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ORIGINAL ARTICLE

Microbial biofilm formation and catheter-associated bacteriuria in patients with suprapubic catheterisation

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Abstract
Purpose Catheter-associated bacteriuria (CAB) with transurethral catheters is almost inevitable. Suprapubic catheters (SPCs) are widely considered to decrease the risk of CAB. However, SPCs are implants similarly prone to microbial biofilm formation. The spectrum of colonising pathogens has not been investigated. The aim of this prospective study was: (1) to assess the diversity of microbial suprapubic catheter colonisation (MSPCC), (2) to identify risk factors and (3) to investigate its association with CAB and catheter-associated urinary tract infection (CA-UTI).
Methods A total of 218 SPCs from 112 patients were studied. Urine specimens were obtained after device replacement or removal. Sonication was performed to dislodge adherent microorganisms. Data of patient sex, age, indwelling time, and underlying disease were recorded.
Results Sonicate-fluid culture (SFC) detected MSPCC in 95 %. Increasing indwelling time correlated with MSPCC ($p < 0.05$). Negative SFC was more frequent when antibiotic prophylaxis was applied at time of catheter placement (15 vs. 2 %, $p < 0.05$). Most commonly isolated were *Enterobacteriaceae* (45.8 %), followed by *Enterococcus* spp. (25.7 %) and *Pseudomonas aeruginosa* (10.3 %). CAB and CA-UTI were observed in 95 and 11 %, respectively.
Conclusions This study provides the first analysis of MSPCC. Indwelling time increases, whereas antibiotic prophylaxis decreases the risk of MSPCC. The spectrum of pathogens is comparable to the one obtained from urethral catheter biofilms. Urine specimens could not demonstrate the microbial diversity of MSPCC. SPCs are not preferable to urethral catheters to reduce CAB. Whether the risk of CA-UTI could be minimised by SPCs remains to be clarified.

Keywords Biofilm · Catheter-associated bacteriuria · Sonication · Suprapubic catheter · Urinary tract infection

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Introduction

Catheter-associated bacteriuria (CAB) with standard urinary catheters is almost inevitable after prolonged catheterisation [1]. Although frequently asymptomatic, CAB often precedes catheter-associated urinary tract infection (CA-UTI) and related complications [2, 3]. Suprapubic catheters (SPCs) are widely considered as an alternative to short-term urethral catheters to lower the risk of CAB or at least delay its onset [4]. However, data are insufficient to make such a recommendation for the long term [5]. In addition, the rate of CAB in patients with suprapubic catheterisation is not as low as expected [5, 6]. This might not be surprising as SPCs offer pathogens from the skin a direct access to the bladder. Little is known on microbial suprapubic catheter colonisation

(MSPCC). The spectrum of colonising pathogens that may be associated with ascending gram-negative bacteria from the bladder or by gram-positive colonisation from the insertion site has not been investigated yet. As microbial biofilms have a major impact on medical devices or foreign bodies placed in the urinary tract, further development of effective methods to prevent their formation and related complications is of utmost importance. However, such innovations are most likely to be effective if they are based on a solid understanding of pathogens involved. Therefore, we designed a prospective observational trial to investigate MSPCC. The rate of MSPCC and the spectrum of pathogens were assessed, just as the association of MSPCC to CAB and indwelling time. In addition, the influence of individual risk factors from patients as well as different indications for SPC placement was evaluated. In this study, we were using both conventional urine culture and sonication as recently published [7–9].

Materials and methods

Study population

All consecutive patients who had their suprapubic catheter removed during the study period (1 March 2010 to 30 November 2010) were eligible for the study. The study was approved by the local human subjects committee and complied with the provisions of the Declaration of Helsinki, Good Clinical Practice guidelines and local laws and regulations. All included patients gave written informed consent.

Laboratory investigations

Suprapubic catheter sonication

Suprapubic catheters (silicone-based) were removed under aseptic conditions in the operating theatre or in the outpatient clinic. The catheter was divided into two segments (distal and proximal). The proximal parts were discarded. The distal segment included the tip of the catheter with the balloon (bladder part) and the adjacent 5 cm. These parts were placed in sterile tubes and processed by the microbiology laboratory within 6 h. Negative controls ($n = 8$) consisted of unused sterile SPCs unpacked in the operation room and sent for sonication to go through the same process as SPCs removed from the patients. Catheter colonisation was detected by sonication as described previously [7–10].

Urine specimens

Urine specimens were obtained after device removal via the freshly placed catheter or a midstream-voided urine specimen if use of the catheter was discontinued. Specimens were analysed by conventional culture methods, outlined by the Manual of Clinical Microbiology, ASM, following guidelines issued by CLSI.

Statistical analysis

Analyses were performed using SPSS version 17 software (SPSS, Inc., Chicago, IL). McNemar's and Chi-square test were applied as appropriate. A p value of less than 0.05 was considered to indicate statistical significance. All tests were two-sided.

Definitions

Positive sonicate-fluid culture (SFC)

Growth of $\geq$100 CFU/ml defined positive SFC. Since no validated cut-off value for MSPCC diagnosed by sonication exists, the threshold of $\geq 10^2$ CFU/ml was chosen according to recommendations for intravascular catheters [11].

Catheter-associated bacteriuria (CAB)

CA bacteriuria refers to catheter-associated urinary tract infection (CA-UTI) and catheter-associated asymptomatic bacteriuria (CA-ASB) combined.

Catheter-associated asymptomatic bacteriuria (CA-ASB)

CA-ASB is defined by the presence of $\geq 10^5$ CFU/mL of $\geq$1 bacterial species in a single catheter urine specimen in a patient without symptoms compatible with UTI [5].

Catheter-associated urinary tract infection (CA-UTI)

CA-UTI is defined by the presence of symptoms or signs (i.e. fever with no alternative source identified, suprapubic pain, urgency, haematuria and catheter obstruction) compatible with UTI along with $\geq 10^3$ CFU/mL of $\geq$1 bacterial species [5].

Results

General characteristics

A total of 218 suprapubic catheters from 112 patients were withdrawn during the study period. Two patients refused consent, and in seven cases, urine at the time of catheter removal was not submitted to the laboratory. Complete

Table 1 Case characteristics

Variables	No. of catheters	SFC positive	SFC negative
Study group	209 (100 %)	199 (95 %)	10 (5 %)
Sex			
Male	172 (82 %)	162 (94 %)	10 (6 %)
Female	37 (18 %)	37 (100 %)	0 (0 %)
CAB			
Yes	199 (56 %)	194 (97 %)	5 (3 %)
No	10 (5 %)	5 (50 %)	5 (50 %)
CA-UTI			
Yes	22 (11 %)	22 (100 %)	0 (0 %)
No	187 (89 %)	177 (95 %)	10 (5 %)
Indwelling time			
Short term (≤14 days)	9 (4 %)	6 (67 %)	3 (33 %)
Long term (>14 days)	200 (96 %)	193 (97 %)	7 (3 %)
Indication for SPC placement			
Voiding dysfunction	81 (39 %)	77 (95 %)	4 (5 %)
Neurogenic voiding dysfunction	12 (6 %)	12 (100 %)	0 (0 %)
Fistula	5 (2 %)	3 (60 %)	2 (40 %)
Urethral stricture	26 (12 %)	23 (88 %)	3 (12 %)
Incontinence	33 (16 %)	33 (100 %)	0 (0 %)
Prostate cancer	22 (11 %)	21 (95 %)	1 (5 %)
Prostate enlargement	30 (14 %)	30 (100 %)	0 (0 %)
ABP[a] at SPC insertion	20 (10 %)	17 (85 %)	3 (15 %)
No ABP at SPC insertion	189 (90 %)	182 (96 %)	7 (4 %)

SPC suprapubic catheter, *SFC* sonicate-fluid culture, *CAB* catheter-associated bacteriuria, *CA-UTI* catheter-associated urinary tract infection, *ABP* antibiotic prophylaxis

[a] Antibiotics applied consisted of a single shot of ciprofloxacin, cefpodoxime or amoxicillin clavunate

data of 209 catheter segments and urine specimens from 107 patients fulfilled the case definition and participated in the study (Table 1).

Sonicate-fluid culture

Sonicate-fluid culture (SFC) detected MSPCC in 97 %. The rate of colonisation increased with time of catheterisation. SPCs in situ ≤14 days were significant less colonised (67 %, $p < 0.05$) compared to SPCs with an indwelling time >14 days (89 %). Antimicrobial prophylaxis was associated with negative SFC (15 vs. 2 %, $p < 0.05$). No significant influence of gender and indication of SPC placement on the incidence of positive SFC could be detected (Table 1). In a subset of paired cultures—urine and sonication of the catheter—the yield of sonication was significantly higher ($n = 85$, $p < 0.05$).

Catheter-associated bacteriuria

CAB was observed in 95 % of the cases. Fifty-six percentage of urine specimens were eligible for further comparative analysis (Table 2) while 39 % were unsuitable due to not identifiable polymicrobial microbial growth. Duration of suprapubic catheterisation increased the risk for CAB (89 % short term vs. 96 % long term, $p = 0.3$).

Catheter-associated urinary tract infection

Overall, 23 cases (11 %) showed at least one symptom of CA-UTI. Amongst these, 22 SPCs were associated with both MSPCC and CAB. Duration of catheterisation (≥14 days) increased the risk for CA-UTI as all cases were observed in long-term catheterised patients. The indication for SPC placement did not influence the incidence of CA-UTI.

Spectrum of organisms

SFC encountered a total of 428 organisms (Tables 2, 3) that were differentiated in eight subgroups. *Enterobacteriaceae* spp. were most commonly cultured (45.8 %), followed by *Enterococcus* spp. (25.7 %) and *Pseudomonas aeruginosa* (10.3 %). A single pathogen grew in 59 %, whereas 41 % of samples showed polymicrobial growth (i.e. on average 2.15 microorganisms were per colonised catheter); 56.5 % of isolated organisms were gram-negative, while 40.2 % were gram-positive and 3.3 % fungi, respectively. Single species was detected in 23 % and polymicrobial growth in 67 %. In cases with positive SFC and positive urine culture SFC observed 81 additional organisms (Table 2).

Table 2 Results of sonicate-fluid culture and urine culture

	No. of cases	No. of organisms	Organism	
Total	202	428[a]		
Positive SFC and UC	116			
Concordant[b]	94	111[a]	*Escherichia coli* (45)	*Candida* spp. (3)
			Enterococcus spp. (31)	*Citrobacter freundii* (2)
			Staphylococcus aureus (9)	*Enterobacter cloacae* (2)
			CoNS (6)	*Actinobaculum schaalii* (1)
			Klebsiella spp. (5)	*Aerococcus urinae* (1)
			Pseudomonas aeruginosa (5)	*Proteus* spp. (1)
Additional organisms detected by SFC		81[a]		
			Enterococcus spp. (15)	*Coryne* spp. (5)
			Escherichia coli (15)	*Candida* spp. (4)
			Pseudomonas aeruginosa (9)	*Citrobacter freundii* (3)
			Staphylococcus aureus (8)	*Enterobacter cloacae* (1)
			Klebsiella spp. (7)	*Actinobaculum schaalii* (1)
			Proteus spp. (7)	*Stenotrophomonas maltophilia* (1)
			CoNS (5)	
Additional organisms detected by UC		18	*Escherichia coli* (12)	*Enterobacter cloacae* (1)
			Enterococcus spp. (2)	*Staphylococcus aureus* (1)
			Pseudomonas aeruginosa (2)	
Discordant	22			
Organisms detected by SFC		45[a]	*Escherichia coli* (13)	*Proteus* spp. (3)
			Enterobacter cloacae (8)	CoNS (2)
			Enterococcus spp. (7)	*Citrobacter freundii* (1)
			Pseudomonas aeruginosa (6)	*Staphylococcus aureus* (1)
			Klebsiella spp. (3)	*Serratia* spp. (1)
Organisms detected by UC		32	*Enterococcus* spp. (12)	*Proteus* spp. (7)
			Escherichia coli (12)	*Klebsiella* spp. (1)
Positive SFC and negative UC	5	10[a]	*Klebsiella* spp. (3)	*Candida* spp. (1)
			Enterobacter cloacae (2)	*Enterococcus* spp. (1)
			Staphylococcus aureus (2)	*Escherichia coli* (1)
Positive SFC and UC[c]	81	181[a]	*Enterococcus* spp. (56)	CoNS (6)
			Escherichia coli (38)	*Coryne* spp. (6)
			Pseudomonas aeruginosa (24)	*Enterobacter cloacae* (4)
			Klebsiella spp. (18)	*Actinobaculum schaalii* (2)
			Proteus spp. (11)	*Citrobacter freundii* (1)
			Staphylococcus aureus (7)	*Serratia* spp. (1)
			Candida spp. (6)	*Stenotrophomonas maltophilia* (1)

Brackets represent numbers

SFC sonicate-fluid culture, *UC* urine culture, *CoNS* coagulase-negative *staphylococcus* spp.

[a] Number of organisms detected by sonication, [b] concordant indicates that the organism was isolated both from sonicate-fluid and urine cultures, [c] polymicrobial microbial growth, not identifiable

Discussion

Suprapubic catheters (SPCs) are widely considered as an alternative to urethral catheterisation. The presumed advantages of SPCs are reduced risk of urethral trauma and stricture formation, ability to attempt normal voiding without the need for recatheterisation, less interference with sexual activity and lower risk of CAB [6]. However,

Table 3 Microorganisms detected by sonicate-fluid culture (male vs. female cases)

Organism	Total no.		Male sex		Female sex	
No. of organisms	428	R	343	R	85	R
Enterobacteriaceae[a]	196 (45.8 %)	1	156 (45.5 %)	1	40 (47.1 %)	1
Enterococcus spp.	110 (25.7 %)	2	88 (25.7 %)	2	22 (25.9 %)	2
Pseudomonas aeruginosa	44 (10.3 %)	3	33 (9.6 %)	3	11 (12.9 %)	3
Staphylococcus aureus	27 (6.3 %)	4	25 (7.3 %)	4	2 (2.4 %)	5
CoNS[b]	19 (4.4 %)	5	16 (4.7 %)	5	3 (3.5 %)	4
Candida spp.	14 (3.3 %)	6	11 (3.2 %)	6	3 (3.5 %)	4
Corynebacterium spp.	11 (2.6 %)	7	8 (2.3 %)	7	3 (3.5 %)	4
Other[c]	7 (1.6 %)	8	6 (1.7 %)	8	1 (1.2 %)	6

R rank

[a] *Escherichia coli* ($n = 112$), *Klebsiella* spp. ($n = 36$), *Proteus* spp. ($n = 22$), *Enterobacter cloacae* ($n = 17$), *Citrobacter freundii* ($n = 7$), *Serratia* spp. ($n = 2$)

[b] Coagulase-negative *staphylococcus* spp.

[c] *Actinobaculum schaalii* ($n = 4$), *Stenotrophomonas maltophilia* ($n = 2$), *Aerococcus urinae* ($n = 1$)

SPCs offer microorganisms a direct access from the insertion site into the bladder, rendering them at risk for microbial colonisation and consecutive biofilm formation. Antimicrobial treatment of biofilm infections is known to be complicated as the microbial inhabitants may be up to a 1,000 times more resistant to antimicrobial therapy than free-floating bacteria of the same species [12]. Antibiotics often fail to penetrate the full depth of the biofilm. Furthermore, pathogens of the biofilm have reduced growth compared to free-floating bacteria. This makes them more resistant against types of antibiotics that has its effect on proliferating bacteria [13]. In contrast to urethral catheters [14–16], data concerning biofilms on the surface of suprapubic catheters (frequency, microbial diversity of pathogens, relation to urine culture, etc.) appear not to exist.

With our sonication system, we predominantly detected *Escherichia coli, Enterococcus* spp. and *Pseudomonas aeruginosa*. The same bacterial species were most commonly identified in urethral catheter biofilms [16]. This is a rather surprising finding of our study, because the insertion site of SPCs are away from the heavily colonised perineum and periurethral skin both regarded to be responsible for predominantly gram-negative biofilms on urethral catheters. We expected to detect mostly growth of gram-positive organisms (i.e. *Staphylococcus* spp.). *Staphylococcus* spp. constitute a significant part of the normal bacterial flora of the human skin and could be easily introduced as a contaminant during the surgical implantation of suprapubic catheters [17, 18]. Our findings might be interpreted as follows: As important protective mechanisms of the urinary tract (i.e. urine flow, regular bladder emptying and valve mechanisms) are disabled in patients with SPCs, gram-negative pathogens ascend via the urethra into the bladder and colonise the surface of the suprapubic catheter. As gram-negative organisms replicate faster than gram-positive (e.g. *Staphylococcus* spp.) due to their thinner murein cell wall, they could easily overgrow the primarily gram-positive colonising flora of SPCs. The spectrum of organisms identified by SFC did not differ between women and men. This is in contrast urine cultures reported by previous studies [19]. *E. coli*, the most common pathogen within the subgroup of Enterobacteriaceae, uses several virulence factors like hemolysins, cytotoxic necrotizing factor, K1 capsule, siderophores as well as adhesins like Type I pili and fimbrial protein fimH [20, 21]. *Enterococcus* spp. were reported to be the most common gram-positive bacteria amongst uropathogens and have been associated with biofilms on various kinds of indwelling medical devices [22]. The formation of pili by *Enterococcus* spp. is necessary for biofilm formation. In addition, the 'enterococcal surface protein' (Esp) has been shown to promote the initial adherence of the bacterial cells to abiotic surfaces [23]. *Pseudomonas aeruginosa* was the third most common isolated pathogen. Virulence of *P. aeruginosa* is multifactorial and has been attributed to cell-associated factors like alginate, lipopolysaccharide (LPS), flagellum, pilus and non-pilus adhesins as well as with exoenzymes or secretory virulence factors [24, 25]. SFC could detect more microorganisms compared to urine culture. This is not surprising as urine culture can only detect free floating, so-called planktonic organisms.

In this study, the frequency of CAB was comparable to published data on patients with transurethral catheters [6, 26, 27]. Therefore, suprapubic catheterisation is not superior over to urethral catheters in the reduction CAB. Whether the rate of CA-UTI could be reduced by the use of SPCs remains speculative. Available literature is rare,

inconsistent and controversial. A number of previous studies used the term CAB without distinguishing CA-UTI or CA-ASB. Moreover, the validity or reliability of symptoms and/or signs distinguishing CA-UTI from CA-ASB has not been critically evaluated yet [5, 6, 28–30].

The ideal interval to perform SPC replacement is difficult to define and might be adjusted on an individual basis. Taking the low complication rate observed into account, a routine change every 2–3 month seems to be justified.

Although we observed a significant effect of antibiotic prophylaxis (ABP) on catheter colonisation, we agree with current guidelines [4, 5] not to apply ABP routinely due to the concern about selection of antimicrobial resistance. In addition, even in cases where AB was applied, the colonisation rate was high. With the increasing number of biomaterial devices used in human medicine, having an effective method for preventing biofilm formation is of utmost importance [13]. Innovations to prevent biofilm formation and related complications are most likely to be effective, if they are based on a solid understanding of pathogens involved. Therefore, our findings could be helpful to improve (i) the development of new biomaterials to reduce biofilm formation on urinary catheters, (ii) the addition or impregnation of antimicrobial agents and (iii) the use of probiotics to delay or prevent urinary catheter colonisation and associated complications. Some limitations of this study should be mentioned: (1) The current lack of a gold standard definition of SPC associated UTI (2), no validated gold standard technique for the diagnosis of MSPCC exist and (3) the cut-off value of SFC for MSPCC was chosen according to a reference for intravascular catheters.

Conclusion

This study provides the first report of microorganisms colonising SPCs. MSPCC is common. The only identified risk factor for MSPCC is indwelling time. Antibiotic prophylaxis reduces MSPCC significantly and, however, could not be recommended for daily practice due to the concern about selection of resistant pathogens. The spectrum of pathogens identified is comparable to urethral catheter biofilms, indicating an infection route from the bladder inside. Urine culture could not demonstrate the microbial diversity of MSPCC. Whether the risk of CA-UTI could be minimised by suprapubic catheterisation remains to be clarified by further well-conducted studies.

Conflict of interest None of the contributing authors has any conflict of interests relevant to the subject matter or materials discussed in the manuscript. No funding or other financial support was received.

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