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

Running Head: Novel *in vitro* urethra model

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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 utilised 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 ($P \leq 0.001$) and hydrophilic coated ($P \leq 0.009$) catheters; no significant difference when a prototype catheter was inserted into the *in vitro* urethra model with either bacterial species tested ($P \geq 0.423$).

Conclusion: The results presented support the hypothesis that a single catheter insertion can initiate a catheter-associated urinary tract infection.

Significance and Impact: The *in vitro* urethra model and associated methodology were found to be reliable and reproducible ($P \geq 0.265$) providing new research tool for the development and validation of emerging technologies in urological healthcare.

Key Words

E.coli (all potentially pathogenic types), Infection, Microbial contamination, Staphylococci, Mechanism of action

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, *e.g.* 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 categorised into two main groups: indwelling and intermittent (Cortese et al., 2018). Indwelling catheters are used for patients that require long term catheterisation 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 *i.e.* transurethral catheterisation, or surgically through the abdominal wall, *i.e.* suprapubic catheterisation (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 pathogens that lead to crystalline biofilm formations, ultimately blocking the catheter and contributing to recurrent infections (Feneley et al., 2002, 2015; Norsworthy and Pearson, 2017). Numerous studies have been carried out to test new catheter materials, antimicrobials, natural compounds, alternative catheter designs, probiotics, and patient care regimes in an effort to reduce or prevent CAUTIs related to indwelling catheters with varying levels of success (Hull et al., 2000; Hentzer et al., 2002; Stickler et al., 2002; Brosnahan et al., 2004;

Mathur et al., 2006; Stickler, 2014; Levering et al., 2016; Cortese et al., 2018). There are several *in vitro* models to test the efficacy of indwelling catheters to prevent either biofilm formation, encrustation, or CAUTI development (Stickler DJ et al., 1987; King et al., 1992; Morris et al., 1997; Stickler et al., 1999; Gaonkar et al., 2003; Barford et al., 2008; Williams and Stickler, 2008; Coenye and Nelis, 2010; Jordan et al., 2015; Chua et al., 2017; Rosenblatt et al., 2017; Cortese et al., 2018). There are two main anatomical *in vitro* models that have been developed to test indwelling catheters *i.e.* the bladder model described by Stickler *et al.*, (Stickler DJ et al., 1987; Stickler et al., 1999) and the urinary tract model developed by Gaonkar *et al.*, (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 (King et al., 1992; Morris et al., 1997; Barford et al., 2008; Williams and Stickler, 2008; Jordan et al., 2015; Chua et al., 2017; Rosenblatt et al., 2017).

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 insertion time (Cortese et al., 2018; 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 (Cortese et al., 2018). 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 (Woodward and Rew, 2003; Hudson and Murahata, 2005; Van Achterberg et al., 2008; Holland and Fish, 2012; Cortese et al., 2018; Goetz et al., 2018). The idea that a CAUTI can be initiated by insertion of a single catheter with a contaminated tip was proposed by Kaye *et al.*, in 1962 (Kaye et al., 1962) and

is supported by the more recent research of Barford *et al.*, (Barford et al., 2008). With the inhibition of bacterial movement, from outside of the body in, acting as a primary target for intermittent catheter CAUTI prevention, an *in vitro* model that can demonstrate the bacterial movement, or lack thereof, during catheter insertion could prove a useful tool in novel device development. With the exception of a meatal model described in a white paper by Holland and Fish (Holland and Fish, 2012), there is currently no validated *in vitro model* to specifically test intermittent catheters and the efficacy of these claims (Cortese et al., 2018).

With this gap in the research previously identified, the purpose of this study was to develop a reproducible and robust *in vitro* urethra model to investigate the hypothesis that the ascension of bacteria along the urethra due to intermittent catheter insertion can contribute to CAUTI initiation (Cortese et al., 2018).

Materials and Methods

IN VITRO URETHRA MODEL

The *in vitro* urethra model consisted of preformed channels in chromogenic agar. The mould for the urethra model was constructed from a polypropylene container with a lid and twin parallel boreholes on either side. Silicone tubing was threaded through the corresponding boreholes (Figure 1). The silicone tubing used was the same diameter as the catheter to be tested to ensure an interference fit, *i.e.* complete surface contact, between the catheter and the agar urethra channel. There were five channels per mould allowing for a sterility control, bacterial growth control, and three test channels. The sterility control, bacterial growth control, and two of the test chambers were 4.667mm in diameter or 14 French and one test channel was 4mm in diameter or 12 French to ensure full surface contact with the catheter samples used. The mould was then sterilised by

115 autoclaving in a paper backed sterilisation pouch. The mould was dried after sterilisation and
116 molten agar added and allowed to cool and set. Once cooled the silicone tubing was removed from
117 the mould by gently pulling on one side of the mould, leaving behind an open channel to model the
118 urethra. The urethra model was then removed from the mould by inversion and flexing of the
119 mould container allowing the model to release onto the container lid. The urethra model's surfaces
120 were then allowed to dry uncovered in a biosafety cabinet for 1 hour. The model's container was
121 then closed and the model was placed back into the sterilisation pouch and stored at 8°C for no
122 more than 1 week before use.
123
124 BACTERIAL STRAINS, MEDIA, MATERIALS, AND URINARY CATHETERS
125 The bacterial stains used were *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* NCTC 12981,
126 both strains were cultured in Tryptone Soy Broth (TSB) and on Tryptone Soy Agar (TSA) prepared
127 according to manufacturer instructions. The chromogenic agars used were Harlequin™ agar
128 (Neogen®) which is selective for *E. coli* and other faecal coliforms, and CHROMagar™ Staph aureus
129 agar (CHROMagar™) which is selective for *S. aureus* and other staphylococcal species. The sample
130 catheters used were an uncoated PVC intermittent catheter (14 French), a hydrophilic coated
131 intermittent catheter (12 French), and a prototype intermittent catheter (14 French). Sterile swabs
132 used were 2mm in diameter, paediatric swabs.
133
134 URETHRA MODEL INTERMITTENT CATHETER TESTING METHODOLOGY
135 TSB was inoculated with an individual strain and incubated at 37°C in an oscillating incubator for 4
136 hours. The inoculum was then diluted to a concentration of 10^5 – 10^6 Colony Forming Units
137 (CFU)/mL. The inoculum was aliquoted to 1mL volumes in sterile Eppendorf tubes. The urethra
138 model's container was opened within a biosafety cabinet to allow access to the urethra channels.

For the sterility control, a sterile swab was inserted ~1cm into a channel of the urethra model and the inner channel surface was swabbed in a counter clockwise motion. For the bacterial growth control, a sterile swab was dipped into a 1mL aliquot of the inoculum for 10 seconds, then inserted ~1cm into the next channel and the inner surface of the channel was inoculated, no catheter was inserted. This process was repeated for the three test channels, the inoculum was then allowed 30 minutes to absorb. After absorption of the inoculum, each test catheter was prepared and inserted following the manufacturers' instructions. Each catheter was inserted on the inoculated side of the channel and once inserted, each catheter was left in place in their respective channels for 30 seconds before being removed from the channel via the inoculated side to best represent the insertion and removal of an intermittent catheter in practice. The urethra model surfaces were allowed to dry and absorb any residual moisture either from the inoculum or from the hydrophilic coated catheter for 30 minutes. The model container was then closed and the model was placed back into the sterilisation pouch the model was then incubated in a stationary incubator at 37°C for 24 hours.

BACTERIAL RECOVERY AND QUANTIFICATION

After 24 hours, the urethra model was removed from the incubator. To visually assess bacterial migration, each channel was aseptically bisected lengthwise and the two halves were separated to allow visualisation of the channel inner surfaces, using a sterile scalpel. Images were recorded of the bisected channel. The channel halves was then dissected transversely into octants *i.e.* eight equal segments. Each pair of halves was aseptically transferred to 10mL of TBS and sonicated for 10 minutes to detach the bacteria from the agar surface. After sonication each sample was vortexed for 60 seconds to evenly disperse bacterial cells, 200µL of each sample was transferred to a 96 well plate and serially diluted from 10^{0} – 10^{-6}. Concentrations 10^{-3} – 10^{-6} were enumerated by drop count

to determine the CFU/mL of each octant. This was repeated for each urethra model channel (Figure 2).

DATA ANALYSIS

Results are presented as the average of 6 independent tests. The reproducibility of the model and methodology was determined via ANOVA analysis. Hypothesis testing was performed with the use of an unpaired T-Test. The Statistical significance was accepted at $P < 0.05$.

Results

The *in vitro* urethra model was developed with the aim of addressing the need for a validated *in vitro* testing method for intermittent catheters as identified in a recent review by Cortese et al., (2018). To validate the model and associated methodology, twelve independent tests were carried out, six with *E. coli* and six with *S. aureus*. 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. The results of the 12 independent tests are shown in Figure 4, with a selection of representative photographs of bisected urethra channels and two graphs displaying the average CFU/mL recovered from each urethra channel octant. The reproducibility of the urethra model was determined by carrying out an ANOVA for the replicates of the bacterial growth control and test samples. The sterility control was omitted as all readings were 0 CFU/mL. There was no significant difference found between replicates when using *E. coli* or *S. aureus*, with the bacterial growth control with all test samples having a $P \geq 0.265$ (Table 1).

To compare the catheter samples to the control channels, an unpaired T-Test was used. It was found that bacterial growth distribution significantly increased when an uncoated or hydrophilic

coated catheter was introduced into the channel with either bacterial strain compared to the bacterial growth control ($P \leq 0.009$, for all comparisons), *i.e.* the bacteria were displaced the length of the artificial urethra when the catheter was inserted compared to no displacement observed in the bacterial growth control. This distribution of bacteria was not found to differ significantly when using the uncoated or hydrophilic coated catheter with either bacterial strain ($P \geq 0.127$). For the prototype catheter, no significant difference from the bacterial growth control was observed ($P \geq 0.423$). A significant difference in bacterial distribution in the channel was observed between the prototype catheter and the uncoated or hydrophilic coated catheters with the bacteria not displaced as far down the artificial urethra as with the other two catheter types ($P \leq 0.042$). These results are represented in Figure 3 and Figure 4.

Discussion

The primary aim of this study was to produce an *in vitro* urethra model to test intermittent catheters and demonstrate that bacteria from the urethral meatus are displaced during catheter insertion. During a previous review of the literature, there was a gap identified wherein no validated *in vitro* models were found for the testing of intermittent catheters, while several for testing indwelling catheters have previously been described (Stickler et al., 1999; Gaonkar et al., 2003; Barford et al., 2008; Jordan et al., 2015; Norsworthy and Pearson, 2017; Cortese et al., 2018). 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 (Cortese et al., 2018; Goetz et al., 2018). Medical device research and development can be complex and costly, thus providing an *in vitro*

model can give industry and researchers a new tool to spur on innovation to better prevent CAUTIs and improve patient experiences (Cortese et al., 2018).

The *in vitro* urethra model described in this study has been validated through 12 independent tests with 2 bacterial species to ensure the reproducibility of 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.

E. coli and *S. aureus* were chosen to validate the model as they are both clinically relevant to CAUTIs, and are isolated from approximately 75% and 3% of patients respectively, whilst also ubiquitous enough to be available in any microbiology laboratory (Flores-Mireles et al., 2015). While *Staphylococcus saprophyticus* and *Enterococcus faecalis* are responsible for the majority of Gram positive UTIs, accounting for 10 – 20%, they may not be widely availability in the average laboratory (Flores-Mireles et al., 2015). The methodology and model was also designed to not require any specialist equipment or skills not available to the average microbiologist, allowing the method/model to be reproduced in any laboratory.

The use of chromogenic agar allows the *in vitro* urethra model to exhibit changes in microbial distribution in a manner that is 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*. By making the model as straightforward as possible, it is hoped that it can help both with innovation for intermittent catheters, and to create a tool that can help scientists better communicate with all contributing parties and end users.

Utilising the *in vitro* urethra model, three intermittent catheter types were tested. The catheters used were an uncoated catheter, a hydrophilic coated catheter, and a prototype catheter. 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 uncoated and hydrophilic coated catheters consistently displaced bacteria along the full length of the artificial urethra (~8cm) 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 prototype catheter did not displace bacteria the full way along the urethra channel with growth stopping at a median value of the 6th octant with *E. coli* and the 4th octant with *S. aureus* (Figures 3 and 4).

When comparing the catheter samples to the bacterial growth control, with either bacterial species, the uncoated and hydrophilic coated catheters were consistently found to significantly displace bacteria further along the length of the artificial urethra when compared to the bacterial growth control ($P \leq 0.042$). In regards to the prototype 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 prototype catheter (Figure 3). 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 (Lane et al., 2005; Kaito and Sekimizu, 2007; Kearns, 2010; Pollitt et al., 2015; Terlizzi et al., 2017). 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 4). Fluctuation can also be seen in the dispersion of bacterial growth along the *in vitro* urethra channel with *E. coli* (Figure 4). *E. coli* growth can move along the artificial urethra from the inoculation site to the end of the channel. This phenomenon can be seen most clearly with the hydrophilic catheter which has a noticeable increase in average bacterial concentration when moving from octant 3 to 4 and then reduces again in octant 5. This fluctuation in bacterial concentration can also be due to the increase moisture introduced into the artificial urethra by the hydrophilic coating, increasing the motility of *E. coli,* as the strain can move more quickly in the presence of moisture (Mitchell and 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 ~8cm length. This is twice the length of the average female urethra which is a mere 4cm, 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 16cm length of the average male urethra as well as a number of anatomical differences due to the inconsistent width of the male urethra (Feneley et al., 2015; Goetz et al., 2018).

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

283 previously investigated with indwelling catheters but the movement of the bacteria in the urethra
284 during insertion of urinary catheters has not been previously studied (Kaye et al., 1962; Garibaldi et
285 al., 1980; Schaeffer and Chmiel, 1983; Barford et al., 2008). It has been proposed in the past by Kaye
286 *et* al., (Kaye et al., 1962) and Barford *et al.,* (Barford et al., 2008) that a CAUTI can be initiated by a
287 single catheterisation and this study further supports this theory, demonstrating that the simple act
288 of inserting a urinary catheter can undermine attempts by either the catheter's design or insertion
289 practice to prevent CAUTIs. In conclusion the model and methodology described provide a useful
290 tool for research and innovations in the field of urinary catheterisation and urology in general.
291

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295

296 ## Conflicts of Interest

297 Yvonne J. Cortese's postgradute research has been joinly funded by Athlone Institute of Technology
298 and Teleflex® as part of a collaborrative industry/academic project.
299

300 Victoria E. Wagner, Morgan Tierney, and David Scully were employed by Teleflex® throughout the
301 duration of the study.
302

303 ## Author Contributions

304 Conceptuallisation, Y. Cortese, D. Scully, M. Tierney; Methodology, Y. Cortese, A. Fogarty;
305 Validation, Y. Cortese; Investigation, Y. Cortese; Writing-Original Draft Preperation, Y. Cortese;

Writing-Review and Editing, Y. Cortese, D. Devine, A. Fogarty; Supervision D. Devine, A. Fogarty, V. Wagner; Funding, D. Devine, M. Tierney, V. Wagner

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410

411 **Abbreviations:** CAUTI, Catheter Associated Urinary Tract Infection; ATCC, American Type Culture
412 Collection; NCTC, National Collection of Type Cultures; TSB, Tryptone Soy Broth; TSA, Tryptone Soy
413 Agar; ANOVA, Analysis of Varience

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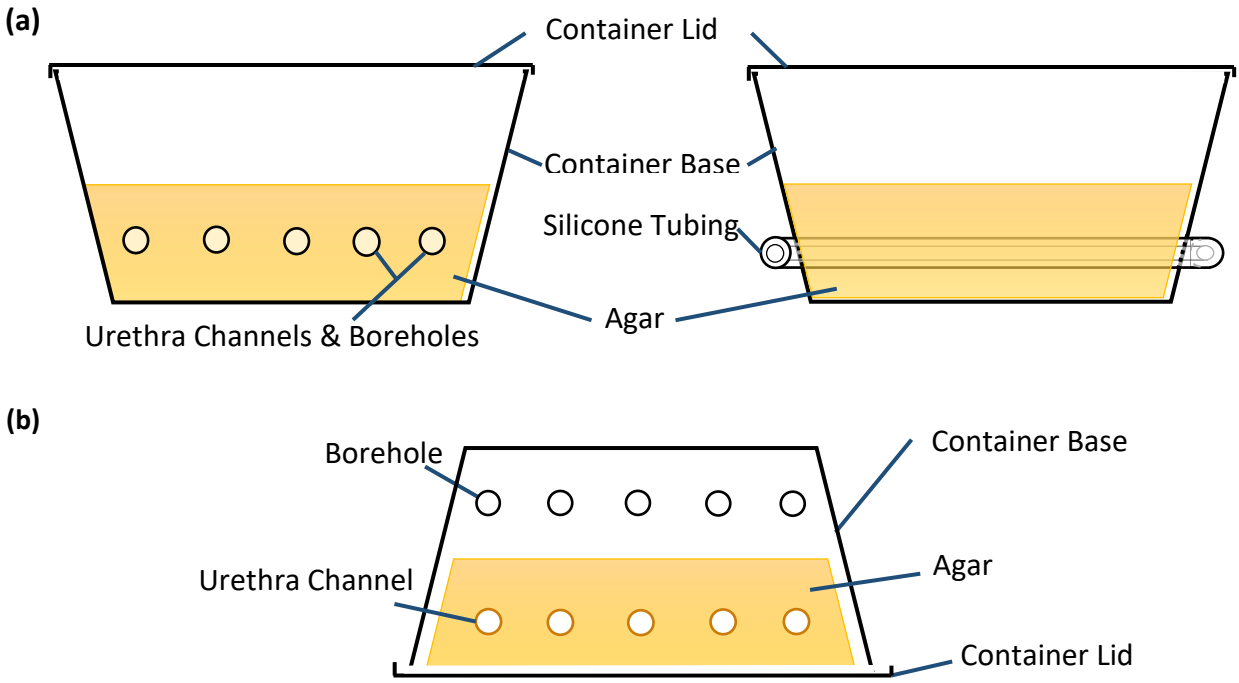
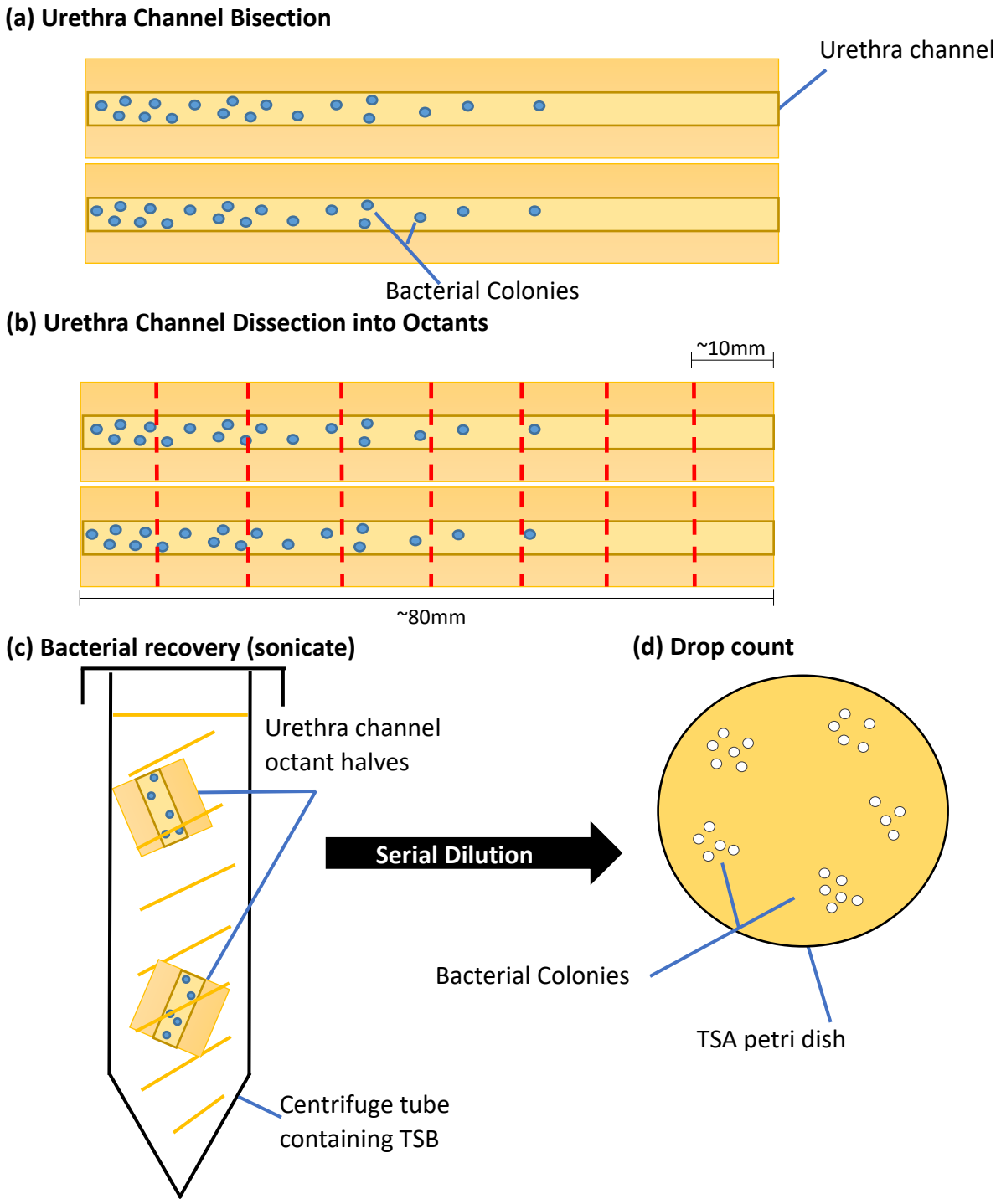


Figure 1 In vitro Urethra Model mould apparatus. (a) Transection of the urethra model mould (left) and end elevation of the urethra model mould with silicone tubing in place (right), (b) inverted mould and released urethra model.



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426 **Figure 2** Bacterial recovery and enumeration methodology for *in vitro* urethra model, (a) bisection
427 of the urethra channel to expose bacterial colonies, (b) dissection of the urethra channel into 8
428 equal segments (octants), (c) bacterial recovery from agar surface by sonication, (d) enumeration of
429 bacteria by drop count.

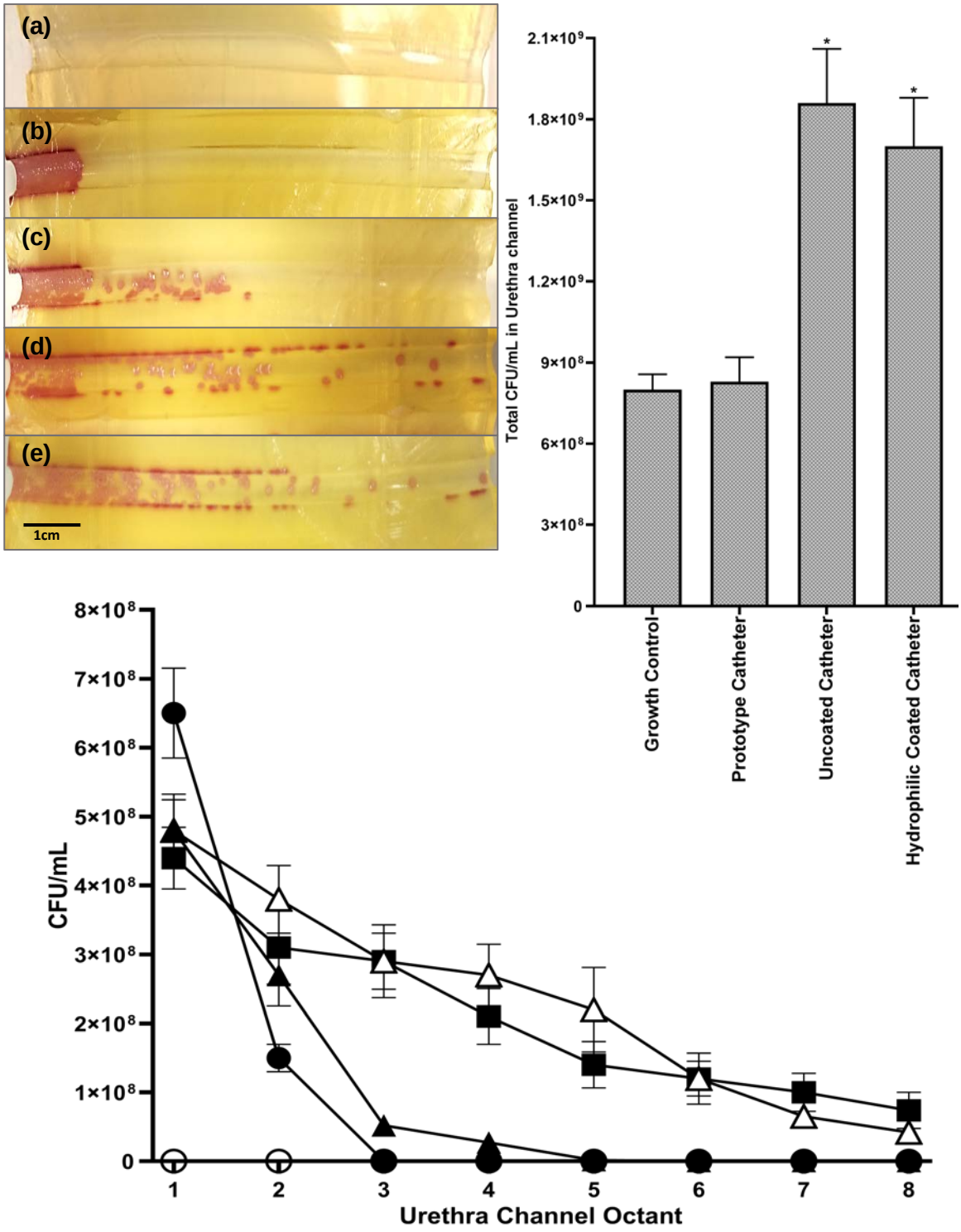

Figure 3 Selection of bisected *in vitro* urethra channels, a scatter plot, and bar chart displaying the average CFU/ml of bacteria recovered from each *in vitro* urethral octant and the total bacteria isolated from the entire urethra channel. Octant 1 and part of 2 represented the site of inoculation. The photographs above display a representative example of urethra channels used for the testing of

435 the sterility control (a, ○), bacterial growth control (b, ●), prototype catheter (c, ▲), uncoated

436 catheter (d, △), and hydrophilic catheter (e, ■). Bacterial colonies are *S. aureus* NCTC 12981 grown

437 on CHROMagar™ Staph aureus agar. n = 6, *P ≤ 0.05

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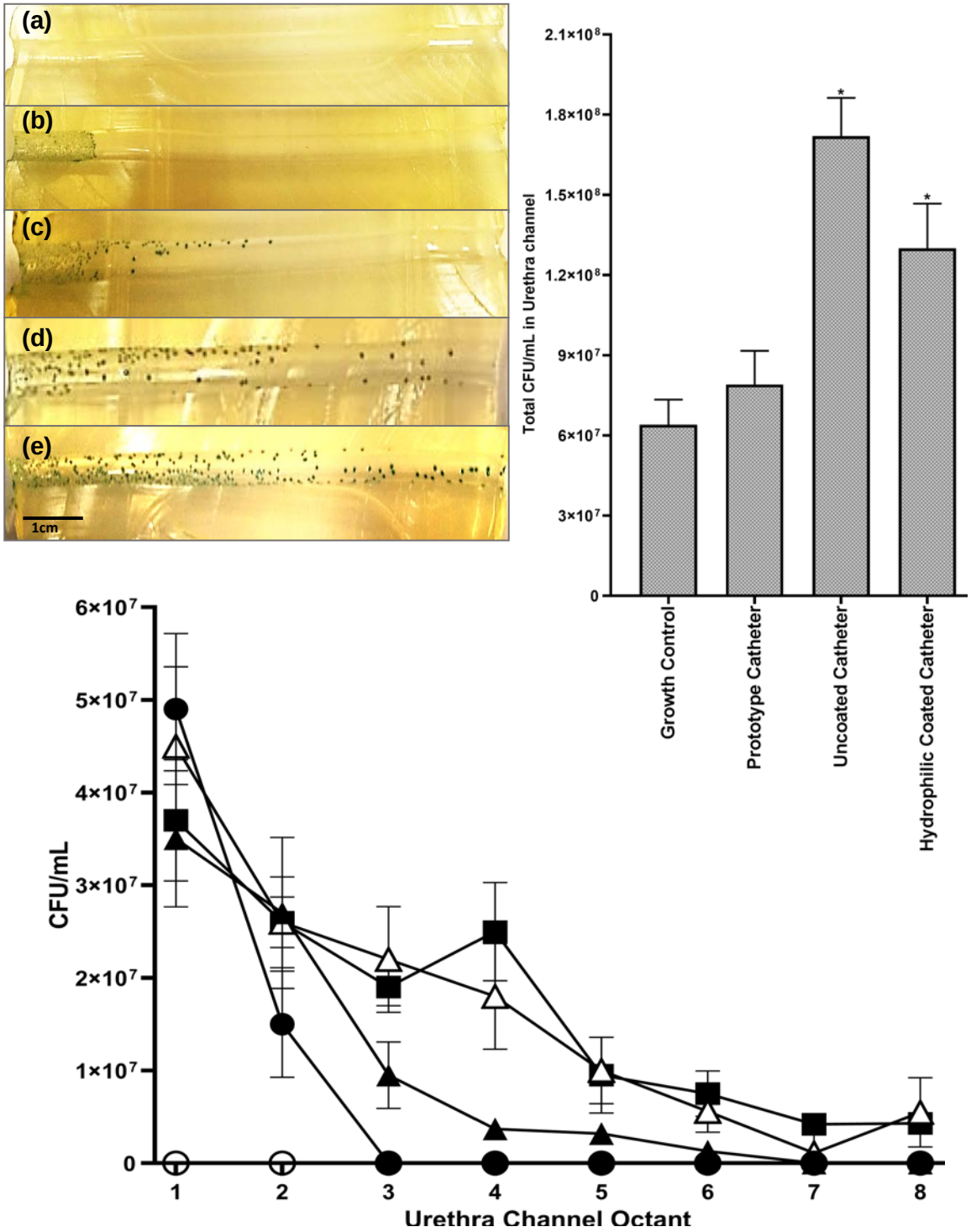

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Figure 4 Selection of bisected *in vitro* urethra channels, a scatter plot, and bar chart displaying the average CFU/ml of bacteria recovered from each *in vitro* urethral octant and the total bacteria isolated from the entire urethra channel. Octant 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, ●), prototype catheter (c, ▲), uncoated catheter (d, △), and hydrophilic catheter (e, ■). Blue colonies shown are *E. coli* ATCC 25922 grown on Harlequin™ agar. n = 6, *P ≤ 0.05

Table 1 Analysis of variance in reproducibility of the *in vitro* urethra model when tested with three sample catheters and two bacterial species. n = 6

	Escherichia coli	***Staphylococcus aureus***
Sterility Control	*	*
Bacterial Growth Control	P = 0.950	P = 0.998
Prototype Catheter	P = 0.732	P = 0.952
Uncoated Catheter	P = 0.819	P = 0.296
Hydrophilic Catheter	P = 0.265	P = 0.265

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