Women with recurrent urinary tract infections: antibiotic resistance and non-antibiotic prophylaxis
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Summary

Urinary tract infections (UTI) are common in women, and about 20-30% of women will have recurrences after an initial UTI. To prevent recurrences, women with recurrent UTIs can use antibiotic prophylaxis. Although effective, this can lead to resistance of not only the causative microorganisms, but also of the commensal flora. Increasing antibiotic resistance has stimulated interest in non-antibiotic alternatives to prevent UTIs.

Therefore, we conducted the “Non-antibiotic versus Antibiotic Prophylaxis for Recurrent Urinary Tract Infections” (NAPRUTI) Study: in two linked randomized clinical non-inferiority trials, we compared the effects of 12-months non-antibiotic prophylaxis with the effects of trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis. In one trial premenopausal women with a history of recurrent UTIs received either cranberry capsules or TMP-SMX, in the other trial postmenopausal women received either lactobacilli oral therapy or TMP-SMX. In both studies we measured the rate of UTI recurrence and the development of antibiotic resistance.

In Chapter 2 we first reviewed evidence from randomized controlled trials on the efficacy and tolerability of non-antibiotic prophylaxis for rUTIs. We searched the electronic databases Pubmed/Medline, Embase, the Cochrane Library, and reference lists of relevant reviews for clinical trials randomizing adults with rUTIs to non-antibiotic prophylaxis or a control group with placebo or no treatment. In seven studies a bacterial extract was used to stimulate the host’s immune system to produce antibodies and cytokines, with the objective to prevent recurrences. Four trials with 891 participants showed efficacy of the oral immunostimulant OM-89 in reducing UTIs (RR=0.61, 95%CI 0.48-0.78), with a good safety profile. In three trials with 220 participants the vaginal mucosal vaccine Urovac, given as suppositories containing heat-killed uropathogenic bacteria, slightly reduced the UTI recurrence rate (RR 0.81, 95%CI 0.68-0.96). Primary immunization followed by booster immunization increased time to re-infection. In two trials vaginal estrogens decreased UTI recurrence rates (RR=0.42, 95%CI 0.16-1.10), but vaginal irritation occurred in 6 to 20% of women. Cranberries decreased UTI recurrence rates in 2 trials (RR 0.53, 95%CI 0.33-0.83). In two open label trials, acupuncture reduced UTI recurrence rates compared to no treatment (RR 0.48, 95%CI 0.29-0.79). Oral estrogens and lactobacilli prophylaxis did not decrease UTI recurrence rates. Based on this meta-analysis we conclude that the evidence for the efficacy of the oral immunostimulant OM-89 is promising. The efficacy of the vaginal vaccine Urovac, cranberry products or acupuncture needs confirmation by larger placebo-controlled studies. To optimally inform clinical decision-making, large head-to-head trials should be performed.

Chapter 3 presents the results of the randomized double-blind, double-dummy non-inferiority trial in which we compared cranberry prophylaxis with trimethoprim-sulfamethoxazole prophylaxis (TMP-SMX, 480 mg once daily) in
221 premenopausal women with rUTIs. Primary end points were the mean number of symptomatic UTIs over 12 months, the proportion of patients with at least 1 symptomatic 1 UTI, the median time to first UTI, and development of antibiotic resistance in indigenous *Escherichia coli* (*E. coli*). After 12 months of prophylaxis the mean number of symptomatic UTIs was 4.0 in the cranberry and 1.8 in the TMP-SMX group. The proportions of patients with at least one symptomatic UTI were 78% and 71%, respectively. Median times to the first symptomatic UTI were 4 and 8 months, respectively. Twenty-four percent of fecal and 28% of asymptomatic bacteriuria *E. coli* isolates were resistant to TMP-SMX after 1 month of cranberry prophylaxis. After 1 month of TMP-SMX prophylaxis these percentages were 86% and 91%, respectively. Similarly, resistance rates for TMP, amoxicillin, and ciprofloxacin increased in the TMP-SMX group. Three months after the discontinuation of TMP-SMX prophylaxis antibiotic resistance rates reached baseline levels. Antibiotic resistance did not increase on cranberries.

We concluded that in premenopausal women TMP-SMX prophylaxis was more efficacious than cranberry capsules to prevent recurrent UTIs. However, unlike TMP-SMX, cranberries did not increase antibiotic resistance rates.

The main study objective of the trial described in Chapter 4 was to determine whether oral capsules with *Lactobacillus rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 were non-inferior to TMP-SMX prophylaxis in postmenopausal women with rUTIs. The outcomes of primary interest were the mean number of symptomatic UTIs, and the development of antibiotic resistance in *E. coli*. The mean number of symptomatic UTIs in the year preceding randomization was 7.0 in the 127 women in the TMP-SMX group, compared to 6.8 in the 125 women in the lactobacilli group. After 12 months of prophylaxis, these numbers were 2.9 and 3.3, respectively. The between-treatment difference of 0.4 UTIs per year and its 95% confidence interval (−0.4 to 1.5 UTIs) included our non-inferiority margin, rendering our trial inconclusive as to the main study objective. The proportion of women with at least one symptomatic UTI was 69.3% in the TMP-SMX group, compared to 79.1% in the lactobacilli group. The median times to the first UTI were 6 and 3 months, respectively.

With regard to antibiotic resistance, already after 1 month of TMP-SMX prophylaxis, resistance to TMP-SMX, TMP, and amoxicillin had increased from approximately 20% to 40% to approximately 80% to 95% in *E. coli* from the feces and the urine of asymptomatic women and in *E. coli* causing a UTI. Resistance levels gradually decreased in the 3 months after TMP-SMX discontinuation. Resistance did not increase during lactobacilli prophylaxis.

In conclusion, in postmenopausal women with rUTIs, *L rhamnosus* GR-1 and *L reuteri* RC-14 did not meet the non-inferiority criteria in the prevention of UTIs when compared with TMP-SMX. However, development of antibiotic resistance is considerably lower with use of lactobacilli.

In Chapter 5 we assessed the contribution of antibiotic use versus patient-related factors on antimicrobial resistance in *E. coli* isolated from urinary and fecal
samples of women with rUTIs participating in the NAPRUTI-study. Therefore, we collected at study baseline 146 urine and 336 fecal *E. coli* strains from 434 asymptomatic women with rUTIs. TMP-SMX use in the previous three months was associated with urine *E. coli* resistance to amoxicillin (OR 3.6, 95% confidence interval: 1.3 - 9.9), amoxicillin-clavulanic acid (OR 4.4, 1.5 – 13.3), TMP (OR 3.9, 1.4 - 10.5) and TMP-SMX (OR 3.2, 1.2 - 8.5), and with fecal *E. coli* resistance to TMP (OR 2.0, 1.0 - 3.7). The number of UTIs in the preceding year was correlated with urine *E. coli* resistance to amoxicillin-clavulanic acid (OR 1.11, 1.01 - 1.22), trimethoprim (OR 1.13, 1.03 - 1.23) and TMP-SMX (OR 1.10, 1.01 - 1.19). Age was predictive for fecal *E. coli* resistance to amoxicillin (OR 1.02, 1.00 - 1.03), norfloxacin and ciprofloxacin (both OR 1.03, 1.01 - 1.06). We concluded that for urinary *E. coli* resistance, previous antibiotic use and a history of UTI were the most important determinants, whereas patient’s age was the most important determinant for fecal *E. coli* resistance. These associations could best be explained by recent and cumulative lifetime use of antibiotics, respectively.

**Chapter 6** focuses on the predictive value of asymptomatic bacteriuria (ASB). We investigated whether ASB is predictive for the development of a UTI and whether the resistance pattern of an *E. coli* ASB strain is predictive for the resistance pattern of the subsequent UTI-causing strain. Therefore, we evaluated additional data obtained from patients included in the trials described in Chapter 3 and 4. The cumulative probability of being UTI-free during the 15 months’ follow-up was not significantly different for women with and without ASB at baseline. Next, we compared the antimicrobial susceptibility and pulsed-field gel-electrophoresis (PFGE) pattern of 50 *E. coli* strains causing a UTI with those of the ASB strain isolated 1 month previously. At least 90% of the patients had a susceptible symptomatic *E. coli* UTI strain when a susceptible asymptomatic *E. coli* was isolated in the preceding month, applying to all antibiotics tested. Also resistant ASB strains had a high predictive value. For the various antibiotics, 76 percent or more of symptomatic *E. coli* isolates were resistant when a resistant ASB strain was isolated one month previously. An exception was the lower predictive value found for ASB strains resistant to amoxicillin-clavulanic acid: Only 34% of the subsequent symptomatic isolates were resistant to this antibiotic. Asymptomatic and symptomatic isolates had similar PFGE patterns in 70% of the patients. We conclude that the presence of ASB is not predictive for the development of UTI in women with rUTI receiving prophylaxis. However, the susceptibility pattern of *E. coli* strains isolated in the month prior to a symptomatic *E. coli* UTI can be used to make informed choices for empirical antibiotic treatment in women with rUTI.

**Chapter 7** we assessed the disease-related knowledge of the women with rUTIs participating in our trials by sending them a knowledge questionnaire. We questioned them about the cause of UTIs, female pelvic anatomy, the incidence of UTIs, preventive practices, antibiotic use and resistance. Among 246 respondents the cause of UTI, and the definition of antibiotic resistance
were well-known. Most women knew that avoiding unnecessary antibiotic use is important to prevent resistance. About one third of women supposed that once resistant bacteria have emerged, they will never get rid of them. Although antibiotics are probably used often in this group of women, almost one third of women thought that these drugs kill bacteria as well as viruses. No more than sixty percent knew that the occurrence of UTIs has nothing to do with a lack of hygiene. Less educated women more frequently gave wrong answers. This study showed that knowledge gaps are present and more common in less educated women. Understanding what women with rUTIs know may help health care providers to give them more tailored information.

**Methodological lessons learned in our randomized controlled trials**

From our trials we learned that trial participants are not just passive recipients of interventions. Many women preferred non-antibiotic prophylaxis above antibiotic prophylaxis and declined to consent to randomization (Chapter 3 and 4). Another factor that contributed to recruitment difficulty were the onerous study requirements (using study medication, monthly questionnaires and collection of urine, feces and vaginal swabs during 15 months). In total, we had to screen 1701 women for both trials to include a total of 473 women.

After enrolment the major reason for withdrawal, besides treatment failures and adverse events, was the burden of study requirements. Especially with a relative benign disease like rUTIs, where most women are asymptomatic most of the time, the burden of taking medication twice daily for a period of 12 months can already seem disproportionate to the benefits of such medication. We can imagine that this may interfere with the women’s normal life, especially in combination with the multiple sample collections required for the trial. Retrospectively, the number of sample collections could have been reduced. Since antimicrobial resistance levels did not change between 1 and 12 months of prophylaxis, collections could have been reduced to the first month and one time point after discontinuation of prophylaxis.

Besides the monthly study requirements, we asked participants to collect a urine dipslide in case of symptoms compatible with a UTI. However, the number of urine dipslides received was smaller than the number of symptomatic UTIs reported in the questionnaires. Nevertheless, in women who did send a dipslide when symptomatic, a UTI was confirmed microbiologically in 85-88%, which is in line with the literature. This indicates that a symptomatic UTI is a reliable outcome in women with rUTI, relevant for patient care.
General discussion: prevention of rUTIs

Antibiotic prophylaxis
Historical data from the nineties showed a 95% success rate in the prevention of rUTIs with TMP-SMX. However, one of the factors possibly limiting its efficacy is emerging antibiotic resistance. It has been estimated that at a resistance level of 0% the UTI recurrence rate is 5%, compared to 12-15% at a resistance level of 20-30%, assuming that about 60% of women still respond to TMP-SMX even though their UTI is caused by a resistant microorganism. In the Netherlands, TMP-SMX resistance of *E. coli* has increased in most patient populations. In outpatients and inpatients of unselected hospital departments the TMP-SMX resistance rate was around 28-29% in 2011. Highest resistance rates were found in *E. coli* isolated from patients admitted to intensive care units (32% in 2010) and urology services (36% in 2010). Only in patients visiting their general practitioners resistance rate decreased from 20% in 2004 to 16% in 2009.

The influence of resistance of microorganisms from the indigenous fecal flora and urine of asymptomatic women on efficacy of TMP-SMX prophylaxis needs further investigation. Nitrofurantoin has a more favorable resistance profile, but long term use can have serious side effects.

Protection by asymptomatic bacteriuria (ASB)
In previous times ASB was considered harmful in any patient. Nowadays ASB is considered a benign condition, similar to commensalism. Recently, Cai et al. reported that ASB could even be protective. In young women with a history of rUTI receiving antibiotic treatment for ASB, only 17% remained asymptomatic in the following year, compared to 76% of untreated women with ASB. This might be explained by the ability of ASB strains to interfere with more virulent uropathogenic strains that can colonize and infect the host (“bacterial interference”). It is not clear whether women with ASB are better protected from recurrences than women without ASB.

In our study the presence of ASB was not predictive for the development of a UTI in women with rUTI using prophylaxis, but not protective either. After adjustment for potential confounders using a Cox regression model, ASB at baseline was still not predictive. Because of the study design the influence of ASB on UTI development in women with rUTIs not receiving prophylaxis could not be assessed. Further studies are needed to address this important question.

Non-antibiotic prophylaxis
In Chapter 2 we summarized the evidence from RCTs evaluating the efficacy and tolerability of non-antibiotic prophylaxis in women with rUTIs. For most forms of non-antibiotic prophylaxis, only a limited number of good quality efficacy studies are available. In addition, studies are heterogeneous, with some limited by a small sample size. As with antibiotic prophylaxis, there is no clear evidence for the optimal duration of the various forms of non-antibiotic prophylaxis, how often they can be repeated and the duration of benefits after stopping the
prophylaxis. In addition, for all forms of non-antibiotic prophylaxis, except estrogens, the exact working mechanism and optimal dose are not clear. Only a few of these forms of non-antibiotic prophylaxis have been directly compared with antibiotic prophylaxis, which in general is considered as the standard of care.8-12

Cranberries
The mechanism of action of cranberries has not been completely elucidated. Based on in vitro studies, cranberries are thought to contain proanthocyanidins (PACs), that can inhibit adherence of P-fimbriated *E. coli* to the uroepithelial cell receptors.13,14 Until recently these in vitro findings were not correlated with clinical outcomes. In 2012 Stapleton et al. demonstrated in a placebo controlled trial that women randomized to cranberry juice had a concurrent but non-significant reduction in numbers of (asymptomatic and symptomatic) P-fimbriated *E. coli* in urine, and in the rate of symptomatic UTIs.15 Larger studies are needed to further evaluate the correlations between in vitro and clinical data.

In addition, the PAC concentration, dosage regimen and formulation to use should be determined scientifically. In early studies with cranberries the chemical composition of available cranberry products was usually not standardized, neither was the dose properly described.16 In more recent studies PAC standardized cranberry products are used. In 2010 Howell assessed ex-vivo anti-adhesion activity in urine following consumption of PAC standardized cranberry powder.17 Anti-adhesion activity was dose dependent and prolonged, and was present for 24 hours after consumption of 72 mg of PACs. The highest anti-adhesion activity was measured 6 hours after ingestion of both 36 and 72 mg. The authors suggest that it may be beneficial to consume cranberry in two split doses of 36 mg. In our trial the women took cranberry capsules twice daily, but the daily amount of PACs consumed was only 9.1 mg. Although the optimal dose to prevent UTIs in vivo is not clear, the dose used in our trial might therefore have been too low. A study that assesses the relation between the dose of cranberry juice, urinary PAC concentration and efficacy is currently underway.18 The results of this study are important for the design of future trials with cranberry products.

Lactobacilli
Evidence for the benefits of lactobacilli in the prevention and treatment of a wide variety of diseases is increasing.19,20 However, the precise interaction of lactobacilli with the commensal flora and the host, and the mechanism of action by which they exert their beneficial effects are still largely unknown. In addition, specific lactobacilli strains have specific effects and the pathophysiology of diseases differs. Finally, the optimal dose and duration of lactobacilli prophylaxis is not clear.21 These factors may explain the lack of effect with the lactobacilli strains used in the trials in our meta-analysis, compared to the apparently more favorable effects of *L. rhamnosus* GR-1 and *L. reuteri* RC-14 used in the trial described in Chapter 4, and of *L. crispatus* given as intravaginal suppositories, which reduced recurrences after antimicrobial treatment of a symptomatic UTI in premenopausal women.22
The research group of Gregor Reid gave the concept to prevent urogenital infections by probiotic treatment a scientific basis by identifying, selecting and testing the most effective strains. Various in vitro and animal studies led to the conclusion that L. rhamnosus GR-1 and L. reuteri RC-14 possess optimal properties to be effective against infections in the urogenital tract. They were selected based on two main criteria: 1) ability to colonize the host (competitive exclusion), 2) and the ability to inhibit the adhesion and growth of urogenital pathogens by the production of several compounds, like hydrogen peroxide and biosurfactant. In previous studies L. rhamnosus GR-1 and L. reuteri RC-14 were able to colonize the vagina, not only after vaginal insertion, but also following oral intake. However, in our trial we could identify L. reuteri in the fecal samples, but not in vaginal specimens of women receiving lactobacilli prophylaxis. Therefore, we can only speculate that a more lactobacilli-dominated fecal flora exhibits a protective effect through the inhibition of the growth of intestinal (uro)pathogenic bacteria. Although the evidence on the beneficial effects by means of gut microbial modulation is growing, the microbiome-modulating abilities of the specific lactobacilli strains are still largely unknown. The advent of genomic profiling will certainly bring probiotic research to a higher level, as data on genome, microbiome and host response become available.

Implications for research
Future RCTs in women with rUTIs should focus on head-to-head comparisons of non-antibiotic prophylaxis with each other and with antibiotic prophylaxis that is standard of care. The results of a study comparing OM-89 with antibiotic prophylaxis are expected in the near future.

The literature suggests that the efficacy of antimicrobial prophylaxis is superior to non-antibiotic alternatives presently available. However, non-inferiority of non-antibiotic prophylaxis would be attractive given their potential to considerably lower the prevalence of antimicrobial resistance (Chapter 3 and 4). Therefore, trials comparing non-antibiotic alternatives to antibiotics should be designed as non-inferiority trials. However, as far as we are aware, there are no validated rules to determine non-inferiority margins. The choice of a 10% non-inferiority margin in our trials was a fairly intuitive mix of weighing pros and cons of cranberries and lactobacilli. As pros we considered less antimicrobial resistance and the fact that women are motivated to avoid antibiotics; as cons possible lower effectiveness and slightly higher costs. A rational approach for planning non-inferiority trials would involve performing a cost-utility analysis to derive the non-inferiority margins before embarking on the trial. Such analyses could incorporate opinions of patients, physicians, investigators, regulators, and payers.

Since UTIs can be relapsing infections and therefore often occur in clusters, intervention and follow-up periods (also post-prophylaxis) in future trials may need to cover a time period of at least one year to take into account the relapsing course of the disease. To prevent withdrawal, study requirements must
be reduced to a minimum. Instead of monthly questionnaires, patients could be asked to keep an (electronic) patient diary and to collect only urine samples when they have symptoms suggestive for a UTI. Since antimicrobial resistance levels did not change between 1 and 12 months of prophylaxis, monthly collecting urine and feces seems superfluous.

Outcomes should include at least the number of symptomatic UTIs, adverse events and cost-effectiveness in the different study arms. To easily compare trials and to draw conclusions, future trials should use uniform outcomes and definitions.

It seems reasonable that particular subgroups might benefit more than others. In postmenopausal women with complicated UTIs (Chapter 4) TMP-SMX prophylaxis appeared to be less effective than lactobacilli prophylaxis, possibly because baseline resistance rates in this patient group were higher. Further research is required to confirm this and to determine whether other variables, like for example comorbidities (e.g. diabetes) or having urinary catheter, are predictors of response.

Implications for practice
In the light of the increasing resistance rates, we argue that some forms of non-antibiotic prophylaxis may already be considered as useful alternatives or helpful adjuncts despite their lower effectiveness. Best studied are cranberries, most promising regarding efficacy are the oral immunostimulant OM-89 and oral capsules with \textit{L. rhamnosus} GR-1 and \textit{L. reuteri} RC-14. For other potential alternatives evidence is based on only a few small studies. Women with rUTIs have to discuss with their clinicians the potential advantages of non-antibiotic prophylaxis.
Chapter 8

References


Summary and general discussion


