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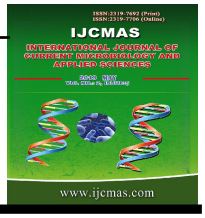

Original Research Article

Identification and quantification of bacteria associated with indwelling urinary catheterization

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Keywords

Urinary catheter; Urine; Bacteria; Nosocomial infections.

A B S T R A C T

The urinary catheterization is one of the causes of nosocomial urinary tract infections. The bacteria that are involved are usually elements of skin, digestive or genital flora. In order to contribute to the improvement of patient safety in hospital setting, a study on the identification and quantification of bacteria colonizing Foley catheters was performed in three departments of the Yaoundé Central Hospital (YCH) in Cameroon. Two hundred and four samples (urine and pieces of catheter tube whose outer surface was disinfected or not) were collected from sixty-eight patients having indwelling urinary catheters. All the catheters were removed sequentially within five (5) or more days. The samples were subjected to qualitative and quantitative bacteriological analysis. The three types of samples studied were colonized by bacteria soon after the installation of catheters. The percentage of colonized samples increased with time. Bacterial concentrations and species isolated from urine and catheters whose outer surface was first disinfected were similar. Antibiotics proved ineffective in preventing bacterial colonization of samples. Five (5) species of bacteria namely *E. coli*, *Klebsiella* sp., *Proteus* sp., *Serratia* sp. and *S. saprophyticus* were identified as urinary pathogenic, following international standards on urinalysis.

Introduction

The urinary catheterization is used to drain urine by aseptic and painless introduction of a catheter into a patient's bladder. It is a medical procedure that is applied for preventive, diagnostic or therapeutic purposes. Its main disadvantage is the possible occurrence of urinary tract severe underlying pathologies is estimated at 30.8% in developed countries (Juan *et al.*, 2001; Mac Leone *et al.*, 2000). In these countries, 5 to 10% of patients admitted into hospitals run the risk of getting a nosocomial infection (OMS, 2008). This rate is above 25% in

developing countries (Bafort and Coates, 2009). The hospital departments that are most affected in descending order are intensive care, surgery, internal medicine, pediatric and psychiatry (Faure, 2002). Most infections are located in the urinary tract (30 to 50% of cases) (Gastmeier, 2000). Contamination of the urinary tract could be in two ways: the transurethral way for peri-urethral germs, and the intra-urethral or endoluminal way for the patient's endogenous flora (80-90% of cases), health care givers or other patients (Caron, 2003; Mac Leone *et al.*, 2000). The perimeatus, the junction between the catheter, the collection bag and the collector drainage site are the microbial points of entry (Jacobsen *et al.*, 2008). The bacteria involved are usually elements of the skin, digestive or genital flora. In order to contribute to the improvement of patient safety in hospitals, the risk posed by urinary catheterization was investigated by identifying and quantifying bacteria which colonize catheter tubes and urine collected from hospitalized patients with Foley catheters.

Materials and Methods

This study was conducted in compliance with the regulation relating to health research involving humans, in force in Cameroon. So, Clearances were obtained from the National Ethics Committee and from the administrative authorities. The subjects were in patients with indwelling urinary catheters in the gynecology and obstetrics, the intensive care and the internal medicine departments of the Yaoundé Central Hospital (YCH). They gave their consent after receiving adequate information about the study. All catheters considered in our analysis were made of siliconized latex. Their sizes ranged between 10 and 18 Fr, and they were placed by medical personnel of the hospital. For each subject, urine samples and 2 pieces of catheter tube were collected. All samples were subjected to qualitative and quantitative bacteriological analysis. Installation conditions of catheters and influence of antimicrobial prophylaxis on bacterial colonization were also examined.

Urinalysis

Urine samples were collected in 10 ml sterile tubes at the drain hose that was clamped to facilitate its accumulation, just before the withdrawal of the catheter by the nursing staff, in accordance with good laboratory practices. Cytological and bacteriological analyses were performed on the total urine and on the pellet, respectively. Isolation and identification of bacteria were performed using conventional methods (Carbonnelle *et al.*, 1991). The results were interpreted using the urinalysis guidelines published by the European Confederation of Laboratory Medicine (2008) which classifies the urinary pathogenic bacteria in 3 groups according to significant bacteriuria values. Following this classification, significant bacterial concentrations in urine are 10^3, 10^4 and 10^5 CFU/ml for group 1, group 2 and group 3, respectively. The first group consists of two bacterial species: *E. coli* and *S. saprophyticus*, while the second is made up of bacteria generally more involved in nosocomial infections i.e. Protae tribe, *Klebsiella* spp., *enterobacte*r spp., *Serratia* spp., *Citrobacter* spp., *Pseudomonas aeruginosa*, *Enterococcus* spp. and *Staphylococcus aureus*. The third group, on the other hand consists of *Streptococcus agalactiae*, other coagulase negative *staphylococci*, *Acinetobacter* spp., *Stenotrophomonas maltophila* and other Pseudomonaceae.

Catheter tube analysis

For each subject, after removal of the catheter, two pieces of 2 cm were cut in its distal part and rinsed in sterile distilled water in the order to remove non-adherent bacteria. One piece was directly immersed in 3.5 ml of saline solution so as to collect the adherent bacteria on the external and internal surfaces. In the following text, it will be referred to as non-disinfected catheter (NDC). The outer surface of the second catheter piece was disinfected using 10 volumes of hydrogen peroxide before immersion in saline solution, in order to retrieve only the adherent bacteria on the inside of the medical device. This one will be referred to as disinfected catheter (DC). In the two situations, the bacterial suspension was obtained by stirring with a Vortex mixer for 2 minutes. Bacterial concentrations of resulting suspensions were determined by the dilution method and agar inclusion (Carbonnelle *et al.*, 1991). Isolation and identification of bacteria were performed according to conventional methods (Carbonnelle *et al.*, 1991).

Data analysis

The data obtained were compared using the Chi-square (X^2) test. A p value < 0.05 was considered statistically significant.

Result and Discussion

Socio demographic and clinical features of study population

Out of the sixty-eight patients who agreed to participate in the study, sixty-three (96.65%) were females and five (7.35%) were males. Their ages ranged from 9 to 92 years, with a modal class that lies between 21 and 26 years. Forty-nine (72.16%) were hospitalized in the gynecology and obstetrics department, twelve (17.66%) in internal medicine department and seven (10.25%) in the intensive care unit. The underlying pathologies identified included: asthma, diabetes, gastritis, hypertension, HIV, malaria, pericarditis and cancer. Indications of the urinary catheterization were the control of diuresis following surgery (91.78%), unconsciousness (5.88%) and urinary incontinence (2.34%). Fifty-nine (86.76%) patients were on antimicrobial prophylaxis viz; aminopenicillins (74.59%), cephalosporins (15.25%), fluoroquinolones (5.08%) and beta lactam + aminoglycoside (5.08%). The used antiseptics were, in 25%, 44% and 31% of cases, povidone-iodine, hexamidine/chlorocresol and unknown product, respectively. Sterile gloves were used to place 50% of the catheters while non sterile gloves were used in 17.65% of cases. The quality of the gloves was not specified in 32.35% of cases. All the catheters were removed sequentially within five (5) or more days. Thus, 30, 15, 2, 5 and 16 catheters were removed after 1, 2, 3, 4 and 5 days or more, respectively.

Cytology and bacteriology of urine

For all the patients who participated in our study, the number of leukocytes observed per field of view of the optical microscope was less than 10. Twenty-nine (42.65%) samples were colonized by single or an association of 2 bacterial species. The isolated bacteria were *Escherichia coli* (31.04%), *Klebsiella* sp. (13.79%), *Proteus* sp. (13.79%), *Pseudomonas* sp. (10.34%), *M. morganii* (10.34%), *Serratia* sp. (6.90%), *Staphylococcus saprophyticus* (6.90%), *Enterococcus* sp. (3.45%), and *Pseudomonas* sp./*Proteus* sp. (3.45%). *Klebsiella* sp. was the most frequently

isolated bacteria (4/5 days). The percentage of colonized samples was found to increase with the duration of catheterization. However, this increase was not found to be statistically significant ($p>0.05$), (Figure 4).

The bacterial concentrations ranged from 10^2 CFU/ml (*M. morganii* on day 1 and 4; *S. Saprophyticus* on day 1) to 10^6 CFU/ml (*E. coli* on day 1 and day 5 or more), as presented in Figure 1. So, the identified pathogens were, on day 1 and 2: *E. coli*, on day 4: *Klebsiella* sp. and *Proteus* sp., on day 5 or more: *E. coli*, *Klebsiella* sp., *Proteus* sp., *Serratia* sp. and *S. saprophyticus.*

Adherent bacteria on disinfected catheters

Out of the sixty-eight DC samples, twenty-eight (41.17%) were colonized by bacteria. *Klebsiella* sp. was also the most frequently isolated (4/5 days).The isolated germs were the eight bacterial species already cited for urine, in similar percentages. The percentage of colonized samples was found to increase with the duration of catheterization. However, this increase was not found to be statistically significant ($p>0.05$). The bacterial concentrations in this case ranged from 0.80 x 10^2 CFU/ml (*M. morganii* on day 2) to 1.28 x 10^7 CFU/ml (*E. coli* on day 5 or more), (Figure 2).

Adherent bacteria on the non disinfected catheters

Out of the sixty-eight NDC samples, sixty-one (89.70%) were colonized by bacteria. Fifty-five samples were each colonized by a single germ, while the remaining 6 were colonized by two germs, each. To the bacteria species already listed for the two first sample types, five others were added. Therefore, isolated bacteria were *E. coli* (21.31%), *Klebsiella* sp. (18.03%), *Enterococcus* sp. (16.39%), *Proteus* sp. (11.47%), *Enterobacter* sp. (4.92%), *M. morganii* (4.92%), *Pseudomonas* sp (3.28%), *Serratia* sp. (3.28%), *S. saprophyticus* (3.28%) *Acinetobacter* sp. (1.64%), *Citrobacter* sp.(1, 64%), *E. coli/S. saprophyticus* (1.64%), *Klebsiella* sp./*S. saprophyticus* (1.64%), M. *morganii/S. epidermidis* (1.64%), *Pseudomonas* sp./*S. aureus* (1.64%), *Enterococcus* sp./*Proteus* sp. (1.64%), and *Pseudomonas* sp./*Proteus* sp. (1.64%). The maximum value of colonized samples was reached on the second day and remained stable until the end of the study period. *Klebsiella* sp. was also the most frequently isolated bacteria (4/5 days).The bacterial concentrations ranged from 0.60 x 10^2 CFU/ml (*Enterococcus* sp. on day 5 or more) to 1.50 x10^7 CFU/ml (*Escherichia coli* on day 5), (Figure 3).

Influence of antibiotic prophylaxis on bacterial colonization

On day 1, the catheters of thirty patients were removed and twenty-eight of them were on antibiotics. In fact, 26, 1 and 1 patients were receiving aminopenicillin, 2^{nd} generation cephalosporin and 3^{rd} generation cephalosporin, respectively. One (3.3%) urine and one DC samples were colonized by *E coli*. The concerned patient was receiving antibiotic (aminopenicillin). In the case of NDC samples, 26 (86.67%) out of the 30 patients were contaminated by *Citrobacter* sp. (1), *Enterobacter* sp. (2), *Enterococcus* sp. (8), *E. coli* (6), *Klebsiella* sp. (3), *M. morganii* (1), *Proteus* sp. (2), S. *saprophyticus* sp. (2), and *Enterococcus* sp./*Proteus* sp. (1). Twenty five (25) of these patients were on antibiotics viz;

Figure.1 Average concentrations of bacteria in urine as a function of duration of catheterization

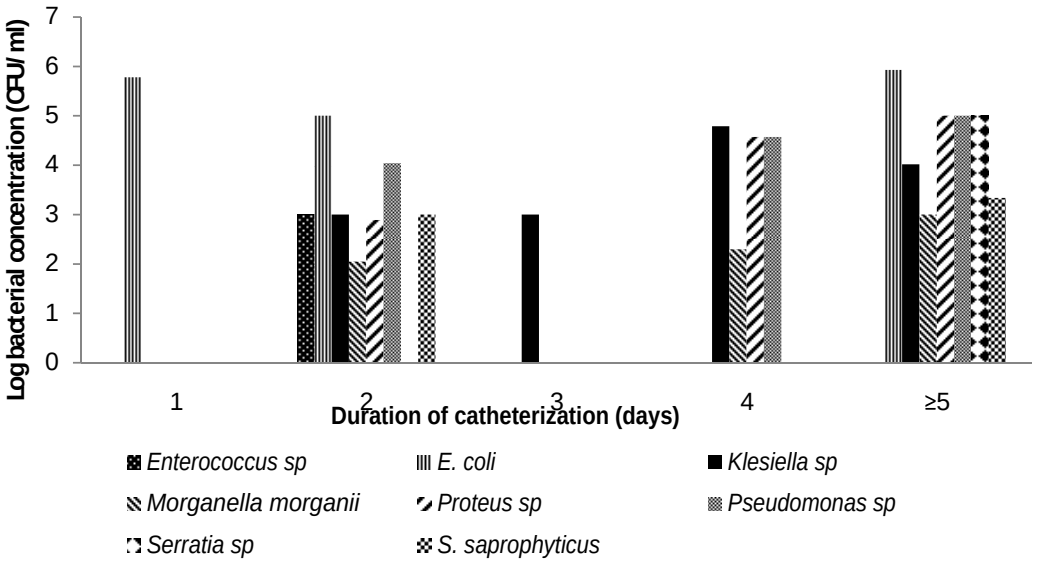


Figure.2 Average concentrations of bacteria on disinfected catheters as a function of duration of catheterization

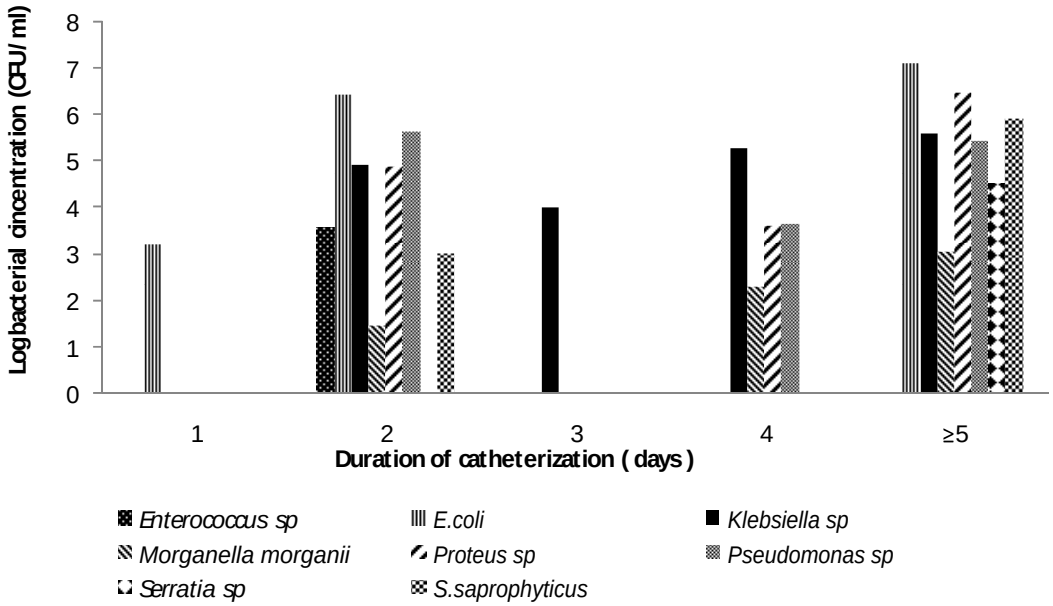


Figure.3 Average concentrations of bacteria on non- disinfected catheters as a function of duration of catheterization

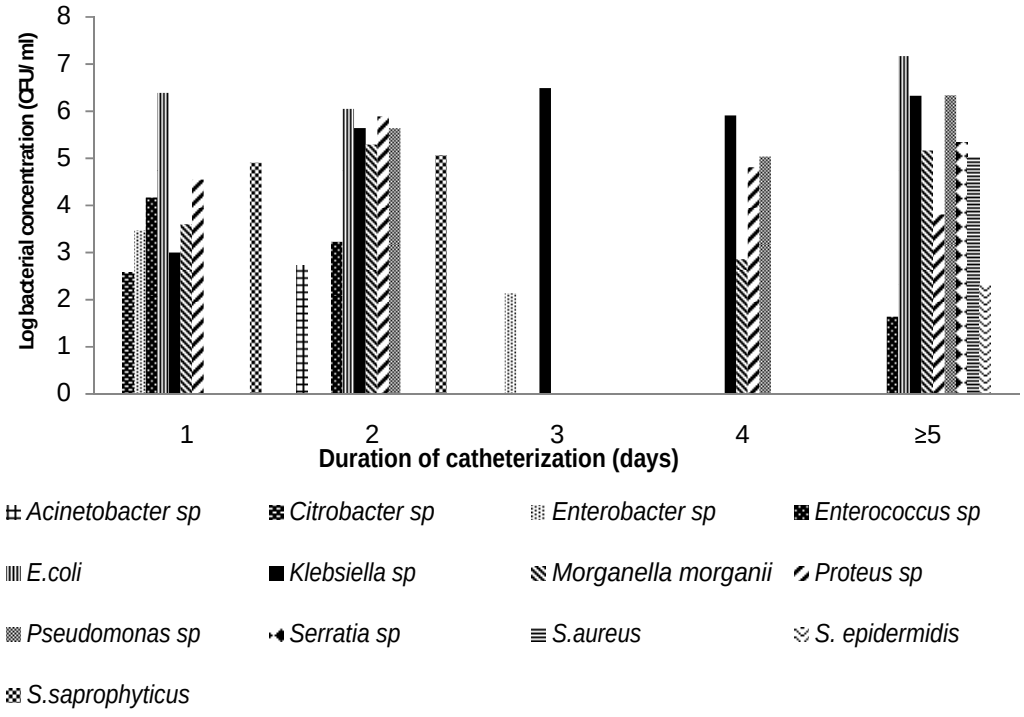
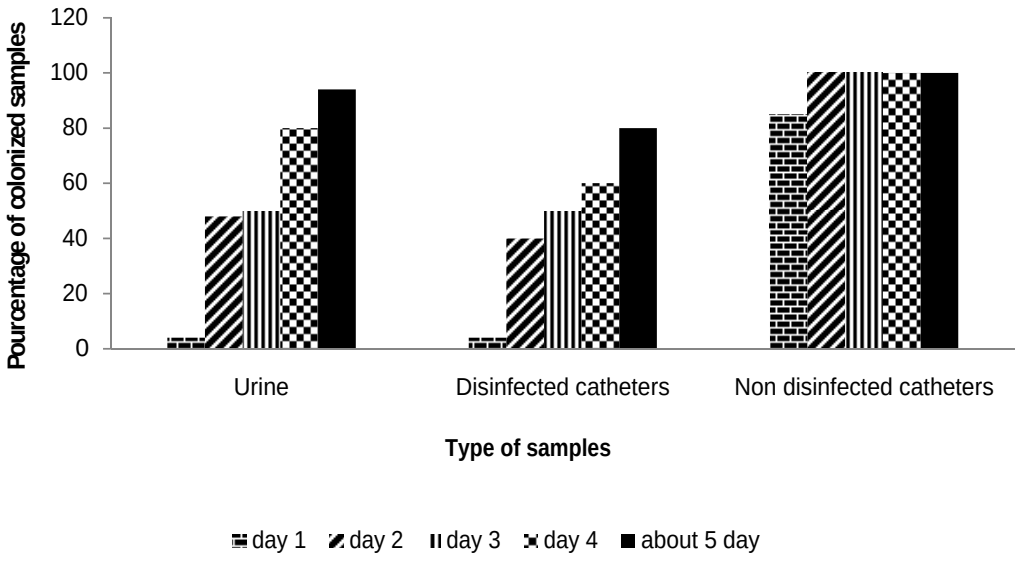


Figure.4 Influence of duration of catheterization on bacterial colonization



aminopenicillin (23), 2nd generation cephalosporin (1) and 3rd generation cephalosporin (1). On day 2, all fifteen patients from whom catheters were removed were on the following antibiotics; aminopenicillin (12), 2nd generation cephalosporin (1), 3rd generation cephalosporin (1) and fluoroquinolone (1). Overall, eight (53.33%) urine, seven (46.67%) DC and twelve (80%) NDC samples were colonized.

On day 3, catheters were removed from two patients, and only one was receiving an antibiotic (aminopenicillin). For this patient, only the NDC sample was colonized by *Enterobacter* sp. While for the other, all the 3 samples types were colonized by *Klebsiella* sp.

On day 4, all the five patients from whom catheters were removed were on antibiotics like on day 2. So, 2, 2 and 1 were receiving amoxicillin, 3rd generation cephalosporin and fluoroquinolone, respectively. 4 (80%) urine samples, 4 (80%) DC samples and all (100%) NDC samples were colonized by bacteria.

Finally, on day 5 or more, catheters were removed from 16 patients, 10 of whom perianal region (Crouzet *et al.*, 2007; Milcent *et al.*, 2003). The percentages of colonized samples depended on the sample type and generally varied with the duration of the catheterization.

Thus, after 24 hours, 86.67% of NDC were colonized versus 3.33% of urine samples and disinfected catheters. After 48 hours, the respective percentages for NDC, DC and urine were 80%, 46.67% and 53.33%. At all durations of catheterization, the percentages of colonized samples and isolated bacteria were broadly similar for urine and DC. were on antibiotics. 3, 3, 1 and 2 were on aminopenicillin, 3rd generation cephalosporin, fluoroquinolone and beta-lactam + aminoglycoside, respectively. 15 (93.75%) urine samples, 15 (93.75%) DC samples and all (100%) NDC samples were colonized by bacteria.

Relation between bacterial colonization of the samples and installation conditions of the catheters

The percentage of colonized catheters was equal to 80.56% when povidone-iodine was used, and 56.44% when hexamidine/chlorocresol was used. However, these differences were not statistically significant ($p > 0.05$). 73.53%, 91.67% and 86.36% of NDC samples were contaminated when catheters were placed with sterile, non sterile and unknown gloves nature, respectively. These differences were not statistically significant ($p > 0.05$).

Urine and catheter samples turned out to be colonized by bacteria at all times considered in this study. The majority of our study subjects being women, gender may have been a contributing factor as the woman's urethra is short and close to the The same germs were found in single species in NDC, or in combination with others. The presence of bacteria in urine and DC could indicate that they were transported to the bladder following the introduction of the catheter despite wearing gloves, use of antiseptic or other best practice. A strict respect of aseptic conditions is generally recommended to prevent nosocomial infections following the placement of a urinary catheter (Mac Leone *et al.*, 2000; Wong and Hodon, 1981; Durion, 2000). In this study, the barrier exerted by sterile gloves against contamination appeared weak; otherwise,

hexamidine/chlorocresol combination was more effective than povidone-iodine. The patients were recruited from three hospitals departments having different antibiotic prescription rates. That way, 100%, 93% and 50% of the subjects had received antibiotics in intensive care, gynecology/obstetrics and internal medicine departments, respectively. These antibiotics were not found to prevent bacterial colonization in this study. So, by the first day, 93.33% patients had received antibiotics. Nevertheless, the single one having colonized urine and DC had received aminopenicillin. The situation was similar on the second day as all the patients were treated with antibiotics; but half of urine and DC were colonized by bacteria. The proportion of colonized samples from patients receiving antibiotics reached 80% by day 4 and more than 90% by the 5th day. The majority of these antibiotics, however, were usually active against the bacteria isolated. This would reflect the ineffectiveness of antibiotics in preventing the fixation of bacterial cells on catheters, and these bacteria once established, proliferate more easily because their adherence has conferred a reduced sensitivity to antibiotics on them (Ntsama Essomba *et al.*, 1997). Once catheters are colonized, the bacteria can easily spread in urine. Sex, age, and underlying disease did not appear to be influential factors in the process of bacterial colonization, in contrast to the results described by Marc Leone *et al*, (2000).

All the eight bacterial species isolated from urine and disinfected catheters belonged to the three categories of microbes involved in urinary tract infections, proposed by the European Confederation of Laboratory Medicine (2008). Five of this bacterial species, namely *E. coli*, *Klebsiella* sp., *Proteus* sp., *Serratia* sp. and *S. saprophyticus* had significant concentrations. *E. coli* and *Proteus* sp. were found in all three departments included in the study. *Klebsiella* sp. was isolated only in the intensive care and internal medicine departments. *Serratia* sp. was found in samples from internal medicine department. The method of urine collection used in this study was considered by Denis *et al* (2007) as ensuring more a reflection of catheter colonizing bacteria than those of the bladder. Nevertheless, the fact remains that the presence of the listed bacteria, at the mentioned concentrations should be considered as a risk even for apparently asymptomatic patients as were the study subjects. This situation is even more serious because the bacteria adhered to catheter surfaces, that is to say they had a reduced sensitivity to antibiotics as previously described and the ability to spread into the bladder. Strategies could be developed to try decrease the risk for urinary tract infection and thereby decrease patient morbidity and health care costs.

Klebsiella sp. was the most frequently isolated bacteria in our study. The presence of this bacterium which is common in the hospital environment could reflect a break in the healthcare chain. The highest bacterial concentrations were reached by *E. coli*. Its concentrations on the catheters at day 5 or more were 100 times more than those determined in the urine. It is commonly believed that uro-pathogenic strains of *E. coli* have several adhesion factors to epithelial cells (Juan *et al*., 2001; Jacobsen *et al.*, 2008; Lee *et al.*, 1997). These factors may also serve for the adherence to inert surfaces.

The three sample types collected in this study were colonized soon after the placement of urinary catheters. The percentages of colonized samples increased with the duration of catheterization. These percentages and the bacterial isolates were broadly similar for urine and disinfected catheters. The antibiotics proved ineffective in preventing the colonization of samples. The risk posed by the urinary catheterization in the YCH, measured in accordance with international standards, was found to take the form of 5 species of bacteria namely *E. coli*, *Klebsiella* sp., *Proteus* sp., *Serratia* sp. and *S. saprophyticus*. Strategies could be developed to prevent the risk for urinary tract infection and thereby decrease patient morbidity and health care cost.

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