JOURNAL OF TRANSLATIONAL MEDICINE

PROCEEDINGS Open Access

Application of a nanotechnology antimicrobial spray to prevent lower urinary tract infection: a multicenter urology trial

Wei He[1], Dongmin Wang[2], Zhangqun Ye[1], Weihong Qian[3], Yan Tao[3], Xiaofeng Shi[3], Ling Liu[4], Jin Chen[5], Ling Qiu[6], Peng Wan[7], Xiaojun Jia[8], Xia Li[9], Caixia Gao[10], Xuexia Ma[11], Biyan Wen[9], Nianzhen Chen[12], Ping Li[13], Zhengzheng Ren[14], Li Lan[15], Siyi Li[16], Yi Zuo[17], Hua Zhang[18], Liming Ma[18], Yueping Zhang[18], Zhicong Li[19], Weiping Su[20], Qing Yang[21], Qingli Chen[22], Xuejing Wang[22], Zhenni Ye[23], JP Chen[24], Wings TY Loo[24,25*], Louis WC Chow[25], Adrian YS Yip[25], Elizabeth LY Ng[25], Mary NB Cheung[25], Zhiping Wang[2†]

From Organisation for Oncology and Translational Research (OOTR) 7th Annual Conference
Hong Kong. 13-14 May 2011

Abstract

Background: Catheter-associated urinary tract infection (CAUTI) is a common nosocomial device-associated infection. It is now recognized that the high infection rates were caused by the formation of biofilm on the surface of the catheters that decreases the susceptibility to antibiotics and results in anti-microbial resistance.
In this study, we performed an *in vitro* test to explore the mechanism of biofilm formation and subsequently conducted a multi-center clinical trial to investigate the efficacy of CAUTI prevention with the application of JUC, a nanotechnology antimicrobial spray.

Methods: Siliconized latex urinary catheters were cut into fragments and sterilized by autoclaving. The sterilized sample fragments were randomly divided into the therapy and control group, whereby they were sprayed with JUC and distilled water respectively and dried before use.
The experimental standard strains of *Escherichia coli* (*E. coli*) were isolated from the urine samples of patients. At 16 hours and 7 days of incubation, the samples were extracted for confocal laser scanning microscopy.
A total of 1,150 patients were accrued in the clinical study. Patients were randomized according to the order of surgical treatment. The odd array of patients was assigned as the therapy group (JUC), and the even array of patients was assigned as the control group (normal saline).

Results: After 16 hours of culture, bacterial biofilm formed on the surface of sample fragments from the control group. In the therapy group, no bacterial biofilm formation was observed on the sample fragments. No significant increase in bacterial colony count was observed in the therapy group after 7 days of incubation.
On the 7th day of catheterization, urine samples were collected for bacterial culture before extubation. Significant difference was observed in the incidence of bacteriuria between the therapy group and control group (4.52% *vs.* 13.04%, $p < 0.001$).

Conclusions: In this study, the effectiveness of JUC in preventing CAUTI in a hospital setting was demonstrated in both *in vitro* and clinical studies.

* Correspondence: wtyloo@gmail.com
† Contributed equally
[24]School of Chinese Medicine, The University of Hong Kong, Hong Kong SAR
Full list of author information is available at the end of the article

Background

Catheter-associated urinary tract infection (CAUTI) is a common nosocomial device-associated infection. Urinary tract infection (UTI) accounts for up to 40% of nosocomial infections and is one of the main types of healthcare-associated infections (HAI). About 80% of UTIs are catheter-associated [1,2]. In the United States, approximately 95% of UTIs were associated with the indwelling catheters [3], and interestingly, 15-25% of patients in short-term hospital care need to be inserted with indwelling urinary catheters [4]. Every year, there are more than 5 million patients necessitating catheterization therapy [5] and approximately 1 million patients suffering from CAUTI [6]. The findings of a European study indicated that 5.4% of patients aged 65 or above required the use of an indwelling urinary catheter [7].

CAUTI is a highly common infection and comes with considerable risk. The duration of hospitalization owing to CAUTI increased from 2.4 to 5.4 days in the United States [8]. On average, the costs of diagnosing and treating CAUTI is US$ 589, excluding extension of hospital costs [9]. Taking into account the expenses of hospitalization, the average cost increases from US$ 2,836 to 3,803 [10,11]. The Centers for Disease Control and Prevention (CDC) pointed out that UTI leads to deaths of over 13,000 patients every year in the United States [12], indicating a growing medical problem.

It is now recognized that the high infection rates were caused by the formation of biofilm on the surface of the catheters that decreases the susceptibility to antibiotics and results in anti-microbial resistance [8,13,14]. The formation of biofilm as a result of extracellular polysaccharide matrix secretions from microorganisms has been demonstrated in clinical studies. Bacterial biofilm is a special honeycomb-shaped structure that forms a very complex ecosystem; magnification of biofilm will reveal microcolonies under the microscope [15-18]. Organisms with biofilm can withstand shear force, pH changes, and antimicrobial agents, and prevent macrophage phagocytosis [13,19]. The proximity of cells allows more frequent genetic information exchange than other free cells [20]. Therefore, antimicrobial resistance genes and strains can be spread easily. With respect to catheters, the formation of biofilm will protect the pathogenic bacteria residing at the urinary tract from antimicrobial medicine and host immune response [15]. It will then facilitate the growth of bacteria which further complicates the problem of CAUTI [13].

Recent research focused on the development of preventive methods for biofilm formation and changes, including furanone, furacilinum, silver-coated catheters, in addition to other techniques. [21-24]. Johnson *et al*. [21,2] discovered that catheters containing silver hydrogel and nitrofurazone coating have excellent effects of inhibiting biofilm formation, but no inhibitory effects for *Pseudomonas aeruginosa*. According to the study conducted by CDC, the results of a comparison of patients inserted with silver-coated catheters and standard catheters for one week revealed no difference in bacteriuria prevention [25]. Silver-bearing catheters can decrease the effect of bacteriuria in a week after indwelling. Burton *et al.*[8] discovered the new oPDM-plus-PS (N, N'-(1,2-phenylene) dimaleimide [oPDM]-plus-protamine sulfate [PS]) coating can inhibit *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* adhering to the catheters, but now these coated catheters can only provide short-term CAUTI prevention upon urinary catheter insertion [13]. Recently, Stickler *et al.*[26] revealed that bacteria on the biofilm of catheters produced quorum-sensing signal that can control the genetic expression of forming biofilm. If the signal is blocked, the formation of biofilm can be impeded. For example, the mutant *Pseudomonas aeruginosa* in the absence of quorum-sensing signal was unable to produce a three-dimensional biofilm [27]. An important finding was established regarding iron and the formation of biofilm. Clinical investigations have detected that elements such as iron are necessary nutrients for biofilm formation. The production of catheters without iron is a new development, but it has not been tested in clinical trials [13]. The use of probiotics can also be considered. Trautner *et al.*[28,29] observed that the rates of pathogenic bacterial infection and CAUTI were reduced if the catheters were inoculated with the non-pathogenic *Escherichia coli* (*E. coli*). Although these methods are considerable, there is no conclusive evidence and the cost-effectiveness remains unclear.

The traditional use of JUC applied to the wounds of post-surgery patients has proven to be effective in the hospital and out-patient setting: application of JUC did not result in drug resistance, nor stimulate serious adverse reactions and reduced the average wound healing time of patients [30]. JUC is composed by nano-manufacture technology, with nano-cations on the nano-scale molecular structure produced and then prepared in water-soluble spray [31]. JUC achieves antibacterial action on skin and wound surface by physical mechanisms and can therefore be regarded as a physical antimicrobial agent [31]. Upon application, JUC prevents bacterial growth by forming an invisible, positively charged protective film on the sprayed surface, isolating and eradicating negatively charged pathogenic micro-organisms including bacteria, fungi and viruses [31,32].

There is no effective way to prohibit biofilm formation clinically; therefore, there is still an unmet need for the establishment of a new clinical application. In this study, we performed an *in vitro* test to explore the mechanism of biofilm formation and subsequently conducted a multicenter clinical trial to investigate the efficacy of CAUTI

prevention with the application of JUC, a nanotechnology antimicrobial spray.

Methods

In vitro testing

Bacteria

The experimental standard strains of *E. coli* were isolated from the urine samples of UTI patients at the Second Hospital of Lanzhou University. Bacteria were cultured in Luria-Bertaini broth [33]. The bacterial suspension was prepared and the bacterial concentration was adjusted to 7.4×10^9CFU/ml.

Preparation of sample fragment

Siliconized latex urinary catheters were cut into sample fragments and sterilized by autoclaving. The sterilized sample fragments were randomly divided between the therapy and control group, with 8 pieces of fragments in each group. The sample fragments were respectively sprayed with JUC and distilled water, and dried before use. The *E. coli* suspension was injected into 24 well plates in which the sample fragments were placed. The plates were then incubated at 37 °C and washed with PBS solution every 48 hours [34]. At 16 hours and 7 days of incubation, the samples were extracted for confocal laser scanning microscopy.

Confocal laser scanning microscopy

The cultured samples were soaked in 1 ml PBS solution. After 50 μg/ml propidium iodide was added, the samples were left for dyeing in a dark area at 4°C for 15 minutes or at room temperature for 30 minutes. The samples were then placed upside down on a glass slide for observing the biofilm formation using laser scanning microscopy [35,36].

Clinical trial

The clinical study commenced in March 2010 and was completed in December 2011. Patients undergoing urological surgery in need of indwelling urethral catheter and more than 7 days of hospitalization were recruited. A total of 1,150 patients (869 male and 281 female), aged from 2 to 82 years, were accrued. Twenty-three hospitals participated in this clinical trial, and every hospital accrued 25 patients each to the control group and therapy group. All patients were operated due to urological diseases, including but not limited to urinary tract stones, tumors, prostatic hyperplasia, ureteral stenosis and hydronephrosis. Indwelling urethral catheter was necessary for patients requiring over 7 days of hospital stay. The midstream urine bacterial culture [15-17] was negative at the time of inclusion in the study. Exclusion criteria of the study included patients with a long-term use of balloon catheter, intermittent self-catheterization, previous treatment of percutaneous paracentetic suprapubic cyctostomy and UTI patients. Patients were randomized according to the order of surgical treatment. The odd array of patients (575 cases) was assigned as the therapy group (the JUC), and the even array of patients (575 cases) was assigned as the control group (normal saline). Patients who were eligible for the trial were explained the nature and purpose of the trial by the investigator, and informed consent was obtained for inclusion in the trial. The Ethics Committee of Tongji Hospital approved the clinical study (Approval Number: 2010006D).

Study design

Therapy group

Prior to the insertion of the catheter into the ureter of the patient at the time of surgery, JUC was sprayed on the surface of the catheter to allow formation of a physical antimicrobial membrane. After surgery, in addition to traditional nursing care, JUC was sprayed onto the skin and mucous membrane around the urethral orifice, the catheter and the drainage tube attachment point. This was done twice a day with 1 ml per spray (approximately 10 sprays) until the catheter was removed on the 7th day.

Control group

The catheter was inserted during surgery. After surgery, conventional nursing care with normal saline was performed until the catheter was removed on the 7th day.

During the study, and according to routine clinical practice, antibiotics were prescribed to patients after surgery. The types, dosage and route of antibiotics prescribed to the patients were carefully and strictly recorded according to the class of antibiotics per institutional guidelines (Table 1).

Class one, class two and class three antibiotics were cumulatively given 521 (40.08%), 572 (44%) and 207 (15.92%) treatment times respectively. There were no restrictions of use for class one antibiotics: they were proven to be safe and effective for long-term clinical application with minimal effects on antimicrobial resistance. The drugs belonging to class one are considered relatively inexpensive antimicrobial agents. Class two antibiotics demonstrated properties of restricted use, with concerned safety, efficacy, and antimicrobial resistance in humans. In comparison, class two drugs were relatively more expensive than the drugs of class one, non-restricted use antibiotics. Class three antibiotics are newly approved, antimicrobial agents with limited safety and efficacy information. There were reported adverse reactions with the use of class three antibiotics. Owing to the concerned safety of class three drugs, they are not recommended for use. Special attention should be made for clinical use to avoid bacterial resistance to antimicrobial agents. Amongst the three classes of antibiotics, class three are relatively more expensive in nature.

The clinical practice on the use of antibiotics differed between all hospitals. A total of 150 patients required the combination use of antibiotics, which were prescribed for

Table 1 Classification of antibiotics used in the clinical trial

Class	Antimicrobial agents	Types	Usage Frequency	Rate of Usage
1	piperacillin, nafcillin, mezlocillin, azlocillin, ticarcillin, mezlocillin, amoxicillin, cefazolin, ceftazidime, cefathiamidine, cefprozil, cefixime, cefotiam, ceftriaxone, cefaclor, Cefonicid sodium, cefamandole sulfate, azithromycin, levofloxacin, ciprofloxacin,Lomefloxacin, enoxacin, gatifloxacin, amikacin, Amikacin' Thiamphenicol, clindamycin	27	521	40.08%
2	ampicillin / sulbactam sodium, timentin / clavulanate, mezlocillin / sulbactam, amoxicillin / clavulanic acid, amoxicillin / sulbactam sodium, piperacillin / sulbactam sodium, cefuroxime sodium, cefmenoxime, cefotaxime sodium,cefpiramide, cefminox, cefodizime, cefpodoxime proxetil, cefetamet pivoxil, cefdinir, aztreonam, latamoxef sodium, cefoxitin sodium, sparfloxacin, moxifloxacin, fleroxacin, antofloxacin hydrochloride, tosufloxacin, etimicin, sisomicin, fusidate sodium, ornidazole	27	572	44%
3	Ceftizoxime, ceftazidime, ceftazidime, cefoperazone, cefoperazone / sulbactam sodium, ceftriaxone / sulbactam sodium, ceftriaxone / sulbactam sodium, cefoperazone / tazobatan, cefepime, cefoselis, imipenem / cilastatin, meropenem, Norvancomycin	13	207	15.92%

0 to 7 days according to the condition of the patient. The percentage use of class one antibiotics in Guangzhou First Municipal People's Hospital and The First Affiliated Hospital of the Sun Yat-sen University was 97.92% and 94.12% respectively, but the use of class two antibiotics was 97.40% in Daping Hospital of the Third Military Medical University. No significant difference was observed in the use of antibiotics between therapy and control groups in each hospital. For example, in the Second Hospital of Xi'an Jiaotong University, class one antibiotics were given to 10 cases in both therapy and control groups, class two antibiotics were given to 12 and 18 cases in treatment and control groups respectively, and class three antibiotics were given to 8 and 6 cases in treatment and control groups respectively. In the General Hospital of Guangzhou Military Command of PLA, class one antibiotics were given to 10 cases in both the therapy and control groups, class two antibiotics were given to 13 and 16 cases in the treatment and control groups respectively, and class three antibiotics were given to 4 and 3 cases in treatment and control groups respectively. Despite the difference in the clinical practice on the use of antibiotics between hospitals, the results were statistically meaningful.

After surgery, the body temperature and UTI symptoms were recorded every day. After 7 days of catheterization, urine samples were collected under aseptic condition for bacterial culture before extubation [37].

Outcome assessment

The collected urine samples with colony count ≥ 10^3 CFU/ml was considered as CAUTI, based on the quantitative urine culture. [9,38,39].

Statistical analysis

Parameters were compared using SPSS version 14.0. The T-test was used to compare the incidence of CAUTI between groups, where $P < 0.05$ was considered as statistically significant.

Results

In vitro test results

After 16 hours of culture, bacterial biofilm formed on the surface of sample fragment in the control group. The bacterial biofilm was dyed red by propidium iodide fluorescent dye (Figure 1A). In the therapy group, no bacterial biofilm formation was observed on the sample fragments. Only small red dots representing a very small number of free bacteria were observed under microscope (Figure 1B).

After 7 days of culture, in the control group with distilled water, the surface of the sample fragments formed a thick, uniform color, dense and darkly stained layer of bacterial biofilm. Due to bacterial overgrowth, the biofilm was cross-linked to form clumps of bacteria and the surface of the sample fragments was rough and uneven (Figure 2A). The surface of sample fragments in the therapy group formed only a small amount of thin membranous structure with smooth surface and light color. No other abnormality was observed (Figure 2B).

Clinical trial results

Significant difference was not observed in demographics including age, gender, etiology, and geographical distribution between the two groups. On the 7th day of catheterization, urine samples were collected for bacterial culture before extubation. In the therapy group, positive bacterial culture was detected in 26 (4.52%) cases, of which 24 cases were *E. coli*, 1 case was *Enterococcus faecalis* and 1 case was *smooth Candida*. In the control group, bacteriuria was detected in 75 (13.04%) cases, of which 69 cases were *E. coli*, 2 cases were *Enterococcus faecalis*, 2 cases were *Enterococcus cloacae*, 1 case was *Candida albicans* and 1 case was *Pseudomonas aeruginosa*. Detailed results were shown in Table 2. Among all 101 cases of infections, 93 (92.08%) cases were *E. coli* infections, 3 (2.97%) cases were *Enterococcus faecalis*, and 2 (1.98%) cases were *Enterococcus cloacae*. Significant difference was observed in the incidence of

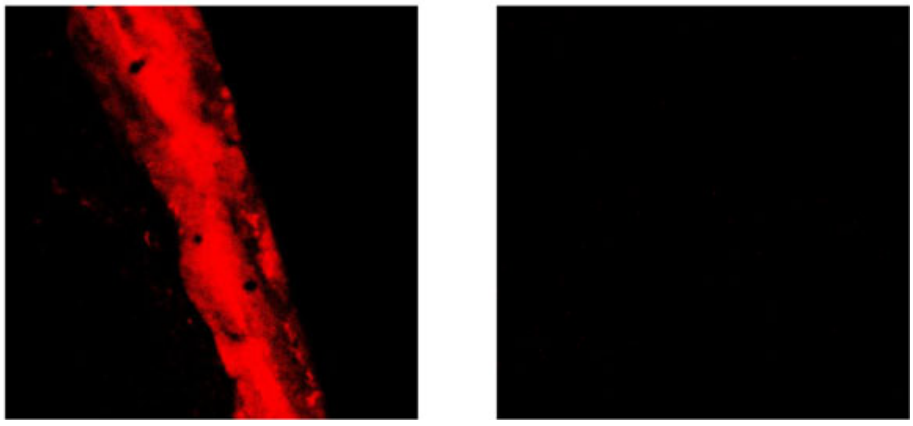

Figure 1 Control and JUC group at 16 hours A: Control group at 16 hours (CSLM 200X). B: JUC group at 16 hours (CSLM 200X)

bacteriuria between the control group and control group (4.52% *vs.* 13.04%, $p < 0.001$).

Discussion

Types of bacteria

UTI is a major nosocomial infection. CAUTI is one of the most common types of bacterial infections [1,2]; ample intestinal bacteria cultivates around the urethra [3,38]. A majority of the short-term CAUTIs were caused by a single strain of bacteria, such as *E. coli*, *Proteus mirabilis and Klebsiella pneumoniae*, whereas long-term CAUTI was caused by multiple microorganisms [3,40,41]. Urethra pathogenic *E. coli* is the most common cause of CAUTI which constitutes 50% of hospital-acquired UTIs [3,42]. In our study, similar results were observed. *E. coli* infection was dominated by 92.08%, while other bacteria such as *Enterococcus faecalis* and *Enterococcus cloacae* constituted a minute proportion of UTIs.

Prevention of catheter-associated infection

Twenty years ago, the U.S. CDC clearly emphasized that hand hygiene, sterile catheterization and closed drainage systems were the necessary elements in preventing CAUTI [43,44]. Recently, the Healthcare-Associated Infections Allied Task Force proposed several frameworks, including infection surveillance, enhancement of education and training in the prevention of CAUTI, the use of appropriate technology for catheter insertion, replacement of indwelling urinary catheter by condoms

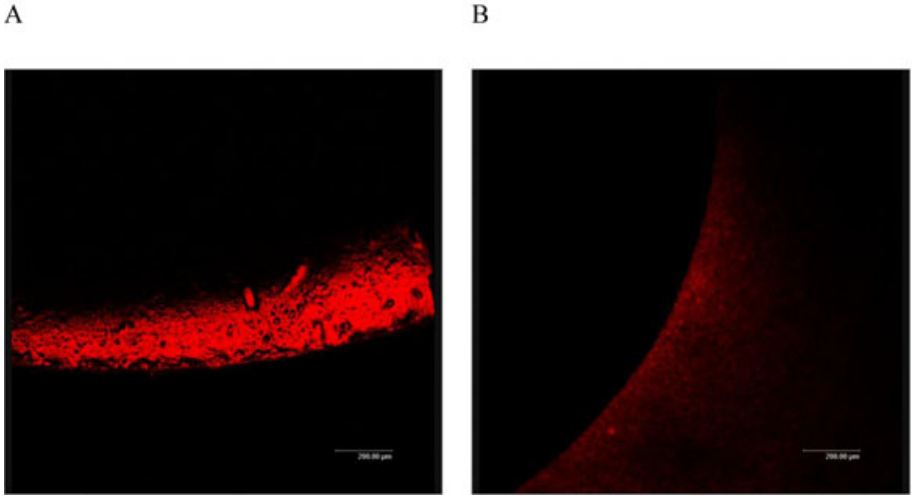

Figure 2 Control and JUC group at 7 days A: Control group at 7 days (CSLM 200X). B: JUC group at 7 days (CSLM 200X).

Table 2 Comparison of post-operative urinary bacterial culture between the control and therapy group

Groups	Number of Case	Before Surgery	Day 7 after surgery	Types of bacteria					
				Escherichia coli	*Enterococcus faecalis*	*Enterococcus cloacae*	*Candida albicans*	*Candida glabrata*	*Pseudomonas aeruginosa*
Therapy	575	0	26 (4.52%)*	24	1	0	0	0	1
Control	575	0	75 (13.04%)	69	2	2	1	0	1

* P<0.001, statistically significant

and intermittent catheterization, immediate removal of the catheter, and other frameworks to prevent the occurrence of CAUTI [43,45,46].

The World Health Organization claimed systemic prophylactic antibiotic, irrigation of bladder, instilling normal saline or antibiotics, sterile drainage bag and other measures are ineffective in preventing the occurrence of CAUTI [1]. The use of anti-microbial drugs, anti-microbial drainage bag and irrigation of bladder can only temporarily reduce the chance of bacteriuria [13]. Furthermore, some studies have shown that the use of soap, skin cleansing foam, povidone iodine or saline in perineal care do not affect the incidence of CAUTI [47]. As for materials of the catheter, the single biological surface coated with silicon, polyurethane, synthetic biomaterials, or hydrogel material, were not proven effective in the prevention of bacterial colonization [5,16].

The formation of biofilm is regarded as one of the major causes of anti-microbial resistance and refractory CAUTI [13]. Therefore, the prevention of biofilm formation has been the research focus toward reducing the incidence of CAUTI. In this study, we investigated the use of new nanotechnology anti-microbial spray JUC, composed of organic silicon quaternary ammonium salt. JUC forms a positively charged film which isolates and kills negatively charged pathogenic micro-organisms including bacteria, fungi and viruses. The physical attraction between the film and the micro-organism would not lead to drug resistance [30].

In the laboratory, the formation of biofilm can be initiated by a small amount of bacteria. The bacteria clump together and form bacterial colonies. It then starts to form a biofilm. When the biofilm matures, it begins to shrink and collapse [48,49]. In the *in vitro* study, the initial stage of biofilm formation was observed at 16 hours of bacterial culture in the control group. After 7 days of incubation, aggregation of bacterial clumps was observed and the surface of sample fragments was unevenly rough, which illustrated the formation of mature biofilm. However, only few free bacteria, represented by red dots under microscope, were also observed in the therapy group. A thin membranous structure was observed which was at a stage between the bacterial colony formation and the initial formation of biofilm. The *in vitro* study clearly demonstrated that the physical anti-microbial film formed by JUC could prevent biofilm formation for 7 days upon application, which was validated in the clinical study. A significantly lower incidence of CAUTI was observed clinically in the therapy group (4.52%) than in the control group (13.04%), which further confirmed the effectiveness of JUC in the prevention of CAUTI.

Comparison between clinical trials

The pre-operative use of anti-microbial drugs as an effective way for the prevention of bacterial infection is widely accepted [50]. In many clinical trials, prophylactic antibiotics were commonly prescribed for the prevention of infection in catheterized patients [5]. The UTI rate did not exclude the factor of antibiotics use. In Tambyah's study [6], the mean antibiotics use was 1.6 ± 1.7 per catheter-day, and the incidence of CAUTI was 14.9% in the urology department. In the surgical unit, 1,162 patients were catheterized patients after surgery. The onset of CAUTI was 6.4 ± 6.1 catheterized days, and the incidence was 11.9% [6]. In Darouiche's study [51], 124 patients were catheterized in place for 14 days with regular silicone bladder catheters or silicone bladder catheters impregnated with minocycline and rifampin after radical prostatectomy. All patients were given a single parental dose of 1g cefazolin as prophylactic antibiotic before anesthesia. The UTI rates measured 7 days after surgery were 15.2% and 39.7% in patients with regular catheters and medicated catheters respectively. The incidence was much higher than the therapy or control group patients of our study.

Compared to the study of 1,497 patients with an overall incidence of CAUTI of 14.9% by Tambyah *et al.* [6], the incidence of CAUTI was higher than the therapy group (4.52%) and slightly higher than the control group (13.04%) of our study. In Tambyah's study, the patients were catheterized with nitrofurazone-impregnated silicone catheters, silver-polyurethane hydrogel catheters or control catheters and obvious differences were not observed in the incidence of UTI between medicated catheters and control catheters. Despite the different practices of the use of antibiotics between Tambyah's study and our study, it seems the duration and types of post-operative antibiotics were not associated with the

incidence of CAUTI. However, a significantly reduced incidence of CAUTI was observed in the therapy group of our study, indicating that the use of JUC, which was effective in preventing the biofilm formation, could be vital to lowering the incidence of CAUTI.

Conclusions

In the clinical trial, only 4.52% of the patients from the therapy group were diagnosed with CAUTI, compared to 13.04% from the control group. *In vitro* testing also showed no obvious biofilm formation in the therapy group sprayed with JUC after 7 days of bacterial incubation. Biofilm began forming after 16 hours of incubation in the control group. The results from the clinical trial and *in vitro* test demonstrated the effectiveness of JUC in the prevention of CAUTI and formation of biofilm.

Acknowledgements

We express our heartfelt thanks for the strong support of Chinese Medical Association Society of Urology. We are also thankful for the close collaborations with Tongji Affiliated Hospital of Tongji Medical College of the Huazhong University of Science & Technology, the Second Hospital of Lanzhou University, Peking University People's Hospital, the Second Military Medical University (Shanghai Changhai Hospital), the First Affiliated Hospital of the Sun Yat-sen University, the Second Affiliated Hospital of the Sun Yat-sen University, the Third Affiliated Hospital of the Sun Yat-sen University, the Second Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, the First Affiliated Hospital of Southern Medical University (Nanfang Hospital), the First Affiliated Hospital of Guangzhou Medical University, General Hospital of Guangzhou Military Command of PLA, Wuhan General Hospital of Guangzhou Military Region, Guangzhou First Municipal People's Hospital, Foshan Hospital of Traditional Chinese Medicine, West China Hospital of the Sichuan University, Daping Hospital of the Third Military Medical University, Xiangya Hospital of the Central-south University, the Second Hospital of Xi'an Jiaotong University, the First Affiliated Hospital of Nanjing Medical University, Nanjing Drum Tower Hospital Affiliated to the Nanjing University Medical School, the Second Affiliated Hospital of Kunming Medical College, Huai'an First Hospital Affiliated to Nanjing Medical University and the Affiliated Hospital of Nantong University.

This article has been published as part of *Journal of Translational Medicine* Volume 10 Supplement 1, 2012: Selected articles from the Organisation for Oncology and Translational Research (OOTR) 7th Annual Conference. The full contents of the supplement are available online at http://www.translational-medicine.com/supplements/10/S1.

Author details

[1]Department of Urology, Tongji Hospital Affiliated to Tongji Medical College, Huazhong University of Science & Technology, Hubei PRC. [2]Institute of Urology, the Second Hospital of Lanzhou University, Lanzhou, PRC. [3]Wuhan General Hospital of Guangzhou Military Region, Guangzhou, PRC. [4]West China Hospital, Sichuan University, Sichuan, PRC. [5]Daping Hospital, Third Military Medical University, Chongqing, PRC. [6]The First Affiliated Hospital of Guangzhou Medical University, Guangzhou, PRC. [7]The Second Military Medical University, Changhai Hospital, Shanghai, PRC. [8]Peking University People's Hospital, Beijing, PRC. [9]The Third Affiliated Hospital, Sun Yat-sen University, Guangdong, PRC. [10]The Second Hospital of Xi'an Jiaotong University, Xi'an, PRC. [11]The Second Affiliated Hospital, Sun Yat-sen University, Guangzhou, PRC. [12]Xiangya Hospital of Central-south University, Changsha, PRC. [13]Nanjing Drum Tower Hospital, The Affiliated Hospital of Nanjing University Medical School, Nanjing, PRC. [14]Huai'an First Hospital Affiliated to Nanjing Medical University, Jiangsu, PRC. [15]The First Affiliated Hospital, Sun Yat-sen University, Guangzhou, PRC. [16]The Second Affiliated Hospital of Guangzhou University of Traditional Chinese Medicine, Guangzhou, PRC. [17]The First Affiliated Hospital of Southern Medical University (Nanfang Hospital), Guangzhou, PRC. [18]Affiliated Hospital of Nantong University, Jiangsu, PRC. [19]Foshan Hospital of Traditional Chinese Medicine, Guangdong, PRC. [20]Guangzhou First Municipal People's Hospital, Guangzhou, PRC. [21]General Hospital of Guangzhou Military Command of PLA, Guangzhou, PRC. [22]The First Affiliated Hospital of Nanjing Medical University, Jiangsu, PRC. [23]The Second Affiliated Hospital of Kunming Medical College, Yunnan, PRC. [24]School of Chinese Medicine, The University of Hong Kong, Hong Kong SAR. [25]UNIMED Medical Institute, Hong Kong SAR.

Authors' contributions

QC, NC, JC, JPC, CG, WH, XJ, LL, ZL, SL, XL, PL, LL, XM, LM, WQ, LQ, ZR, XS, WS, YT, PW, XW, DW, ZW, BW, QY, ZY, ZY, YZ, HZ, YZ equally conducted clinical test planning and performance. LWCC, WTYL, MNBC, AYSY and ELYN participated in the writing of the manuscript.

Competing interests

The authors state they have no competing interests to declare.

Published: 19 September 2012

References

1. **Prevention of hospital-acquired infections. A practical guide**, 2 2002, WHO/CDS/CSR/EPH/2002.12.
2. Haley RW, Culver DH, White JW, Morgan WM, Emori TG: **The nationwide nosocomial infection rate: a new need for vital statistics.** *AM J Epidemiol* 1985, **121**:159-67.
3. Trautner BW, Darouiche RO: **Catheter-associated infections: pathogenesis affects prevention.** *Arch Intern Med* 2004, **164**:842-85.
4. Johnson JR, Kuskowski MA, Wilt TJ: **Systemic review: Antimicrobial urinary catheters to prevent catheter-associated urinary tract infection in hospitalized patients.** *Ann Intern Med* 2006, **144**:116-26.
5. Maki DG, Tambyah PA: **Engineering out the risk for infection with urinary catheters.** *Emerg. Infect. Dis* 2001, **7**:342-347.
6. Tambyah PA, Maki DG: **Catheter-associated urinary tract infection is rarely symptomatic: a prospective study of 1,497 catheterized patients.** *Arch. Intern. Med* 2000, **160**:678-682.
7. Sorbye LW, Finne-Soveri H, Ljunggren G, Topinkova E, Bernabei R: **Indwelling catheter use in home care; elderly, aged 65+, in 11 different countries in Europe.** *Age Ageing* 2005, **34**:377-381.
8. Burton E, Gawande PV, Yakandawala N, LoVetri K, Zhanel GG, Romeo T, Friesen AD, Madhyastha S: **Antibiofilm activity of GlmU enzyme inhibitors against catheter-associated uropathogens.** *Antimicrob Agents Chemother* 2006, **50**:1835-1840.
9. Tambyah PA, Knasinski V, Maki DG: **The direct costs of nosocomial catheter-associated urinary tract infection in the era of managed care.** *Infect Control Hosp Epidemiol* 2002, **23**:27-31.
10. Saint S: **Clinical and economic consequences of nosocomial catheter-related bacteriuria.** *Am J Infect Control* 2000, **28**:68-75.
11. Greene L, Marx J, Oriola S: **Guide to the Elimination of Catheter-Associated Urinary Tract Infections (CAUTIs).** The Association for Professionals in Infection Control and Epidemiology (APIC) Washington, DC; 2008.
12. **Catheter-Associated Urinary Tract Infection (CAUTI) Event.** CDC; 2009.
13. Trautner BW, Darouiche RO: **Role of biofilm in catheter-associated urinary tract infection.** *AM J Infect Control* 2004, **32**:177-183.
14. Costerton JW, Stewart PS, Greenberg EP: **Bacterial biofilms: a common cause of persistent infections.** *Science* 1999, **284**:1318-1322.
15. Donlan RM, Costerton JW: **Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms.** *Clin. Microbiol. Rev* 2002, **15**:167-193.
16. Donlan RM: **Biofilms and device-associated infections.** *Emerg. Infect. Dis* 2001, **7**:277-281.
17. Schaudinn C, Stoodley P, Kainovic A, O'Keefe T, Costerton B, Robinson D, Baum M, Erlich G, Webster P: **Bacterial biofilms, other structures seen as mainstream concepts.** *Microbe* 2007, **2**:231-237.
18. Flemming HC, Neu TR, Wozniak DJ: **The EPS Matrix: The "House of Biofilm Cells".** *J. Bacteriol* 2007, **189**:7945-7947.
19. Costerton J, Geesey G, Cheng K: **How bacteria stick.** *Sci Am* 1978, **238**:86-95.
20. Hausner M, Wuertz S: **High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis.** *Appl. Environ. Microbiol* 1999, **65**:3710-3713.

21. Johnson JR, Delavari P, Azar M: **Activities of a nitrofurazone-containing urinary catheter and a silver hydrogel catheter against multidrug-resistant bacteria characteristic of catheter-associated urinary tract infection.** *Antimicrob. Agents Chemother* 1999, **43**:2990-2995.
22. Johnson JR, Berggren T, Conway AJ: **Activity of a nitrofurazone matrix urinary catheter against catheter-associated uropathogens.** *Antimicrob. Agents Chemother* 1993, **37**:2033-2036.
23. Tenke P, Kovacs B, Bjerklund Johansen TE, Matsumoto T, Tambyah PA, Naber KG: **European and Asian guidelines on management and prevention of catheter-associated urinary tract infections.** *Int J Antimicrob Agents* 2008, **31(Suppl 1)**:S68-78.
24. Kunin CM: **Nosocomial urinary tract infections and the indwelling catheter: what is new and what is true?** *Chest* 2001, **120**:10-12.
25. Gould CV, Umscheid CA, Agarwal RK: **Guideline for prevention of catheter-associated urinary tract infections 2009 (CDC).** *Infect Control Hosp Epidemiol* 2010, **31**:319-326.
26. Stickler D, Morris N, McLean R, Fuqua C: **Biofilms on indwelling urethral catheters produce quorum-sensing signal molecules in situ and in vitro.** *Appl Environ Microbiol* 1998, **64**:3486-90.
27. Davies D, Parsek M, Pearson J, Iglewski B, Costerton J, Greenberg E: **The involvement of cell-to-cell signals in the development of a bacterial biofilm.** *Science* 1998, **280**:295-8.
28. Trautner B, Hull R, Darouiche R: **Escherichia coli 83972 inhibits catheter adherence by a broad spectrum of uropathogens.** *Urology* 2003, **61**:1059-62.
29. Trautner B, Darouiche R, Hull R, Hull S, Thornby J: **Pre-inoculation of urinary catheters with Escherichia coli 83972 inhibits catheter colonization by Enterococcus faecalis.** *J Urol* 2002, **167**:375-379.
30. Zeng Y, Runzhi Deng R, Yeung BH, Loo WTY, Cheung MN, Chen JP, Zhou B, Fu Y, Huang L, Lu M, Wang M: **Application of an antibacterial dressing spray in the prevention of post-operative infection in oral cancer patients: A phase 1 clinical trial.** *African Journal of Biotechnology* 2008, **7**:3827-3831.
31. Li W, Ma X, Peng Y, Cao J, Loo TY, Hao L, Cheung MN, Chow LW, Jin L: **Application of a nano-antimicrobial film to prevent ventilator-associated pneumonia: A pilot study.** *African Journal of Biotechnology* 2011, **10**:1926-193.
32. Wan KA, Ng MY, Wong T: **New horizon on community-acquired methicillin resistant Staphylococcus aureus (CA-MRSA) skin and soft tissue infection: nanotechnology antimicrobial spray.** *Hong Kong Journal of Emergency Medicine* 2011, **18**:432-436.
33. Viana ES, Campos ME, Ponce AR: **Biofilm formation and acyl homoserine lactone production in Hafnia alvei isolated from raw milk.** *Biol Res* 2009, **42**:427-436.
34. Pearson MM, Laurence CA, Guinn SE, Hansen EJ: **Biofilm Formation by Moraxella catarrhalis In Vitro: Roles of the UspA1 Adhesin and the Hag Hemagglutinin.** *Infect Immun* 2006, **74**:1588-1596.
35. Oasgupta MK, Bettcher KB, Ulan RA, Burns V, Lam K, Oossetor JB, Costerton WJ: **Relationship of adherent bacterial biofilms to peritonitis in chronic Ambulatory Peritoneal dialysis.** *Perit Dial Int* 1987, **7**:168-73.
36. Gorman SP, Mawhinney WM, Adair CG, Issoukis M: **Confocal laser scanning microscopy of peritoneal catheter surfaces.** *J Med Microbiol* 1993, **38**:411-7.
37. **Guidelines on Standard Operating Procedures for MICROBIOLOGY – Chapter 19- Urinary Tract Infection.** WHO SEARO; 2006.
38. Saint S, Lipsky Benjamin A: **Preventing Catheter-Related Bacteriuria. Should We? Can We? How?** *Arch Intern Med* 1999, **159**:800-808.
39. Stensballe J, Tvede M, Looms D, Lippert FK, Dahl B, Tønnesen E, Rasmussen LS: **Infection risk with nitrofurazone-impregnated urinary catheters in trauma patients: a randomized trial.** *Ann Intern Med* 2007, **147**:285-293.
40. Vejborg RM, Klemm P: **Blocking of Bacterial Biofilm Formation by a Fish Protein Coating.** *Appl Environ Microbiol* 2008, **74(11)**:3551-3558.
41. McTaggart LA, Rigby RC, Elliott TS: **The pathogenesis of urinary tract infections associated with *Escherichia coli*, *Staphylococcus saprophyticus* and *S. epidermidis*.** *J Med Microbiol* 1990, **32**:135-141.
42. Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME: **Complicated Catheter-Associated Urinary Tract Infections Due to Escherichia coli and Proteus mirabilis.** *Clin Microbiol Rev* 2008, **21**:26-59.
43. Saint S, Meddings JA, Calfee D, Kowalski CP, Krein SL: **Catheter-associated urinary tract infection and the Medicare rule changes.** *Ann Intern Med* 2009, **150**:877-884.
44. Wong ES: **Guideline for prevention of catheter-associated urinary tract infections.** *Am J Infect Control* 1983, **11**:28-36.
45. Yokoe DS, Mermel LA, Anderson DJ, Arias KM, Burstin H, Calfee DP, Coffin SE, Dubberke ER, Fraser V, Gerding DN, Griffin FA, Gross P, Kaye KS, Klompas M, Lo E, Marschall J, Nicolle L, Pegues DA, Perl TM, Podgorny K, Saint S, Salgado CD, Weinstein RA, Wise R, Classen D: **A compendium of strategies to prevent healthcare-associated infections in acute care hospitals.** *Infect Control Hosp Epidemiol* 2008, **29(Suppl 1)**:S12-21.
46. Lo E, Nicolle L, Classen D, Arias KM, Podgorny K, Anderson DJ, Burstin H, Calfee DP, Coffin SE, Dubberke ER, Fraser V, Gerding DN, Griffin FA, Gross P, Kaye KS, Klompas M, Marschall J, Mermel LA, Pegues DA, Perl TM, Saint S, Salgado CD, Weinstein RA, Wise R, Yokoe DS: **Strategies to prevent catheter-associated urinary tract infections in acute care hospitals.** *Infect Control Hosp Epidemiol* 2008, **29(Suppl 1)**:S41-50.
47. Jeong I, Park S, Jeong JS, Kim DS, Choi YS, Lee YS, Park YM: **Comparison of Catheter-associated Urinary Tract Infection Rates by Perineal Care Agents in Intensive Care Units.** *Asian Nursing Research* 2010, **4**:142-150.
48. Douglas LJ: **Medical importance of biofilms in *Candida infections*.** *Rev Iberoam Micol* 2002, **19**:139-143.
49. Xi C, Marks DL, Schlachter S, Luo W, Boppart SA: **High-resolution three-dimensional imaging of biofilm development using optical coherence tomography.** *J Biomed Opt* 2006, **11**:34001.
50. Antibiotic Action Group Aintree Hospitals NHS Trust, Aintree University Hospitals NHS Foundation Trust, The Walton Centre: **Guidelines For Antimicrobial Therapy.**, Eighth 2007.
51. Darouiche RO, Smith JA Jr, Hanna H, Dhabuwala CB, Steiner MS, Babaian RJ, Boone TB, Scardino PT, Thornby JI, Raad II: **Efficacy of antimicrobial-impregnated bladder catheters in reducing catheter-associated bacteriuria: a prospective, randomized, multicenter clinical trial.** *Urology* 1999, **54**:976-981.

doi:10.1186/1479-5876-10-S1-S14
Cite this article as: He *et al.*: Application of a nanotechnology antimicrobial spray to prevent lower urinary tract infection: a multicenter urology trial. *Journal of Translational Medicine* 2012 **10**(Suppl 1):S14.

应用纳米技术抗微生物喷雾剂来预防低尿路感染：多中心泌尿实验

何玮[1] 王东敏[2] 叶章群[1] 钱卫红[3] 陶燕[3] 史晓凤[3] 刘玲[4] 陈锦[5] 邱玲[6] 万蓬[7] 贾晓君[8] 栗霞[9] 高彩霞[10] 马雪霞[11] 文碧燕[9] 程念珍[12] 李萍[13] 任琤琤[14] 蓝丽[15] 李思逸[16] 左翼[17] 张华[18] 马利名[18] 张跃平[18] 黎智聪[19] 苏为平[20] 杨青[21] 陈庆丽[22] 王雪静[22] 叶珍妮[23] JP Chen[24], Wings TY Loo[24,25*], Louis WC Chow[25], Adrian YS Yip[25], Elizabeth LY Ng[25], Mary NB Cheung[25], 王志平[2+]

选自癌转译研究组织（OOTR）第七届年会
香港，2011 年 5 月 13-14 日

摘要

背景：CAUTI 是常见的院内器械相关感染。现发现导致其感染率居高不下的原因是导尿管表面形成的生物膜阻碍了抗生素对细菌的作用，导致了抗微生物耐药性。在本研究中，我们进行了体外试验来研究生物膜形成的机理，然后转向临床多中心研究，观察应用 JUC，一种纳米技术抗微生物喷雾剂预防 CAUTI 的效果。

方法：将硅化乳胶导尿管切割为若干样品片段，并进行高压灭菌。把灭菌后的样品片段随机分为治疗组和对照组，分别喷洒 JUC 和蒸馏水，均干燥后待用。

实验用的大肠杆菌的标准菌株由患者尿液样本中分离获得。在培养 16 小时和 7 天时取出样本以备激光共聚焦显微镜观察。

本临床研究共招募了 1150 名患者。患者根据治疗入选的次序，随机分组，奇数组入选治疗组（JUC），偶数组入选对照组（生理盐水）。

结果：经过 16 小时培养，对照组样品片段表面形成细菌生物膜。治疗组样品片段上未观察到细菌生物膜形成。7 天培养后，治疗组未观察到细菌定殖的显著增加。

第 7 天拔管前采集尿液作细菌培养。治疗组和对照组菌尿率有显著差异。(4.52% *vs.* 13.04%, $p < 0.001$)。

结论：在本研究中，JUC 在医院环境中预防 CAUTI 的有效性在体外和临床研究中均得到证实。

*通信：wtyloo@gmail.com

[24] 香港大学中医药学院，香港特别行政区。

全部作者信息见本文结尾。

背景

CAUTI 是常见的院内感染，UTI 占院内感染的比例高达 40%，成为院内感染（HAI）的重要原因之一，约 80%的 UTI 与导尿管有关[1,2]。在美国，约 95%的尿路感染与留置导尿管有关[3]。医院内短期护理的患者中有 15-25%无法自主排尿的患者需要留置尿管[4]。每年只在美国需插管治疗的患者就超过 500 万[5]，而患 CAUTI 的患者则有 100 万[6]。而欧洲的研究表明，大于 65 岁的老人使用留置尿管比例是 5.4%[7]。

CAUTI 是一种常见感染，引起的危害很大，在美国 CAUTI 使住院时间增加 2.4 到 4.5 天[8]，但因诊断和治疗 CAUTI 的平均花费为 589 美元（不包括延期出院的费用）[9]，加上住院等费用，每人平均增加 2836~3803 美元[10,11]。美国疾病预防和控制中心（CDC）指出每年美国 UTI 导致超过 13000 位患者死亡[12]，因而 CAUTI 对全世界的医疗有巨大的影响。

现发现导致其感染率居高不下的原因是导尿管表面形成的生物膜阻碍了抗生素对细菌的作用[8,13,14]。研究表明生物膜是以细胞外基质围绕包裹细菌形成。细菌生物膜为特殊的蜂巢形结构，有非常复杂生态系统。生物膜的放大倍数会在显微镜下显示微菌落[15-18]。形成生物膜后细菌可以抵御剪力、pH 变化、抗菌药物，并阻止噬菌作用 [13,19]。且遗传信息交换的频率远远大于游离细胞[20]，因此耐抗菌素的基因和菌株得以传播。一旦生物膜形成，就会保护尿道病原菌对抗抗菌药物和宿主的免疫应答，而从生长的细菌或者成熟的生物膜上脱落的子细胞团，还能入侵其它的地方[15]。生物膜阻碍了抗生素对细菌的作用，才导致 CAUTI 难治[13]。

现有的研究旨在预防、改变生物膜形成的新方法，其中包括呋喃酮、呋喃西林、含银导尿管等[21-24]。Johnson 等人[21,2]发现含呋喃西林或含银水凝胶涂层的导尿管在抑制生物膜形成方面有一定效果，但是对对铜绿假单胞杆菌则没有作用[22]。美国 CDC 的研究指出，表面有银涂层的导管与标准导尿管在预防菌尿症上没有区别[25]，银合金导管在留置 1 周内有减少菌尿症的作用。而 Burton 等人[8]发现新型 N,N-(1,2-亚苯基)双马来酰乙胺加硫酸鱼精蛋白(oPDM-plus-PS)涂层可以抑制铜绿假单胞杆菌和表皮葡萄球菌对导管的粘附[14]，但这些带涂层的导管目前只能在短期导尿管植入中才能起到预防 CAUTI 的作用[13] 。最近 Stickler 等人[26]发现导尿管上的生物膜细菌产生微生物的群体感应信号(quorum-sensing signal)，可控制生物膜形成的基因表达，如果破坏这些信号的产生，就能阻止生物膜形成。例如，不能产生群体感应信号的绿脓杆菌的突变柱无法成熟为 3 维结构的生物膜[27]。还有人发现一些元素如铁对生物膜形成是必须的营养，而去铁导管也是发展的方向，但是现在还没有临床试验[13]。益生菌也是一个研究方向，Trautner 等人发现利用非致病型大肠杆菌可以减少致病菌和 CAUTI 的感染率 [28,29]。虽然这些方法也有一定的前景，但目前还没有确证，而且在成本-效果方面也有问题。

将 JUC 用于术后患者伤口的传统应用已在医院和门诊环境中被证明是有效的：应用 JUC 不会导致耐药性，也不会引起严重不良反应，并缩短了患者的平均创面愈合时间[30]。JUC 采用纳米制造技术，产生具有纳米级分子结构的纳米阳离子，然后制备成水溶性喷雾剂[31]。 JUC 通过物理机理达到皮肤和伤口表面的抗菌作用，因此可以被视为一个物理抗菌剂[31]。应用时，JUC 通过在喷洒表面形成隐形、带正电荷的保护膜来防止细菌生长，隔离和消除带负电荷的病原微生物包括细菌，真菌和病毒[31,32]。

由于临床工作中还未出现有效阻止生物膜形成的方法，我们力求在寻找一种革命性的有效阻止生物膜的形成的创新方法。首先从由实验室物理抗微生物膜体外细菌生物膜形成的机理，然后转向临床多中心研究，观察其在中国地区临床试验中预防 CAUTI 的效果。

方法

体外试验

细菌制备

实验用的大肠杆菌的标准菌株由兰州大学第二医院临床尿路感染患者分离获得。将细菌在 LB 培养基中培养[33]。制备细菌悬液，并调整菌液浓度至 7.4×10^9CFU/ml。

样品片段制备

将硅化乳胶导尿管切割为若干样品片段，并进行高压灭菌。把灭菌后的样品片段随机分为实验组和对照组各 8 片，实验组表面分别喷洒 JUC 长效抗菌材料形成物理抗微生物膜，对照组喷洒蒸馏水，均干燥后待用。将大肠杆菌菌液注入 24 孔板，放入样品片段，在 37℃恒温箱中孵育，每 48h 更换 PBS 液一次[34]。在培养 16 小时和 7 天时取出样本以备激光共聚焦显微镜观察。

激光共聚焦显微镜观察

把培养的样本浸泡于 1mlPBS 液中，加入 50ug/ml 碘化丙锭 4℃避光染色 15min 或常温染色 30min 后取出，倒置于载玻片上，通过激光共聚焦显微镜观察生物膜形成情况[35,36]。

临床试验

本研究开始于 2010 年 3 月，结束于 2011 年 12 月。进行了泌尿外科手术，需留置导尿管及住院 7 天以上的患者被招募。共计 1150 例患者（其中男 869 例，女 281 例），年龄 2-82 岁。23 家医院参与了此次临床实验，每个医院对照组和治疗组各招募 25 位患者。所有患者因泌尿疾病而进行手术，包括但不限于尿路结石、肿瘤、前列腺增生、肾盂输尿管狭窄和肾积水。对于需住院 7 天以上的病人来说，留置导尿管是必要的。入组时患者中段尿细菌培养[15-17]阴性，且排除了长期使用气囊导尿管、自我间歇导尿术、耻骨上经皮穿刺膀胱造瘘术的患者和有 UTI 的患者。患者根据治疗入选的次序，随机分组，奇数组入选治疗组（JUC）575 例，偶数组入选对照组（生理盐水）575 例。两组在年龄、性别、发病原因、地区分布等方面均具可比性，无显著性差异（P>0.05）。研究者通过与每位患者或家属谈话，在充分理解的基础上，签订知情同意书。本研究方案由同济医院伦理委员会批准（批准号：2010006D）。

研究设计

治疗组

手术中留置导尿，导尿前用 JUC 长效抗菌材料喷洒导管外表面，使导管表面形成物理抗微生物膜。手术后在常规护理的基础上，再用 JUC 喷洒尿道口周围皮肤和粘膜、导尿管与引流管连接处，2

次/日，1ml/次（掀压喷头约 10 次），直到第 7 天导尿管拔除。

对照组

手术中留置导尿，手术后用生理盐水进行常规护理，直到第 7 天导尿管拔除。

所有受试者术后根据实际情况应用抗生素，并按公共机构指南中规定的分级标准，仔细、严格记录抗生素种类及用法用量（表 1）。一级抗菌药物应用 521 人次（40.08%），二级抗菌药物使用 572 人次（44%），三级抗菌药物应用 207 人次（15.92%）。一级抗生素非限制使用：经临床长期应用证明安全、有效，对细菌耐药性影响小，价格相对较低的抗菌药物。二级抗生素限制使用：与非限制使用抗菌药物相比较，这类药物在疗效、安全性、对细菌耐药性影响、药品价格等方面存在局限性，不宜作为非限制药物使用。三级抗生素为新上市的抗菌药物；其疗效或安全性任何一方面的临床数据尚较少，已报告不良反应。由于对三级药物安全性的担心，不推荐使用。临床需要加倍保护以免对抗菌药物产生耐药性。三级抗生素在三种抗生素中价格最贵。

每家医院在应用抗菌药物的种类和级别之间有应用的差异，而且有 150 例患者出现抗菌药物联合用药的情况，根据患者情况选用抗生素抗菌药物应用的天数为 0-7 天不等。广州第一人民医院和中山大学附属第一医院应用的抗菌药物中一级抗菌药物各占其使用的 97.92%和 94.12%，而第三军医大学第三附属医院大坪医院应用的抗菌药物中的二级抗菌药物占绝对优势为 97.40%。但是每个医院对照组和治疗组之间均无统计意义的差异。如西安交通大学第二附属医院治疗组的一级抗菌药物对照组和治疗组均有 10 例使用，二级抗菌药物对照组和治疗组分别有 12 例和 18 例使用，三级抗菌药物对照组和治疗组分别有 8 例和 6 例使用。广州军区广州总医院治疗组的一级抗菌药物对照组和治疗组均有 10 例使用，二级抗菌药物对照组和治疗组分别有 16 例和 13 例使用，三级抗菌药物对照组和治疗组分别有 3 例和 4 例使用。因而虽每个医院应用的抗菌药物种类和等级不同，但具有统计意义。

手术后，每日记录患者的体温、UTI 症状，受试者于导尿管留置的第 7 天拔管前在严格无菌操作下采集尿液并作尿液的细菌培养[37]。

疗效评定

受试者手术后尿样本定量培养计数，菌落计数 $\geq 10^3$CFU/ml，判为 CAUTI[9,38,39]。

统计学方法

研究结果采用配对 T 检验进行差异显著性分析，应用 SPSS14.0 统计软件包进行。

结果

体外试验结果

经过 16 小时培养，对照组样品片段表面形成生物膜，生物膜被碘化丙锭荧光染剂染为红色（见图 1A）。治疗组样品片段上无生物膜形成，显微镜视野中仅见少量游离细菌呈现出红色亮点（见图 1B）。

经过 7 天培养，对照组样品片段表面形成较厚的生物膜，其着色均匀、致密、深染，由于细菌过度繁殖，交联聚集形成细菌团块，样品片段表面粗糙，凹凸不平（见图 2A）。实验组样品片段表面只形成少量微薄的膜状结构，表面光滑、色浅，其余无异常（见图 2B）。

临床试验结果

两组受试者在第 7 天拔管前采集尿液作细菌培养，治疗组中有 26 例出现菌尿症，其中 24 例的阳性细菌为大肠杆菌，1 例为粪肠球菌，1 例为光滑念球菌，菌尿率为 4.52%；对照组中有 75 例出现菌尿症，其中 69 例的阳性细菌为大肠杆菌，2 例为粪肠球菌，2 例为阴沟肠肠球菌，1 例为白色念球菌，1 例为铜绿假单胞杆菌，总菌尿率为 13.04%，详细结果见表 2。在所有共计 101 例感染情况中，大肠杆菌感染占 92.08%，粪肠球菌占 2.97%，阴沟肠肠球菌占 1.98%。治疗组和对照组菌尿率有显著差异。(4.52% vs. 13.04%, $p < 0.001$)。

讨论

细菌种类

尿路感染是一种主要的院内感染。CAUTI是最常见的细菌性感染之一[1,2]；尿道周围区域存在大量的肠道细菌[3,38]，大多短期的CAUTI由单一的细菌引起，例如大肠杆菌，奇异变形杆菌，肺炎克雷伯菌，而长期的则有多重微生物引起[3,40,41]。尿道致病的大肠杆菌是最常见，由其导致的CAUTI占50%的院内获得性尿路感染[3,42]。在本研究中，我们也看到类似结果，大肠杆菌的感染占绝对优势92.08%，其它细菌有粪肠球菌和阴沟肠球菌等，都占的比例很小。

预防导尿管感染的方法

20 多年前，美国 CDC 就明确提出为了预防 CAUTI 强调采用手部卫生、无菌插管和应用封闭式引流装置的方法[43,44]；近期院内感染联合工作组提出了几个框架，包括：对感染的监察，加强教育和培训，采用适当的插入技术，取代留置尿管的方法（如避孕套和间歇导尿），实时移除导尿管等框架来预防 CAUTI 的发生[43,45,46]。

世界卫生组织认为，全身预防性应用抗生素、膀胱冲洗、灌输生理盐水或抗生素、无菌引流袋和其它方法在预防 CAUTI 的发生上都是无效的[1]。抗菌药物的使用、抗菌引流袋和膀胱冲洗只能暂时减少菌尿症的发生[13]。而且，有研究证明不论是应用肥皂水、皮肤清洁泡沫、聚维酮碘、生理盐水护理会阴并不影响 CAUTI 的发生率[47]。至于导尿管的材料，涂有硅、聚亚安酯、合成生物材料或水凝胶材料的单一生物表面在预防细菌定殖上被证明是无效的[5,16]。

在实验室里，生物膜的形成是起源于少量的细菌。这些细菌聚集在一起，形成了菌落。然后，它们开始形成生物膜。生物膜成熟时就开始萎缩和塌陷[48,49]。在体外研究中，观察对照组 16 小时细菌培养下生物膜形成的初始阶段。7 天培养后，观察到细菌团块聚集和样品片段的表面粗糙不均，这说明已经形成成熟的生物膜。但同时在治疗组，只观察到少数游离细菌在显微镜下表现为红色小点。观察到薄膜结构处于细菌集落形成和生物膜初步形成之间的阶段。体外研究清楚地表明，JUC 形成的物理抗微生物膜可以在应用后 7 天内预防生物膜形成，这在临床研究中得以验证。临床观察发现治疗组(4.52％)的 CAUTI 发病率显著低于对照组(13.04％)，这进一步证实了 JUC 预防的 CAUTI 的有效性。

临床试验之间的对比

术前使用抗菌药物作为预防细菌感染的一种有效方法已被广泛接受[50]。在许多临床试验中，预防性应用抗生素常用于导尿患者的感染预防[5]。尿路感染率不排除抗生素的使用因素。在 Tambyah 的研究中[6]，抗生素的平均使用率是每个导尿日 1.6±1.7 个，泌尿科 CAUTI 的发病率为 14.9％。在手术室，1162 例患者为术后导尿患者。CAUTI 的发病为 6.4±6 个导尿日，发病率为 11.9％[6]。在 Darouiche 的研究中[51]，124 例患者在前列腺癌根治术后使用普通硅膀胱导尿管或浸渍有米诺环素和利福平的硅膀胱导尿管插管 14 天。所有患者均在麻醉前给予单一亲代剂量的 1g 头孢唑啉作为预防性抗生素。术后 7 天测得使用普通导尿管和含药导尿管患者的尿路感染率分别为 15.2％和 39.7％。发病率比我们研究中的治疗组或对照组患者高得多。

与 Tambyah 等人有关 1497 例患者 CAUTI 总发病率 14.9％的研究相比[6]，CAUTI 发病率高于我们研究中的治疗组(4.52％)，略高于对照组(13.04％)。在 Tambyah 的研究中，患者使用呋喃西林浸渍的硅导尿管、银聚氨酯水凝胶导尿管或对照导尿管插管，但是含药导尿管和对照导尿管之间在尿路感染的发病率上未观察到有显著差异。尽管 Tambyah 研究和我们研究之间在抗生素使用操作方面有所不同，但是术后使用抗生素的持续时间和类型似乎与 CAUTI 的发病率并不相关。然而，在我们的研究中观察到治疗组的 CAUTI 发病率显著降低，表明使用 JUC 可以有效地预防生物膜的形成，这对于降低 CAUTI 的发病率至关重要。

结论

在临床试验中，仅 4.52％的治疗组患者被确诊有 CAUTI，而对照组有 13.04％。体外试验也显示，细菌培养 7 天后，治疗组喷洒 JUC 后没有明显的生物膜形成情况。对照组培养 16 小时后生物膜开始形成。临床试验和体外试验的结果证明了 JUC 可以有效地预防 CAUTI 和生物膜的形成。

致谢

感谢中华医学会泌尿外科学分会的大力支持；感谢华中科技大学同济医学院附属同济医院、兰州大学第二医院、北京大学人民医院、第二军医大学第一附属医院（上海长海医院）、中山大学附属第一医院、中山大学附属第二医院、中山大学附属第三医院、广州中医药大学第二附属医院、南方医科大学第一附属医院（南方医院）、广州医学院第一附属医院、广州军区广州总医院、广州军区武汉总医院、广州第一人民医院、广州中医药大学附属医院(佛山市中医院)、四川大学附属华西医院、第三军医大学大坪医院、中南大学湘雅医院、西安交通大学医学院第二附属医院、南京医科大学第一附属医院、南京大学医学院附属鼓楼医院、昆明医学院第二附属医院、南京医科大学附属淮安第一医院、南通大学附属医院的积极配合。本文已刊登于《转化医学杂志》2012 年卷 10 增刊 1：选自癌转译研究组织（OOTR）第七届年会的文章。增刊所有内容可在线浏览：

http://www.translationalmedicine.com/supplements/10/S1

作者信息

1 华中科技大学同济医学院附属同济医院，中国湖北。2 兰州大学第二医院，中国兰州。3 广州军区武汉总医院，中国广州。4 四川大学附属华西医院，中国四川。5 第三军医大学大坪医院，中国重庆。6 广州医学院第一附属医院，中国广州。7 第二军医大学长海医院，中国上海。8 北京大学人民医院，中国北京。9 中山大学附属第三医院，中国广州。10 西安交通大学医学院第二附属医院，中国西安。11 中山大学附属第二医院，中国广州。12 中南大学湘雅医院，中国长沙。13 南京大学医学院附属鼓楼医院，中国南京。14 南京医科大学附属淮安第一医院，中国江苏。15 中山大学附属第一医院，中国广州。16 广州中医药大学第二附属医院，中国广州。17 南方医科大学第一附属医院（南方医院），中国广州。18 南通大学附属医院，中国江苏。19 广州中医药大学附属医院(佛山市中医院)，中国广东。20 广州第一人民医院，中国广州。21 广州军区广州总医院，中国广州。22 南京医科大学第一附属医院，中国江苏。23 昆明医学院第二附属医院，中国云南。24 香港大学中医药学院，香港特别行政区。25 仁康医疗乳腺综合诊治中心，香港特别行政区。

作者贡献

QC, NC, JC, JPC, CG, WH, XJ, LL, ZL, SL, XL, PL, LL, XM, LM, WQ, LQ, ZR, XS, WS, YT, PW, XW, DW, ZW, BW, QY, ZY, ZY, YZ, HZ, YZ 同样进行了临床试验的设计和执行。LWCC, WTYL, MNBC, AYSY 和 ELYN 参与了手稿的撰写。

竞争利益

各位作者无竞争利益。

发表：2012年9月19日

参考文献

1. Prevention of hospital-acquired infections. A practical guide. 2nd edition 2002, WHO/CDS/CSR/EPH/2002.12.
2. Haley RW, Culver DH, White JW, Morgan WM, Emori TG. The nationwide nosocomial infection rate: a new need for vital statistics. AM J Epidemiol 1985;121:159-67
3. Trautner BW, Darouiche RO. Catheter associated infections: pathogenesis affects prevention. Arch Intern Med, 2004.164:842-850
4. Johnson JR., Kuskowski MA, Wilt.TJ. Systemic review: Antimicrobial urinary catheters to prevent catheter-associated urinary tract infection in hospitalized patients. Ann Intern Med. 2006;144:116-26
5. Maki, D. G., and Tambyah P. A.. Engineering out the risk for infection with urinary catheters. Emerg. Infect. Dis. 2001.7:342–347.
6. Tambyah, P. A., and Maki.D. G. Catheter-associated urinary tract infection is rarely symptomatic: a prospective study of 1,497 catheterized patients. Arch. Intern. Med. 2000.160:678–682.
7. Sorbye LW, Finne-Soveri H, Ljunggren G, Topinkova E, Bernabei R. Indwelling catheter use in home care; elderly, aged 65+, in 11 different countries in Europe. Age Ageing 2005; 34: 377–81.
8. Burton E., Gawande P. V., Yakandawala N., et al .Antibiofilm Activity of GlmU Enzyme Inhibitors against Catheter-Associated Uropathogens. ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, 2006, 50:5:1835–1840
9. Tambyah PA, Knasinski V, Maki DG. The direct costs of nosocomial catheter-associated urinary tract infection in the era of managed care. Infect Control Hosp Epidemiol. 2002;23:27-31
10. Saint, S. 2000. Clinical and economic consequences of nosocomial catheter-related bacteriuria. Am. J. Infect. Control 28:68–75
11. Greene L., Marx J., Oriola S. Guide to the Elimination of Catheter-Associated Urinary Tract Infections (CAUTIs) The Association for Professionals in Infection Control and Epidemiology (APIC) Washington, DC
12. Catheter-Associated Urinary Tract Infection (CAUTI) Event. CDC .March, 2009
13. Trautner BW, Darouiche RO. Role of biofilm in catheter-associated urinary tract infection. AM J Infect Control 2004;32:177-83
14. Costerton, J.W., P. S. Stewart, and E. P. Greenberg. 1999. Bacterial biofilms: a common cause of persistent infections. Science 284:1318–1322.
15. Donlan, R.M. and Costerton, J.W., Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms, Clin. Microbiol. Rev., 2002, 15：167–193.
16. Donlan, R. M. Biofilms and device-associated infections. Emerg. Infect. Dis. 2001.7:277–281.
17. Schaudinn, C., P. Stoodley, A. Kainovic, T. O'Keefe, B. Costerton, D. Robinson, M. Baum, G. Erlich, and P. Webster. Bacterial biofilms, other structures seen as mainstream concepts. Microbe 2007. 2:231-237.
18. Flemming H.-C., Neu T. R., and Wozniak D. J. The EPS Matrix: The "House of Biofilm Cells" J. Bacteriol., 2007; 189(22): 7945 - 7947.
19. Costerton J, Geesey G, Cheng K. How bacteria stick. Sci Am.1978;238:86-95.
20. Hausner, M., and S. Wuertz. High rates of conjugation in bacterial biofilms as determined by quantitative in situ analysis. Appl. Environ. Microbiol. 1999.65:3710–3713.
21. Johnson JR, Delavari P, Azar M: Activities of a nitrofurazone-containing urinary catheter and a silver hydrogel catheter against multidrug-resistant bacteria characteristic of catheter-associated urinary tract infection.Antimicrob. Agents Chemother. 1999.43:2990–2995.
22. Johnson, J. R., T. Berggren, and A. J. Conway. Activity of a nitrofurazone matrix urinary catheter against catheter-associated uropathogens. Antimicrob. Agents Chemother. 1993. 37:2033–2036
23. Tenke P, Kovacs B, Bjerklund Johansen TE, et al.; European and Asian guidelines on management and prevention of catheter-associated urinary tract infections.; Int J Antimicrob Agents; 2008; Vol. 31 Suppl 1; pp. S68-78

24. Kunin CM. Nosocomial urinary tract infections and the indwelling catheter: what is new and what is true? Chest. 2001;120:10–12.
25. Gould CV, Umscheid CA, Agarwal RK, et al. Guideline for prevention of catheter-associated urinary tract infections 2009（CDC）. Infect Control Hosp Epidemiol. 2010;31:319-326
26. Stickler D, Morris N, McLean R, Fuqua C. Biofilms on indwelling urethral catheters produce quorum-sensing signal molecules in situ and in vitro. Appl Environ Microbiol 1998;64:3486-90.
27. Davies D, Parsek M, Pearson J, Iglewski B, Costerton J, Greenberg E. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 1998;280:295-8.
28. Trautner B, Hull R, Darouiche R. Escherichia coli 83972 inhibits catheter adherence by a broad spectrum of uropathogens. Urology 2003;61:1059-62.
29. Trautner B, Darouiche R, Hull R, Hull S, Thornby J. Pre-inoculation of urinary catheters with Escherichia coli 83972 inhibits catheter colonization by Enterococcus faecalis. J Urol 2002;167:37
30. Zeng Y, Runzhi Deng R, Yeung BH, Loo WTY, Cheung MN, Chen JP, Zhou B, Fu Y, Huang L, Lu M, Wang M: Application of an antibacterial dressing spray in the prevention of post-operative infection in oral cancer patients: A phase 1 clinical trial. African Journal of Biotechnology 2008, 7:3827-3831.
31. Li W, Ma X, Peng Y, Cao J, Loo TY, Hao L, Cheung MN, Chow LW, Jin L: Application of a nano-antimicrobial film to prevent ventilator-associated pneumonia: A pilot study. African Journal of Biotechnology 2011, 10:1926-193.
32. Wan KA, Ng MY, Wong T: New horizon on community-acquired methicillin resistant Staphylococcus aureus (CA-MRSA) skin and soft tissue infection: nanotechnology antimicrobial spray. Hong Kong Journal of Emergency Medicine 2011, 18:432-436.
33. Viana ES, Campos ME, Ponce AR: Biofilm formation and acyl homoserine lactone production in Hafnia alvei isolated from raw milk. Biol Res 2009, 42:427-436.
34. Pearson MM, Laurence CA, Guinn SE, et al . Biofilm Formation by Moraxella catarrhalis In Vitro: Roles of the UspA1 Adhesin and the Hag Hemagglutinin. INFECTION AND IMMUNITY, 2006 ：1588–1596
35. Oasgupta MK, Bettcher KB, Ulan RA, et al. Relationship of adherent bacterial biofilms to peritonitis in chronic Ambulatory Peritoneal dialysis. Perit Dial Int , 1987; 7: 168－73
36. Gorman SP, Mawhinney WM, Adair CG, Issoukis M. Confocal laser scanning microscopy of peritoneal catheter surfaces. J Med Microbiol 1993; 38: 411-7.
37. Guidelines on Standard Operating Procedures for MICROBIOLOGY -- Chapter 19- Urinary Tract Infection. WHO SEARO. 2006 April
38. Saint S, Lipsky Benjamin A: Preventing Catheter-Related Bacteriuria. Should We? Can We? How? Arch Intern Med 1999, 159:800-808.
39. Stensballe J, Treve M, Looms D, et al. Infection risk with nitrofurazone impregnated urinary catheters in trauma patients. Ann Intern Med. 2007;147:285-293.
40. Vejborg R. M., Klemm P. Blocking of Bacterial Biofilm Formation by a Fish Protein Coating. A PPLIED AND ENVIRONMENTAL MICROBIOLOGY, 2008, 74:11: 3551–3558
41. McTaggart L A, Rigby R C, Elliott T S. The pathogenesis of urinary tract infections associated with *Escherichia coli*, *Staphylococcus saprophyticus* and *S. epidermidis.* J Med Microbiol. 1990;32:135–141.
42. Jacobsen S. M., Stickler D. J., Mobley H. L. T., Shirtliff M. E. et al. Complicated Catheter-Associated Urinary Tract Infections Due to Escherichia coli and Proteus mirabilis CLINICAL MICROBIOLOGY REVIEWS, 2008, 21:1 :26–59
43. Saint S, Meddings JA, Calfee D, et al. Catheter-associated urinary tract infection and the Medicare rule changes. Ann Intern Med 2009; 150:877–884.
44. Wong ES. Guideline for prevention of catheter-associated urinary tract infections. Am J Infect Control. 1983;11:28-36.
45. Yokoe DS, Mermel LA, Anderson DJ, Arias KM, Burstin H, Calfee DP, et al. A compendium of strategies to prevent healthcare-associated infections in acute care hospitals. Infect Control Hosp Epidemiol. 2008;29 Suppl 1:S12-21.
46. Lo E, Nicolle L, Classen D, Arias KM, Podgorny K, Anderson DJ, et al.Strategies to prevent catheter-associated urinary tract infections in acute care hospitals. Infect Control Hosp Epidemiol. 2008;29 Suppl 1:S41-50.
47. Jeong, I.; Park, S.; Jeong, J.S.; Kim, D.S, et al. Comparison of Catheter-associated Urinary Tract Infection Rates by Perineal Care Agents in Intensive Care Units. Asian Nursing Research 2010;4(3):142–150
48. Douglas L. J. Medical importance of biofilms in Candida infections. Rev. Iberoam. Micol. 2002. 19:139-143
49. Xi C, Marks D L, Schlachter S, Luo W, Boppart S A. High-resolution three-dimensional imaging of biofilm development using optical coherence tomography J. Biomed. Opt. 2006，11(3):034001-1-134001-6
50. Antibiotic Action Group Aintree Hospitals NHS Trust , Aintree University Hospitals NHS Foundation Trust And The Walton Centre , Guidelines For Antimicrobial Therapy (Eighth Edition) , September 2007
51. Darouiche RO, Smith JA Jr, Hanna H, ,et al. Efficacy of antimicrobial-impregnated bladder catheters in reducing catheter-associated bacteriuria: a prospective, randomized, multicenter clinical trial. Urology. 1999 Dec;54(6):976-81.

表 1 试验应用的抗生素分级表

分级	分级原则	抗菌药物	种类	使用人次	使用比例
一级	非限制使用：经临床长期应用证明安全、有效，对细菌耐药性影响小，价格相对较低的抗菌药物	呱拉西林、奈夫西林、、美洛西林、阿洛西林、替卡西林、美罗西林、阿莫西林、头孢唑啉、头孢拉定、头孢硫咪、头孢丙烯、头孢克肟、头孢替安、头孢曲松、头孢克洛、头孢尼西钠、头孢孟多酯钠、阿奇霉素、左氧氟沙星、环丙沙星、洛美沙星、依诺沙星、加替沙星、阿米卡星、丁胺卡那霉素、甲砜霉素、克林霉素	27 种	521	40.08%
二级	限制使用：与非限制使用抗菌药物相比较，这类药物在疗效、安全性、对细菌耐药性影响、药品价格等方面存在局限性，不宜作为非限制药物使用	氨苄西林/舒巴坦钠、替卡西林/克拉维酸、美罗西林 / 舒巴坦、阿莫西林/克拉维酸、阿莫西林/舒巴坦钠、呱拉西林/舒巴坦钠、头孢呋辛钠、头孢甲肟、头孢噻肟钠、头孢匹胺、头孢米诺、头孢地嗪、头孢泊肟酯、头孢他美酯、头孢地尼、氨曲南、拉氧头孢钠、头孢西丁钠、司帕沙星、莫西沙星、氟罗沙星、盐酸安妥沙星、妥舒沙星、依替米星、西索米星、夫西地酸钠、奥硝唑、	27 种	572	44%
三级	不良反应明显，不宜随意使用或临床需要加倍保护以免细菌过快产生耐药而导致严重后果的抗菌药物；新上市的抗菌药物；其疗效或安全性任何一方面的临床数据尚较少，或并不优于现用药物者；药品价格昂贵。	头孢唑肟、头孢他啶、头孢呱酮、头孢美唑、头孢呱酮/舒巴坦钠、头孢曲松/他唑巴坦、头孢曲松/舒巴坦钠、头孢呱酮/他唑巴坦、头孢吡肟、头孢噻利、亚胺培南/西司他丁、美罗培南、去甲万古霉素	13 种	207	15.92%

表 2 两组术后尿细菌培养病例数比较

组别	例数	术前	第 7 天	种类					
				大肠杆菌	粪肠球菌	阴沟肠球菌	白色念珠菌	光滑念珠菌	铜绿假单胞杆菌
治疗组	575	0	26（4.52%）*	24	1	0	0	0	1
对照组	575	0	75（13.04%）	69	2	2	1	0	1

* P＜0.001，差异有显著意义。

A B

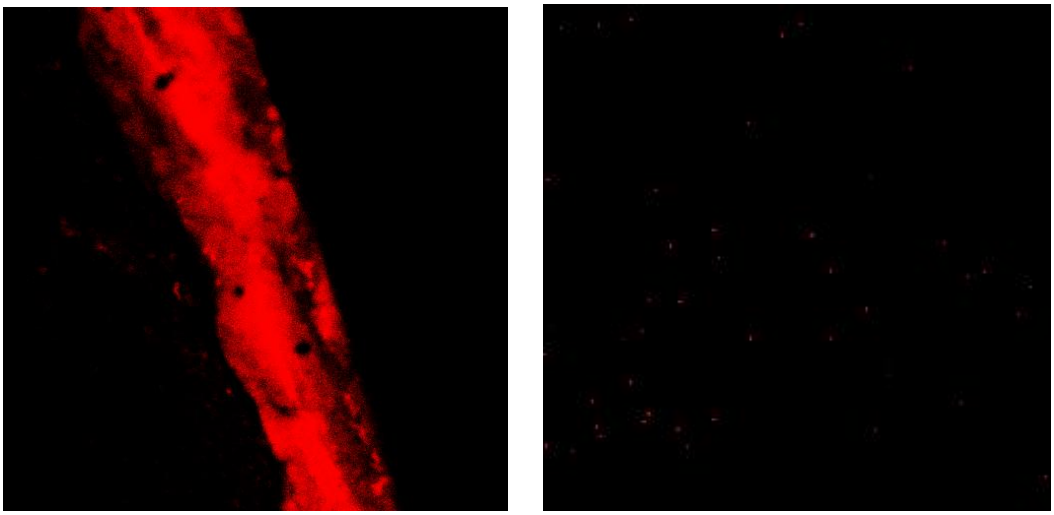

图 1 对照组和 JUC 组 16 小时 A：对照组 16 小时（CSLM 200X） B：JUC 组 16 小时（CSLM 200X）

A B

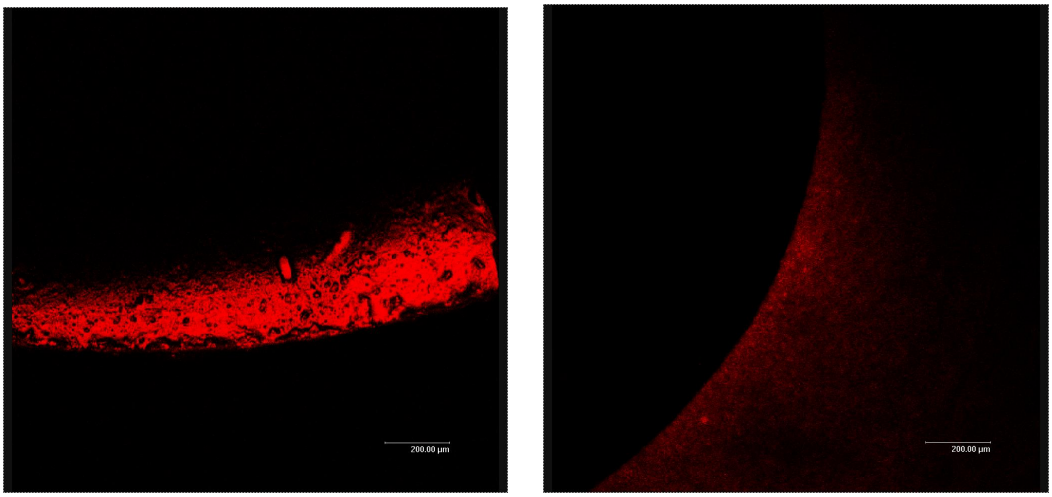

图 2 对照组和 JUC 组 7 天 A： 对照组 7 天（CSLM 200 X） B：JUC 组 7 天（CSLM 200X）