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Establishment of urinary catheter materials antibacterial efficacy evaluation platform in pigs

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Abstract

Urinary catheters are one of the common invasive medical catheters, mostly composed of high-polymer materials such as polyurethanes. During clinical use, the high friction between the catheter material surface and tissues can lead to tissue damage, patient discomfort, and pain during insertion or removal. Moreover, due to the hydrophobic nature of the polymer material surface, there is a risk of urinary tract infections (CTIs) caused by problems such as protein adsorption and bacterial infection upon insertion into the body. Prolonged indwelling time of urinary catheters in the body increases the probability of other complications, potentially leading to life-threatening consequences and imposing medical burdens. Therefore, the purpose of this study was focused on the insertion of the *Escherichia coli* (*E. coli*, 1×10^5 CFU/mL)-coated urinary catheter to implant into a female pig's urethra for establishing the urinary catheter materials antibacterial efficacy evaluation platform in pigs. Results were shown that during the experimental period, the week 1 and week 2 average pig' body temperature were 39.4 ± 0.6°C and 39.1 ± 0.2°C, respectively. The average pig's body temperature was normal and there was no significant differences in body temperature at week 1 and week 2 urinary catheter implanted-experiment period. Moreover, the clinical observation of the pigs showed that their vitality, appetite, and excretion were all normal, and they survived until the end of the experiment without presenting any abnormal clinical symptoms. In addition, *E. coli* in urine culture respectively showed $0.95 \pm 0.03 \times 10^5$ CFU/mL, $1.23 \pm 0.03 \times 10^5$ CFU/mL, $1.43 \pm 0.04 \times 10^5$ CFU/mL, $1.75 \pm 0.08 \times 10^5$ CFU/mL, and $1.91 \pm 0.09 \times 10^5$ CFU/mL on day 0, 3, 7, 10, and 14 of urinary catheter implantation. As time progressed after urinary catheter implantation, there was an increasing trend in *E. coli* counts. Staining with crystal violet for urinary catheter showed that the urinary catheter wall was positive for crystal violet staining. *E. coli* isolation and counts from biofilm on inside and outside of urinary catheter respectively showed $5.10 \pm 0.10 \times 10^5$ CFU/mL and $7.50 \pm 1.08 \times 10^5$ CFU/mL on day 14 of urinary catheter implantation. As time progressed after urinary catheter implantation, there was an increasing trend in *E. coli* counts. Finally, the pathological interpretation of the urinary tract indicates that in the tissue morphology of the bladder, there is observed a localized mild shedding of superficial and intermediate layer cells in the transitional epithelium of the ureteral stent group, with only basal cells remaining connected to its submucosa. There are no significant pathological changes observed in the tissue morphology of the urethra and kidneys. According to the results of this study, a urinary catheter materials antibacterial efficacy evaluation platform in pigs has successfully established, which can be provided for R&D of urinary catheter antibacterial materials.

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Keywords: Antibacterial Efficacy; Evaluation Platform; Pig; Urinary Catheter Materials

1. Introduction

A urinary catheter is a common method of urine drainage used in clinical settings, often employed in patients undergoing general anesthesia or those suffering from conditions such as dementia or Parkinson's disease, which impair their ability to urinate independently. It involves inserting a tube into the bladder through which urine is drained directly into a collection bag, aiding in the elimination of urine. However, the use of urinary catheters increases the risk of urinary tract infections (UTIs), especially with prolonged placement, making proper care essential for individuals with catheters [1-5].

There are two main types of urinary catheters: intermittent catheterization and indwelling catheters. The key difference between them lies in whether the catheter remains continuously in the body after insertion. Intermittent catheterization is primarily used for bladder training, aiming to help patients overcome the challenges associated with long-term indwelling catheter use. Daily care of the catheter involves maintaining hand hygiene to reduce infection risk. One of the primary concerns post-catheterization is infection or catheter blockage, thus adequate fluid intake is crucial, with adults typically needing to drink 1,500 mL to 2,000 mL of water per day. Additionally, positioning the urinary bag lower than the patient's bladder helps prevent the backflow of contaminated urine, reducing the risk of urinary tract infections. Furthermore, catheters should be regularly replaced, with silicone catheters needing replacement every 30 days and rubber catheters every 14 days, as failure to do so can easily lead to urinary tract infections [6-10].

UTIs are among the most common healthcare-associated infections. The majority of healthcare-associated infections are related to catheter use, with up to 95% of UTIs in intensive care units being catheter-associated. Most catheter-associated urinary tract infections (CAUTIs) are caused by bacteria from endogenous flora around the perineum, entering the bladder via the outer surface of the catheter through the urethra. A small portion of bacteria may come from exogenous sources, contaminating the closed catheter system. In rare cases, bloodstream transmission may occur, often involving *Staphylococcus aureus*, though it is not a common causative agent of CAUTIs. When isolated from urine, it may suggest bacteremia or infective endocarditis. According to surveys, common bacteria causing CAUTIs include intestinal bacteria such as *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Candida* spp., and *Enterococcus* spp. The most common bacterial species causing CAUTIs due to prolonged catheterization include *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Proteus mirabilis* [11-15].

Due to the hydrophobic nature of the polymer material surface, there is a risk of UTIs caused by problems such as protein adsorption and bacterial infection upon insertion into the body. Prolonged indwelling time of urinary catheters in the body increases the probability of other complications, potentially leading to life-threatening consequences and imposing medical burdens [16-20]. Therefore, the purpose of this study was focused on the insertion of the *E. coli*-coated urinary catheter to implant into a female pig's urethra for establishing the urinary catheter materials antibacterial efficacy evaluation platform in pigs.

2. Materials and Methods

2.1 Experimental Reagents

Experimental reagents included as saline, phosphate buffered saline (PBS; No. P3813, Sigma-Aldrich®), Zoletil 50 (Vibac Laboratories, Carros, France), azaperone (Stresnil®; Elanco Animal Health, USA), urinary catheters, and *E. coli* (kindly provide by Dr. Meng-Hwan Lee).

2.2 Bacterial Culture

E. coli or samples (urine and biofilms of urinary catheters) were cultured in sterilized LB broth (Luria or Lenox broth) at 37°C with shaking at 300 rpm overnight.

2.3 Animal Care

All animal experiments were approved by the Institutional Animal Care and Use Committee of the Animal Technology Research Center, Agricultural Technology Research Institute (ATRI), Taiwan. One twelve-week-old specific pathogen-free (SPF) female pig [two breed (LY) crossbred pigs] was ordered from ATRI, Taiwan (the ATRI approval No.: 112086) and experimented in the GLP Animal Trial Facility, Animal Drugs Inspection Branch (ADIB), Animal Health Research Institute, Council of Agriculture, Executive Yuan, Miaoli, Taiwan (the ADIB approval No.: 112-T07). The pig was housed

under a 12-h light/dark cycle at 22-24°C and 70-75% humidity. Normal laboratory diet (FWUSOW industry, Taichung, Taiwan) and fresh water were supplied to pigs continuously ad libitum.

2.4 Procedure of Urinary Catheter Implantation into Pig's Bladder

Video recording when inserting and removing the urinary catheter. The urinary catheter was inserted from the pig's urethral opening into the bladder, followed by inflation of the catheter's distal balloon with air. The external catheter was then sutured to the vulva using 1.0 sterile sutures to prevent the urinary catheter from slipping out of the urethra. Next, a urinary catheter coated with *E. coli* solution (1×10^5 CFU/mL) was implanted.

2.5 Monitor of Clinical Symptoms and Survival, and Detection of Body Temperature

In this study, the monitoring of clinical behavior and survival, and the detection of body temperature for a pig once per day. Three indexes of clinical behavior as vitality, appetite, and excretion are used (Table 1).

2.6 Collection of Urine and Urinary Catheter for Bacterial Counting

Urine samples were collected and cultured on days 0, 3, 7, 10, and 14 for the detection of *E. coli* counts triple repetition per day. After removing the urinary catheter, scraping of the biofilm inside the catheter is conducted for bacterial LB culture and counting (CFU/mL). Urinary catheter stained with crystal violet.

2.7 Gross and Histopathologic Examination

After 14 days urinary catheter implantation, the urinary catheter was removed from pig and collected. The pigs were sacrificed and collected urinary system organs included the bladder, urethra, and kidneys. The organs were placed in 10% neutral buffered formalin solution for fixation for at least 24 hours. Then, tissue trimming is performed by longitudinal or transverse sectioning. The trimmed tissues are placed in embedding boxes and processed into paraffin tissue blocks through dehydration, paraffin infiltration, and embedding procedures. The tissue blocks of paraffin are cut into 4 μm thick tissue sections using a paraffin microtome (RM 2145, Leica). Subsequently, Hematoxyl in & Eosin (H&E) staining is performed, and tissue pathological changes of each organ are observed under an optical microscope (BX51, Olympus).

2.8 Statistical Analysis

The data of animals are represented by mean and standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (one-way ANOVA) via SPSS 12.0 statistical software. When $p < 0.05$, it indicates a significant difference.

3. Results

3.1 Changes in Body Temperature at Week 1 and Week 2 Urinary Catheter Implanted-Experiment in Pigs

During the experimental period, the week 1 and week 2 average pig's body temperature were 39.4 ± 0.6°C and 39.1 ± 0.2°C, respectively. The average pig's body temperature was normal and there was no significant differences in body temperature at week 1 and week 2 urinary catheter implanted-experiment period.

3.2 Clinical Observation of a Pig after Urinary Catheter Implantation

Clinical observation results of the pig showed that its vitality, appetite, and excretion was all normal, and they survived until the end of the experiment without presenting any abnormal clinical symptoms.

3.3 Bacterial Isolation and Counts from the Urine and Inner and Outer Walls of Urinary Catheter

E. coli in urine culture respectively showed $0.95 \pm 0.03 \times 10^5$ CFU/mL, $1.23 \pm 0.03 \times 10^5$ CFU/mL, $1.43 \pm 0.04 \times 10^5$ CFU/mL, $1.75 \pm 0.08 \times 10^5$ CFU/mL, and $1.91 \pm 0.09 \times 10^5$ CFU/mL on day 0, 3, 7, 10, and 14 of urinary catheter implantation (Table 1). As time progressed after urinary catheter implantation, there was an increasing trend in *E. coli* counts.

Staining with crystal violet for urinary catheter showed that the urinary catheter wall was positive for crystal violet staining (data not shown). *E. coli* isolation and counts from biofilm on inside and outside of urinary catheter respectively showed $5.10 \pm 0.10 \times 10^5$ CFU/mL and $7.50 \pm 1.08 \times 10^5$ CFU/mL on day 14 of urinary catheter implantation (Table 2). As time progressed after urinary catheter implantation, there was an increasing trend in *E. coli* counts.

Table 1 *E. coli* isolation and counts (CFU/mL) from a pig's urine

Days	Urinary catheter group
0	$0.95 \pm 0.03 \times 10^5$ CFU/mL
3	$1.23 \pm 0.03 \times 10^5$ CFU/mL
7	$1.43 \pm 0.04 \times 10^5$ CFU/mL
10	$1.75 \pm 0.08 \times 10^5$ CFU/mL
14	$1.91 \pm 0.09 \times 10^5$ CFU/mL

Table 2 *E. coli* isolation and counts (CFU/mL) from biofilm on inside and outside of urinary catheter

Location	Urinary catheter group
Inner wall	$5.10 \pm 0.10 \times 10^5$ CFU/mL
Outer wall	$7.50 \pm 1.08 \times 10^5$ CFU/mL

3.4 Histopathological Exanimation on Bladder, Urethra, and Kidney in a Pig

The pathological interpretation of the urinary tract indicates that in the tissue morphology of the bladder, there was observed a localized mild shedding of superficial and intermediate layer cells in the transitional epithelium of the ureteral stent group, with only basal cells remaining connected to its submucosa. There were no significant pathological changes observed in the tissue morphology of the urethra and kidneys (data not shown).

4. Discussion

According to the previous study [4], two out of three Landrace × Yorkshire (LY) crossbred pigs and one out of two Gottingen Minipigs (GM) developed significant bacteriuria, defined as $>10^3$ CFU/mL, 24 hours post-inoculation, with the uropathogenic *E. coli* (UPEC) at 10^1 CFU/mL. The remaining GM pig developed bacteriuria after UPEC inoculation with 10^2 CFU/mL, while the last LY pig remained uninfected. Despite using only one LY pig (SPF degree) in our study, we achieved 100% induction of bacteriuria in swine. *E. coli* in urine culture showed counts of $0.95 \pm 0.03 \times 10^5$ CFU/mL, $1.23 \pm 0.03 \times 10^5$ CFU/mL, $1.43 \pm 0.04 \times 10^5$ CFU/mL, $1.75 \pm 0.08 \times 10^5$ CFU/mL, and $1.91 \pm 0.09 \times 10^5$ CFU/mL on days 0, 3, 7, 10, and 14 of urinary catheter implantation, respectively. There was an increasing trend in *E. coli* counts over time post-implantation. Additionally, crystal violet staining of the urinary catheter showed positive staining on the catheter wall. *E. coli* isolation and counts from biofilm on the inner and outer walls of the urinary catheter respectively showed $5.10 \pm 0.10 \times 10^5$ CFU/mL and $7.50 \pm 1.08 \times 10^5$ CFU/mL on day 14 of urinary catheter implantation, with an increasing trend over time post-implantation.

The pig model used in the study [4] was costly, particularly in GM pigs, with total costs reaching roughly USD $3,000 per animal. However, the study found no apparent differences in UTIs susceptibility or inflammatory response between LY pigs and GM, suggesting that the choice of breed may not significantly impact this disease. In our study, although the LY pig model (SPF degree) incurred higher costs, reaching total study costs of roughly USD $4,800 per animal, we were able to mitigate other factors affecting the experiment. Thus, we successfully established 100% *E. coli*-infected UTIs in swine.

Pigs are gaining attention as biomedical models for vaccine research and pharmacological studies across various fields of human health, but their use in cystitis research has only recently emerged. This study, along with previous work using the model, highlights the potential of pigs as suitable UTI models. A urinary catheter is a medical device used to assist with urination, typically inserted through the urethra into the bladder to allow urine drainage. At the catheter's tip, there is a balloon used to secure it within the bladder, preventing slippage. This study provides valuable insights into the infectious inoculum of UPEC in a pig, which was previously challenging to study using traditional rodent models. Given the high anatomical and physiological similarity between pigs and humans in the urinary tract, the susceptibility observed in pigs likely mirrors that in humans [21-26].

5. Conclusion

Based on the results of body temperature, *E coli* isolation and counts in urine culture and on the urinary catheter wall, and pathological interpretation of urinary tract, the urinary catheter implanted-platform in a pig has been successfully established.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare no conflict of interest.

Statement of ethical approval

The Institutional Animal Care and Use Committee (IACUC) of Agricultural Technology Research Institute and Animal Drugs Inspection Branch of Animal Health Research Institute, Council of Agriculture, Executive Yuan inspected all animal experiments and this study comply with the guidelines of protocol IACUC 112086 and IACUC 112-T07 approved by the IACUC ethics committee.

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