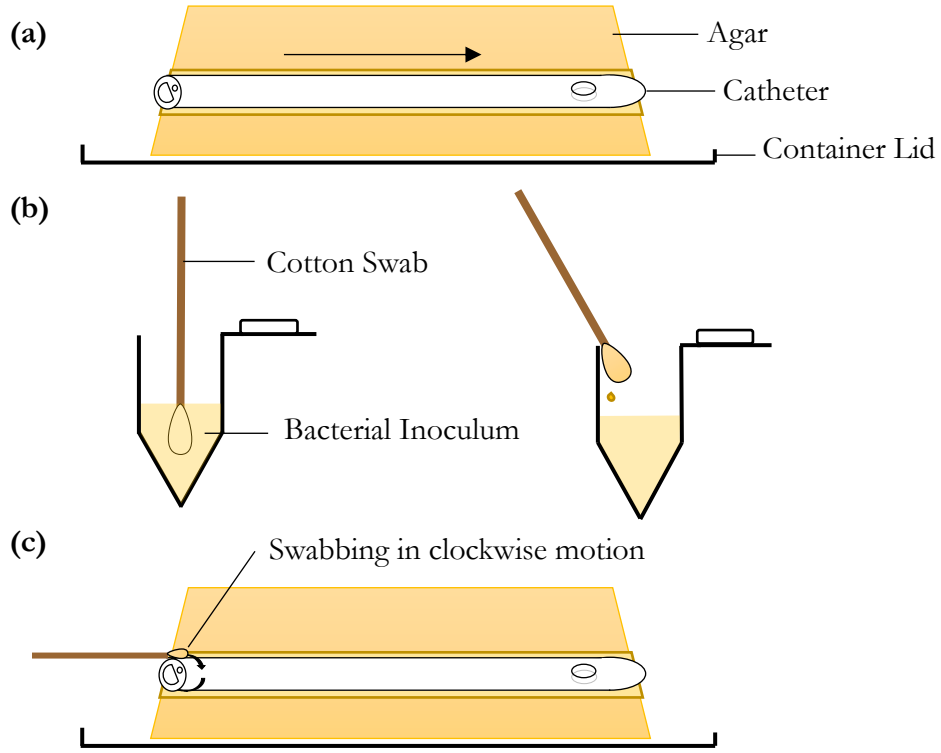


Figure 2.5 Illustration of the urethra migration model testing methodology; (a) insertion of indwelling urinary catheter, (b) inoculation of sterile swab, (c) inoculation of catheter and urethra channel.

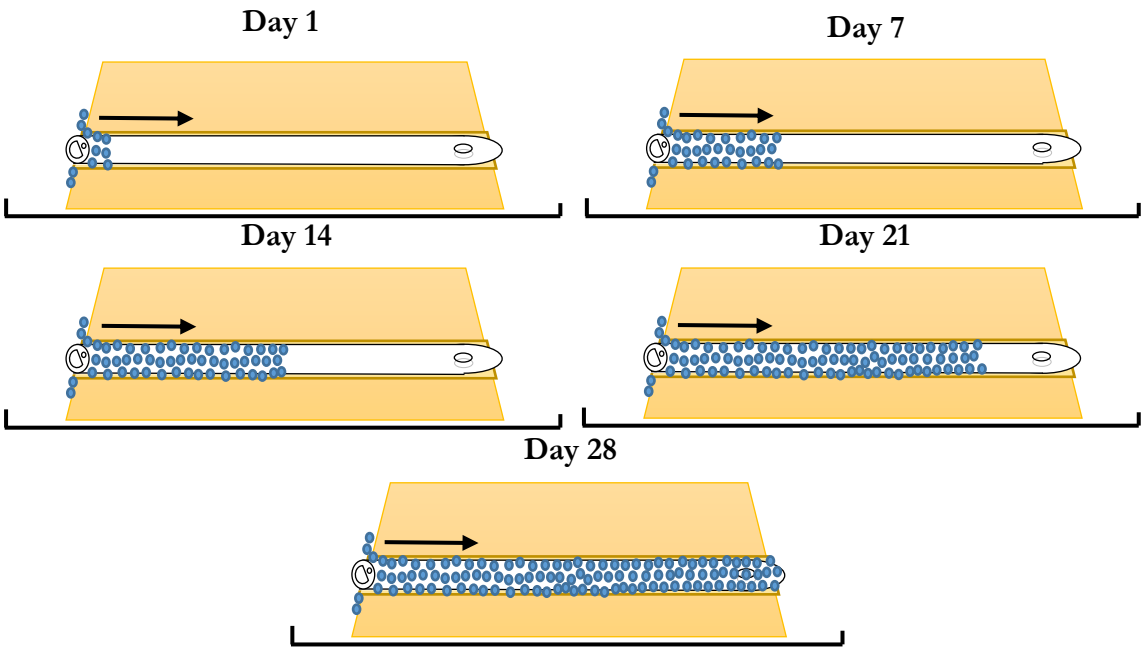


Figure 2.6 Illustration of bacterial migration over the extraluminal surface of an indwelling urinary catheter within the *in vitro* urethra extraluminal migration model over a month-long period. Arrow indicates direction of bacterial migration; blue circles represent bacterial colonies.

2.5 Antimicrobial Analysis and Drug Release

Chlorhexidine diacetate (CHX) was the antimicrobial compound used in the coating or banding of all indwelling catheters used in this study. The antimicrobial ability and diffusion of CHX from the coating was investigated to provide supportive evidence to its ability to inhibit bacterial growth and potentially prevent CAUTI. Three assays were undertaken to provide this support and the methods involved are covered in this section.

2.5.1 Minimum Inhibitory/Bactericidal Concentration

Two bacterial inoculums ~$1.5x10^8$ CFU ml^{-1} of *E. coli* and *S. aureus* were prepared in Mueller Hinton Broth (MHB). This was serially diluted to a final in-well bacterial density of $5x10^5$ CFU ml^{-1}. A 0.8 μg ml^{-1} CHX stock solution was prepared in sterile deionised water (dH_2O) and later diluted in MHB in two-fold dilutions to form an in-well range of 0.4 μg ml^{-1} to 0.0007 μg ml^{-1} CHX. In a 96-well plate, one column acted as a sterility control with 100 μL of sterile MHB added to each well. For the growth control, 50 μL of MHB and 50 μL of inoculum were added. To each test well, 50 μL of inoculum was added and then 50 μl of the corresponding concentration of CHX. This was repeated in quadruplicate wells for each strain and repeated in two independent tests. The plates were then sealed in Parafilm to prevent evaporation and incubated at 37°C for 18 h in an oscillating incubator. After incubation, 10 μl of 0.02% resazurin (indigo) was added to each well and incubated with oscillation for 2 h at 37°C (Elshikh et al., 2016). The fluorescence of each well was then quantified by a microplate reader set for excitation at 520 nm and emission at 590 nm to detect the metabolically reduced fluorescent resorufin (pink). All wells containing CHX were compared to the growth controls to calculate reduction in cellular viability. The concentration of CHX that reduced cellular viability to 0% was considered to be the Minimum Inhibitory Concentration (MIC).

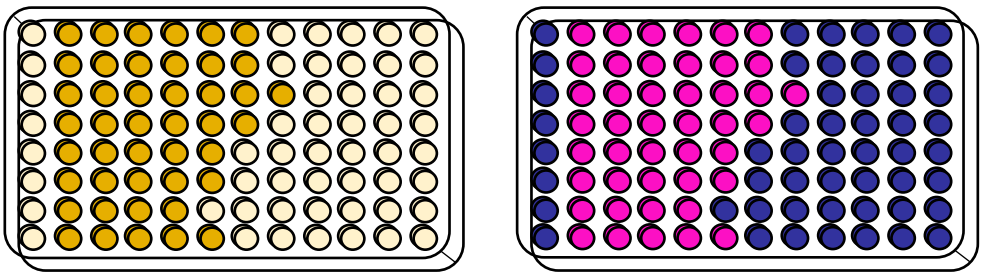


Figure 2.7 Illustration of minimum inhibitory concentration assay when using resazurin as a growth indicator; Left: a 96-well plate with darker wells containing live bacteria and lighter wells containing inhibited growth or no bacteria, Right: a 96-well plate after resazurin addition with pink wells containing live bacteria and indigo wells containing inhibited growth or no bacteria.

To determine the Minimum Bactericidal Concentration (MBC), the contents of the wells above and below the MIC and the MIC itself were transferred to a new 96-well plate and serially diluted down to 10^{-8} in TSB (Koeth, 2016). Each dilution was then plated onto TSA in a modified 6x6 method wherein a multichannel pipette was used to aliquot 10 µl drops onto the agar surface with 5 rows by 5 columns (Figure 2.8, Chen et al., 2003). The drops absorbed into the agar surface before plates were inverted and incubated at 37°C for 18 hours after which the colonies in each drop were counted and CFU ml^{-1} was calculated with the following formula:

$$CFU\ ml^{-1} = Total\ Colonies\ in\ 5\ Drops\ \times \frac{1}{Dilution\ Factor} \times 20$$

The concentration at which no bacterial growth was observed was determined to be the MBC. This process was repeated in two independent tests each for both bacterial species tested.

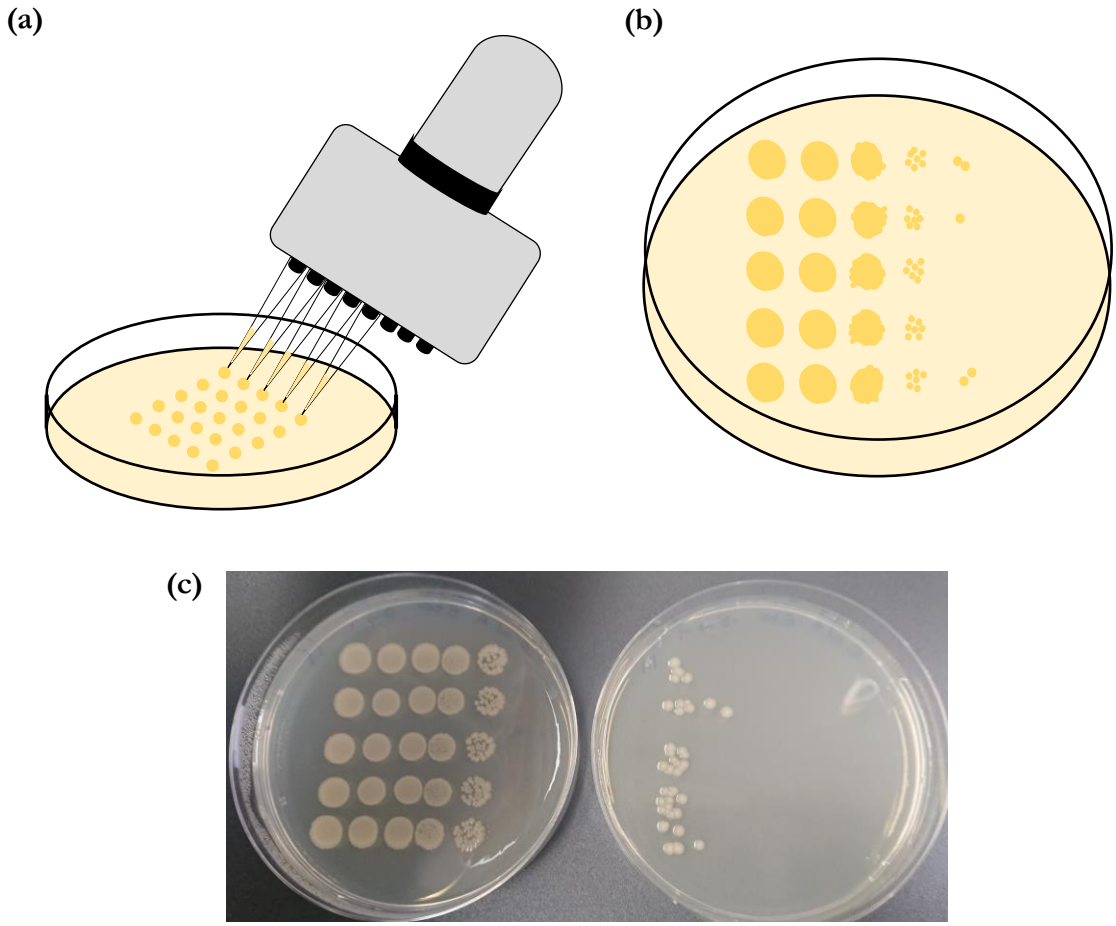


Figure 2.8 Illustration of the modified 6x6 method of bacterial enumeration with 5x5 drops; (a) method of dotting the agar surface with the use of a multichannel pipette, (b) after incubation colonies are counted where discrete colonies are visible, (c) example image of 5x5 bacterial enumeration method with dilutions of 10^{0}-10^{-10} from left to right.

2.5.2 Serial Plate Transfer test

Mueller Hinton agar plates were prepared, and the agar surfaces were fully dried. Sextuplet 10 mm long, 4 mm Ø, ID +CHX catheter samples were aseptically prepared. A bacterial inoculate at a density of ~1.5x10^8 CFU ml^{-1} or a 0.5 McFarland, of either *E. coli* or *S. aureus,* was also prepared in phosphate buffered saline by the McFarland method. A sterile swab was dipped in the inoculum for 30 seconds then used to swab the entire plate surface. Sextuplet 4 mm Ø wells were created in the agar surface and samples were aseptically placed in wells. The plates were not inverted before being placed in a stationary incubator at 37°C. Plates were incubated for 24 hours after which the zones of inhibition around each disc or sample were recorded in millimetres (Figure 2.9). This process was repeated each day for 30 days transferring the same catheter samples to freshly inoculated plates each day and the zones of inhibition recorded each subsequent day (Saini et al., 2016).

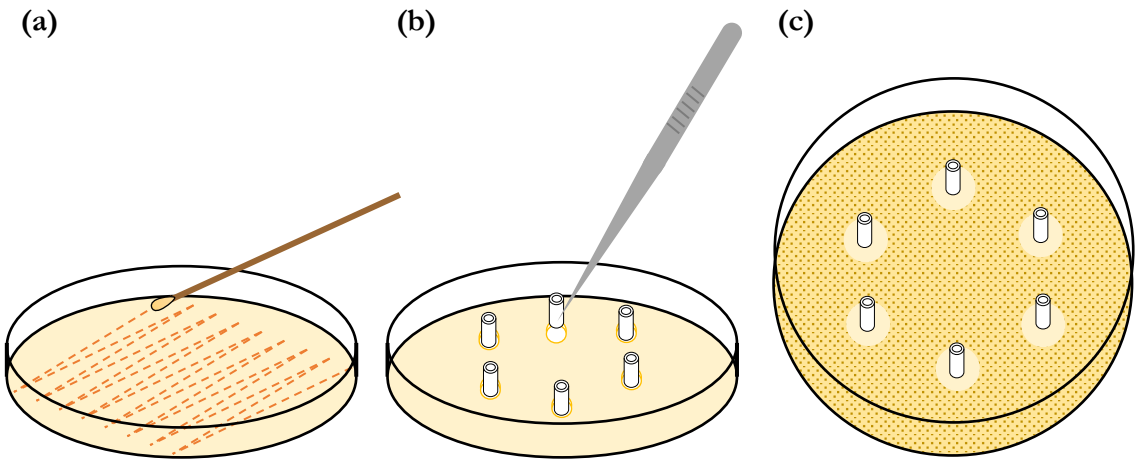


Figure 2.9 Illustration of the sterial plate transfer method; (a) inoculation of entire agar surface, (b) placing of catheter samples into wells in agar surface, (c) zones of inhibition around samples after incubation.

2.5.3 Drug Release Study

The peak absorbance (λ_{max}) of CHX was first determined by wavelength scan of a stock solution at 2 ng ml^{-1} CHX dissolved in dH_2O (Shimadzu UV-1280). The λ_{max} wavelength was then used for all further readings. A set of CHX standards were then prepared with a range of 2 ng ml^{-1} to 0.22 ng ml^{-1}. The absorbance of each standard was then read at 230 nm (λ_{max}). A standard curve was plotted and used to calculate drug concentration from absorbance values. To assess the release rate of CHX from the polymer coating, 10 mm triplicate samples of ID UnC, ID -CHX, and ID +CHX catheters were prepared. Each sample was placed in 10 ml of dH_2O in a T25 culture flask. The flasks were incubated in darkness at 37°C for 30 d in an

oscillating incubator. Each day, 1 ml was removed from each flask to read the drug concentration in the dH_2O by UV absorption and, 1 ml of fresh dH_2O was then added to each flask before being replaced into the incubator. The concentration of the drug on each day versus the cumulative release was then plotted.

2.6 Statistical Analysis

Statistical analysis of results from the *in vitro* urethra model was performed on Graphpad Prism® 8 and Minitab® 18. Results are presented as the average of 6 independent tests. Normality of the data was determined with the Anderson- Darling test or the Shapiro-Wilk Test. The reproducibility of the models and methodology was determined using a one-way analysis of variance (ANOVA) or the Kruskal-Wallis test. Hypothesis testing was performed with the use of an unpaired T-Test or the Mann-Whitney U-Test. All non-parametric tests were adjusted for ties. The Statistical significance was accepted at $P \leq 0.05$.

Null hypothesis:	All means are equal
Alternative hypothesis:	Not all means are equal
Significance level:	$\alpha = 0.05$

Chapter 3 *In vitro* Urethra Friction Model

3.1 Introduction

Urinary catheters are one of the most common devices used in modern medical treatment; however, they are also implicated in the majority of nosocomial infections. There are several *in vitro* models to test the efficacy of indwelling catheters to prevent either biofilm formation, encrustation, or CAUTI development as previously discussed in section 1.10.2. There are two main *in vitro* models that have been developed to test indwelling catheters, that is, the bladder model described by (Stickler *et al.,* 1999; 1987) and the urinary tract model developed by Gaonkar *et al.,* (2003). Both of these models have been adapted and inspired a multitude of derivative models that have aided in the advancement of indwelling catheter design and CAUTI management.

Intermittent catheters, while still implicated in CAUTIs, are often recommended as an alternative to indwelling catheters due to less complex associated infections, with biofilm formation not possible as a result of limited *in situ* time (Goetz *et al.,* 2018). This lower risk of serious complications may be a contributory factor in the relative stagnation in intermittent catheter research and innovation when compared to that of indwelling catheters. CAUTI prevention for intermittent catheters, traditionally and currently, focuses on preventing the movement of bacteria into the urethra and ultimately the bladder, or preventing pathogens coming into contact with the catheter by utilising closed "no touch" systems (Goetz *et al.,* 2018; Holland & Fish, 2012; Van Achterberg *et al.,* 2008; Hudson & Murahata, 2005; Woodward & Rew, 2003). The idea that a CAUTI can be initiated by insertion of a single catheter with a contaminated tip was first proposed by Kaye *et al.,* (1962) and is supported by the more recent research of Barford *et al.,* (2008). With the inhibition of bacterial movement, from outside of the body to inside, acting as a primary target for intermittent catheter CAUTI prevention. An *in vitro* model that can demonstrate the friction-mediated bacterial movement, or lack thereof, during catheter insertion could prove a useful tool in novel device development. In reviewing the literature, it was identified that there was no validated *in vitro* model to specifically test intermittent catheters.

An initial urethra model was developed from gelatine, produced by an engineer in Teleflex® before the start of this project. Their model only served as a visual representation of the urethra. The model described in this thesis was formulated and adapted to sustain bacterial growth and quantify the movement of bacteria due to friction during the insertion of a catheter.

3.2 Stage 1: Agar Formulation, Container Selection, and Channel Formation

Agar Formulation

In the first stage of urethra model development, investigation into the optimum agar formulation was undertaken. The characteristics desired comprised of a formula that was both as translucent as possible while still capable of supporting bacterial growth, and firm enough to allow catheter insertion into a moulded channel (Figure 3.1).

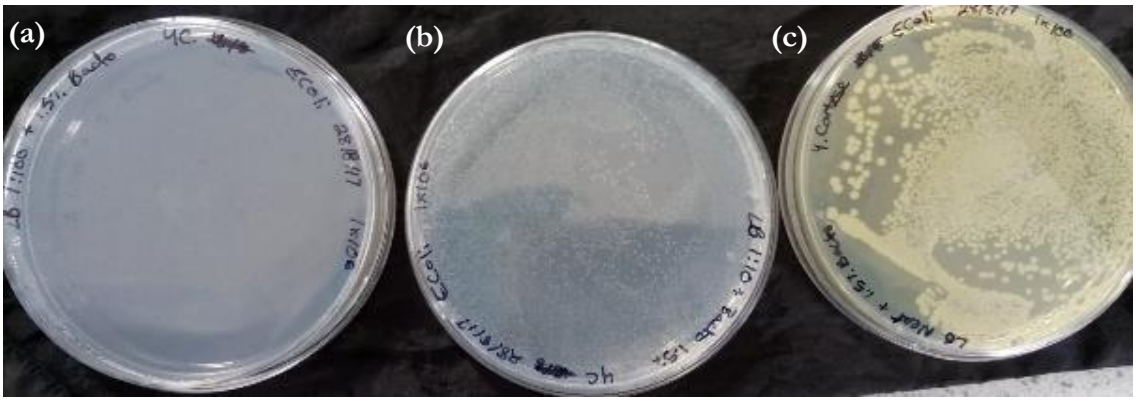


Figure 3.1 Agar formulation plate comparison with three different concentrations of Luria broth combined with a 1.5% agar solution and inoculated with *E. coli* ATCC 25922 to visually assess agar clarity and bacterial growth, (a) 0.2g L^{-1} Luria broth and 1.5% bacteriological agar; (b) 2g L^{-1} Luria broth and 1.5% bacteriological agar; (c) 20g L^{-1} Luria broth and 1.5% bacteriological agar.

It was initially decided to introduce *E. coli* ATCC 25922, a standard quality control species, into the model. A Luria broth-based agar was trialled as it provided essential nutrients for the non-selective growth of *E. coli* and numerous other bacterial species. The Luria broth was supplemented with bacteriological agar which is a purified agar to act as a gelling agent. Different ratios of Luria broth combined with bacteriological agar were trialled. The criteria for selection of the final solution was an agar that was both translucent enough to observe the growth through the agar and supportive of bacterial growth. For the concentration of Luria broth, it was found that a 1:10 dilution or 2g L^{-1} sustained bacterial growth but limited it enough to allow the growth of small discrete colonies (Figure 3.1). The neat (20g L^{-1}) and 1:100 (0.2g

L^{-1}) concentrations of Luria broth were discarded as at 1:100, visible colonies did not grow and at a neat concentration the colonies were too large with single colonies indistinguishable.

The required concentration of bacteriological agar was also tested. Solutions containing 1% (10g L^{-1}), 1.5% (15g L^{-1}), and 2% (20g L^{-1}) were investigated to see which maintained the clarity of the agar whilst also creating a channel that was structurally sound and did not collapse or tear when a catheter was inserted. The optimal agar concentration was found to be 1.5% agar, which created a well-formed channel and allowed smooth insertion of test catheters (Table 3.1).

Table 3.1 Determination of agar and media formulation for the development of the *in vitro* urethra agar model. LB: Luria broth.

Media Ratio	Observations	Suitable for Urethra Model
1.5% Agar and Neat LB	Agar too opaque, Agar channel well formed Bacterial growth normal, discrete colonies not visible	No
2.0% Agar and Neat LB	Agar too opaque Agar too brittle, channel splits on catheter insertion Bacterial growth normal, discrete colonies not visible	No
2.5% Agar and Neat LB	Agar too opaque Agar too brittle, channel splits on catheter insertion Bacterial growth normal, discrete colonies not visible	No
1.5% Agar and 1:10 LB	**Agar translucent** **Agar channel well formed** **Bacterial growth reduced; discrete colonies visible**	**Yes**
2.0% Agar and 1:10 LB	Agar translucent Agar too brittle, channel splits on catheter insertion Bacterial growth reduced; discrete colonies visible	No
2.5% Agar and 1:10 LB	Agar translucent Agar too brittle, channel splits on catheter insertion Bacterial growth reduced; discrete colonies visible	No
1.5% Agar and 1:100 LB	Agar translucent Agar channel well formed Bacterial growth not visible	No
2.0% Agar and 1:100 LB	Agar translucent Agar too brittle, channel splits on catheter insertion Bacterial growth not visible	No
2.5% Agar and 1:100 LB	Agar translucent Agar too brittle, channel splits on catheter insertion Bacterial growth not visible	No

Container selection

The container selection process was based on the cost of the containers and their ability to withstand steam autoclave sterilisation. Unbranded polypropylene (PP) containers were chosen as PP can withstand traditional autoclave cycles at 121°C (Maier & Calafut, 1998). The containers were chosen for their ease of availability and physical handling characteristics *i.e.,* their structure was firm enough for handling when filled but flexible enough to allow ease of model removal. PP is one of the most common plastics used for heat safe food storage containers and are relatively inexpensive. Initially, containers were reused after each test to reduce cost and environmental waste however this led to problems with temporal contamination and ultimately, they were replaced with a lower cost disposable container (Figure 3.6). Containers were originally autoclaved wrapped in aluminium foil with tubing in place.

Urethra Channel Formation

To form the urethra channel of the model, silicone tubing was used as it is thermally stable at autoclave temperatures of 121°C (Singha et al., 2017; Jerschow, 2002). Silicone is also flexible enough to create a seal with the boreholes in the sides of the container whilst also stiff enough to form a consistent channel when agar was poured over top (Figure 3.2).

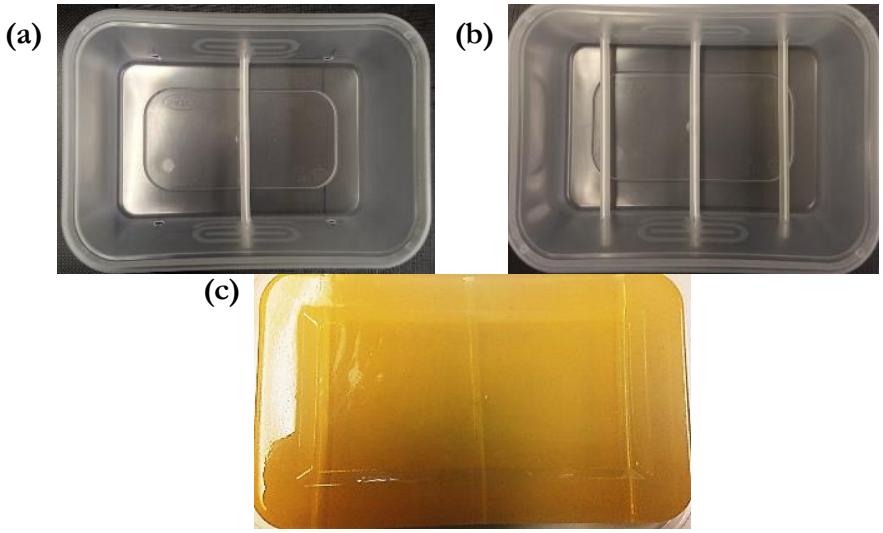


Figure 3.2 Urethra model mould containers and formed urethra model: (a) Urethra model mould container with single silicone tube to create single channel urethra model; (b) Urethra model mould container with three silicone tubes to create three channel urethra model; (c) Formed agar urethra model with three *in vitro* urethra channels visible from above.

To form the urethra channel in the urethra model mould, a drill was used to prepare parallel boreholes on each side of the container through which a tube would be threaded. The drilling process is simple but delicate, pressure must be controlled when drilling to avoid cracking of the plastic container. Fine cracks were covered with a small piece of autoclave tape to ensure the container remained watertight, however any containers with large cracks were discarded.

At this stage in development it was decided to form a channel slightly larger than the test catheter *i.e.* a 5 mm Ø channel for a 4.667 mm Ø catheter or a 15 Ch and 14 Ch on the French gauge respectively (Iserson, 1987). A larger channel was used to prevent the agar cracking or splitting when inserting a test catheter. If the agar tears or small cracks form in the agar during catheter insertion, the bacteria can and will move along the aberrations and grow outside of the channel, ultimately affecting the quantification and reproducibility of the model.

Plan View

Front Elevation

Figure 3.3 Examples of urethra agar channel splitting leading to bacterial growth outside of the urethra channel. Areas encircled in red highlight areas where excessive splitting of the agar channel occurred due to insertion of a catheter with an insertion tip wider in diameter than the diameter of the agar urethra channel.

3.3 Stage 2: Initial Testing Methodology, Visualisation, and Temporal Contamination

Initial Testing Methodology

The initial testing methodology was based on work carried out by Hudson and Murahata, (2005) in which they inoculated their gloves and tested the transfer of bacteria from their gloved hands to a catheter during insertion. Although Hudson and Murahata, (2005) do not elaborate what they insert the test catheters into, they do describe the bacterial recovery procedure to enumerate the bacteria on the catheter surface.

Enumeration of the bacteria on the catheter surface after insertion into the *in vitro* urethra model was the first method investigated to obtain quantitative data, to support the urethra model. This methodology was later changed, as the aim of the urethra model is to demonstrate the friction-mediated movement of bacteria along the urethra channel induced by the insertion of the catheter. From this point it was decided to quantify the bacteria in the channel itself rather than on the catheter.

Focusing on the quantification of the bacteria within the agar channel instead of those on the catheter surface also proved more reproducible. Differences in catheter materials, surface coatings, and physical design can cause bacteria to adhere to the catheter dissimilarly or not release equally from the catheter surface during recovery, a problem also noted by Hudson and Murahata, (2005).

Colony Visualisation

The original agar formulation chosen to create the *in vitro* urethra model was a combination of a 1:10 dilution of Luria Broth (2g L^{-1}) supplemented with bacteriological agar to a final agar concentration or 1.5%. The urethra mould was firm enough to form an open urethra channel whilst translucent enough to see through. However, as *E. coli*'s colonies were the same colour as the surrounding agar, they were not readily visible (Figure 3.4 a). The agar was changed to a chromogenic agar formulation, which allowed easier visualisation of coloured bacterial colonies (Figure 3.4 b). A 1:5 dilution of Harlequin™ E. coli/Coliform agar (Neogen®) was supplemented with bacteriological agar to a final agar concentration of 1.5% to remain translucent whilst also producing small discrete colonies (Figure 3.4 c).

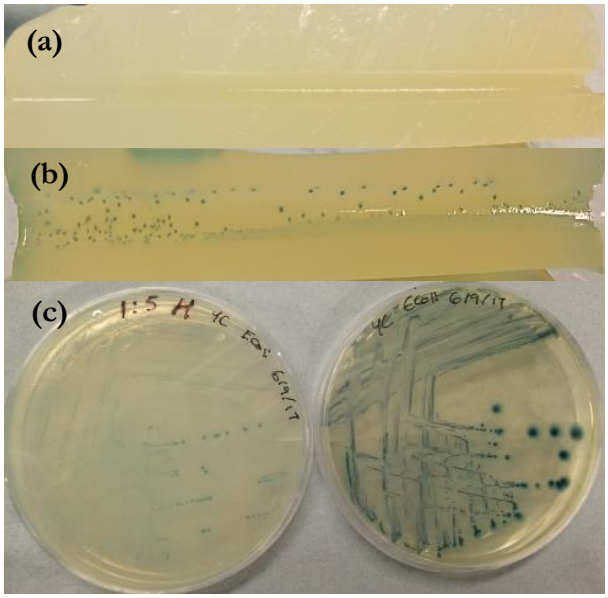


Figure 3.4 Changing the agar formulation to allow chromogenic visualisation of bacterial colonies. (a) Urethra model formed with Luria broth and bacteriological agar; *E. coli* colonies present inside channel but not visible. (b) Urethra model formed with Harlequin™ E. coli/Coliform agar, *E. coli* colonies visible as blue growth. (c) Test of various Harlequin™ E. coli/Coliform agar concentrations effect on growth of *E. coli*.

Originally, it was decided to use a combination of Luria broth and bacteriological agar to formulate the urethra model, whilst the model formed from this ratio was transparent and structurally sound, visually identifying the bacterial colonies was not possible as they grew an off white to straw colour, which was too similar to the surrounding agar to distinguish them (Figure 3.4). Different possible solutions were investigated such as staining the colonies, however this was impossible without also colouring the agar. The use of a fluorescent bacteria strain that could be visualised by confocal or fluorescent microscopy and quantified by spectroscopy was also investigated, however this would require specialist bacterial strains, analytical equipment, and expertise that not all labs possess consequently making the urethra model less user accessible.

Ultimately chromogenic agar was chosen as solution that would allow easier visualisation of bacterial colonies either by changing colour of the colonies or the agar itself in response to bacterial growth. The chromogenic agar chosen to grow *E. coli* was Harlequin™ E. coli/Coliform agar. Harlequin™ E. coli/Coliform agar is a selective chromogenic agar meaning it will only grow coliform bacteria and those that do grow are coloured due to the breakdown of either X-glucuronide or magenta-β-galactoside (Neogen, 2019; R. D. González *et al.,* 2003). In the case of *E. coli*, X-glucuronide is hydrolysed by β-D-glucuronidase which

creates a precipitate that causes the colonies to grow a dark blue or violet colour (R. D. González *et al.,* 2003).

With the contrast of the blue colonies of *E. coli* on the straw-coloured Harlequin™ E. coli/Coliform agar it is possible to visualise growth patterns in the *in vitro* urethra channel without the use of any specialist equipment or techniques. The use of chromogenic media allowed for the use of any bacterial strain within the model as long as the agar was changed to a chromogenic media that allowed visualisation of the desired species.

The use of chromogenic agar allowed the *in vitro* urethra model to exhibit changes in microbial distribution in a manner that is highly visual and accessible to a wide range of people which is important when explaining results to those outside of the field of microbiology *i.e.,* patients, clinicians, engineers, *etc.*

Temporal Contamination

The containers chosen to form the urethral model mould purchased during the first stage of development were intended to be reusable for multiple tests. This was not possible as temporal contamination occurred, *i.e.,* the containers became more contaminated with bacteria over each subsequent use regardless of sterilisation or cleaning method employed thus in the latter stages of experimentation the containers were used once and discarded (Figure 3.5 and Figure 3.6).

Figure 3.5 Early *in vitro* urethra model with evidence of extensive temporal contamination. Blue colour, highlighted by the arrows, is due to *E. coli* growth on chromogenic Harlequin™ E. coli/Coliform agar and is evident throughout the entirety of the model.

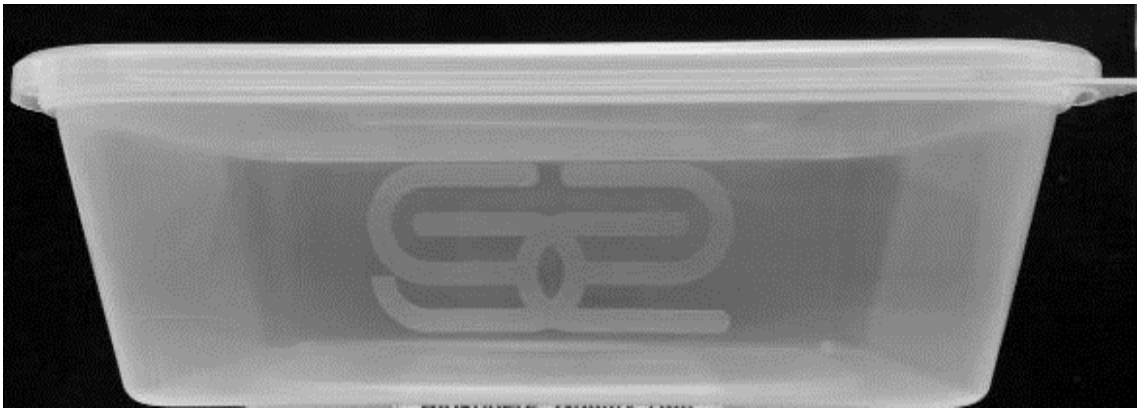


Figure 3.6 Disposable polypropylene containers used to create the *in vitro* urethra model mould.

The initial containers chosen to form the mould of the *in vitro* urethra model were intended to be reused to reduce cost and plastic waste. The containers were drilled, tubing installed, agar filled, tubing removed, used for testing, and finally cleaned/sterilised before being used for testing again. To clean the containers, they were first decontaminated by autoclave sterilisation at 121°C for 15 minutes. They were then soaked overnight in Virkon™ before being rinsed and thoroughly dried in a 60°C oven and finally autoclaved again in preparation for future testing.

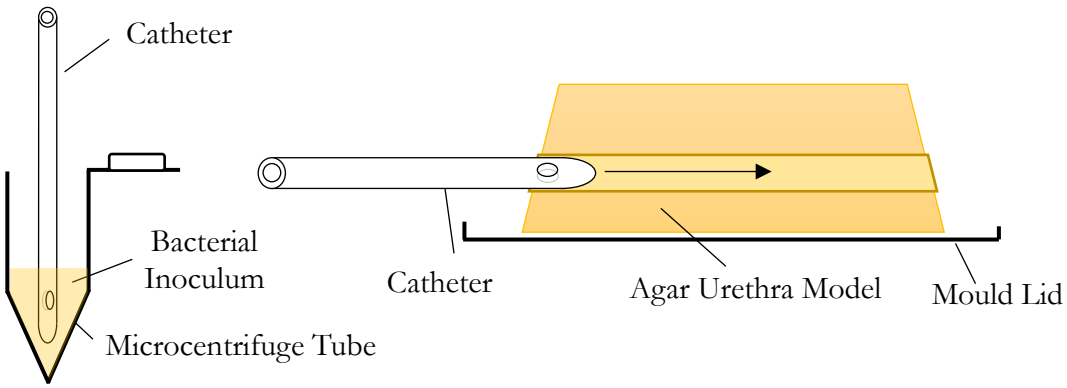
Despite meticulous cleaning, the containers became contaminated over time, which was evident with increased incidence of contamination over several weeks until all urethra models were extensively contaminated. The problem of temporal contamination was furthermore highlighted by the use of the chromogenic agar that tinted the entirety of the model blue with *E. coli* flourishing on all surfaces of the model (Figure 3.5). Although it should not be possible for *E. coli* to survive both steam sterilisation and chemical disinfections there is reported cases of *E. coli* strains becoming resistant to both disinfectants and temperatures as high as those found in autoclaves when grown under environmental stress (Markova *et al.,* 2010; El-Naggar *et al.,* 2001).

To remove the source of contamination, the containers used to form the *in vitro* urethra model were discarded after each use (Figure 3.6). While there is a greater financial cost and environmental impact to using single use containers, this was necessary to reduce the risk of wasting materials and time on tests that are invalid due to recurring contaminants.

3.4 Stage 3: Change in Inoculation Method

In the initial draft of the methodology for the *in vitro* urethra model, the catheter to be tested was inoculated by dipping the catheter tip into a bacterial inoculum (Figure 3.7 a). This was changed as it was deemed too variable. A new method was developed wherein a sterile swab was dipped into the inoculum and the model itself was inoculated (Figure 3.7 b).

(a) Old Inoculation Method



(b) New Inoculation Method

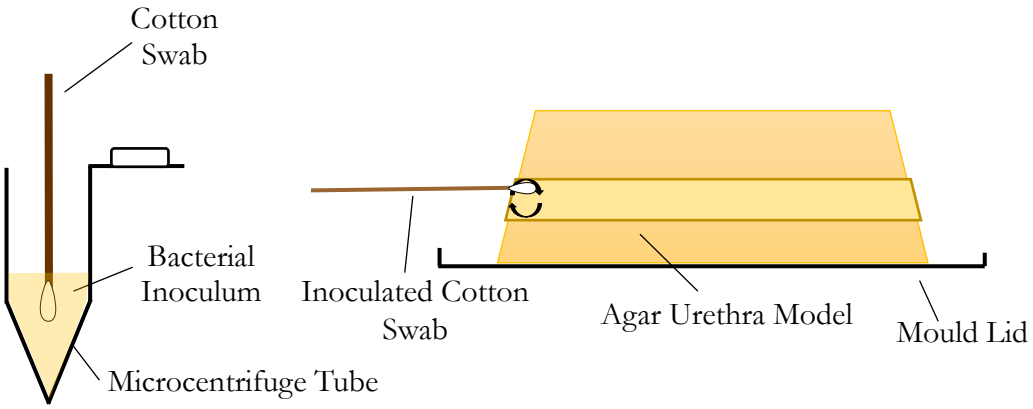


Figure 3.7 Illustration of the inoculation methodology of the *in vitro* urethra model: (a) original inoculation method, (b) the new inoculation method.

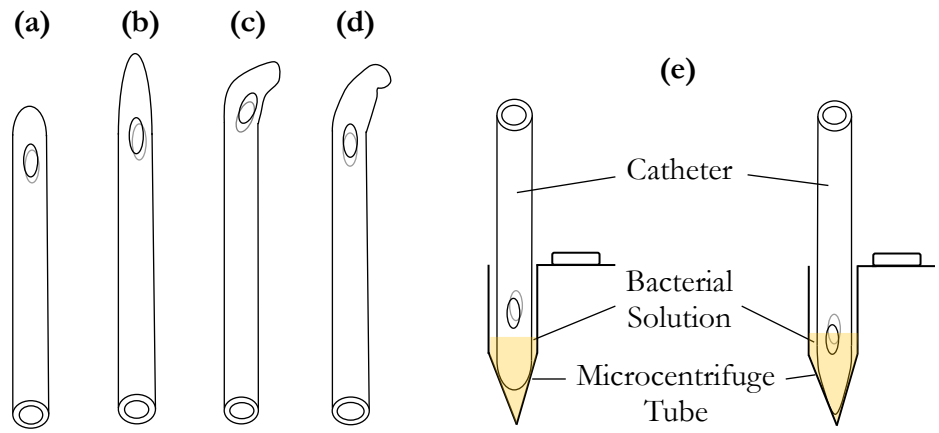


Figure 3.8 Illustration of different intermittent catheter tip designs and inoculation of a test catheter tip. (a) Rounded tip, (b) Tapered tip, (c) Coudé tip, (d) Coudé Olive tip, (e) comparison of the inoculation of a rounded tip *vs.* a tapered tip.

Inoculation of the catheter itself presented two main problems. Firstly, different catheters have varying eyelet heights, which allowed the inoculum to get trapped in the tip if the eyelets were lower than the liquid level. This introduced varying volumes of bacteria into the *in vitro* urethra channel (Figure 3.8 a-d). Secondly, the other problem was the differing shape of various catheter tips, *i.e.,* when the inoculum was contained in a microcentrifuge tube, a catheter with a tapered tip would dip lower into the inoculum when compared to a catheter with a rounded tip (Figure 3.8 e). Changing the inoculation method to using a sterile swab and inoculating the urethra model itself, reduced variation in the bacterial densities observed between replicates and improved reproducibility, as per the method outlined in section 2.4.1 (Figure 2.2).

3.5 Stage 4: Condensation, Bacterial Swarming, and Model Inversion

Initially condensation was an extensive issue with the model as a wet agar surface allowed for bacterial swarming in non-swarming species (Damton *et al.,* 2010). The condensation mediated swarming was identified by the presence of coloured growth outside of the urethra channel of the chromogenic model and poor colony formation (Figure 3.9 a, b). Several attempts were made to control or eliminate the condensation including drying in a biosafety cabinet, slow cooling of the agar, replacing the aluminium foil wrapping with air permeable sterilisation pouches, and warming the urethra moulds at 37°C before testing. Ultimately condensation was controlled by a combination of drying in the biosafety cabinet, the use of air permeable sterilisation pouches, and most importantly inversion of the model (Figure 3.9 c, d and Figure 3.10).

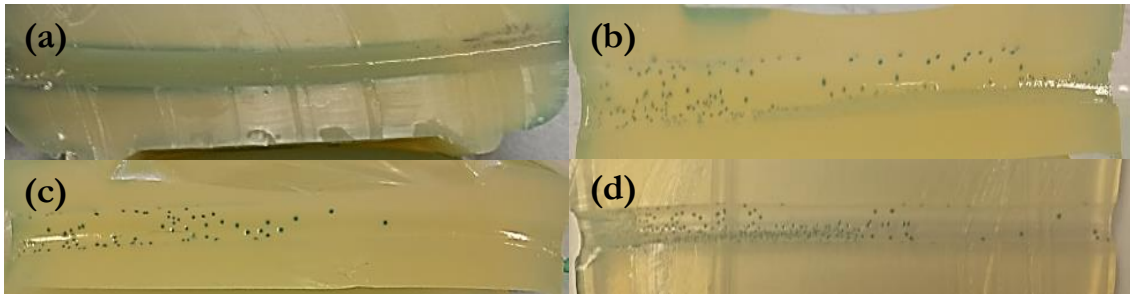


Figure 3.9 Comparison of urethra models before and after inversion. Before inversion: growth outside of the channel can be observed by the change of colour I the surround agar from a straw colour to blue and/or no defined colonies visible (a, b). After inversion: colonies are defined, and no growth observed outside of the *in vitro* urethra channel (c, d).

Condensation was the major issue with the *in vitro* urethra friction model, many solutions were tested but ultimately it took approximately 6 months to solve this problem. The final agar formulation consisted of 7.32g L^{-1} of Harlequin™ medium and 12g L^{-1} Bacteriological agar meaning that the formed agar models were ≥98% water. This high-water content led to significant condensation within the model containers and on all agar surfaces. The primary reason that this presented a major issue was that on a wet agar surface bacterial species such as *E. coli* become increasingly motile and can exhibit swarming motility (Damton *et al.,* 2010; Ren *et al.,* 2001). Before the condensation was resolved, the bacteria were found to be moving out of the urethra channel and swarming along the outside surfaces of the channel which was highlighted by the use of chromogenic agar as the entire model would be tinted blue as *E. coli* grew in a thin film along the outside of the entire model (Figure 3.5 and Figure 3.9). In the worst cases of condensation, discrete colonies didn't form in the channel at all (Figure 3.9 a).

Numerous different approaches were taken in an attempt to control the condensation with varying levels of success. The first method tried to control the condensation was to pour the urethra models in a biosafety cabinet so that they could be allowed to set with the lids on. As the lids of the urethra models collected the most condensation much like a petri dish, extra container lids were autoclaved separately and once the agar had cooled the lid of the container was swapped with a new dry one. When this proved ineffective, the models were allowed to cool in the laminar flow with the lids off entirely to allow the steam to escape. The moulds were also left in the laminar flow overnight after setting to allow the exposed agar surfaces to air dry. Formerly the models were left to dry in the biosafety cabinet under a UV light to prevent possible contamination of the exposed agar however after sterility control plates left open in the cabinet showed no signs of contamination in the absence of UV irradiation, the UV light was no longer deemed necessary. The overnight drying time was also deemed too

long and eliminated as the agar would often desiccate and crack. The method of open cooling in the laminar flow until the agar solidified, was continued as it eliminated the condensation formed on the lid and sides of the model mould while the molten agar set and dried any exposed agar surfaces, this however was not fully effective on its own.

Originally, the moulds for the urethra models were autoclaved covered in aluminium foil to preserve their sterility, once removed from the autoclave. The aluminium foil was found to retain moisture and was later replaced with air permeable sterilisation pouches. These pouches were suitable for autoclave sterilisation and allowed moisture to evaporate from the model mould whilst remaining self-contained and aseptic. The air permeable pouches, while an improvement to the original model mould production, did not eliminate the problem of condensation on their own.

At one stage the use of a clay desiccant was trialled, in that individual reusable montmorillonite clay desiccant pouches were place inside the aluminium foil but outside of the model moulds. The desiccant was not effective and although other stronger desiccants were researched, the idea was ultimately dropped as the desiccants would require constant maintenance and were a potential source of contamination if in contact with the model moulds.

Slow cooling was also trialled. It was thought that if the agar was allowed to cool slowly, it would reduce the condensation formed. The model moulds were heated to 60°C and the agar was poured at 60°C, then the models-in-mould were left in the 60°C oven for 1 hour, the models were then transferred each hour to a lower temperature *i.e.,* 50°C, 40°C, 30°C. Once the models were at 20°C they would be used for testing. This slow cooling method was not effective, nor was the idea to keep the models at 37°C before testing, regardless of configuration or temperature, the condensation was not prevented.

Ultimately to solve the condensation and swarming problem, the entire urethra model and mould had to be flipped upside down (Figure 3.10). The original idea was that by flipping the model mould a greater surface area of the model would be exposed to the air allowing improved drying of the agar surfaces. Additionally, the drill holes used to form the channels now became ventilation holes in the mould to allow any leftover excess moisture to escape. Flipping the model/mould, also solved a problem that hadn't yet been considered. By flipping the model, the sides *i.e.,* the inoculation sites, were no longer in direct contact with the plastic. A study by Williams & Stickler, (2008b), which used the Gaonkar *et al.,* (2003) model discussed earlier in section 1.10.2, demonstrated bacteria that normally don't swarm like *E. coli* can move

along small gaps between surfaces especially in the presence of moisture. They used the Gaonkar *et al.,* (2003) model but substituted the TSA in the model with a chromogenic agar which highlighted that the bacteria were not only travelling to the bladder from the meatus via the catheter, but also between the agar's edge and the glass tube encasing it. Williams & Stickler, (2008b) found that when they didn't include liquid in the bladder portion of the model, the swarming ceased. They believed, this was due to the moisture allowing a film to form between the glass tube and agar channel that the bacteria could use for transport.

Regarding the *in vitro* urethra friction model, it is possible this issue was the same problem involved in the swarming observed. With the agar dry and no longer in contact with the plastic container sides, the problem of the bacteria growing outside of the channels was eradicated. The solution to condensation and bacterial swarming was the combined effect of encasing the moulds in air permeable pouches, drying the models in a biosafety cabinet, and most importantly inversion of the models.

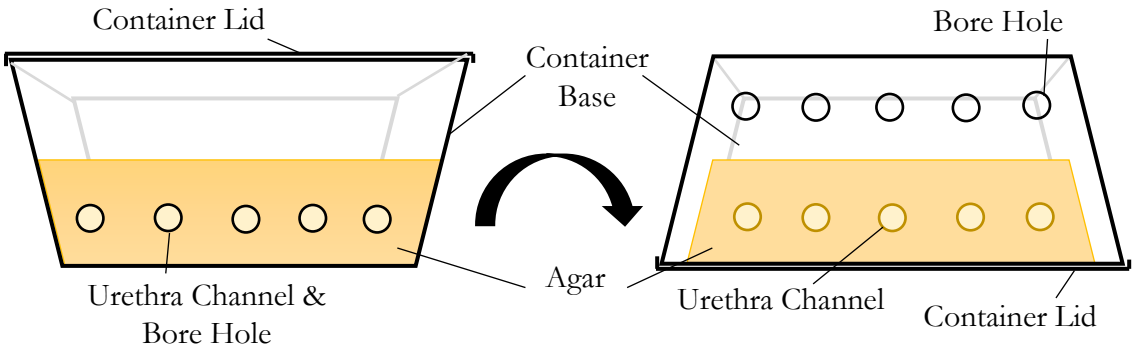


Figure 3.10 Illustration of the inversion of the *In vitro* Urethra Friction Model.

3.6 Stage 5: Multiple Channels and Model Quantification

Multiple Channels

Originally the urethra model contained one formed urethra channel. As development progressed, the number of channels in the model increased to three so that each mould could contain triplicate replication and to reduce on the overall materials needed *i.e.,* less agar and containers needed to produce fewer models (Figure 3.11). The increase to three channels was only possible once the condensation problem was solved.

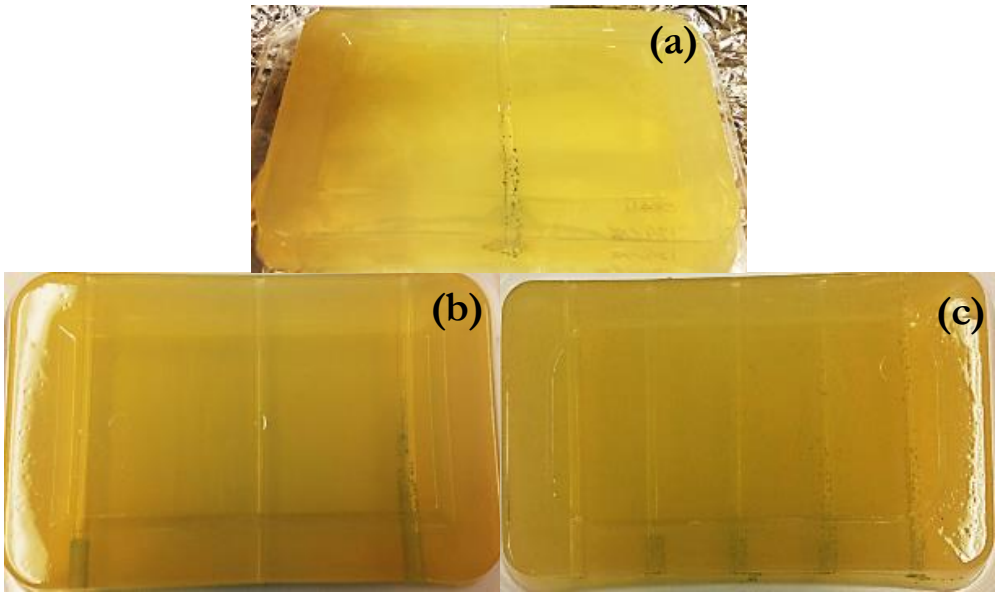


Figure 3.11 Comparison of a single channel (a), triple channel (b) and quintuple channel (c) *in vitro* urethra model.

In the initial testing stages, each control and catheter type were tested in triplicate during each independent test. It was later decided instead of doing triplicate replication that the focus should be on multiple independent tests. This decision led to the formation of the five channel model in the final model design (Figure 3.11). Having five urethra channels per model allowed for the testing of a sterility control, growth control, and three different test catheters per model/independent test. By focusing on multiple contained independent tests, the true reproducibility of the model could be better determined than with the use of replicates alone (Vaux *et al.,* 2012). Each five-channel model represented a single independent test.

Quantification

During the initial development of the methodology to quantify the *in vitro* urethra fiction model, the bacterial density on the catheter surface after insertion/removal was the focus due to influence of the paper by Hudson and Murahata, (2005) as discussed in section 1.10.2. This methodology was later discarded. The focus then shifted to specifically the changes in bacterial density throughout urethra channel caused by catheter insertion.

The methodology to quantify the bacteria within the urethra channel first focused on four data collection points, with the urethra channel being dissected into four quadrants and the bacteria within each quadrant recovered. The bacteria were recovered by sonication of the channel quadrant in TSB to dislodge the bacteria from the agar surface. The quadrant in TSB was then vortexed to disperse the bacteria evenly in the growth medium. The bacterial suspension was then serially diluted and enumerated by drop count to determine the CFU ml^{-1} present at that point in the channel. This quantification approach was found to be successful, and the data produced can be seen in Figure 3.12.

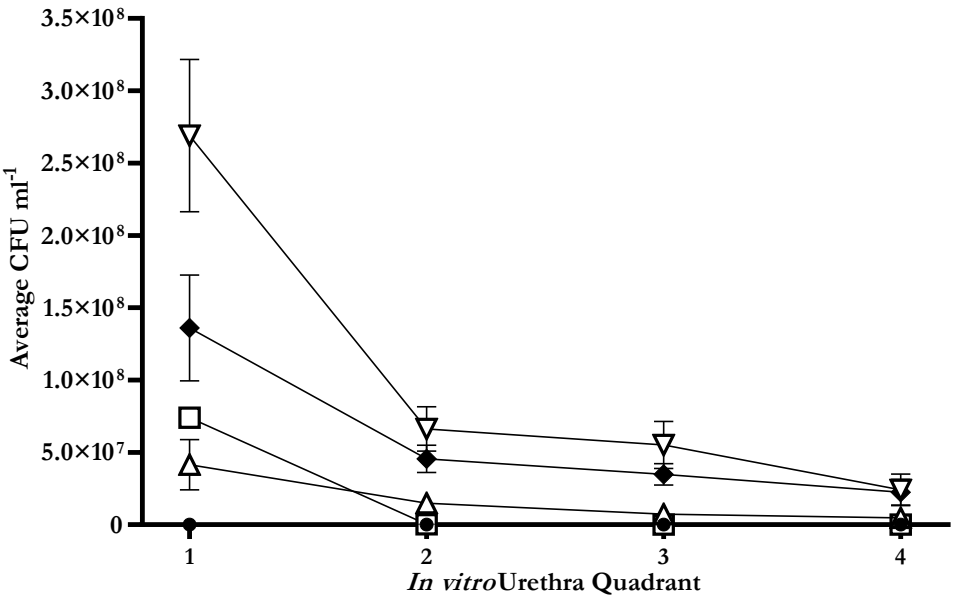


Figure 3.12 Enumeration of the average *E. coli* ATCC 25922 Colony Forming Units per millilitre (CFU ml^{-1}) recovered along the *in vitro* urethra channel using quadrants; sterility control (●), growth control (□), uncoated catheter (IC UnC, ▽), prototype catheter (△, IC Proto), hydrophilic coated (◆, IC Hydro). Error bars represent standard error of the mean, n=6.

Once the methodology was found to be reproducible, it was decided to further dissect the urethra channel into eight segments or octants to double the number of data points and provide a better representation of the changes in bacterial density along the *in vitro* urethra channel (Figure 3.13). Eight data collection points also allowed the stopping point of the bacterial movement in the channel to be better identified.

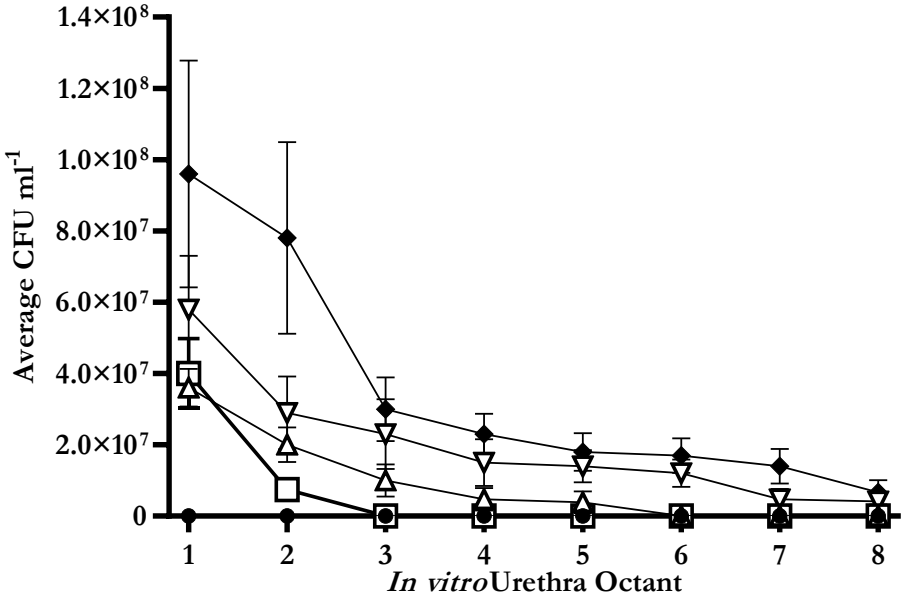


Figure 3.13 Enumeration of the average *E. coli* ATCC 25922 Colony Forming Units per millilitre (CFU ml^{-1}) recovered along the *in vitro* urethra channel using octants; sterility control (●), growth control (□), uncoated catheter (IC UnC, ▽), prototype catheter (△, IC Proto), hydrophilic coated (◆, IC Hydro). Error bars represent standard error of the mean, n=6.

3.7 Stage 6: Reducing Variability

At this stage, results obtained from the *in vitro* urethra friction model were still presenting significant variability, especially when working with a prototype catheter developed by the projects industry co-funder. After much consideration, the problem was traced back to the width of the urethra channel. Up until this point the channel had been slightly wider than the catheter to be inserted *i.e.,* a 5 mm Ø channel for a 4.667 mm Ø catheter. The slightly wider channel did not allow complete surface contact between the catheter and the internal surface of the agar channel, as such the movement of the bacteria in response to catheter-mediated friction was inconsistent. This inconsistent movement of the bacteria can be seen in Figure 3.14 (a), where the bacterial growth in the channel has a large, skipped area between two areas of dense bacterial growth. It was assumed that gap in growth observed was the result of incomplete contact between the catheter and the internal surfaces of the *in vitro* urethra. These

inconsistencies with bacterial growth led to variability in quantifying the bacterial densities throughout the channel and as such undermined the reproducibility of the entire model.

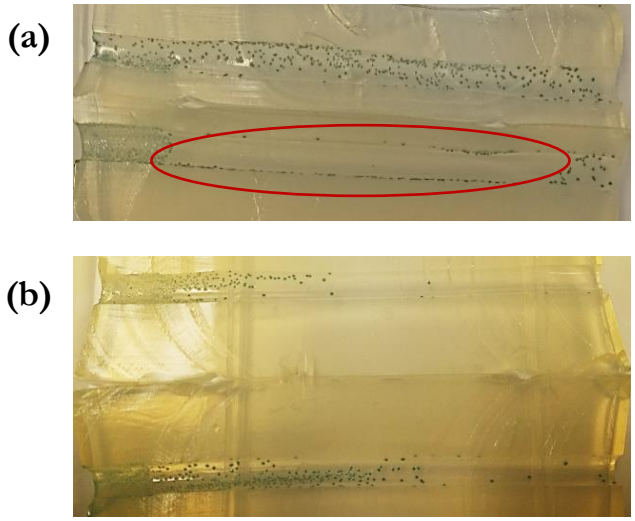


Figure 3.14 Comparison of the use of an agar channel wider in diameter than the test catheter (a) and the use of a channel of the same diameter as the test catheter (b). The zone encircled in red highlights an area with which the catheter was not in direct contact with the internal surface of the *in vitro* urethra channel.

The wider channel use was an artefact of the original gelatine model developed by a Teleflex® engineer. They used a wider channel to prevent the gelatine from splitting due to the insertion of the catheter. This is a phenomenon also experienced in the agar model however, after experimentation with varying channel widths, it was found that the channel can be the same width as the catheter. If the catheter was inserted carefully the agar did not split. Having the channel, the same width as the catheter to be inserted ensured that the catheter was in complete surface contact with the inner channel walls and allowed consistent friction-mediated movement of bacteria along the channel, (Figure 3.14 b).

3.8 Stage 7: Urethra Model Validation and Statistical Analysis

To validate the model and associated methodology, twelve independent tests were carried out, six with *E. coli* ATCC 25922 and six with *S. aureus* NCTC 12981. Each independent test was performed in a five channel urethra model containing a channel each for a bacterial growth control, sterility control, and three test channels (Table 3.2, Table 3.3). The results of the 12 independent tests are shown in Figure 3.17 and Figure 3.18, with selected representative photographs of bisected *in vitro* urethra channels, graphs displaying the average CFU ml^{-1}

recovered from each urethra channel octant, and bar charts of the average total CFU ml^{-1} recovered from the full length of the *in vitro* urethra.

Table 3.2 Average Colony Forming Units ml^{-1} of *E. coli* isolated in each octant of the *in vitro* urethra Friction model channel, n = 6.

Octant	1	2	3	4	5	6	7	8
Sterility Control	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Growth Control	6.5E+08	1.5E+08	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
IC Proto	4.8E+08	2.7E+08	5.2E+07	2.7E+07	1.5E+06	2.5E+05	2.0E+05	0.0E+00
IC Hydro	4.4E+08	3.1E+08	2.9E+08	2.1E+08	1.4E+08	1.2E+08	1.0E+08	7.4E+07
IC UnC	4.8E+08	3.8E+08	2.9E+08	2.7E+08	2.2E+08	1.2E+08	6.5E+07	4.2E+07

Table 3.3 Average Colony Forming Units ml^{-1} of *S. aureus* isolated in each octant of the *in vitro* urethra Friction model channel, n = 6.

Octant	1	2	3	4	5	6	7	8
Sterility Control	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
Growth Control	4.9E+07	1.5E+07	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00	0.0E+00
IC Proto	3.5E+07	2.7E+07	9.5E+06	3.7E+06	3.2E+06	1.3E+06	0.0E+00	0.0E+00
IC Hydro	3.7E+07	2.6E+07	1.9E+07	2.5E+07	9.5E+06	7.5E+06	4.2E+06	4.3E+06
IC UnC	4.5E+07	2.6E+07	2.2E+07	1.8E+07	1.0E+07	5.6E+06	8.5E+05	5.5E+06

To compare the different test catheter types to the bacterial growth control and assess their efficiency in preventing friction-mediated bacterial displacement, initially the data was analysed by comparing the CFU ml^{-1} in each octant between each test catheter and the bacterial growth control. This entailed first assessing the distribution of the data through the use of the Anderson-Darling Normality test (Figure 3.15). The Anderson-Darling test clarified that the data was not normally distributed ($P \leq 0.005$, for all data sets) thus to determine the reproducibility of the *in vitro* urethra Friction model and testing methodology the Kruskal-Wallis test was used, the results of which can be seen in Table 3.4. To compare the test catheter samples to the growth control, the nonparametric Mann Whitney U test was used, the results of which are outlined in Table 3.5.

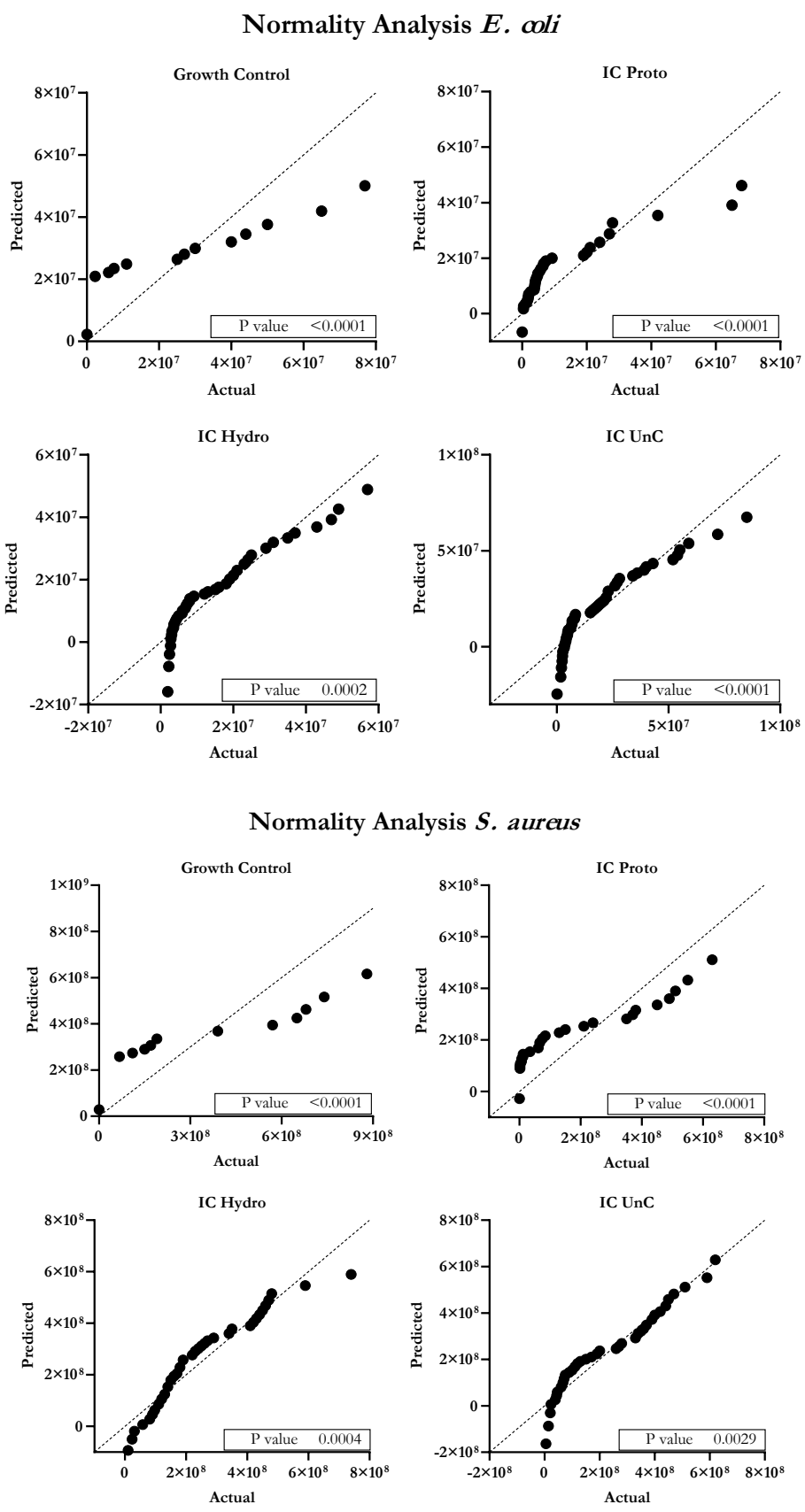


Figure 3.15 Distribution of bacteria recovered from octants within the *in vitro* urethra friction model for *E. coli* and *S. aureus* determined by the Anderson darling test, n = 48.

Table 3.4 Analysis of variance in reproducibility of the *in vitro* urethra Friction model *via* the Kruskal-Wallis test. n = 48.

	E. coli	***S. aureus***
Sterility Control	*	*
Growth Control	$P \geq 0.9999$	$P \geq 0.9999$
IC Proto	$P = 0.8684$	$P = 0.8338$
IC UnC	$P = 0.5691$	$P = 0.6619$
IC Hydro	$P = 0.2972$	$P = 0.2222$

*Results for the sterility control could not be statistically analysed as all colony counts were zero

Table 3.5 Null hypothesis testing by use of the Mann-Whitney U Test to compare the changes of bacterial distribution in the *in vitro* urethra octant due to catheter insertion, n = 48.

	E. coli	***S. aureus***
Positive Control *v.* IC Proto	$P = 0.002$	$P = 0.064$
Positive Control *v.* IC UnC	$P \leq 0.001$	$P \leq 0.001$
Positive Control *v.* IC Hydro	$P \leq 0.001$	$P \leq 0.001$
IC Proto *v.* IC UnC	$P \leq 0.001$	$P \leq 0.001$
IC UnC *v.* IC Hydro	$P = 0.339$	$P = 0.750$
IC Hydro *v.* IC Proto	$P \leq 0.001$	$P \leq 0.001$

Table 3.6 Null hypothesis testing by use of an unpaired T-Test to compare the changes of bacterial density distribution in the entire *in vitro* urethra channel due to friction during catheter insertion, n = 6.

	E. coli	***S. aureus***
Positive Control *v.* IC Proto	$P = 0.422$	$P = 0.818$
Positive Control *v.* IC UnC	$P \leq 0.001$	$P \leq 0.001$
Positive Control *v.* IC Hydro	$P = 0.009$	$P \leq 0.002$
IC Proto *v.* IC UnC	$P = 0.001$	$P = 0.002$
IC UnC *v.* IC Hydro	$P = 0.127$	$P = 0.515$
IC Hydro *v.* IC Proto	$P \leq 0.041$	$P = 0.003$

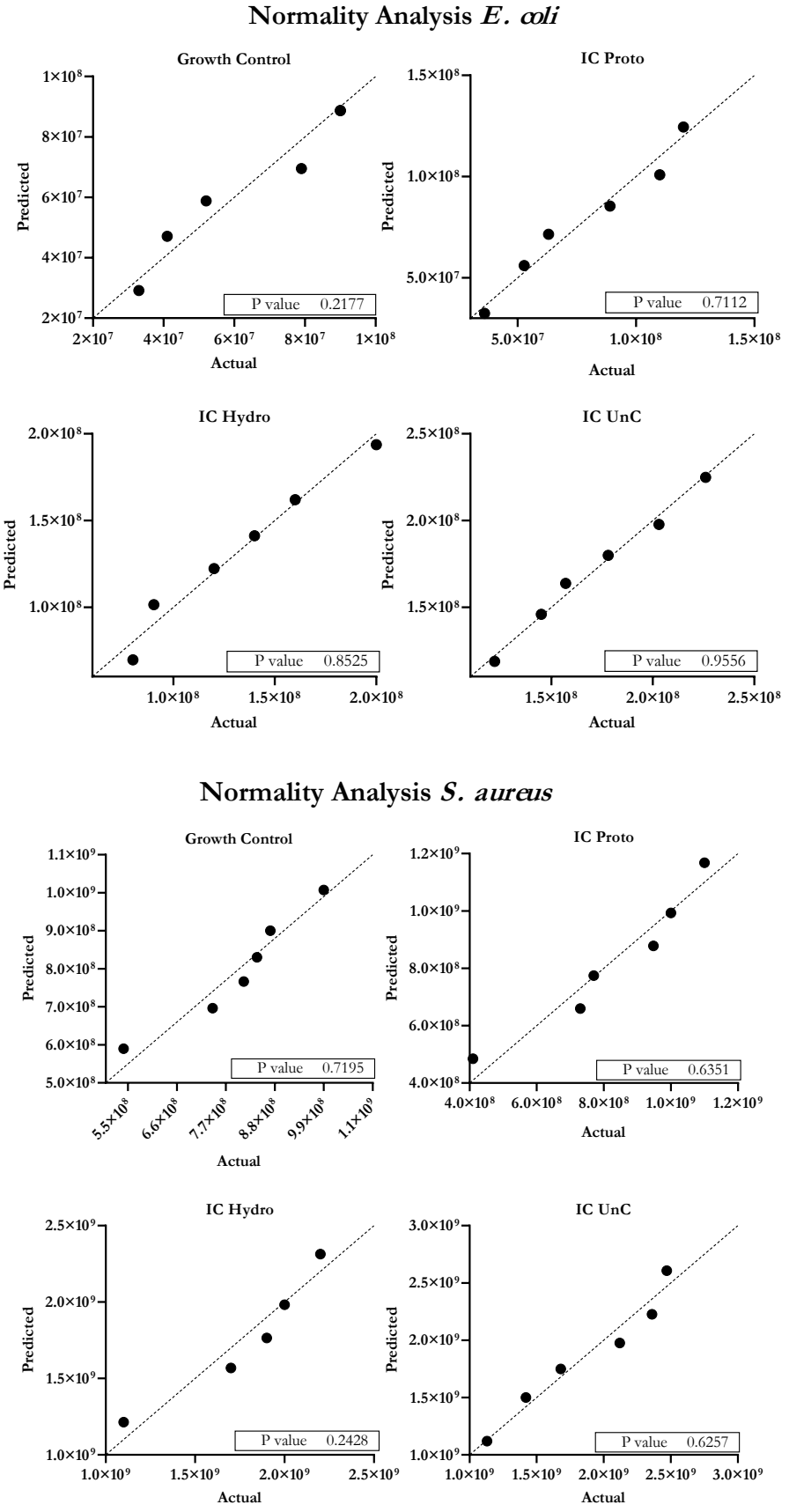


Figure 3.16 Distribution of total bacteria recovered from *in vitro* urethra friction model for *E. coli* and *S. aureus* determined by the Shapiro-Wilk test, n = 6.

As the results of the nonparametric tests, comparing the individual octants proved ambiguous between the bacterial strains tested, the data was re-analysed. Instead of comparing the individual octants between the growth control and test catheters, the total CFU ml^{-1} recovered from the entire *in vitro* urethra channel was assessed first for normality *via* the Shapiro-Wilk Test then compared using an unpaired T-Test, the results of which are outlined in Figure 3.16 and Table 3.6.

When comparing the results for both bacterial species tested in the 12 independent tests, no significant difference was found between replicates for each bacterial strain ($P \geq 0.2222$) indicating that the methodology and model was reproducible.

Utilising the *in vitro* urethra model, three intermittent catheter types were tested. The catheters used were an uncoated catheter (IC UnC), a hydrophilic coated catheter (IC Hydro), and a prototype catheter (IC Proto). Each catheter was inserted, as per the manufacturer's instructions, into an *in vitro* urethra channel that was inoculated on one side to act as a contaminated urethral meatus. It was found that all three types of catheters tested displaced the bacteria along the *in vitro* urethra channel as they were inserted when compared to the bacterial growth control, which was inoculated with bacteria, but no catheter was inserted. The IC UnC and IC Hydro catheters consistently displaced bacteria along the full length of the artificial urethra (~80 mm) and there was no significant difference in their performance when compared to each other with either *E. coli* ($P = 0.127$) or *S. aureus* ($P = 0.515$). Conversely, the IC Proto catheter did not displace bacteria the full way along the urethra channel with growth stopping at a median value of the 6^{th} octant with *E. coli* and the 4^{th} octant with *S. aureus* (Figure 3.17, Figure 3.18).

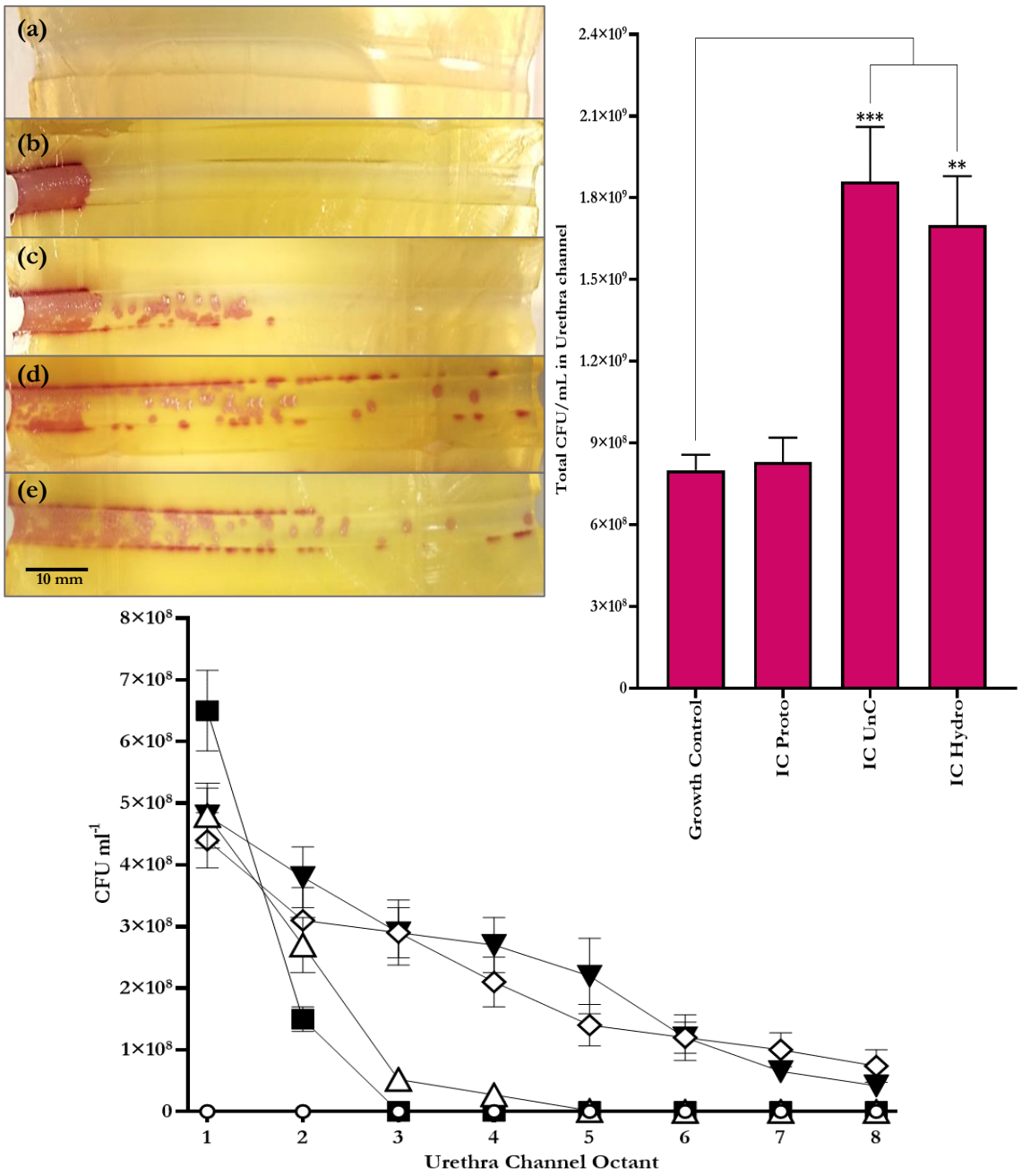


Figure 3.17 Selection of bisected *in vitro* urethra channels, a scatter plot, and bar chart displaying the average CFU ml^{-1} of bacteria recovered from each *in vitro* urethral octant and the total bacteria isolated from the entire urethra channel. Octant 1 and part of 2 represented the site of inoculation. The photographs above display a representative example of urethra channels used for the testing of the sterility control (a, ○), bacterial growth control (b, ●), prototype catheter (c, ▽, IC Proto), uncoated catheter (d, △, IC UnC), and hydrophilic catheter (e, ■, IC Hydro). Bacterial colonies are *S. aureus* NCTC 12981 grown on CHROMagar™ Staph aureus agar. n = 6, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, bars represent standard error of the mean.

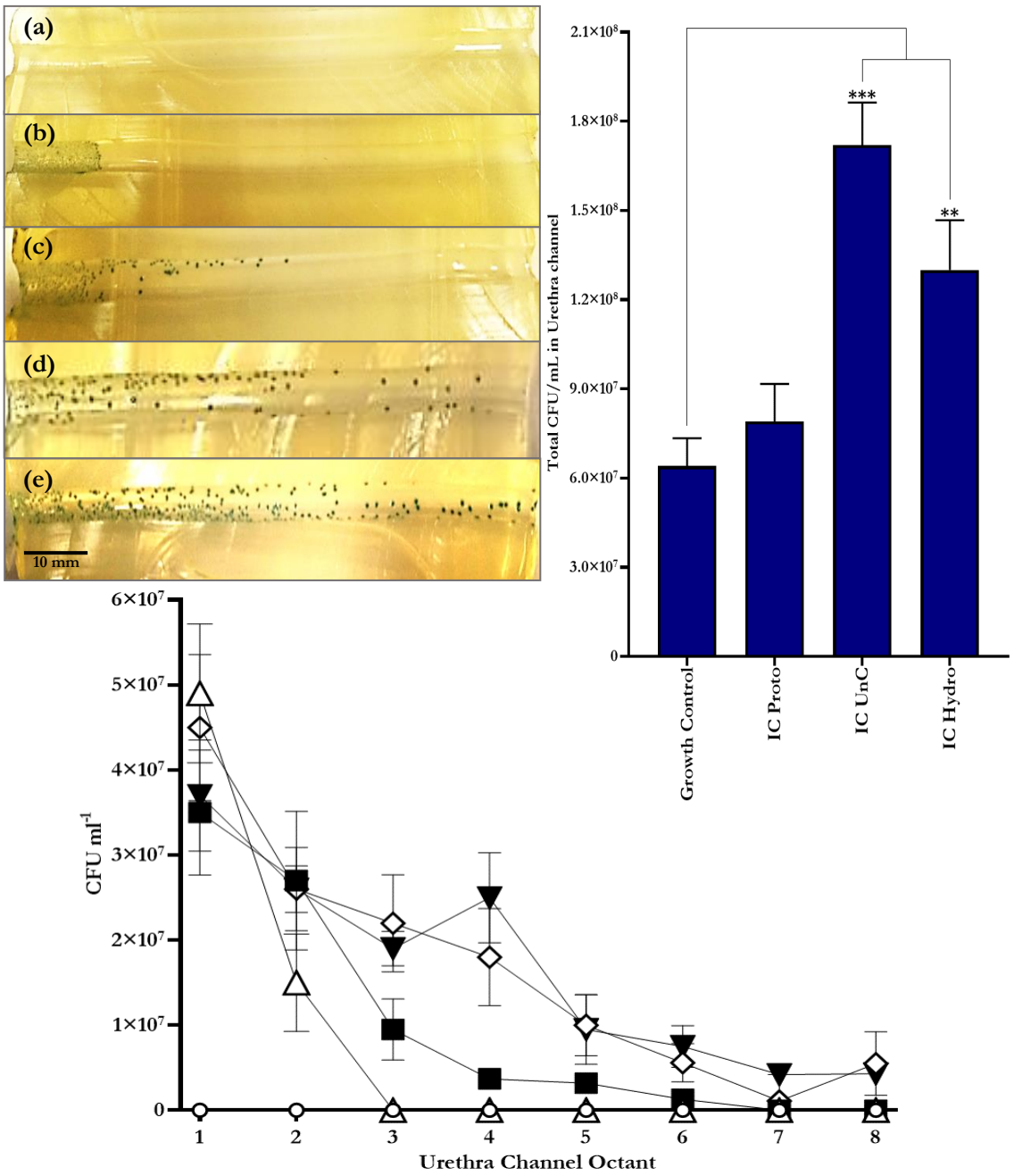


Figure 3.18 Selection of bisected *in vitro* urethra channels, a scatter plot, and bar chart displaying the average CFU ml^{-1} of bacteria recovered from each *in vitro* urethral octant and the total bacteria isolated from the entire urethra channel. Octant 1 and part of 2 represented the site of inoculation. The photographs above display a representative example of urethra channels used for the testing of the sterility control (a, ○), bacterial growth control (b, ●), prototype catheter (c, ▽, IC Proto), uncoated catheter (d, △, IC UnC), and hydrophilic catheter (e, ■, IC Hydro). Blue colonies shown are *E. coli* ATCC 25922 grown on Harlequin™ agar. n = 6, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, bars represent standard error of the mean.

When comparing the catheter samples to the bacterial growth control, with either bacterial species, the IC UnC and IC Hydro catheters were consistently found to significantly displace bacteria further along the length of the artificial urethra when compared to the bacterial growth control ($P \leq 0.042$). Regarding the IC Proto catheters, there was no significant difference found from the bacterial growth control with either bacteria strain ($P \geq 0.423$). There is a marginal increase in the average bacteria recovered from the entire *in vitro* urethra channel with *E. coli* when compared to *S. aureus* after insertion of the IC Proto catheter. The active motility of *E. coli* versus the passive motility of *S. aureus* may be responsible for this increase in bacterial growth in the *in vitro* urethra channel (Terlizzi *et al.,* 2017; Pollitt *et al.,* 2015; Kearns, 2010; Kaito & Sekimizu, 2007; Lane *et al.,* 2005). This difference in motility may also be responsible for the larger standard error seen in the colony counts from the *E. coli* tests as the higher motility may have made the strain less predictable in its displacement in the artificial urethra channel (Figure 3.18). Fluctuation can also be seen in the dispersion of bacterial growth along the *in vitro* urethra channel with *E. coli* (Figure 3.18). This phenomenon can be seen most clearly with the IC Hydro catheter which has a noticeable increase in average bacterial concentration when moving from octant 3 to 4 and then reducing again in octant 5. This fluctuation in bacterial concentration can also be due to the increased moisture introduced into the artificial urethra by the hydrophilic coating, increasing the motility of *E. coli,* as the strain can move quickly in the presence of moisture (A. J. Mitchell & Wimpenny, 1997).

The results clearly indicate that the insertion of a urinary catheter can displace pathogenic microorganisms from the meatus further into the urethra. In the context of the model presented, the channel in the *in vitro* urethra model is ~80 mm length. This is twice the length of the average female urethra which is typically 40 mm; with this in mind each catheter type tested in this study would have introduced bacteria into the female bladder (Feneley *et al.,* 2015). To ascertain the same conclusion for the male urinary tract, a much longer artificial urethra channel would be required to represent the 160 mm length of the average male urethra as well as a number of anatomical differences due to the inconsistent width of the male urethra (Goetz *et al.,* 2018; Feneley *et al.,* 2015).

3.9 Stage 9: Dissemination

The *in vitro* urethra friction model was published in an original article in the Journal of Applied Microbiology and is cited as: Cortese, Y. J., Wagner, V. E., Tierney, M., Scully, D., Devine, D. M., & Fogarty, A. (2020). Pathogen displacement during intermittent catheter insertion: a novel *in vitro* urethra model. Journal of Applied Microbiology, 128(4), 1191–1200. https://doi.org/10.1111/jam.14533.

The development stages and validation of the *in vitro* urethra model was also disseminated in an oral presentations at the Bioengineering in Ireland (BINI) 2018 and 2019 conferences, as well as several meetings of the ENIUS Cost action group. Different posters were presented at the SURE 2018 conference, the Athlone Institute of Technology (AIT) Research Days 2018/2019/2020, and the Federation of Microbiological Societies (FEMS) 8th congress of European microbiologists 2019.

3.10 Conclusion

The primary aim of developing the *in vitro* urethra friction model was to test intermittent catheters and demonstrate that microorganisms from the urethral meatus are displaced during catheter insertion due to friction between the extraluminal catheter surface and the urethra channel walls. Intermittent catheters are often recommended as an alternative to indwelling catheters as users experience less severe complications and lower occurrence of CAUTIs, however there has been limited research and innovation in regards to intermittent catheters especially when compared to the numerous studies focused on indwelling catheters (Goetz *et al.,* 2018). Medical device research and development can be complex and costly, thus providing an *in vitro* model can provide industry and researchers a new tool to spur on innovation to better prevent CAUTIs and improve patient experiences.

The results presented in this study demonstrate that movement of bacteria from a contaminated meatus into the urinary tract is likely due to the insertion of urinary catheters. The principle that meatal contamination acts as a source of pathogens that can lead to CAUTI development has been previously investigated with indwelling catheters, however the friction-mediated movement of pathogens in the urethra during insertion of urinary catheters has not been previously studied (Barford *et al.,* 2008; Schaeffer & Chmiel, 1983; Garibaldi *et al.,* 1980; Kaye *et al.,* 1962). It has been proposed in the past by Kaye *et al.,* (1962) and Barford *et al.,* (2008) that a CAUTI can be initiated by a single catheterisation. This study further supports

this theory, demonstrating that the simple act of inserting a urinary catheter can undermine attempts by either the catheter's design or insertion practice to prevent CAUTIs. In conclusion this model and methodology provide a useful tool for research and innovations in the field of urinary catheterisation and particularly novel intermittent catheter design.

Chapter 4 *In vitro* Urethra Diffusion Model

4.1 Introduction

Models and methods to investigate the diffusion of antimicrobial agents have existed since the 1920s when the first method to analyse antibiotic diffusion was described by Fleming, (1929). Sometime after, the standardised method of antimicrobial disc diffusion, still used today and considered a gold standard in antimicrobial testing, was introduced by Bauer *et al.,* (1966). The antimicrobial disc diffusion assay, colloquially known as the Kirby-Bauer disc diffusion assay, is routinely used in research and clinical settings to screen microorganisms for antimicrobial susceptibility (Z. A. Khan *et al.,* 2019).

The disc diffusion assay is technically simplistic with antimicrobial impregnated discs applied to an inoculated agar surface before incubation. After incubation, if the potential antimicrobial inhibits microbial growth, a clear zone of no growth is observed around the discs and the diameter measured to ascertain the "zone of inhibition" (Z. A. Khan *et al.,* 2019). Regarding urinary catheters, the disc diffusion assay has been modified in several ways to assess the efficacy of antimicrobial materials or coatings used to prevent CAUTIs. The well diffusion assay is one adaptation where instead of an antimicrobial disc, wells are cut into the agar surface for the placement of catheter segments. Zones of inhibition are then measured around the catheters segments (Divya *et al.,* 2019). Alternatively a study by Yassin *et al.,* (2019) placed catheter segments directly on the agar surface with the outer antimicrobial coating in contact with the agar creating an oblong zone of inhibition around the ~10 mm long pieces.

Whilst all of these methods are effective and validated techniques, the major drawback is that they provide limited data in a single dimension around the disc or sample. When in use catheters are in a three-dimensional environment and as such it is desirable to know the diffusion of antimicrobial coatings not only in the diameter around them but in all directions. With this limitation in mind, the aim of developing the *In vitro* urethra diffusion model was to provide a method to observe the diffusion of antimicrobial coatings or materials in three dimensions.

4.2 Stage 1: Conceptualisation

The *in vitro* urethra diffusion model builds on the knowledge obtained from the *in vitro* urethra friction model described in Chapter 3. With the basic structure of the *in vitro* urethra model mould already developed, the focus for the diffusion model development was on adapting the testing methodology of the existing model to test antimicrobial coated urinary catheters.

Teleflex®, industry partners of this project, in the past developed an antimicrobial coating containing chlorhexidine for indwelling catheters. This coating was found to be too expensive when coating the entire catheter and ultimately it was unmarketable. To address this, they devised a possible solution in coating the catheter in discrete bands of the coating to reduce overall cost. It was then necessary to determine if the coating would be as effective as separate bands when compared to a fully coated catheter to prevent bacterial migration is discussed later in Chapter 5.

During the design of the banded catheter another consideration was how far apart the antimicrobial bands could be whist still providing consistent protection from bacterial migration. To answer this question the diffusion model was developed. The original concept hinged on the idea that, within the *in vitro* urethra channel, if the zones of inhibition around the antimicrobial bands overlapped then the protection along the catheters would be sufficient to prevent bacterial migration (Figure 4.1).

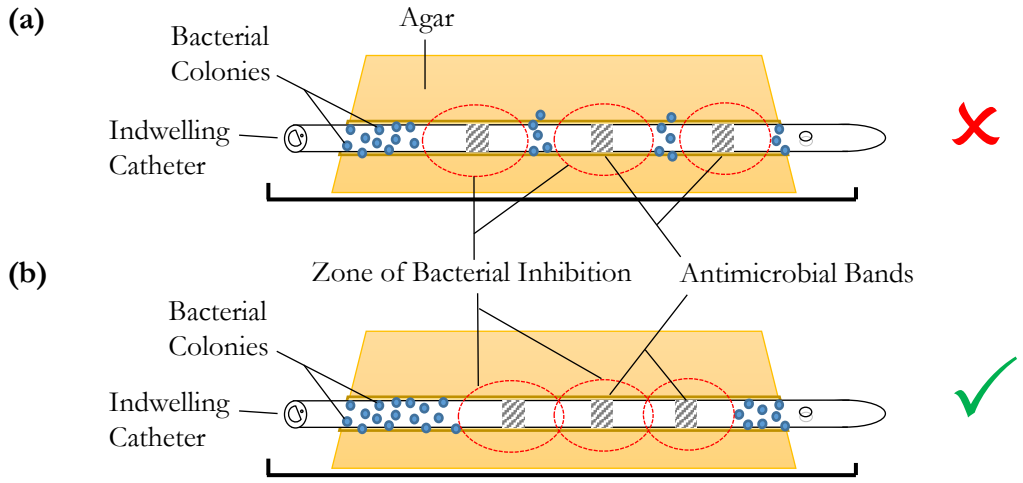


Figure 4.1 *In vitro* urethra diffusion model original concept illustration; (a) zones of inhibition do not overlap – antimicrobial bands are too far apart, (b) zones of inhibition overlap – antimicrobial bands correctly spaced.

4.3 Stage 2: Agar Formulation

When performing the traditional disc diffusion assay, Mueller Hinton Agar (MHA) is the standard media of choice as it demonstrates general reproducibility batch-to-batch; there is low/no inhibition of common antimicrobials; supports the growth of most non-fastidious bacteria or can be supplemented to support fastidious species; and there is years of clinical experience and data to support its use (Weinstein *et al.,* 2018; Jorgensen & Turnidge, 2015). For these reasons, MHA was the obvious choice to form the *in vitro* urethra diffusion model. Initial testing however, demonstrated that the traditional formulation of MHA would not be suitable for a three-dimensional medium. This was due to the colony morphology of the test strains, *E. coli* and *S. aureus*, when grown on MHA. *E. coli* colonies, when grown on MHA, were observed to be off-white or milky in colour, while *S. aureus* grew in smaller white colonies. Both species were difficult to visualise within the three-dimensional *in vitro* channel, even when observed over a black background (Figure 4.2).

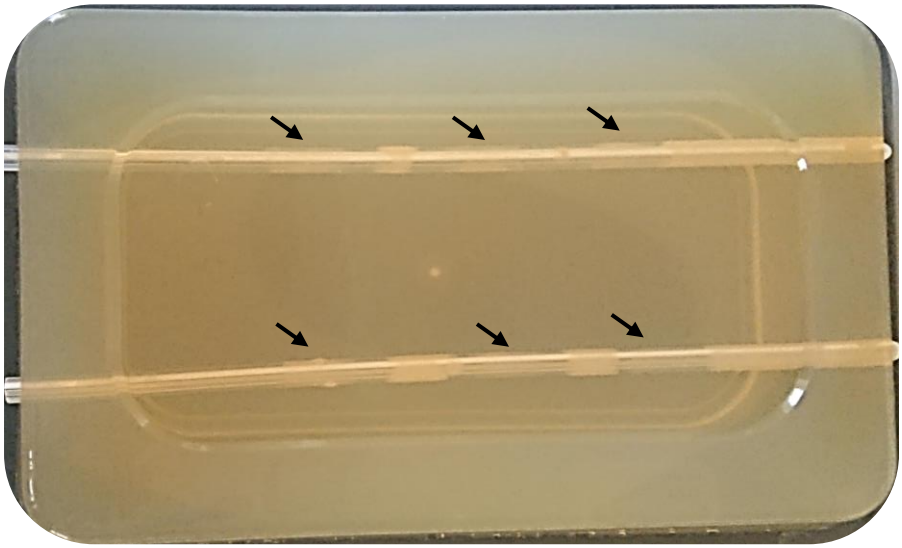


Figure 4.2 *In vitro* urethra diffusion model made with Mueller Hinton agar containing two triple banded catheters; arrows indicate location of the antimicrobial bands. Opaque areas within the urethra channel are *E. coli* ATCC 25922.

To address the issue of poor visualisation, a chromogenic MHA formulation was procured. CHROMagar™ MH Orientation agar was determined to be the best available chromogenic MHA locally available that would provide better visualisation within a three-dimensional model. When grown on CHROMagar™ MH Orientation agar, *E. coil* colonies grow dark pink or red and *S. aureus* colonies grow as a golden yellow with an opaque halo.

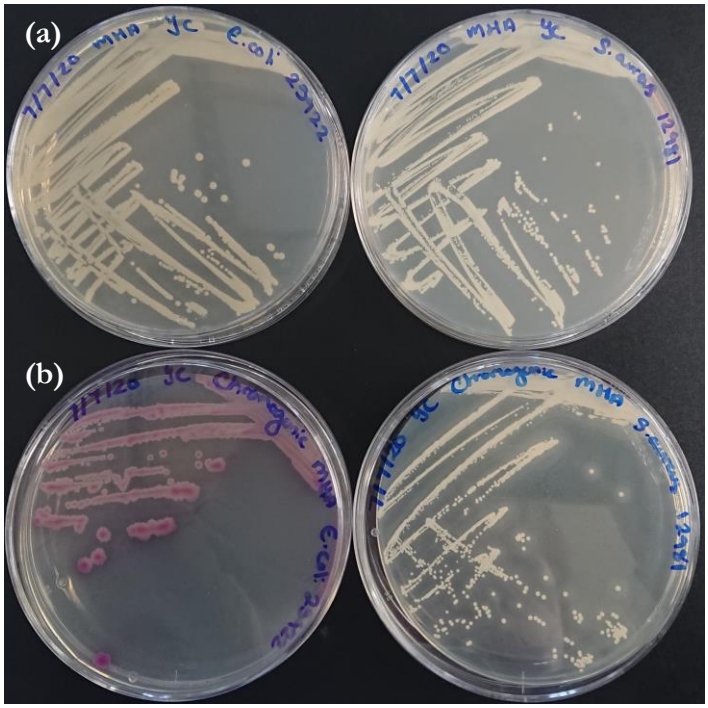


Figure 4.3 Difference in colony colour of *E. coli* ATCC 25922 and *S. aureus* NCTC 12981 grown on Mueller Hinton agar *vs.* CHROMagar™ MH Orientation agar: (a) *E. coli* (left) and *S. aureus* (right) grown on Mueller Hinton agar; (b) *E. coli* (left) and *S. aureus* (right) grown on CHROMagar™ MH Orientation agar.

The chromogenic media provided improved contrast between areas of bacterial growth and the straw-coloured agar allowing for better visualisation of inhibition zones within the *in vitro* urethra channel. It should be noted, that while *S. aureus* colonies grow a similar colour in both MHA and the chromogenic formula; CHROMagar™ MH Orientation agar presented a more translucent formula than MHA allowing for improved visualisation overall as seen in Figure 4.3. While this change in media did improve visualisation of bacteria within the *in vitro* urethra channel it did highlight another issue. As can be seen in Figure 4.4, the growth of bacteria was inconsistent within the channel when antimicrobial bands were used in comparison to control bands with no antimicrobial.

Figure 4.4 *In vitro* urethra diffusion model composed of CHROMagar™ MH Orientation agar containing three triple banded catheters within three urethra channels; arrows indicate location of the antimicrobial bands (ID +CHX3B, top) or control bands (ID -CHX3B, bottom). Red/Pink areas within the urethra channel are *E. coli* ATCC 25922.

4.4 Stage 3: Inoculation Method

When performing a traditional disc diffusion assay, the entire surface of the agar plate is inoculated with bacteria. To replicate this technique, within the channel of the antimicrobial diffusion model, consideration of the inoculation method was necessary. Sterile swabs dipped in a prepared bacteria inoculum were used to inoculate the channel. Initially, standard swabs (~5 mm Ø) were used and inserted from one end of the channel to the centre, before being removed then a second swab was inserted from the other side to the centre and removed to accommodate the full length of the channel. This method proved unsuccessful as it would split the agar of the 4 mm Ø channel as well as cause dripping of the inoculum as the narrower

channel created friction which pressed the inoculum out of the cotton of the swab tip (Figure 4.5).

To remedy this problem, standard swabs were replaced with paediatric swabs (~2 mm Ø). When using a paediatric swab, the inoculation method was amended to ensure full inoculum surface coverage which was achieved by repeatedly moving the swab in and out of the channel four times at the 12:00, 3:00, 6:00, and 9:00 positions in a clockwise pattern. This method was repeated from both sides of the channel to the centre to ensure full surface coverage throughout the length of the channel.

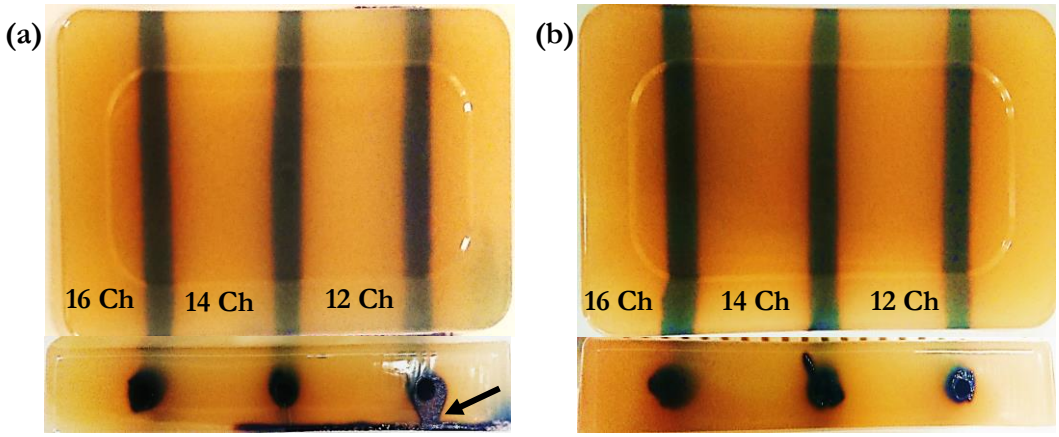


Figure 4.5 Plan and elevation images of triple channel *in vitro* urethra diffusion models with varying channel widths; (a) model swabbed with standard swab, (b) model swabbed with paediatric swab. Arrow highlights area where inoculum dripped from the channel to the underside of the model. Dark Blue/black growth is *E. coli* ATCC 25922 grown in Harlequin™ E. coli/Coliform agar.

4.5 Stage 4: Channel Diameter

During the development of the *in vitro* urethra diffusion model, it was important to examine the relationship between channel diameter and the diameter of the catheter samples. In the previously described *in vitro* urethra friction model, channel diameter greatly influenced the functionality and reproducibility of the model. Regarding the friction model, it was important that the channel and catheter were the same diameter to allow full surface contact resulting in consistent friction around the catheter (Section 3.7).

In respect to the *in vitro* urethra diffusion model, there were three potential problems to consider in relation to channel diameter: (1) would a channel that is too narrow inhibit bacterial growth due to creation of an anaerobic environment; (2) would a channel that is too wide,

prevent consistent diffusion of the antimicrobial from the catheter bands; and (3) would a channel that is too narrow, cause shearing forces that could damage the antimicrobial coating?

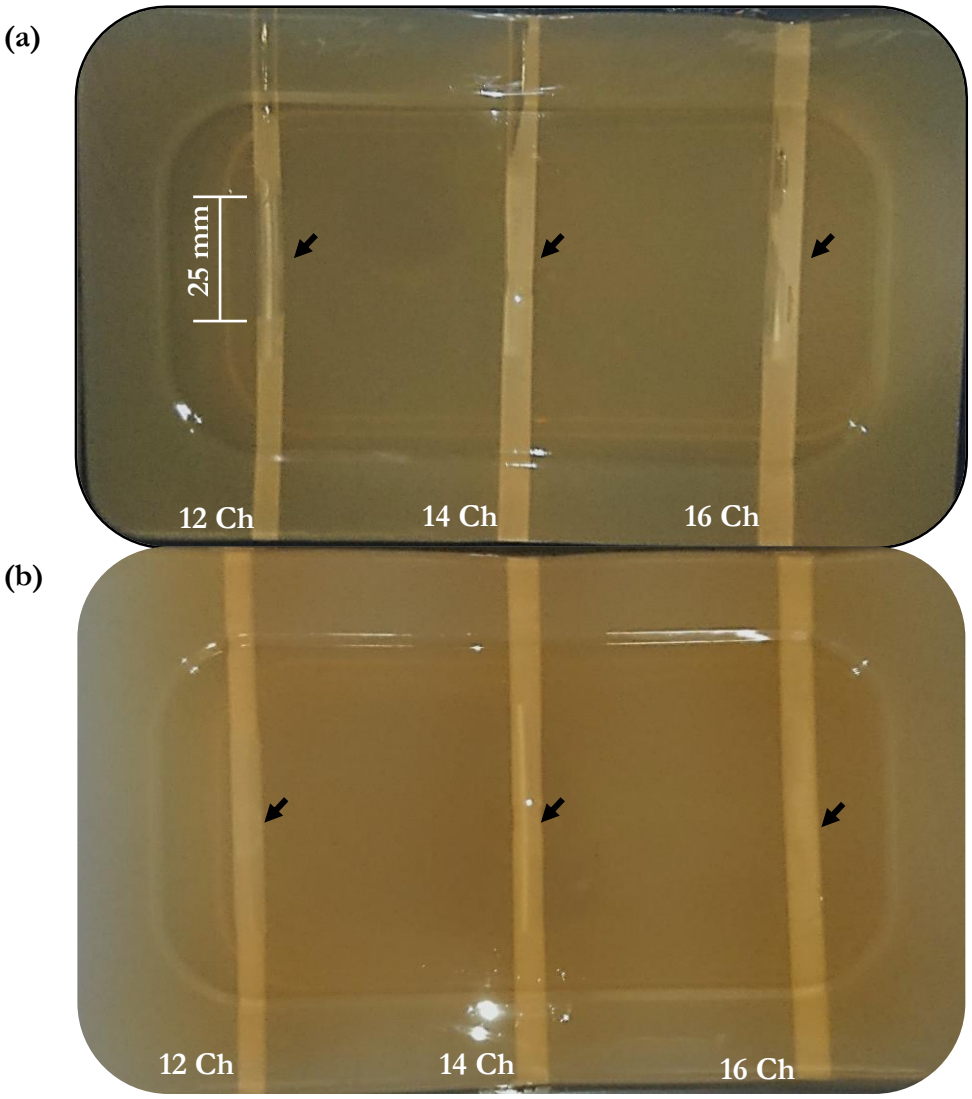


Figure 4.6 *In vitro* urethra diffusion model containing three channels with differing diameters of 12 Ch (4 mm), 14 Ch (4.67 mm), and 16 Ch (5.3 mm). All test sample catheters were 12 Ch in diameter. Opaque areas within the urethra channel are *E. coli* ATCC 25922 grown in Mueller Hinton agar. Arrows indicate the location of either antimicrobial (a) or non-antimicrobial (b) bands.

An investigation into channel diameter within the *in vitro* urethra diffusion model was undertaken using two models formed with Mueller Hinton agar, each containing three channels of differing diameters *i.e.,* 12 Ch (4 mm), 14 Ch (4.67 mm), and 16 Ch (5.3 mm). Two sets of triplicate catheter samples were then prepared, three with 10 mm antimicrobial bands and three with 10 mm bands of the base polymer coating. Each channel was fully inoculated with *E. coli* before the catheter samples were placed within the channels. After overnight

incubation at 37°C, any zone of inhibition around the bands was measured in millimetres. The results of this trial experiment, represented in Figure 4.6, demonstrated that channel diameter was a crucial variable for inhibition zone visibility. In channels of a 14 Ch or 16 Ch Ø, zones of inhibition were absent or unclear in areas where the catheter samples were not in direct contact with the agar. The only sample that presented a clear zone of inhibition was the 12 Ch catheter within a 12Ch Ø channel. This experiment illustrated that an interference fit was needed to ensure full surface contact and consistent diffusion in all directions, however, this also led to increased friction which in turn, caused more problems than it solved.

4.6 Stage 5: Problems with Friction

Friction was the major challenge during the development of the *in vitro* urethra diffusion model. As discussed in the previous section, to allow for the formation of consistent zones of inhibition, an interference fit between the catheter and the channel was needed. This tight fit also led to high amounts of friction during insertion of the catheter. The results of Chapter 3 clearly elucidated a link between catheter insertion friction and the movement of bacteria. During the development of the *in vitro* urethra diffusion model another issue related to friction was observed. This was the shearing forces on the catheter surface and in particular on the catheter coating. The chlorhexidine (CHX) containing coating used in the study was not chemically bound to the catheter surface and as such was vulnerable to shearing forces. Early experiments, with triple banded catheters highlighted a problem wherein, the zones on inhibition nearest the side of catheter insertion, were increasingly unreadable (Figure 4.7 a). This phenomenon was only observed when using catheters banded with CHX (ID +CHX3B), indicating that the problem was not due to friction alone moving bacteria during insertion but due to the CHX bands themselves (Figure 4.7 b).

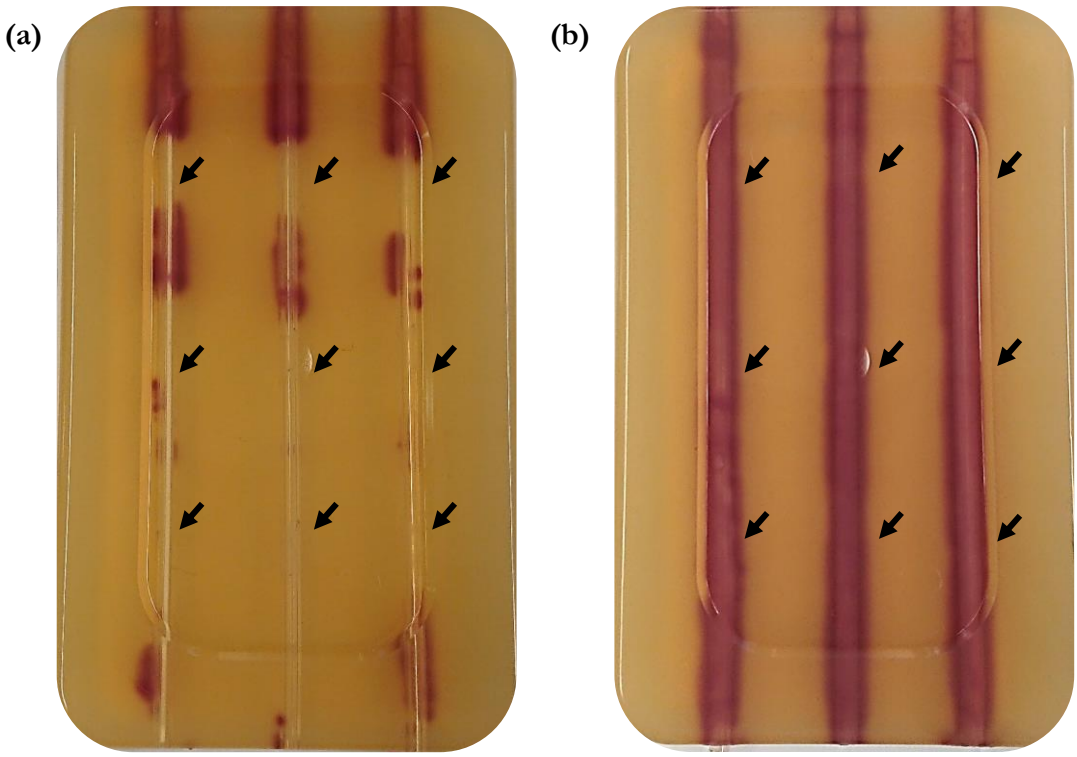


Figure 4.7 *In vitro* urethra diffusion model containing three channels. All test sample catheters were 12 Ch in diameter. Red/pink areas within the urethra channel is *E. coli* ATCC 25922 grown in CHROMagar™ MH Orientation agar. Arrows indicate the location of either antimicrobial (a, ID +CHX3B) or non-antimicrobial (b, ID -CHX3B) bands. Catheters inserted from bottom of models as pictured.

In an attempt to reduce/eliminate friction during insertion, three solutions were trialled. The first idea was to inoculate the catheter itself instead of the channel. This proved entirely unsuccessful as it did not provide a consistent inoculation of the channel due to beading of the inocula on the hydrophobic catheter surface. This approach also led to bacterial swarming outside of the channel due to the introduction of excess moisture during catheter insertion (Figure 4.8 a). The second idea was to use a sterile water based lubricant. The lubricant was applied to the whole length of the catheter with extra care given to the bands *via* sterile swab. Use of the lubricant provided only a minor improvement but was not sufficient to negate friction during insertion and also introduced excess moisture leading to bacterial swarming (Figure 4.8 b).

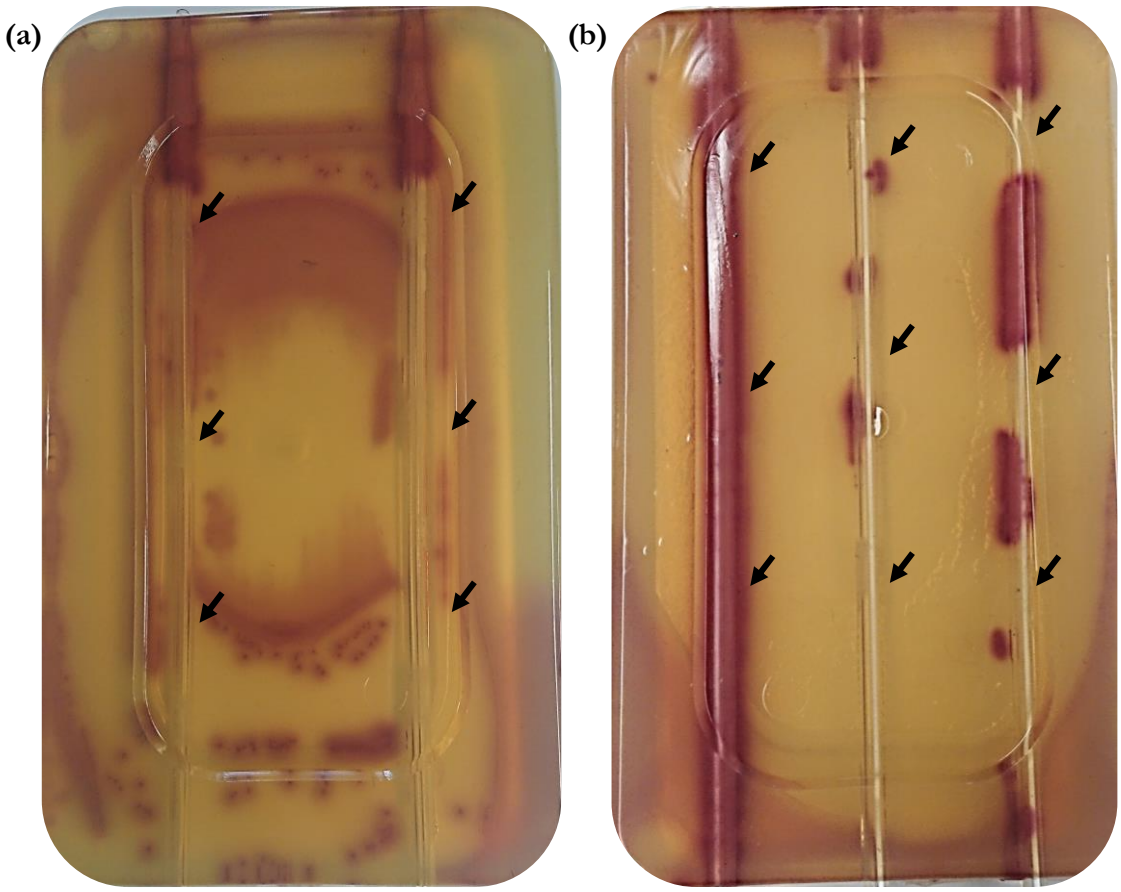


Figure 4.8 *In vitro* urethra diffusion models used to test inoculation of catheter (a), or the use of water based lubricant (b) to prevent friction disruption of inhibition zones. All test sample catheters were 12 Ch in diameter. Red/pink areas within the urethra channel is *E. coli* ATCC 25922 grown in CHROMagar™ MH Orientation agar. Arrows indicate the location of either antimicrobial (ID +CHX3B) or non-antimicrobial (ID -CHX3B) bands. Catheters inserted from bottom of models as pictured.

The third idea was to study singular isolated bands *i.e.,* instead of trying to measure the zones of inhibition of multiple bands, singular bands could be used to optimise the model methodology. This however, also proved fruitless as the problem of friction remained. In previous tests, usually at least the first band formed a readable zone of inhibition with the problem of unclear zones of inhibition evident with the second and third bands (Figure 4.7). As such, the idea of simplifying to a single band was trialled, with the thought being that if the results presented with high reproducibility the findings could be extrapolated to estimate the distances needed for multiple band spacing. This unfortunately was unsuccessful as a singular band also entirely disrupted the growth pattern on the insertion side within the channel (Figure 4.9).

This was probably the result of the coating's durability and delamination of the coating due to poor bonding with the substrate (Feneley *et al.,* 2015). In regard to vascular catheters, delamination can be extremely dangerous, increasing morbidity rates, as fragments of coating can lead to inflammation and clot formation (Roth & Lewitus, 2020). For urinary catheters, this does not present the same dangers as coating fragments can be flushed from the body through micturition or *via* secretions of the urothelial mucosa (Summers & Goeres, 2019). Minor delamination of the coating may also be of benefit in regard to CAUTI prevention, as small fragments may eliminate local colonisation by pathogens however this may not prove true in an *in vivo* setting thus further studies are needed (Awasthi *et al*., 2020).

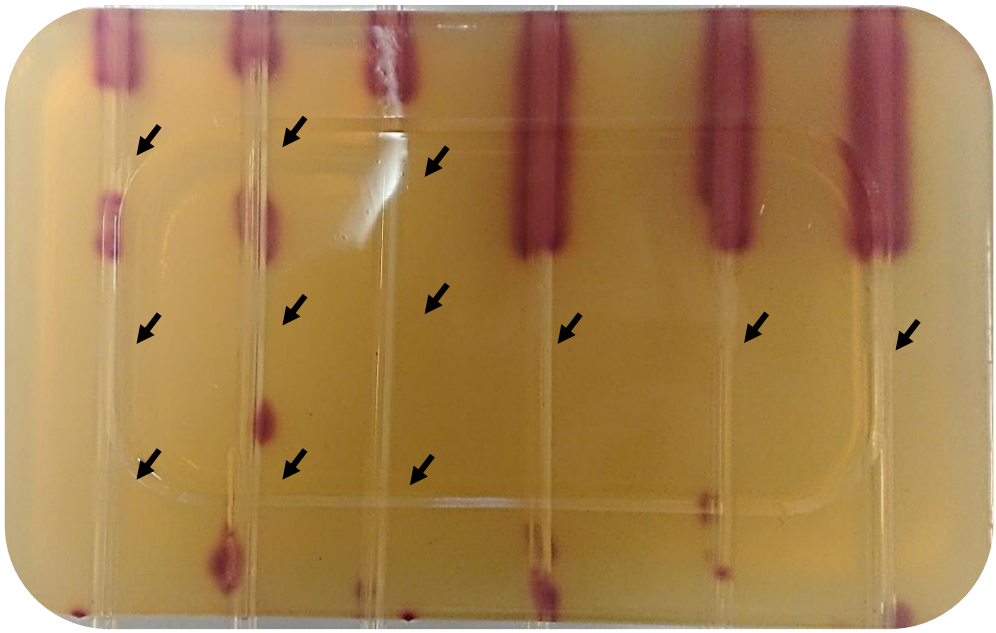


Figure 4.9 *In vitro* urethra diffusion model used to assess the difference in zone of inhibition formation when using triple (left) or single (right) chlorhexidine banded catheters. Red/pink areas within the urethra channel is *E. coli* ATCC 25922 grown in CHROMagar™ MH Orientation agar. Arrows indicate the location of antimicrobial bands. Catheters inserted from bottom of models as pictured.

4.7 Stage 6: Discontinuation of Development, Future Considerations, and Conclusions

After eight months of trials, improvements, and ultimately insurmountable challenges, the development of the *in vitro* diffusion model was discontinued. The problem of friction-induced shearing of the coating could not be overcome in a satisfactory way. It was not possible to validate the model or methodology as inter test results were either unreadable or highly variable. It is possible that with different catheter samples impregnated with antimicrobial or catheters with a more durable coating that the model may have proved reproducible. However, regarding the antimicrobial coating developed by Teleflex®, the *in vitro* urethra diffusion model did not perform as envisaged.

Another factor that led to the discontinuation of the *in vitro* urethra diffusion model development was a change in the coating strategy. The concept of multiple discrete bands was discarded after it was found that it did not prevent bacterial migration on a catheter long term as discussed later in Chapter 5, section 5.7.

For these reasons, the development process was abandoned indefinitely to focus on more promising work. The development process for the *in vitro* urethra diffusion model presented many unique challenges, and while it unfortunately was not completely successful, the knowledge gained could prove useful in future test development. A model and method that would allow visualisation and quantification of antimicrobial diffusion from medical device coatings in three dimensions, could aid innovation in future medical device design. The *in vitro* urethra diffusion model could be used in the future to investigate other impregnated devices or device coatings in a wide array of medical fields and should not be discarded entirely as a failure. Indeed, it could be a starting point for future *in vitro* model development.

Chapter 5 *In vitro* Urethra Extraluminal Migration Model

5.1 Introduction

Indwelling urinary catheters are responsible for the majority of severe CAUTI complications including septicaemia, biofilm formation, bladder/kidney stones, pyelonephritis, and sceptic shock (Agarwal & Radera, 2019; Jordan *et al.,* 2015). When used long-term in particular, they can undermine the natural immune defences of the host causing localised tissue inflammation while providing a route for bacterial infection and facilitate the formation of crystalline biofilms (Jeffery & Mundy, 2020; Agarwal & Radera, 2019).

Indwelling urinary catheters when used long-term, provide a direct means of travel for microorganisms to enter the urinary tract from outside of the body. Previous studies have shown that microorganisms can move along the intra- and extraluminal surface of urinary catheters by capillary action and through their own innate motility to enter the urinary tract and manifest a CAUTI (Gould *et al.,* 2017; Melo *et al.,* 2016; Jacobsen *et al.,* 2008; Maki & Tambyah, 2001; Darouiche *et al.,* 1997). Infections initiated by bacterial migration on intraluminal surfaces typically originate from a contaminated urine collection system however, with the advent of closed sterile urine collection systems in the 1950s, this occurs in the minority of cases (Summers & Goeres, 2019; Miller *et al*., 1958). In the majority of cases, extraluminal migration of bacteria is responsible for the ascension of bacteria from the urethral meatus along the periurethral mucous sheath to the bladder (Summers & Goeres, 2019). The urethral meatus plays host to its own diverse microbiota with a number of potentially pathogenic microbial species, both enteric and skin borne species, that can cause CAUTI including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcal* sp., *Enterococcal* sp., *Proteus* sp., *Klebsiella* sp., *Citrobacter freundii*, *Providentia rettgeri*, and *Candida* sp., (Summers & Goeres, 2019; Chatterjee *et al*., 2014). The extraluminal surface of a catheter can become contaminated during insertion due to poor hand/meatal hygiene or due to the continued colonisation of the meatus from which pathogenic organisms can migrate (Thomas-White *et al.,* 2016; Barford *et al.,* 2008).

In the past, several models have been described to test indwelling catheters such as those described in section 1.10.2. There is however no model that quantifies and allows consistent visualisation of the bacteria growth along the extraluminal catheter surface long-term. The closest model that exists was first described by Gaonkar *et al.,* (2003), later modified by Williams & Stickler, (2008b) and Vargas-Cruz *et al.,* (2019). These models utilised an agar channel to qualify bacterial migration along an indwelling catheter.

While each model was successful, they limited the data collection to only if the bacteria moved from one side of the urethra to the other and did not specifically quantify the rate of bacterial

migration between these points. There are also less complex *in vitro* assays that asses the swarming migration of bacteria on catheter surfaces such as the "Catheter Bridge model", described by Kazmierska *et al.,* (2010). In this model, a catheter segment is placed in a channel cut from an agar plate and on one side of the remaining agar, bacteria were inoculated. This method was used to assess if the bacteria was able to move along the catheter surface to the other side of the channel. This straightforward model does allow quick assessment of potential antimicrobials but is limited by its simplicity.

The aim of developing the *In vitro* Urethra Extraluminal Migration model was to provide a further tool to build on previous existing models thus providing a way to quantify and visualise bacterial migration along an indwelling catheter during long-term use. Once developed, the model was used to assess novel antimicrobial urinary catheters, coated in a proprietary novel coating containing chlorhexidine. This chapter outlines and discusses each stage of development for the *in vitro* urethra extraluminal migration model.

5.2 Stage 1: Conceptualisation

The *in vitro* urethra extraluminal migration model builds on the knowledge obtained from the *in vitro* urethra friction model described in Chapter 3. With the basic structure of the *in vitro* urethra model mould already developed, the focus for the extraluminal migration model development was on adapting the testing methodology of the existing model to test indwelling urinary catheters.

Teleflex®, industry partners of this project, in the past developed an antimicrobial coating containing chlorhexidine for indwelling catheters. This coating was found to be too expensive when coating the entire catheter and ultimately it was unmarketable. They devised a possible solution in coating the catheter in discrete bands of the coating to reduce overall cost. It was then necessary to determine if the coating was as effective in the form of separate bands when compared to a fully coated catheter. To assess the efficacy of the antimicrobial bands versus full coating, an initial concept was developed to adapt the existing *in vitro* urethra model (Figure 5.1).

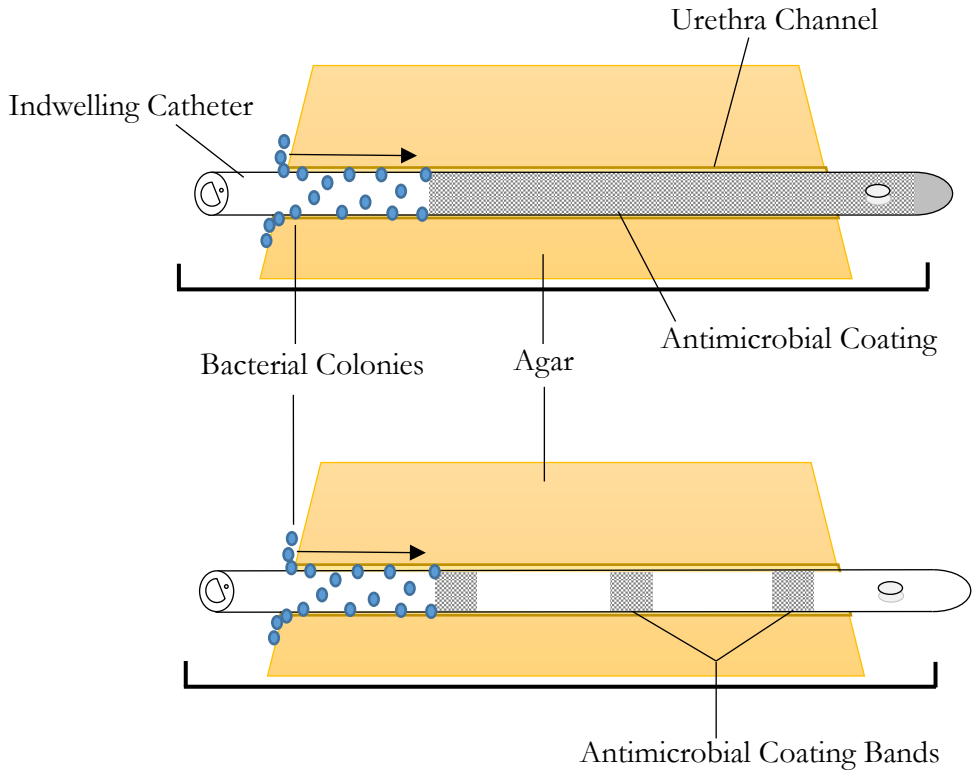


Figure 5.1 *In vitro* urethra migration model original concept illustration, arrow indicates direction of extraluminal bacterial migration.

It was at the conceptualisation stage that the agar formulations to be used was also determined. In the past with the friction model, some agar formulations were altered from the manufacturer's instructions. In the case of the extraluminal migration model, the manufacturers' instructions for both Harlequin™ E. coli/Coliform agar and CHROMagar™ Staph aureus agar was followed. For the friction model the Harlequin™ E. coli/Coliform agar was diluted 1:5 and supplemented with bacteriological agar do allow for discrete colony formation. As discrete colony formation was no longer necessary, no dilution of agar solutions was performed. The agar concentrations used were also not diluted to avoid negatively altering the nutritional composition of the agar. During this stage of development, it was also decided to continue using the bacterial strains *E. coli* ATCC 25922 and *S. aureus* NCTC 12981 as they are both normally non-swarming and are relevant to CAUTIs.

5.3 Stage 2: Inoculation Method Selection

As with the friction model, it was determined that the best site of inoculation would be the urethral meatus. *In vivo*, this is the area in which the highest concentration of pathogenic bacteria naturally occurring in the urinary tract (Goetz *et al.,* 2018). Three strategies to inoculate the *in vitro* urethra channel meatus were devised: (1) inoculation of the catheter end before full

insertion, (2) over insertion of the catheter followed by inoculation of the channel behind the catheter before pushing the catheter back into place, and (3) inoculation of the inner circumference of the meatus after the catheter is fully inserted flush with the meatal end (Figure 5.2).

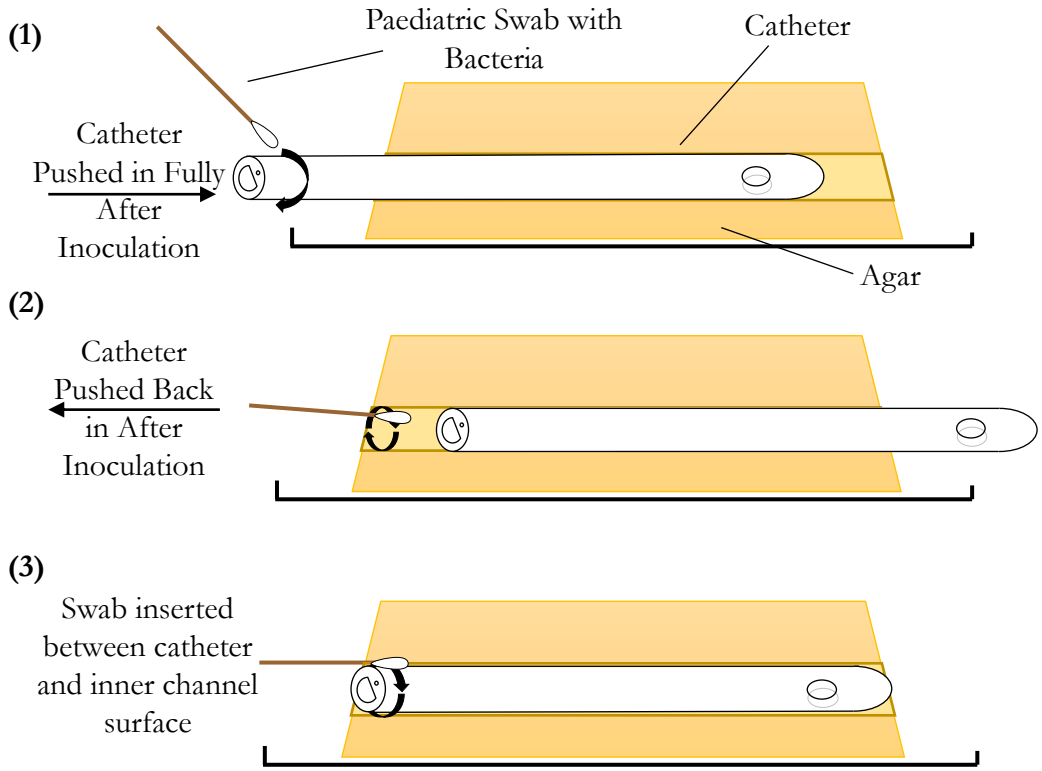


Figure 5.2 Illustration of different inoculation techniques trialled during in vitro urethra migration model development.

Regarding the first inoculation method tried, a silicone indwelling catheter was inserted partially into the urethra channel until 10 mm was protruding from the meatal side of the channel. A sterile paediatric swab (2 mm Ø) was then dipped into a broth containing ~10^6 CFU ml^{-1} bacteria for 10 seconds and the protruding end of the catheter was then inoculated by swabbing the exposed sides in a clockwise rotation. The catheter was then fully inserted until flush with the meatal side of the channel, see Figure 5.2 (1). After incubation, this method was found to be unsuitable as the bacterial growth at the meatal site was found to be inconsistent with low reproducibility. Gaps in the growth were noted as opposed to the consistent lawn of growth desired (Figure 5.3). This inconsistency was most likely caused by the hydrophobicity of silicone which caused the inoculum to bead on the catheter surface; thus this inoculation method was discontinued (Owen, 2017).

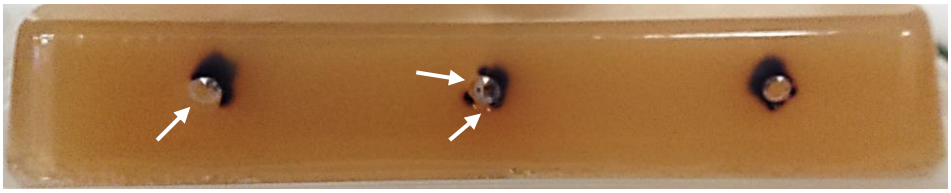


Figure 5.3 Example of poor inoculation consistency at *in vitro* urethral meatus, arrows highlight areas where no bacteria was transferred to the agar. Dark blue growth is *E. coli* grown on Harlequin™ E. coli/Coliform agar.

For the second inoculation method tested, a silicone indwelling urinary catheter was over inserted into the *in vitro* urethra *i.e.,* the catheter was inserted fully then pushed a further 10 mm into the channel, so the catheter tip extended out from the terminal end of the channel. A sterile paediatric swab (2 mm Ø) was then dipped into a broth containing ~10^6 CFU ml^{-1} bacteria for 10 seconds before the inner surface of the meatal side of the channel ~10 mm in was swabbed in a clockwise motion. The catheter was then pushed from the tip side back into the channel until flush with the meatal side, see Figure 5.2 (2). After incubation, this method was also found to be unsuitable as, when pushing the catheter back into place after inoculation, a small amount of the inoculum was pushed from the channel (Figure 5.4). This small amount of liquid would follow gravity down the side of the model and contact the plastic at its base. Once in contact with the plastic, the bacteria transferred would grow under and around the model as they move under their own motility and/or through capillary action. This result undermined the aims of the model to only observe growth along the extraluminal catheter surface thus this method was abandoned.

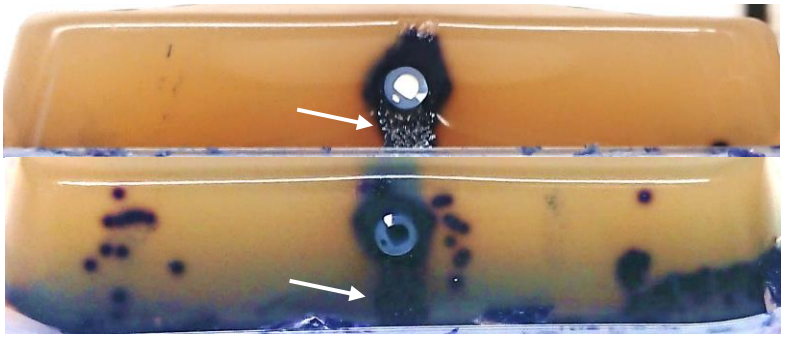


Figure 5.4 Examples of *in vitro* urethra model meatuses when the back-up inoculation method was used *i.e.,* the catheter was over inserted into the channel then the meatus was inoculated before the catheter was pushed back into place. Arrows highlight the path of inoculum out of the channel and contacting the plastic below allowing bacteria to grow out of the channel. Dark Blue growth is *E. coil* grown on Harlequin™ E. coli/Coliform agar.

The final inoculation method tested proved the most successful and consistent. For this method a silicone indwelling catheter was inserted fully into the channel until the cut end was flush with the meatal side. A sterile paediatric swab (2 mm Ø) was then dipped into a broth containing ~10^6 CFU ml^{-1} bacteria for 10 seconds before being carefully inserted into the space between the extraluminal catheter surface and the inner wall of the *in vitro* urethra. The meatus was then inoculated by swabbing along the inner circumference of the meatus and outer circumference of the catheter in a clockwise motion, Figure 5.2 (3). After incubation, this inoculation method was found to produce a concise ring of growth on the inner surface of the *in vitro* urethra (meatus) around the catheter end. This method was found to be reproducible as long as care was taken to be precise during application, ensuring that the inoculum was only applied to a small consistent area and even pressure was applied to avoid splits forming in the surrounding agar. When using this method, no growth was observed outside of the urethra channel (Figure 5.5).

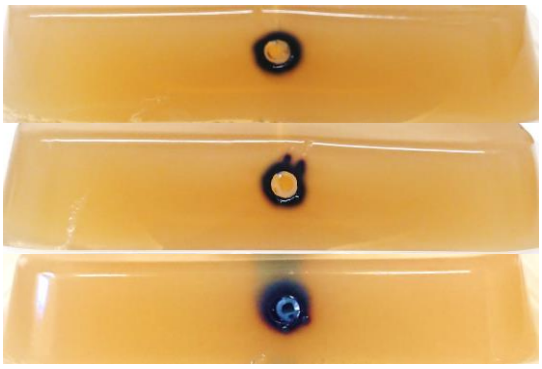


Figure 5.5 Examples of replicate *in vitro* urethra model meatuses inoculated by insertion of a swab between the agar and extraluminal catheter surface. Dark blue growth in *E. coil* grown on Harlequin™ E. coli/Coliform agar.

5.4 Stage 3: Condensation and Bacterial Swarming

As discussed in the previous section, a major problem experienced during development was bacteria growing uncontrolled outside of the *in vitro* urethra channel. While optimising the inoculation method greatly reduced the problem of bacteria growing in undesired areas, it was still an enduring issue. This issue was also a problem with the *in vitro* urethra friction model as seen in Chapter 3, Stage 4: Condensation, Bacterial Swarming, and Model Inversion. As with the friction model, the migration model was also inverted to remove the model from the container base of the mould eliminating contact between the inoculation site and the plastic.

Bacteria coming into contact with the plastic was also prevented by careful inoculation, trimming all catheters to the exact length of the channel so as not to touch the insides of the

container, and reducing condensation within the container as much as possible. Ensuring that the bacteria never touch the plastic prevented the bacteria from moving along the outsides of the agar model and along the plastic of the container. The bacteria when in contact with the plastic can move either through their own motility or through capillary action which is exacerbated by condensation within the container during model formation and water loss during incubation (Figure 5.6 a). In the worst cases of bacterial growth outside of the channel, the bacteria travelled on the plastic over the top of the model and drop down onto the model through high condensation droplets and established new isolated colonies on top of the model as shown in Figure 5.6 (b).

Controlling the condensation in the model container during incubation presented an interesting challenge as agar by nature is >98% water. Substantial evaporation from the model was also observed when the incubation was extended over several days to a month. It was found through trial and error that the ideal way to control the condensation required consideration of a number of factors. Like the friction model, the migration model was contained in air permeable sterilisation pouches which allowed excess moisture to escape. The models also needed to be incubated in a single layer to allow airflow around the entire pouch. Calcium chloride dehumidifiers were also utilised in the incubator to control the humidity when multiple models were incubated together, due to intensification of the condensation problem. Finally, when measuring bacterial migration each day, the models were opened in a laminar flow hood and any present condensation was allowed to evaporate.

In combination, these techniques adequately controlled the condensation and resulting swarming problems allowing for control and one directional movement of the bacteria within the *in vitro* urethra.

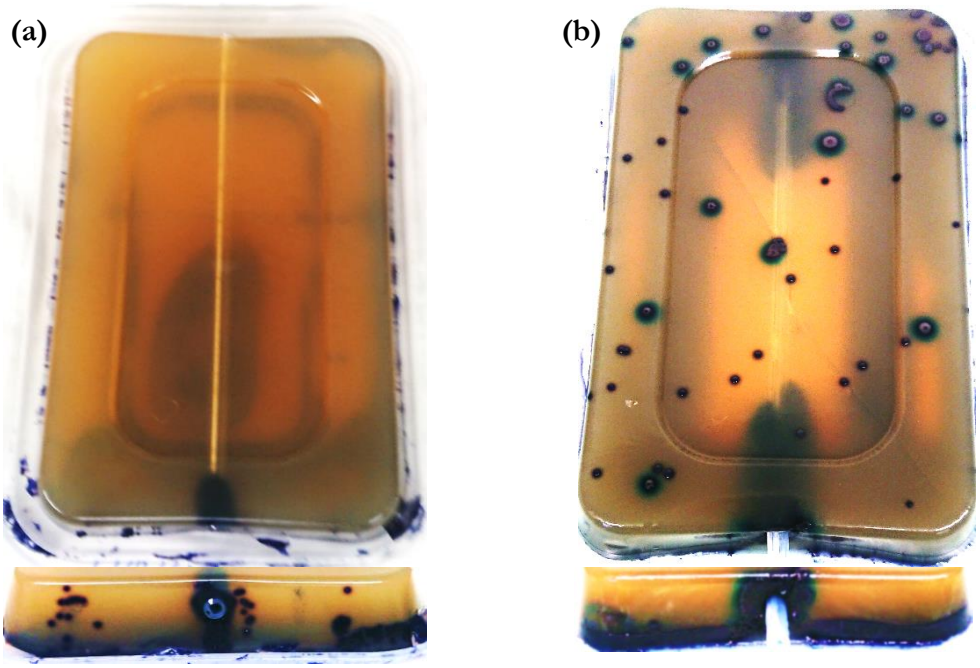


Figure 5.6 Examples of bacterial swarming and condensation observed during the development of the *in vitro* urethra extraluminal migration model; (a) bacterial growth under and around model facilitated by bacterial contact with plastic, (b) individual colonies on top of model caused by bacteria suspended in condensation droplets. Dark blue bacterial growth is *E. coli* grown on Harlequin™ E. coli/Coliform agar.

5.5 Stage 4: Aerobic *vs.* Anaerobic Environment

Taking on board the knowledge gained during the development of the *in vitro* urethra friction model, when constructing the migration model, the *in vitro* channel was first formed with a silicone tube the same diameter as the catheter to be tested *i.e.,* a 4 mm Ø channel to test a 4 mm Ø catheter. This was crucial for the friction model, as an interference fit was essential to ensure consistent and reproducible friction-mediated movement of the bacteria. For the extraluminal migration model, using an interference fit resulted in a unique problem. It was found that during incubation, bacterial progress along the catheter was notably slow. The aim of the method was to observe the bacterial migration along the catheter over a period of one month, however it was found in early tests that after 29 days the bacteria had progressed only halfway down the channel (Figure 5.7). At that rate, it would have potentially taken two months to reach the bladder end of the channel, if at all. This would have made the method excessively long time wise, with concomitant desiccation of the agar over the extended time.

To investigate the problem of slow growth in the channel, at 29 days the model seen in Figure 5.7 was dissected to expose the channel and a sample of the bacteria found furthest in the channel was taken and streaked onto a fresh agar plate. This was done to ensure that the

bacteria were still viable, which it was found to be. A Gram stain found no changes to cellular morphology. It was at this stage that channel *versus* catheter width was investigated. The idea being that if the channel was the same diameter as the catheter, potentially, an anaerobic or microaerophilic environment was being created within the channel. Previous studies have shown that there is variation in oxygen availability in solid medias as thin as 5 mm, so it is possible that the microenvironment of the *in vitro* urethra channel created a gradient of oxygen availability as the bacteria progressed deeper into the channel (Jeanson *et al.,* 2015). While *E. coli* and *S. aureus* are facultative anaerobes, both organisms will grow faster in oxygen rich environments due to the disparity of energy production in anaerobic (~2 – 3 ATP) *versus* aerobic (~30 – 38 ATP) environments (Madigan *et al.,* 2019; Balasubramanian *et al.,* 2017; Finn *et al.,* 2017).

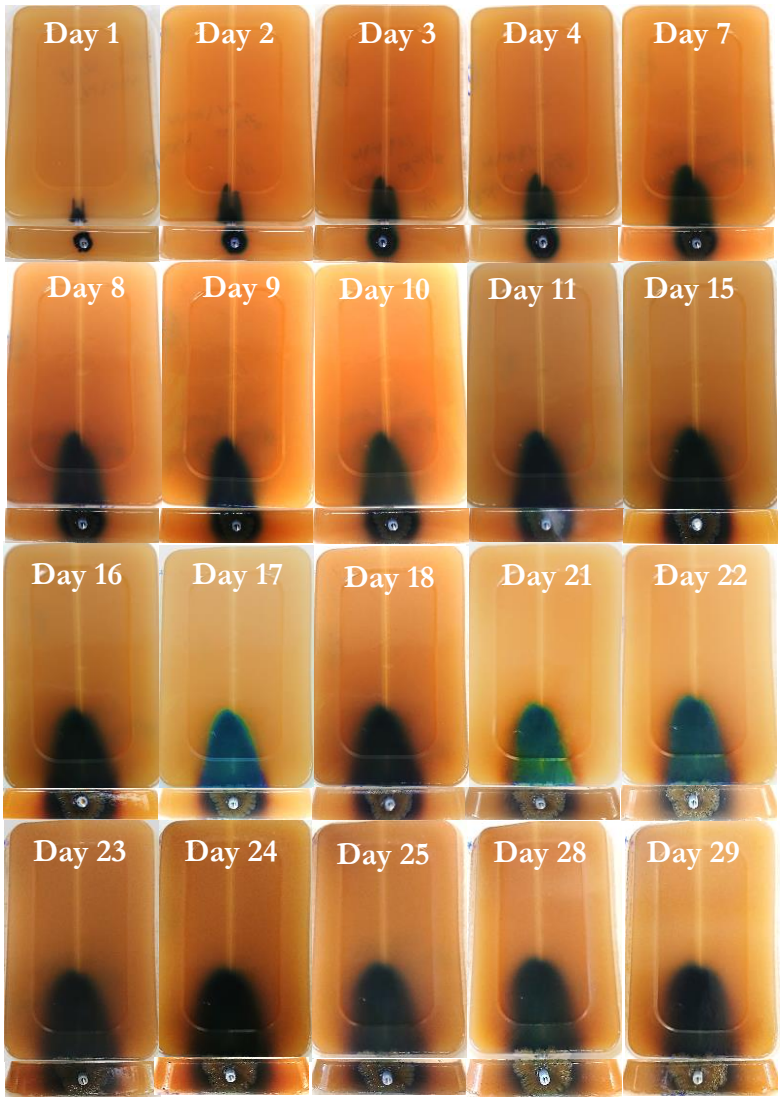


Figure 5.7 *In vitro* urethra migration model incubated over 29 days, plan and front elevation of model imaged each day. Channel diameter 12 Ch (4 mm), catheter diameter 12 Ch (4 mm). Dark blue growth is *E. coli* grown on Harlequin™ E. coli/Coliform agar.

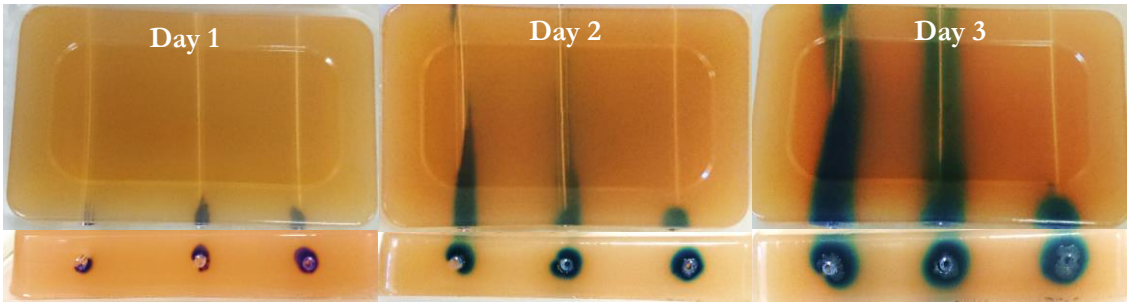


Figure 5.8 *In vitro* urethra migration model incubated over 3 days, plan and front elevation of model imaged each day. Catheters were all 12 Ch (4 mm) in width. Channel diameter was left 16 Ch (5.3 mm), centre 14 Ch (4.67 mm), and right 12 Ch (4 mm). Dark blue growth is *E. coli* grown on Harlequin™ E. coli/Coliform agar.

To test this hypothesis, a new model was produced containing three channels with varying diameters. As can be seen in Figure 5.8, the three channel diameters used were 16 Ch (5.3 mm), 14 Ch (4.67 mm) and 12 Ch (4 mm). Each channel was inoculated after a 12 Ch uncoated silicone catheter (ID UnC) was inserted. The model was incubated only for 3 days as the result was quickly evident that increasing the channel diameter greatly impacted bacterial growth and migration speed along the catheter. While the difference in results between the 16 Ch and 14 Ch catheters were negligible, as in both instances the bacterial growth reached the other end of the channel within one day, it was evident that the most probably cause of slow bacterial movement in previous models was due to a lack of oxygen in the channel with little bacterial movement observed in the 12 Ch channel. From this observation it was decided that a larger channel diameter would be used with consideration of the test catheters diameter in all future models to allow adequate oxygenation of the *in vitro* urethra.

5.6 Stage 5: Multiple Channels

Once bacterial swarming and condensation were controlled, the idea of producing models with multiple channels was investigated. Urethra models with multiple channels was desired as it would allow for testing multiple samples within one model. This would reduce the overall number of models needing to be produced, consequently reducing the resources required to produce them and the incubator space needed to store them during long-term incubation.

The main concern with having multiple channels within the same model was that the bacteria in one channel could migrate between channels if the colonies from the initial inoculation site spread on the outside of the channel. Whilst in normal conditions *E. coli* and *S. aureus* are not motile on agar surfaces, during the extended time that the models are incubated for, bacteria

at the inoculation site were observed spreading outward from the *in vitro* urethra channel inoculation site (Kearns, 2010). This most likely occurred as a result of nutrition depleted over time at the original inoculation site and possible sliding motility as the initial inoculation colony grew (Pollitt & Diggle, 2017; Pollitt et al., 2015).

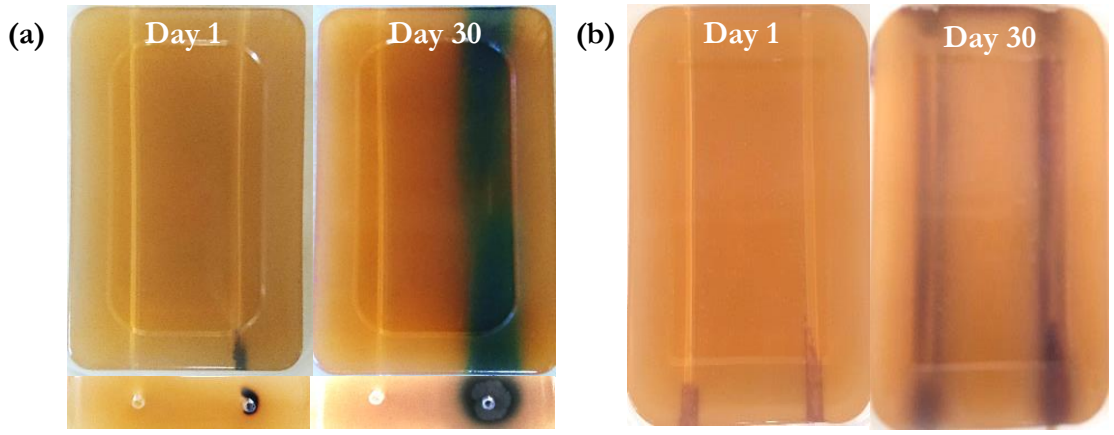


Figure 5.9 Dual channel *in vitro* urethra extraluminal migration models: (a) *E. coli* grown on Harlequin™ E. coli/Coliform agar with sterility control (left) and growth control (right); (b) *S. aureus* grown on CHROMagar™ Staph aureus agar with two growth control replicates.

Another concern with multiple channels was the spread of the chromogenic pigment produced by the bacterial growth. When using Harlequin™ E. coli/Coliform agar *E. coli*, X-glucuronide in the agar is hydrolysed by β-D-glucuronidase, produced by *E. coli,* which forms a precipitate that causes *E. coli* colonies to grow a dark blue or violet colour (Manafi, 2015; R. D. González *et al.,* 2003). This blue precipitate can be seen in the right channel of the model seen in Figure 5.9 (a). While it is unlikely that the bacteria in the channel could move through the solid agar between channels as the agar concentration was 1.5%, it is possible for the blue precipitate to diffuse into the agar surrounding the channel as the bacteria within continue to grow and metabolise the X-glucuronide. The diffusion of the precipitate can be seen in Figure 5.9 (a), where there is dark blue colouration visible around the expanded meatal inoculation colony at day 30. If the precipitate diffused far enough, it could overlap with an adjacent channel and obstruct visualisation or interfere with the quantification of bacterial migration distance measurements. Regarding *S. aureus* grown on CHROMagar™ Staph aureus agar, the mechanism of the chromogenic effect is not known as the manufacturer has not released this information. The accumulation of precipitated pigments was lower with this agar. However, a colourless, slightly opaque precipitate formed over time that could have similarly posed a risk

to visibility within the model thus this needed to be taken into consideration when using multiple channels (Figure 5.9 b).

In order to evaluate the influence multiple channels could have on the validity of the migration model long-term, replicate models were produced with two channels each. The left channel was a sterility control wherein a catheter was inserted, and the meatus was swabbed with a sterile swab dipped in sterile TSB (Figure 5.9 a). The right channel acted as a growth control in which a catheter was inserted and then the meatal end was swabbed with ~10^6 CFU ml^{-1} *E. coli* (Figure 5.9 a). The replicate models were incubated for 30 days and observed each day to determine if the bacterial growth in one channel would spread to the other. It was found after 30 days that while both the *E. coli* growth on the meatal side and the chromogenic precipitate did spread from their initial sites, they did not spread in a substantial quantity to affect the adjacent channel. This finding supported the use of multiple channels being viable in the *in vitro* urethra extraluminal migration model so long as consideration was taken to spacing the channels to allow sufficient room bacterial colony expansion and pigment precipitation.

5.7 Stage 6: Antimicrobial Catheter Coating Inconsistencies

The prototype indwelling urinary catheters that the *in vitro* urethra migration model was developed to test were provided by the industry partner of the project (Teleflex®). The catheters were produced by the company and coated by their engineers with a proprietary polymer coating containing the antimicrobial agent chlorohexidine (CHX). They provided four catheter types to be tested in the *in vitro* extraluminal migration model: uncoated silicone Foley catheters (ID UnC), silicone Foley catheters fully coated in a polymer matrix containing CHX (ID +CHX), silicone Foley catheters with three 10 mm bands of a polymer matrix with no antimicrobial (ID -CHX3B), and silicone Foley catheters with three 10 mm bands of a polymer matrix containing CHX (ID +CHX3B). The aim of testing these catheter variations was to determine firstly if CHX could prevent extraluminal migration. The second objective was to ascertain if the coating could be applied in discrete bands without decreasing the ability of the coating to prevent extraluminal migration. This would reduce the overall quantity of coating needed and cost per catheter.

During preliminary trials of the *in vitro* urethra extraluminal migration model, problems were identified with the ID +CHX3B catheters. Two independent tests were carried out with similar results both times. These models were inoculated as discussed earlier in section 5.3 and all four

catheter types were tested with *E.* coli. The models were incubated and observed over a 14-day period.

The results of these preliminary tests highlighted a problem specifically with the ID +CHX3B samples. When observing the models, it was found that by day 2, *E. coli* had migrated the full length of the ID UnC. In comparison, *E. coli* travelled a max of 27.5 mm over the 14 days when using a ID +CHX catheter, demonstrating the coated catheter was effective at preventing bacterial movement along the catheter (Figure 5.10). The results were less promising with the ID +CHX3B catheters as there was little difference in the performance of either ID +CHX3B catheters or the controls (Figure 5.11). Upon examination of the models, a problem with the application of the antimicrobial bands by Teleflex's engineers became evident. There appeared to be inconsistencies with the application method of the bands as the bacterial growth along the channel appeared to narrow substantially as it crossed the bands (Figure 5.12 a). This indicated the possibility that there were gaps in the bands that may have created a narrow bridge for the bacteria to transverse whilst avoiding the antimicrobial. With this observation in mind, the engineers at Teleflex® refined the application method for the antimicrobial bands.

With a change from hand application of bands to a mechanical application method, it was hoped that any inconsistencies with application would be eradicated. This was not the case and consistently the bacteria would pass through the bands with little hinderance. It was suspected that the bands were spaced too far apart, however moving the bands closer together was no more successful at preventing bacterial migration (Figure 5.12 b). From these observations, the idea of multiple bands was discarded as they consistently failed. There was also growing suspicion that if the bacteria were able to pass the first band, they may become resistant to CHX negating the antimicrobial effect of subsequent bands. Resistance to CHX would not only render the coating ineffective but also endanger patients who then may ultimately become carriers of antimicrobial resistant species.

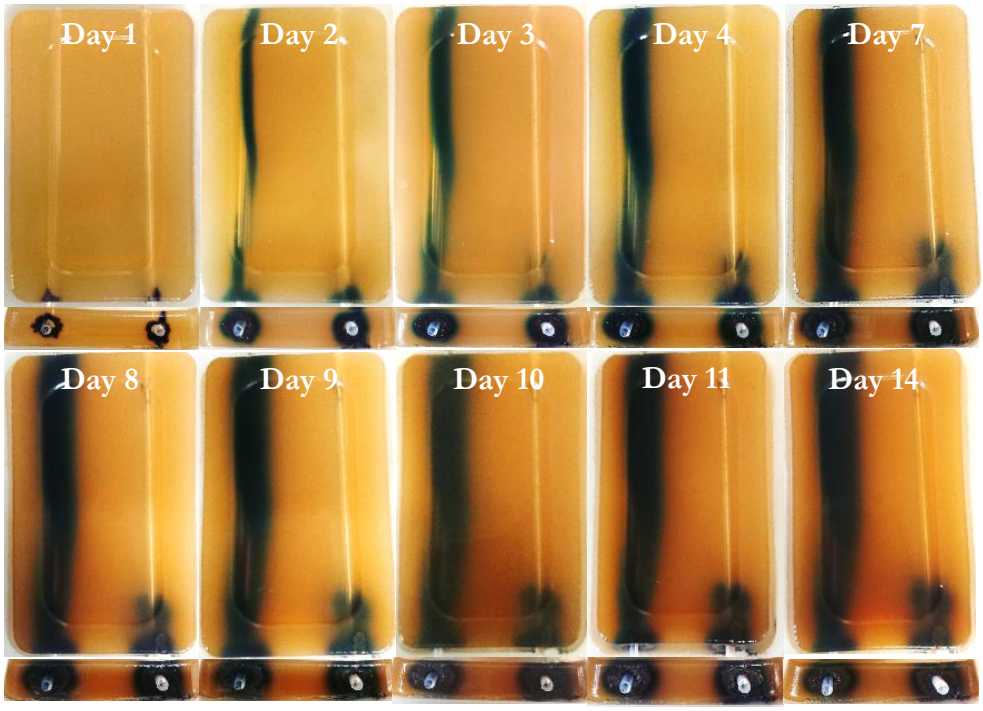


Figure 5.10 Dual channel *in vitro* urethra extraluminal migration model with an uncoated (ID UnC, left) and fully coated with chlorhexidine (ID +CHX, right) catheters. Dark blue growth is *E. coli* in Harlequin™ E. coli/Coliform agar.

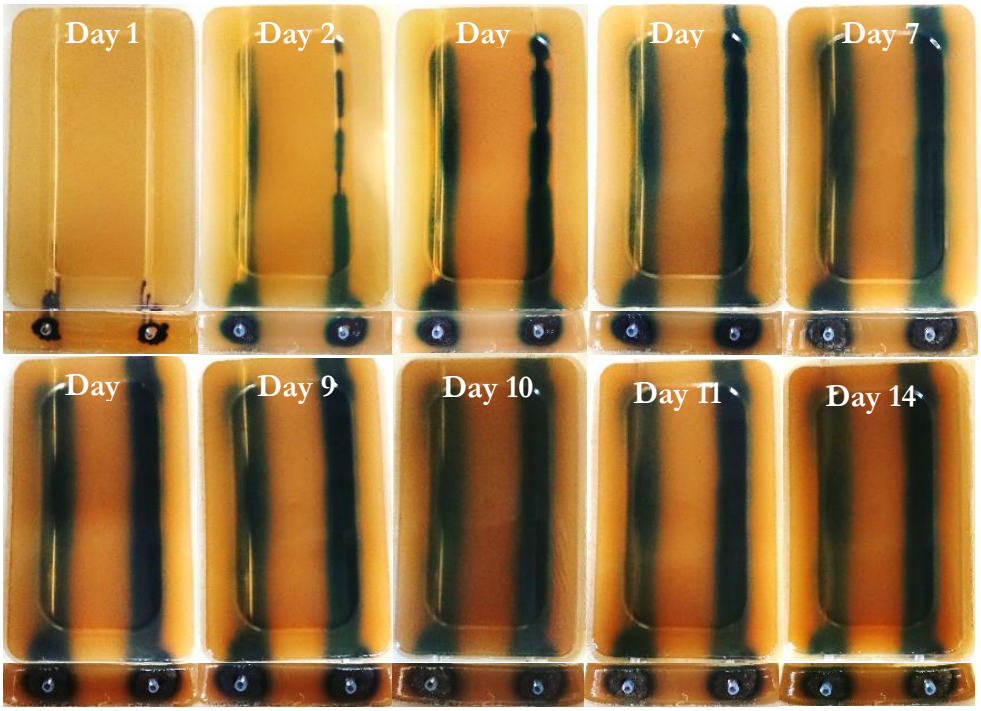


Figure 5.11 Dual channel *in vitro* urethra extraluminal migration model with banded non-antimicrobial (ID -CHX3B, left) banded with chlorhexidine (ID +CHX3B, right) catheters. Dark blue growth is *E. coli* in Harlequin™ E. coli/Coliform agar.

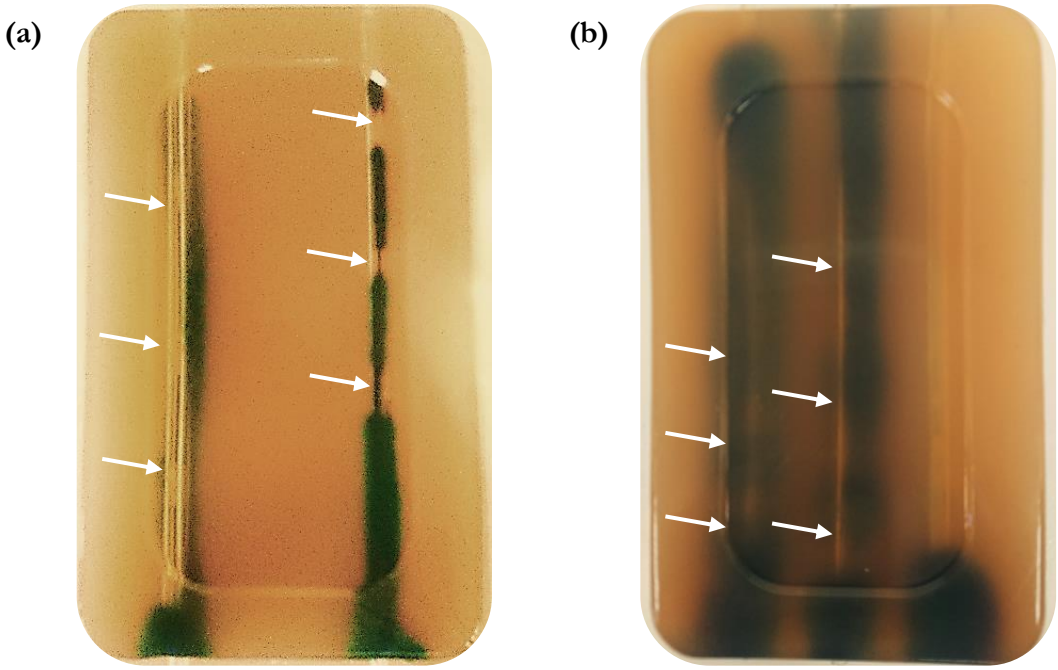


Figure 5.12 Images of *in vitro* urethra extraluminal migration models: (a) catheters with triple 10 mm bands containing no antimicrobial (ID 3B-CHX, left) or containing Chlorhexidine (ID +CHX3B, right); (b) catheters with 10 mm bands 10 mm apart containing CHX (left), 10 mm bands 20 mm apart containing CHX (centre), or fully coated in CHX (ID +CHX, right). Arrows indicate position of antimicrobial bands. Dark blue growth is *E. coli* in Harlequin™ E. coli/Coliform Agar.

In consideration of all previous observations, the decision was made to change strategies. To avoid both the possibility of gaps during coating with short bands, and the possibility of antimicrobial resistance due to exposure to short bands, new catheters were coated with a single larger band (the length of which cannot be disclosed as it would infringe on Teleflex's intellectual property rights). To determine the optimal band length, various catheters were trialled with incremental band lengths until one was identified that was as short as possible whilst also consistently preventing extraluminal bacterial migration. Although the final length cannot be reported, it can be stated that the singular band, used in the final prototype catheters for validation of the model represented a ≥50% reduction in the overall quantity of coating needed for each catheter.

5.8 Stage 7: Quantification Method

Two methods were investigated to quantify the extraluminal migration of the bacteria within the *in vitro* extraluminal migration model. The first involved quantifying the bacteria themselves within the channel after 30 days. This was done on day 30, by either dissecting each channel into octants or taking the entire channel and recovering the bacteria via sonification. The viable bacteria were then enumerated by the drop count method. The results were recorded as total CFU ml^{-1} per channel whether the channel was dissected into octants or not. This was quickly abandoned as whether, quantifying bacterial density in the channel by dissection into octants or by recovering the bacteria from the channel as a whole, the bacterial counts were found to be inconsistent and not reproducible. This was primarily due to the duration of the study, as over the 30 day incubation period, the bacteria in areas of dense growth were no longer viable most likely due to depletion of nutrients in the surrounding agar. Other methods to quantify the total bacteria were investigated. However, difficulty in isolating the bacteria from the agar as well as interference from the chromogenic pigments ruled out spectrophotometric, microscopy, and other such analytical quantification techniques.

The second method to quantify the extraluminal bacterial migration was to visually measure the distance of migration as indicated by the chromogenic pigments. This was done each day over the 30 day incubation period and distance was measured in millimetres with a digital calliper. This method proved simple and reproducible whilst also providing additional data throughout the study as opposed to only on the end date (Figure 5.13). By measuring the distance migrated each day, two useful data points were collected. The first being the final migratory distance after 30 days, allowing insight into how long an antimicrobial band may need to be to prevent full catheter migration. The second being, the estimated speed at which a species can traverse a catheter in different circumstances. It is for these reasons; measurement of visual distance was chosen as the quantification method for the *in vitro* extraluminal migration model. Details of the final quantification method can be found in section 2.4.3.

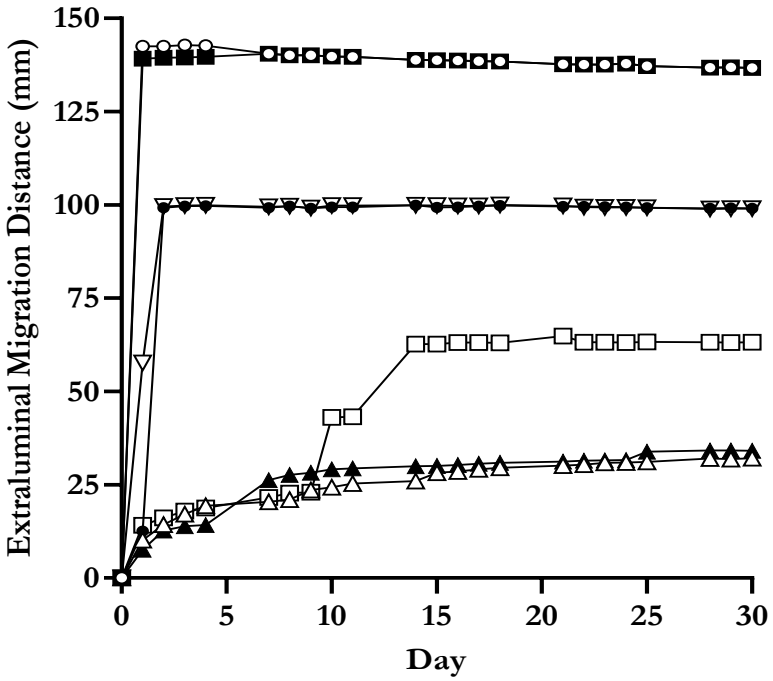


Figure 5.13 Results of a pilot study to determine the viability of visual distance measurement for quantification of the *in vitro* urethra extraluminal migration model. Catheter types: uncoated (○, ID UnC), coated with no Chlorhexidine (■, ID -CHX), fully coated in CHX (▲, ID +CHX), catheters with undisclosed CHX band lengths (▽,●,□,▲).

5.9 Stage 8: Migration Model Validation and Statistical Analysis

To validate the *in vitro* urethral extraluminal migration model and associated methodology, twelve independent tests were carried out, six with *E. coli* ATCC 25922 and six with *S. aureus* NCTC 12981. Each independent test was performed in two dual channel urethra models containing a channel each for two controls and two test catheters. Regarding the catheter samples, two acted as controls, first the uncoated catheter (**ID UnC**) acted as a base growth control to elucidate the speed of microbial migration on an unaltered silicone surface. The fully coated catheter with no CHX (**ID -CHX**), acted as a control for the base prototype polymer coating, ensuring that its chemical constituents were not involved in the migration prevention effect desired. There were two antimicrobial test catheters. The first was a catheter fully coated in the CHX containing prototype polymer coating (**ID +CHX**), the second was a catheter with a single continuous band of coating representing ≥50% of the full catheter length (**ID +CHX50**).

The results of the 12 independent tests are shown in Figure 5.15 and Figure 5.16, with selected representative photographs of the models at different dates during the incubation period;

graphs displaying the daily progression of the bacterial migration over the 30 day period; and bar charts recording the final distance of extraluminal migration after 30 days. To assess the distribution of the data collected over the 30 day period, the Anderson-Darling Normality test was used (Figure 5.14). It was found that the data was not normally distributed ($P \leq 0.0065$) thus the non-parametric Kruskal–Wallis test was used to determine reproducibility between replicate independent tests (Table 5.1). To compare the test catheters to the control catheters, the non-parametric Mann–Whitney U test was utilised (Table 5.2).

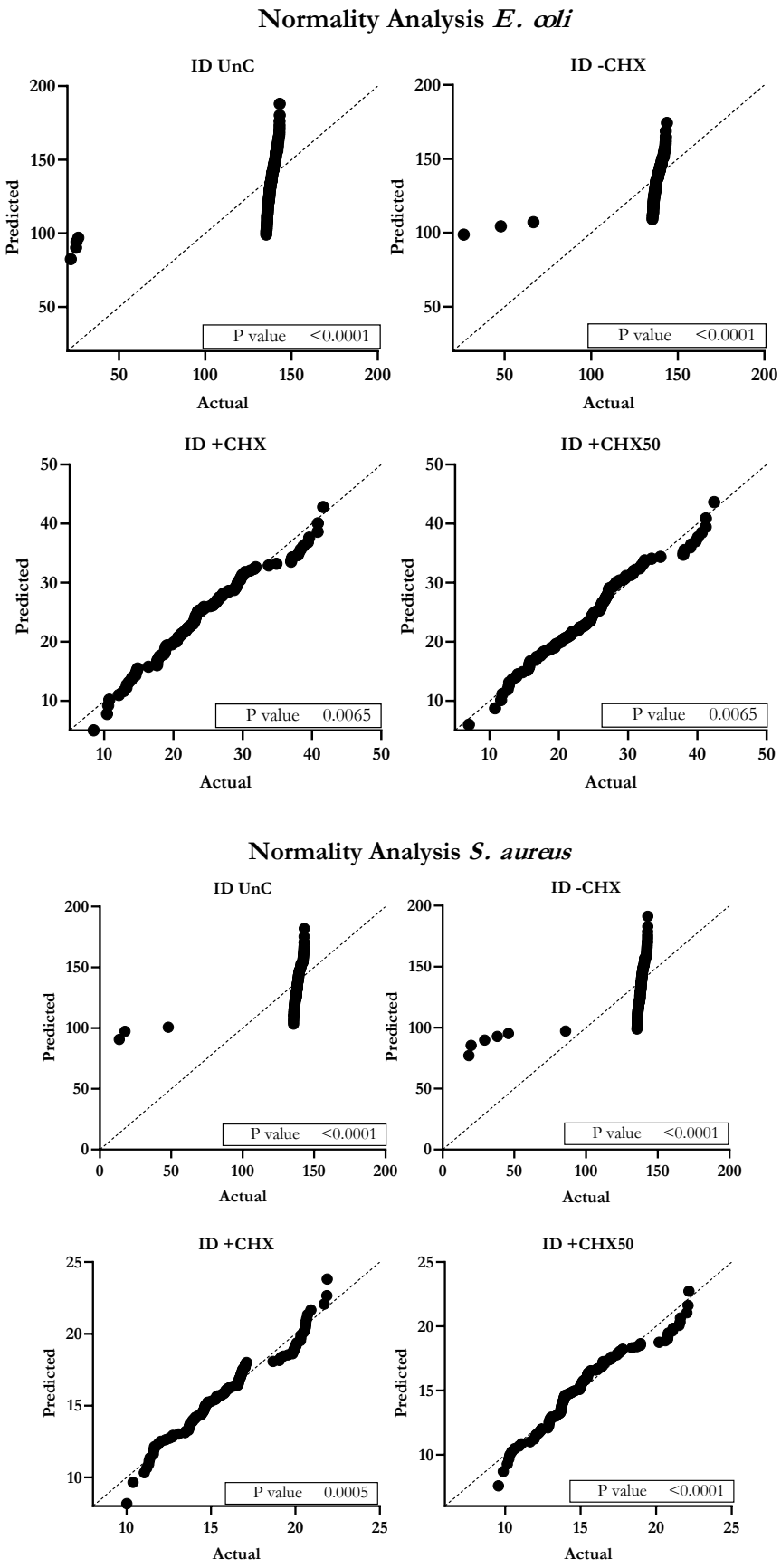


Figure 5.14 Distribution of bacterial migration within the *in vitro* urethra extraluminal migration model over 30 days for *E. coli* and *S. aureus*; n = 6.

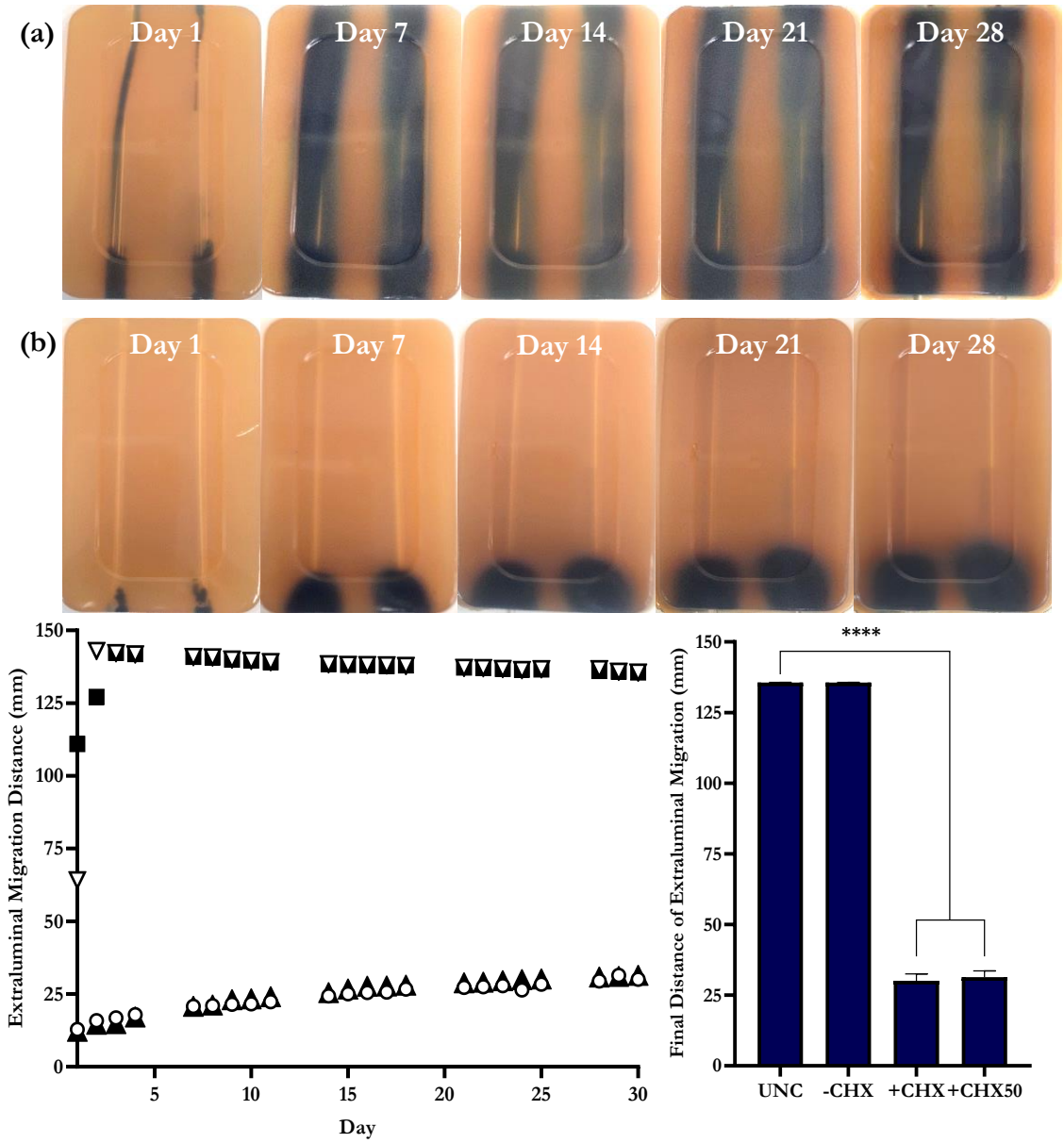


Figure 5.15 Extraluminal migration of *E. coli* ATCC 25922 on catheters over 30 days: ID UnC (a, left, ▼), ID -CHX (a, right, ■), ID +CHX (b, left, ●), and ID +CHX50 (b, right, ▲). Bacterial growth is *E. coli* grown in Harlequin™ E. coli/Coliform Agar (a, b). Error bars represent standard error of the mean (n = 6), ****$P \leq 0.0001$.

Table 5.1 Results of Kruskal-Wallis test used to determine reproducibility of the *in vitro* urethra extraluminal migration model when tested with four sample catheters and two bacterial species, n = 6.

	E. coli	***S. aureus***
ID UnC	$P = 0.8510$	$P = 0.9998$
ID -CHX	$P = 0.9885$	$P = 0.9999$
ID +CHX	$P \leq 0.0001$	$P \leq 0.0001$
ID +CHX50	$P \leq 0.0001$	$P \leq 0.0001$

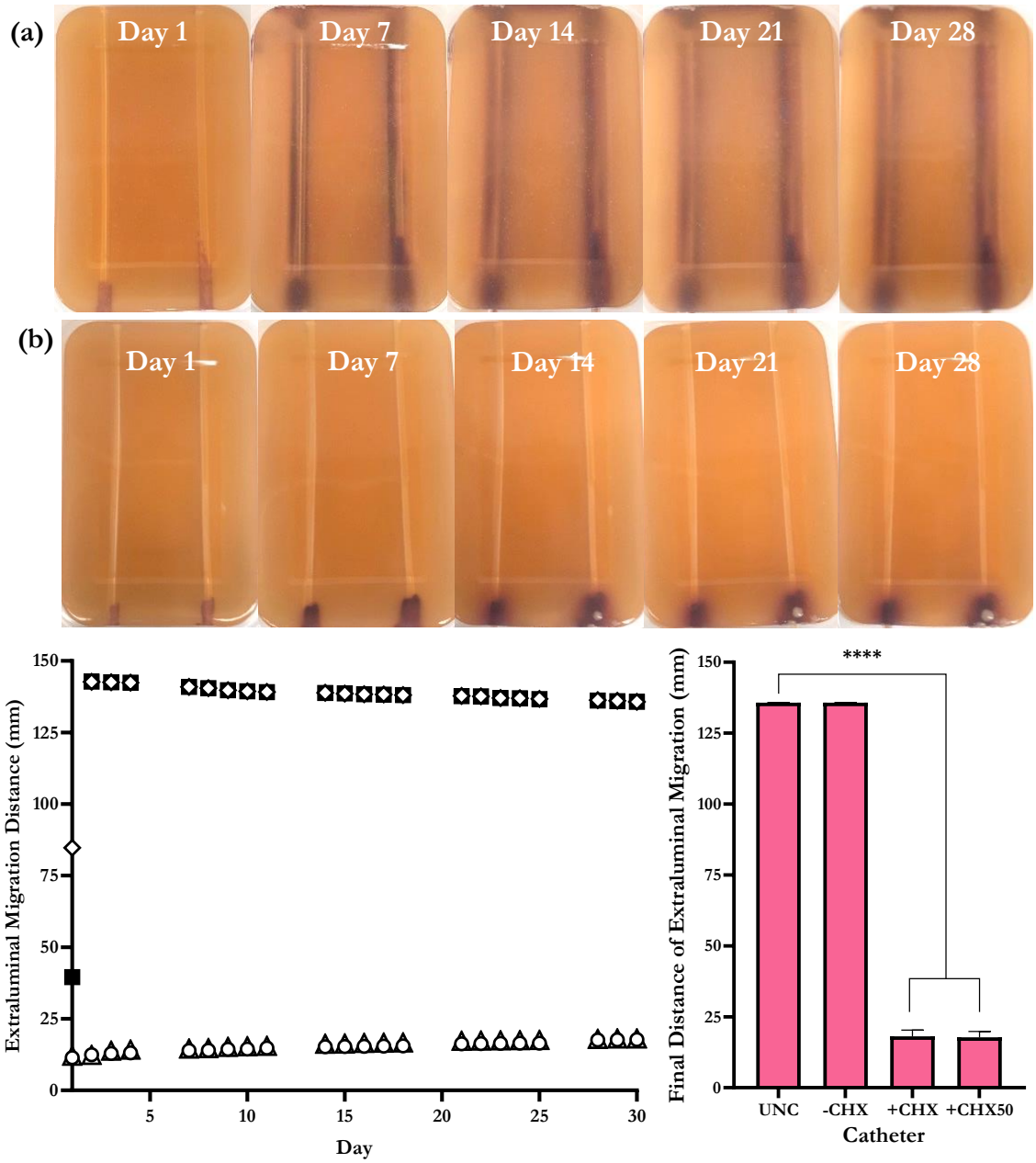


Figure 5.16 Extraluminal migration of *S. aureus* NCTC 12981 on catheters over 30 days: ID UnC (a, left, ▼), ID -CHX (a, right, ■), ID +CHX (b, left, ●), and ID +CHX50 (b, right, ▲). Bacterial growth is *S. aureus* grown in CHROMagar™ Staph aureus agar (a, b). Error bars represent standard error of the mean (n = 6), ****$P \leq 0.0001$.

Table 5.2 Null hypothesis testing by use of the Mann-Whitney U Test to compare the changes of bacterial migration in the *in vitro* urethra extraluminal migration model in response to chlorahexidine exposure, n = 6.

	E. coli	*S. aureus*
ID UnC *v.* ID -CHX	$P = 0.5890$	$P = 0.9955$
ID UnC *v.* ID +CHX	$P \leq 0.0001$	$P \leq 0.0001$
ID UnC *v.* ID +CHX50	$P \leq 0.0001$	$P \leq 0.0001$
ID -CHX *v.* ID +CHX	$P \leq 0.0001$	$P \leq 0.0001$
ID -CHX *v.* ID +CHX50	$P \leq 0.0001$	$P \leq 0.0001$
ID +CHX *v.* ID +CHX50	$P = 0.1084$	$P = 0.3698$

No significant difference was detected between replicates of the growth controls or non-antimicrobial samples (catheters ID UnC and ID -CHX) with either species tested ($P \geq 0.8510$). While there was a statistically significant difference detected between the replicates of catheters ID +CHX and ID +CHX50, there was no noticeable difference in overall growth patterns. The statistically significance differences, between replicates, observed with catheters ID +CHX and ID +CHX50 are most likely due to biological variance in response to CHX exposure. *E. coli* and *S. aureus* were chosen to validate the model as they are clinically relevant to CAUTI, accounting for ~75% and 3% of clinical CAUTI isolates respectively, whilst also being non-specific and widely cultured in most labs ensuring the accessibility of the model (Flores-Mireles *et al.,* 2015).

Utilising the *in vitro* extraluminal migration model, extraluminal migration in the absence of CHX from the meatal side of the model to the bladder side was observed to occur within 24-48 hours when using either *E. coli* or *S. aureus* (catheters ID UnC and ID -CHX). A study by Letica-Kriegel *et al.,* (2019) of indwelling catheter patients found that in 7.8% of cases patients are at risk of developing asymptomatic bacteriuria (bladder bacterial concentrations $\geq 10^5$ CFU ml^{-1}) within 24 hours of catheter insertion and this increases by 8.1% for each day the catheter remains in place. This clinical study established a similar time period to potential infection observed in the *in vitro* extraluminal migration model. While the speed at which *E. coli*, an actively motile species, traversed the catheter within the *in vitro* urethra was unsurprising, *S. aureus*, a passively motile species, was also consistently observed to migrate the full distance within the same time period (Jacobsen *et al.,* 2008). Traditionally, *S. aureus* has been classified as a passively motile species, with movement associated with sliding motility. Recent studies have observed some strains of *S. aureus*, in specific environments, engaging in active gliding motility with organised directional movement from a central colony (Pollitt & Diggle, 2017; Pollitt *et al*., 2015). While this form of active motility could be responsible for the relatively quick ascension of *S. aureus* within the *in vitro* urethra, another factor to consider is capillary action which could have exacerbated passive sliding motility.

In the presence of the novel CHX coating, complete extraluminal migration to the bladder side was prevented for 30 days with either species. There was a significant reduction in final bacterial migration distance on both catheters ID +CHX and ID +CHX50 when compared to either catheters A or B ($P \leq 0.0001$). In the presence of CHX, on either catheters ID +CHX or ID +CHX50, *E. coli* migrated an average of 30.72 ± 11.72 mm over the 30 day period and *S. aureus* reached an average of 17.94 ± 4.23 mm in the same time span. This result gives an

estimated migration speed of ~1 mm day^{-1} for *E. coli* and ~0.6 mm day^{-1} for *S. aureus*. There were no significant differences in final migration distances between catheters ID +CHX or ID +CHX50 indicating that fully coating a catheter in the CHX coating is not necessary to achieve migration prevention for 30 days ($P \geq 0.1084$). Reducing the amount of coating by ≥50% (catheter ID +CHX50) could allow for an overall cheaper product for patients and a reduction CHX exposure without compromising on infection protection.

5.10 Stage 9: Supporting Experiments

To support the findings of the *in vitro* extraluminal migration model, three experiments were undertaken. The first was the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) concentration assay to determine the lowest concentration of CHX required to inhibit or eradicate *E. coli* and *S. aureus*. The second was the Serial Plate Transfer Test (SPTT), which was used to determine how long CHX diffused from the coating and if it was at a high enough concentration to continually diffuse for ≥ 30 days. Finally, a small scale drug release trial was preformed to quantitatively measure the CHX release rate over 30 days and at which point it reached the MIC/MBC concentration and how long it stayed above this concentration.

The MIC and MBC of CHX was determined to be 0.2 μg ml^{-1} for both bacterial species tested and in contradiction to the SPTT, *E. coli* was found to have a higher susceptibility to CHX. There was no significant drop in *S. aureus* viability in any concentration below 0.2 μg ml^{-1}, *E. coli* on the other hand, exhibited a 40% reduction at a CHX concentration of 0.1 μg ml^{-1} (Figure 5.17**,** Table 5.3**)**.

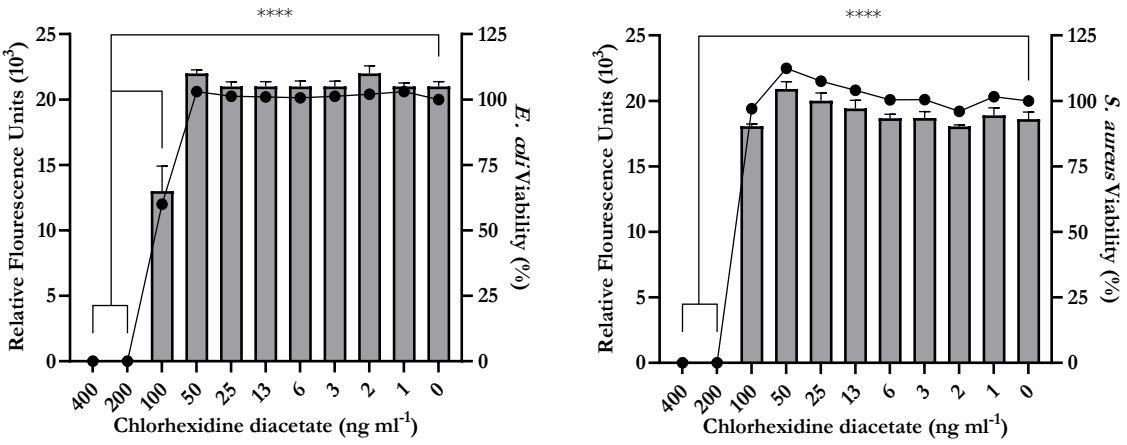


Figure 5.17 Minimum Inhibitory Concentration (MIC) of chlorhexidine diacetate for *E. coli* ATCC 25922 (left) and *S. aureus* NCTC 12981 (right) determined by the reduction of resazurin to resorufin correlated to reduction of cellular viability (●). Error bars represent standard deviation, n = 8.

Table 5.3 Average CFU ml^{-1} counts from the Minimum Bactericidal Concentration (MBC) assay of chlorhexidine diacetate (CHX).

CHX Concentration (μg ml^{-1})	*E. coli*	*S. aureus*
400	0.00E+00	0.00E+00
200	0.00E+00	0.00E+00
100	2.48E+06	3.12E+07
50	7.40E+07	7.07E+07
25	5.45E+07	5.34E+07
12	4.09E+07	4.84E+07
6	4.14E+07	2.30E+07
3	4.15E+07	2.90E+07
2	6.79E+07	1.07E+07
1	5.50E+07	5.85E+07
0	4.15E+07	4.52E+07

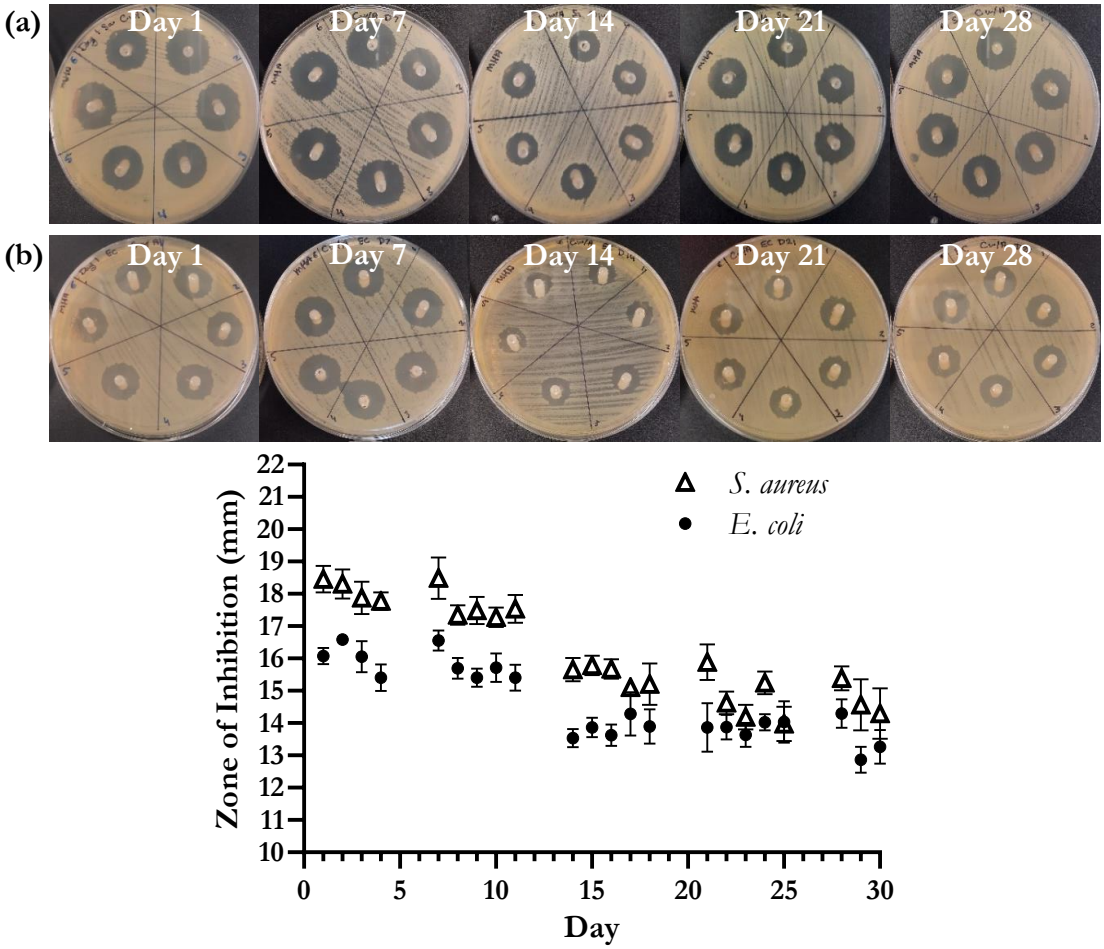


Figure 5.18 Images of the serial plate transfer test for catheter ID +CHX when exposed to *S. aureus* NCTC 12981 (a, △) and *E. coli* ATCC 25922 (b, ◆). Error bars represent standard deviation, n = 6.

The results of the SPTT demonstrated that the antimicrobial coated catheter segments exhibited antimicrobial activity for at least 30 days. In the SPTT, *S. aureus* was observed to have a greater sensitivity to CHX which may be responsible for the ~13 mm lower average final migration distance of *S. aureus* in the *in vitro* extraluminal migration model (Figure 5.18).

The drug release trial revealed the extended release of chlorhexidine for at least 30 days. It should be noted that the drug release trial was limited to 10 mm segments of coating. During the 30 days, the level of chlorhexidine released, on any given day, did not reach or exceed the MIC/MBC of both bacterial species tested, 0.2 μg ml^{-1} (Figure 5.19 c). However, as there was no significant difference between replicates observed (P = 0.8092), the results obtained could be used to predict the release of chlorhexidine when larger sections of the catheter are coated. To that end, both catheter ID +CHX and ID +CHX50 were predicted to reach the MIC within 24 - 48 hours if the experiment were repeated (Figure 5.19 d).

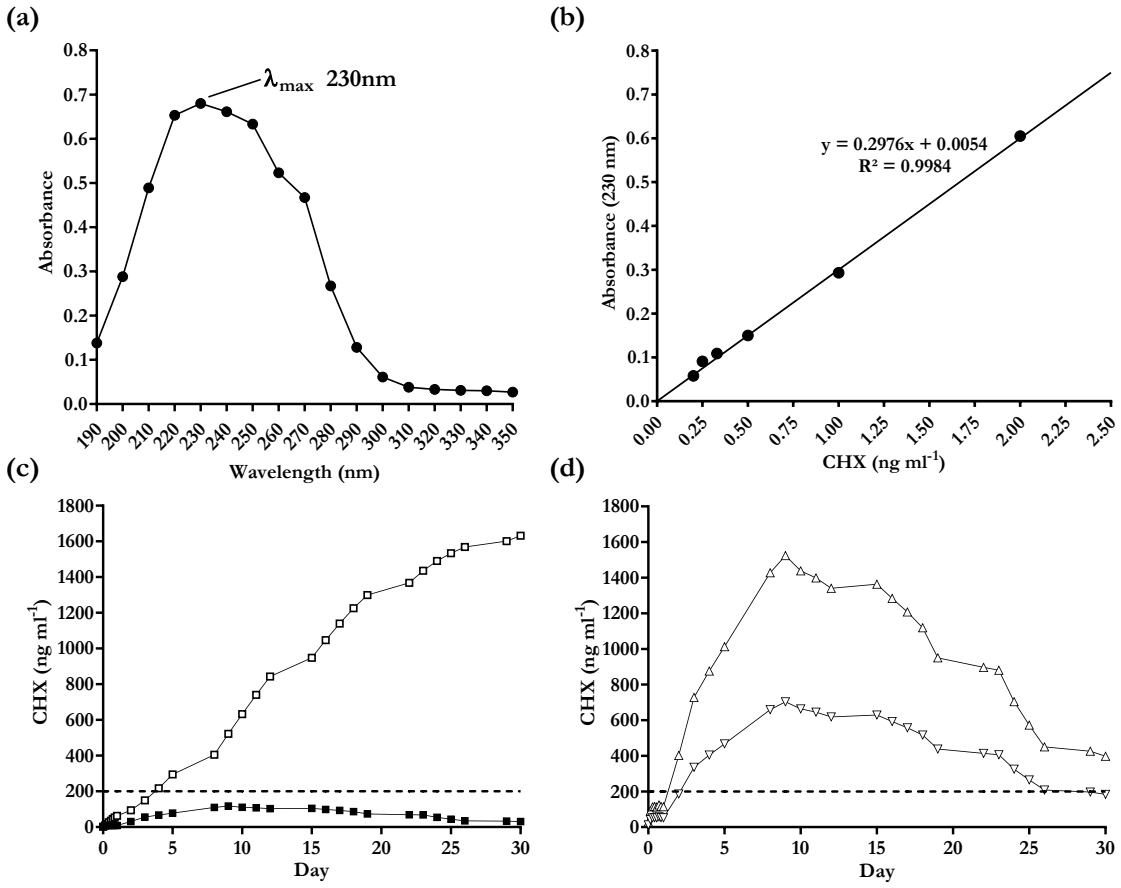


Figure 5.19 Drug Release trial of chlorhexidine diacetate (CHX): (a) determination of λ_{max}, (b) standard curve, (c) 30-day real-time (■) and cumulative (□) release of CHX from coated 10mm samples, (d) predicted 30 day CHX release from fully coated catheter (Catheter ID +CHX, △) and 50% coated catheters (Cather ID +CHX50, ▲). Dashed line indicates the minimum inhibitory concentration of CHX.

In combination, the data obtained from the serial plate transfer test and the drug release trial corroborated the 30 day release of CHX from the prototype coating. These results positively indicate that, at least within an *in vitro* setting, that the prototype CHX coating can be effective in inhibiting extraluminal migration and general bacterial growth for ≥ 30 days.

5.11 Stage 10: Dissemination

An original research article about the *in vitro* urethra extraluminal migration model and methodology is currently under review for publication and is entitled: "Prevention of extraluminal migration on indwelling urinary catheters: a novel *in vitro* model and method".

The developmental stages of this model were disseminated in an oral presentation at the Federation of European Microbiological Societies (FEMS) Online Conference on Microbiology (2020) in a presentation entitled: "A Novel *In vitro* Urethra Model to Visualise Bacterial Migration During Long-Term Urinary Catheter Insertion".

A poster entitled "Visualisation of Extraluminal Bacterial Migration on Indwelling Urinary Catheters: Development of a Novel *In vitro* Urethral Migration Model" was presented at the Microbiology Society's annual conference online (2021).

An interactive iposter presentation entitled "Prevention of Catheter-associated Urinary Tract Infections: A Novel *In vitro* Urethra Model to Observe Extraluminal Bacterial Motility" was presented at the World Microbe Forum (2021). This conference was a collaborative online conference held by the American Society for Microbiology (ASM) and the Federation of European Microbiological Societies (FEMS).

5.12 Conclusion

The primary aim of this study was to develop an *in vitro* model focused on the visualisation and quantification of extraluminal bacterial migration on urinary catheters with the ultimate aim of CAUTI prevention. In Chapter 3 the primary focus was the movement of bacteria from the meatus to the bladder by friction-mediated pathogen displacement during catheter insertion. The results of which, demonstrated that meatal contamination during catheter placement can directly contribute to CAUTI manifestation, and that reducing/eliminating friction could be a target to prevent CAUTIs. With this in mind, the *in vitro* extraluminal migration model was

designed to further study meatal colonisation as an origin for CAUTI and the role that catheters play in promoting movement of bacteria into the urinary tract.

The results obtained from the *in vitro* urethra extraluminal migration model, illustrate that a catheter provides a bridge for relatively fast movement of bacteria from a colonised meatus to the bladder. A urinary catheter, by nature of being a foreign entity, undermines the bodies innate defences to infections. In regard to extraluminal migration, the redirection of micturition through the catheter, prevents the regular flushing of the periurethral space providing an uninterrupted path for pathogens along the extraluminal catheter surface and the urethra epithelium (Vargas-Cruz *et al.,* 2019).

If early extraluminal migration can be delayed or prevented, the length of time a patient can be safely catheterised before infection occurs could be improved, or in a best-case scenario, the initiation of the infection can be avoided entirely improving clinical outcomes for patients. It is envisaged that the *in vitro* urethra extraluminal migration model will provide a robust tool to further study bacterial migration on urinary catheters and other medical devices as a target to prevent Healthcare Associated infections.

Chapter 6 Conclusions, Implications, and Future Considerations

6.1 Conclusions and Implications

In a world where great leaps in medical science, to fight a sudden pandemic, are entirely possible, common healthcare associated infections (HCAIs) continue to be problematic. Urinary catheters are ubiquitous in modern healthcare and catheter-associated urinary tract infections (CAUTIs) are one of the most common HCAIs. Unchanged for the majority of their history, urinary catheters have been described as "*healthcare's hidden scandal of neglect*" due to their prodigious impact on patient morbidity and mortality (Feneley *et al.*, 2015). Urinary catheters have been in use since 1500 B.C.E. according to historical records from Ancient China, Egypt, and Greece (Jeffery & Mundy, 2020). While the base materials have improved, with malleable materials first introduced in 1779; the advent of polymers and antimicrobials in modern medicine, led to contemporary catheters, which have remained predominantly unchanged since the first half of the 20th century (Singha *et al.*, 2017).

Innovation in medical devices can be stifled for any number of reasons be they regulatory, financial, ethical, or time constraints (Myers *et al.*, 2017). Oftentimes, the exorbitant cost of animal and clinical trials can prevent novel devices progressing from the design stage as there is no guarantee of a return on investment. *In vitro* testing offers a relatively fast and inexpensive alternative to *in vivo* testing and clinical trials (Myers *et al.*, 2017). While *in vitro* tests are not capable of fully representing the complexity of an *in vivo* setting, they can provide useful data to study specific mechanisms of infection within controlled settings. *In vitro* models are also highly adaptable and facilitate high throughput, allowing for changes to suit a variety of samples and multiple replicates with little increase in time or expense.

With urinary catheters specifically in mind, *in vitro* models facilitate the testing of emerging technologies and antimicrobials with relative ease permitting the screening of new ideas before committing to difficult and costly *in vivo*/clinical trials. This early screening can protect medical device manufacturers from wasting time and resources developing a dysfunctional new device by highlighting problems early. It can also reduce animals and potential clinical trial subjects by reducing the overall amount of testing needed and by ensuring that at least, within an *in vitro* setting, that the device/coating/additive is effective, non-toxic, and not likely to cause physical injury. The research strategies employed in this thesis are strongly informed by the Alternatives to Laboratory Animals (ATLA) philosophies which are enshrined in the European Directive on the protection of animals used for scientific purposes (2010/63) which can be summarised by the 3Rs principles *i.e.* (1) the reduction of animal testing, (2) the refinement of animal testing, (3) in the case of this thesis the replacement of animal testing.

Having comprehensively reviewed the extant literature on CAUTIs, the essence of this thesis emerged with the primary aim of developing an *in vitro* model of the urethra thus facilitating innovation in the field of urinary catheters, ultimately aiding Teleflex® with the design of two novel urinary catheters with the intention to prevent CAUTIs. The novel *in vitro* urethra model, and its adaptations, were created to study specific mechanisms of infection initiation as well as providing a tool to guide the development process for industry. Each model was intended to present industry and academia with a straightforward production and testing procedure that would require no specialist equipment and/or training whilst also producing accessible data that could be easily understood by any person be they professional or lay person. In the modern age of the internet, misinformation, and "fake news", accurate and accessible dissemination of research in imperative (Ross-Hellauer *et al.*, 2020). For this reason, in was important to create a model that did not only provide valuable quantitative data but that was also highly visual so that the results could be presented to and understood by anyone, not just microbiologists. Beyond the importance of communicating scientific research to the public, when developing a medical device, a litany of people needs to understand why your new product deserves their attention. This includes engineers, in house management, financiers, regulators, clinicians, and most importantly patients. It is easy to say the numbers go up or down so that proves ours is better, but to show someone an image where your device literally and visually stops an infection can be truly powerful. As scientists, we often get bogged down in the numbers, the words, the stats, when a picture can say a thousand words.

The *in vitro* urethra model provides an open canvas that could be adapted in the future to test any number of medical devices. While designed for urology, the basic design could be adapted to test medical devices in any number of medical fields such as cardiovascular vascular devices, orthopaedic hardware, endotracheal devices, *etc.* Basically, any medical device that connects the outside world to the inside of the body, or connects two internal microbiota, where microbial movement between the two environments in undesired. Adaptations to the physical structure of the model is as easy as adjusting the channel width or even geometry to suit the test device. The channel can be lengthened or shortened and is only limited by the size of the mould. The mechanism of infection to be studied and desired end point can also be modified to suit any study with considerations to the overall limitations of *in vitro* testing in general. The versatility of the base *in vitro* urethra model is only restricted by the imagination of the researcher and scope of their work. With this in mind, the IP associated with the *in vitro* urethral model was made publicly available in the interest of the advancement of public health microbiology and future improvements in CAUTI prevention.

While the primary aim of this project was the development of an *in vitro* urethra model, the secondary aim of elucidating the effects friction and extraluminal migration on CAUTI initiation became increasingly important. The results of the *in vitro* urethra friction model validation displayed a direct relationship between meatal colonisation, catheter-mediated friction, and potential CAUTI manifestation. It has been proposed in the past, that a CAUTI can be initiated by a single catheterisation, and the data produced by the *in vitro* urethra friction model this study further supports this theory (Barford *et al.*, 2008; Kaye *et al.*, 1962). The *in vitro* urethra friction model demonstrated that the simple act of inserting a urinary catheter can undermine attempts, by either the catheter's design or insertion practice, to prevent CAUTIs.

Meatal contamination is also critical to extraluminal migration. The *in vitro* urethra extraluminal migration model provides a highly visual method to study extraluminal migration and bacterial migratory patterns/speeds on implanted and *in situ* medical devices. Delay or prevention of extraluminal migration could increase the length of time a patient can be safely catheterised before infection occurs. In a best-case scenario, the initiation of the infection can be avoided entirely improving clinical outcomes for patients. The results obtained from the *in vitro* extraluminal migration model adds to the body of evidence that meatal contamination is a crucial target for CAUTI prevention.

In summary, the primary conclusions of this research study and its implications toward the fields of urology and healthcare engineering are:

- Pathogenic colonisation of the urethral meatus provides a reservoir of bacterial species whose introduction into the urinary tract by insertion of medical devices directly contributes to the manifestation of CAUTIs.

- Friction-mediated movement of bacteria during urinary catheter insertion introduces pathogenic organisms from the outside of the body into the urinary tract. The **novel *in vitro* urethra friction model** provides a robust tool to quantitatively study this phenomenon and direct the development of next-generation catheters to minimise or eliminate this route to infection.

- Reduction or elimination of fraction during urinary catheter insertion could prevent the initiation of CAUTIs directly impacting patient morbidity and mortality.

- Indwelling urinary catheters provide a bridge for pathogenic bacterial species to migrate from outside of the body into the bladder where an infection can then take hold. **The novel**

***in vitro* urethra extraluminal migration model** was developed to provide a model and method to visualise and quantify bacterial migration as a target to prevent CAUTI.

- Prevention of extraluminal migration on the surface of indwelling urinary catheters could prevent the initiation of CAUTIs directly impacting patient morbidity and mortality.
- The development of next-generation urinary catheters should focus on these critical control points to prevent the introduction of pathogenic bacterial species into the urinary tract, consequently preventing infections.

6.2 Future Considerations

In the future, it would be interesting to combine the methodologies of the *in vitro* urethra friction and extraluminal migration models as each model studies only one aspect of infection initiation in isolation. To increase the relevance and translational potential of the models, relevant uropathogenic strains could be used *e.g.*, *P. mirabilis*, *C. albicans*, *P. aeruginosa*, *etc.* in isolation or combination to study polymicrobial infections more consistent with clinical observations.

Regarding meatal contamination as a target for CAUTI prevention, more research is needed into non-antimicrobial solutions. Is there a non-antimicrobial way to prevent extraluminal migration long-term? Is there a non-antimicrobial way to control or modify the meatal microbiota minimising pathogenic colonisation? The increasing prevalence of antimicrobial resistant microorganisms is having a compounding impact on worldwide healthcare. The overuse of antimicrobials both within and outside of healthcare is of major concern to the World Health Organization and numerous national health organisations worldwide. The One Heath approach is focused the reduction of disease in animal and human ecosystems; present and emerging antimicrobial resistance is a key target of the One Health concept. With this in mind, the future of CAUTI research and urinary catheter innovation should strive to focus on non-antimicrobial infection prevention and mitigation rather than antimicrobial treatment.

In the future, it is envisaged that the novel *in vitro* urethra models described in this study, can have an impact on not just innovation in future catheter design, but also furthering the study of CAUTI to the ultimate benefit of all catheterised patients.

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Appendices

Appendix A Journal Publications

Hindawi
Journal of Healthcare Engineering
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Hindawi

Review Article

Review of Catheter-Associated Urinary Tract Infections and *In Vitro* Urinary Tract Models

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Catheter-associated urinary tract infections (CAUTIs) are one of the most common nosocomial infections and can lead to numerous medical complications from the mild catheter encrustation and bladder stones to the severe septicaemia, endotoxic shock, and pyelonephritis. Catheters are one of the most commonly used medical devices in the world and can be characterised as either indwelling (ID) or intermittent catheters (IC). The primary challenges in the use of IDs are biofilm formation and encrustation. ICs are increasingly seen as a solution to the complications caused by IDs as ICs pose no risk of biofilm formation due to their short time in the body and a lower risk of bladder stone formation. Research on IDs has focused on the use of antimicrobial and antibiofilm compounds, while research on ICs has focused on preventing bacteria entering the urinary tract or coming into contact with the catheter. There is an urgent need for *in vitro* urinary tract models to facilitate faster research and development for CAUTI prevention. There are currently three urinary tract models that test IDs; however, there is only a single very limited model for testing ICs. There is currently no standardised urinary tract model to test the efficacies of ICs.

1. Introduction

The urinary system is one of the main routes through which the human body excretes liquid waste. The urinary tract is divided into two sections: the upper tract consists of the kidneys and ureters, where liquid wastes from the body are converted into urine and other products; and the lower tract includes the bladder and urethra, where urine is stored in the bladder before being expelled from the body through the urethra [1]. The outermost section of the urethra and the tissue surrounding the urethral opening are known as the urethral meatus [2].

When functioning normally, the lower urinary tract flushes out the urethra as the bladder empties, preventing the movement of bacteria up from the periurethral skin into the urethra and then into the bladder [3]. If bacteria manage to enter the bladder of a healthy individual, they will usually be expelled during micturition. However, if they remain, the bladder's internal surface is resistant to bacterial attachment as it is lined with urothelial cells that are covered in a glycosaminoglycan mucin that prevents bacteria adhering to the internal bladder surface [3]. In the event that a bacterium bypasses this first line of defences, the immune system should be able to eliminate the bacteria as long as the patient is healthy. If the immune system fails, a urinary tract infection (UTI) can occur and possibly lead to serious illness [3]. When problems arise in the lower urinary tract such as nerve damage or muscle atrophy leading to incontinence, or by prostate enlargement or urethral stricture resulting in urinary retention, the use of a urinary catheter becomes a necessity [1, 3].

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ORIGINAL ARTICLE

Pathogen displacement during intermittent catheter insertion: a novel *in vitro* urethra model

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Keywords
E. coli (all potentially pathogenic types), infection, mechanism of action, microbial contamination, staphylococci.

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Abstract

Aim: To develop a novel *in vitro* urethra model and use it to determine if insertion of an intermittent urinary catheter (IC) displaces pathogenic bacteria from the urethral meatus along the urethra.
Methods: Displacement of microbial growth after catheter insertion was assessed using a novel *in vitro* urethra model. The *in vitro* urethra model utilized chromogenic agar and was inoculated with bacteria at one side of the artificial urethra channel, to act as a contaminated urethral meatus, before an IC was inserted into the channel. Three ICs types were used to validate the *in vitro* urethra model and methodology.
Results: When compared to the bacterial growth control, a significant difference in bacterial growth was found after insertion of the uncoated ($P \leq 0{\cdot}001$) and hydrophilic coated ($P \leq 0{\cdot}009$) catheters; no significant difference when a prototype catheter was inserted into the *in vitro* urethra model with either bacterial species tested ($P \geq 0{\cdot}423$).
Conclusion: The results presented support the hypothesis that a single catheter insertion can initiate a catheter-associated urinary tract infection.
Significance and Impact of the Study: The *in vitro* urethra model and associated methodology were found to be reliable and reproducible ($P \geq 0{\cdot}265$) providing new research tool for the development and validation of emerging technologies in urological healthcare.

Introduction

Urinary catheters are one of the most common devices used in modern medical treatment; however, they are also implicated in the majority of nosocomial infections, specifically, catheter-associated urinary tract infections also referred to as CAUTIs. The lower urinary tract consists of the bladder, urethra and urethral meatus (Gaonkar *et al.* 2003; Chapple 2011). When problems arise in the lower urinary tract, for example, urinary retention or incontinence, the use of a catheter can become an unavoidable necessity (Chapple 2011; Feneley *et al.* 2015). CAUTIs can cause a number of complications including catheter encrustation and bladder stones in the lower urinary tract; pyelonephritis and kidney stones in the upper urinary tract; and systemic complications including septicaemia and endotoxic shock (Jordan *et al.* 2015; Cortese *et al.* 2018).

Urinary catheters are categorized into two main groups: indwelling and intermittent (Cortese *et al.* 2018). Indwelling catheters are used for patients that require long-term catheterization and can remain in place for anywhere from a couple of hours to a maximum of 12 weeks in ideal conditions in the absence of infection (Cortese *et al.* 2018). Indwelling catheters can be inserted via the urethra into the bladder, that is, transurethral catheterization, or surgically through the abdominal wall, that is, suprapubic catheterization (Feneley *et al.* 2015; Cortese *et al.* 2018). Indwelling catheters historically have been plagued by CAUTIs and many of these issues are caused by bacterial biofilms by urease producing

Prevention of extraluminal migration on indwelling urinary catheters: a novel *in vitro* model and method

Running Head: Extraluminal migration model

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Abstract
Aim: To develop a novel *in vitro* extraluminal migration model to observe the migration of bacteria along indwelling urinary catheters within the urethra and assess the efficacy of a prototype chlorhexidine diacetate (CHX) coating to prevent this migration.
Methods: The *in vitro* urethra model utilised chromogenic agar, a catheter was inserted into each *in vitro* urethra. One side of the urethra was then inoculated with bacteria, to replicate a contaminated urethral meatus. The models were then incubated for 30 d, with the migration distance was recorded each day. Four indwelling catheter types were used to validate the *in vitro* urethra model and methodology.
Results: In the absence of CHX, *E. coli* and *S. aureus* migrated the entire length of the catheter within 24-48 h. In the presence of CHX, full migration of the channel was prevented for 30 d.
Conclusion: The results presented support the hypothesis that catheter-associated urinary tract infections (CAUTIs) could be prevented by targeting catheter-mediated extraluminal microbial migration from outside of the urinary tract into the bladder.
Significance and Impact: The *in vitro* extraluminal migration model and associated methodology were found to be reliable and reproducible ($P \geq 0.8510$), providing a new research tool to study extraluminal migration as a target to prevent CAUTIs.

Appendix B Conference Abstracts

Bioengineering in Ireland 2018

Development of an *in vitro* agar based urethral model for testing novel medical devices

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INTRODUCTION

Urinary catheters are one of the most commonly used medical devices, however throughout their history of use there has been difficulties in controlling concomitant bacterial infections. Catheter associated-urinary tract infections (CAUTIs) are one of the most common hospital-acquired infections (Nicolle, 2014). All catheter types and brands are vulnerable to pathogen contamination (Jacobsen *et al.*, 2008; Jordan *et al.*, 2015). CAUTIs can lead to numerous medical complications from mild which includes bacteriuria, catheter encrustation and bladder stones, to severe complications such as septicaemia, endotoxic shock, and pyelonephritis (Jordan *et al.*, 2015).

There is currently no standard established *in vitro* urethral model to test the efficacy of medical devices to prevent or control introduction/growth of bacteria into the urethra. This study aims to address this issue by developing a reproducible agar based urethral model.

MATERIALSAND METHODS

The urethral model consists primarily of a preformed channel within an agar based matrix. The urethral model utilises chromogenic agar, which indicates bacterial growth by changing the colour of the agar or the bacterial colonies themselves. Due to current intellectual property agreements, specific information of the urethral model production is not available to disseminate.

RESULTS

Following several trial and error experiments reproducible results were obtained using IP protected methodologies. Initial difficulties using the *in vitro* urethral models included contamination due to temporal exposure, condensation and bacterial swarming. After these issues were addressed, the *in vitro* model was used to test the efficacies of several commercially available and novel catheters to control bacterial growth (Figure 1).

DISCUSSION

Preliminary results from this model indicate that it performs better than a similar model developed by Gaonkar and Modak, (2003) which utilised soft agar. The model investigated venous catheters and is comparable it methodology and materials.

Figure 1 In vitro urethral models: Upper model inoculated with E.coli ATCC 25922. Lower model acted as a negative control. Entry point of catheter insertion indicated by Star.

Another study reported in a monograph by Holland and Fish, (2012) only represents the outermost portion of the urethra leading to the urethral meatus and tested the efficacies of their proprietary catheters preventing transfer of external microflora into the urethra. The model developed at Athlone Institute of Technology represents the entire length of the female urethra and shows the distribution of microflora throughout the urethra following the insertion of urethral catheters

The IP protected *in vitro* models are currently being extended to include ureter devices. It is hoped that this *in vitro* technology will provide a realistic model to aid the development and test the efficacies of urinary medical devices to reduce/eliminate CAUTIs.

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Bioengineering in Ireland 2019

IN VITRO MODEL OF BACTERIAL MIGRATION DURING URINARY CATHETER INSERTION

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INTRODUCTION

Urinary catheters are one of the most commonly used medical devices, however throughout their history of use there has been difficulties in controlling concomitant bacterial infections. Catheter associated-urinary tract infections (CAUTIs) are one of the most common hospital-acquired infections (Nicolle, 2014). All catheter types and brands are vulnerable to pathogen contamination (Jacobsen et al., 2008; Jordan et al., 2015). CAUTIs can lead to numerous medical complications from mild which includes bacteriuria, catheter encrustation and bladder stones, to severe complications such as septicaemia, endotoxic shock, and pyelonephritis (Jordan et al., 2015).

There is currently no standard established *in vitro* urethral model to test the efficacy of medical devices, in particular intermittent catheters, to prevent or control introduction/movement of bacteria into the urethra during device insertion. This study aims to address this issue by developing a reproducible agar based *in vitro* urethral model (Cortese et al., 2018).

MATERIALS AND METHODS

The urethral model consists primarily of a preformed channel within an agar based matrix. The urethral model utilises a specific agar formulation which indicates bacterial growth. The model producation and associated methodology are IP protected.

RESULTS

Major difficulties developing the *in vitro* urethral model have now been addressed. The in vitro model and associated methodology has been optimised with several commercially available catheters. Validation of the urethral model and methodology is currently ongoing and a paper is undergoing preparation for submission to a peer-review journal (Figure 1).

DISCUSSION

Numerous problems have been encountered during the development process. Poor visualisation of microbial growth was an initial problem which was solved with a change in media formulation. Temporal contamination was also a significant challenge and was solved by a redesign of the urethra model mould. Condensation and bacterial swarming were also significant issues, affecting primarily the reproducibility of the model. After 6 months of improvements, reproducibility issues have also been resolved. Finally, the channel width has been customised to accommodate the specific dimensions and structural differences of various intermittent catheter models to further lower variability. The urethra model is now generating reproducible data and is currently under validation with the aim to publish within one month.

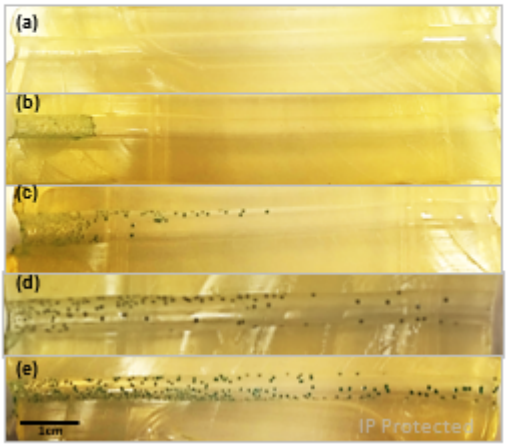

Figure 1 Cross sections of five *in vitro* Urethra channels. Bacterial growth can be identified in channels as blue colonies.
(a) Negative control – Sterile swab inserted
(b) Positive control – Inoculated with bacteria, no catheter inserted
(c) Inoculated with bacteria, Prototype catheter inserted
(d) Inoculated with bacteria, Un-coated PVC catheter inserted
(e) Inoculated with bacteria, Hydrophilic coated catheter inserted

With the exception of a meatal model reported in a monograph by Holland and Fish, (2012), there is currently no validated *in vitro* model to quantify the movement of bacteria in the urethra due to medical device insertion. The model developed at Athlone Institute of Technology aims to address this issue and provide a robust model to aid in the improvement of future urological devices focused toward preventing hospital acquired infections.

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FEMS Online Conference on Microbiology 2020

A Novel *In vitro* Urethra Model to Visualise Bacterial Migration During Long-Term Urinary Catheter Insertion

Background:
Urinary catheters are one of the most commonly used medical devices, however they are a common cause of hospital-acquired infections *i.e.,* Catheter Associated-Urinary Tract Infections (CAUTIs). All catheter types/brands are vulnerable to pathogen contamination. A primary source of pathogenic contamination is from the area just outside of the urethra known as the urethral meatus. From the meatus, bacteria can travel into the urinary tract by friction during insertion or migration along a catheter by either self-motility or capillary action.

Objective:
This study aimed to develop a reproducible urethral model to aid in the visualisation of bacterial migration on an indwelling urinary catheter and assess the efficacy of prototype antimicrobial catheters to prevent this migration.

Method:
The urethral model consists of a preformed channel within an agar-based matrix. The urethral model utilises chromogenic agar to visualise bacterial growth before quantification. The model production and associated methodology are IP protected.

Results:
Numerous problems have been overcome during the *in vitro* model development process. Bacterial swarming and condensation were addressed by a redesign of the urethra model mould and incubation conditions. Problems with poor oxygenation within the *in vitro* urethra was managed by a change in channel structure within the model. The urethra model is now generating reproducible data and is currently under validation with the aim to publish by the end of the year. The bacterial migration urethra model developed aims provide a robust model to aid in the improvement of future urological devices focused toward preventing hospital-acquired infections.

AIT Research Day 2018, and SURE 2018

Development of an *in vitro* agar based urethral model for testing novel medical devices

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INTRODUCTION

Urinary catheters are one of the most commonly used medical devices, however throughout their history of use there has been difficulties in controlling concomitant bacterial infections.

Catheter associated-urinary tract infections (CAUTIs) are the most common hospital-acquired infections. All catheter types and brands are vulnerable to pathogen contamination. CAUTIs can lead to numerous medical complications from mild which includes bacteriuria, catheter encrustation and bladder stones, to severe complications such as septicaemia, endotoxic shock, and pyelonephritis.

There is currently no standardised *in vitro* urethra model to test the efficacy of intermittent catheters to prevent CAUTIs. The aim of this project is to develop a robust, anatomically realistic, and reproducible model that can aid in the development and testing of novel urological devices.

MATERIALS AND METHODS

The urethral model consists primarily of a preformed channel within an agar based matrix. The urethral model utilises a specific agar formulation which indicates bacterial growth by colour of the agar or the bacterial colonies themselves.

Due to current intellectual property (IP) agreements specific information of the urethral model production or associated methodology is not available to disseminate.

RESULTS

Following several months of experimentation reproducible results were obtained using IP protected methodologies. Initial difficulties using the *in vitro* urethral model have now been addressed. The *in vitro* model and associated methodology has been optimised with several commercially available catheters.

DISCUSSION

There is currently no standardised *in vitro* urethra model to test the efficacy of intermittent catheters to prevent CAUTIs. The aim of this project is the development of a robust, anatomically realistic, and reproducible model that can aid in the development and testing of novel urological devices.

Numerous problems have been encountered during the development process. Poor visualisation was an initial problem solved with a change in media formulation. Temporal contamination was also a significant challenge and was solved by a redesign of the novel urethra model mould. Finally, condensation and bacterial swarming was a significant issue, affecting primarily the reproducibility of the model. After 6 months of improvements, this problem has also been resolved.

The urethra model is now generating reproducible data and is currently under validation with the aim to publish this year.

FEMS 8th Congress of European Microbiologists 2019

Demonstration of Bacterial Migration due to Medical Device Insertion in a Novel *In vitro* Urethral Model

Cortese, Y. [1, 2, *], Wagner, V.[3], Tierney, M.[3], Scully, D.[3], Devine, D.[1], Fogarty, A.[2]
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Background: Urinary catheters are commonly used medical devices, however throughout their history there has been difficulties in controlling concomitant bacterial infections. Catheter associated-urinary tract infections (CAUTIs) are common hospital-acquired infections. All catheter types and brands are vulnerable to pathogen contamination.

Objective: There is currently no standard established *in vitro* model to test the efficacy of medical devices, in particular intermittent catheters, to prevent or control introduction/movement of bacteria into the urethra during device insertion. This study aims to address this issue by developing a reproducible agar based *in vitro* urethral model.

Materials/Method: The urethral model consists primarily of a preformed channel within an agar based matrix. The urethral model utilises a specific agar formulation which indicates bacterial growth. The model production and associated methodology are IP protected.

Results: Numerous problems have been encountered during the *in vitro* model development process. Poor visualisation of microbial growth was an initial problem which was solved with a change in media formulation. Temporal contamination was also a significant challenge and was solved by a redesign of the urethra model mould. Condensation and bacterial swarming were also significant issues, affecting primarily the reproducibility of the model. After 6 months of improvements, reproducibility issues have been resolved. The urethra model is now generating reproducible data and is currently under validation with the aim to publish within one month. The model developed at Athlone Institute of Technology aims provide a robust model to aid in the improvement of future urological devices focused toward preventing hospital acquired infections.

Microbiology Society's Annual Conference 2021

Visualisation of Extraluminal Bacterial Migration on Indwelling Urinary Catheters: Development of a Novel *In vitro* Urethral Migration Model

Cortese, Y. J.; Fayne, J.; Devine, D.; Fogarty, A

Catheter-Associated Urinary Tract Infections (CAUTIs) are one of the most common healthcare-associated infections in the world and can lead to increased patient morbidity and mortality. Regarding indwelling urinary catheters, infection is regularly caused by bacterial migration along the intra- and extraluminal surfaces of the *in situ* catheter. Extraluminal migration allows bacteria to ascend, by either self-motility or capillary action, from the urethral meatus along the catheter surface to the bladder. It was the aim of this study to develop an *in vitro* model to study and visualise this extraluminal migration, and to provide a tool for the development of novel catheters and coatings to prevent extraluminal bacterial migration. The *in vitro* urethral migration model consists of a preformed channel within an agar-based matrix. The urethral model utilises chromogenic agar to visualise bacterial migration over an extended incubation period to mimic longterm catheterisation. During the early stages of model development, bacterial swarming and condensation presented a challenge which was addressed by a redesign of the urethra model mould and incubation conditions. Inadequate oxygenation within the *in vitro* urethra was addressed by modification of the urethra channel within the model. Preliminary tests have demonstrated reproducible results. The *in vitro* urethral migration model is currently under validation. The bacterial migration urethral model aims provide a robust tool to aid in the development of future urological devices focused toward preventing CAUTIs and improving clinical outcomes for patients.

World Microbe Forum 2021

Prevention of Catheter-associated Urinary Tract Infections: A Novel *In vitro* Urethra Model to Observe Extraluminal Bacterial Motility

Background

Catheter-associated urinary tract infections (CAUTI) are ubiquitous in worldwide healthcare settings(Cortese *et al.,* 2018). These infections negatively affect patient mortality and morbidity. Indwelling catheter infections are often initiated by bacterial migration on intra- or extraluminal surfaces from either a contaminated urine collection system or the urethral meatus(Summers & Goeres, 2019). In the majority of cases, extraluminal migration of bacteria is responsible for the ascension of bacteria from the urethral meatus to the bladder resulting in the initiation of a CAUTI.

Objectives

The aim of this study was to develop a novel *in vitro* model and methodology to visualise extraluminal migration on catheters, thus providing a robust tool to ultimately investigate the effects of antimicrobial treated catheters in preventing CAUTIs. If early extraluminal migration can be delayed or prevented, the length of time a patient can be safely catheterised before infection occurs could be improved, or in a best-case scenario, the initiation of the infection can be avoided entirely.

Methods

The *in vitro* urethra migration model comprises of preformed channels within a chromogenic agar-based matrix based on the model previously described by Cortese, *et., al* (Cortese *et al.,* 2020). Either a control or prototype indwelling catheter was inserted into each *in vitro* urethra channel. One side of the urethra channel was then inoculated along the outer rim to simulate pathogen colonisation of the urethral meatus. The model was then incubated for 30 days with observations recorded each day to assess the progress of bacteria along the extraluminal surface of the catheter.

Results

Challenges in the model development such as condensation, oxygen diffusion, and unintended bacterial swarming were controlled by modifications of the incubation environment and redesign of the model. Preliminary validation tests demonstrated reproducible results ($P \geq 0.147$). In a pilot study using catheters that were uncoated; coated with no antimicrobial; or coated with an antimicrobial additive; migration from the meatal side of the model to the bladder was observed to occur within 24-48 hours when using *E. coli* ATCC 25922 in the presence of no antimicrobial. In the presence of a novel catheter antimicrobial coating, complete extraluminal migration to the bladder side was prevented for 30 days. It is envisaged that this model will aid innovation in indwelling catheter design focused on improving clinical outcomes for patients and preventing CAUTIs.

Microbiology Society Annual Conference 2020

MICROBIOLOGY SOCIETY

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Meeting Report | Open Access

A novel *in vitro* urethra model to demonstrate bacterial displacement during urinary catheter insertion

Yvonne J. Cortese[1], Victoria E. Wagner[2], Morgan Tierney[3], David Scully[3], Declan M. Devine[1], Andrew Fogarty[4]

View Affiliations

First Published: 10 July 2020 | **https://doi.org/10.1099/acmi.ac2020.po0509**

Background: There is currently no standard established *in vitro* model to test the efficacy of intermittent catheters to prevent or control introduction/movement of bacteria into the urethra during device insertion. This study aimed to address this issue by developing a reproducible agar based *in vitro* urethral model.

Method: A novel *in vitro* model and testing method was developed to quantify the displacement of bacterial growth after intermittent catheter insertion.The urethral model consists primarily of a preformed channel within a specifically formulated agar based matrix. The urethra model was inoculated at one side of the channel to act as the urethral meatus, a catheter was then inserted. After incubation the bacteria within the urethra channel was quantified.

Results: Once optimised, the model produced reliable and reproducible results with both *E. coli* and *S. aureus* ($P \geq 0.265$). The model was used to test three different intermittent catheter types. When compared to the growth control there was a significant difference in bacterial distribution when inserting an uncoated ($P \leq 0.001$) or hydrophilic coated ($P \leq 0.009$) catheter; there was no significant difference when a prototype catheter was inserted with either bacterial species used ($P \geq 0.423$).

Conclusion: These findings support the hypothesis that a single catheter insertion can initiate a catheter-associated urinary tract infection. The *in vitro* urethra model and associated methodology provide a new research tool for the development and validation of emerging technologies in urological healthcare.

Published Online: 10/07/2020

Appendix C Conference Posters

FEMS 8th Congress of European Microbiologists 2019

Demonstration of Bacterial Migration due to Medical Device Insertion in a Novel In vitro Urethral Model

Yvonne J. Cortese [1, 2,*], Declan Devine [1], Victoria E. Wagner [4], David Scully [4], Morgan Tierney [4], Andrew Fogarty [2,3]

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Introduction

Urinary catheters are one of the most commonly used medical devices, however throughout their history of use there has been difficulties in controlling concomitant bacterial infections. Catheter associated-urinary tract infections (CAUTIs) are one of the most common hospital-acquired infections [1].

All catheter types and brands are vulnerable to pathogen contamination [2,3]. CAUTIs can lead to numerous medical complications from mild including: bacteriuria, catheter encrustation and bladder stones; to severe complications such as: septicaemia, endotoxic shock, and pyelonephritis [3].

There is currently no standard established *in vitro* urethral model to test the efficacy of intermittent catheters designed to prevent/control introduction of bacteria into the urethra and bladder [4]. It was the aim of this study to address this issue by the development of a reproducible *in vitro* agar based urethral model and to evaluate the hypothesis that insertion of a urinary catheter can push bacteria from the urethral meatus up the urethra channel.

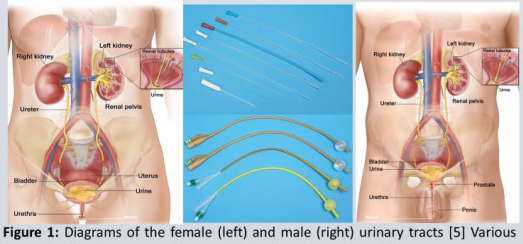

Figure 1: Diagrams of the female (left) and male (right) urinary tracts [5] Various intermittent catheter types (top centre), various indwelling catheter types (bottom centre) [6,7].

Model Development

The *in* vitro urethra model consisted of a preformed channel in an agar based matrix. Numerous challenges were encountered during the development process, as can be seen in **Figure 2**. Once these were overcome, the model was validated with three different catheter types.

To mimic urethral meatal contamination, one side of each positive control and test channel was inoculated with bacteria, while the negative control did not contain bacteria. For each test channel, a catheter was then inserted into the channel. After incubation, the *in vitro* urethra model was dissected and the bacteria were recovered from the agar surface before quantification. The bacterial growth patterns for each catheter were then compared to the positive control to determine if introduction of the catheter changed bacterial distribution throughout the urethra.

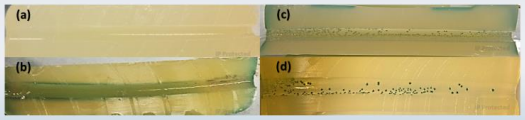

Figure 2: The four main developmental milestones of the *in vitro* urethra model in chronological order.

Acknowledgements
Funding for this project was provided by the Athlone Institute of Technology President Seed Fund in conjunction with an industry partnership with Teleflex.

Results

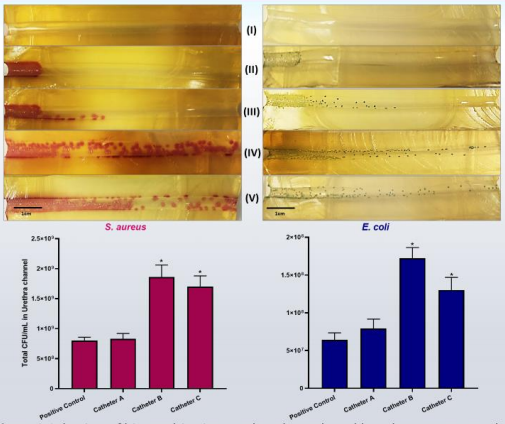

Figure 3 Selection of bisected *in vitro* urethra channels and bar chart represents the average CFU/mL recovered from entire length of the in vitro urethra, i.e. the sum total from all octants, for three intermittent catheter types. Statistically significant difference from the positive control, i.e. $P \leq 0.05$, is indicated by an asterisk (*). Pink bacterial colonies are *S. aureus*, blue colonies shown are *E. coli*. The photographs above display an example of urethra channels used for the testing of negative controls (I), positive controls (II), Catheter A (III), Catheter B (IV), and Catheter C (V). Scale bars represent ~1cm. N = 6

Discussion

Numerous problems were encountered during the development process. As can be seen in **Figure 2** in photo **(a)**, the poor visualisation of bacterial growth was solved with a change in media formulation. Temporal contamination **(b)** was also a significant challenge and was solved by a redesign of the novel urethra model mould. Finally condensation and bacterial swarming **(b,c)** were the main issues, affecting primarily the reproducibility of the model. After 6 months of improvements, this problem has also been resolved, **(d)**.

Urethra model and methodology have now been validated with 2 bacterial species and 3 catheter types, in 12 independent tests, **Figure 3**. The reproducibility of the model and methodology was assessed and there was no significant difference found between replicates for each bacterial strain ($P \geq 0.265$).

When comparing the bacterial distribution in the *in vitro* urethra channels between the positive control and the test samples, it was found that the catheters A and B changed the bacterial growth patterns significantly when using either bacterial strain ($P < 0.009$). The results for catheter C revealed no significant difference from the positive control was found when a prototype catheter was inserted into the in vitro urethra model with either bacterial species tested ($P \geq 0.423$).

Previous studies have proposed that a CAUTI can be initiated by a single catheter insertion and this study further backs that theory by demonstrating that catheter insertion does move bacteria from the meatus, along the urethra, and potentially into the bladder [8,9]. Hopefully this model can be a useful tool in the future development of urinary catheters and other devices that can prevent or eliminate the movement of bacteria during medical device insertion.

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AIT Research Day, April 2019

Catheter Type Affects Bacterial Migration During Insertion Into A Novel *In Vitro* Urethral Model

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Introduction

Urinary catheters are one of the most commonly used medical devices, however throughout their history of use there has been difficulties in controlling concomitant bacterial infections. Catheter associated-urinary tract infections (CAUTIs) are one of the most common hospital-acquired infections, accounting for 22.5% of all healthcare acquired infections (HCAI) in Ireland and 30% in other reporting European countries in 2006 [1,2].

All catheter types and brands are vulnerable to pathogen contamination [3,4]. CAUTIs can lead to numerous medical complications from mild including: bacteriuria, catheter encrustation and bladder stones; to severe complications such as: septicaemia, endotoxic shock, and pyelonephritis [4].

There is currently no standard established *in vitro* urethral model to test the efficacy of intermittent catheters designed to prevent/control introduction of bacteria into the urethra and bladder [5]. The aim of this study is to address this issue by the development of a reproducible *in vitro* agar based urethral model and to evaluate the hypothesis that insertion of a urinary catheter can push bacteria from the urethral meatus up the urethra channel.

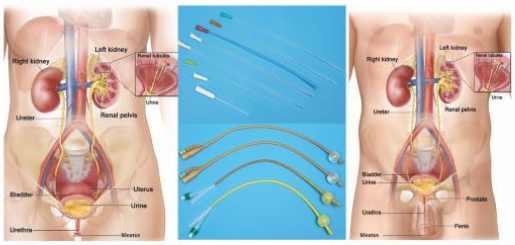

Figure 1: Diagrams of the female (left) and male (right) urinary tracts [6] Various intermittent catheter types (top centre), various indwelling catheter types (bottom centre) [7,8].

Model and Methodology Development

The *in* vitro urethra model consisted of a preformed channel in an agar based matrix. Numerous challenges were encountered during the development process, as can be seen in **Figure 2**. Once these were overcome, the model was validated with three different catheter types.

To mimic urethral meatal contamination, one side of each positive control and test channel was inoculated with bacteria, while the negative control did not contain bacteria. For each test channel, a catheter was then inserted into the channel. After incubation, the *in vitro* urethra model was dissected and the bacteria were recovered from the agar surface before quantification. The bacterial growth patterns for each catheter were then compared to the positive control to determine if introduction of the catheter changed bacterial distribution throughout the urethra.

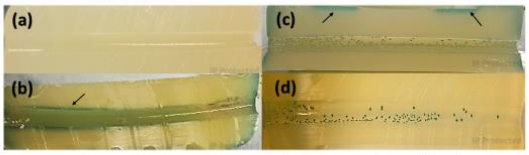

Figure 2: The four main developmental milestones of the *in vitro* urethra model in chronological order. **(a)** The poor visualisation of bacterial growth was solved with a change in media formulation. **(b)** Temporal contamination was also a significant challenge and was solved by a redesign of the novel urethra model mould. **(c)** Condensation and bacterial swarming were the main issues, affecting primarily the reproducibility of the model, see arrows. **(d)** Validation of the model was carried out.

Results

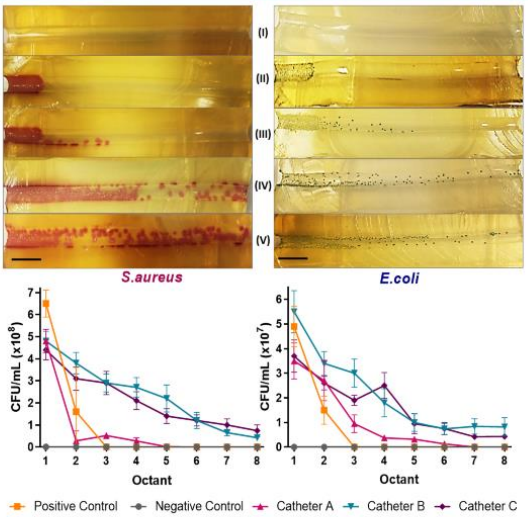

Figure 3 Selection of bisected *in vitro* urethra channels and scatter plots displaying the average CFU/ml of bacteria recovered from each *in vitro* urethral octant. Octant 1 and part of 2 is the site of inoculation. Pink bacterial colonies are *S. aureus*, blue colonies shown are *E. coli*. The photographs above display an example of urethra channels used for the testing of negative controls (I), positive controls (II), Catheter A (III), Catheter B (IV), and Catheter C (V). N = 6, scale bars represent ~1cm.

Discussion

Urethra model and methodology have now been validated with 2 bacterial species and 3 catheter types, in 12 independent tests, **Figure 3**. The reproducibility of the model and methodology was assessed and there was no significant difference found between replicates for each bacterial strain ($P \geq 0.265$).

When comparing the bacterial distribution in the *in vitro* urethra channels between the positive control and the test samples, it was found that the catheters A and B changed the bacterial growth patterns significantly when using either bacterial strain ($P < 0.000$). The results for catheter C were comparatively variable, with a significant difference in bacterial growth patterns found when using *E. coli* ($P = 0.001$) and no significant difference when using *S. aureus*, when not adjusted for ties ($P = 0.064$).

Previous studies have proposed that a CAUTI can be initiated by a single catheter insertion and this study further backs this theory by demonstrating that catheter insertion does move bacteria from the meatus, along the urethra, and potentially into the bladder [9,10]. It is envisaged that this model will be a useful tool in the future development of urinary catheters and other devices that can prevent or eliminate the movement of bacteria during medical device insertion.

Acknowledgements

Funding for this project was provided Jointly by Athlone Institute of Technology and Teleflex through the AIT Industrial President Seed Fund.

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AIT Research

AIT Research Day, November 2019

In vitro urethra model to demonstrate bacterial migration during medical device insertion

Yvonne J. Cortese [1, 2,*], Victoria E. Wagner [4], David Scully [4], Morgan Tierney [4], Declan M. Devine [1,*], Andrew Fogarty [2,3]

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Introduction

Catheter associated-urinary tract infections (CAUTIs) are one of the most common hospital-acquired infections [1]. There is currently no standard established *in vitro* urethral model to test the efficacy of intermittent catheters designed to prevent/control introduction of bacteria into the urethra and bladder [2].

It was the aim of this study to address this issue by the development of a reproducible *in vitro* agar based urethral model and to evaluate the hypothesis that insertion of a urinary catheter can push bacteria from the urethral meatus up the urethra channel.

Model Development

The *in vitro* urethra model consisted of a preformed channel in an agar based matrix. Numerous challenges were encountered during the development process, as can be seen in **Figure 2**. Once these were overcome, the model was validated with three different intermittent catheter types.

To mimic urethral meatal contamination, one side of each positive control and test channel was inoculated with bacteria, while the negative control did not contain bacteria. For each test channel, a catheter was then inserted into the channel. After incubation, the *in vitro* urethra model was dissected and the bacteria were recovered from the agar surface before quantification. The bacterial growth patterns for each catheter was then compared to the growth control to determine if introduction of the catheter changed bacterial distribution throughout the urethra.

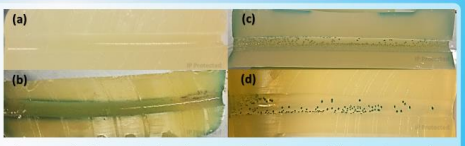

Figure 1: The four main developmental milestones of the *in vitro* urethra model in chronological order.

Results

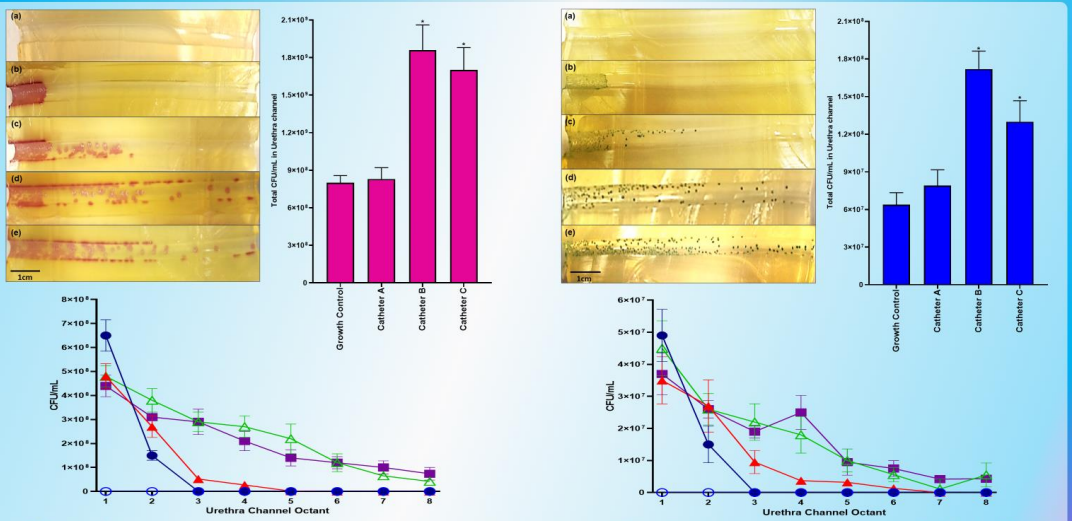

Figure 2 Selection of bisected *in vitro* urethra channels, a scatter plot, and bar chart displaying the average CFU/ml of bacteria recovered from each *in vitro* urethral octant and the total bacteria isolated from the entire urethra channel. Octant 1 and part of 2 represented the site of inoculation. The photographs above display a representative example of urethra channels used for the testing of the sterility control (a, ○), bacterial growth control (b, ●), catheter A (c, ▲), catheter B (d, △), and catheter C (e, ■). All data and images to the left were produced with *S. aureus*. All data and images to the right were produced with *E. coli*. n = 6, $*P \leq 0.05$

Discussion

Numerous problems were encountered during the development process. As can be seen in **Figure 1 (a)**, the poor visualisation of bacterial growth was solved with a change in media formulation. Temporal contamination **(b)**, was also a significant challenge and was solved by a redesign of the novel urethra model mould. Finally condensation and bacterial swarming **(b, c)**, were the main issues, affecting primarily the reproducibility of the model. After 6 months of improvements, this problem was resolved, **(d)**.

The urethra model and methodology have now been validated with 2 bacterial species and 3 catheter types, in 12 independent tests, **Figure 2**. The reproducibility of the model and methodology was assessed and there was no significant difference found between replicates for each bacterial strain ($P \geq 0.265$).

When comparing the bacterial distribution in the *in vitro* urethra channels between the growth control and the test samples, it was found that the **catheters B** and **C** changed the bacterial growth distribution significantly when using either bacterial strain ($P < 0.009$). The results for **catheter A** revealed no significant difference in bacterial growth distribution from the growth control with either bacterial species tested ($P \geq 0.423$).

It is envisaged that this model will be a useful tool in the future development of urinary catheters and other devices that can prevent or eliminate the movement of bacteria during medical device insertion.

Acknowledgements
Funding for this project was provided by the Athlone Institute of Technology President Seed Fund in a collaborative industry partnership with Teleflex.

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Digital AIT Research Day 2020

Development of a novel in vitro urethra model for improved medical use and research innovation

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Background

Urinary tract infections are one of the most common hospital-acquired infections (HAIs) at ~30% within the EU with Catheter-Associated Urinary Tract Infections (CAUTIs) accounting for 80% of these infections [1,2]. There is currently no standardestablished *in vitro* urethral model to test the efficacy of intermittent catheters designed to prevent/controlintroduction of bacteria into the urinary tract system and prevent CAUTIs [3]. **It was the aim of this cross-disciplinary study to address this issue by the development of a reproducible *in vitro* agar based urethral model and to evaluate the hypothesis that insertion of a urinary catheter can push bacteria from the urethral meatus up the urethra channel.**

Model Design and Methodology

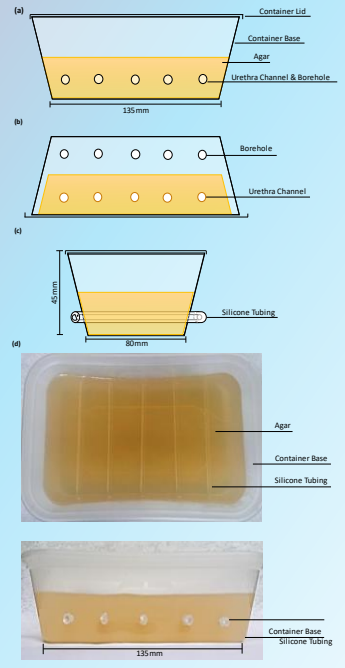


Figure 1 Model Design – The *in vitro* urethra model consisted of a preformed channel in an agar based matrix. To form the model mould, parallel holes were bored into a polypropylene container and silicone tubing was threaded through the parallel bore holes (a, c). The model mould was sterilised before molten agar was poured into the mould (d). Once the agar was set the silicone tubing was removed and the mould was inverted (b) to allow release of the *in vitro* urethra model from the mould.

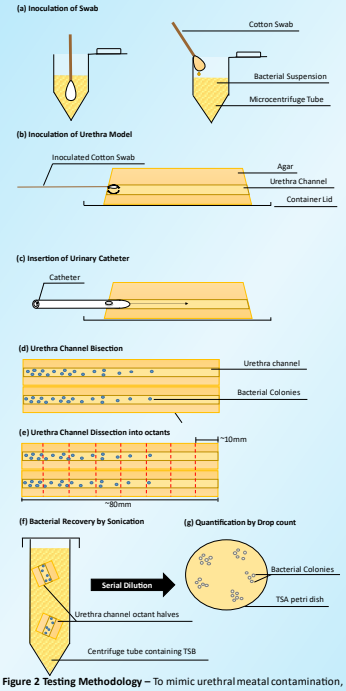
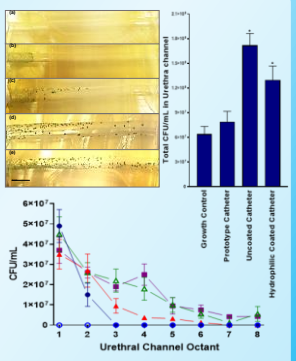


Figure 2 Testing Methodology – To mimic urethral meatal contamination, one side of each positive control and test channel was inoculated with bacteria, while the negative control was swabbed with sterile media (a, b). For each test channel, a catheter was then inserted into the channel, left in place form 30 seconds then removed and discarded (c). All models were incubated at 37°C. After incubation, the *in vitro* urethra model was dissected (d, e) and the bacteria were recovered from the agar surface (f) before quantification (g).

Results



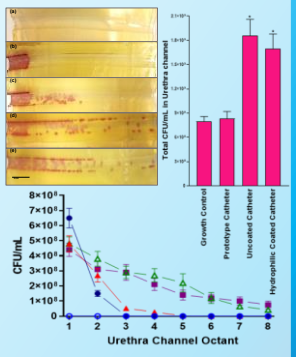


Figure 3 Selection of bisected *in vitro* urethra channels, a scatter plot, and bar chart displaying the average CFU/ml of bacteria recovered from each *in vitro* urethral octant and the total bacteria isolated from the entire urethra channel. The photographs above display a representative example of urethra channels used for the testing of the sterility control (a, ○), bacterial growth control (b, ●), prototype catheter (c, ▲), uncoated PVC catheter (d, △), and hydrophilic coated catheter (e, ■). All data and images in the top set were produced with *E. coli*. All data and images in the bottom set were produced with *S. aureus*. n = 6, *$P \leq 0.05$

Discussion

The *in vitro* urethra model described in this study has been validated through 12 independent tests with 2 bacterial species to ensure the reproducibilityof the model and methodology,and versatility with both Gram positive and negative species. There was no significant difference found between replicates for each bacterial strain ($P > 0.200$) indicating that the methodology and model was reproducible. When comparing the bacterial distribution in the *in vitro* urethra channels between the growth control and the test samples, it was found that the **uncoated** and **hydrophilic coated catheters** changed the bacterial growth distribution significantly when using either bacterial strain ($P < 0.009$). The results for the **prototype catheter** revealed no significant difference in bacterial growth distribution from the growth control with either bacterial species tested ($P \geq 0.423$).

The results presented in this study demonstratethat movement of bacteria from a contaminatedmeatus into the urinary tract is likely due to the insertion of urinary catheters **(Figure 3)**. It has been proposed in the past that a CAUTI can be initiated by a single catheterisation and this study further supports this theory, demonstrating that the simple act of inserting a urinary catheter can undermine attempts by either the catheter's design or insertion practice to prevent CAUTIs [4, 5].

It is envisaged that this model will be a useful tool for industry/academiain the future developmentof urinary catheters and other devices that can prevent or eliminatethe movement of bacteria during medical device insertion. This model and methodology has been publishedin the Journal of Applied Microbiology [5].

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[6] Y. J. Cortese, V. E. Wagner, M. Tierney, D. Scully, D. M. Devine, and A. Fogarty, "Pathogen displacement during intermittent catheter insertion: a novel in vitro urethra model," *Appl. Microbiol.*, vol. 128, no. 4, pp. 1191-1200, 2020, doi: 10.1111/jam.14533.

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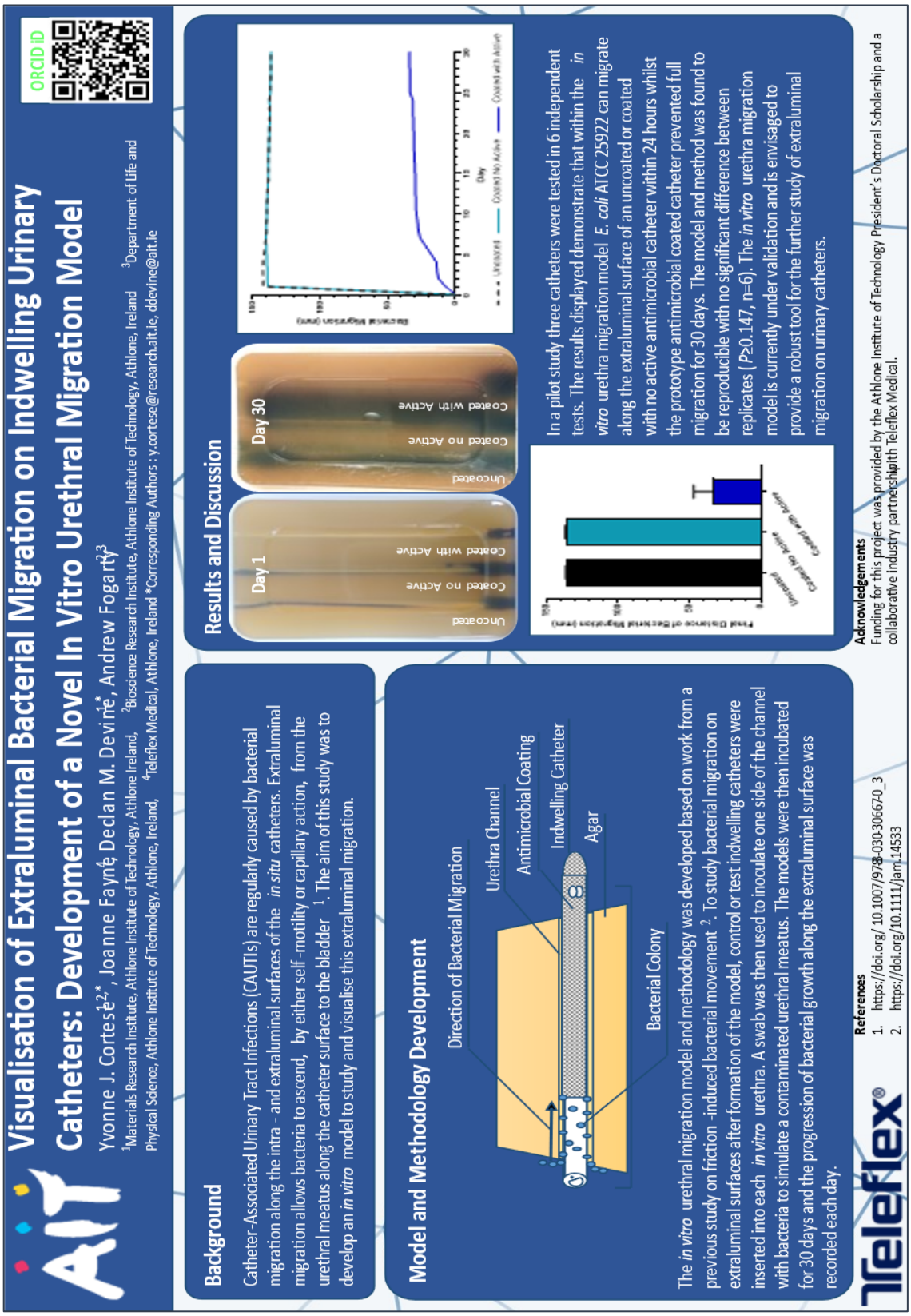
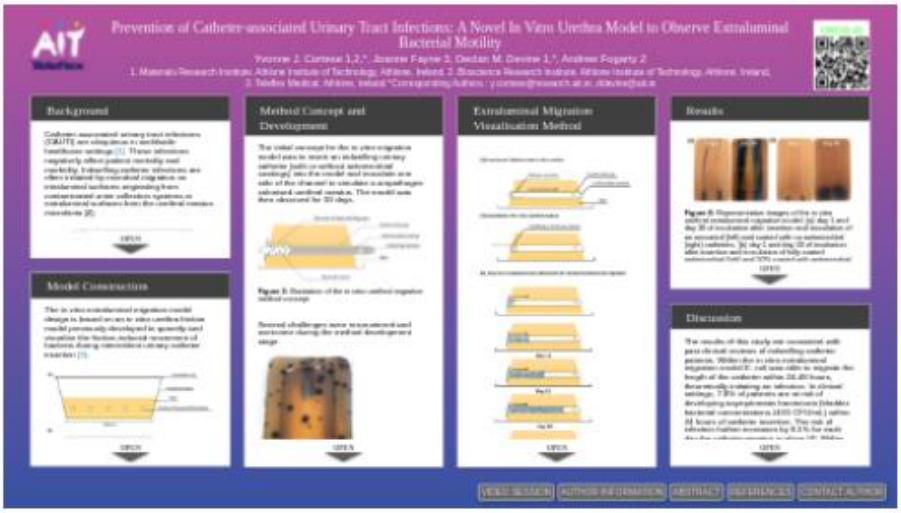
Microbiology Society's Annual Conference 2021



World Microbe Forum

Prevention of Catheter-associated Urinary Tract Infections: A Novel In Vitro Urethra Model to Observe Extraluminal Bacterial Motility



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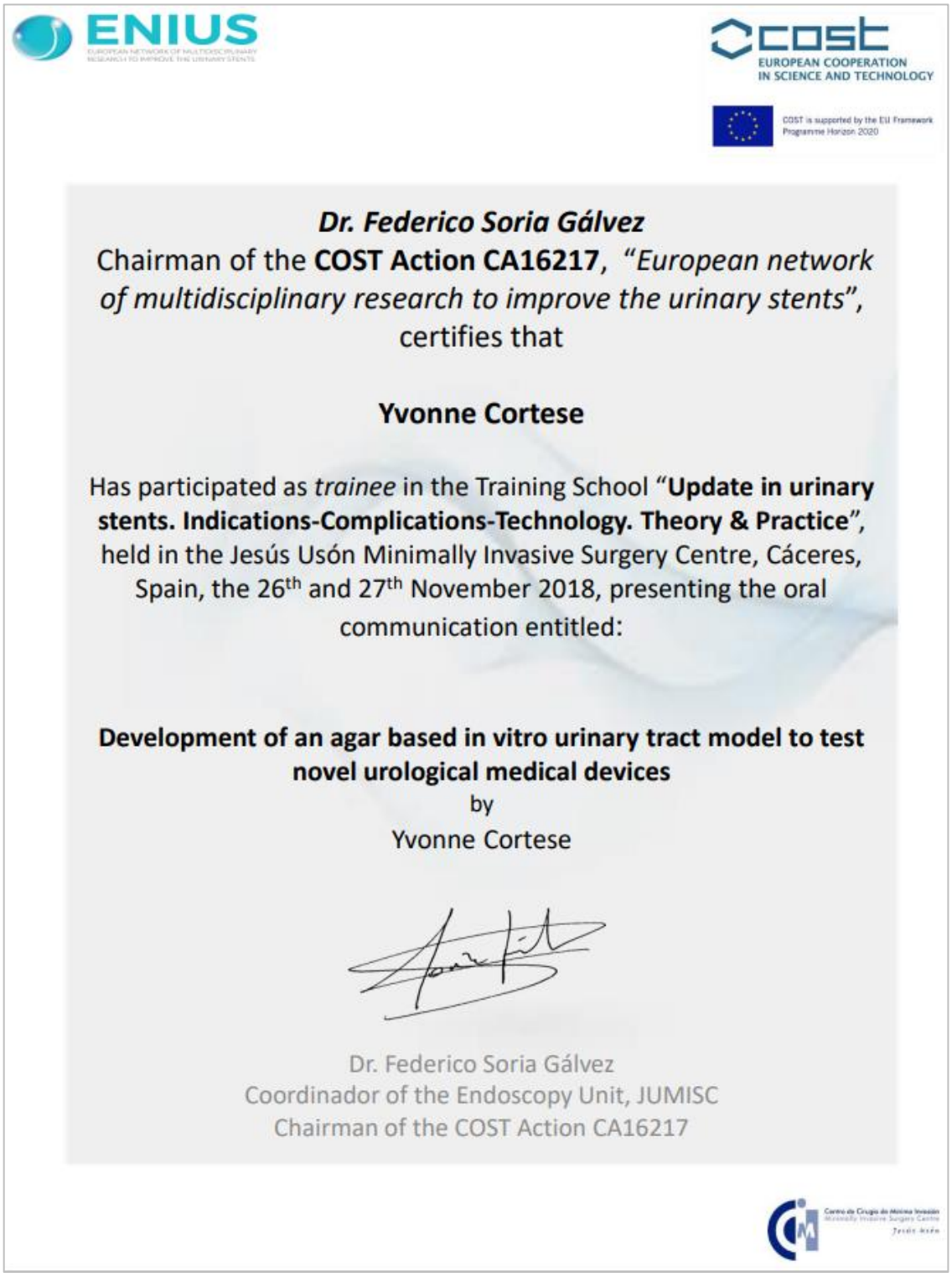
Appendix D Certificates



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Dr. Federico Soria Gálvez

Chairman of the **COST Action CA16217**, *"European network of multidisciplinary research to improve the urinary stents"*, certifies that

Yvonne Cortese

Has participated as *trainee* in the Training School "**Update in urinary stents. Indications-Complications-Technology. Theory & Practice**", held in the Jesús Usón Minimally Invasive Surgery Centre, Cáceres, Spain, the 26th and 27th November 2018, presenting the oral communication entitled:

Development of an agar based in vitro urinary tract model to test novel urological medical devices

by

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Yvonne Cortese

Has participated as *trainee* in the Virtual Workshop "***Latest advances in Urinary Stents. Biomaterials, Technology and Coatings.***", held from 23rd-24th March 2021, presenting the oral communication entitled:

Extraluminal Migration of Pathogens on Urological Medical Devices: A Novel *In Vitro* Model

by

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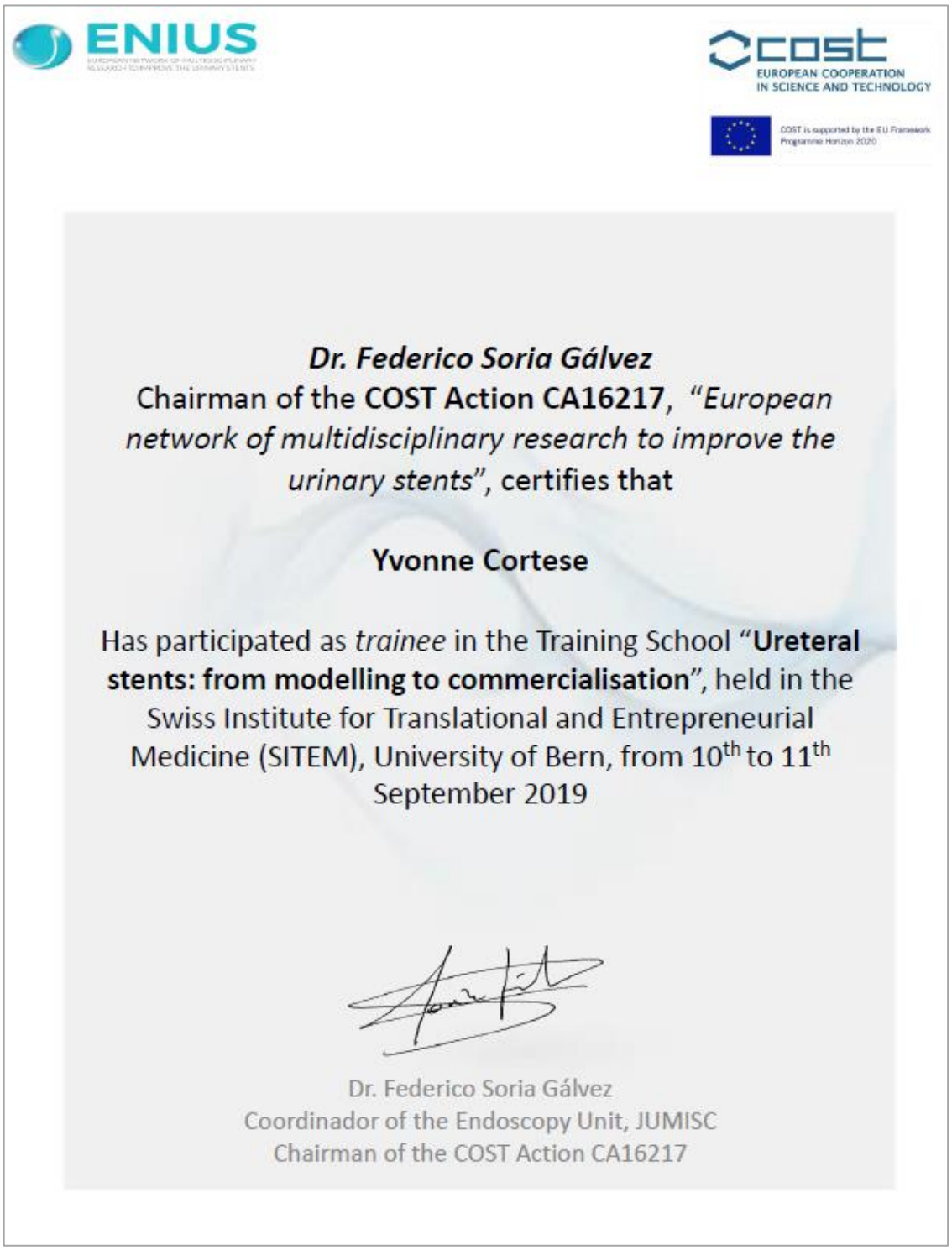

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Yvonne Cortese

Has participated as *trainee* in the Training School "**Ureteral stents: from modelling to commercialisation**", held in the Swiss Institute for Translational and Entrepreneurial Medicine (SITEM), University of Bern, from 10th to 11th September 2019

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This is to certify that

yvonne cortese

Presented an iPoster at World Microbe Forum on June 20-24, 2021

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AMiCI Training School

Assessment of the effectivity of antimicrobial coatings

Riga, Latvia

Certificate of Attendance

This is to certify that

Cortese, Yvonne

has attended the AMiCi (CA15114 - Anti-Microbial Coating Innovations to prevent infectious diseases) Training School "Assessment of the effectivity of antimicrobial coatings (AMCs)" in Riga, Latvia 29-31 January 2020.

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