Jarmo Salo

LONG-TERM CONSEQUENCES AND PREVENTION OF URINARY TRACT INFECTIONS IN CHILDHOOD
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Academic dissertation to be presented with the assent of the Doctoral Training Committee of Health and Biosciences of the University of Oulu for public defence in Auditorium 12 of the Department of Paediatrics, on 31 August 2012, at 12 noon.
Abstract

Urinary tract infections (UTIs) are among the most common bacterial infections in childhood and have a tendency to recur. The ability of uropathogens to form biofilm may be important in the pathogenesis of UTI. Although childhood UTIs are thought to increase the risk of chronic kidney disease (CKD), evidence showing this association is scarce. Cranberry juice has been shown to prevent UTIs in women, but evidence of its efficacy in children is lacking. The aim of this work was to evaluate the significance of biofilm formation for the clinical presentation of UTI, the long-term consequences of UTI in childhood and the efficacy, safety and acceptability of cranberry juice in the prevention of UTIs in children.

The formation of biofilm in clinical samples was assessed in vitro with optical density measurements and verified by scanning electron microscopy and confocal scanning laser microscopy. A systematic literature search on the association between childhood UTIs and CKD was conducted, and data on patients with CKD treated or monitored at Oulu University Hospital were reviewed. The efficacy of cranberry juice in preventing recurrences of UTIs in children and its effects on the normal flora were evaluated with two randomized controlled trials.

About one third of the uropathogenic E. coli strains were capable of forming biofilm, and the strains isolated from patients having pyelonephritis formed biofilm better than those from cystitis cases. We did not find any cases among the 1576 reviewed in the literature search or the 366 in our patient series who had structurally normal kidneys in their first kidney imaging and in whom childhood UTIs could have been the cause of subsequent CKD. The aetiological fraction of childhood UTIs as a cause of CKD was at most 0.3%. The administering of cranberry juice did not reduce the number of children who experienced a recurrence of UTI, but it did reduce the number of recurrences per person year at risk by 39% (0.25 vs. 0.41 episodes, 95% CI for difference -0.31 to -0.01) and the number of days on antimicrobials per patient year by 34% (11.6 vs. 17.6 days, 95% CI for difference -7 to -5). Cranberry juice was well accepted and tolerated by the children and did not cause harmful changes in the normal flora.

The ability of bacteria to persist and grow in a biofilm seems to be one of the significant factors in the pathogenesis of UTIs. A child with normal kidneys is not at risk of developing CKD because of UTIs in childhood, so that imaging procedures after the first UTI can be focused on finding severe urinary tract abnormalities. Taking into account the relatively innocent nature of childhood UTIs, cranberry juice offers an alternative to antimicrobials for preventing UTIs in children. The mechanism of action of cranberry juice may be associated with biofilm formation by uropathogens.

Keywords: biofilms, child, chronic kidney insufficiency, cranberry, Escherichia coli, urinary tract infections
Salo, Jarmo, Lasten virtsatieinfektioiden pitkäaikaisseuraukset ja ennaltaehkäisy.

Oulun yliopiston tutkijakoulu; Oulun yliopisto, Lääketieteellinen tiedekunta, Kliinisen lääketieteen laitos, Lastentaudit; Oulun yliopistollinen sairaala, PL 5000, 90014 Oulun yliopisto

Oulu

Tiivistelmä


Kolmasosa E. coli -kannoista muodosti biofilmiä, ja pylonefriittipotilaita eristetyt kannat olivat parempia biofilmimuodostajia kuin kystitiipipotilaita eristettyjä. Kirjallisuuskatsauksen 1576:n ja OYS:n 366:n tapauksen joukossa ollut potilaat, joilla oli ensimmäisessä uusintainfektion sairaalavaikutus, mutta se vähensi uusintaepisodeja 39 % (0,25 vs. 0,41/paivi) ja antibioottitippahtumia 34 % (11,6 vs. 17,6/paivi). Lapset joivat karpalomehua mielellään, eikä sillä ollut haitallisia vaikutuksia normaaliflooraan.

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Asiakasarät: biofilm, Escherichia coli, karpalo, krooninen munuaisten vajaatoiminta, lapsi, virtsatieinfektiot
To my family
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Oulu, June 2012

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<table>
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<tr>
<th>Abbreviation</th>
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<tr>
<td>ABU</td>
<td>Asymptomatic bacteruria</td>
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<tr>
<td>CFU</td>
<td>Colony forming unit</td>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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<tr>
<td>CKD</td>
<td>Chronic kidney disease</td>
</tr>
<tr>
<td>CL</td>
<td>Confidence limit</td>
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<tr>
<td>CP</td>
<td>Chronic pyelonephritis</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSLM</td>
<td>Confocal scanning laser microscopy</td>
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<tr>
<td>DMSA</td>
<td>Dimercaptosuccinic acid</td>
</tr>
<tr>
<td>FSGS</td>
<td>Focal segmental glomerulosclerosis</td>
</tr>
<tr>
<td>GLC</td>
<td>Gas liquid chromatography</td>
</tr>
<tr>
<td>IVU</td>
<td>Intravenous urography</td>
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<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PAC</td>
<td>Proanthocyanidins</td>
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<tr>
<td>PCT</td>
<td>Procalcitonin</td>
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<tr>
<td>PYR</td>
<td>Person years at risk</td>
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<tr>
<td>RCT</td>
<td>Randomized controlled trial</td>
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<td>RRT</td>
<td>Renal replacement therapy</td>
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<td>SD</td>
<td>Standard deviation</td>
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<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
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<tr>
<td>SPA</td>
<td>Suprapubic aspiration</td>
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<tr>
<td>UPEC</td>
<td>Uropathogenic <em>Escherichia coli</em></td>
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<tr>
<td>UTI</td>
<td>Urinary tract infection</td>
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<td>VUR</td>
<td>Vesico-ureteral reflux</td>
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1 Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections in childhood, and recurrences are common (Winberg et al. 1974, Uhari & Nuutinen 1988, Mårild & Jodal 1998, Nuutinen & Uhari 2001, Craig et al. 2009). Dietary factors are associated with susceptibility to UTIs but the exact pathomechanisms leading to UTI and its recurrences in children are not well known (Kontiokari et al. 2004). Several Escherichia coli (E. coli) strains are capable of forming biofilms (Reisner et al. 2006, Hancock et al. 2007), but the clinical significance of bacterial biofilms for the level of UTI and for its recurrences is largely unknown (Kanamaru et al. 2006, Soto et al. 2007).

Childhood UTIs cause kidney scarring and are said to lead to impaired kidney function later, especially in the presence of vesicoureteral reflux (VUR) (Jakobsson et al. 1999b, Lahdes-Vasama et al. 2006, Swerkersson et al. 2007). Children are therefore normally subjected to radiological imaging after the first UTI and long-term antibiotic prophylaxis if grade III to V VUR is found. The most severe consequence of childhood UTIs has been thought to be chronic kidney disease (CKD), but evidence for an association between childhood UTIs, renal damage and later CKD in children with structurally normal kidneys is scarce (Hellerstein 2000). Accurate diagnosis is the basis of all actions taken after UTI, so it is essential that the diagnosis should be made correctly and using adequate methods.

Many children with recurrent UTI need antimicrobial prophylaxis for years, which is not without problems (Uhari et al. 1996b, Williams et al. 2001). Adverse reactions and cessation of therapy are common during this long-term antibiotic regimen, and there is an increasing problem of bacterial resistance (Uhari et al. 1996b). Cranberry products appear to be able to prevent UTI recurrences in adult women, but evidence for their efficacy in children is lacking (Blatherwick 1914, Avorn et al. 1994, Schlager et al. 1999, Kontiokari et al. 2001, Stothers 2002, Jepson et al. 2004, Wing et al. 2008). The acceptability of cranberry juice has been questioned as well, and the broad in vitro antimicrobial spectrum of cranberry raises concerns about the possibility of adverse events during continuous use (Jepson et al. 2004).
2 Review of the literature

2.1 Urinary tract infections in childhood

2.1.1 Definitions

A urinary tract infection (UTI) is defined as the presence of microbial pathogens within one or more structures in the urinary system, including the urethra, bladder and kidneys, in a symptomatic patient. Cystitis is an infection of the lower urinary tract (urethra, urinary bladder), pyelonephritis an infection of the upper urinary tract (ureter, kidneys) and pyelitis an infection of the renal pelvis. Asymptomatic bacteruria is defined as the significant growth of bacteria in urine without symptoms of UTI. A diagnosis of urosepsis is recorded if the patient has clinical sepsis and bacteraemia that have originated from bacteruria caused by bacteria capable of inducing inflammation in the urinary tract. UTI in patients with a normal urinary tract is regarded as uncomplicated, whereas complicated UTI is associated with urinary tract abnormalities, catheters or operations. Recurrent UTIs are classified as relapses or reinfections, relapses being caused by the same bacterial strain as the initial episode and occurring within a few months of the cessation of therapy, while reinfections are caused by a new bacterial strain.

2.1.2 Epidemiology

About 2–8% of children have at least one symptomatic UTI before puberty (Winberg et al. 1974, Hellström et al. 1991, Mårild & Jodal 1998, Conway et al. 2007, Shaikh et al. 2008), and almost one percent have UTI before the age of six (Conway et al. 2007). The occurrence is highest in infancy, when about 2/3 of the UTI patients are boys, after which time the majority of patients are girls (Ginsburg & McCracken 1982, Jakobsson et al. 1999a, Foxman 2003, Conway et al. 2007). UTI is a diagnostic challenge, especially among young children. About 5% of infants with a fever of unknown origin at admission have UTI, and about 5% of children less than two years of age with UTI have bacteraemia (Hobberman et al. 1993a, Hoberman et al. 1999). UTI is cause of 15% of all cases of fever of unknown origin in girls under two years of age (Shaw et al. 1998). After the first episode, 10–30% of patients experience a recurrence or reinfection, mostly within six months of the initial UTI episode (Uhari et al. 1996b, Nuutinen & Uhari 2001,
The incidence of UTI in childhood is 0.2%-0.3% per year and has been decreasing since the 1970s, presumably in response to changes in diet, the quality of napkins and child-care practices in general (Dickinson 1979, Uhari & Nuutinen 1988). The incidence of UTI and its diagnostic rate differ between populations and centres in addition to true differences in incidence, partly because of variations in diagnostic methods, diagnostic criteria, UTI awareness and the organisation of health-care systems (Jakobsson et al. 1999a).

2.1.3 Symptoms and diagnosis

Accurate diagnosis is the basis of all reactions to UTI, so it is essential that the diagnosis should be correct and made using adequate methods. The diagnosis of UTI is based on clinical signs and/or symptoms, urinalysis (leucocytes, nitrite) and the presence of a significant growth of bacteria in the urine. The symptoms depend on the level of infection and the age of the patient, but typical manifestations include fever, dysuria, frequency, urgency, enuresis, abdominal pain, flank back pain and incontinence. The symptoms are less specific in infants than in older children, however. Urosepsis and pyelonephritis cause similar symptoms and are clinically indistinguishable from each other (Honkinen et al. 2000). Decision rules based on symptoms and patient characteristics have been developed and studied in order to select high-risk patients for urinalysis and treatment and low-risk patients for follow-up (Gorelick et al. 2003, Shaikh et al. 2007). In girls younger than two years, for example, a fever of 39.0 °C or more in the absence of any other potential cause and fever for two days or more entail with a high risk of UTI (Gorelick et al. 2003). Children with UTI cannot be distinguished from non-infected children on the basis of symptoms alone, however (Pylkkanen et al. 1979, Shaikh et al. 2007).

A urine collection pad or bag are reliable and easy methods for taking samples for urinalysis, and in the case of young children urinalysis can be performed on a urine bag or cushion/pad sample for use in assessing the likelihood of UTI (Liaw et al. 2000, Macfarlane et al. 2005, McGillivray et al. 2005, Schroeder et al. 2005, American Academy of Pediatrics 2011). A urine collection pad should be changed every 30 minutes in order to reduce the possibility of a false positive result (Rao et al. 2004). Bag samples are especially prone to contamination of the urine culture (Al-Orifi et al. 2000), and thus the diagnosis of UTI children who are unable to control their bladder should be
established from a sample collected by suprapubic aspiration (SPA), or by transurethral catheterization if the SPA method is unsuccessful (Pylkkanen et al. 1979, American Academy of Pediatrics 2011). Beyond infancy a midstream urine sample can be taken, whereupon the number of false positive results can be reduced by positioning a sterile cup in the front part of the urinal (Huttunen et al. 1970).

SPA is the gold standard for taking samples for urine culture (American Academy of Pediatrics 2011). It is safe and complications are rare, but timing of the procedure is not always easy because of irregular voiding by infants (Polnay et al. 1975, O'Callaghan & McDougall 1987, Moustaki et al. 2007). The success rate of SPA can be improved by real-time ultrasound guidance (O'Callaghan & McDougall 1987, Chu et al. 2002). A urine sample taken by SPA can be stored and cultivated later when necessary without risk of contamination, because any growth of bacteria in such a sample is significant (Bailey & Little 1969). In children aged less than two months SPA is a more painful way of taking urine samples than catheterization (Kozer et al. 2006). In the classic study by Pryles et al. the sensitivity and specificity of transurethral catheterization for diagnosing UTI were excellent by comparison with SPA, but in a recent study more than 10% of the catheterized urine samples were contaminated, and the rate of contamination was especially high in uncircumcised boys (Pryles et al. 1959, Wingerter & Bachur 2011).

The rapid screening tests available for UTI are based on dipstick tests, gram staining, microscopic analysis or various combinations of these. Their sensitivities and specificities for UTI depend on the selection of the patients for testing, the methods by which the urine samples are taken, the processing of the samples and the cut-off points used (Gorelick & Shaw 1999, Huicho et al. 2002). Hoberman et al. found that the presence of more than 10 leukocytes/ml had a sensitivity of 91% and a false positivity rate of 3.4% for identifying significant growth in cultures of urine samples taken by catheter, while the presence of either leucocytes or bacteria in an enhanced urinalysis had a sensitivity of 95% for finding positive urine cultures (Hoberman et al. 1994, Hoberman et al. 1996). When a positive urinalysis is defined as entailing the presence of both bacteruria and pyuria, enhanced urinalysis (using an uncentrifuged specimen) has a better sensitivity and positive predictive value (85% and 93%, respectively) than the standard analysis (66% and 81%, respectively), and both of them have a specificity and negative predictive value of about 99% (Hoberman et al. 1993b). The sensitivity of urinalysis is better among older children than in children under
two years of age (Doley & Nelligan 2003). Standard urinalysis has a sensitivity of 82% and a specificity of 92% for diagnosing UTI in febrile children aged less than two years (Bachur & Harper 2001), while the sensitivity of pyuria for diagnosing UTI in samples taken by transurethral catheterization in febrile infants was 30% (≥10 WBC/high-power field) to 80% (any WBC/HPF) and specificity 47% (any WBC/HPF) to 99% (≥10 WBC/HPF) (Hoberman et al. 1993a). The sensitivity of urinalysis is better when the samples are taken with urine bags than with a catheter but the specificity is poorer (McGillivray et al. 2005).

Most of the bacteria causing UTI can reduce dietary nitrate to nitrite, a phenomenon that can be measured and utilized diagnostically. The sensitivity of the nitrite test for symptomatic UTI is less than 50%, however, although the specificity is 99% (Powell et al. 1987, Hoberman et al. 1994). The sensitivity of the nitrite test is higher for asymptomatic bacteruria (ABU) than for UTI (Kunin & DeGroot 1977, Powell et al. 1987). Inclusion of the nitrite test in urinalysis enhances the sensitivity of the latter for UTI, and when both leukocyte esterase and nitrite tests are used the sensitivity is more than 90% (Conway et al. 2007).

Urine is a good growth medium for bacteria, and bacteria can grow to a maximum density of about 10^8/ml in a few hours in sterile urine (Kass 1956, Wheldon & Slack 1977). On the other hand, bacteria from the distal urethra and perineum also grow well and rapidly in urine (Wheldon & Slack 1977), so that in order to avoid contamination, the sample should be cultured immediately or after only brief storage at 5 °C. When diagnosing UTI it is important to distinguish between contamination and true bacteruria. In the classic study by Kass et al. 95% of the women with pyelonephritis had a bacterial growth of 10^5 or more per ml in a urine sample taken by catheter, and 3% had 10^4–10^5 bacteria per ml (Kass 1956). In addition, 80% of the women with a growth of 10^5 or more bacteria per ml had a positive gram stain for their urine (Kass 1956). This would imply that the definition of bacteruria in adults is 10^5 or more colony forming units (CFU) of a single bacterial strain in a clean voided urine sample. The result of a urine culture is more reliable if the urine has remained in the bladder for at least four hours and when two separate samples are taken. Based on the study by Kass et al., significant growth in paediatric urine samples is usually defined as the growth of a single bacterial strain to 10^5 or more CFU/ml in a midstream urine sample, any growth in a suprapubic bladder aspirate sample, or growth of at least 50 000 CFU/ml in a catheter sample (Kass 1956, Hoberman et al. 1994, American Academy of Pediatrics 2011). This definition is not based on firm evidence, however, and it should be noted that as about 20% infants with true UTI have
bacterial counts of less than $10^5$ CFU/ml, the use of SPA or catheterization for sampling is important in order to avoid both the under-diagnosing and over-diagnosing of UTI (Hansson et al. 1998).

Cystitis has no long-term consequences, but pyelonephritis is thought to cause kidney scarring, which may lead to chronic kidney disease (CKD) and hypertension. Consequently, pyelonephritis is considered to require a more active approach to diagnosis, therapy and follow-up than lower urinary tract infections. There is thus a need for a reliable method for distinguishing between infections of the upper and lower urinary tract. Dimercaptosuccinic acid (DMSA) scanning is commonly used to determine the level of UTI, and power Doppler ultrasonography correlates well with DMSA in diagnosing the level of UTI (sensitivity and specificity both about 90%) (Jakobsson et al. 1992, Halevy et al. 2004). The significance of the findings obtained from a DMSA scan is somewhat unclear, however (consequences of UTI vs. congenital abnormalities) and may be dependent on the age of the patients (Ilyas et al. 2002, Shaikh et al. 2010). There are no direct methods for determining the level of UTI in clinical practise. Pyelonephritis is usually defined as UTI associated with a fever of over 38.5 °C and/or a C-reactive protein value greater than 40 mg/l (Jodal et al. 1975). Procalcitonin (PCT) may be better than C-reactive protein (CRP) for diagnosing the level of UTI (Gervaix et al. 2001, Pecile et al. 2004). Benador et al. (1998) reported the sensitivity and specificity of PCT for finding renal lesions in DMSA to be 70% and 83%, respectively, whereas CRP had sensitivity of 100% but a specificity of 26% (Benador et al. 1998). Patients are diagnosed as having urosepsis if they have UTI and a blood culture positive for the same pathogen. Such patients have higher CRP, but the range is wide, so that CRP is of little use for differential diagnosis between pyelonephritis and urosepsis (Honkinen et al. 2000).

ABU has no marked consequences, and screening and treating bacteruria does not help prevent either UTI episodes or renal scarring (Kemper & Avner 1992, Harding et al. 2002). Furthermore, screening of ABU would result in a great number of false positive tests (Kemper & Avner 1992, Kramer et al. 1994). Thus ABU should not be treated or screened for in children (American Academy of Pediatrics 2011).
2.1.4 Treatment

Standard antibiotic therapy aimed at *E. coli* is very effective, and fails to eradicate bacteruria in only 1–3% of UTI episodes in children (Winberg *et al.* 1974, Craig *et al.* 1998). Short-term oral antibiotic therapy (2–4 days) is as effective as the standard therapy (7–10 days) for treating cystitis in children (Michael *et al.* 2012), and a single dose of trimethoprim or trimethoprim-sulphamethoxazole is as effective as a 5 or 7-day course for this same purpose, but the risk of bacteruria after cessation of the therapy is higher (Pitt *et al.* 1982, Nolan *et al.* 1989, Lidefelt *et al.* 1991). Oral and intravenous treatment are equally as effective for pyelonephritis in children, and oral therapy is a safe and effective alternative when hospitalization is not necessary (Hodson *et al.* 2007). The recommended duration of therapy is 10–14 days, this is not based on firm evidence (Hodson *et al.* 2007). Oral and initial intravenous therapies are also equally effective for treating UTIs in 1–24-month-old children provided that the absorption of antibiotics in the gastrointestinal tract is sufficient (Hoberman *et al.* 1999). The urine cultures become sterile at a similar time (24 h), and there is no difference in the reinfection rate (5%–9%) or in the extent of scarring (8%–9% of the renal parenchyma). Even infants under two months of age with febrile UTI can be treated safely with brief hospitalization and close follow-up without any marked risk of complications, provided they are not clinically ill on admission (Schnadower *et al.* 2010). Recent antimicrobial therapy increases the risk of UTI caused by a resistant pathogen (Paschke *et al.* 2010). ABU should only be treated in pregnant women (Nicolle *et al.* 2005, American Academy of Pediatrics 2011).

2.1.5 Imaging of the urinary tract after the first UTI

According to widespread recommendations, the urinary tract should be routinely imaged after the first UTI in childhood, but, again, these recommendations are not based on firm evidence (Dick & Feldman 1996, American Academy of Pediatrics 2011). The need for invasive radiological examinations after UTI has been questioned, and the number of voiding mictiocystographies etc. performed in clinical practise has been decreasing during the last two decades (South 2009, Venhola & Uhari 2009). Ultrasonography performed after the first UTI in childhood is a sufficient method for detecting clinically significant abnormalities in the urinary tract, such as obstructive disorders (Jahnukainen *et al.* 2006, American Academy of Pediatrics 2011, Hannula *et al.* 2011, Pennesi *et al.* 2012).
Over 80% of all major congenital malformations and minor abnormalities of the urinary tract are found in antenatal ultrasonography (Grandjean et al. 1999). In addition, some authors have maintained that the findings in ultrasonography do not alter the management of a case after the first UTI (Hoberman et al. 2003, Zamir et al. 2004, Miron et al. 2007, Montini et al. 2009). It should be noted that many functional factors, e.g. constipation, may cause reversible abnormalities identifiable in imaging examinations (Dohil et al. 1994). DMSA scanning is considered to be a reliable method for detecting or ruling out abnormalities in the kidney parenchyma, and some experts recommend it for detecting renal scars after an acute infection, but the clinical significance of these findings is not well established (Jakobsson et al. 1992, Stokland et al. 1999, Ilyas et al. 2002, Montini et al. 2009, Shaikh et al. 2010).

2.2 Pathogenesis of urinary tract infections in childhood and risk factors

UTI is almost always an ascending infection, so that the bacterial strain causing it can be found in the patient’s faeces (Jantunen et al. 2001a). In order to colonize the urinary tract and cause infection, bacteria from the faecal or periurethral flora must ascend to the bladder, survive the defence mechanisms, adhere to the uroepithelial cells and proliferate (Hooton 2000, Finer & Landau 2004). In spite of the excretion of bactericidal compounds, bacteria grow well in urine. Uroepithelial cells have several defence mechanisms against bacteria (physical barrier, defensins, cytokines, chemokines, recruitment of neutrophils and other leucocytes) which are activated by the attachment of bacteria (Jahnukainen et al. 2005), but in addition to protecting the urinary tract, these mechanisms also cause damage to the renal parenchyma and disturbances in renal function through inflammatory responses (Jahnukainen et al. 2005, Gil-Ruiz et al. 2011). If the host defence mechanisms and inflammatory response are not activated, invasion of the urinary tract by bacteria may lead to asymptomatic bacteruria.

After becoming attached to the uroepithelium, uropathogens may invade the uroepithelial cells and form biofilm-like structures inside them (Reid et al. 1993, Mulvey et al. 2001, Anderson et al. 2003). Most recurrences of UTI in infants are caused by the same bacterial strain as the initial episode, and reinfections are in the minority (Jantunen et al. 2002). On the other hand, recurrences caused by an *E. coli* strain identical to that implicated in the first UTI occur earlier than those caused by a different strain (Foxman et al. 2000). Thus it seems that a significant
proportion of recurrent UTIs may be of endogenous origin (Jantunen et al. 2002). The finding of biofilm-like structures in the uroepithelium supports this theory (Reid 1994, Mulvey et al. 2001, Anderson et al. 2003).

2.2.1 Pathogens and virulence factors

*Escherichia coli* (*E. coli*) is the leading pathogen causing UTIs in childhood, being implicated in about 80% of cases (Honkinen et al. 1999, Conway et al. 2007, Craig et al. 2009). Also, UTI episodes caused by *E. coli* are more likely to recur than those caused by other bacteria (Foxman et al. 2000). Bacteria with low virulence have better chances of causing UTI in children who have functional or anatomical abnormalities in the urinary tract (Honkinen et al. 1999), and if UTI is caused by a less virulent pathogen, the probability of urinary tract abnormalities will be higher than when UTI is caused by uropathogenic *E. coli* (UPEC) (Jantunen et al. 2001b).

The ability of bacteria to attach to the uroepithelium and cause UTI is determined by virulence factors such as adhesins, toxins, the capsule, siderophore and lipopolysaccharides (Kaper et al. 2004). The adhesins associated with UPEC strains include type 1 pili, P pili, S pili and Dr adhesins (Kaper et al. 2004), the blocks of genes encoding these virulence factors being situated in pathogenicity islands (Hacker & Kaper 2000). The two most important adhesins of *E. coli* are type 1 fimbria (or pili) and P fimbria (or pili). UPEC adheres to the uroplakin in uroepithelial cells by means of type 1 fimbriae (Connell et al. 1996, Mulvey et al. 1998), while P fimbriae are associated with acute pyelonephritis (Roberts et al. 1994). Most UPEC strains have the ability to express both adhesins in response to growth conditions (Xia et al. 2000). Flagella are responsible for the motility of UPEC and are important for colonization of the urinary tract (Lane et al. 2005, Lane et al. 2007). In addition to colonization, type 1 fimbriae also have an essential function in the process of intracellular biofilm formation (Pratt & Kolter 1998, Mulvey et al. 2001, Wright et al. 2007). Bacteria causing ABU can persist in the urinary tract without adhesins and resist host responses by virtue of their high growth rate, and can even protect the subject from UTI by out-competing the uropathogenic bacteria for colonization of the urinary tract (Roos et al. 2006). This mechanism is probably associated with their better iron acquisition, achieved through upregulation of the genes involved (Roos et al. 2006). The ability of bacteria to form a biofilm is also important in the colonization process (Jefferson
2004), the *E. coli* strains causing ABU being better biofilm formers than the UPEC strains (Hancock et al. 2007).

Bacterial biofilms are sessile bacterial communities attached to a surface and forming organized structures surrounded by an exopolysaccharide matrix (Costerton et al. 1999, Webb et al. 2003, Branda et al. 2005, Tenke et al. 2006). Biofilms protect bacteria from harmful conditions and host defences, and the bacteria in a biofilm benefit from co-operation, as they are able to act together in the manner of a multi-cellular organism. Also a biofilm can be a source of new bacterial colonies (Jefferson 2004). Living bacteria are constantly being released from a biofilm, which can therefore give rise to infection (Figure 1). Several *E. coli* strains are capable of forming biofilms, and these have been found in catheters, the prostate and kidney stones (Adams & McLean 1999, Danese et al. 2000a, Danese et al. 2000b, Banning et al. 2003, Wakimoto et al. 2004, Tenke et al. 2006, Hancock et al. 2007). Bacteria have also been found in the uroepithelium of patients with a previous history of UTI (Elliott et al. 1985). Bacteria in intracellular biofilms are safe from antibiotics because of inadequate penetration and other mechanisms associated with the architecture of a biofilm, the low metabolic rate of bacteria in a biofilm, etc. (Stewart & Costerton 2001, Zheng & Stewart 2002). Some antibiotics, such as aminoglycosides, may even induce biofilm formation (Hoffman et al. 2005). There is evidence that the elimination of bacteria in a biofilm can be induced with agents derived from plants, such as forskolin (Bishop et al. 2007).
Fig. 1. Stages of biofilm formation: A) initial attachment of planktonic bacteria, B) irreversible adhesion and formation of the biofilm matrix, C) growth and multiplication of bacteria in the biofilm, D) a mature biofilm, and E) dispersion of the planktonic bacteria and formation of new colonies.

2.2.2 Anatomical factors

Urinary tract abnormalities affecting the flow of urine and emptying of the bladder increase the risk of UTI. Male circumcision reduces the risk of UTI substantially (odds ratio 0.13, 95% CI 0.08 to 0.20, p<0.001) (Singh-Grewal et al. 2005), so that the reported incidence of UTI in infancy is greater in non-circumcised boys than in circumcised boys (1–2% vs. 0.1–0.2%) (Wiswell & Roscelli 1986, Schoen et al. 2000). Also, children with UTIs caused by pathogens other than *E. coli* have more functional or anatomical abnormalities in the urinary tract than those with UTIs caused by *E. coli* (Honkinen et al. 1999). Urinary tract obstructions and high-grade reflux are more common among patients with urosepsis than with pyelonephritis (9% vs. 1% and 40% vs. 22%, respectively) (Honkinen et al. 2000).

2.2.3 Functional factors

Dysfunctional elimination syndrome (bladder and bowel incontinence and withholding) diagnosed after the age of two increases the risk of recurrent UTIs (Shaikh et al. 2003, Mingin et al. 2004a), and it is this dysfunctional elimination that causes children aged 3 to 5 years to have a higher risk of recurrent UTI than
either younger and older children (Conway et al. 2007). Recurrent UTIs are more common among children who have daytime wetting and high voiding frequency (Bakker et al. 2004). Also, the starting of potty training after the age of 18 months and excessively active potty training are associated with a higher risk of recurrent UTIs (Bakker et al. 2004). Voiding dysfunction, e.g. detrusor instability or any other factor affecting the flow of urine, will increase the risk of UTI (Hellerstein & Linebarger 2003), the most common cause of detrusor instability being constipation (Hellerstein & Linebarger 2003). Although there is an association between bladder dysfunction and UTIs, it is not clear which comes first (Hellström et al. 1991). Constipation is more common in children with UTI than in controls (Dohil et al. 1994, Blethyn et al. 1995), and it is known to increase the risk of UTIs, whereas its treatment prevents the recurrence of UTIs (Neumann et al. 1973, Loening-Baucke 1997). Constipation increases the residue after micturition, causes functional obstruction and affects the flow of urine (Neumann et al. 1973, Dohil et al. 1994, Blethyn et al. 1995, Loening-Baucke 1997).

The concept of vesicoureteral reflux (VUR) as a cause of renal damage was based on the findings among paraplegic adult patients (Hutch 1952). Vesicoureteral reflux (VUR) is found in 20–40% of children after the first UTI (Dick & Feldman 1996, Honkinen et al. 1999, Hannula et al. 2010, Shaikh et al. 2010), and contrary to the earlier understanding, it seems to be common among normal children and to occur at similar rates among children with or without UTI (Köllerman & Ludwig 1967, Ataei et al. 2004, Hannula et al. 2010). VUR of grades 1 to 3 has no effect on the risk of UTI recurrence but grades 4 and 5 carry a higher risk (Nuutinen & Uhari 2001, Conway et al. 2007). High-grade VUR is more common among patients with urosepsis than with pyelonephritis (Honkinen et al. 2000). VUR alone has no severe long-term consequences and is neither a necessary nor a sufficient cause for renal scars (Smellie et al. 1998, Shaikh et al. 2010). Operative treatment is effective in correcting VUR, but does not reduce the incidence of UTI, nor does it improve the prognosis in the case of children (Mor et al. 2003, Venhola et al. 2006, Hannula et al. 2010).

2.2.4 Other risk factors

Dietary factors are associated with susceptibility to UTIs (Kontiokari et al. 2003, Kontiokari et al. 2004). Poor fluid intake and even mild hydration are risk factors, but the evidence for this association is not consistent (Beetz 2003, Gray &
Krissovich 2003). There seems to be a genetic predisposition to UTIs (Hopkins et al. 1999b, Scholes et al. 2000, Zaffanello et al. 2010), with a history of UTI in the mother (odds ratio 2.3, 95% CI, 1.5–3.7) and the first UTI in childhood (odds ratio 3.9, 95% CI, 1.9–8.0) acting as risk factors for recurrent UTIs in early adulthood (Scholes et al. 2000). The attachment of *E. coli* to uroepithelial cells is determined by the receptors of the epithelial cells, and is higher in the uroepithelial cells of ABH blood group antigen non-secretors than those of secretors (Lomberg et al. 1986). However, neither the ABO phenotype nor the Lewis blood cell antigen phenotype entails a risk of recurrent UTIs (Hopkins et al. 1998). The risk of UTI seems not to be related to the use of nappies, the type of nappy used or other nursing habits (Nuutinen et al. 1996).

2.3 Long-term consequences of urinary tract infections in childhood

Childhood UTIs cause substantial morbidity and are thought to increase the risk of chronic kidney disease and hypertension, especially if VUR is present (Hodson & Edwards 1960, Ransley et al. 1984, Risdon et al. 1994). The causal relationship between childhood UTIs and their severe long-term consequences and the rationale involved in evaluating their significance have nevertheless been questioned (Chambers 1997, Stark 1997, Round et al. 2012).

2.3.1 Chronic kidney disease

Childhood UTIs are found to cause kidney scarring, especially when VUR is present, and it is claimed that they may lead to impaired kidney function (Jakobsson et al. 1999b, Wennerstrom et al. 2000, Lahdes-Vasama et al. 2006, Swerkersson et al. 2007). The presence of scars in the first intravenous urography (IVU) after the first UTI is more common in boys than in girls (Wennerstrom et al. 2000), but the majority of the scars detected in the acute or early phase disappear within two years (Jakobsson et al. 1994). Less than 40% of the children have permanent scars in the kidneys after the first episode of pyelonephritis, and it is rare for scars to be found at new sites in follow-up DMSA scans (McKerrow et al. 1984, Jakobsson et al. 1994, Vernon et al. 1997, Smellie et al. 1998, Hoberman et al. 1999, Ilyas et al. 2002, Shaikh et al. 2010). The most severe consequence of childhood UTI has been thought to be CKD, but evidence for an association between childhood UTIs, renal damage and CKD in children with structurally
normal kidneys is scarce (Hellerstein 2000, Round et al. 2012). The aetiological role of childhood UTIs as a cause of CKD is not well known, and there are only a few follow-up reports available on patients who had had UTIs decades earlier (Hellerstein 2000). The active treatment of children with VUR has not reduced the incidence of end-stage renal disease caused by reflux nephropathy (Craig et al. 2000).

2.3.2 Other consequences

Diastolic blood pressure in adulthood is higher in patients who had had febrile UTIs and kidney scars in childhood than in controls or patients without scars, but the scars do not affect systolic blood pressure and hypertension is rare (Jacobson et al. 1989, Martinell et al. 1990, Martinell et al. 1996, Lahdes-Vasama et al. 2006). Thus UTIs in childhood are not a significant risk factor for hypertension (Wennerström et al. 2000a). Women with scarred kidneys after UTIs or ABU in childhood seem to have more pregnancy complications such as arterial hypertension and pre-eclampsia than those without such a history (Sacks et al. 1987, Jacobson et al. 1989, McGladdery et al. 1992, Smellie et al. 1998).

2.4 Prevention of urinary tract infections in childhood

The objectives of the prevention of UTI are to reduce the burden of the related symptoms and to protect the kidneys from damage caused by recurrent episodes of pyelonephritis.

2.4.1 Antimicrobials

Antimicrobials are effective to a limited degree in preventing recurrences of UTI in children (Uhari et al. 1996b, Roussey-Kesler et al. 2008, Craig et al. 2009, Williams & Craig 2009). Craig et al. (2009) have reported recently that trimethoprim-sulphamethoxazole reduced the absolute risk of UTI by 6 percentage points (13% vs. 19%), that the number it was necessary to treat to eliminate one case was 14, and that 67% of the infections in the antibiotic group were caused by resistant bacteria, the use of prophylactic antibiotics having increased the risk of infections caused by these. Likewise, there are reports of antimicrobials having no effect on the recurrence of UTI but increasing the risk of infections caused by resistant bacteria (Garin et al. 2006, Conway et al. 2007,
Montini et al. 2008). Differences in compliance between the antibiotic and placebo groups do not explain the poor efficacy revealed by these studies. Furthermore, prophylactic antibiotics are not effective in preventing UTIs or the progression of renal scars in patients with VUR (Garin et al. 2006, Montini et al. 2008, Pennesi et al. 2008, Craig et al. 2009). In a small series (N=18) studied by Lohr et al. (1977) nitrofurantoin was more effective than a placebo for preventing UTIs (0 vs. 1.7 episodes/PYR) and bacteruria (0.2 vs. 2.5 episodes/PYR) in girls with a history of recurrent UTIs (Lohr et al. 1977). Similarly, nitrofurantoin is more effective in preventing recurrences of UTI in children than are sulphonamides (9.4 vs. 27 episodes/100 person years at risk) (Uhari et al. 1996b).

2.4.2 Dietary factors

Many plants produce antimicrobial compounds in response to microbial invasion, and when ingested, these compounds may modulate the bacterial flora in the gut. Changes in cattle nutrition have been shown to result in changes in their colonic bacterial flora (Callaway et al. 2003). Berries of the Vaccinium family (bilberries, lingonberries and cranberries) and their extracts have marked activity against many human bacteria in vitro (Ofek et al. 1991, Howell et al. 1998, Burger et al. 2000, Ho et al. 2001, Puupponen-Pimiä et al. 2001, Reid et al. 2001, Cavanagh et al. 2003, Ho et al. 2001, Puupponen-Pimiä et al. 2001, Reid et al. 2001, Cavanagh et al. 2003, Ho et al. 2001, Puupponen-Pimiä et al. 2001, Reid et al. 2001, Cavanagh et al. 2003, Nogueira et al. 2003, Weiss et al. 2004). The ingestion of fermented milk products containing probiotics has also been said to reduce the risk of UTI (Kontiokari et al. 2003).

2.4.3 Cranberry

Cranberries, like other berries of the Vaccinium family, have a wide antimicrobial spectrum in vitro (Table 1). Cranberry products appear to be able to prevent UTI recurrences and bacteruria in adults, but evidence for their efficacy in children is lacking (Blatherwick 1914, Avorn et al. 1994, Schlager et al. 1999, Kontiokari et al. 2001, Stothers 2002, Jepson et al. 2004, Wing et al. 2008). Three randomized controlled trials (RCT) have shown that cranberry juice can prevent bacteruria in elderly people and UTIs in women, and it has been suggested as an alternative to antimicrobials for UTI prevention (Avorn et al. 1994, Kontiokari et al. 2001, Stothers 2002). In a recent RCT, however, cranberry juice was not effective in reducing the number of UTI recurrences among college women (Barbosa-Cesnik et al. 2011).
The mechanism of action of cranberry juice is uncertain. It does not reduce the pH of the urine significantly nor does it produce enough hippuric acid to explain its antibacterial activity (Avorn et al. 1994, Di Martino et al. 2006, Naves et al. 2008a). It is thought to act by inhibiting the adhesion of E. coli to uroepithelial cells (Sobota 1984, Howell et al. 1998), since cranberry extract and its urinary metabolites have been shown to reduce bacterial adherence in urine in a dose-dependent manner (Di Martino et al. 2006, Gupta et al. 2007, Lavigne et al. 2008, Howell et al. 2010). This effect has been demonstrated in urine a few hours after the ingestion of cranberry juice and it is not dependent on the expression of genes encoding type P pili (Howell et al. 2005, Di Martino et al. 2006, Tao et al. 2011).

Cranberries have been shown to contain two compounds that have anti-adherence activity, fructose and proanthocyanidins (PAC). The inhibition of type 1 fimbria-mediated adherence by cranberries has been attributed to fructose, but the effect has only been demonstrated in vitro, and other fruits and berries containing comparable amounts of fructose do not have the same anti-adhesion activity (Zafriri et al. 1989, Howell et al. 2005). Cranberry PACs with A-type linkage inhibit the adherence of P-fimbriated strains of E. coli (Zafriri et al. 1989, Howell et al. 1998, Foo et al. 2000, Howell et al. 2005), and PACs have been shown in animal models to prevent UPEC from invading the kidney cells (Tufenkji et al. 2010). Cranberry-derived PACs are also known to inhibit the flagellum-mediated motility of UPEC in vitro (Hidalgo et al. 2011a). Furthermore, cranberries and their PACs act as iron chelators and inhibit the growth of UPEC by inducing a state of iron depletion in bacteria (Hidalgo et al. 2011b, Lin et al. 2011). The drinking of cranberry juice has not been found to lead to any significant increase in the concentration of PACs in the urine, however (Valentova et al. 2007).

2.4.4 Other preventive measures

Patients are often advised to increase their fluid intake in order to control or prevent UTIs, but there is no evidence to support such a recommendation (Beetz 2003, Gray & Krissovich 2003). Treating constipation is effective in correcting voiding dysfunction and preventing UTI recurrences in children (Neumann et al. 1973, Loening-Bauke 1997), while recurrent UTIs in adults can be prevented by vaginal mucosal immunization with vaccines containing killed uropathogens, the efficacy of this vaccination being dependent on the HLA-DR phenotype of the patient, for example (Hopkins et al. 1999a). Circumcision reduces the risk of UTI in boys and may be cost-effective, but as only 1% of boys with a normal urinary tract have UTI in childhood, the number of normal boys that it is necessary to treat to prevent one UTI is more than 100 (To et al. 1998, Schoen et al. 2000, Singh-Grewal et al. 2005). On the other hand, the number of boys with recurrent UTIs or significant urinary tract abnormalities that is was necessary to treat per prevention was 4–10, so that they may benefit from circumcision (Singh-Grewal et al. 2005). Colonization of the periurethral area with Lactobacilli may reduce the risk of recurrent UTIs, but oral administration of probiotics is not effective (Reid et al. 1992, Raz & Stamm 1993, Kontiokari et al. 2001).
Table 1. *In vitro* antimicrobial effects of *Vaccinium* berry extracts on certain bacteria and fungi.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Example of diseases in humans</th>
<th>Growth or adhesion inhibition in <em>vivo</em> by Blueberry</th>
<th>Cranberry</th>
<th>Lingonberry</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Actinobacillus actinomyces</em></td>
<td>Dental caries</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Ho et al. 2001</td>
</tr>
<tr>
<td><em>Bifidobacterium lactis</em></td>
<td></td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Puupponen-Pimiä et al. 2001</td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>Oral thrush</td>
<td>-</td>
<td>No</td>
<td>No</td>
<td>Cavanagh et al. 2003</td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>Gas gangrene</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Cavanagh et al. 2003</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>UTI, sepsis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Puupponen-Pimiä et al. 2001, Cavanagh et al. 2003, Ofek et al. 1991,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Howell et al. 1998, Nogueira et al. 2003, Reid et al. 2001</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Peritonitis</td>
<td>No</td>
<td>Yes/No</td>
<td>No</td>
<td>Cavanagh et al. 2003, Puupponen-Pimiä et al. 2001</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>Otitis, sinusitis</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>McRea et al. 2002</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Gastritis</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Burger et al. 2000</td>
</tr>
<tr>
<td><em>Lactobacillus sp.</em></td>
<td></td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>Weiss et al. 2004</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Miscarriage</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Nogueira et al. 2003</td>
</tr>
<tr>
<td><em>Mycobacterium phlei</em></td>
<td></td>
<td></td>
<td>Yes</td>
<td>-</td>
<td>Cavanagh et al. 2003</td>
</tr>
<tr>
<td><em>Porphyromonas gingivalis</em></td>
<td>Dental caries</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Ho et al. 2001</td>
</tr>
<tr>
<td><em>Prevotella intermedia</em></td>
<td>Dental caries</td>
<td>-</td>
<td>-</td>
<td>Yes</td>
<td>Ho et al. 2001</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Wound infection</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Cavanagh et al. 2003</td>
</tr>
<tr>
<td><em>Salmonella sp.</em></td>
<td>Enteritis</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>Cavanagh et al. 2003, Nogueira et al. 2003</td>
</tr>
<tr>
<td><em>Shigella sonnei</em></td>
<td>Enteritis</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Cavanagh et al. 2003</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Sepsis, impetigo</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Cavanagh et al. 2003</td>
</tr>
<tr>
<td><em>Streptococcus mutans</em></td>
<td>Dental caries</td>
<td>-</td>
<td>Yes</td>
<td>-</td>
<td>Weiss et al. 2004</td>
</tr>
</tbody>
</table>

- = not tested
3 Aims of the research

The specific aims of the research were:

1. To find out whether the formation of an *Escherichia coli* biofilm is important for the pathogenesis of UTI, and how it is associated with the level of UTI (I).
2. To determine the aetiological fraction of UTI in childhood as a cause of CKD (II).
3. To evaluate whether cranberry juice is effective, safe and acceptable for the prevention of UTIs in children (III and IV).
4  Biofilm formation by uropathogens as an aspect of pathogenesis (I)

4.1  Subjects

We identified 70 patients with acute symptomatic UTI (34 children and 36 adults) from whom we had causative *E. coli* strains available (Table 2). Twenty-one of these patients had participated in our previous clinical trial to evaluate the use of cranberry-lingonberry juice for the prevention of UTIs in women (Kontiokari et al. 2001). The diagnosis of UTI was based on clinical signs and/or symptoms and bacteria growing in a urine culture obtained by suprapubic aspiration or more than $10^5$ CFU/ml of a single pathogen in a urine culture obtained by clean voiding or transurethral catheterization. If the sample had been taken by the urine pad/bag technique the criteria for UTI diagnosis were pyuria (> 5 leukocytes/field) and more than $10^5$ CFU/ml of a single pathogen growing in two consecutive urine cultures (American Academy of Pediatrics 1999). Growth of $10^4$–$10^5$ CFU/ml of a single pathogen was considered diagnostic if the urine nitrite test was positive. Pyelonephritis was defined as UTI associated with a fever of over 38.5 °C and/or a C-reactive protein value greater than 40 mg/l (Jodal et al. 1975). Patients were diagnosed as having urosepsis if they had UTI and a blood culture positive for the same pathogen during the same disease period.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cystitis (n=43)</th>
<th>Pyelonephritis (n=11)</th>
<th>Urosepsis (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female/Male</td>
<td>43/0</td>
<td>8/3</td>
<td>11/5</td>
</tr>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>19.8</td>
<td>3.6</td>
<td>63.7</td>
</tr>
<tr>
<td>Range</td>
<td>1.8-57.8</td>
<td>0.1-13.5</td>
<td>0.5-97.3</td>
</tr>
</tbody>
</table>

All the pyelonephritis patients were children and all but one of the urosepsis patients were adults. Twenty-seven of the 34 patients younger than sixteen years of age had undergone diagnostic imaging of the urinary tract, 20/27 of these (74%) having no urinary tract abnormalities, 4/27 having grade 1–2 vesicoureteral reflux (VUR), 2/27 having grade 3–4 VUR and one having a neurogenic bladder. The frequency of VUR among the child UTI patients in this population (22%) is in accordance with previous figures (Jacobson et al. 1999). Imaging results were available for eight adult patients, all of whom had normal findings.
4.2 Methods

**Bacterial strains and growth conditions**

The uropathogenic *E. coli* strains were frozen and stored in skimmed milk at −20 °C (strains isolated from recidive infections at −80°C), revived on blood agar and grown overnight in Luria broth at 35 °C. The susceptibility of these strains to antimicrobials was determined by the plate diffusion method from urine (all strains) and blood samples (strains causing urosepsis). A strain was classified as having antimicrobial resistance if it was resistant to at least one of the antibiotics tested (Table 3). The strains with intermediate resistance were included in the susceptible group. This classification into resistant and susceptible strains was based on the findings in urine samples. One patient was excluded because of missing susceptibility data. The frequency of antimicrobial resistance is in accordance with figure published for Finland (Finnish Study Group for Antimicrobial Resistance 2008).

**Table 3. Antibiotics against which the resistance of *E. coli* strains was tested, and their mean optical density (OD) values by susceptibility group (69 samples)**

<table>
<thead>
<tr>
<th>Antimicrobials</th>
<th>Susceptible</th>
<th>Resistant</th>
<th>Intermediate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>OD</td>
<td>n</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>68</td>
<td>0.51</td>
<td>1</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>59</td>
<td>0.52</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacain²</td>
<td>36</td>
<td>0.47</td>
<td>0</td>
</tr>
<tr>
<td>Mecillinam</td>
<td>66</td>
<td>0.51</td>
<td>1</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>69</td>
<td>0.51</td>
<td>0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>58</td>
<td>0.54</td>
<td>11</td>
</tr>
<tr>
<td>Trimethoprim-sulphamethoxazole³</td>
<td>30</td>
<td>0.57</td>
<td>4</td>
</tr>
</tbody>
</table>

¹ 1 patient’s urine sample was missing. ² Not tested in paediatric samples (except 1 for urosepsis sample). ³ tested only in paediatric samples

**Verification of biofilm formation**

The formation of organized biofilm structures was verified by scanning electron microscopy (SEM) in 22 selected samples and the viability of the bacteria with LIVE/DEAD Baclight® staining followed by confocal scanning laser microscopy (CSLM) in 29 selected samples. Biofilm formation was assessed by subculturing
20 µl of an overnight Luria broth in 800 µl of Dulbecco’s modified Eagle’s medium (0.45% glucose) in each well of a 24-well flat-bottomed polystyrene microtitre plate containing removable plastic coverslips. Incubation was carried out anaerobically at 37 °C. For practical reasons the incubation time was 48 h. After the incubation period the samples were rinsed once with sterile water and fixed in 4% formaldehyde overnight for SEM. The fixed samples were dehydrated with 25%, 50%, 75% and 96% ethanol for 20 minutes at room temperature and finally air-dried. SEM was carried out on a Jeol JSM-6400 microscope in the Department of Electron Optics, University of Oulu. The samples for CSLM were rinsed once with sterile water after incubation, following which 2 µl of LIVE/DEAD Baclight® stain was added and they were scanned for 30 minutes or less with a Zeiss LSM 510 microscope at the Department of Anatomy, University of Oulu.

Optical density as a measure of biofilm formation

The biofilm formation capacity of all the *E. coli* strains was tested by subculturing 5 µl of an overnight Luria broth in 200 µl of Dulbecco’s modified Eagle’s medium (0.45% glucose) in each well of a 96-well flat-bottomed polystyrene microtitre plate. *E. coli* ATCC 10798 was used as a positive control for biofilm formation and Luria broth as a negative control (Rediske et al. 1999).

Three wells were used for each sample. The 72 h incubation was carried out anaerobically at 37 °C. After discarding the medium, the microtitre plate wells were rinsed once with sterile water, stained with 1% crystal violet for 15 minutes and rinsed twice with sterile water. The presence of a biofilm was assessed after eluting the attached crystal violet with 96% ethanol and measuring the optical density (OD) of the eluted material at 570 nm (Multiscan MCC/340 P). The results are presented as means of three parallel samples. The mean OD of the biofilm-positive control sample (5 measurements performed on different cultures, *E. coli* ATCC 10798) was 0.72 (range 0.46–1.31) and that of the negative control sample (3 measurements on different cultures, Luria broth) was 0.22 (range 0.15–0.29). In order to test the validity of the method, we compared the measured ODs with the findings in the SEM and CSLM images. The *E. coli* attached to the polystyrene microtitre plates were alive and had formed organized biofilm structures when the OD of the strain was higher than 0.50 (Figure 2).
Fig. 2. SEM and confocal CSLM images of biofilm-negative (A and B, optical density 0.48) and biofilm-positive (C and D, optical density 1.27) *E. coli* strains. Almost all the stained material in the CSLM images (B and D) is green, indicating live bacteria.

**Statistical analysis**

The differences in optical density between the two susceptibility groups were tested using the Mann-Whitney U test and those between the three diagnostic groups using the Kruskal-Wallis one-way analysis of variance. If the Kruskal-Wallis analysis indicated statistically significant differences, post hoc comparisons between the pairs were carried out. The differences in the proportions of biofilm formation between the two susceptibility groups were tested using the SND test and those between the three diagnostic groups using the chi-square test. In the case of a statistically significant P-value, post hoc comparisons of multiple pairs were performed using the SND test according to our a priori hypothesis (Armitage & Matthews 2002). The statistical analyses were carried out with SPSS 15.0 for Windows and the StatsDirect statistical software, version 2.5.6.
4.3 Results

The mean OD of all the *E. coli* strains was 0.50 (range 0.21 to 1.45, SD 0.30). Taking this figure as the cut-off point for *in vitro* biofilm formation on the basis of findings in the SEM images, 22/70 (31%) of the *E. coli* strains could be said to have been biofilm-positive. The ODs of the causative strains did not correlate with the age of the patient \((r=-0.12, P=0.32)\), and the severity of infection was similarly not dependent on the age of the patient (the mean age of the patients with invasive infections was 39 years and that of the patients with cystitis 20 years, \(P=0.32\)). The mean ODs of the three diagnostic groups differed significantly \((P=0.03)\). The strains isolated from the patients with pyelonephritis had significantly higher ODs than those from the patients with cystitis (difference in the means 0.19, 95% confidence limits (CL) 0.06 to 0.32, \(P=0.02\)) (Figure 3), but the urosepsis patients did not differ significantly in this respect from either the cystitis patients (difference in the means 0.12, 95% CL -0.06 to 0.13, \(P=0.68\)) or the pyelonephritis patients (difference in the means 0.07, 95% CL -0.04 to 0.32, \(P=0.28\)). Altogether 11/43 (26%) of the *E. coli* strains isolated from cystitis patients, 6/11 (55%) from pyelonephritis patients and 5/16 (31%) from urosepsis patients were positive for biofilm formation. When the children and adults were tested separately, the difference in the mean OD between the strains causing invasive infections and those causing cystitis was 0.14 (95% CL -0.02 to 0.31, \(P=0.06\)) in the children and 0.16 (95% CL -0.03 to 0.15, \(P=0.22\)) in the adults.

![Fig. 3. Biofilm-forming capacity of *E. coli* strains cultured from patients with different levels of urinary tract infection. The dotted line indicates the cut-off optical density value for biofilm formation.](image)

43
Altogether 8/43 (19%) of the cystitis episodes, 1/11 (9%) of the pyelonephritis episodes and 6/15 (40%) of the urosepsis episodes were caused by a resistant *E. coli* strain as defined in the plate diffusion assay. The *E. coli* strains collected from the urine and blood of the urosepsis patients were similar in terms of their susceptibility. The susceptible strains had significantly higher ODs than the resistant strains (difference in the means 0.21, 95% CI 0.03 to 0.27, *P*=0.016), but there was no difference in age between the patients from whom the susceptible and resistant strains had been isolated (25.7 vs. 30.7 years, difference 5 years, 95% CI -20.1 to 10.8, *P*=0.61) (Figure 4). One out of fifteen (7%) of the resistant *E. coli* strains and 21/54 (39%) of the susceptible strains formed biofilms (difference 32%, 95% CI 5% to 48%, *P*=0.015). The only biofilm-positive resistant strain had an OD of 0.53.

![Fig. 4. Biofilm-forming capacity of antimicrobially resistant and susceptible *E. coli* strains. The dotted line indicates the cut-off optical density value for biofilm formation.](image-url)
5 Long-term consequences of urinary tract infections in childhood (II)

5.1 Methods

Data sources

We sought evidence of a causal relationship between childhood UTIs and CKD by performing a systematic literature review and analysing the causes of CKD in patients who were monitored or treated at Oulu University Hospital or who had died while undergoing renal replacement therapy (RRT) before enrolment in the present series. To identify congenital kidney anomalies, we reviewed the first kidney images systematically for structural abnormalities. VUR was not considered a structural abnormality.

Literature review

A systematic literature search for the period from January 1966 to August 2009 was conducted with the PubMed database, using the Medical Subject Headings search terms “kidney failure, chronic,” “renal insufficiency, chronic,” or “uremia” and “urinary tract infections,” “pyelonephritis,” or “cystitis” (Figure 5). The searches were limited to publications in English and to human subjects, and the results were combined. All of the titles and abstracts found were reviewed, and papers that might only possibly have dealt with the relationship between childhood UTIs and chronic kidney damage were read and evaluated. Papers were included if they reported on childhood UTI in patients with CKD or on clinically significant long-term renal consequences of childhood UTIs. Papers concerning only patients with congenital malformations were excluded. The reviews and articles included were searched manually for additional references. The focus of the review was on the number of childhood UTIs and kidney imaging findings in patients with CKD without a definitive non-infectious cause. We followed the PRISMA guidelines and used the PRISMA checklist when conducting and reporting the literature review (Moher et al. 2009).


Local patients

The hospital records of all 366 patients who had been treated or monitored on account of CKD at Oulu University Hospital (which serves an area with 384 000 inhabitants) in 2005–2006 were reviewed (Figure 6 and Table 4). We estimated glomerular filtration rates by the Modification of Diet in Renal Disease method and defined CKD on the basis of an estimated glomerular filtration rate of <40 mL/minute per 1.73 m² or kidney disease necessitating RRT (haemodialysis, peritoneal dialysis, or kidney transplantation) for >3 months (Levey et al. 1999).

The 308 patients who had a defined non-infectious cause of CKD (e.g. glomerulonephritis or diabetic nephropathy) were excluded from the more thorough investigations (Figure 6). Patients with VUR, nephrosclerosis, or diseases or malformations affecting only one kidney were included in the review analysis. The remaining 58 patients were asked for interviews, and 54 (92%) of them gave their informed consent and completed a structured questionnaire about childhood UTI. The hospital records of the four patients who declined to be interviewed were reviewed (Figure 6). The patient records of the 54 patients who gave their informed consent were obtained from the community health centres where they had been treated or monitored in childhood. The patients and their records were identified on the basis of individual social security numbers, which have been issued to every Finnish citizen since 1964.
Fig. 5. Results of the literature search on childhood UTI in patients with chronic kidney disease.
Fig. 6. Patients treated or monitored at Oulu University Hospital on account of chronic kidney disease (CKD) and their UTIs in Childhood.
Table 4. Patients monitored at Oulu University Hospital on account of CKD.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Definitive non-infectious cause for CKD&lt;sup&gt;1&lt;/sup&gt; (n=308)</th>
<th>No definitive non-infectious cause for CKD in childhood (n=45)</th>
<th>Symptomatic UTI in childhood (n=13)&lt;sup&gt;2&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (range), y</td>
<td>54.1 (1.4-85.2)</td>
<td>61.2 (35.1-92.6)</td>
<td>54.7 (39.9-71.1)</td>
</tr>
<tr>
<td>Born after 1960, no. (%)</td>
<td>93 (30)</td>
<td>17 (38)</td>
<td>4 (31)</td>
</tr>
<tr>
<td>Female, no. (%)</td>
<td>124 (40)</td>
<td>19 (42)</td>
<td>11 (85)</td>
</tr>
<tr>
<td>Current therapy, no. (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predialysis</td>
<td>57 (19)</td>
<td>7 (16)</td>
<td>2 (15)</td>
</tr>
<tr>
<td>Peritoneal dialysis</td>
<td>21 (7)</td>
<td>3 (7)</td>
<td>-</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>93 (30)</td>
<td>19 (42)</td>
<td>1 (8)</td>
</tr>
<tr>
<td>Renal transplant</td>
<td>137 (44)</td>
<td>16 (36)</td>
<td>10 (77)</td>
</tr>
</tbody>
</table>

<sup>1</sup> Childhood UTI history was not reviewed.

<sup>2</sup> For more detailed information, see Table 7.

Abbreviations: UTI=Urinary tract infection, CKD=Chronic kidney disease

Registry data

Data on patients undergoing RRT were collected from the Finnish Registry for Kidney Diseases, which has been estimated to cover 97% to 99% of all Finnish RRT patients since 1964 (Finnish Registry for Kidney Diseases 2008). Diagnoses for patients who had died before the beginning of the present work were collected from this source.

Ethics approval

The protocol for the investigation was evaluated and approved by the ethics committee of the Northern Ostrobothnia Hospital District (Oulu, Finland).

5.2 Results

Literature review

Our systematic literature search yielded a total of 781 papers, the titles and abstracts of which led to the selection of 39 for further evaluation (Figure 5). Finally, we found 10 papers reporting results regarding childhood UTIs for 1576 patients with CKD or other clinically significant long-term consequences of
childhood UTI (Table 5). None of the studies had been designed to evaluate the aetiological fraction of UTI in childhood as a cause of CKD, and none of the reports enabled population-based occurrence figures be calculated. The patient series were in all cases based on tertiary hospitals serving large populations.

Four of the papers described prospective follow-ups of patients with UTI and the risk of complications (e.g. scars, VUR) in childhood (Table 5) (Jacobson et al. 1989, Martinell et al. 1996, Smellie et al. 1998, Wennerström et al. 2000b). Patients with obstructive malformations were excluded. These four papers included a total of 435 patients, whose ages at the end of the follow-up periods were between 7 and 52 years. Five of these patients (patients A–E) developed CKD before the end of the follow-up period (Table 6), all five having had severe bilateral scars at their first kidney examination and 3 having already had impaired renal function in childhood. In addition, 1 girl with severe bilateral scars and impaired renal function in childhood had died as a result of cerebral haemorrhage attributable to uncontrolled hypertension at the age of 25 years (not included in Tables 5 and 6) (Smellie et al. 1998).

The remaining six papers examined the causes of CKD for a total of 1141 consecutive patients referred to the renal units of hospitals (Table 5) (Schechter et al. 1971, Murray & Goldberg 1975, Stewart et al. 1975, Huland et al. 1979, Esbjörner et al. 1997, Sreenarasimhaiah & Hellerstein 1998). Of these patients, 934 had a definitive non-infectious cause of CKD, whereas only 17 out of the remaining 207 were reported to have experienced symptomatic UTIs at any time. Seven of the 17 patients (patients F–L) had experienced symptomatic UTIs in childhood (Table 6), four of them having exhibited structural abnormalities in their first kidney images. The remaining 3 patients had chronic pyelonephritis diagnosed in adulthood, by means of a biopsy in 2 cases. The structures of their kidneys were not evaluated or reported in childhood. In conclusion, only 3 of the 1576 patients identified in the literature search (patients J–L) had childhood UTIs as a possible cause of CKD, and unfortunately there were no data available on the structure of their kidneys before the recurrences of UTI.

Local patients

In our review of the data for patients with CKD treated at Oulu University Hospital, 13 of the 58 patients without a specific non-infectious cause of CKD had a history of symptomatic UTIs in childhood (Table 7). They all had structural abnormalities found in their first kidney imaging examination, of which had
mostly been performed in childhood (5 patients) or early adulthood (4 patients). VUR (which was not considered a structural abnormality) had been diagnosed in 3 patients. Six of the 13 patients exhibited urethral obstructions or congenital anomalies severe enough to cause kidney failure without other contributing factors, and four of the remaining 7 patients demonstrated dysplastic or hypoplastic kidneys in their first kidney images and had experienced only 1 or 2 UTIs during childhood but recurrent UTIs in adulthood. Recurrent UTIs during childhood may have contributed significantly to the later development of CKD in 3 patients (patients 8, 10, and 11) whose kidney structures had been abnormal when first examined, in such a way that the abnormalities could have been observed by ultrasonography.

Among these 3 patients was a 46-year-old woman (patient 8) who had experienced recurrent febrile UTIs during childhood and recurrent UTIs that required antimicrobial prophylaxis in early adulthood. Her left kidney was hypoplastic and the right kidney was seen to be scarred in her first kidney examination, at the age of 20 years. She received a renal transplant at the age of 39 years. The second patient was a 52-year-old woman (patient 10) who had experienced 3 UTIs in childhood. She had exhibited a hypoplastic left kidney and hydronephrosis and deformed calices in the right kidney in her first urographic study, at the age of 11 years. She had also suffered from hypertension since the age of 11 years, and her left kidney had been removed because of uncontrolled hypertension when she was 13 years of age. She had received a renal transplant at the age of 44 years. The third patient was a 60-year-old woman (patient 11) who had experienced recurrent UTIs in childhood and had exhibited a shrunken kidney in her first imaging examination, at the age of 30 years. She had received a renal transplant at the age of 55 years. If we regard childhood UTIs as a factor contributing to CKD in this third patient, then the aetiological fraction of recurrent childhood UTIs as a cause of CKD would be at most 1 in 366 (0.3%).
### Table 5. Papers on patients with UTIs in childhood and later CKD

<table>
<thead>
<tr>
<th>Study</th>
<th>Population</th>
<th>Age at end of follow-up period</th>
<th>Patients with symptomatic UTIs in childhood and CKD without any definitive non-infectious cause¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Prospective studies</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smellie et al. 1998</td>
<td>226 patients with UTI and non-obstructive VUR in childhood</td>
<td>26-52 years</td>
<td>2 girls</td>
</tr>
<tr>
<td>Martinell et al. 1996</td>
<td>111 girls with UTI or ABU and risks in childhood</td>
<td>16-33 years</td>
<td>None</td>
</tr>
<tr>
<td>Wennersström et al. 2000</td>
<td>68 patients with UTI and non-obstructive scars in childhood</td>
<td>7-34 years</td>
<td>None</td>
</tr>
<tr>
<td>Jacobson et al. 1989</td>
<td>30 patients with UTI and non-obstructive scars in childhood</td>
<td>22-41 years</td>
<td>1 man and 2 women</td>
</tr>
<tr>
<td><strong>Retrospective studies on patients with CKD</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Esbjömer 1997</td>
<td>All patients with CKD in childhood in Sweden</td>
<td>0-16 years</td>
<td>None</td>
</tr>
<tr>
<td>Sreenarasimhaiah et al. 1998</td>
<td>102 children</td>
<td>0-20 years</td>
<td>1 girl</td>
</tr>
<tr>
<td>Stewart et al 1975</td>
<td>403 consecutive patients</td>
<td>15-55 years</td>
<td>None (timing of UTI in 3 patients with uncertain diagnosis was not reported)</td>
</tr>
<tr>
<td>Murray et al. 1975</td>
<td>320 patients</td>
<td>10-81 years</td>
<td>None (timing of UTI in 1 patient with nephrosclerosis was not reported)</td>
</tr>
<tr>
<td>Schechter et al. 1971</td>
<td>173 consecutive patients</td>
<td>9-61 years</td>
<td>2 women</td>
</tr>
<tr>
<td>Huland et al. 1979</td>
<td>25 consecutive patients (all had VUR in adulthood)</td>
<td>7-61 years</td>
<td>4 women</td>
</tr>
<tr>
<td><strong>Total N</strong></td>
<td>1576</td>
<td></td>
<td>12</td>
</tr>
</tbody>
</table>

¹ For more detailed information, see Table 6
Table 6. Twelve patients with CKD of no definitive non-infectious cause identified in the literature review as having had UTIs in childhood.

<table>
<thead>
<tr>
<th>Patient</th>
<th>The first kidney imaging</th>
<th>Clinical data</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Designation</strong></td>
<td><strong>Gender</strong></td>
<td><strong>Reference</strong></td>
</tr>
<tr>
<td>A</td>
<td>Female</td>
<td>Smellie <em>et al.</em> 1998</td>
</tr>
<tr>
<td>B</td>
<td>Female</td>
<td>Smellie <em>et al.</em> 1998</td>
</tr>
<tr>
<td>C</td>
<td>Male</td>
<td>Jacobson <em>et al.</em> 1989 and 1992</td>
</tr>
<tr>
<td>D</td>
<td>Female</td>
<td>Jacobson <em>et al.</em> 1989 and 1992</td>
</tr>
<tr>
<td>E</td>
<td>Female</td>
<td>Jacobson <em>et al.</em> 1989 and 1992</td>
</tr>
<tr>
<td>F</td>
<td>Female</td>
<td>Sreenarasarimhaiah <em>et al.</em> 1998</td>
</tr>
<tr>
<td>G</td>
<td>Female</td>
<td>Schechter <em>et al.</em> 1971</td>
</tr>
<tr>
<td>H</td>
<td>Female</td>
<td>Schechter <em>et al.</em> 1971</td>
</tr>
<tr>
<td>I</td>
<td>Female</td>
<td>Huland <em>et al.</em> 1979</td>
</tr>
<tr>
<td>J</td>
<td>Female</td>
<td>Huland <em>et al.</em> 1979</td>
</tr>
<tr>
<td>K</td>
<td>Female</td>
<td>Huland <em>et al.</em> 1979</td>
</tr>
<tr>
<td>L</td>
<td>Female</td>
<td>Huland <em>et al.</em> 1979</td>
</tr>
</tbody>
</table>

CP indicates chronic pyelonephritis; NR = not reported; *VUR diagnosed at the age of 48 y*
<table>
<thead>
<tr>
<th>Patient Designation</th>
<th>Gender</th>
<th>Age, y</th>
<th>First kidney imaging</th>
<th>No. of symptomatic UTIs</th>
<th>Clinical data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypoplasia (a)</td>
<td>Other features</td>
<td>Age, y</td>
</tr>
<tr>
<td>Obstructive uropathy</td>
<td></td>
<td></td>
<td>Right</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>1 Female 40</td>
<td>No</td>
<td>No</td>
<td>Urethral stenosis</td>
<td>Recurrent</td>
<td>Recurrent</td>
</tr>
<tr>
<td>2 Male 52</td>
<td>Yes</td>
<td>Yes</td>
<td>Urethral valve</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>3 Male 51</td>
<td>No</td>
<td>Yes</td>
<td>Urethral valve</td>
<td>1,5</td>
<td>A few</td>
</tr>
<tr>
<td>Congenital anomaly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Female 41</td>
<td>No</td>
<td>Not present</td>
<td>Scars</td>
<td>Aplasia</td>
<td>37</td>
</tr>
<tr>
<td>5 Female 61</td>
<td>Not present</td>
<td>NR</td>
<td>Aplasia</td>
<td>Ectopic</td>
<td>19</td>
</tr>
<tr>
<td>6 Female 76</td>
<td>Not present</td>
<td>Yes</td>
<td>Aplasia</td>
<td>Horseshoe</td>
<td>61</td>
</tr>
<tr>
<td>Hypoplastic/dysplastic kidneys</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 Female 52</td>
<td>Yes</td>
<td>Yes</td>
<td>Dilated ureter</td>
<td>None</td>
<td>12</td>
</tr>
<tr>
<td>8 Female 46</td>
<td>Yes</td>
<td>Yes</td>
<td>Scars</td>
<td>Scars</td>
<td>20</td>
</tr>
<tr>
<td>9 Female 60</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>23</td>
</tr>
<tr>
<td>Designation</td>
<td>Gender</td>
<td>Age, y</td>
<td>First kidney imaging</td>
<td>No. of symptomatic UTIs</td>
<td>Clinical data</td>
</tr>
<tr>
<td>-------------</td>
<td>--------</td>
<td>--------</td>
<td>----------------------</td>
<td>------------------------</td>
<td>---------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypoplasia*</td>
<td>Other features</td>
<td>Age, y</td>
</tr>
<tr>
<td>10(b)</td>
<td>Female</td>
<td>50</td>
<td>Yes</td>
<td>Yes</td>
<td>Hydronephrosis</td>
</tr>
<tr>
<td>11(b)</td>
<td>Female</td>
<td>44</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>Female</td>
<td>69</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>Female</td>
<td>71</td>
<td>Yes</td>
<td>Yes</td>
<td>None</td>
</tr>
</tbody>
</table>

FSGS indicates focal segmental glomerulosclerosis; NR, not reported.

* Includes dysplasia.

\(b\) Recurrent childhood UTIs possibly contributed significantly to the development of CKD.
Registry data

According to the Finnish Registry for Kidney Diseases, nineteen RRT patients born in the Oulu University Hospital area after 1960 had died before the beginning of the present work (Table 8) (Finnish Registry for Kidney Diseases 2008), 16 of whom had a specific non-infectious cause of CKD, while of the remaining three patients (all male), one with probable VUR during childhood had died of alcohol-related liver cirrhosis at the age of 39, one with CKD had died of ileus due to a complication of sepsis at the age of 40 and one with congenital kidney malformation and obstruction in the urinary tract had refused dialysis and died of terminal uraemia at the age of 36. None of these three patients had had UTIs during childhood.

Table 8. Patients monitored in the Northern Ostrobothnia Hospital District who had been born after 1960 and had died before the commencement of the present work.

<table>
<thead>
<tr>
<th>Renal diagnosis</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic nephropathy</td>
<td>8</td>
</tr>
<tr>
<td>Rheumatoid disease</td>
<td>6</td>
</tr>
<tr>
<td>Reflux nephropathy</td>
<td>2</td>
</tr>
<tr>
<td>Glomerulonephritis</td>
<td>2</td>
</tr>
<tr>
<td>Unspecified uraemia</td>
<td>1</td>
</tr>
<tr>
<td><strong>Total N</strong></td>
<td><strong>19</strong></td>
</tr>
</tbody>
</table>
6 Cranberry juice for the prevention of urinary tract infections (III and IV)

6.1 Subjects

A group of 263 children aged 1–16 years who had been referred to the paediatric department of one of four university hospitals in Finland (Oulu, Tampere, Kuopio and Helsinki) or one of the three central hospitals (Joensuu, Lahti, and Kemi) on account of verified UTI in the previous two months were recruited for a prevention study (III) to be carried out over the period 2001–2008. Urinary tract imaging was performed after the first UTI in the manner recommended in Finland at the time, that is using urogenital ultrasonography and voiding cystography in all cases aged <2 years and ultrasonography alone on older children initially, followed by voiding cystography if the ultrasonography findings were abnormal. Children with severe genitourethral abnormalities and children requiring antimicrobial prophylaxis for any reason were excluded (Figure 7).

For the colonization study we recruited a total of 341 children attending day-care centres in the city of Oulu. Children receiving continuous antimicrobial treatment or having marked immunological defects were excluded. After signed, informed consent had been obtained, the children were screened by means of tympanometry and/or pneumatic otoscopy and only those with normal ear status were accepted (Figure 8).
Fig. 7. Design of the prevention trial (III).
Recruitment at day-care centers (gave informed consent) (n=341)

Random allocation

Cranberry juice for 3 months (n=171)
Placebo juice for 3 months (n=170)

Delivery of juices and follow up sheets, first faecal and nasopharyngeal sample

Diary for 3 months, family physician consulted if symptoms of infection occurred, second faecal and nasopharyngeal sample at 3 months

4 protocol violations
4 did not start drinking the juice

2 protocol violations
2 did not start drinking the juice

20 patients dropped out*
15 did not accept the juice
2 lost from follow-up

11 patients dropped out*
11 did not accept the juice

Cranberry (n=147)
Placebo (n=157)

Comparison

Fig. 8. Design of the colonization trial (IV).
6.2 Methods

Definition of UTI in the prevention trial

UTI was defined on entry as fever and/or local urinary tract symptoms (dysuria, abdominal/back pain, strong-smelling urine, enuresis) and growth of a single bacterial strain of $10^5$ or more CFU/ml in a midstream urine sample or a sample taken with a urine bag or catheter, or any growth in a suprapubic bladder aspirate sample. The same criteria for UTI were used during the trial except that two consecutive midstream or bag samples with the same bacterial growth were required. The UTI symptoms were defined in the information sheet and a study diary was given to the parents, who were asked to show it to the attendant physicians. An interval of ten days or more was required for two consecutive UTIs to be recorded as separate events. Significant bacteruria was defined as growth of a single bacterial strain of $10^5$ CFU/mL in a midstream urine sample or a sample taken with a urine bag.

Randomization

On receipt of a signed declaration of informed consent from the parents, the children were randomized with a block size of four (prevention study) or six (colonization study) to receive either cranberry juice or a placebo juice 5 ml/kg up to 300 ml a day in one or two daily doses for six months (prevention study) or in three daily doses for three months (colonization study) (Figures 7 and 8). The code was kept sealed until all the data had been collected and processed. Each centre (prevention study) received sealed envelopes for randomization purposes before the beginning of the recruitment phase, so that the clinicians were unaware of the allocation of individual patients.

Study design

At the first visit the parents completed a questionnaire providing background data, nutritional status and a medical history and were requested not to give the children any other Vaccinium products during the period of the trial.

In the prevention study the children were followed up for one year, during which their consumption of the juice and daily symptoms compatible with UTI
were recorded in a diary kept by the parents. This one-year follow-up period was chosen on the basis of our earlier experience in a trial among adult women (Kontiokari et al. 2001). The parents were advised to take the child to their own family physician whenever a fever or local symptoms suggestive of UTI occurred. When UTI was diagnosed, it was treated with a standard antimicrobial regimen, while the child continued taking the juice. If the child had three or more UTI recurrences during the trial, antimicrobial prophylaxis for six months was recommended. The physicians were asked to record their diagnosis and the resulting treatment on a follow-up sheet, which was returned to the centre concerned at the last visit. The results of all urine tests carried out during the follow-up were collected from the laboratory databases. Compliance with the protocol was determined on the basis of the self-report sheets and by counting the empty juice cartridges returned by the parents at the end of the trial.

In the colonization study the parents were advised to take the child to their own family physician every time he/she had any symptoms of infection such as fever, cough, rhinitis, earache, sore throat, vomiting, diarrhoea, dysuria, night restlessness, irritability, rash, poor appetite or conjunctival symptoms. The physicians were asked to record their diagnosis and the resulting treatment on a follow-up sheet. When any bacterial infection was diagnosed it was treated with the standard antimicrobial regimen and the child continued taking the juice. Symptoms, diagnoses, treatments and doses of the products were recorded on a self-report sheet by the parents and attending physicians and this sheet was returned at the last visit.

**Products and controls**

The commercially available cranberry juice used (Cranberry Classic) contained 41 g of cranberry concentrate, including flavouring, in one litre of juice, while the placebo drink was almost identical in appearance, smell, taste and colour but did not contain either fruit or berry extracts. The juices were supplied in similar white, coded cartridges containing 200 ml. The nutritional values of the drinks resembled those of commonly available fruit and berry juices. Both products were manufactured and provided by Ocean Spray Cranberries (Lakeville, MA, USA).
Sampling and bacterial identification

Stool samples for the colonization study were taken into dry vials at the start and at three months, and stored at −70 °C until analysed. Before gas liquid chromatography (GLC), the samples were processed to separate the bacteria from other faecal material, as described previously (Moss & Nunez-Montiel 1982, Eerola & Lehtonen 1988). A GLC profile represents all the bacterial cellular fatty acids in the sample and thus reflects its microflora and can be used to detect changes, differences or similarities in bacterial flora between individual samples or sample groups (Peltonen et al. 1992). The fibrous material from the diet and eukaryotic cells from the gut wall were removed and the methylated fatty acids were extracted from the remaining sample. GLC was performed using an HP 6890 gas chromatograph with an Ultra 2 (cross linked 5% PH ME Silicone) 25m x 0.2mm column (HP 19091B) combined with HP ChemStation analysis software.

In the colonization study nasopharyngeal samples for bacterial culture were taken at the start and at three months using a calcium alginate swab, which was immediately transferred to a transport medium (ST-GG tube, National Public Health Institute, Helsinki, Finland) (Kaijalainen et al. 2004). The samples were stored at −70 °C until analysed. The four most common respiratory pathogens, i.e. Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and Streptococcus pyogenes, were identified at the National Public Health Institute, Oulu, Finland, by routine methods that involved comparing their colony morphology and substrate utilization on plate cultures.

Sample size

A 50% reduction in recurrences was considered to be clinically important in the prevention study, being an effect that was regarded as attainable on the basis of our earlier trial (Kontiokari et al. 2001). The sample size calculations were based on earlier reports in which up to 30% of children were said to experience a new episode of UTI within twelve months (Mangiarotti et al. 2000, Nuutinen & Uhari 2001). To detect a reduction in recurrences to 15% with a two-tailed α of 0.05 and a power of 80%, and estimating that 10% of the subjects will drop out, it was decided that 130 children were needed in each group.

The assumptions used for the sample size calculations in the colonization study were based on differences in nasopharyngeal carriage. We knew the
baseline carriage rates of the most common respiratory pathogens to be around 30% in children attending day care centres and assumed that active treatment would reduce this to 15% (Uhari et al. 1996a). A reduction of this size with a two-tailed $\alpha$ of 0.05 and a power of 90% would require a sample size of 152 children in each group. Given an estimated a dropout rate of 10%, a total sample size of 336 was deemed to be needed.

**End points**

The primary end point in the prevention study was the occurrence of the first UTI episode during the 12-month follow-up. Secondary end points were the UTI incidence density and antimicrobial use.

In the colonization study the primary end points for the trial were differences in the nasopharyngeal carriage of respiratory pathogenic bacteria, faecal fatty acid composition and compliance, while the secondary end points were the total numbers of infection episodes in the groups and the duration of their symptoms. A new episode of infection was diagnosed only after the child had had at least three asymptomatic days following the previous episode.

**Statistical methods**

In the prevention study we used the Kaplan–Meier method to analyse the time elapsing before the first UTI recurrence and the log-rank test to assess the differences in cumulative survival functions between the groups. The occurrence of all episodes of UTI was expressed in terms of incidence density, calculated by summing the number of UTI attacks and the time at risk in each group and then calculating the rate of UTI episodes per person-year at risk (PYR). Each patient contributed days at risk until the point of dropping out or receiving antimicrobials for any reason (if for UTI, at least 10 days were subtracted), or until the follow-up ended. For the children who received antibiotic prophylaxis the intervention ceased at that point. The differences in UTI incidence density between the groups were tested on the assumption that the occurrence of UTI follows a Poisson distribution. The differences between the groups in the proportion of children with at least one UTI recurrence, antimicrobial days per year, antimicrobial prophylaxis and dropouts were tested with the binomial SND (Standardized Normal Deviate) test. The data were analysed with PASW Statistics for Windows (version 18.0) and Stats- Direct software (version 2.7.2).
In the colonization study the number of children carrying nasopharyngeal bacteria at each time point was calculated and the proportions of children showing no change, changing from positive to negative, or vice versa were determined and compared between the groups. The fatty acid profiles were analysed by computerized correlation and cluster analysis to calculate mean correlation values between isolates belonging to the same or different species, and to establish cluster analysis dendrograms (Peltonen et al. 1997). The cumulative drop-out rate was calculated and the children were assigned to three dosage groups, >90%, 70–90% or <70% of the recommended doses taken, and the proportions in each group counted. The numbers of episodes with any symptoms of infection (fever, cough, rhinitis, earache, sore throat, vomiting, diarrhoea, irritability or conjunctival symptoms), symptoms suggesting respiratory infection (fever, cough, rhinitis, earache, sore throat, irritability or conjunctival symptoms) and symptoms suggesting enteric infection (vomiting, diarrhoea) were counted and the mean duration of these symptoms was calculated per child and the mean of the means compared between the groups. Similarly, the diagnoses given by physicians were counted and grouped as possible bacterial (acute otitis media, pneumonia, conjunctivitis, sinusitis, bacterial tonsillitis, skin abscess, impetigo, balanitis or UTI) or viral (laryngitis, pharyngitis, bronchitis, rhinitis, upper respiratory infection, varicella, viral tonsillitis or viral rash) infections. The incidence densities of infection episodes and diagnoses were then calculated by summing the numbers of episodes and times at risk in each group and then calculating the rate of episodes per person year (365 days) at risk (PYR). Differences, standard normal deviations and 95% confidence intervals (CI) were calculated between the proportions and tested with the standard normal deviation test, t-test or Mann Whitney U-test, depending on the variable.

Ethics

The Ethical Committee of the Medical Faculty of the University of Oulu evaluated and accepted the protocols of both studies.
6.3 Results

Efficacy

The baseline characteristics of the UTI prevention groups were similar (Table 9). Eight out of the 263 children were omitted from the analysis because of protocol violation at entry, so that 255 children were included in the final analyses (Figure 7).

Table 9. Baseline characteristics of the subjects in the cranberry juice and placebo juice groups in the prevention study.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cranberry (n=126)</th>
<th>Placebo (n=129)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Girls (%)</td>
<td>115 (91)</td>
<td>117 (91)</td>
</tr>
<tr>
<td>Age (years) mean (SD), y</td>
<td>3.8 (2.5)</td>
<td>4.5 (2.9)</td>
</tr>
<tr>
<td>No. of toddlers (aged 1-3 years)</td>
<td>56</td>
<td>47</td>
</tr>
<tr>
<td>Previous UTI morbidity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of UTIs, mean (SD)</td>
<td>1.6 (1.3)</td>
<td>1.6 (1.3)</td>
</tr>
<tr>
<td>Children with at least two UTIs</td>
<td>38 (30)</td>
<td>38 (30)</td>
</tr>
<tr>
<td>Frequency of intake of berry or fruit juices¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 2 times a week</td>
<td>75 (61)</td>
<td>78 (62)</td>
</tr>
<tr>
<td>1-2 times a week</td>
<td>16 (13)</td>
<td>21 (17)</td>
</tr>
<tr>
<td>Less than once a week</td>
<td>32 (26)</td>
<td>26 (21)</td>
</tr>
<tr>
<td>Frequency of intake of berries²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>More than 2 times a week</td>
<td>16 (13)</td>
<td>29 (23)</td>
</tr>
<tr>
<td>1-2 times a week</td>
<td>51 (43)</td>
<td>45 (36)</td>
</tr>
<tr>
<td>Less than once a week</td>
<td>52 (44)</td>
<td>50 (40)</td>
</tr>
<tr>
<td>Constipation³</td>
<td>2 (2)</td>
<td>8 (6)</td>
</tr>
<tr>
<td>Vesicoureteral reflux grade I-II</td>
<td>1 (0.8)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>Duplex system</td>
<td>0</td>
<td>1 (0.8)</td>
</tr>
</tbody>
</table>

All data are no. (%) unless otherwise specified.

Abbreviations: SD, standard deviation; UTI, urinary tract infection.

¹ Missing data: 3 children in the cranberry group and 6 children in the placebo group,
² Missing data: 7 children in the cranberry group and 5 children in the placebo group,
³ Less than 3 bowel movements a week

A total of 27 (11%) children dropped out: 16 (13%) in the cranberry group and 11 (9%) in the placebo group (difference 4%, 95% CI −4% to 12%, P=0.24)
10). The most common reason was the child’s reluctance to drink the juice (7 vs. 6 children in the cranberry and placebo groups, respectively).

There were 20 (16%) children in the cranberry group and 28 (22%) in the placebo group who had at least one recurrent UTI during the 12-month follow-up (difference −6%, 95% CI −16 to 4%, P=0.21) (Table 10). There was no significant difference in the timing of the first UTI recurrence (P=0.32) (Figure 9). A total of 74 episodes of UTI were recorded (Table 10). The UTI incidence density per PYR was significantly lower in the cranberry group, but the proportion of children having more than one recurrence was similar in both groups (Figure 10, Table 10). The difference between the groups became apparent 5–6 months after starting the juice (Figure 10).

Table 10. UTI recurrences and compliance of the subjects in the cranberry juice and placebo juice groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cranberry (n=126)</th>
<th>Placebo (n=129)</th>
<th>Difference</th>
<th>95% CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children with recurrent UTI, no. (%)</td>
<td>20 (16)</td>
<td>28 (22)</td>
<td>6%</td>
<td>-16% to 4%</td>
<td>0.21</td>
</tr>
<tr>
<td>1 episode</td>
<td>16</td>
<td>17</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 episodes</td>
<td>2</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 episodes</td>
<td>1</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 episodes</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of UTI episodes</td>
<td>27</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence density of UTI per PYR</td>
<td>0.25</td>
<td>0.41</td>
<td>-0.16</td>
<td>-0.31 to -0.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Antimicrobial days per year</td>
<td>11.6</td>
<td>17.6</td>
<td>-6</td>
<td>-7 to -5</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antimicrobial prophylaxis, no. (%)</td>
<td>4 (3)</td>
<td>7 (5)</td>
<td>-2%</td>
<td>-8% to 32%</td>
<td>0.38</td>
</tr>
<tr>
<td>Compliance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doses taken</td>
<td>64%</td>
<td>80%</td>
<td>-16%</td>
<td>-25% to -7%</td>
<td>0.001</td>
</tr>
<tr>
<td>&gt;90 % of doses taken, no. (%)</td>
<td>58 (46)</td>
<td>79 (62)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-90 % of doses taken, no. (%)</td>
<td>21 (17)</td>
<td>27 (21)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50 % of doses taken, no. (%)</td>
<td>47 (37)</td>
<td>21 (17)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dropouts</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dropouts, No. (%)</td>
<td>16 (13)</td>
<td>11 (9)</td>
<td>4%</td>
<td>-4% to 12%</td>
<td>0.24</td>
</tr>
<tr>
<td>Follow-up days, mean (range)</td>
<td>111 (7-259)</td>
<td>122 (26-259)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; PYR, person-year at risk; UTI, urinary tract infection.
Fig. 9. Timing of the first UTI recurrence during the 12-month follow-up in children receiving cranberry juice or placebo juice. The difference between the groups was not significant (P=0.32).
The children in the cranberry group had significantly fewer antimicrobial days, and antimicrobial prophylaxis was started on account of UTI recurrences in 4 of 126 (3%) cases in the cranberry group and 7 of 129 (5%) in the placebo group (Table 10). The incidence densities of UTI during the cranberry prevention regimen were 0.36 per PYR in the cranberry group and 0.54 per PYR in the placebo group (difference -0.18, 95% CI, -0.42 to 0.06, P=0.15), and those recorded after that were 0.12 per PYR in the cranberry group and 0.26 per PYR in the placebo group (difference -0.14, 95% CI, -0.31 to 0.02, P=0.09).

Three of the 23 boys (cranberry 2, placebo 1) and 45 of the 232 girls (cranberry 18, placebo 27) had at least 1 recurrent UTI. The differences in the proportions between the cranberry and placebo groups were not statistically significant. There were a total of 3 recurrent UTI episodes among the boys (cranberry 2, placebo 1) and 71 among the girls (cranberry 25, placebo 46). The UTI incidence density per PYR among the girls was significantly lower in the cranberry group (0.25 vs. 0.46 episodes per PYR; difference 0.20, 95% CI 0.24 to 0.20, P<0.01). When the toddlers (aged 1–3 years) and older children were
analysed separately, the results were consistent with the whole group results but the differences between the cranberry group and placebo group were no longer statistically significant.

*E. coli* was the dominant bacterial pathogen, found in 79% of the isolates in both groups (P=0.96). All the baseline samples were negative, and only one control sample had significant bacterial growth at 12 months. This child had no symptoms, however, and the growth was considered to represent asymptomatic bacteruria and was not treated.

Table 11. Baseline subject characteristics and compliance.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cranberry (n=171)</th>
<th>Placebo (n=170)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of girls, no. (%)</td>
<td>87 (51)</td>
<td>82 (48)</td>
</tr>
<tr>
<td>Mean age, years (SD)</td>
<td>4.4 (1.6)</td>
<td>4.1 (1.6)</td>
</tr>
<tr>
<td>Breast feeding at any time, no. (%)</td>
<td>154 (95)</td>
<td>162 (9)</td>
</tr>
<tr>
<td>Mean duration of day care (mo)</td>
<td>25.1</td>
<td>22.2</td>
</tr>
<tr>
<td>Previous history of acute otitis media, no. (%)</td>
<td>138 (87)</td>
<td>144 (88)</td>
</tr>
<tr>
<td>Smoking parent, no. (%)</td>
<td>57 (35)</td>
<td>46 (28)</td>
</tr>
<tr>
<td>Drop-outs, no. (%)</td>
<td>18 (11)</td>
<td>11 (7)</td>
</tr>
<tr>
<td>Refused to drink the juice</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>Parents tired of the trial</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Rash</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gastric symptoms</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Illness during the trial</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Reason not known</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Compliance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90% of doses taken, no. (%)</td>
<td>139 (84)</td>
<td>129 (77)</td>
</tr>
<tr>
<td>70–90% of doses taken, no. (%)</td>
<td>17 (10)</td>
<td>31 (18)</td>
</tr>
<tr>
<td>&lt;70% of doses taken, no. (%)</td>
<td>9 (6)</td>
<td>8 (5)</td>
</tr>
</tbody>
</table>

Percentages are calculated from the number of subjects giving current information.

**Effects on normal flora**

The baseline characteristics were essentially the same in both groups in the colonization study (Figure 8, Table 11). Six children did not start taking the juice at all and two were lost from the trial without any data on their consumption of the juice. Samples were taken from all the children at the start and from those still taking the juice at three months who were available for sampling. This gave us a total of 341 (100%) baseline nasopharyngeal samples and 304 (89%) control samples after three months, and similarly 189 (55%) and 156 (46%) faecal
samples. The mean age of the children in the colonization study was 4.3 years, ranging from 1 to 7 years, and the background characteristics of the two groups were similar (Table 11). The number of dropouts during the three months was 18 (11%) in the cranberry group and 11 (7%) in the placebo group (Figure 8, Table 11).

Fig. 11. Changes in the nasopharyngeal carriage of respiratory pathogenic bacteria during 3 months of receiving cranberry or a placebo drink. The number of children carrying nasopharyngeal bacteria at each point was calculated and the proportions showing no change, changing from negative to positive (+/-), or vice versa (+/-) were counted and a P-value calculated for the difference between the groups. All differences were statistically non-significant (P=0.12–0.92).

There were no statistically significant changes in the nasopharyngeal carriage of respiratory pathogenic bacteria within or between the groups (Figure 11). The overall carriage and variation in S. pneumoniae was greatest, ranging from 27% to 46%, and the carriage of M. catarrhalis also varied greatly, from 7% to 25%,
compared with the stable carriage of *H. Influenzae* (11–16%) and *S. pyogenes* (2%).

The variation in bacterial fatty acid composition was greater within the groups than between them, i.e. the bacterial flora changed in both groups over time (P<0.001) but did not differ markedly between the groups (P>0.05).

### Table 12. Infectious symptoms.

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Cranberry (n=165)</th>
<th>Placebo (n=168)</th>
<th>Difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any infection symptom¹</td>
<td>12.0</td>
<td>12.6</td>
<td>-0.7 (-2.3 to 0.9)</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean number of episodes/PYR</td>
<td>8.5</td>
<td>9.2</td>
<td>-0.7 (-3.4 to 1.9)</td>
<td>0.52</td>
</tr>
<tr>
<td>Mean duration of symptoms (days)</td>
<td>11.1</td>
<td>11.6</td>
<td>-0.55 (-2.1 to 1.0)</td>
<td>0.48</td>
</tr>
<tr>
<td>Mean number of episodes/PYR</td>
<td>8.7</td>
<td>9.4</td>
<td>-0.7 (-3.4 to 1.9)</td>
<td>0.46</td>
</tr>
<tr>
<td>Symptom of respiratory infection²</td>
<td>1.9</td>
<td>2.3</td>
<td>-0.45 (-1.1 to 0.2)</td>
<td>0.18</td>
</tr>
<tr>
<td>Mean number of episodes/PYR</td>
<td>0.62</td>
<td>0.66</td>
<td>-0.04 (-0.3 to 0.2)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

The number of episodes is given per person-year at risk (PYR). The mean duration of symptoms was calculated per child and the means of these means were compared.

¹Fever, cough, rhinitis, earache, sore throat, vomiting, diarrhoea, irritability, conjunctival symptoms,
²Fever, cough, rhinitis, earache, sore throat, irritability, conjunctival symptoms,
³Vomiting, diarrhoea.

There were equal numbers of infection episodes in both groups, and the duration of symptoms was similar (Table 12). Respiratory symptoms accounted for most of the symptoms (Table 12). The clinical diagnoses reached by the attending physicians were also quite similar in the two groups, with acute otitis media, upper respiratory infection, acute bronchitis and acute conjunctivitis the four most common diagnoses, accounting for 71% of all the diagnoses (Table 13). Accordingly, the need for antimicrobials did not differ between the groups, either.

### Acceptability

An average of 64% of the doses of cranberry juice and 80% of the doses of the placebo in the prevention study were actually taken (P=0.001), indicating that compliance was better among the children receiving the placebo (Table 10). Only seven children (5%) in the cranberry group and six (4%) in the placebo group did not accept the juice. In the colonization study 18 children in the cranberry group
(11%) and 11 children in the placebo group (6%) stopped taking the juice before the end of the trial (Figure 8, Table 11). About 80% of children in both groups took more than 90% of the doses as instructed (Table 11).

Table 13. Incidence densities of clinical diagnoses, given as the mean number of diagnoses per person-year at risk (PYR)

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Cranberry (n=165)</th>
<th>Placebo (n=168)</th>
<th>Difference (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Possible bacterial infections(^1)</td>
<td>2.1</td>
<td>1.9</td>
<td>0.2 (-0.5 to 0.8)</td>
<td>0.6</td>
</tr>
<tr>
<td>Possible viral infections(^2)</td>
<td>1.1</td>
<td>1.3</td>
<td>0.2 (-0.7 to 0.3)</td>
<td>0.4</td>
</tr>
<tr>
<td>Four most common infections(^3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute otitis media</td>
<td>1.5</td>
<td>1.3</td>
<td>0.2 (-0.3 to 0.8)</td>
<td>0.4</td>
</tr>
<tr>
<td>Bronchitis</td>
<td>0.2</td>
<td>0.3</td>
<td>0.1 (-0.3 to 0.2)</td>
<td>0.6</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>0.1</td>
<td>0.4</td>
<td>-0.2 (-0.5 to 0)</td>
<td>0.05</td>
</tr>
<tr>
<td>Upper respiratory infection</td>
<td>0.6</td>
<td>0.8</td>
<td>-0.2 (0.6 to 0.1)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

\(^1\) Otitis media, pneumonia, conjunctivitis, sinusitis, bacterial tonsillitis, skin abscess, impetigo, balanitis, UTI, \(^2\) Laryngitis, pharyngitis, bronchitis, rhinitis, upper respiratory infection, varicella, viral exanthema, viral tonsillitis, \(^3\) Accounting for 71% of all diagnoses.
7 Discussion

7.1 Biofilm formation by uropathogens as an aspect of pathogenesis (I)

We found that one third of the *E. coli* strains isolated from clinical urine samples formed a biofilm *in vitro*. The strains from the patients with pyelonephritis were more capable of forming a biofilm than those isolated from the cystitis patients. The biofilm-forming *E. coli* strains were more susceptible to antibiotics than those that did not form a biofilm. In two previous studies comparing *E. coli* strains isolated from prostatitis, pyelonephritis and cystitis patients by similar methods no differences in biofilm-forming capacity were found between the strains causing cystitis and pyelonephritis, probably because of the use of a qualitative method for determining biofilm formation (Kanamaru *et al.* 2006, Soto *et al.* 2007). The proportions of biofilm-positive uropathogenic *E. coli* isolates and their susceptibilities to antibiotics reported by us are similar to those found by Soto *et al.* (Soto *et al.* 2007).

We validated our method for measuring biofilm by adjusting the quantitative optical density values to the qualitative biofilm findings in the SEM and CSLM images (Costerton *et al.* 1999, Danese *et al.* 2000b, Donlan & Costerton 2002, Branda *et al.* 2005, Kanamaru *et al.* 2006, Reisner *et al.* 2006, Rosen *et al.* 2007, Soto *et al.* 2007), and were thus able to present images of well-organized multi-layer biofilm structures, including the water channels formed by vital bacteria. Measurement of the optical density of bacteria attached to microtitre plates appeared to be a simple and reliable method for quantifying the biofilm formation ability of *E. coli*.

The biofilm formation detected in an *in vitro* setting may be different from that occurring *in vivo*. In addition to the phenotype and genotype of the bacteria, the biofilm-forming capability of *E. coli* is also associated with the growth media, growth surfaces and other environmental factors (Pratt & Kolter 1998, Reisner *et al.* 2006, Naves *et al.* 2008b). In addition to this, the storage of the bacterial isolates, and especially serial cultures, may affect their genotype and alter their mode of growth (Fux *et al.* 2005). We were interested in the ability of *E. coli* strains to form a biofilm in favourable circumstances, however, and used clinical strains, which had been frozen, stored and revived only once. Our results demonstrate an association between biofilm formation and clinical presentation.
with UTI, which indicates that we were able to measure a real biological phenomenon.

*E. coli* has been shown to form persistent intracellular biofilm-like reservoirs in uroepithelial cells (Mulvey et al. 2000, Anderson et al. 2003, Soto et al. 2006, Rosen et al. 2007). Living bacteria are occasionally released from the biofilm, and some of these may start to grow in planktonic form (Hancock et al. 2007). If the released bacteria are virulent enough they may reinvade the uroepithelium and cause recurrent cystitis, or ascend to the kidneys and cause pyelonephritis (Soto et al. 2006). Uropathogens frequently gain access to the lower urinary tract, but their persistence in the bladder and further ascent to the kidneys are more restricted (Nicolle et al. 2005). The ability of *E. coli* to form a biofilm provides the uropathogens with more opportunities to invade those parts of the urinary tract that are more difficult to reach (Soto et al. 2007). In our material most of the *E. coli* strains isolated from pyelonephritis patients formed a biofilm and all the strains with a very low biofilm forming ability were isolated from cystitis patients.

Bacteria in biofilms are protected from antimicrobial agents by reduced penetration and/or active efflux of the antimicrobial drugs from the biofilm (Costerton et al. 1999, Stewart & Costerton 2001, Smith 2005, Lynch et al. 2007), and they are therefore not under the same selection pressure as planktonic bacteria and do not develop resistance by mechanisms that are manifested in the laboratory tests used to assess the minimal inhibitory concentration of planktonic bacteria. The lower penetrance of antibiotics into biofilms may lead to suboptimal antibiotic therapies, which, apart from causing clinical failures, may also promote the production of more biofilm, which will further reduce the possibilities for eradicating the bacteria from the urinary tract (Hoffman et al. 2005).

### 7.2 Long-term consequences of urinary tract infections in childhood (II)

Our literature search failed to identify any papers designed to evaluate the aetiological fraction of UTI in childhood as a cause of CKD, or any in which population based occurrence figures could be calculated. Many of the items on the PRISMA checklist were not applicable (Moher et al. 2009), and taking into account the variability in the methods used during a period going back 50 years, we found just ten reports on childhood UTIs in CKD patients or on clinically significant long-term renal consequences of childhood UTIs. There were no
patients among the 1576 cases reviewed in these reports for whom childhood UTIs were the main cause of subsequent CKD, i.e. all of the patients who had experienced childhood UTIs and developed CKD either had a specific non-infectious cause for their CKD or else had structural kidney anomalies in their first kidney imaging examination, although there was admittedly no information on the kidney imaging results for three of the patients who had had UTIs in childhood. Of the 366 living adult patients with CKD who were monitored in our hospital, only three had experienced recurrent UTIs in childhood that might have contributed to the development of CKD, and these had had structurally abnormal kidneys in their first examinations. When population-based occurrence figures were calculated from our hospital data, the aetiological fraction of childhood UTIs as a cause of CKD was less than one percent.

We analysed the data on childhood UTIs for the cohort of all patients treated or monitored on account of CKD in the geographically defined area of northern Finland. As the patient records for Finnish children have been available since the 1930s (when infant welfare clinics were introduced), and their reliability and comprehensiveness were improved under the Primary Health Care Act of 1972, the Finnish health care system provides reasonably reliable clinical data on childhood UTIs even for patients who were children 50 years ago. The significance of UTIs in general, and especially in childhood, as a cause of CKD was nevertheless substantially smaller in our material than could have been expected on the grounds of previous registry data. There are several possible explanations for this. In the first place, we were able to get more detailed information on the aetiology of CKD and the history of UTIs in childhood than could be derived from registers, and we were thus able to evaluate the significance of childhood UTIs alone more specifically and take into account the existence of structural kidney abnormalities etc. On the other hand, advancements in diagnostic methods and the treatment of UTIs have also had an impact on the long-term prognosis for UTI in childhood.

As it is thought that recurrent UTIs in childhood, especially together with VUR, can lead to development of renal scars, hypertension and kidney failure, imaging is routinely recommended and performed after the first UTI in order to identify urinary tract abnormalities and VUR (Jacobson et al. 1989, Jakobsson et al. 1994, Smellie et al. 1998, American Academy of Pediatrics 1999, Vallee et al. 1999, Orellana et al. 2004, Swerkersson et al. 2007). The existing concept of childhood UTIs as a risk factor for kidney failure is nevertheless based on analyses of selected patient series. According to our results, VUR with UTI
without structural abnormalities in the kidneys seems not to cause CKD, and so, understandably, active treatment of VUR does not reduce the occurrence of CKD and large prospective follow-up studies have shown renal function to be well preserved in patients with VUR (Smellie et al. 1998, Craig et al. 2000, Jodal et al. 2006, Venhola et al. 2006). The renal problems associated with primary VUR are more probably sequelae of congenital abnormalities such as renal dysplasia than of VUR itself (Risdon et al. 1993). VUR is a self-limiting, age-related phenomenon, the prevalence of which among otherwise healthy children is significantly higher than traditional estimates would lead us to believe, and it is not associated with urinary tract infections (Köllerman & Ludwig 1967, Hannula et al. 2010).

It is difficult to differentiate between infectious scars and hypoplasia or dysplasia associated with congenital malformations. A hypoplastic kidney is defined as a morphologically normal kidney with nephrons reduced in either number or size, while a dysplastic kidney is one with a disorganized renal parenchyma resulting from a failure to undergo normal cellular differentiation during organogenesis. As a dysplastic kidney is often associated with malformations in other parts of the urinary tract, renal dysplasia is best regarded as an abnormality of the whole urinary tract (Risdon et al. 1975). Also, because it is rare for scars to develop at new sites during follow-up and the scarring rate is independent of the age at which the kidneys are imaged, it seems that the scars are signs of congenital abnormalities rather than consequences of UTI (McKerrow et al. 1984, Jakobsson et al. 1994, Coulthard et al. 1997, Vernon et al. 1997, Smellie et al. 1998, Hoberman et al. 1999). As the dysplastic features may be microscopic and the dysplastic tissue may be located as islets among normal tissue, renal dysplasia is often impossible to differentiate from renal hypoplasia or infectious scars by imaging alone (Risdon et al. 1975). Shrunken or hypoplastic kidneys found in paediatric patients with CKD by imaging are probably a sign of renal dysplasia rather than a consequence of UTIs (Risdon et al. 1993). In the case of all three patients in our series with childhood UTIs that could have contributed to the development of CKD, hypoplastic kidneys had been seen at their first imaging examination, and, consistent with earlier suggestions, we think that the patients had most probably had these changes before the recurrent UTIs occurred (Craig et al. 2000).

The significance of childhood UTIs and the need for interventions to reduce the risk of their rare long-term consequences are both matters that depend on the
point of view adopted by the patient, parents, doctor, health care system etc. (Chambers 1997). There are many widespread practices in medicine that are not based on evidence but include unpleasant and possibly harmful procedures which may lead to unnecessary treatment and follow-up. Because 2% to 8% of all children experience UTIs at some stage and 20% to 30% of those children have VUR, many children are subjected to repeated radiological imaging and long-term antibiotic prophylaxis. The mean effective radiation dose in one fluoroscopic voiding cystoureterography is 0.5 to 1.5 mSv, the organ-specific radiation dose for the ovaries is 0.5 to 1.5 mGy, and the mean radiation dose for the supporting adult is 0.14 mGy (Perisinakis et al. 2006, Sulieman et al. 2007). The effective doses depend on the age and size of the patient and correspond to more than 50 native chest X-ray examinations or up to 6 months of environmental radiation in Finland (Perisinakis et al. 2006, Kiljunen et al. 2009).

Since all the patients with UTIs in childhood and subsequent CKD considered in this work had abnormal findings in their first kidney imaging examination which could have been observed by ultrasonography, ultrasonography performed after a UTI in childhood seems to be sufficient to identify patients at risk of developing CKD. It may be that not even ultrasound is necessary for all children after the first UTI, but more evidence is needed before recommendations can be given (Hoberman et al. 2003, Zamir et al. 2004, Miron et al. 2007, Montini et al. 2009). Furthermore, as children with normal kidneys have no significant risk of CKD, the objective of preventing UTIs should be to reduce the distress caused by recurrent episodes. The current recommendation to search actively for VUR and to protect the kidneys from damage caused by UTIs by means of long-term antibiotics leads to unnecessary radiation exposure, imposes a burden on patients and families, and implies unnecessary use of the health care system.

### 7.3 Cranberry juice for the prevention of urinary tract infections (III and IV)

Regular drinking of cranberry juice reduced the number of UTI recurrences by 43%, but did not significantly reduce the number of children experiencing at least one recurrence after the initial episode. Among adults, cranberry juice has been reported to reduce both the number of patients affected and the number of episodes (Avorn et al. 1994, Kontiokari et al. 2001, Stothers 2002). We had a lower than expected recurrence rate among our patient, but as we based our
sample size calculations on an expected recurrence rate of 30% by reference to earlier studies, our trial lacked statistical power (Uhari et al. 1996b, Nuutinen & Uhari 2001). Nevertheless, only 22% of the control children in our trial experienced at least one recurrence, which was comparable to the rate of 19% recorded in a recent UTI prevention trial (Craig et al. 2009). Even though the UTI incidence density was a secondary outcome, our figures indicate a true protective effect of cranberry juice against UTI in children comparable to that found in adults. The preventive effect was evident in the second half of the period, a finding that is in line with the observation in our earlier trial that cranberry-lingonberry juice maintained its protective effect against UTI for at least six months after the women ceased to consume it (Kontiokari et al. 2001).

We also found in our trial that the UTI recurrence rate of the children was lower than that of the adults, and that the recurrences occurred later (Kontiokari et al. 2001). Whereas 30% of the adult women in the control group had had at least one recurrence at 3 months, this figure was only 10% in the children’s placebo group, while at 12 months the figures were 39% and 22%, respectively (Kontiokari et al. 2001). It seems that children develop recurrences of UTI at a constant rate over time, an observation supported by findings in other studies (Nuutinen & Uhari 2001, Craig et al. 2009). The slower and lower UTI recurrence rate in children may be due to differences in UTI risks and pathogenesis, which are quite well characterized in adults but not so well in children (Stull & LiPuma 1991, Mårild & Jodal 1998, Nuutinen & Uhari 2001, Conway et al. 2007, Shaikh et al. 2008, Craig et al. 2009). Uropathogenic bacteria are able to create intrauroepithelial pods where they rest in biofilm form and cause recurrences or relapses when circumstances are suitable (Anderson et al. 2003). This has been suggested as an explanation for the numerous recurrences that occur in adults soon after an initial UTI.

Cranberry products are thought to act against uropathogenic bacteria, mostly E. coli, by inhibiting their growth and P-pilia-mediated adhesion, and possibly by reducing their biofilm production (Sobota 1984, Zafiri et al. 1989, Foo et al. 2000, Reid et al. 2001, Howell et al. 2005, Valentova et al. 2007, Lin et al. 2011). Our concept of its mechanism of action, however, is based on in vitro studies. The beneficial effects of cranberry may be experienced in the urinary tract, in the gut or in both. The selection pressure created in the stool by the presence of cranberry metabolites may induce a shift towards a less uropathogenic bacterial flora, and hence prolonged protection against UTI while changes in the composition of the
gut flora and the virulence of the pathogens present may take place slowly, so that
the efficacy of cranberry juice for the prevention of UTI will not be seen
immediately. On the other hand, starting the juice 1–2 months after a UTI episode
is too late for preventing the causative pathogens from forming a biofilm in the
uroepithelium during the acute infection. The above mechanism would be in
accordance with our finding that cranberry juice does not prevent the first
recurrence but does reduce the number of subsequent episodes. Thus the
preventive effect would be optimal if the juice was started as soon as possible,
preferably at the same time as antimicrobial treatment, which may itself induce
biofilm formation (Hoffman et al. 2005). The current evidence on the effect of
ingested cranberry on biofilm formation by UPEC does not support the latter
hypothesis, however (Reid et al. 2001, Valentova et al. 2007, Laplante et al. 2012,
Tapiainen et al. 2012), so that, since biofilm formation and the expression of the
genesis involved are highly dependent on growth conditions, further studies are still
needed (Naves et al. 2008b).

In a recent RCT, cranberry juice had no effect on recurrences of UTI among
college women (Barbosa-Cesnik et al. 2011). The recurrence rate in that series
was about 50% of that reported by Kontiokari et al. (16% vs. 30%) and the
patients had had fewer previous UTIs (3.7 vs. 6) (Kontiokari et al. 2001, Barbosa-
Cesnik et al. 2011). It may be that the difference in the preventive effect of
cranberry juice between the two trials is associated with differences in the diets of
the populations concerned. If the diet includes factors that induce similar
beneficial changes in bacterial flora to cranberry, the effect of a cranberry
intervention will be less evident. Also, Kontiokari et al. used cranberry-
lingonberry juice, and the lingonberry compound may have had an effect on the
recurrences (Kontiokari et al. 2001).

According to the urine samples taken at the start and end of the trial there was
only one child with asymptomatic bacterial growth, so that we believe that our
results were not compromised by the presence of asymptomatic bacteruria cases.
In addition, we excluded those children who would most urgently need
antimicrobial prophylaxis, because we considered it unethical to leave them
without medication. The group included those with severe VUR, who have the
highest risk of UTI recurrences (Nuutinen & Uhari 2001). The majority of
children who suffer from UTIs have no underlying urinary tract pathology and
may benefit from cranberry juice.

Antimicrobial prophylaxis is of limited efficacy in preventing UTIs (Montini
large placebo-controlled trial found a modest reduction in the number of children having at least one recurrence a year, from 19% to 13%, i.e. a decrease of 6 percentage points, or a relative reduction of 65%, implying that 14 children had to be treated to prevent one case of UTI (Craig et al. 2009). Long-term prophylactic antimicrobials may induce antimicrobial resistance, and their use may similarly involve problems of compliance (Uhari et al. 1996b, Craig et al. 2009, Williams & Craig 2009). The use of cranberry juice for children seems to share some of these problems, such as limited efficacy and low compliance but even so, cranberry juice has many advantages over antimicrobials, as it does not induce antimicrobial resistance (McMurdo et al. 2009). On the contrary, antimicrobial consumption was reduced by 34%, or 6 days per patient-year, in the cranberry group.

Most of the bacteria affected by cranberry and other berries of the Vaccinium family are harmful to humans, while bacteria considered to belong to the normal beneficial bacterial flora are not affected. Clinically, cranberry juice, capsules and/or powder have been shown to reduce UTI recurrences and salivary counts of bacteria causing dental caries (Avorn et al. 1994, Kontiokari et al. 2001, Stothers 2002, Weiss et al. 2004). We were unable to see any change in oral bacterial carriage, even though cranberries have been found to act anti-adhesively on some of these bacteria in vitro. Our trial similarly showed that drinking cranberry juice did not markedly affect the bacterial composition of the stools, and gastric symptoms, which might be related to disturbed faecal bacterial balance, were rare, occurring in only 1% of the cranberry group during the three months of the trial.

Most bacteria causing human diseases, especially colonic bacteria, arise from our own bacterial flora, which is in constant contact with ingested food items. It would be logical to assume that the risk of contracting infectious diseases could be affected by our diet, but only a few attempts have been made to evaluate this association (Peltonen et al. 1997). The consumption of berries, fruits and dairy products containing probiotic bacteria, for example, has been found to be associated with a decreased risk of UTI (Kontiokari et al. 2003, Kontiokari et al. 2004). In our trial, adding cranberry juice to the normal diet did not alter the frequency of common infectious diseases in the children over a period of three months. Most of the infectious episodes were respiratory infections, and there were no differences in the number of episodes or the duration of the symptoms with respect to any of the diseases evaluated. Acute otitis media was the most common bacterial disease and upper respiratory infection the most common viral
Some infections such as enteric infections or UTIs were so rare that no comparisons could be made. The children had an average of three infectious episodes during the three months, and each one lasted an average of 9 days. This means that they were sick for 1/3 of the time they spent at the day care centre, a proportion consistent with our earlier findings (Uhari & Mottonen 1999).

We did not see any major changes in the colonic bacterial flora in our children receiving cranberry juice when analysed in terms of the fatty-acid composition of the stool (Kontiokari et al. 2005). This is a crude analysis of the gram-negative bacterial flora of the gut and thus does not indicate that there cannot be changes in the ability of the bacteria to cause UTI.

Cranberry juice at a dose of 5 ml/kg was well accepted by the children, and their compliance was as good or better than with the long-term antimicrobial regimen prescribed for UTI prophylaxis (Uhari et al. 1996b, Craig et al. 2009). There was no single reason compromising its use. The children and their families easily adopted the regimens of doses one to three times a day for several months, so that in this sense cranberry juice is a feasible alternative for preventing UTIs.
8 Conclusions

1. A substantial proportion of uropathogenic *E. coli* strains are capable of forming a biofilm *in vitro*, and the strains isolated from patients having pyelonephritis form a biofilm better than those from cystitis cases. Given that a biofilm protects bacteria from antibiotics, the ability of bacteria to persist and grow in a biofilm seems to be one of the significant factors in the pathogenesis of UTIs, and it should be taken into account when creating strategies for prevention of UTIs.

2. In the absence of serious congenital anomalies, the aetiological fraction of childhood UTIs as a cause of CKD after the first UTI in childhood is very small. A child with normal kidneys has no significant risk of developing CKD because of UTIs. Imaging procedures after the first UTI can be focused on finding severe urinary tract abnormalities, for which purposes ultrasonography would be sufficient.

3. Dietary elements are significant factors in susceptibility to UTIs. Taking account of the relatively innocent nature of UTI recurrences in children who do not have any marked urinary tract pathology, cranberry juice is a feasible alternative to antimicrobials for preventing UTI in children. Cranberry juice is well tolerated and accepted by children, and it does not cause harmful changes in the normal flora.
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Original publications


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