Escherichia coil 83972 Bacteriuria Protects Against Recurrent Lower Urinary Tract Infections in Patients With Incomplete Bladder Emptying

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Deliberately induced *E. coli* 83972 bacteriuria protects against recurrent lower urinary tract infections in patients with incomplete bladder emptying. A blinded randomized placebo-controlled cross-over study.

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ABSTRACT

Purpose: To determine if deliberate establishment of asymptomatic bacteriuria with *E. coli* 83972 in patients with incomplete bladder emptying and recurrent UTI protects against recurrences.

Materials and methods: In a first phase patients were randomized to blinded inoculations with *E. coli* 83972 or saline. Cross-over was after monitoring for 12 months, or after a UTI. The outcome was the time to the first UTI in patients with and without *E. coli* 83972 bacteriuria. In a second phase patients were subjected to additional blinded inoculations to extend periods with and without *E. coli* 83972 bacteriuria. The outcome was the number of UTI during 12 months with, and 12 months without *E. coli* 83972 bacteriuria.

Results: 20 patients completed the study. In the first phase the time to the first UTI was longer with, than without *E. coli* 83972 bacteriuria (median 11.3 versus 5.7 months, p = 0.0129, Sign test). The second phase was analyzed after patients had spent a total of 202 months with, and 168 months without *E. coli* 83972 bacteriuria. There were fewer reported UTI episodes with, than without *E. coli* 83972 bacteriuria (13 versus 35 episodes, p= 0.009, CI 0.31-1.89, paired t-test). There was no febrile UTI episode in either of the study-arms, and no significant side effects of intravesical bacterial inoculation were reported.

Conclusion: Deliberately induced *E. coli* 83972 bacteriuria protected UTI prone patients with incomplete bladder emptying from recurrent UTI, as determined by delaying the time to UTI, and by reducing the number of UTI episodes.

INTRODUCTION

Urinary tract infections (UTI) are among the most common infectious diseases in man, [1] with a subset of susceptible individuals developing recurrent episodes. Treatment with antibiotics is initially effective, but the rapid increase in antibiotic resistance in gram-negative uropathogens, [2, 3] is a strong rationale to develop therapeutic alternatives. This study discusses bacterial interference, where bacteria of low virulence inhibit symptomatic infection.

Patients with asymptomatic bacteriuria (ABU) carry $>10^5$ CFU/ml of urine without developing symptoms, and is harmless in patients without risk-factors.[4-6] In addition, ABU may be protective as shown in paediatric populations, where the eradication of ABU increased the risk for symptomatic UTI.[7] This protective effect has been attributed to "bacterial interference" by competition for nutrients and by bacterial production of toxic molecules.[8] Regardless of the mechanism, the ABU strains appear to prevent more virulent *E. coli* strains to cause ascending infection.

Based on this observation, a protocol to deliberately establish bacteriuria of the lower urinary tract was created as an alternative to conventional therapy in patients with recurrent complicated UTI.[9, 10] The strain used for colonization, *E. coli* 83972, was originally isolated from a girl with long term ABU.[9, 11] It lacks expressed virulence factors as well as a defined O:K:H serotype,[12] is sensitive to all common antibiotics used for UTI and carries a small plasmid (1.2 KB) enabling identification through plasmid test.[9] Early colonization studies identified incomplete bladder emptying as a pre-requisite for *E. coli* 83972 establishment, and long-term observational follow-up has shown the approach to be safe, that ABU is achieved and that symptomatic recurrences seem to be hindered.[9, 10, 13, 14]

The aim of the present study was to investigate if *E. coli* 83972 bacteriuria protects against symptomatic infections in UTI prone patients with incomplete bladder

emptying. In a first phase of the study we compared the time to the first UTI in the same patients with and without *E. coli* 83972 bacteriuria. In a second phase, the number of UTI in the same patients during about 12 months with, and about 12 months without *E. coli* 83972 bacteriuria, was compared.

METHODS

Participants

Patients with incomplete bladder emptying and with recurrent lower UTI were invited to participate in the study. All patients were on optimal conservative therapy, and the majority treated with Clean Intermittent Catheterization (CIC). The patients were accustomed to using a self-reporting system for the diagnosis of UTI.[15] Criteria for study participation is listed in table 1, patient flow-chart is shown in figure 1, and characteristics of the 20 patients who fulfilled the protocol are given in table 2.

Study intervention, objectives and outcomes

Phase 1 was a blinded, randomized and placebo-controlled cross-over study. Patients were inoculated with *E. coli* 83972, or with saline, according to the randomization. The outcome measure was the time to the first UTI after establishment of *E. coli* 83972, compared with the time to the first UTI after a placebo, or a failed *E. coli* 83972 inoculation. Cross-over was after the first UTI report, or after approx. 12 months if no UTI was reported. Patients who did not establish *E. coli* 83972 bacteriuria during phase 1 were excluded from the study (figure 1).

Phase 2. All patients who fulfilled phase 1 proceeded to phase 2 which was blinded, observational and placebo-controlled. The outcome measure was the total number of UTIs over an optimal of 12 months with *E. coli* 83972 bacteriuria versus an optimal of 12 months without *E. coli* 83972 bacteriuria. Patients who had spent less than approx. 12 months in any of the study arms during phase 1 were given additional saline or *E. coli* 83972 inoculations (figure 1). A minimum of 3 months in both study-arms were required for evaluation in this phase.

Inoculation protocol. The patient was given antibiotics to sterilize the urine and after an antibiotic free interval the patient was catheterized and the bladder emptied[16].

According to the randomization 30 ml of *E. coli* 83972 (10^5 cfu/ml) or saline was instilled in the bladder, and the catheter removed. The procedure was repeated once daily for three days.

Monitoring and re-inoculations. Urine cultures were surveilled by the study monitor (BW), who otherwise did not participate actively in the study team. If an *E. coli* 83972 colonization attempt failed the patient then entered the control-arm without established *E. coli* 83972 bacteriuria. If spontaneous clearance of the strain was detected after bacterial establishment, a re-inoculation was performed. The patients were informed at inclusion that random re-inoculations could be performed in each study-arm and always after an antibiotic treatment of a UTI episode or an intercurrent infection.

Urine sampling, symptom-score and patient follow-up. Urine samples for culture were obtained once monthly and whenever the patients reported symptoms from the lower urinary tract. Patients scored their subjective symptoms, indicating being symptom-free, experiencing minor symptoms from the lower urinary tract without the need for further evaluation, or having symptoms of UTI with the need for antibiotic treatment, on each sampling occasion (table 3). If *E. coli* was identified, the strain was saved, the antibiotic susceptibility pattern was recorded and the presence of a 1.2 KB plasmid was tested on approximately every third isolate. The patients had access to the research nurse (EL) by telephone and e-mail, also during non-office hours.

Definition of UTI. If patients reported subjective symptoms of UTI [1], [15] urine sampling was arranged and antibiotic therapy was prescribed. The decision of antibiotic therapy was thus taken by the patient him/herself, based only on subjective symptoms and without the knowledge of any previous or present urinary findings.

Sample size

Assuming a mean difference of 1.0 and a standard deviation of 1.5 for the paired differences a sample size of 26 was needed to reach a significant difference with 90 % power using a two-sided paired *t*-test at 5 % significance level. With power set to 80 % a sample of 20 was required.

Randomization

Patients were randomized on entry into phase 1 of the study. Because *E. coli* 83972 generally establishes bacteriuria in about 2/3 of inoculated cases a computer generated randomization list in a 3:1 ratio to the bacteriuria arm was used. In the control-arm, patients were subjected to the identical protocol but given intra-vesical inoculations with saline.

Blinding

The complete study (phase 1+2) was patient-blind regarding the nature of the inoculum and double-blind regarding results of urine cultures. This implied that the study team planned and performed inoculations with the patients uninformed about what inoculum was given, and that the study team and the patients were unaware of the urine culture results during the entire study.

Statistics

The Sign-test was used to analyze the time to the first UTI episode and the Kaplan Meier approach was used to describe survival functions. The paired t-test was used for analyzing differences in the number of UTI. A p value < 0.05 was considered significant.

RESULTS

Twenty patients completed the study and fulfilled the criteria for evaluation in phase 1 and 2 (table 1, figure 1). There were no significant side effects reported from patients with established *E. coli* 9372 bacteriuria, and there were no febrile UTI episodes reported in either of the study arms.

Phase 1; E. coli 83972 bacteriuria delays UTI recurrences

In this phase patients were randomized using a blinded, placebo-controlled protocol with cross-over, to *E. coli* 83972 or saline inoculations. The time to the first UTI was compared in the same patients after established *E. coli* 83972 bacteriuria, and after saline or failed *E. coli* 83972 inoculations.

In all 23 UTI reports were made from the 20 patients who fulfilled the phase 1 protocol. 8 patients reported a UTI from both study-arms, one patient only after established *E. coli* 83972 bacteriuria and 6 patients only after saline, or failed *E. coli* 83972 inoculations. The median time to UTI was significantly longer after *E. coli* 83972 establishment, as compared to after saline or failed *E. coli* 83972 inoculations (11. 3 versus 5. 7 months, $p = 0.0129$, Sign test, figure 2).

Phase 2; E. coli 83972bacteriuria reduces the number of UTI episodes

All patients from phase 1 proceeded to phase 2 and were further analyzed regarding the number of UTI over 12 months with, and 12 months without, *E. coli* 83972 bacteriuria, using a blinded, observational protocol. Additional inoculations with *E. coli* 83972 or saline were given patients with less than 12 months in either study-arm during phase 1. Evaluation was performed when the patients had spent 202 months (mean 10.1/patient) with *E. coli* 83972 bacteriuria, and 168 months (mean 8.4 months /patient) without *E. coli* 83972 bacteriuria. The number of reported UTIs was

significantly lower in the same patients with, than without *E. coli* 83972 bacteriuria (13 and 35 UTI reports, respectively, p= 0.009, CI 0.31-1.89, paired t-test, table 4).

Analysis of reported UTI episodes

A urine sample was collected in connection with all UTI reports. In patients without *E. coli* 83972 bacteriuria 31/35 (89%) of these cultures showed uropathogenic growth. In all patients with *E. coli* 83972 bacteriuria, the presence of *E. coli* 83972 was verified in urine samples 1-3 weeks prior to the 13 UTI reports. In 11 of these cases a urine culture was obtained on the same day as the UTI report, of which three showed growth of *Proteus sp.*, *Pseudomonas sp.* and *Enterococcus faecalis,* respectively. In the eight remaining cases *E. coli* growth was verified. Four isolates were saved and two were, by the plasmid test, identified as *E. coli* 83972.

Analysis of symptom free periods and reported minor episodes

The patients' symptom-scores in conjunction to the monthly urine sampling were compared between the two study-arms. The scoring from asymptomatic periods did not differ between patients with or without established *E. coli* 83972 bacteriuria (figure 3). The frequency of minor symptoms was halved in patients with established *E. coli* 83972 bacteriuria, as compared to patients without *E. coli* 83972 establishment, but this difference was not statistical significant (p = 0.13, paired t-test), table 5.

DISCUSSION

Patients with incomplete bladder emptying and UTI susceptibility were shown to benefit from deliberate establishment of bacteriuria with the ABU isolate *E. coli* 83972 in this study. It has previously been shown that eradication of spontaneously developed ABU increases the risk for a subsequent symptomatic UTI episode.[7] Here, this concept is carried one logical step further, by showing that deliberate bacteriuria with a model ABU strain can be established and that it carries a similar protective effect as naturally selected strains, and that UTI prone individuals benefit from this approach.

There are three possible weaknesses of the study that we are aware of. First, patients who did not develop bacteriuria after inoculation were excluded from the study. These patients might eliminate the inoculated bacteria due to a more active antimicrobial host defense in the urinary tract[16] than the patients who developed *E. coli* 83972 bacteriuria, who might have an attenuated antibacterial response. The second possible weakness is our choice of a patient self-reporting definition of UTI episodes. The lack of reliable objective markers of UTI in patients with lower urinary tract dysfunctions, and on CIC, is well known. In our opinion the self-reporting was the most adequate method to use in this study, well adjusted to the every-day clinical practice of these patients and providing an excellent non-biased marker of UTI episodes. The high accuracy of self diagnosing recurrent UTIs has previously been shown in selected patient groups[15], and the reliability was verified in this study by the high rate of uropathogenic growth in urine cultures from UTI reports in patients without *E. coli* 83972 bacteriuria.

A third weakness might be the fact that re-inoculation was required in a few patients after spontaneous clearance of the *E. coli* 83972 bacteriuria, or after antibiotic treatment due to other infections. However; all participants were prepared at inclusion to enter random re-inoculations in both study arms and colonization

failures or sporadic spontaneous clearance of the *E. coli* 83972 bacteriuria made it impossible for the patient and the study team to know whether or not *E. coli* 83972 was present in the urine or not. To our opinion, this suggests that all UTI reports from both study-arms were un-biased.

This study shows that the *E. coli* 83972 bacteriuria approach is safe, as reflected by the absence of serious side effects as pyelonephritis. The safety of the approach, including the protective effect of *E. coli* 83972 bacteriuria, has been suggested in several open trials.[9, 10, 13, 14] One study on male patients with neurogenic bladders randomized patients to *E. coli* 83972 inoculation or placebo and followed the patients until the first UTI event. Most patients in that study were treated with indwelling catheters or supra-pubic drainage, and the study did not include a cross over. Only a minority of the patients had monocultures of *E. coli* 83972, making the contribution of *E. coli* 83972 and its protective effect difficult to evaluate.[17] The present study strictly analyses verified periods of *E. coli* 83972 bacteriuria as a monoculture and demonstrates that this results in protection against UTI. In addition, the cross-over approach allowed for intra-individual comparison, showing the same effect.

The study supports the notion that *E. coli* 83972 causes asymptomatic bacteriuria, with a few exceptions. A detailed analysis of the thirteen UTI episodes reported by patients with *E. coli* 83972 bacteriuria revealed eight cases of *E. coli* growth in urine at the time of the report. Four of these strains were saved, and two showed a positive plasmid test, verifying *E. coli* 83972. This suggests that the main cause of these UTI episodes were super-infecting strains outcompeting *E. coli* 83972. However, the question remains if *E coli* 83972 may cause UTI symptoms also after long-term symptom free carriage. The genome of *E. coli* 83972 has been fully sequenced, but there may be genetic variation arising during carriage, which needs to be investigated. There is also variation in host susceptibility, based on the innate immune response in the urinary tract.[18] As *E. coli* 83972 is extremely well characterized[19, 20] the human inoculation model is ideal for further *in vivo* studies of the host response to bacterial carriage. We have previously used genetically

transformed variants of *E. coli* 83972 to study the role of bacterial adhesion factors (P- and Type 1 fimbriae) in early bacterial establishment.[16, 21, 22] Future studies are needed to further define individual variation and host response genetics, and phenotypic and genotypic adaptation of *E. coli* 83972 in individual hosts. The aim would be to enhance *E. coli* 83972 persistence in the human lower urinary tract, and to define those patients who are suitable for this therapeutic approach.

CONCLUSION

Patients with dysfunctional voiding and recurrent UTI can benefit from the protective effect of spontaneously developed ABU, but if this does not occur, protective ABU may be induced by deliberate inoculation with *E. coli* 83972. Due to increasing microbial multi-resistance, driven by the antibiotic usage, alternative therapies, including the *E. coli* 83972 bacteriuria approach, might become more widely used in the future.

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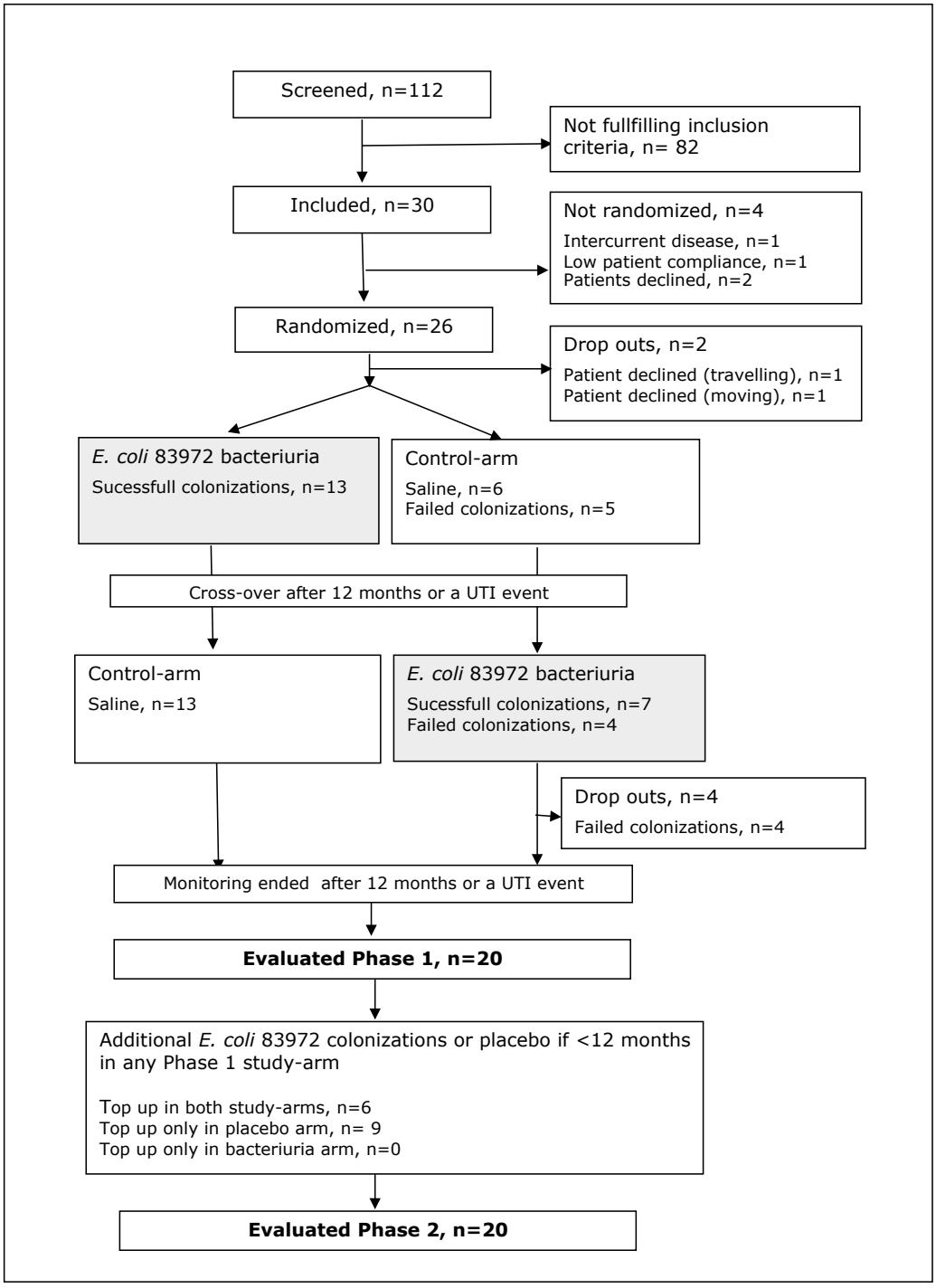

Figure 1. Participiant flow through trial.

Repeated inoculation during phase 1 was performed at 11 occasions: spontaneous loss of *E. coli* 83972 after established bacteriuria (5 occasions), random re-inoculations in the placebo-arm (3 occasions), and after antibiotic treatment due to intercurrent infection (1 and 2 occasions in the bacteriuria-arm and the placebo-arm, respectively).

The study was carried out at the Urology Out-Patient Department, Lund. The first patient was recruited March 2003, and the study was closed in December 2006.

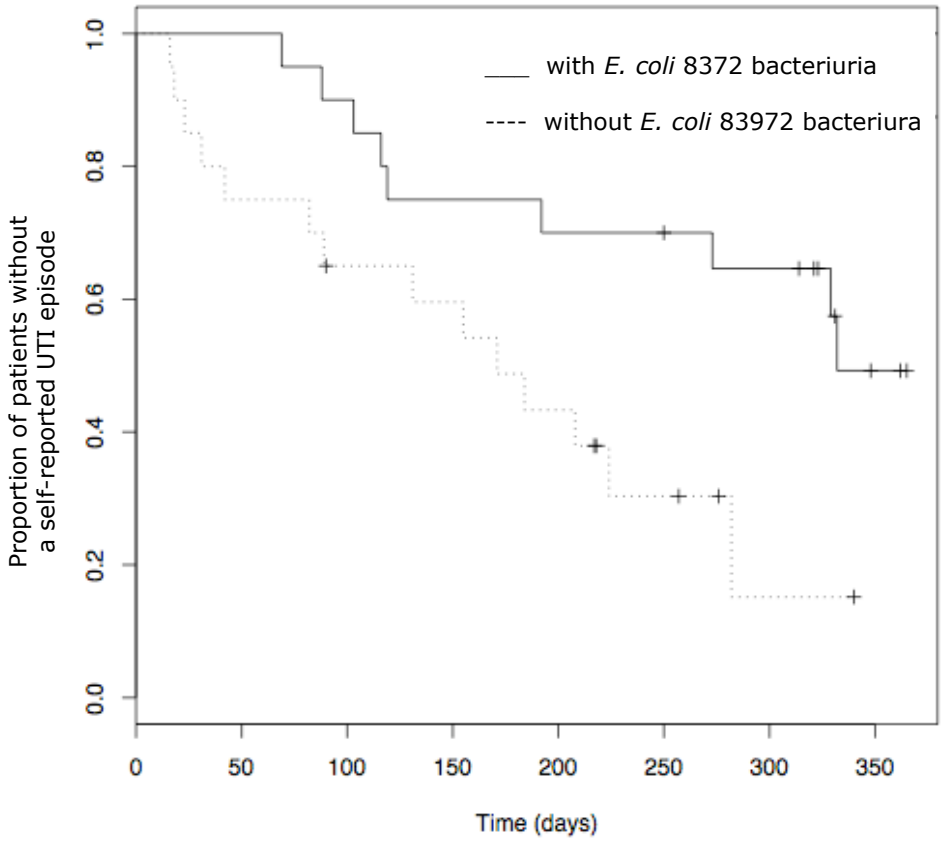

Figure 2. Kaplan-Meyer estimate of the Phase 1 part of the study; risk of UTI in the 20 patients who fulfilled the protocol.

Patients were randomized to *E. coli* 83972 or saline incoulations and were followed for twelve months, or until the self-report of a UTI. Cross over was performed after either a completed follow up period, or after a UTI. There was a significant delay of the time to a UTI episodes in patients with *E. coli* 83972 bacteriuria, as compared to when the same patients were without *E. coli* 83972 bacteriuria (median 11,3 versus 5,7 months, respectively, p = 0.0129, Sign test).

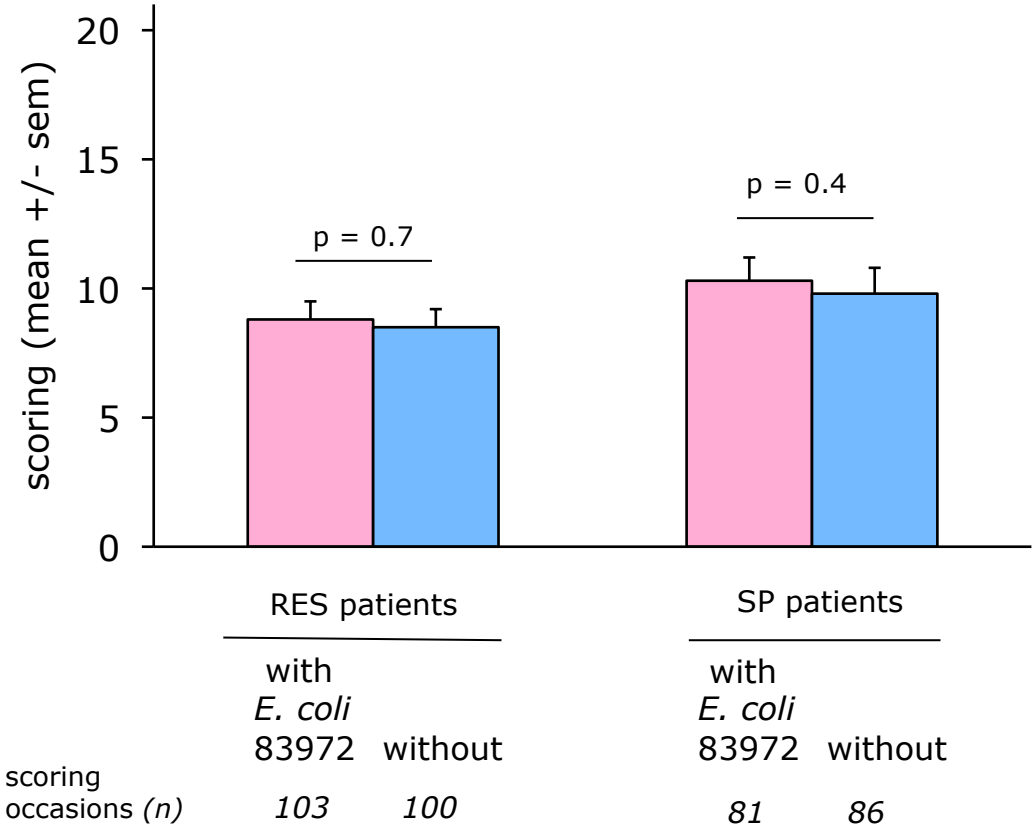

Figure 3. Mean monthly scoring from patients with residual urine due to lower motor neuron lesions (RES), and from spinal lesion patients (SP) with *E. coli* 83972 bacteriuria (with *E. coli* 83972) and without *E. coli* 83972 bacteriuria (whithout). In the patients without *E. coli* 83972 bacteriuria, transient growth of gram-negative uropathogens, *Ent. faecalis* and *Beta-streptocci* were found in 68% of the urine samples.

The scoring protocol described in table 2 was used. All sampling occasions had been defined as "asymptomatic" by the patients. There was no significant difference between the scorings in either group of patients regardless of study-arm (mean scoring from each patient, paired t-test, $p > 0.05$).

Table 1. Criteria for study participation

Inclusion criteria	
	incomplete bladder emptying
	≥ 3 microbiologically proven UTI/ year two years prior to the study
	Optimal conservative measurements incl. Clean Intermittent Catherization
Exclusion criteria	
	Upper urinary tract infections
	Renal detoriation
	Hydronephrosis
	Untreated bladder outflow obstruction
	Urinary calculi
	Immunosuppression (incl. corticosteroid medication)
	Urological malignancies

Before inclusion patients underwent renal function tests, upper urinary tract imaging, urodynamic assesment and cystoscopy.

Table 2. Patient characteristics and number of UTI episodes prior to the study

Pat ID[1]	Sex[2]	Age	UTI episodes 12 months prior to inclusion (*n*)	CIC[3]	Diagnosis, comment
SP 2	M	39	*4*		Tetraplegia; spinal cord injury
SP 4	M	47	*7*	x	Tetraplegia; spinal cord injury, diabetes mellitus type 2, sphincterotomy.
SP 5	F	60	*4*	*x*	Tetraplegia; epidural hematoma
SP 7	F	61	*3*	*x*	Paraplegia; slipped disc, epidural hematoma
SP 8	M	51	*6*	*x*	Tetraplegia; spinal cord injury
SP 11	M	38	*4*	*x*	Tetraplegia; spinal cord injury
SP 12	M	55	*3*	*x*	Paraplegia; epidural hematoma
SP 13	M	45	*5*	*x*	Paraplegia; spinal cord injury
RES 2	M	77	*3*	x	Idiopatic detrusor insufficiency
RES 4	F	59	*4*	*x*	Idiopatic detrusor insufficiency
RES 9	F	66	*5*	*x*	Residual urine after urethropexia
RES 10	F	46	*4*		Detrusor insufficiency; after borreliainfection
RES 11	F	84	*3*		Idiopatic detrusor insufficiency
RES 12	F	82	*3*	*x*	Idiopatic detrusor insufficiency
RES 13	F	77	*5*		Idiopatic detrusor insufficiency, coronary by pass surgery
RES 15	F	45	*4*		Detrusor insufficiency; diabetes mellitus type 1
RES 16	F	76	*4*		Idiopatic detrusor insufficiency
RES 17	F	64	*3*		Residual urine after urethropexia, cystocele
RES 18	M	72	*3*	*x*	Detrusor insufficiency; after encephalitis
RES 19	F	32	*3*		Idiopatic detrusor insufficiency

All patients had incomplete bladder emptying (residual urine ≥ 100 ml) and UTI susceptibility with a history ≥ 3 UTI/ year with urinary cultures showing uropathogenic growth, two years prior to the study.

1) SP= Spinal lesion, RES= Residual urine due to lower motor neuron lesions
2) M= Male, F= Female
3) Clean Intermittent Catheterization. All patients had been instructed to use CIC regularly. Of the 8 patients who did not use CIC during the study 2 patients refused because of practical reasons and the remaining 6 patients had residual urine < 300 ml, and had not experienced any improvement from previously performed regular CIC.

Table 3. Symptom score-questionaire used in this study

All patients
Supra-pubic pain/ discomfort
Malaise
Fever, chills
Urgency, leakage
Abnormal urine properties (smell, colour, turbidity)
Only spinal lesion patients
Autonomous dysreflexia
Spasticity

Questions scored 1-3; 1 being no symptoms. The minimum score was 5 (7; spinal lesion patients), the maximum score was 15 (21; spinal lesion patients). The patients defined further (yes/no) if he/she was in symptom-free period, experienced minor irritative symptoms or significant symptoms of UTI in the need for antibiotic treatment.

Table 4. Phase 2; number of self reported UTI with and without *E. coli* 83972 bacteriuria

	With *E. coli* 83972 bacteriuria			Without *E. coli* 83972 bacteriuria		
Pat ID	top up[1]	months	UTI (*n*)	top up[2]	months	UTI (*n*)
SP 2	--	11.1	*1*	yes	7.7	*1*
SP 4	--	11.0	*0*	--	7.2	*0*
SP 5	yes	10.4	*2*	yes	9.6	*2*
SP 7	--	10.8	*0*	yes	10.8	*2*
SP 8	--	4.0	*1*	yes	12.2	*1*
SP 11	--	12.2	*0*	--	7.3	*0*
SP 12	yes	12.2	*1*	yes	9.7	*2*
SP 13	--	11.0	*1*	--	9.2	*0*
RES 2	yes	9.4	*1*	yes	12.2	*1*
RES 4	yes	11.8	*1*	yes	7.8	*2*
RES 9	--	12.2	*0*	yes	10.0	*2*
RES 10	--	12.1	*0*	--	8.6	*0*
RES 11	--	10.5	*0*	--	3.6	*0*
RES 12	--	12.0	*0*	yes	5.7	*6*
RES 13	--	8.3	*0*	yes	10.5	*2*
RES 15	yes	5.2	*3*	yes	5.1	*4*
RES 16	yes	3.8	*2*	yes	7.6	*3*
RES 17	--	12.0	*0*	yes	7.4	*4*
RES 18	--	11.6	*0*	--	11.3	*0*
RES 19	--	10.7	*0*	yes	5.4	*3*
Total of UTI episodes (n)			*13*			*35*

All patients who fullfilled phase 1 of the study proceeded to phase 2. Patients who had spent < 12 months in any study-arm during phase 1 were subjected to additional inoculations with *E. coli* 83972 or with saline resulting in a mean of 10.1 months / patient with *E. coli* 83972 bacteriuria, and a mean of 8.4 months/patient without *E. coli* 83972. The number of UTI episodes differed significantly between the two study-arms, p= 0.009, CI 0.31-1.89, paired samples T-test.

1) additional inoculations resulting in *E. coli* 83972 bacteriuria
2) additional saline or failed *E. coli* 83972 inoculations

Table 5. Number of minor irritative symptoms in the completed study

Pat ID	Minor symptoms (*n*) with *E. coli* 83972 bacteriuria[1]	Minor symptoms (*n*) without *E. coli* 83972 bacteriuria [2]
SP 2	*1*	*3*
SP 4	*2*	*0*
SP 5	*2*	*3*
SP 7	*1*	*5*
SP 8	*0*	*3*
SP 11	*2*	*2*
SP 12	*2*	*1*
SP 13	*0*	*1*
RES 2	*1*	*0*
RES 4	*0*	*1*
RES 9	*0*	*0*
RES 10	*0*	*3*
RES 11	*0*	*0*
RES 12	*0*	*1*
RES 13	*3*	*2*
RES 15	*0*	*0*
RES 16	*1*	*2*
RES 17	*2*	*1*
RES 18	*0*	*0*
RES 19	*0*	*0*
Total	*17*	*28*

Minor irritative symptoms from the lower urinary tract, not needing antibiotic treatment, were reported less frequently from patients with *E. coli* 83972, than from patients without *E. coli* 83972 bacteriuria (p=0.13, paired t-test)

1) On all occasions the accompanying urine culture demonstrated growth of *E. coli* 83972.
2) 18/28 (64%) of the accompanying urine culture were sterile, the remaining showing significant bacterial growth.