
A Biphasic Response From Bladder Epithelial Cells Induced by Catheter Material and Bacteria: An In Vitro Study of the Pathophysiology of Catheter Related Urinary Tract Infection

Jessica M. T. Barford,[†] Yanmin Hu, Ken Anson and Anthony R. M. Coates

From the Department of Medical Microbiology, Cellular and Molecular Medicine, St. George's, University of London and Department of Urology, St. George's Hospital (KA), London, United Kingdom

Purpose: Catheter associated urinary tract infection is the most common type of hospital acquired infection. An understanding of catheter associated urinary tract infection pathogenesis is needed to improve the control and treatment of these infections. We investigated the relationship among catheter material, bacteria and bladder epithelial cell reaction.

Materials and Methods: Urinary catheter sections and a clinical isolate of *Escherichia coli* were added to human bladder carcinoma epithelial cells in vitro in combination or independently. The catheters were rotated for 30 seconds over the cells, followed by incubation. The cytokines interleukin-6 and 8 were measured by enzyme-linked immunosorbent assay as indicators of inflammation and cell membrane disruption was assessed using a lactate dehydrogenase assay.

Results: The levels of lactate dehydrogenase release and cytokine production depended on the number of bacteria added. Bacteria grown for 3 days caused greater secretion of cytokines than bacteria grown overnight. Silicone catheter material alone caused immediate damage to cells with increased lactate dehydrogenase in the supernatant but little interleukin-6 or 8 production. Silicone catheters caused significantly less cytokine secretion from bladder cells than latex catheters. Conversely bacteria caused little immediate damage to cells but stimulated cytokine production after 12 hours.

Conclusions: Disruption of bladder epithelial cell membranes in vitro occurred immediately as a result of physical abrasion caused by catheters but delayed inflammation occurred in response to bacterial infection.

Key Words: bladder, urinary tract infections, cross infection, urinary catheterization, *Escherichia coli*

Urinary tract infections are usually the most common type of hospital acquired infection in developed countries^{1,2} and most of them are associated with urinary catheters.³ These catheters are generally well tolerated, although there is huge variability in patient tolerance and the causes of this are difficult to elucidate. Catheter related bacteriuria is often asymptomatic⁴ but symptoms arise in some patients, of whom a few show serious complications, such as bacteremia. The appearance of white blood cells in urine is not directly related to symptoms⁵ but it is associated with the presence of bacteria, although this association is less strong than in noncatheter associated UTIs.⁶

Catheter materials, particularly latex, can be toxic in vitro^{7,8} and cause inflammation in vivo,⁹ while long-term catheterization can lead to polypoid cystitis.¹⁰ It has also been demonstrated that bacteria can stimulate the production of the cytokines IL-6 and IL-8 in bladder epithelial cells in vitro.¹¹ Bacteria have also been shown to induce the transepithelial migration of neutrophils via an IL-8 concentration gradient in vitro.¹² However, to our knowledge catheters have not been shown to cause cytokine production in vitro.

We investigated the effect of catheter material and bacteria on markers of epithelial cell disruption and inflammation. The relative contribution of these factors to bladder damage and inflammation may provide some information about how symptomatic catheter associated UTIs are initiated.

MATERIALS AND METHODS

Bacteria

Escherichia coli (SGH031) from a UTI isolated at St. George's Hospital, London and stored at -80°C was inoculated into 10 ml Bacto™ Tryptic soy broth and incubated at 37°C overnight or for 3 days with shaking.

Cell Culture

EJ138 human bladder epithelial cells (No. 85061108, European Collection of Cell Cultures, Salisbury, United Kingdom) were cultured in EMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1% nonessential amino acids and 100 U/ml penicillin-streptomycin solution at 37°C with 5% CO₂.

Effect of Bacteria on Bladder Cells

EJ138 cells were seeded at 1 to 2 × 10⁶ cells into each well (9.6 cm²) of 6-well plates with 3 ml PRF growth medium (Sigma-Aldrich™) supplemented with 10% fetal bovine serum, 0.22% sodium bicarbonate, 2 mM L-glutamine and 100 U/ml penicillin-streptomycin solution, and allowed to settle

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Correspondence: Department of Cellular and Molecular Medicine, St. George's, University of London, Cranmer Terrace, London SW17 0RE, United Kingdom (telephone: +44 (0)20 8672 9944).

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overnight. Overnight and 3-day cultures of *E. coli* were washed with phosphate buffered saline, resuspended in 1 or 10 ml BPRF containing 0.22% sodium bicarbonate and 2 mM L-glutamine, and further diluted.

Medium covering the cells was replaced with 3 ml BPRF containing 50 mg/l gentamicin (Sigma-Aldrich). *E. coli* culture (10 ILI or 100 ILI) was added to the wells. The plates were swirled on a rotary shaker for 30 seconds and then incubated for 24 hours, as described. The plates were centrifuged for 4 minutes at 250 × gravity. Supernatants from each well were placed into clean 96-well microtiter plates for LDH assay and for storage at -80C, enabling cytokine ELISA to be performed when convenient.

Effect of Bacteria and Silicone Catheters on Bladder Cells With Time

The method described was performed using only overnight cultures of *E. coli* resuspended in 10 ml BPRF. *E. coli* culture (100 ILI) and/or 2.5 cm sections of uncoated 16Fr silicone catheters (Bactiguard, Stockholm, Sweden) with the ends coated in wax were added to the wells. Silicone catheters were used to assess physical effects because they are known to be nontoxic.^{7,8} Catheter sections were removed from the wells, and LDH, IL-6 and IL-8 assays were done using replicate plates for multiple time points.

Effect of Bacteria, Silicone and Latex Catheters on Bladder Cells

The method described was performed using only overnight cultures of *E. coli* resuspended in 10 ml BPRF. *E. coli* culture (100 ILI), and/or 2.5 cm sections of uncoated 16Fr silicone or uncoated latex catheters (Bactiguard) with the ends coated in wax were added to the wells. After 24 hours of incubation the catheter sections were removed from the wells, and LDH, IL-6 and IL-8 assays were performed.

LDH Assay

A Tox-7 lactate dehydrogenase assay kit (Sigma-Aldrich) was used according to manufacturer instructions. Briefly, equal volumes of substrate solution, enzyme preparation and dye solution were mixed, and 30 ILI were added to each well containing supernatants. Incubation was done at room temperature for 20 to 30 minutes in the dark. Absorbance was read at 490 and 630 nm.

IL-6 ELISA

Nunc™ MaxiSorp™ immunoplates were coated with antibody at 4C overnight. Samples and standards were incubated at 4C overnight. Biotinylated antibody was applied for 2 to 3 hours at room temperature. Avidin peroxidase was incubated for 25 minutes at room temperature and color was developed using o-phenylenediamine dihydrochloride buffer (Sigma-Aldrich). Reactions were stopped with H₂SO₄ (VWR®). Absorbance was read at 490 nm. The standard curve was plotted and concentrations were calculated using software (Revelation™ Software) with an MRX microtiter plate reader (Thermo Labsystems, Ashford, United Kingdom).

IL-8 ELISA

IL-8 ELISA was performed according to manufacturer recommendations except plates were always coated with the

first antibody at 4C, and avidin peroxidase and o-phenylenediamine dihydrochloride buffer were used, as described.

Data Analysis

Statistical analysis was done using SPSS®, and the non-parametric Mann-Whitney and Kruskal-Wallis tests.

RESULTS

Effect of Bacteria on Bladder Cells

Figure 1, A shows that an increasing number of bacteria caused increasing LDH release from bladder cells, particularly between 10⁸ and 10⁹ bacteria per well. Calculating the AUC demonstrated no difference between overnight and 3-day stationary phase cultures (p = 0.275). In contrast, a peak of IL-6 and 8 occurred between 5 × 10⁸ and 10⁹ bacteria per well, after which levels decreased (fig. 1, B and C). Three-day cultures induced increased cytokine release from bladder epithelial cells compared to overnight cultures for IL-6 and 8 (each p = 0.050).

Effect of Bacteria and Silicone Catheters on Bladder Cells With Time

Catheter sections alone and catheters with bacteria caused massive LDH release after rotation (fig. 2, A), which remained constant after incubation. When bacteria alone were added to the bladder cells, a significant increase in LDH was seen compared to that in the control only after 24 hours of incubation (p = 0.021). Significant differences in IL-6 and 8 levels between tests and controls were only seen after 12 hours of incubation (p = 0.041 and 0.034, respectively, fig. 2, B and C). After 24 hours of incubation IL-6 and 8 levels when bacteria or bacteria with catheters were present were significantly higher than in the control (bacteria only p = 0.021 and 0.043, and catheter with bacteria p = 0.021 and 0.021), although not when catheters alone were present (p = 0.386 and 0.149, respectively). IL-6 and 8 levels were slightly higher when catheters as well as bacteria were present compared to bacteria alone but this was not statistically significant (at 24 hours p = 0.386 and 0.149, and at 36 hours p = 0.275 and 0.275, respectively).

Effect of Bacteria, and Silicone and Latex Catheters on Bladder Cells

LDH levels increased significantly above that of controls when latex or silicone catheters were rotated on cells with or without bacteria (all 4 comparisons p = 0.050, fig. 3, A). There was a small but significant decrease in LDH levels when silicone catheters were used compared to latex catheters (p = 0.050). Latex catheters alone caused higher IL-6 and 8 levels than in the control but silicone catheters only caused higher IL-6 levels (each p = 0.050, fig. 3, B and C). Compared to bacteria alone latex catheters with bacteria caused an increase in IL-6 but silicone catheters did not (p = 0.050 and 0.275, respectively). Without bacteria significantly less IL-6 and IL-8 was produced in response to silicone catheters compared to latex catheters (each p = 0.050). However, there was only a significant difference in IL-6 levels but not IL-8 levels when bacteria were also present (p = 0.050 and 0.127, respectively).

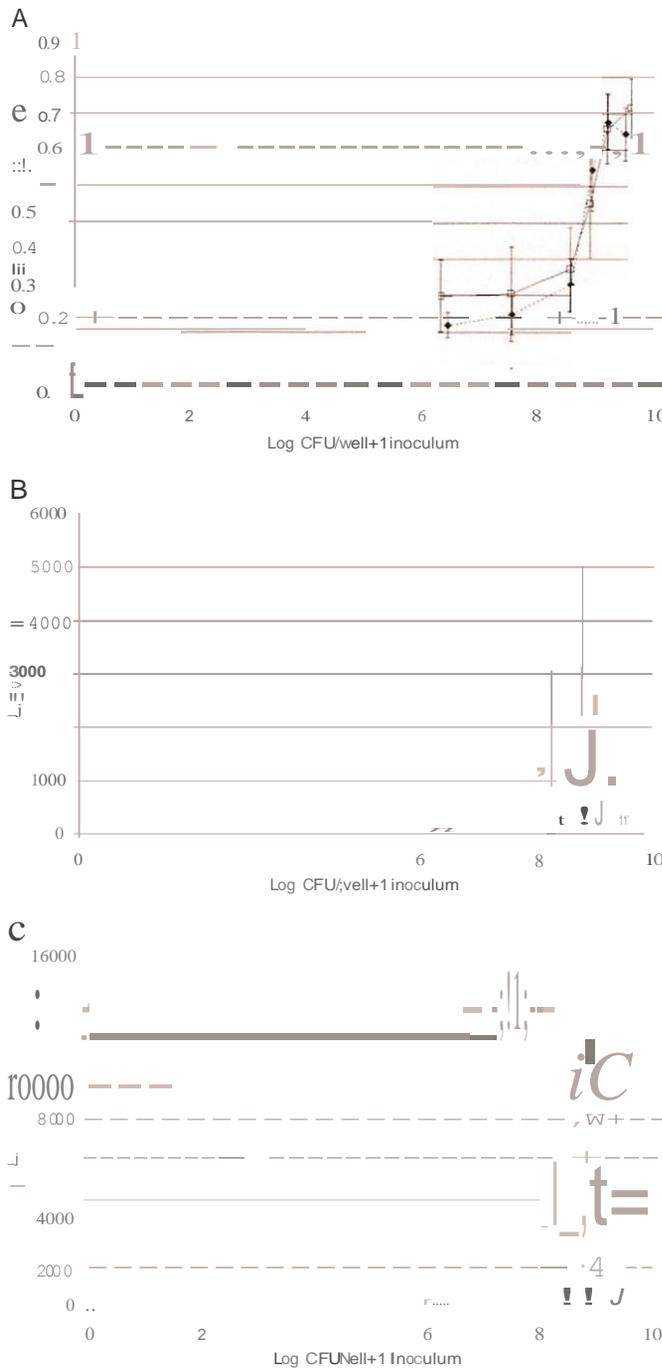


FIG. 1. Effect of different concentrations of overnight and 3-day E. coli cultures on EJ138 bladder epithelial cells, as assessed by LDH assay (A), and IL-6 (B) and IL-8 (C) ELISA after 30-second rotation and 24-hour incubation. Results represent mean of 3 experiments. Diamonds indicate overnight culture. Squares indicate 3-day culture. Triangle indicates control. Cross indicates medium control. Error bars indicate SD.

DISCUSSION

When bacteria were added to bladder epithelial cells in vitro, cell membrane disruption occurred in a dose dependent manner with a significant increase in LDH occurring at approximately 10^9 cfu per well. As measured by cytokine ELISA, inflammation peaked at approximately the same concentration of bacteria. When the cfu counts per well exceeded 10^9 , cytokine secretion decreased, probably because bladder epithelial cell viability was significantly de-

creased as a result of bacterial damage and, therefore, IL-6 and 8 synthesis could not occur. It is thought that uropathogenic E. coli cytotoxic necrotizing factor type 1¹³ or type 1 fimbriae-mediated binding to uroepithelial cells¹⁴ stimulates bladder cell apoptosis and exfoliation as a defense mechanism, which may explain the high levels of LDH re-

lease, and low levels of IL-6 and IL-8 after exposure to a large number of E. coli. A smaller number of E. coli may stimulate only an inflammatory response, possibly because

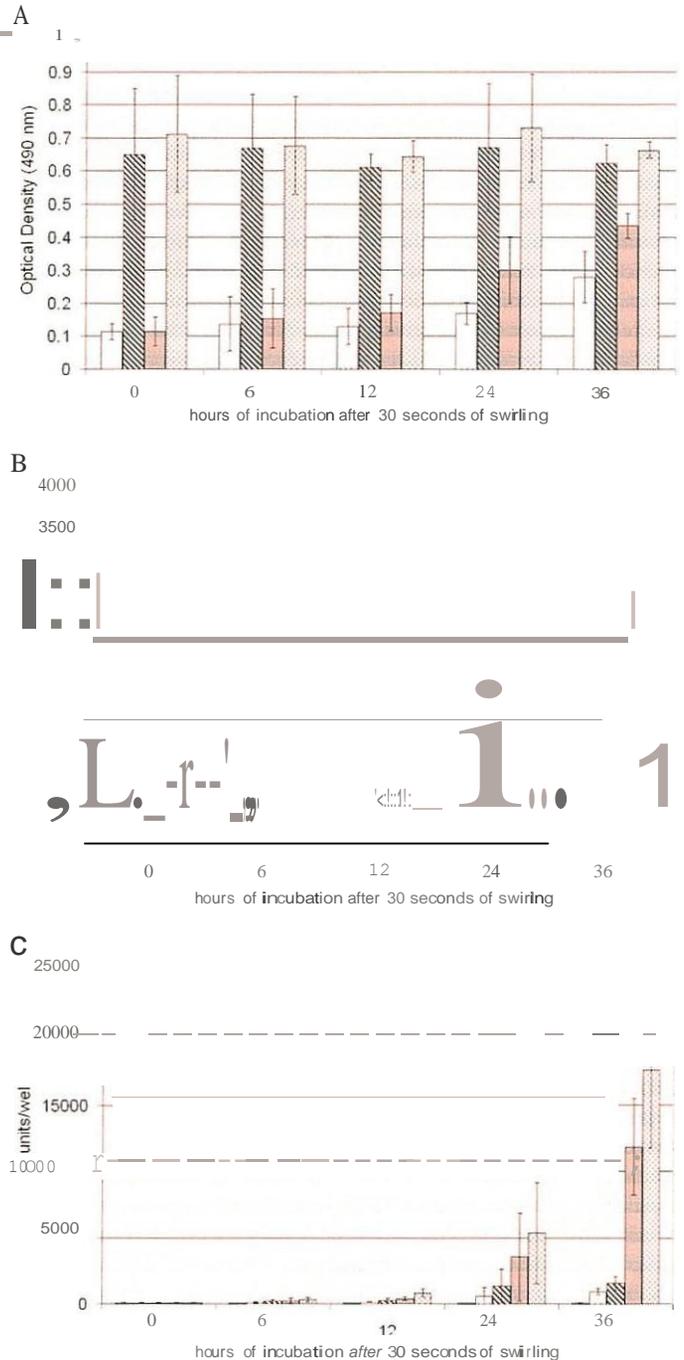


FIG. 2. Effects of overnight E. coli culture and silicone catheter sections on EJ138 bladder epithelial cells, as assessed by LDH assay (A), and IL-6 (B) and IL-8 (C) ELISA after 30-second rotation and up to 36-hour incubation. Results represent mean of 4 experiments.

Black bars represent medium control, spotted bars control, hatched bars catheter, gray bars bacteria, and cross-hatched bars catheter and bacteria.

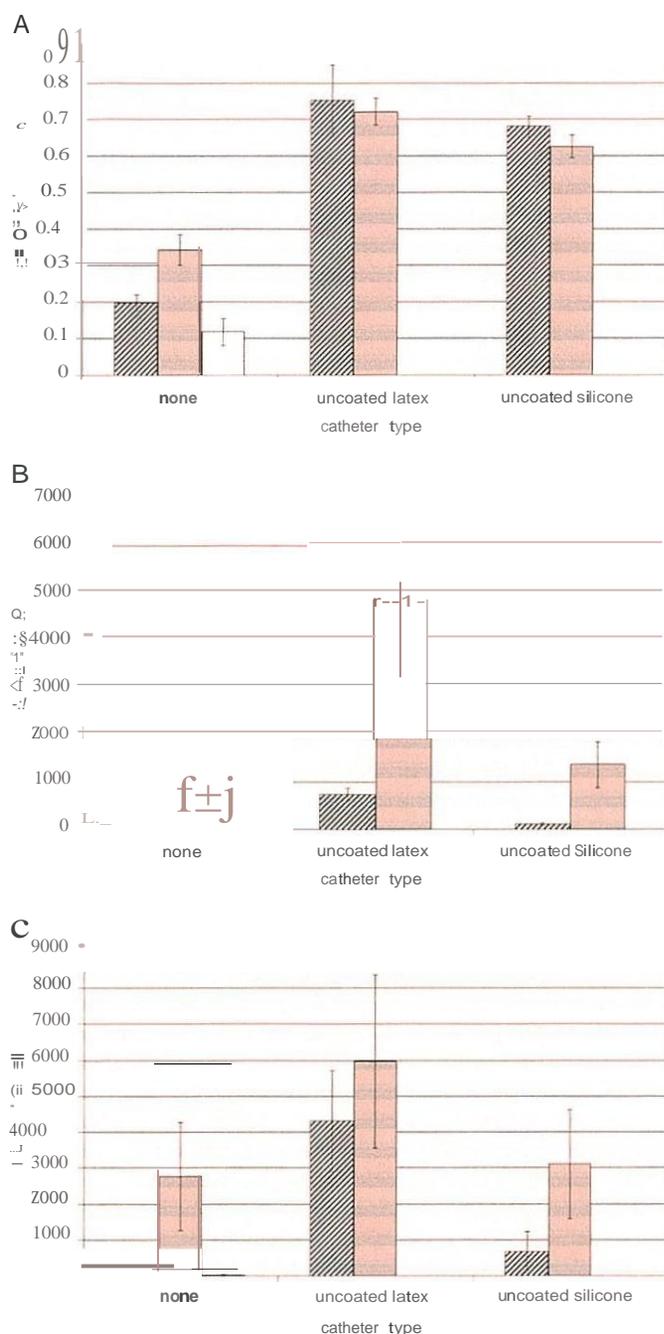


FIG. 3. Effects of overnight *E. coli* culture, and silicone and latex catheter sections on EJ138 bladder epithelial cells, as assessed by LDH assay (A), and IL-6 (B) and IL-8 (C) ELISA after 30-second rotation and 24-hour incubation. Results represent mean of 3 experiments. Hatched bars indicate no bacteria. Gray bars indicate bacteria. Spotted bars indicate medium control. Error bars represent SD.

the bladder cells in this case do not need to die to limit the infection.

When bacteria and/or silicone catheters were rotated on bladder epithelial cells and then incubated, a biphasic response emerged with time. Catheters caused an immediate release of LDH, demonstrating that the rotation of catheters had physically damaged the cell membranes. This was not affected by adding bacteria or by subsequent incubation. When bacteria alone were added to the cells, an increase in

LDH was only seen after 24 hours, and a significant increase in IL-6 and 8 levels was seen after 12 hours. Results suggest that catheters and bacteria affect bladder epithelial cells in different ways but each may have detrimental effects. Catheters cause immediate physical damage, whereas bacteria cause delayed damage and inflammation.

Three-day stationary phase cultures of *E. coli* caused the secretion of higher levels of cytokines from bladder epithelial cells than overnight cultures. Although a difference in cytokine secretion stimulated by different growth phases of bacteria has been seen before,^{5, 16} to our knowledge there are no reports of this phenomenon caused by *E. coli* stimulation of bladder epithelial cells. There was no difference in viable counts between the 2 cultures of *E. coli* after they were added to medium containing gentamicin (data not shown), which suggests that there may be a difference in bacterial surface molecules that favors the stimulation of bladder epithelial cells to produce IL-6 and 8 when *E. coli* is in stationary phase. However, the possibility of more dead cells being present in the initial inoculum of stationary phase bacteria compared to those in the overnight culture must be excluded before this conclusion can be confirmed. It has been demonstrated that biofilms form on the outside of catheters within a few days after insertion and biofilms contain stationary phase bacteria.^{17, 18} Therefore, biofilms on catheters may cause clinically significant inflammation *in vivo*.

To our knowledge cytokine secretion from bladder epithelial cells caused by catheters *in vitro* has not been described previously, although inflammation due to catheters has been reported.⁹ This study suggests that the latex catheters alone may account for at least part of this inflammation because higher levels of cytokines were secreted in response to latex than to silicone catheters and controls. Latex catheters have been demonstrated to be toxic *in vitro*^{7, 8} and it is possible that the same characteristics that cause toxicity may also cause inflammation.

The results support the hypothesis that latex catheters alone may be responsible for the production of cytokines, the presence of pyuria, inflammation in the bladder, polypoid cystitis and urinary symptoms in some patients. It is likely that silicone catheters alone might produce fewer of these complications *in vivo*. In this situation the impact of bacteria becomes more important. Therefore, we suggest that symptomatic catheter associated UTIs may be caused by a combination of the immediate physical damage caused by catheters, and the delayed damage and inflammation caused by bacteria.

Our study has a number of limitations, including the use of only 1 strain of bacterium and a cancer cell line that may differ from bladder cells *in vivo*. Normal urothelial cells were not used because a noncancer cell line was not readily available. Cancer cell lines are easy to work with and they were considered appropriate for these preliminary studies. However, this model could prove useful for assessing new catheter materials or coatings in the research setting. Preliminary tests, including hydrogel coated catheters, were done but the results were inconclusive and these catheters must be investigated more thoroughly using this model. Silver alloy coated catheters should also be assessed. It would also be interesting to investigate the effect of adding catheter sections coated with mature biofilms to epithelial cells, in contrast to adding stationary phase and log phase planktonic bacteria, and catheter sections separately. The study

was performed in vitro and correlations to the clinical setting should be viewed with caution. However, we believe that these findings are interesting and they should be further explored in clinical trials.

CONCLUSIONS

We report a novel model for assessing the influence of catheter material and bacteria on cell membrane disruption and cytokine secretion from urothelial cell lines. It appears that catheter material and bacteria stimulate a biphasic response from epithelial cells in vitro that is characterized by immediate physical damage from catheter material, and delayed damage and inflammation from bacteria. These findings may help improve our understanding of the pathophysiology of catheter related UTIs.

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Abbreviations and Acronyms

BPRF	basic PRF
ELISA	enzyme-linked immunosorbent assay
EMEM	minimum essential medium with Earl's salts
IL	interleukin
LDH	lactate dehydrogenase
PRF	phenol red-free EMEM
UTI	urinary tract infection

REFERENCES

- Gravel D, Taylor G, Ofner M, Johnston L, Loeb M, Roth VR et al: Point prevalence survey for healthcare-associated infections within Canadian adult acute-care hospitals. *J Hosp Infect* 2007; 66: 243.
- Pellizzer G, Mantoan P, Timillero L, Allegranzi B, Fedeli U, Schievano E et al: Prevalence and risk factors for nosocomial infections in hospitals of the Veneto region, north-eastern Italy. *Infection* 2008; 36: 112.
- Bouza E, San Juan R, Munoz P, Voss A and Kluytmans J: A European perspective on nosocomial urinary tract infections II. Report on incidence, clinical characteristics and outcome (ESGNI-004 study). *European Study Group on Nosocomial Infection. Clin Microbiol Infect* 2001; 7:532.
- Tambyah PA and Maki DG: Catheter-associated urinary tract infection is rarely symptomatic: a prospective study of 1,497 catheterized patients. *Arch Intern Med* 2000; 160:678.
- Steward DK, Wood GL, Cohen RL, Smith JW and Mackowiak PA: Failure of the urinalysis and quantitative urine culture in diagnosing symptomatic urinary tract infections in patients with long-term urinary catheters. *Am J Infect Control* 1985; 13: 154.
- Tambyah PA and Maki DG: The relationship between pyuria and infection in patients with indwelling urinary catheters: a prospective study of 761 patients. *Arch Intern Med* 2000; 160:673.
- Liedberg Hand Lundeberg T: Assessment of silver-coated urinary catheter toxicity by cell culture. *Urol Res* 1989; 17: 359.
- Ruutu M, Alfthan O, Talja M and Andersson LC: Cytotoxicity of latex w"inary catheters. *Br J Urol* 1985; 57: 82.
- Edwards LE, Lock R, Powell C and Jones P: Post-catheterisation urethral strictures. A clinical and experimental study. *Br J Urol* 1983; 55: 53.
- Goble NM, Clarke T and Hammonds JC: Histological changes in the urinary bladder secondary to urethral catheterisation. *Br J Urol* 1989; 63: 354.
- Funfstuck R, Franke S, Hellberg M, Ott U, Knofel B, Straube E et al: Secretion of cytokines by uroepithelial cells stimulated by *Escherichia coli* and *Citrobacter* spp. *Int J Antimicrob Agents* 2001; 17: 253.
- Godaly G, Proudfoot AE, Offord RE, Svanborg C and Agace WW: Role of epithelial interleukin-8 (IL-8) and neutrophil IL-8 receptor A in *Escherichia coli*-induced transuroepithelial neutrophil migration. *Infect Immun* 1997; 65:3451.
- Mills M, Meysick KC and O'Brien AD: Cytotoxic necrotizing factor type 1 of uropathogenic *Escherichia coli* kills cultured human uroepithelial 5637 cells by an apoptotic mechanism. *Infect Immun* 2000; 68: 5869.
- Mulvey MA, Lopez-Boado YS, Wilson CL, Roth R, Parks WC, Heuser J et al: Induction and evasion of host defenses by type 1-piliated uropathogenic *Escherichia coli*. *Science* 1998; 282: 1494.
- Graham GM, Fan-ar MD, Cruse-Sawyer JE, Holland KT and Ingham E: Proinflammatory cytokine production by human keratinocytes stimulated with *Propionibacterium acnes* and *P. acnes* GroEL. *Br J Dermatol* 2004; 150: 421.
- Zhou X, Gao DQ, Michalski J, Benitez JA and Kaper JB: Induction of interleukin-8 in T84 cells by *Vibrio cholerae*. *Infect Immun* 2004; 72:389.
- Barford JM, Anson K, Hu Y and Coates AR: A model of catheter-associated urinary tract infection initiated by bacterial contamination of the catheter tip. *BJU Int* 2008; 102: 67.
- Ander! JN, Zahller J, Roe F and Stewart PS: Role of nutrient limitation and stationary-phase existence in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. *Antimicrob Agents Chemother* 2003; 47:1251.