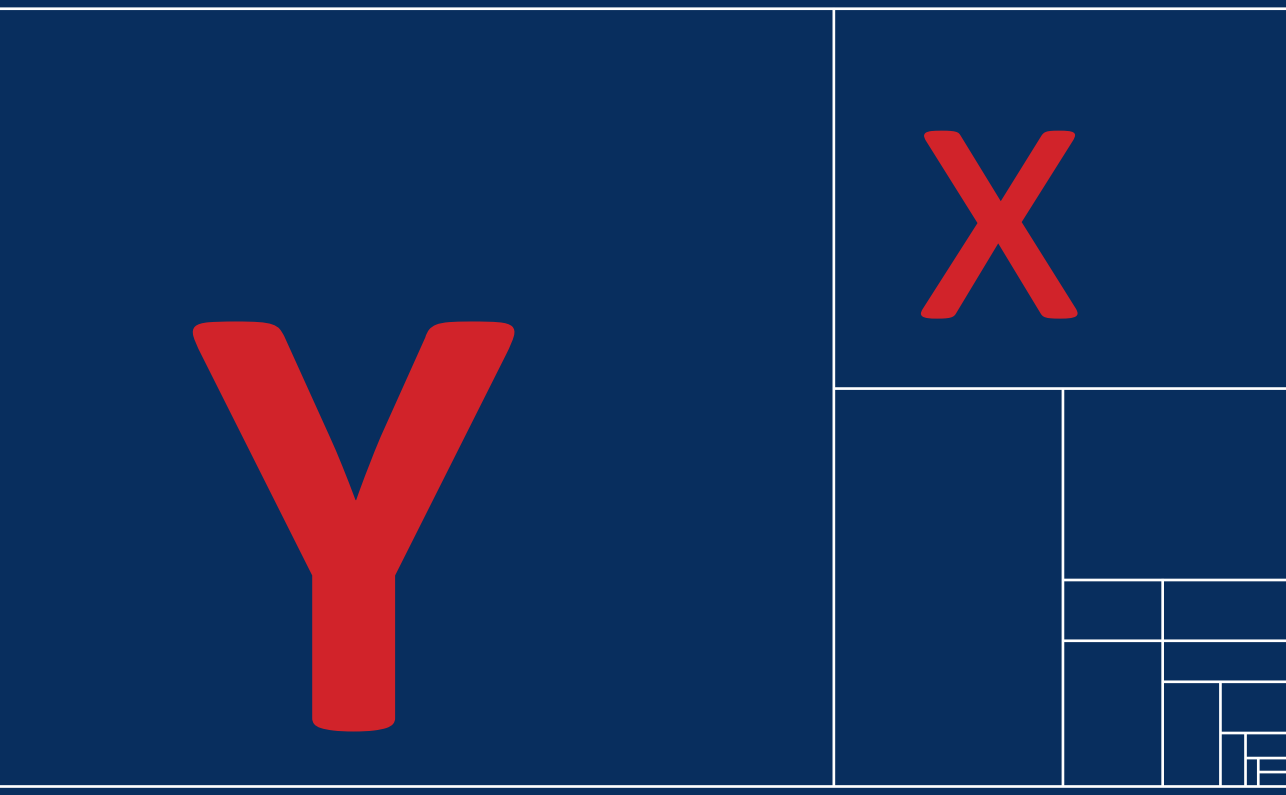
OLD MEANS FOR NEW ENDS

Optimizing the treatment of urinary tract infections



Thijs ten Doesschate

Old Means for New Ends

Optimizing the treatment of urinary tract infections

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Old means for new ends. Optimizing the treatment of urinary tract infections.

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Old Means for New Ends

Optimizing the treatment of urinary tract infections.

Oude middelen voor nieuwe doeleinden. Optimalisatie van de behandeling van urineweginfecties
(met een samenvatting in het Nederlands)

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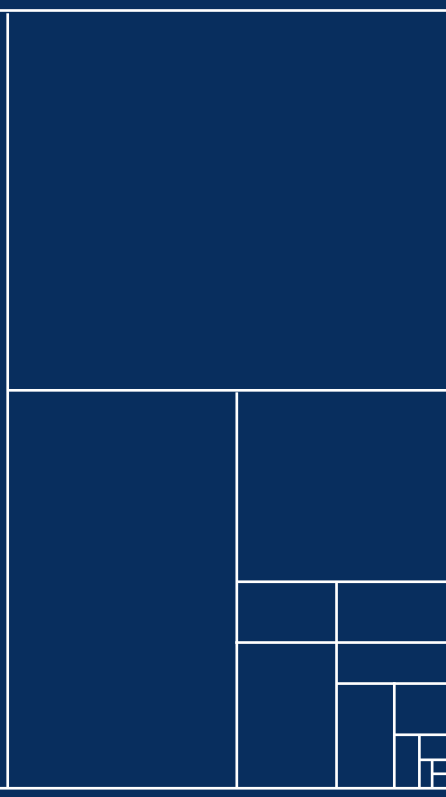
Copromotor:	Dr. C. H. van Werkhoven

Alleen ga je sneller, samen kom je verder (Nelson Mandela)

TABLE OF CONTENTS **Page**

CHAPTER 1

GENERAL INTRODUCTION



AN INTRODUCTION IN URINARY TRACT INFECTIONS

Urinary tract infections (UTIs) are common bacterial infections that are at most inconvenient for some patients while they seriously impede health for others.[1] Its high incidence leads to a substantial societal and economic burden on a population level.[1–7] This thesis addresses the management of UTI from two perspectives, that of the general practitioner and from the hospital perspective. To provide a context for this thesis, the definition, classification, incidence, pathogenesis, diagnosis and treatment of UTI in adults are summarized, after which we elaborate on the chapters in this thesis. The summary is also represented as a graphical abstract, with the chapters of this thesis involved.

Definitions, manifestations and classifications

Definitions and classifications serve to provide tools for clinical decision-making, scientific standards, quality measurement and education. Unfortunately, there is no international consensus on the exact definition and classification of UTI.[8–10] Recently, a new lexicon has been proposed by the recently originated UTIGA society (Urinary Tract Infection Global Alliance), which aims to provide clarity.[11]

At large, UTIs are defined on the presence of symptoms with bacteriuria. Herein, the ambiguous definition of bacteriuria and the low specificity of some UTI symptoms put doubt on the assessment of UTI. Being symptomatic requires symptoms or signs that result from either inflammation of the urinary tract, or from invasion of surrounding tissues or the bloodstream.[12] Inflammation of the urinary tract provokes local symptoms, i.e. dysuria, urinary frequency, urgency, incomplete bladder emptying, incontinence, haematuria, and suprapubic or lower abdominal pain. Invasion of surrounding tissue or bloodstream could result in flank pain (suggestive for pyelonephritis), perianal pain (suggestive for prostatitis), fever (≥38.0 °C), under-temperature (<35 °C), and delirium or chills (as signs of systemic inflammation).[13] Attribution of symptoms to UTI can be very difficult due to the low specificity of systemic symptoms for the presence of UTI, as are local symptoms in patients with indwelling urinary catheters.[14–17]

Bacteriuria is defined as the isolation of a specified quantitative count of bacteria from urine specimen, without contamination. There is an ongoing debate about which species in which density (colony forming units per mL) should be considered uropathogenic. In general, lowering this density threshold increases the sensitivity of establishing bacteriuria at the expense of a lower specificity. Traditionally, bacteriuria was defined on the bacterial growth from urine in a density of $\geq 10^5$ CFU/mL with little distinction between uropathogens. It has become known that the specificity of this density largely depends on the species involved, host factors and the collection method. The presence of *E.coli* in a density of 10^2 CFU/mL from midstream urine in women could be highly specific for bacteriuria, while densities up to 10^4 CFU/mL of

Enterococcus spp. or *Group B Streptococcus* regularly represent contamination.[18,19] A certain density of *Candida* spp. or *Enterococcus* spp. species could be considered uropathogenic in immunosuppressed hosts but contaminants in immunocompetent persons.[1,20] Contamination is defined variably in literature as a urine culture revealing lower colony counts, mixed bacteria, or skin flora.[21] The risk of contamination is lower if urine is collected and stored appropriately. Reference procedures for urine collection are by single catheterization or suprapubic aspiration, although these cannot be used structurally in routine care. Obtainment of midstream urine with cleansing is recommended for non-invasive routine testing, although the underlying evidence for cleansing is low.[22] Some methods to collect urine are accompanied with a high risk of contamination, e.g. urine collected from catheter bags or diapers.[19,23,24]

In the classification of UTI, a distinction is made between cystitis (or lower-UTI) with inflammation of the urinary tract only and febrile UTI (or upper-UTI or UTI with systemic symptoms) with invasion of the prostate, pyelum and/or bloodstream, causing prostatitis, pyelonephritis and/or sepsis.[20] Cystitis is a common, benign yet burdensome disease that is mainly treated in general practice, while febrile UTI is a potentially lethal disease if not treated quickly with appropriate antibiotics, often in the hospital.[1,8,25] By definition, patients with cystitis do not exhibit signs or symptoms of tissue invasion or systemic infection. Bacteriuria without any symptom or sign of UTI is called asymptomatic bacteriuria.

Cystitis has been further sub-classified into uncomplicated or complicated cystitis on the estimated risk of the host on a complicated course, i.e. on the development of recurrent or febrile UTI or other complications. Uncomplicated cystitis is defined as a cystitis episode in non-pregnant women above 12 years of age that are not immunocompromised, and do not have anatomical or functional abnormalities of the urogenital tract or diabetes mellitus.[20] The risk of progression to sepsis is very low for uncomplicated cystitis and even if treated with only painkillers prognosis is good.[26] Predisposing host factors for a complicated course are, next to the above mentioned compromised host factors, male gender and urinary catheter use.[27,28] Prognosis in patients with complicated cystitis is principally determined by the underlying host factors rather than the episode of cystitis.[29]

Particularly in clinical registry trials, a more general subdivision has been made between complicated and uncomplicated UTI, wherein uncomplicated UTI comprises cystitis or pyelonephritis in non-pregnant women above 12 years of age with normal host defences, while complicated UTI comprehends a large spectrum of disease from cystitis in hosts with predisposing risk factors as febrile UTI. Another classification exists between community and healthcare acquired UTI. Community acquired UTI is acquired outside or within 48 hours after admission to a hospital or nursing home, without being a direct

consequence of medical treatment. It is associated with a different spectrum of uropathogens than health care acquired UTI.[1,30]

Etiology and incidence

Cystitis is mostly caused through invasion of the urethra and bladder by adjacent bacteria on the perineum that originate from the bowel.[31] The interaction between pathogen and host ultimately determines whether UTI will develop. Under normal circumstances, natural defense mechanisms exist in the bladder and urethra to protect for, or to eradicate, uropathogens. Besides, urine has antimicrobial properties, as it contains few nutrients, a low pH, high nitrates and high urea. Furthermore, bacteria are diluted by increased fluid intake and cleared by frequent urination. UTI develops if defence mechanisms are hampered, either structurally or incidentally, e.g. non-secretor blood type, intercourse, dehydration. Indwelling urinary catheters pave a direct route, promoting direct migration of pathogens to the bladder.[25,27]

Cystitis is the most common reason for contacting GPs among women (3.7% of all consultations).[32] Women are disproportionally affected with about 70/1000 new cystitis episodes per year, compared to 10/1000 in men.[34] In the Netherlands this implies that among 50% of women experience at least one UTI in their lifetime, and 2-10% of women experience recurrent UTI, defined as at least 3 UTIs in the prior 12 or at least 2 UTIs in the prior 6 months.[1,34,35] Incidence peaks are observed in women of 20 to 30 years of age, related to intercourse, and in postmenopausal women between 50 and 70 years of age, often resulting from pelvic floor dysfunction, oestrogen deficiency and changes in the vaginal microbiome.[16,25,35,36]

Pyelonephritis arises if uropathogens migrate from the bladder to the kidneys, or via direct routes, i.e. through nephrostomy catheter. The ureter contains valves that prevent vesicoureteral reflux. Bacterial migration is encouraged in case of ureter obstruction through calculi or damaged valves after kidney transplantation.[37,38] The overall incidence of febrile UTI in the general population is much lower than that of cystitis, but its occurrence is clustered in aforementioned risk populations with compromised host defences.[1,8,25] In populations where multiple risk factors exist, such as in kidney transplant recipients, febrile UTI can have a high incidence and can lead to frequent hospitalizations.[39]

Health-care acquired UTIs are the fifth most common health care infection in the hospital,[40] of which 75% can be attributed to urinary catheters.[41] Duration of urinary catheterization and catheter-hygiene are important risk factors for the development of catheter associated UTI.[42]

Diagnosis

Symptoms or signs of UTI warrant the confirmation of bacteriuria, and in case of not finding bacteriuria, this should prompt for an alternative diagnosis, such as a sexually transmitted disease.[1] On the other hand, the recommendation is to not seek for bacteriuria without UTI symptoms or signs, nor if an alternative explanation already exists, as demonstration of asymptomatic bacteriuria may lead to unnecessary prescription of antibiotics.

Except for uncomplicated cystitis, diagnosis of UTI warrants a direct treatment. As it takes 1-3 days for urine culture results to be known, urine screening methods are used for direct testing of urine. Screening methods indicate UTI on the presence of bacteria or inflammation in urine. Examples are urine dipstick testing for leukocyte esterase, nitrite and erythrocytes, urine dip slide, urine sediment, urine Gram staining and urine particle flow cytometry.[43,44] In the initial approach of cystitis in Dutch general practice urine culture is considered not cost effective, so UTI diagnosis fully relies on dipstick testing with urine dip slide or urine sediment for confirmation. Multiple screening methods are sometimes needed to diagnose UTI. The presence of nitrite in dipstick testing has a high positive predictive value, but because not all bacteria form nitrite, the negative predictive value is poor. In contrast, a positive leucocyte esterase test has a high negative predictive value but a low positive predictive value. If only leucocyte esterase is positive, it therefore needs to be followed by a dip slide or urine sediment for confirmation, for which specificity lies above 90% in most studies.[34,45] Gram staining of the urine is performed in some Dutch hospitals; in non-neutropenic patients the sparse literature reports a high sensitivity and specificity, although their routine diagnostic value has not yet been sufficiently investigated.[46] Flow cytometry of urine particles has a high sensitivity but a low specificity.[47,48] In some hospitals, laboratory capacity and costs are prevented by not processing urine cultures in case of negative urine particle flow cytometry or dipstick testing, which approach is called reflex urine testing.[47,49,50]

Pathogens and antibiotic resistance

Uropathogenic *Escherichia coli* (UPEC) is responsible for 70-80% of UTI. More than other bacteria, UPEC possess virulence factors that promote the development of UTI. It has flagella, pili and adhesins that facilitate adherence to the epithelium of the urethra and bladder, and some strains possess the capacity to infiltrate, multiply, to form biofilms and to internalize into host cells, where they cause inflammation, e.g. the production of toxins, to acquit iron and to evade the immune system.[1,38] Other common uropathogens are *Klebsiella pneumoniae* and *Proteus mirabilis*. Certain uropathogens are found in specific conditions, e.g. *Enterococcus faecalis* in patients with catheter-related UTI, *Proteus mirabilis* in patients

with calculi, *Pseudomonas auruginosa* in hospitalized patients.[1,18] Novel molecular microbiological techniques have illustrated the diversity of the urine microbiome, which might play an important role in the pathogenesis of UTI, especially given the differences in microbiomes between healthy women, patients with UTI and patients with predisposing risk factors for UTI. Although this might lead to new treatment perspectives, research on this is still in its infancy.[51,52]

Antibiotic resistance may hamper the treatment of patients with recurrent febrile UTI.[7,53] Highly Resistant Enterobacterales (HRE) are defined as being resistant to ceftriaxone, this is often a result of the production of the enzyme beta-lactamase (so-called ESBL-producing Enterobacterales; ESBL-E), to carbapenem and/or to both fluoroquinolones and aminoglycosides. Within the Netherlands, HRE are more prevalent in hospitals (8% of first cultured *E.coli* in a patient) than in general practice (5% of first cultured *E.coli*).[54] *E.coli* isolates from hospitalized patients were ESBL-E in 6.1%, carbapenem resistant in 0.02% and resistant to both fluoroquinolones as aminoglycosides in 3.4%.[54] The prevalence of HRE has been more or less stable in the past five years.[8,55] Possibly, this has been the result of infection control measures and antibiotic stewardship.[55–57] Structural problems in some countries, but sporadic challenges in the Netherlands, are the high incidence of colistin resistant Enterobacterales, further .[7,65,66] In some European countries, prevalence of CRE among *K.pneumoniae* is up to 60%, while this is only 0.2% in the Netherlands.[7,67] Some populations require frequent use of broad-spectrum antimicrobials, which facilitates selection of antibiotic-resistant bacteria and diminishes the arsenal of antibiotic options for UTI.[7,58,59] Examples of such populations are kidney transplant recipients or patients with urologic malformations.[8,60] Once a patient is colonized with HRE, it takes around a half year (median) to decolonize.[61,62] According to the current guideline, ESBL-E should be covered in the empiric antibiotic treatment during the period of one year after ESBL-E colonization.[8,62–64]

Treatment

Antimicrobials for the treatment of UTI should possess antimicrobial activity against common uropathogens and reach sufficiently high concentrations in the kidney and bladder. Based on pharmacokinetic properties, guidelines distinguish antimicrobials recommended for the treatment of cystitis only and antimicrobials that can also be applied to treat febrile UTI.[8,34] For antimicrobial treatment of cystitis sufficient concentrations should be achieved in urine,[68] while for treatment of febrile UTI antimicrobials should reach invaded tissues, such as the kidneys for pyelonephritis, the prostate for prostatitis, and the bloodstream for urosepsis.[68–71]

To date, nitrofurantoin, fosfomycin (available as fosfomycin-trometamol) and trimethoprim are recommended for the treatment of cystitis in the Netherlands.[34] Some other antibiotics are recommended internationally, but we will not address these here.[72] For uncomplicated cystitis, the Dutch guideline recommends a regimen consisting of five days of normal release nitrofurantoin (Furadantin®) or extended release nitrofurantoin (Furabid®), a single-dose of fosfomycin-trometamol or a three-day treatment with trimethoprim. For complicated cystitis in non-pregnant women, the 2020 guideline recommends seven days of nitrofurantoin as first, a single dose of fosfomycin as second and seven days of trimethoprim as third choice.[33,34] Asymptomatic bacteriuria does not require antimicrobial treatment as it does not seem to affect the outcome, except for some populations in which it could probably avert complications, i.e. in preparation for endo-urological procedures, in the first month after kidney transplantation and during pregnancy[15] , although for the latter population this has been questioned.[15,73]

Regarding nitrofurantoin, consumption of the recommended dose leads to sufficient high antimicrobial concentrations in urine to eliminate *E.coli.* Due to its hydrophilic nature, nitrofurantoin does not reach sufficient concentrations in surrounding tissues or systemically, prohibiting its use for other indications than cystitis.[68,74] The resistance rate among urine *E.coli* isolates from GP patients to nitrofurantoin has been stable for decennia at about 2%, despite being used frequently.[54] Presumably, one of the reasons for this is that acquisition of resistance to nitrofurantoin among *E.coli* seems to be accompanied by loss of fitness, so that the mutated strains are less virulent.[75] Reliability of nitrofurantoin susceptibility measurement in *K.pneumoniae* is low, and *P.mirabilis* are usually resistant to nitrofurantoin.[76]

Fosfomycin-trometamol (FT) as a single dose of 3000 mg reaches urinary concentrations above the epidemiological cut-off value (ECOFF) of *E.coli* for about 48 hours, with large inter-individual variability.[77–79] The same dose results in peak concentrations in serum and prostate above the ECOFF of *E.coli*.[79–85] With the current susceptibility methods and according to EUCAST standards, around 1-2% of *E.coli* isolates is resistant to fosfomycin in the Netherlands, while this is much higher for *K.pneumoniae* (28%) and *P.mirabilis* (16%), without cross-resistance to other antibiotics or ESBL.[54] FT is currently registered for the treatment of uncomplicated cystitis in women and, since the updated NHG guideline in 2020, as second choice for the treatment of cystitis in women with diabetes mellitus, that are immunocompromised, or that have renal or urological disorders, although little evidence supports this recommendation.[34]

Oral consumption of trimethoprim leads to relatively high concentrations in urine, surrounding tissues and the bloodstream.[70] The limitation of its use for UTI mainly results from high antibiotic resistance among Enterobacterales, which has increased sharply in the Netherlands in the past two decades to above 20% for *E.coli* in general practice.[34,54] For indications other than cystitis, trimethoprim is mainly used in

combination with sulfamethoxazole, which combination broadens its antimicrobial spectrum to Gram-positive bacteria, but does not substantially increase activity against *E.coli* species.[86]

Treatment options for febrile UTI are often categorized into empirical and pathogen-directed treatment options. Empirical options are started without knowledge of the responsible pathogen or the antibiotic resistance profile, and should possess broad-spectrum antimicrobial activity. The exact coverage and composition of empirical treatment should be adapted to local epidemiology of antimicrobial resistance. For treatment of febrile UTI, ciprofloxacin (or other fluoroquinolones), amoxicillin with clavulanic acid and trimethoprim-sulfamethoxazole can be used as oral medication, against which resistance rates among *E.coli* isolates in Dutch hospitals were 14%, 36% and 22%, respectively in 2019.[54] Consequently, only fluoroquinolones are recommended as empirical treatment of febrile-UTI and only if no risk factors exist for resistance, i.e. not in patients from urology departments or that have been treated with fluoroquinolones in the prior 6 months.[8] Intravenous antibiotic regimens for the empirical treatment of febrile UTI in the Netherlands consist of a second or third generation cephalosporin or amoxicillin with clavulanic acid or a second generation cephalosporin in combination with an aminoglycoside. For patients that are colonized with ESBL-E within the prior year, the empirical treatment must be targeted to the ESBL-E resistance pattern.[8] Antimicrobials registered for treatment of febrile UTI should not be used as first line treatment for cystitis, as its use is associated with the development of resistance.[59,87] In addition to the use of antimicrobials, the treatment of UTI includes other interventions.[16] Treatment of catheter-associated UTI should include catheter removal under antibiotic coverage, and obstruction of the urinary tract requires catheter placement or other interventions to clear obstruction.[16] Patients with febrile UTI regularly require fluid administration, and sometimes intensive care monitoring.[88]

The prevention of UTIs partly consists of the treatment of underlying risk factors, e.g. bladder prolapse, obstruction, urinary retention.[16] In women these should be followed by generally accepted lifestyle advices, such as urinating after intercourse and increased water intake.[34] Recently, it was found in a randomized controlled trial that an increased water intake in women with recurrent UTI reduces the incidence of cystitis with on average 1.5 episodes in 12 months.[89] If these measures do not suffice, non-antimicrobial options, such as cranberries or hormone replacement, should be considered.[34] New non-antimicrobial treatments are available or are being developed to prevent UTI, such as dietary supplements,[90] vaccination,[91] decolonization[92] and meatal cleaning before urinary catheter insertion.[93] In the Netherlands, nitrofurantoin or trimethoprim are recommended as prophylaxis for UTI in case non-antimicrobial options fail.

AIM AND THESIS OUTLINE

The challenges in the treatment of UTI differ between the GP practice and the hospital. Studies in this thesis are subdivided into the empirical treatment and prevention of cystitis in general practice (**chapters 2 to 4**) and the treatment of UTI in view of antibiotic resistance in the hospital (**chapters 5 to 11**).

Treatment of cystitis in general practice

Nitrofurantoin, fosfomycin and trimethoprim are eliminated by glomerular filtration. In patients with renal impairment all three antibiotics are associated with lower urinary concentrations, but the effect of this on clinical effectiveness is unknown.[74,94–99] In **chapter 2**, we evaluate the effect of renal function on clinical failure after using nitrofurantoin, fosfomycin and trimethoprim for treatment of cystitis.

The Dutch guideline does not distinguish between the recommendation of four times daily 50 mg normal-release nitrofurantoin (Furadantine®) or twice daily 100 mg extended-release nitrofurantoin (Furabid®) for the treatment of cystitis.[34] Pharmacokinetic studies suggest a higher bioavailability of Furabid® and, intuitively, adherence is higher with the use of a twice instead of a four times daily regimen.[100] In **chapter 3**, we compare the clinical effectiveness of these dosing schedules in patients with cystitis.

Although nitrofurantoin is considered highly efficacious for the treatment and prevention of cystitis,[101] concerns exist about the development of lung toxicity.[102] General practice guidelines make no distinction between the use of 50 mg or 100 mg nitrofurantoin as daily prophylaxis for UTI, which implies that some patients receive twice the dose of nitrofurantoin than others for a long period of time. In **chapter 4**, we compare the effectiveness and safety between 50 mg and 100 mg nitrofurantoin as daily prophylaxis for UTI.

Urinary tract infections and antibiotic resistance among Gram-negative bacteria in the hospital

The global increase in antibiotic resistance among *Enterobacterales* fuels the continuous search for new antimicrobials. For a long time, carbapenems were considered as a refuge for the treatment of febrile UTI, having a high efficacy and good safety profile. The emergence of carbapenem resistant Enterobacterales (CRE) created the need to investigate and to develop alternative treatments. **Chapter 5** provides a systematically review of randomized controlled trials that evaluated alternatives to carbapenems with *in vitro* activity to ESBL-E for the treatment of complicated UTI.

New treatment options for UTI may arise from the development of new drugs or from the investigation of new applications of existing drugs. In **chapters 6 to 10**, we investigate several aspects in the use of fosfomycin-trometamol (FT) that is currently only registered for the treatment of uncomplicated cystitis.

The oral administration of FT and its high *in vitro* activity against multiresistant uropathogens favours its use for various unregistered indications, such as for chronic prostatitis and pyelonephritis. [103–105]

Efficacy partly depends on the measured susceptibility of the pathogen. Fosfomycin susceptibility testing in Enterobacterales is challenging in routine setting. [106] The fosfomycin Etest is used routinely to determine fosfomycin susceptibility, but the reading of MICs can be hampered by the interpretation of macrocolonies in the inhibition zone. In **chapter 6**, we investigate the inter-observer and inter-laboratory agreement of the fosfomycin Etest against the reference susceptibility test agar dilution.

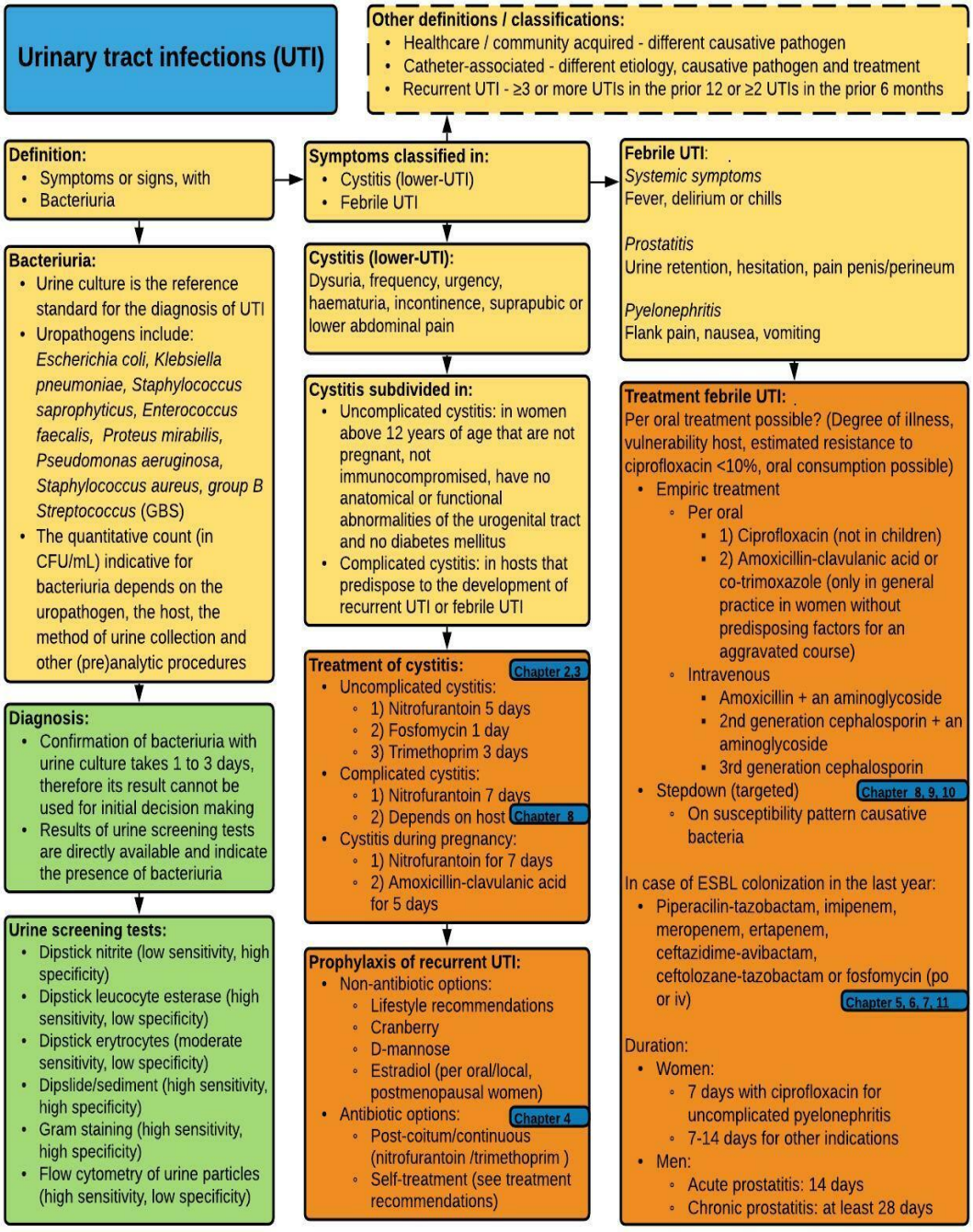
The increasing use of FT requires knowledge about acquisition of resistance to fosfomycin, in particular because a rapid increase of resistance to fosfomycin has been observed in populations with high fosfomycin use. [78,107] The case report in **chapter 7** illustrates how FT use can be accompanied with the acquisition of resistance to fosfomycin.

The use of FT for a new indication ideally requires a thorough investigation of its efficacy and safety with a randomized controlled trial (RCT). In clinical practice, the potential benefits for the unregistered use of FT may outweigh the long period required for extensive clinical evaluation. It is then important to determine its effectiveness in routine practice. An example of this has been the use of fosfomycin for cystitis in kidney transplant recipients. Antimicrobial resistance is a burden in kidney transplant recipients and regularly, no oral treatment options exist for cystitis, necessitating intravenous antibiotics and thus hospitalization for these patients whose life is already dominated by frequent hospital visits. In **chapter 8** we retrospectively determine the effectiveness of FT to treat cystitis in kidney transplant recipients.

FT is already being used for pathogen-directed stepdown treatment of febrile UTI. Regularly, hospital admission is required for the full duration of intravenous treatment if oral antibiotic alternatives do not exist. The FORECAST randomized controlled trial was designed to provide FT as an extra oral stepdown treatment for febrile UTIs that are caused by *E.coli* in women. **Chapter 9** and **chapter 10** contain the protocol and results of the FORECAST trial.

In addition to the need for new antibiotics, it is essential to limit the use of broad spectrum antibiotics. A reliable estimate of the duration of carriage of highly resistant Enterobacterales among hospital care patients is relevant for the empirical antimicrobial treatment of subsequent UTIs and other Gram negative infections. **Chapter 11** estimates the persistence of rectal carriage with highly resistant Enterobacterales. [108]

GRAPHICAL ABSTRACT



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CHAPTER 2

The effectiveness of nitrofurantoin, fosfomycin and trimethoprim for the treatment of cystitis in relation to renal function

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ABSTRACT

Objectives: We evaluated the effect of renal function on clinical failure rates of nitrofurantoin, fosfomycin and trimethoprim for treatment of cystitis in primary care.

Methods: Data was retrospectively obtained from 78 Dutch general practitioner (GP) practices between 2013 and 2019. Episodes in patients (>11 years) were eligible that required five days nitrofurantoin (NF5), single-dose fosfomycin-trometamol (FT1), three days trimethoprim (TMP3) for uncomplicated cystitis, or seven days nitrofurantoin (NF7) or trimethoprim (TMP7) for complicated cystitis. Clinical failure was defined as second antibiotic prescription for cystitis or pyelonephritis within 28 days post-prescription. Mixed effects regression analysis was used, with patient and GP practice as random effects and demography, comorbidity, cystitis history as fixed effects.

Results: Adjusted odds ratios (aOR) for clinical failure per 10 mL/min decrease in eGFR were 1.05 (95%CI 1.01-1.09) for NF5 (n= 24,591), 0.96 (95%CI 0.92-1.01) for FT1 (n=5,359), 0.98 (95%CI 0.89-1.08) for TMP3 (n= 1,064), 1.05 (95%CI 1.02-1.09) for NF7 (n= 10,628) and 1.02 (95%CI 0.93-1.14) for TMP7 (n= 831). In uncomplicated cystitis and eGFR ≥60mL/min, clinical failures occurred in 14.6% (1,895/12,980) of NF5-treated, 20.7% (266/1,283) of FT1-treated (aOR versus NF5 1.37, 95%CI 1.18-1.59) and 20.8% (66/318) of TMP3-treated patients (aOR 1.42, 95%CI 1.07-1.87 versus NF5). In uncomplicated cystitis and eGFR <60 mL/min, FT1 resulted in 16.0% (39/244) and NF5 in 23.3% clinical failures (110/472, aOR 0.61,95%CI 0.39-0.95).

Conclusions: In eGFR ≥60 mL/min treatment with fosfomycin or trimethoprim for uncomplicated cystitis was associated with more clinical failure than nitrofurantoin, while in eGFR <60 mL/min nitrofurantoin was associated with more clinical failure than fosfomycin-trometamol. Renal function, if known, should be considered in the clinical decision-making for cystitis treatment.

INTRODUCTION

Cystitis is a common bacterial infection with an annual incidence of approximately 70 per 1000 in adult women and 10 per 1000 in adult men.[1] In primary care in the Netherlands, nitrofurantoin is recommended as first-choice oral treatment for acute uncomplicated cystitis, with fosfomycin-trometamol (fosfomycin) as second choice and trimethoprim as third choice.[1] Extended regimen of nitrofurantoin and trimethoprim are first and second choice in patients with complicated cystitis, respectively, defined as having risk factors for a complicated course, i.e. male gender, diabetes mellitus (DM), urologic abnormalities and immunosuppression.[1]

The efficacy of antimicrobial treatment for cystitis largely depends on its antimicrobial activity against the pathogen and the achieved concentration in urine.[2] Nitrofurantoin, fosfomycin and trimethoprim are active against most uropathogens and are eliminated by renal excretion resulting in high concentrations in urine.[2–4] Lower urinary concentrations have been reported for all three antibiotics in patients with impaired renal function.[2–5] The concern is that efficacy declines if insufficient drug concentrations are achieved in urine, although strong, pharmacokinetic-based evidence for this concern is lacking.[4,5] Retrospective cohort studies failed to demonstrate clear effects of impaired renal function on the clinical effectiveness of nitrofurantoin or trimethoprim for the treatment of cystitis.[6,7] To the best of our knowledge, no such studies have been conducted for fosfomycin. Consequently, little evidence exists to guide the choice of antibiotic treatment of cystitis for patients with impaired renal function.

The aim of this study was to evaluate the effect of renal function on the occurrence of clinical failure when using nitrofurantoin, fosfomycin or trimethoprim for the treatment of cystitis. Furthermore, the effectiveness of nitrofurantoin, fosfomycin and trimethoprim for cystitis was compared for normal and decreased renal function.

METHODS

Design and data collection

Data were retrospectively obtained from the Julius General Practitioners' Network (JGPN), consisting of 444,782 patients receiving care from 78 general practitioner (GP) practices in the province of Utrecht, the Netherlands, in 2018.[8] The database consists of all antibiotic prescriptions to treat cystitis and includes information on patient characteristics, comorbidities and co-medication. Diagnoses were coded according to the International Classification of Primary Care (ICPC). Medication prescriptions were coded according to the Anatomical Therapeutic Chemical (ATC) classification system. Episodes were selected between January 2013 and June 2019 (see the online supplementary material S1 for definitions).

Study population

Episodes were eligible for analysis if antibiotic therapy was prescribed by the GP for the treatment of cystitis according to the Dutch guideline in patients of at least 12 years of age. Diagnoses were classified as uncomplicated or complicated according to the duration of treatment. For uncomplicated cystitis, the guideline recommends a regimen consisting of five days nitrofurantoin 100 mg extended release (Furabid®) every 12 hours or 50 mg normal release (Furadantin®) every 6 hours (NF5), a single-dose of fosfomycin 3000 mg (FT1) or a three-day treatment with trimethoprim 300 mg every 24 hours (TMP3). We excluded cystitis episodes that were treated as uncomplicated cystitis despite the presence of one of the following risk factors which define it as complicated cystitis: male gender, pregnancy, DM, urologic abnormalities and immunosuppression. For complicated cystitis, the guideline recommends nitrofurantoin and trimethoprim in an extended duration of seven days (NF7 and TMP7). Patients without documented risk factors but receiving an extended course were considered as complicated cystitis. Prescriptions occurring within 28 days of a UTI episode were considered to represent treatment failures and were therefore not included as a new cystitis episode.

Renal function was based on the most recent estimated Glomerular Filtration Rate (eGFR) value measured within six months before or after the prescription date. Episodes were excluded from the main analysis if no eGFR was measured in this period. The eGFR was calculated with the Chronic Kidney Disease Epidemiology (CKD-Epi) formula using plasma creatinine values, age and gender.[9]

Outcome

Clinical failure was defined as the prescription of one of the following antibacterial agents within 28 days of the initial prescription: nitrofurantoin, fosfomycin and trimethoprim, with exclusion of prophylactic use of trimethoprim or nitrofurantoin (>7-day use), or one of the following antimicrobials in combination with an ICPC code for cystitis or pyelonephritis: ciprofloxacin, co-trimoxazole or amoxicillin-clavulanic acid. The secondary outcome, pyelonephritis, was defined as a prescription of ciprofloxacin, co-trimoxazole or amoxicillin-clavulanic acid with an ICPC code for pyelonephritis within 28 days of the initial prescription.

Statistical analysis

Effect of renal function on clinical failure per treatment regimen

Odds ratios were calculated to determine the association between the patients' renal function and the risk of clinical failure (crude analysis) within each of the treatment regimens. Renal function was analysed in the model as a continuous variable. The linearity assumption was tested by visual inspection of residuals

plots. eGFR values ≥90 mL/min were truncated, as no effect is expected across the range of normal glomerular filtration rates on the effectiveness of these antibiotics.[10] For the multivariable analysis, a logistic model with mixed effects was used, that incorporated the correlation among repeated episodes within one patient and within one GP practice using a random intercept. The adjusted model was corrected for fixed variables, in the population of uncomplicated cystitis: age, socio-economic status, number of cystitis prescriptions in the previous year, year of prescription, a history of dementia, cognitive impairment other than dementia, depression, a consultation because of a (presumed) sexually transmitted disease (STD) within the prior 6 months, and oral contraceptives use. In the population of complicated cystitis, we additionally added gender, pregnancy, solid organ transplantation, diabetes mellitus, anatomic/functional deficits in the urinary tract or kidney, and immunosuppressive medicine use as confounders. For nitrofurantoin, the dosing regimen (50 mg normal release every 6 hours vs. 100 mg slow release every 12 hours) was included as confounding variable. Fixed variables were predefined as (potential) risk factors for clinical failure. Depression and dementia seemed to be associated with a higher risk of clinical failure in our own data. Oral contraceptive use and STD presume active sexual behaviour, which is associated with the occurrence of urinary tract infections. Moreover, STD could mimic and be misclassified as cystitis and vice versa. Year of prescription as confounder was included because the use of fosfomycin has increased since 2013, as a consequence of a guideline change (online supplementary material S2) with a possible effect on the risk of clinical failure. Linearity to the log odds was observed for the continuous values, socio-economic status and age. Missing data were imputed using multiple imputation. Two sensitivity analyses were performed for the primary endpoint in which we applied the same multivariable model as for the first model. In sensitivity analysis A we additionally included patients with unknown serum creatinine, for which we set the eGFR at 90 mL/min. In sensitivity analysis B, we selected episodes in which the eGFR was measured before or at the day of prescription.

Effect of treatment regimen on clinical failure rate within strata of renal function

To compare the effect of antibiotic classes on clinical failure within strata of renal function, a crude and multivariable mixed effects logistic regression model was used with only first cystitis episodes per patient included for analysis. The same fixed effects as described above were used with additionally eGFR as a continuous variable and without patient as random effect. We compared the short regimens for uncomplicated cystitis (NF5, FT1, TMP3) and extended regimens for complicated cystitis (NF7, TMP7) in patients with normal to mild decreased renal function (eGFR ≥60 mL/min; Kdigo stage G1 or G2) and in patients with moderately decreased renal function to kidney failure (eGFR <60 mL/min; Kdigo stage G3-

G5).[11] We performed the same as above sensitivity analysis A for the population with eGFR equal or above 60 mL/min in which we included a factor indicating whether eGFR had been measured or not. Sensitivity analysis B was performed on both renal function populations (eGFR<60mL/min and eGFR≥60 mL/min). The models were fit to maximum likelihood using the Laplace approximation. In all cases, P-values less than 0.05 were considered statistically significant. All analyses were performed using R software (version 3.4.1), using the lme4 package (version 1.1-21).

Ethics

Approval for the study was obtained from the ethical board of the University Medical Centre Utrecht, the Netherlands, with reference WAG/mb/18/022909.

RESULTS

Study population

The complete dataset consisted of 164,589 episodes of nitrofurantoin, fosfomycin and trimethoprim prescriptions. After applying the exclusion criteria, 42,473 episodes in 21,891 patients remained for analysis, of which 31,014 (73%) consisted of a short regimen for uncomplicated cystitis and 11,459 (27%) of an extended regimen for complicated cystitis (Figure 1). Table 1 gives the patient characteristics at baseline in the five treatment regimens. Online supplementary material S2 indicates the frequency of antibiotic use over the past years in this period, with the patient characteristics at baseline of the population without known renal function (sensitivity analysis A).

Figure 1. Flowchart for inclusion of episodes from the Julius General Practitioners' Network (JGPN) consisting of data from 78 general practitioner practices (GP practices') in the province of Utrecht, the Netherlands, between January 2013 and June 2019.

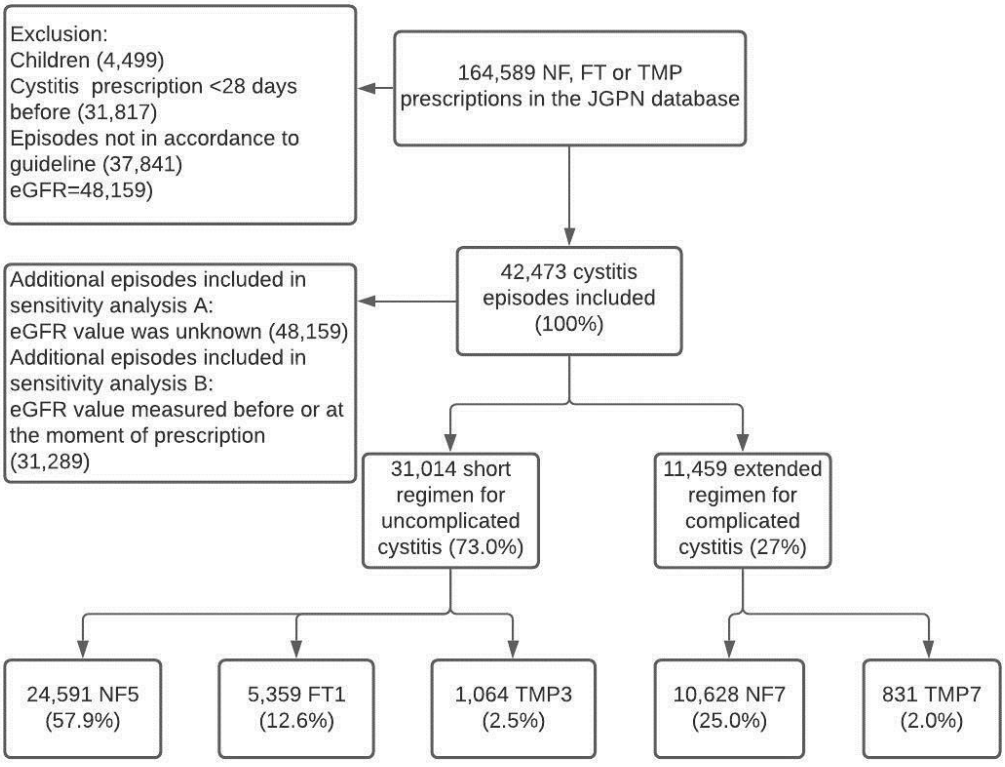

Table 1. Baseline characteristics of cystitis episodes for each antimicrobial regimen.

Patient characteristics	Cystitis episodes (n=42,473)				
	Uncomplicated cystitis			Complicated cystitis	
	NF5 (n=24,591)	FT1 (n=5,359)	TMP3 (n=1,064)	NF7 (n=10,628)	TMP7 (n=831)
Age (years)					
Median	61	72	73	71	77
Interquartile range	43 to 75	58 to 83	55 to 84	59 to 81	67 to 85
Gender					
Male (%)	NA	NA	NA	3,367 (31.7%)	209 (25.2%)
eGFR (mL/min)					
Mean ± SD	85.5± 10.1	80.0 ± 17.0	79.4 ± 17.8	78.6 ± 16.1	73.2 ± 19.5
eGFR levels					
≥90	17,686 (71.9%)	3,009 (56.1%)	625 (58.7%)	5,434 (51.1%)	333 (40.1%)
60-90	5,840 (23.7%)	1,630 (30.4%)	278 (26.1%)	3,586 (33.7%)	295 (35.5%)
30-60	1,026 (4.2%)	580 (10.8%)	128 (12.0%)	1,501 (14.1%)	172 (20.7%)
0-30	39 (0.2%)	140 (2.6%)	33 (3.1%)	107 (1.0%)	31 (3.7%)
Pregnancy (%)	NA	NA	NA	413 (3.9%)	13 (1.6%)
STD (%)	1,176 (4.8%)	348 (6.5%)	56 (5.3%)	612 (5.8%)	66 (7.9%)
Cognitive impairment* (%)	29 (0.12%)	3 (0.06%)	5 (0.47%)	23 (0.22%)	1(0.12%)
Dementia (%)	435 (1.8%)	181 (3.4%)	20 (1.9%)	326 (3.1%)	45 (5.4%)
Use of OAC (%)	2,637 (10.7%)	420 (7.8%)	93 (8.7%)	379 (3.6%)	19 (2.3%)
Depression (%)	1,868 (7.6%)	410 (7.7%)	94 (8.8%)	691 (6.5%)	53 (6.4%)
Diabetes Mellitus (%)	NA	NA	NA	5,089 (47.9%)	448 (53.9%)
Urologic abnormalities (%)	NA	NA	NA	418 (3.9%)	20 (2.4%)
Use of immunosuppressants (%)	NA	NA	NA	372 (3.5%)	36 (4.3%)
Socio-economic status score#					
Median	0.19	0.19	0.32	0.19	0.19
Interquartile range	-0.19 to 1.24	-0.12 to 1.10	-0.48 to 1.31	-1.16 to 0.97	-1.16 to 0.97
N episodes of cystitis previous year$					
Median	0	1	1	0	1
Interquartile range	0 - 1	0 - 2	0 - 2	0 - 1	0 - 2

eGFR = estimated glomerular filtration rate, STD = sexually transmitted diseases, OAC = oral anticonception, NF5 = nitrofurantoin five-day treatment, FT1 = fosfomycin-trometamol one day treatment, TMP3 = trimethoprim three-day treatment, NF7 = nitrofurantoin seven-day treatment, TMP7 = trimethoprim seven-day treatment

*other than dementia

Socio-economic state ranges from -7 to +7 and is estimated on the neighbourhood the patient lives (postal code) (271 missings)

$ Number of prescriptions for cystitis or pyelonephritis in the past 365 days before the episode (4 missing values)

Effect of renal function on clinical failure per antibiotic regimen

After adjusting for confounders, every 10 mL/min decrease in eGFR resulted in significantly more clinical failures when using NF5 and NF7, but not when using FT1, TMP3 or TMP7 (Table 2 and online supplementary material S3 for a graphical display). Results were similar for sensitivity analyses A and B. Decreasing renal function also tends to higher rates of pyelonephritis for NF5, although there is wide uncertainty around the estimate (Table 2).

Table 2. The effect of every 10 mL/min decrease in eGFR on the odds ratio of clinical failure within 28 days post-prescription.

Therapy	Patients	Clinical failure	Crude analysis	Multivariable analysis#	Sensitivity analysis A#	Sensitivity analysis B#	Sec. outcome pyelonephritis#
	Number	Number (%)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
NF5	24,591	4,245 (17.3)	1.02 *** (1.02 to 1.03)	1.05 ** (1.01 to 1.09)	1.05** (1.01 to 1.09)	1.04 * (1.00 to 1.09)	1.34 (0.96 to 1.87)
FT1	5,359	1,235 (23.0)	1.00 (0.99 to 1.00)	0.96 (0.92 to 1.01)	0.97 (0.93 to 1.02)	0.93 * (0.88 to 0.98)	0.83 (0.58 to 1.20)
TMP3	1,064	248 (23.3)	1.00 (0.98 to 1.01)	0.98 (0.89 to 1.08)	0.96 (0.88 to 1.05)	0.97 (0.87 to 1.08)	1.12 (0.89 to 1.41)
NF7	10,628	2,113 (19.9)	1.01 *** (1.01 to 1.02)	1.05 ** (1.02 to 1.09)	1.01 (0.98 to 1.04)	1.05 * (1.01 to 1.10)	1.00 ## (0.93 to 1.06)
TMP7	831	201 (24.2)	0.99 (0.98 to 1.01)	1.02 (0.93 to 1.14)	0.99 (0.93 to 1.05)	0.99 (0.88 to 1.12)	0.86 (0.73 to 1.02)

NF5 = nitrofurantoin five-day treatment, FT1 = fosfomycin-trometamol one day treatment, TMP3 = trimethoprim three-day treatment, NF7 = nitrofurantoin seven-day treatment, TMP7 = trimethoprim seven-day treatment, significance levels: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

Adjusted for the following confounding variables: age, year of prescription, a consultation because of sexual transmitted disease in the prior half year, cognitive impairment other than dementia, oral contraceptive use, depression, dementia, socio-economic status, number of episodes of cystitis in the previous year, the use of normal or slow release nitrofurantoin formulation, with as random effects the patient and the general practitioners practice. For complicated cystitis additionally for gender, pregnancy, diabetes mellitus, urologic abnormalities, use of immunosuppressants, solid organ transplantation.

Due to convergence problems when adjusting for both patient and the general practitioners practice as random effects, only first episodes were analysed with only general practitioners practice as random effect.

Sensitivity analysis A = multivariable analysis using the same confounders as above with addition of patients with unknown serum creatinine, for whom we set the eGFR to 90 mL/min.

Sensitivity analysis B = multivariable analysis using the same confounders as above with selection of patients in whom the eGFR was measured before or at the moment of prescription. Patients with unknown eGFR were not included.

Outcome pyelonephritis = multivariable analysis using the same confounders as above with the outcome pyelonephritis as manifestation of clinical failure.

Effect of antibiotic class on clinical failure rate within strata of renal function

In first episodes per patient, clinical failure occurred in 16.3% (3,578/21,891) of all episodes: 14.9% after using NF5, 20.0% after using FT1, 21.4% after using TMP3, 18.1% after using NF7, and 19.7% after using TMP7 (online supplementary material S4). Pyelonephritis as a manifestation of clinical failure occurred in 4.0% (886/21,891) of all episodes: 2.8% after using NF7, 4.1% after using FT1,3.6% after using TMP3, 6.6% after using NF7, and 6.4% after using TMP7. In first episodes in patients in whom no renal function was measured, the clinical failure rate was 11.0% (3,286/29,768) overall and 2.2% (660/29,768) for pyelonephritis. The probability of clinical failure in patients with eGFR below 60 mL/min was significantly higher when treated with NF5 in comparison to FT1 for uncomplicated cystitis (Table 3). In patients with eGFR above or equal to 60 mL/min significantly more clinical failures occurred when using FT1 or TMP3 instead of NF5. Results were similar in sensitivity analysis A and B, and with pyelonephritis as outcome, although the latter with larger confidence intervals.

Table 3. The odds ratio on clinical failure at 28 days within strata of renal function.

			Crude analysis	Multivariable analysis	Sensitivity analysis A#	Sensitivity analysis B#	Sec. outcome pyelonephritis#
eGFR	**Population**	**Therapy**	**Odds ratio (95% CI)**	**Odds ratio (95% CI)**	**Odds ratio (95% CI)**	**Odds ratio (95% CI)**	**Odds ratio (95% CI)**
<60 mL/min	**Uncomplicated cystitis**	**FT1 vs. NF5**	0.63* (0.42 to 0.94)	0.61* (0.39 to 0.95)	NA	0.73 (0.46 to 1.12)	0.67 (0.25 to 1.76)
		TMP3 vs. NF5	1.05 (0.58 to 1.92)	0.96 (0.51 to 1.82)	NA	0.53 (0.25 to 1.16)	0.28 (0.04 to 2.23)
		TMP3 vs. FT1	1.68 (0.87 to 3.25)	1.59 (0.79 to 3.21)	NA	0.73 (0.32 to 1.68)	0.42 (0.05 to 3.62)
	Complicated cystitis	**TMP7 vs. NF7**	0.89 (0.49 to 1.59)	0.89 (0.48 to 1.65)	NA	0.62 (0.32 to 1.22)	1.50 (0.66 to 3.42)
≥60 mL/min	**Uncomplicated cystitis**	**FT1 vs. NF5**	1.53*** (1.33 to 1.77)	1.37*** (1.18 to 1.59)	1.29*** (1.14 to 1.46)	1.35*** (1.16 to 1.58)	1.52* (1.13 to 2.06)
		TMP3 vs. NF5	1.53** (1.16 to 2.02)	1.42* (1.07 to 1.87)	1.55*** (1.26 to 1.92)	1.48*** (1.08 to 2.02)	1.32 (0.74 to 2.36)
		TMP3 vs. FT1	1.00 (0.74 to 1.36)	1.03 (0.76 to 1.40)	1.20 (0.95 to 1.53)	1.09 (0.78 to 1.53)	0.87 (0.47 to 1.63
	Complicated cystitis	**TMP7 vs. NF7**	1.15 (0.82 to 1.62)	0.99 (0.69 to 1.41)	0.99 (0.72 to 1.35)	1.06 (0.74 to 1.51)	0.83 (0.44 to 1.56)

NF5 = nitrofurantoin five-day treatment, FT1 = fosfomycin-trometamol one day treatment, TMP3 = trimethoprim three-day treatment, NF7 = nitrofurantoin seven-day treatment, TMP7 = trimethoprim seven-day treatment, significance levels: * p < 0.05 ** p < 0.01 *** p < 0.001

Adjusted for the following confounding variables: age, year of prescription, a consultation because of sexual transmitted disease in the prior half year, cognitive impairment other than dementia, oral contraceptive use, depression, dementia, socio-economic status, number of episodes of cystitis in the previous year, the use of normal or slow release nitrofurantoin formulation, with a random effects general practitioners practice. For complicated cystitis additionally for gender, pregnancy, diabetes mellitus, urologic abnormalities, use of immunosuppressants, solid organ transplantation.

Sensitivity analysis A = multivariable analysis using the same confounders as above with addition of patients with unknown serum creatinine, for whom we set the eGFR to 90 mL/min.

Sensitivity analysis B = multivariable analysis using the same confounders as above with selection of patients in whom the eGFR was measured before or at the moment of prescription.

Outcome pyelonephritis = multivariable analysis using the same confounders as above with the outcome pyelonephritis as manifestation of clinical failure.

DISCUSSION

In patients with uncomplicated and complicated cystitis treated with nitrofurantoin, an association was seen between decreased renal function and clinical failure, which was not seen in those treated with fosfomycin or trimethoprim. Treatment with a single-dose of fosfomycin for uncomplicated cystitis resulted in less clinical failures in patients with eGFR below 60 mL/min compared to five days of nitrofurantoin. In contrast, in patients with eGFR above or equal to 60 mL/min nitrofurantoin appeared more effective than fosfomycin or trimethoprim for uncomplicated cystitis. The latter is in line with the results of a trial in which five days of nitrofurantoin was more efficacious than a single-dose of fosfomycin for uncomplicated cystitis, although a nitrofurantoin schedule of three times daily 100mg with normal release was used in this trial.[12]

Based on the pharmacokinetic profile, it could have been expected that renal function has an impact on the efficacy of nitrofurantoin more than that of fosfomycin or trimethoprim. All three antibiotics are eliminated by glomerular filtration,[13–16] however, in contrast to nitrofurantoin, high urinary concentrations of trimethoprim and fosfomycin are reached after administration of the registered dose.[17–19] The effect of decreased renal function on the efficacy of cystitis treatment has not been thoroughly investigated as prior randomized controlled trials excluded patients with decreased renal function.[12,20–24] A retrospective cohort study investigated the effect of eGFR on the effectiveness of nitrofurantoin compared to trimethoprim; eGFR <80 mL/min/1.73m^2 was not associated with decreased effectiveness of nitrofurantoin or trimethoprim, although confidence intervals were wide.[7] In the same study, a significant association between decreased renal function (eGFR <50 mL/min/1.73m^2) and the occurrence of pulmonary reactions leading to hospitalization was found for nitrofurantoin. In another retrospective cohort study on the effectiveness of nitrofurantoin for cystitis in males the odds ratio of clinical failure was 1.13 (95%CI 1.04-1.23) for every 10 mL/min decrease in eGFR, which is in line with the odds ratio of 1.05 (95%CI 1.01-1.09) derived in this study.[6] We are not aware of clinical studies that evaluated the effect of renal function on the effectiveness of fosfomycin for cystitis.

The population in the current study is relatively old, with substantial comorbidities, and therefore serum creatinine levels were available. The overall clinical failure rate was high (16.3%), as compared to episodes in generally younger patients with unknown renal function (11.0%). We do not suggest routine testing of renal function in all patients with cystitis, but only in those cases were renal impairment is suspected. In patients with uncomplicated cystitis, if renal impairment is not suspected, the renal function may be assumed adequate; in fact, our results did not change when patients with unknown eGFR were included in the normal renal function group.

Our study has limitations, the most important being its retrospective design. Although the JGPN database provides reliable quantitative estimates of demographic data, drug prescriptions (ATC codes), symptoms (ICPC codes) and laboratory values, detailed information on dipstick results, treatment compliance, microbiological cultures, and considerations for clinical decision making are missing.[8] However, Dutch GPs usually confirm the presence of cystitis with dipstick before prescribing antibiotics, which increases the likelihood that true cystitis episodes were treated.[25] Especially severe clinical failure rates may have been underestimated as prescription data from hospitals and out-of-office GP services were lacking. However, in the Netherlands only 6% of total antibiotic prescriptions in primary care occur in out-of-office hours.[26,27] Second, confounding by indication could not be excluded when comparing the different treatment options, as nitrofurantoin is the first choice for cystitis according to the treatment guideline and

nitrofurantoin is contraindicated in patients with a severely decreased renal function (GFR <30 mL/min).[1] Although we only included first episodes of cystitis and we adjusted for the number of cystitis prescriptions in the previous year, residual confounding is possible. If so, we expect bias in favour of NF5 and NF7. Third, the treatment regimens and definitions of cystitis as used in this study comply with Dutch primary care guidelines. Generalizability of the results may therefore be limited for countries or health care settings where different definitions apply. Nevertheless, we expect that the effect of renal function on the clinical effectiveness is to some extent generalizable to the therapeutic use of nitrofurantoin, fosfomycin and trimethoprim for urinary tract infections. Fourth, in some patients the renal function was estimated on eGFR values that were measured after the prescription date, which is inconsistent with the etiologic nature of the study and does not represent the clinical decision-making. Therefore, sensitivity analysis B was performed that only included episodes eGFR measured within 6 months prior to the episode, which resulted in similar findings. Fifth, the results of our study were not corrected for multiple testing, because distinct hypotheses were tested for separate antibiotic regimens, and the comparisons between the effectiveness of cystitis regimens were a derivative of these results. There might however be a risk of type I errors and independent confirmation of our results is required.

In conclusion, the results of this study indicate that impaired renal function reduces the effectiveness of nitrofurantoin for treatment of cystitis, which could have clinically relevant implications for the patient. Consequently, fosfomycin might be more effective than nitrofurantoin in patients with eGFR below 60 mL/min. These findings should be considered in the clinical decision-making for treatment of cystitis. New trials, including patients with impaired renal function are needed to confirm these findings. Next, more studies are warranted investigating the PK/PD profile of nitrofurantoin, fosfomycin and trimethoprim for treatment of cystitis in patients with impaired renal function.

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Reply to: 'The effectiveness of nitrofurantoin, fosfomycin and trimethoprim for the treatment of cystitis in relation to renal function' by ten Doesschate et al.

S. Karakonstantis

To the editor

I read with interest the recently published article by Doesschate et al.[1] suggesting that fosfomycin may be a more effective treatment option than nitrofurantoin for uncomplicated cystitis in patients with an eGFR below 60 mL/min. However, according to other studies, mild/moderate reduction in eGFR (30–60 mL/min) was not associated with decreased efficacy of nitrofurantoin.[2–4]

As the authors acknowledge, the lack of microbiological data is an important limitation of their study, especially considering that chronic kidney disease may be associated with a higher risk of infection by resistant pathogens.[5] In one study, for example, most treatment failures with nitrofurantoin (five of eight) in patients with uncomplicated cystitis and eGFR <60 mL/min were due to infections by bacteria intrinsically resistant to nitrofurantoin (e.g. Proteus spp).[3] When comparing antibiotics in patients with decreased renal function, differences in antimicrobial resistance rates between the compared antibiotics can be an important source of bias. For example, if with decreasing renal function the rate of resistance to nitrofurantoin is higher than the rate of resistance to fosfomycin, this could have significantly biased the results in favor of fosfomycin. Therefore, conclusions regarding the effect of renal function on the efficacy of antibiotics for cystitis cannot be drawn in the absence of antimicrobial susceptibility data.

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Author's response to: Antimicrobial resistance may be an important confounder when assessing the effect of renal function on the efficacy of antibiotics in cystitis

T. ten Doesschate

To the editor

We thank our colleagues for their interest in our publication. We disagree with their suggestion that antimicrobial resistance is a potential confounder when assessing the effect of renal function on the efficacy of antibiotics in cystitis. Intuitively, it is possible that patients with renal impairment have a higher chance of hospitalization and acquisition of resistance, although to the best of our knowledge there is limited empirical evidence to support a difference in resistance to nitrofurantoin, fosfomycin or trimethoprim in this population.[1] We assume that the observed higher frequency of *P. mirabilis* is driven by more frequent catheter use or kidney stones in this population.[2] In our uncomplicated cystitis population, patients with urinary catheters and kidney stones were excluded, while we still observe an association between decreased renal function and clinical failure when using nitrofurantoin.

Yet, if it is true that patients with impaired renal function more frequently present with nitrofurantoin resistant pathogens, it follows that impaired renal function is the first cause and antibiotic resistance is the second, intermediate cause of treatment failure. Stated in epidemiological terms, antibiotic resistance might be an intermediate in the causal pathway between renal function and treatment failure. Therefore, the term confounder is inappropriate to describe this relationship. More evidence exists for our hypothesis that the negative relationship between creatinine clearance and urinary nitrofurantoin concentration is primarily responsible for this association.[3] However, we do acknowledge that multiple causal pathways may play a role.

Most important, the precise causal pathway and relative importance of the different routes are of less relevance for general practitioners, who have to decide which treatment to start for cystitis without knowledge of causing pathogen or individual antibiotic concentration that will be reached. The results of our study suggest that fosfomycin has a higher effectiveness than nitrofurantoin in the treatment of patients with cystitis with a eGFR below 60ml/min.[4] Therefore, the results of our study warrant consideration of renal function in the choice of antibiotics for patients with cystitis.

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CHAPTER 3

The effectiveness of extended versus normal release nitrofurantoin for cystitis: an instrumental variable analysis

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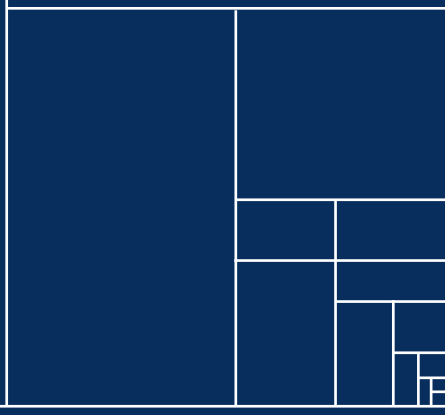


ABSTRACT

Background: It is unknown whether nitrofurantoin 50 mg every 6 h (NF50) and 100 mg extended-release every 12 h (NF100) are equally effective for treating cystitis in primary care. In the Netherlands, GP prescription of either option largely depends on pharmacy procurement, rather than on patient-related factors.

Methods: GP data between January 2013 and July 2018 were retrospectively collected. Inclusion criteria were the use of nitrofurantoin for uncomplicated cystitis, complicated cystitis or cystitis in pregnancy. Criteria for early and late failure were a second antibiotic prescription for cystitis or pyelonephritis within 14 and 28 days post-prescription, respectively. Crude and confounder adjusted (CA) risk differences (RD) were estimated using linear regression. Instrumental variable analysis and CA instrumental variable analysis used GP practice proportion of NF50 versus NF100 use the instrumental variable.

Results: For uncomplicated cystitis (n=46,855), treatment with NF50 and NF100 resulted in late failure in 9.7% and 9.6%, respectively. The CA RD, instrumental variable RD and CA instrumental variable RD were 0.2% (95%CI -0.5 to 0.8), -0.7% (95%CI -1.7 to 0.3) and 0.0% (95%CI -0.9 to 1.0), respectively. In complicated cystitis (n=10,767), late failure occurred in 10.9% and 11.1% after using NF50 and NF100, respectively [CA RD: 0.5% (95%CI -1.2 to 1.8), instrumental variable RD: -0.8% (95%CI -3.4 to 1.8), CA instrumental variable RD:-0.3 (95%CI -3.0 to 2.4)]. For cystitis in pregnancy (n=1,087), NF50 and NF100 resulted in late failure in 13.4% and 7.8%, respectively [CA RD: -5.4% (95%CI -10.0 to -1.4), instrumental variable RD: -8.9% (95%CI -16.0 to -1.8), CA-instrumental variable RD: 8.9% (95%CI -16.0 to -1.7)]. No differences were observed in early failure.

Conclusions: In patients with cystitis in pregnancy, NF100 was associated with a lower incidence of late clinical failure compared to NF50. We found no differences in clinical failure between NF50 and NF100 for uncomplicated and complicated cystitis.

INTRODUCTION

Nitrofurantoin is the first-choice treatment for cystitis in the Netherlands, and in most countries.[1,2] The antimicrobial activity derives from metabolites that are formed by reduction of nitrofurantoin, but the exact structure and antimicrobial activity of each metabolite is still unclear.[3] Despite the widespread use of nitrofurantoin, reported resistance rates to nitrofurantoin among *Enterobacterales* are low.[2,4,5] Nitrofurantoin reaches high concentrations in urine, with very low concentrations in plasma, and nitrofurantoin is therefore exclusively used to treat or prevent cystitis.

The Dutch guideline for GPs recommends treatment of uncomplicated cystitis with a 5 day nitrofurantoin regimen in patients of 12 years and older, and treatment for 7 days in patients with complicated cystitis or cystitis in pregnancy.[1] Cystitis is defined as complicated in the presence of risk factors for worse outcome, such as being male, having diabetes mellitus, using immunosuppressive drugs or having urogenital abnormalities. The guideline does not distinguish between the use of normal-release macrocrystal formulation of 50 mg of nitrofurantoin, available as Furadantine® (NF50), and the extended-release formulation of nitrofurantoin 100 mg, available as Furabid® (NF100). The extended release formulation consists of 25 mg nitrofurantoin in the form of macrocrystals, and 80.7 mg in the form of monohydrate, corresponding to 75 mg of anhydrous nitrofurantoin. The NF50 formulation has been available since 1969 and needs to be taken every 6 hours, while the NF100 formulation was registered in 1994, and can be taken every 12 hours.[6] Pharmacokinetic studies suggest that the NF100 extended release formulation could be superior to NF50.[3] Moreover, compliance with a twice-daily regimen may be higher as compared with a four-times-daily regimen. However, the comparative effectiveness of these two regimens for the treatment of cystitis has not been assessed in clinical studies.[1]

In the Netherlands, the GP prescription of either NF50 or NF100 depends on the pharmacy's procurement, which is based on economic or logistic reasons. We made an inventory of this procurement and encountered that 4/10 pharmacies only procured NF100, 2/10 only procured NF50 and 4/10 procured both NF100 and NF50. Consequently, GP practice prescription of either option does not primarily depend on patient-related factors, creating a natural experiment. Next to multivariable regression analysis this study used instrumental variable analysis with GP practice as instrumental variable to compare the effectiveness of NF50 with NF100 for the treatment of cystitis.

METHODS

Population

Data were collected from the Julius General Practitioners' Network (JGPN) consisting of 806,105 patients from 64 GP practices in the province of Utrecht, the Netherlands, between January 2013 and July 2018. [7] Diagnoses were coded according to the International Classification of Primary Care (ICPC) and medication prescriptions were coded according to the Anatomical Therapeutic Chemical (ATC) classification system (see online Supplementary data).

Episodes were included if nitrofurantoin was prescribed according to the Dutch treatment guideline for patients ≥12 years of age. An episode was defined as uncomplicated if nitrofurantoin was prescribed for 5 days in the absence of pregnancy or the following risk factors: male gender, diabetes mellitus, anatomic/functional deficits in the urinary tract or kidney, having used immunosuppressive drugs within 6 months before the prescription, or solid organ transplantation. For complicated cystitis, patients who received a 7 day NF regimen in the absence of pregnancy were included. Since prescription of nitrofurantoin for 7 days is not common practice for GPs, we assumed that this duration reflected the presence of complicating factors. Therefore, patients without documented risk factors and receiving the extended course were included in the complicated cystitis group. In accordance with the Dutch guideline, cystitis during pregnancy was interpreted and analyzed as a separate population, and was defined as prescriptions of nitrofurantoin for 7 days with a pregnancy-related consultation within the 9 months prior to the episode. Cystitis episodes were not included if they occurred within 28 days of a previous therapeutic antibiotic prescription for cystitis, i.e. short course nitrofurantoin, trimethoprim or fosfomycin/trometamol, as these are assumed to represent treatment failures of a prior cystitis episode. In- and exclusion criteria, as structured on ATC and ICPC codes, are provided in the online Supplementary data. Socio-economic status was estimated on aggregated level of the patient's postal code and based on education, income and labor market position of that neighborhood.[8]

Endpoints

The endpoints in this study were clinical failure within 14 days post-prescription (early failure) and clinical failure within 28 days post-prescription (late failure). Clinical failure was defined as a new antibiotic prescription for cystitis (nitrofurantoin, trimethoprim or fosfomycin/trometamol, with exclusion of prophylactic prescriptions based on the number of prescription days) or pyelonephritis, i.e. ciprofloxacin, amoxicillin/clavulanic acid or trimethoprim/sulfamethoxazole, combined with an ICPC code for urinary tract infections (see the online supplementary data).

Statistical analysis

Crude and multivariable linear regression (adjusted) models were used to estimate the risk difference (RD) of clinical failure between NF50 and NF100. A sandwich estimator was used to obtain robust standard errors and 95% CIs. Prior to data analysis, the following set of confounding variables was selected to be included in the multivariable model: age, renal insufficiency, previous antibiotic use for cystitis (nitrofurantoin, trimethoprim or fosfomycin/trometamol), dementia, other forms of cognitive impairment, depression, sexually transmitted disease, oral contraceptive use, and socioeconomic status. For complicated cystitis, we additionally corrected for sex, diabetes mellitus, anatomic/functional defects in the urinary tract or the kidney, using immunosuppressive drugs, and solid organ transplantation. For cystitis during pregnancy, we corrected for the same factors as for complicated cystitis, except for sex and the use of oral contraceptives. The potential confounding variables were defined by ATC and ICPC codes (see the online Supplementary data). Renal insufficiency was categorized with three levels, based on the estimated glomerular filtration rate [≥60 mL/min or < 60 mL/min, as calculated by the Chronic Kidney Disease epidemiology collaboration (CKD-epi) formula using serum creatinine, gender and age] or as 'unknown' in the case of serum creatinine not being measured. Two sensitivity analyses were performed to check the robustness of the results, using multivariable linear regression analyses as above. The first looked at severe late clinical failure defined as: 'a new prescription for pyelonephritis within 28 days'. In the second model only unique patients were analyzed, with one random episode included per patient.

Next, instrumental variable analysis was performed, which has the potential to control for unobserved confounding.[9] We used GP practice preference as instrumental variable, where GP practice preference was defined as the proportion of NF50 prescriptions for cystitis compared with all (NF50 and NF100) prescriptions for cystitis per GP practice. With instrumental variable analysis, treatment failure rates per GP practices are regressed on GP practice preference. The observed relation is then extrapolated to provide an estimate of treatment failure rates if only NF50 was prescribed versus if only NF100 was prescribed among those patients in whom there is an option to choose for either of the two.

The three assumptions of instrumental variable analysis are explained in Figure 1. Whether GP practices differ in their preference for either NF100 or NF50 (instrumental variable assumption 1) was tested using the F-statistic of a linear regression of actual NF50/NF100 prescription on GP practice. In our study, the prescription of NF50 or NF100 per GP practice largely depends on the pharmacy procurement, which is assumed to be independent of the effect of GP practice itself on the outcome clinical failure (instrumental variable assumption 2), e.g. through differences in advice or tendency to prescribe antibiotics. Next, we assumed this prescription per GP practice to be independent of patient characteristics (instrumental

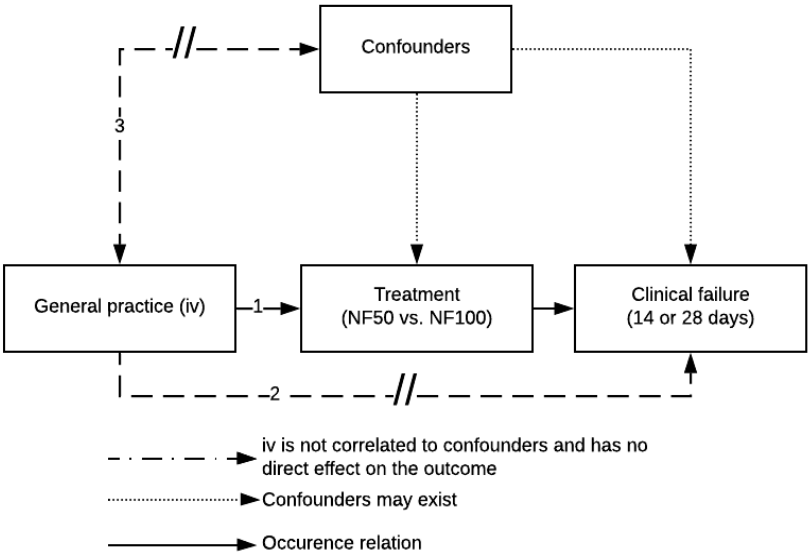
variable assumption 3), which was checked for measurable confounders by calculating F-statistics with multivariable linear regression analysis. Additionally, we performed instrumental variable analysis with adjustment for the same measured confounders as in the multivariable linear regression model (adjusted instrumental variable analysis).

As treatment failure rates were expected to be low with either option, and as both options yield an identical cumulative nitrofurantoin dose, we defined equivalence as a difference in clinical failure of less than 2%, which was tested using the 95% CI of the RD. R-software version 3.4.1 was used for data analysis, with R package 'ivpack' version 1.2 to perform instrumental variable analysis and applying the sandwich method.

Ethics

Ethical approval was obtained from the ethical board of directors of the University Medical Centre Utrecht, the Netherlands, with a waiver for informed consent, with reference WAG/mb/18/022909, on 26 June 2018. Individuals are not traceable as all data were provided coded.

Figure 1. Graphical representation of the model and the assumptions for instrumental variable analysis. GP practice preference is assumed to affect clinical failure only through prescription of NF50 or NF100. This instrumental variable analysis is unbiased if (1) GP practices differ in their preference for either NF100 or NF50, (2) GP practice itself has no direct effect on the outcome clinical failure, (3) GP practices do not differ in the type of patients they prescribe NF50 or NF100.

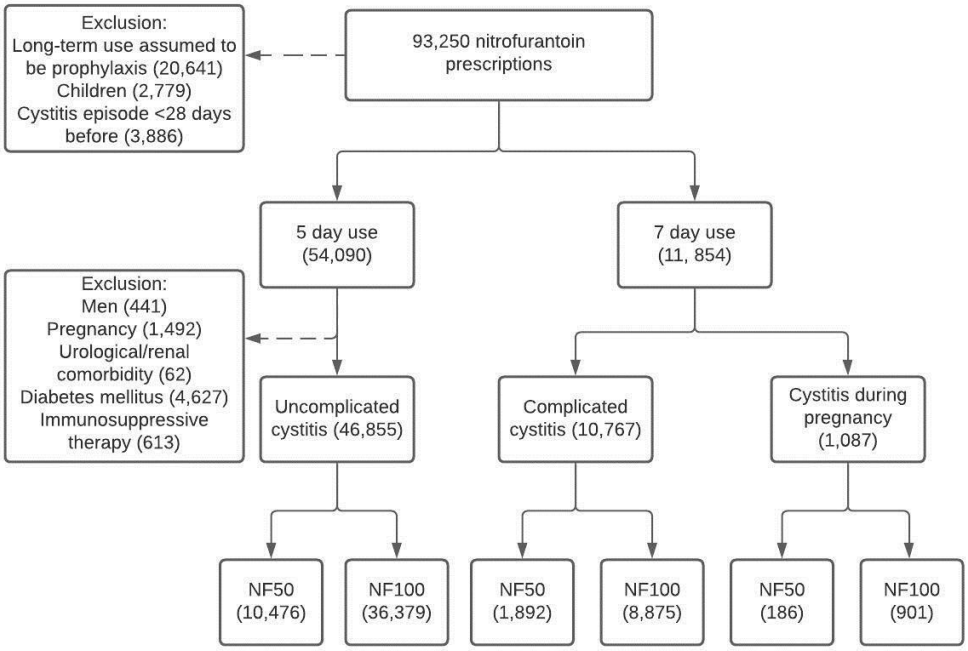


RESULTS

Data collection

A total of 119,342 cystitis episodes were identified during the study period. Of these, nitrofurantoin was prescribed 93,250 times (78% of all cystitis prescriptions). After exclusion of 34,451 episodes, we analyzed 58,709 episodes in 36,439 patients, of which 46,855 episodes (80%) of uncomplicated cystitis, 10,767 (18%) of complicated cystitis and 1,087 (2%) of cystitis during pregnancy (Figure 2). Of included cystitis episodes 46,155 (79%) were treated with NF100 and the remainder with NF50. The number of patients under care per GP practice in 2017 ranged from 1,936 to 44,079. Nitrofurantoin was prescribed in 64 GP practices in a range of 256 to 3,059 times per GP practice in the study period, and for a median of 18 times per 1000 person years with an IQR of 14 to 34 between GP practices.

Figure 2. Flowchart for inclusion of uncomplicated cystitis, complicated cystitis and cystitis during pregnancy collected from the JGPN consisting of 806,105 patients from 64 GP practices in the province of Utrecht, The Netherlands, between January 2013 and July 2018. NF50, 50 mg of nitrofurantoin every 6 h; NF100, 100 mg of nitrofurantoin every 12 h.



Assumptions instrumental variable analysis

For uncomplicated cystitis, complicated cystitis, and cystitis during pregnancy, GP practices used NF50 in a median of 10% (IQR= 5% to 43%, range 1% to 95%), 6% (IQR 3% to 23%, range 0% to 96%), and 8% (IQR 0% to 58%, range 0% to 100%) of the episodes, respectively (Figure S1, available online as Supplementary data). The F-statistics of the linear models predicting prescribed nitrofurantoin dosage based on GP practice preference were 656.5, 96.8 and 11.4 for uncomplicated cystitis, complicated cystitis and cystitis during pregnancy, respectively. The F-statistics of the linear model predicting GP practices' preference based on measured confounders were 79.4, 8.9 and 1.5 for uncomplicated cystitis, complicated cystitis and cystitis during pregnancy, respectively. The approximately 8 to 10-fold higher F-statistic of the first model as compared to the second demonstrates that the prescription of NF100 or NF50 is much better explained by the GP practice then by measured confounders, confirming the appropriateness of GP practice as an instrumental variable.

Uncomplicated cystitis

Patients that received NF100 were younger and had a higher socio-economic state compared to those receiving NF50 (Table 1). Clinical failure within 14 days post-prescription was observed in 606/10,476 (5.8%) and 2,150/36,379 (5.9%) patients receiving NF50 and NF100, respectively. Clinical failure within 28 days post-prescription was observed in 1,014/10,476 (9.7%) in the NF50 and 3,487/36,379 (9.6%) in the NF100 group. Crude, adjusted, instrumental variable analysis and adjusted instrumental variable analysis revealed no differences in clinical failure rate between NF50 and NF100 (Table 2). In the sensitivity analysis no differences were found between NF50 and NF100 when analyzing unique patients [RD -0.3% (95%CI -1.1 to 0.5)] or when looking at severe failure [RD 0.1% (95%CI -0.2 to 0.4)].

Table 1. Baseline characteristics of patients receiving NF50 and NF100 for cystitis in primary care in the Netherlands

	Uncomplicated cystitis		Complicated cystitis		Cystitis during pregnancy	
Variable	**NF50 (n=10,476)**	**NF100 (n=36,379)**	**NF50 (n=1,892)**	**NF100 (n=8,878)**	**NF50 (n=186)**	**NF100 (n=901)**
Age (years), median (IQR)	46 (28 to 64)	40 (26 to 60)	67 (49 to 78)	66 (46 to 78)	30 (27 to 34)	31 (28 to 34)
SES*, median (IQR)	0.50 (0.27 to 1.50)	0.96 (0.19 to 1.63)	0.39 (0.39 to 1.36)	0.78 (0.44 to 1.59)	0.68 (0.27 to 1.35)	0.78 (0.13 to 1.54)
Glomerular filtration rate (mL/min), mean (SD)	97.3 (17.9)	98.4 (18.1)	85.8 (23.2)	86.0 (24.0)	128 (12.2)	125 (13.1)
Men, n (%)	NA	NA	737 (39.0)	2,940 (33.1)	NA	NA
Diabetes Mellitus, n (%)	NA	NA	866 (45.8)	3,789 (42.7)	13 (7.0)	26 (2.9)
Urologic/renal comorbidity, n (%)	NA	NA	3 (0.2)	40 (0.5)	0	0
Immunosuppressive drugs, n (%)	NA	NA	89 (4.7)	230 (2.6)	0	1 (0.1)
Cognitive impairment, n (%)	12 (0.1)	44 (0.1)	4 (0.2)	10 (0.1)	0	0
Dementia, n (%)	70 (0.7)	300 (0.8)	56 (3.0)	206 (2.3)	0	0
Depression (ICPC) < 6 months, n (%)	851 (8.1)	2,878 (7.9)	234 (12.4)	1,094 (12.3)	7 (3.8)	25 (2.8)
Oral contraceptive use <6 months, n (%)	1,978 (18.9)	6,107 (16.8)	139 (7.3)	577 (6.5)	NA	NA
Number of prescriptions for cystitis or pyelonephritis in the past 365 days before the episode, median, (IQR)	0 (0 to 1)	0 (0 to 1)	0 (0 to 1)	0 (0 to 1)	0 (0 to 0)	0 (0 to 0)
Sexually transmitted disease< 6 months, n (%)	293 (2.8)	1,223 (3.4)	75 (4.0)	420 (4.7)	4 (2.2)	25 (2.8)

NA = not applicable, NF50 = 50 mg nitrofurantoin every 6 h, NF100 = 100 mg nitrofurantoin every 12 h.
*Socio-economic state ranges from -7 to +7 and is estimated on the neighborhood the patient lives (postal code).

Complicated cystitis

Of complicated cystitis episodes, in 7,276 of 10,767 episodes (68%) we found at least one risk factor for a complicated course. Patients that used NF100 instead of NF50 had a higher mean socio-economic status and more often used medication for Diabetes Mellitus. The use of NF50 and NF100 was associated with clinical failure rates of 127/1,892 (6.7%) and 579/8,875 (6.5%) within 14 days and of 207/1,892 (10.9%) and 986/8,875 (11.1%) within 28 days, respectively. No differences were found in clinical failure rate

between 50mg and 100mg in crude, adjusted, instrumental variable and adjusted instrumental variable analysis (Table 2). The sensitivity analyses on unique patients [RD -0.5% (95%CI -1.2 to 0.7)] and on severe failure [RD -0.2%, 95%CI -1.9 to 1.9)] revealed no differences between NF50 and NF100.

Cystitis during pregnancy

No large differences existed between women that received either NF50 or NF100 (Table 1). Using NF50 and NF100 led to clinical failure within 14 days in 15/186 (8.1%) and 48/901 (5.3%), respectively. Clinical failure within 28 days post therapy was found in 25/186 (13.4%) and 70/901 (7.8%) in the NF50 and NF100 users, respectively, which was statistically significant in crude, adjusted, instrumental variable analysis and adjusted instrumental variable analysis (Table 2). The sensitivity analyses on unique patients [RD -5.0% (95%CI -9.6 to -0.3)] and severe failure [RD -4.2% (95%CI -7.9 to -1.1)] resulted in significant differences in favour of NF100. In a post-hoc analysis we looked whether an enhanced renal function that is observed in this population could explain this effect. For this, a multivariable linear regression model was performed in 1,973 patients with an enhanced renal function (eGFR>120mL/min) with any form of cystitis, i.e. uncomplicated cystitis, complicated cystitis or cystitis during pregnancy. There was a non-significant trend towards less late failures in patients using NF100 compared to NF50 (RD -5.4%, 95%CI -14.1 to 3.2).

Table 2. Risk difference of clinical failure between NF50 and NF100 in cystitis patients in primary care

Entity	Time horizon	NF50, number of failures/total episodes (%)	NF100, number of failures/total episodes (%)	Crude analysis, RD (95%CI)	Multi-variable analysis*, RD (95%CI)	Instrumental variable analysis, RD (95%CI)	Adjusted instrumental variable analysis*, RD (95%CI)
Uncomplicated cystitis	Day 14	606/10,476 (5.8)	2,150/36,379 (5.9)	0.1% (-0.4 to 0.6)	0.3% (-0.2 to 0.9)	-0.5% (-1.2 to 0.3)	0.0% (-0.7 to 0.8)
	Day 28	1,014/10,476 (9.7)	3,487/36.379 (9.6)	-0.1% (-0.8 to 0.5)	0.2% (-0.5 to 0.8)	-0.7% (-1.7 to 0.3)	0.0% (-0.9 to 1.0)
Complicated cystitis	Day 14	127/1,892 (6.7)	579/8,875 (6.5)	-0.2% (-1.4 to 1.1)	0.0% (-1.4 to 1.1)	-0.3% (-2.3 to 1.8)	0.3% (-1.8 to 2.4)
	Day 28	207/1,892 (10.9)	986/8,875 (11.1)	0.3% (-1.3 to 1.8%)	0.5% (-1.2 to 1.8)	-0.8% (-3.4 to 1.8)	-0.3% (-3.0 to 2.4)
Cystitis during pregnancy	Day 14	15/186 (8.1)	48/901 (5.3)	-2.7% (-6.4 to 1.0)	-2.4% (-6.2 to 1.2)	-3.7% (-9.6 to 2.1)	-3.3% (-9.3 to 2.6)
	Day 28	25/186 (13.4)	70/901 (7.8)	-5.6% (-10.1 to -1.2)	-5.4% (-10.0 to -1.4)	-8.9% (-16.0 to -1.8)	-8.9% (-16.0 to -1.7)

NA = not applicable, RD = Risk Difference, NF50 = 50mg of nitrofurantoin every 6 h; NF100 = 100mg of nitrofurantoin every 12 h. A positive RD is in favour of NF50 and a negative RD is in favour of NF100.

*for uncomplicated cystitis adjusted for age, socio-economic state, Glomerular Filtration Rate, depression, mental disorder, dementia, number of cystitis prescription in past 365 days, sexually transmitted disorders and using oral anticonceptive medicines, for cystitis in pregnancy additionally for sex, diabetes mellitus, urologic comorbidity and immunosuppressive medicines and in complicated cystitis additionally for sex and oral anti-conceptive medicine use

DISCUSSION

In the treatment of cystitis during pregnancy, the use of NF50 was associated with a significantly higher incidence of late clinical failure than when using NF100, as estimated by crude, multivariable, instrumental variable and adjusted instrumental variable analysis, and in the multivariable sensitivity analyses on unique patients and severe clinical failure. The 95%-confidence intervals for the RD between NF50 and NF100 for uncomplicated cystitis were within the predefined 2%, demonstrating clinical equivalence. For complicated cystitis, the upper and lower margin of the 95%-CIs exceeded this margin in some analyses, which means no statement can be made about clinical equivalence. Previous studies comparing different antibiotic classes for cystitis used a higher margin for equivalence of 5%-10%. [11,12] To the best of our knowledge, the clinical effectiveness of NF50 and NF100 for treating cystitis has not been compared before. Our findings suggest that NF50 is suboptimal for cystitis in pregnancy, whereas especially in pregnancy, effective antimicrobial treatment for cystitis is important to prevent pyelonephritis, which may affect pregnancy outcome. [13]

Nitrofurantoin should be administered with food, is absorbed in the upper part of the small intestine and is bound to plasma-albumin, after which it is excreted mainly in urine, where it has its highest activity. [12] The extended release form (NF100) is 1.3 to 1.45 times better absorbed as a consequence of the prolonged residence in the gastrointestinal (GI) tract, and, therefore, yields higher total urinary excretion

rates as compared to the normal formulation (NF50).[3] This higher bioavailability of NF100 could result in a better efficacy. It is unclear why this was only observed in pregnancy. Potentially the physiologic changes that occur in pregnancy, with the combination of an increased glomerular filtration rate and an increased total body volume, necessitate this higher GI uptake, which may not be clinically relevant in non-pregnant patients.[14] According to our post hoc analysis results an enhanced renal function might explain the difference observed in pregnancy. Although less likely, an alternative explanation could be that the difference in compliance to the 'every 6 h' instead of the 'every 12 h' regimen is more pronounced in pregnant women as compared to the other patient groups.

Even in the non-pregnant patient, a four-times daily regimen is considered not very patient-friendly. One of the reasons why not all pharmacies or patients choose for the 100mg extended release is the limited availability of the extended release formulations and the extra costs of this regimen. In the Netherlands, the NF100 formulation costs 0.76€ per day and the NF50 0.51-0.61€ per day, and the difference of around 2.50€ per regimen has to be paid directly by the patient. Patients are free to choose their own pharmacy and some pharmacies have both the NF50 and the NF100 formulation available. This could possibly explain why, on an individual level, patients with a lower socio-economic status more often used the NF50 formulation. However, on GP practice level, the ratio between NF50 and NF100 was not associated with socio-economic status and therefore we may assume it did not confound the instrumental variable analysis.

Some potential limitations of this study need to be mentioned. First, retrospective data collection may suffer from inaccurate data. However, the JGPN database that was used provides reliable demographic data, drug prescriptions (ATC codes), symptoms (ICPC codes) and laboratory values.[7] The cystitis diagnosis in our database is based on the GP's decision to prescribe a 5 or 7 days nitrofurantoin regimen as no other indications exist for these regimens. Yet, we were unable to confirm that both the actual existence of cystitis and episodes that resemble cystitis, for which nitrofurantoin was prescribed, could have been included. However, there is no reason to expect imbalances of cystitis-like episodes between the NF50 and NF100 group. In addition, it is known that Dutch GPs frequently use a dipstick test to confirm the presence of cystitis and prescribe antibiotics once they are convinced of the diagnosis, as compared to other European countries, meaning that the entity of cystitis in the Netherlands is considered concordant to its definition.[15] In the Netherlands, urine screening during pregnancy is not routine thus we expect that the majority of cystitis episodes in pregnancy are symptomatic. Second, there may exist misclassification between episodes of uncomplicated cystitis, complicated cystitis and cystitis during pregnancy. We excluded a substantial amount of episodes for which a 5 day nitrofurantoin regimen was started, while,

based on registered comorbidity and the Dutch guideline, they should have received a 5 days course for complicated cystitis. Episodes could have been misclassified as complicated cystitis if a GP wrongly prescribes an extended 7 day regimen for uncomplicated cystitis, although we expect this to occur rarely. We decided to not exclude episodes that were treated with 7 days in which no risk factors were found for a complicated course. First, because not all risk factors are available in the JGPN database. Next, GPs are not used to prescribe a seven day nitrofurantoin prescription, as this deviates from their normal prescription behavior. This implies that, if they do, they must have considered a risk factor for a complicated course. Of included complicated cystitis episodes, we found that 68% had at least one risk factor for a complicated course. The existence of cystitis in pregnancy was based on two arguments, a consultation for pregnancy within the prior nine months and the use of an extended 7 day nitrofurantoin regimen. We excluded patients with a pregnancy related consultation within the prior 9 months that were treated with nitrofurantoin for 5 days, as these were either treated wrongly, or they were not pregnant at the moment of cystitis. Consequently, we would not have been able to interpret these results. According to the literature, in more than 50% of the pregnancies the GP is not consulted, however this fraction is probably lower if the patient had to consult the GP for cystitis anyway.[16] Cystitis episodes during pregnancy, in which no pregnancy related consultation was registered, are probably misclassified as uncomplicated or complicated cystitis, depending on the duration of therapy. As the number of these episodes is probably small in comparison to the population of uncomplicated or complicated cystitis, the effect of these misclassified episodes is probably negligible. Colloquially, women with a recent pregnancy may have been included as cystitis during pregnancy if treated for 7 days. The majority of these will have been treated with 5 instead of 7 days and will have been excluded from the analysis based on this treatment duration. However, those with complicated cystitis and recent pregnancy may have biased the estimate for the cystitis during pregnancy group towards zero, given the absence of an effect in the complicated cystitis group. Third, the endpoint clinical failure is based on a new prescription for cystitis or pyelonephritis by the GP. Therefore, clinical failures were missed if prescriptions were made during out of office hours due to the severity of the disease, either by the GP urgent care center, or in the hospital. Yet, the occurrence of pyelonephritis after using nitrofurantoin for uncomplicated cystitis was 0.4% in a recent study.[12] Additionally, only 6% of total antibiotic prescriptions occur out of office hours in primary care in the Netherlands.[17] We consequently expect to have missed only few outcomes. In a recent trial investigating uncomplicated cystitis, the late clinical failure rate for nitrofurantoin thrice daily 100mg was around 30%, which is much higher than the around 10% in the current study.[12] This may have resulted from the strict definition for clinical response in that trial, taking into account residual symptoms and not

only antibiotic use. The clinical failure rate in our study corresponds to other trials investigating nitrofurantoin for this indication.[18] Fourth, we have no information about the reason for clinical failure. Theoretically, a patient could require a new antibiotic prescription as a consequence of relapse or recurrence of infection or due to adverse events. The latter is less likely as nitrofurantoin is tolerated well as a short course.[12] Theoretically, it is not expected that the tolerability structurally differs between the NF50 and NF100 regimen, as they contain an identical cumulative dose. Perhaps, differences in tolerability could arise from the better absorption of NF100 than NF50. Fifth, the validity of the instrumental variable analysis is based on the assumption that the GP practice ratio to prescribe NF50 or NF100 is not correlated to the average prognosis of cystitis patients of that GP practice. These assumptions are based on the underlying theory, and were strengthened by the fact that differences in baseline characteristics between those receiving NF50 and NF100 that were found on an individual level were not seen at the GP practice level and results of the adjusted instrumental variable analysis did not differ from the unadjusted instrumental variable analysis. However, it is not possible to demonstrate that this also holds for non-observed confounders. The multivariable regression analysis on the other hand has the assumption that there are no unobserved confounders. Therefore, the combined analyses should be considered as complementary as both have assumptions that cannot be tested. Sixth, we evaluated the clinical failure rate for three cystitis entities. To not increase the type-II error rate, we decided to not correct for multiple testing. However, this increases the risk of type I errors. Therefore, as with any research finding, independent confirmation of our results is mandated.

Based on the results of this study, it would be interesting to study the pharmacokinetic profile of both regimens in pregnant women with cystitis. A randomized controlled trial would provide an unbiased estimate of the efficacy of both regimens for all cystitis entities. Until that time, we would recommend prescribing NF100 instead of NF50 for pregnant women with cystitis, and we argue for reimbursement of the full price for this patient group. Unfortunately the extended release formulation is not available in all countries and some national guidelines (e.g. France and Belgium) recommend the normal release formulation of nitrofurantoin 100mg thrice daily while the European and American guidelines recommend the NF100 extended release formulation twice daily for cystitis.[2,12] Investigating the pharmacokinetic profile and the efficacy between twice daily NF100 extended release and thrice daily original formulation of NF100 would be interesting especially in pregnancy.

In conclusion, we found no differences between NF50 and NF100 in the treatment of patients with uncomplicated and complicated cystitis for early and late treatment failure. NF100 was superior in patients with cystitis during pregnancy, yielding a lower rate of late treatment failures.

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CHAPTER 4

Nitrofurantoin 100 mg versus 50 mg prophylaxis for urinary tract infections, a cohort study

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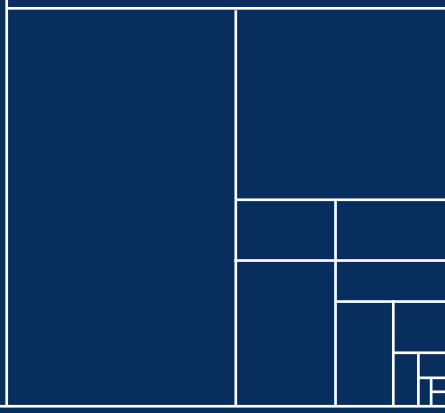


ABSTRACT

Objectives: Guidelines do not distinguish between 50 mg or 100 mg nitrofurantoin as daily prophylaxis for recurrent urinary tract infection (UTI), although 50 mg might have a better safety profile. Our objective was to compare the effectiveness and safety of both regimens.

Methods: Data were retrospectively collected from 84 Dutch GP practices between 2013 and 2020. Nitrofurantoin prescriptions of 100 mg and 50 mg every 24 hours in women were included. Cox-proportional-hazard regression analysis was used to calculate hazard ratios (HRs) on first episode of UTI, pyelonephritis and (adverse) events. Patients were followed for the duration of consecutive repeated prescriptions, assuming non-informative right censoring, up to one year.

Results: Nitrofurantoin prophylaxis was prescribed in 1,893 patients. Median lengths of follow-up were 90 days (IQR 37-179) for 100 mg (n=551) and 90 days (IQR 30-146) for 50 mg (n=1,342) with few differences in baseline characteristics between populations. Under 100 mg and 50 mg, 82/551 (14.9%) and 199/1,342 (14.8%) developed UTI and 46/551 (8.3%) and 81/1,342 (6.0%) developed pyelonephritis, respectively. Adjusted HRs of 100 mg versus 50 mg were 1.01 (95%CI 0.78-1.30) on first UTI, 1.37 (95%CI 0.95-1.98) on first pyelonephritis episode, 1.82 (95%CI 1.20-2.74) on first consultation for cough, 2.68 for dyspnea (95%CI 1.11-6.45) and 2.43 for nausea (95%CI 1.03-5.74).

Conclusion: Daily prophylaxis for recurrent UTI with 100 mg instead of 50 mg nitrofurantoin was associated with an equivalent hazard on UTI or pyelonephritis, and a higher hazard on cough, dyspnea and nausea. We recommend 50 mg nitrofurantoin as daily prophylaxis.

INTRODUCTION

Nitrofurantoin is frequently used as prophylaxis for recurrent urinary tract infection (UTI).[1,2] Although non-antimicrobial strategies to prevent UTI recurrences are preferable with regard to antimicrobial resistance and adverse events, the use of antimicrobial prophylaxis is still needed in daily practice.[3–6] Nitrofurantoin is the first choice antimicrobial for uncomplicated UTI due to persisting low resistance rates among uropathogens.[7–11] The efficacy of nitrofurantoin as daily prophylaxis has been well established in multiple randomized controlled trials with an estimated reduction in the risk of UTI of more than 50% as compared to placebo.[8,9,11]

Nitrofurantoin as prophylaxis is sometimes being used for years at a time.[8] The Dutch guideline advises to prescribe nitrofurantoin for no longer than 12 months,[1] because prolonged use seems to be associated with the occurrence of severe adverse events, among which pulmonary toxicity.[8,12,13]

International and Dutch guidelines make no distinction between their recommendation to use 50 mg or 100 mg normal-release macrocrystalline nitrofurantoin for continuous prophylaxis of UTI.[1,2,14] Since a daily dose of 50 mg nitrofurantoin results in half the cumulative dose, we hypothesized that this might be accompanied with a better safety profile. The objective of this retrospective cohort study is to compare the effectiveness and safety of nitrofurantoin 100 mg versus 50 mg as continuous prophylaxis of recurrent UTI in women.

METHODS

Design and data collection

An extensive description of the methods could be found in online supplementary material S1. Data were retrospectively obtained from the Julius General Practitioners' Network (JGPN) that comprises information on patient characteristics, diagnoses, prescriptions, requested investigations and laboratory values from general practitioners (GP) practices in the Netherlands.[15] Online supplementary material S2 provides the classifications of episodes, endpoints and confounders that were used. Episodes were selected between January 2013 and November 2020.

Study population

First episodes of nitrofurantoin prophylaxis in women of at least 12 years of age who had been prescribed nitrofurantoin (50 mg or 100 mg) every 24 hours for at least 15 days were eligible, according to the guideline.[1]

Endpoints

First episode of UTI was defined as a registration of UTI with a prescription of an appropriate antibiotic. Therapeutic prescriptions with nitrofurantoin were excluded from the main analysis, but in a sensitivity analysis, nitrofurantoin prescriptions were included in the endpoint. First episode of pyelonephritis was defined as a registration of pyelonephritis with a therapeutic prescription. The safety endpoints consisted of first consultations for common or serious (adverse) events during nitrofurantoin use, listed in Table 2.

Duration of follow-up

The patients' duration of follow-up was estimated by the number of days between the start date of the first prescription to the end date of the last repeated prescription. Consecutive repeated prescriptions were included in follow-up if the start date fell within 30 days of the end-date of the prior prescription. Because uncertainty exists about the estimated follow-up duration, two sensitivity analyses were performed for the endpoints first episode of UTI and first episode of pyelonephritis, in which different follow-up durations were estimated, with an interval of respectively 1 and 75 days between the start date of a consecutive prescription and the end date of the prior prescription, instead of the above 30 days. For the endpoint first pulmonary event, a sensitivity analysis was performed with a fixed follow-up duration of 365 days after initiation of nitrofurantoin, irrespective the estimated duration of use, because pulmonary toxicity can occur after discontinuation of nitrofurantoin.

Statistical analysis

Kaplan–Meier estimates were plotted for time to first UTI and for first pulmonary event for the populations that used 100 mg and 50 mg nitrofurantoin. Mixed effects cox regression was used to calculate hazard ratios for all endpoints, using 50 mg nitrofurantoin as the reference category. The confounders that were used are described in the footnote of Table 2. A sensitivity analysis was conducted using propensity matching in which 551 patients using 50 mg nitrofurantoin were matched with 551 patients using 100 mg nitrofurantoin, on covariates that were either imbalanced between populations or that were associated with the endpoint. The covariates that were used for matching are described in the footnote of Table 2.

Ethics

The study was designated as not being subject to the Medical Research Involving Human Subjects Act by the ethical board of the University Medical Centre Utrecht, the Netherlands.

RESULTS

Study population

Figure 1 provides the flowchart for inclusion. In the end, 551 patients receiving 100 mg and 1,342 patients receiving 50 mg nitrofurantoin were eligible. For the main analysis, 3,333 consecutive prescriptions (42%) were excluded because the start-date fell more than 30 days after the prior prescriptions' stop date. The estimated median follow-up duration was 90 days (Inter Quartile Range: 30-152), which was equal between the 100 mg population (median 90 days, IQR 37-179) and the 50 mg population (median 90 days, IQR 30-146). For the sensitivity analysis with an interval of 1 day, 5,023 (63%) prescriptions were disregarded, with a median follow-up duration of 89 days (IQR 30-90, 100 mg: 89 days, IQR 30-92, 50 mg: 89 days, IQR 30-90). For the sensitivity analysis with an interval of 75 days, 2,518 (31%) prescriptions were disregarded, with a median follow-up duration of 90 days (IQR 40-181, 100 mg: 90 days, IQR 40-197, 50 mg: 90 days, IQR 40-180). A large variety existed in the proportional use of 100 mg versus 50 mg nitrofurantoin prophylaxis per GP practice, illustrated in online supplementary material S3. The baseline characteristics of the study population are summarized in Table 1.

Figure 1. Flowchart of nitrofurantoin (NF) as continuous prophylaxes from 84 Dutch GP practices between January 2013 and November 2020

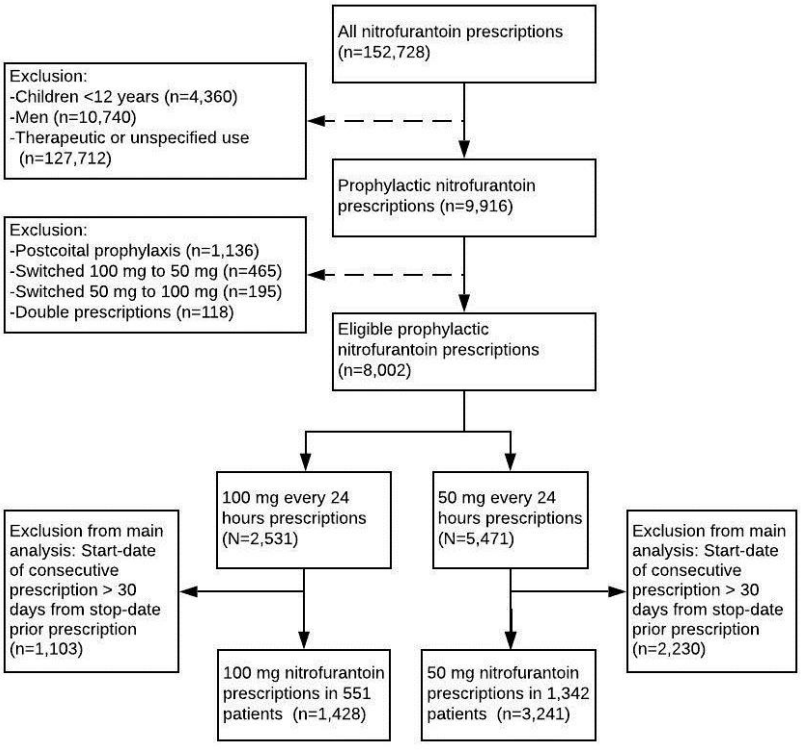


Table 1. Baseline characteristics in the population that received nitrofurantoin 100 mg or 50 mg as continuous prophylaxis for urinary tract infections in women.

Patient characteristics	Prophylactic nitrofurantoin (n=1,893)		
	100 mg every 24 hours (n=551)	50 mg every 24 hours (n=1,342)	P-value
Age (years)			
Mean (SD)	57 (22)	57 (23)	0.74
Socio-economic status score#			
Not available (%)	1 (0)	2 (0)	
Mean (SD)	0.34 (1.2)	0.25 (1.2)	0.14
eGFR (mL/min)			
Not available (%)	306 (56)	714 (53)	
Mean (SD)	90 (22)	92 (22)	0.45
Pregnancy (%)	65 (12)	219 (16)	0.01
STD (%)	48 (9)	130 (10)	0.51
Use of OAC (%)	98 (18)	253 (19)	0.59
Diabetes Mellitus (%)	101 (18)	194 (14)	0.04
Hba1C (SD)	50 (15)	54(12)	
Renal or urologic disorders* (%)	19 (3)	58 (4)	0.38
Nephrolithiasis (%)	23 (4)	58 (4)	0.89
Immunosuppressive state (%)	18 (3)	23 (2)	0.04
N of UTI episodes in previous year$			
Mean (SD)	2.6 (3.5)	2.7 (2.8)	0.57
N of UTI episodes in previous half year$			
Mean (SD)	1.8 (2.2)	1.9 (1.9)	0.61
Recurrent UTI^ (%)	283 (51)	748 (56)	0.08
Obstructive lung disease&	61 (11)	156 (12)	0.73
IBD/peptic ulcer	46 (8)	115 (9)	0.61

SD = standard deviation, IQR = interquartile range, mL/min = millilitre per minute, N = number, NA = not available, eGFR = estimated glomerular filtration rate, STD = sexually transmitted diseases, OAC = oral anticonception, UTI=urinary tract infection, IBD = inflammatory bowel disease
# socio-economic state ranges from -7 to +7 and is estimated on the neighbourhood the patient lives (postal code)
* other than nephrolithiasis
$ number of prescriptions for cystitis or pyelonephritis in the previous 183 or 365 days before the episode, respectively
^ defined as having had three or more UTIs in the last year or two in the last 6 months
& asthma or chronic obstructive pulmonary disease

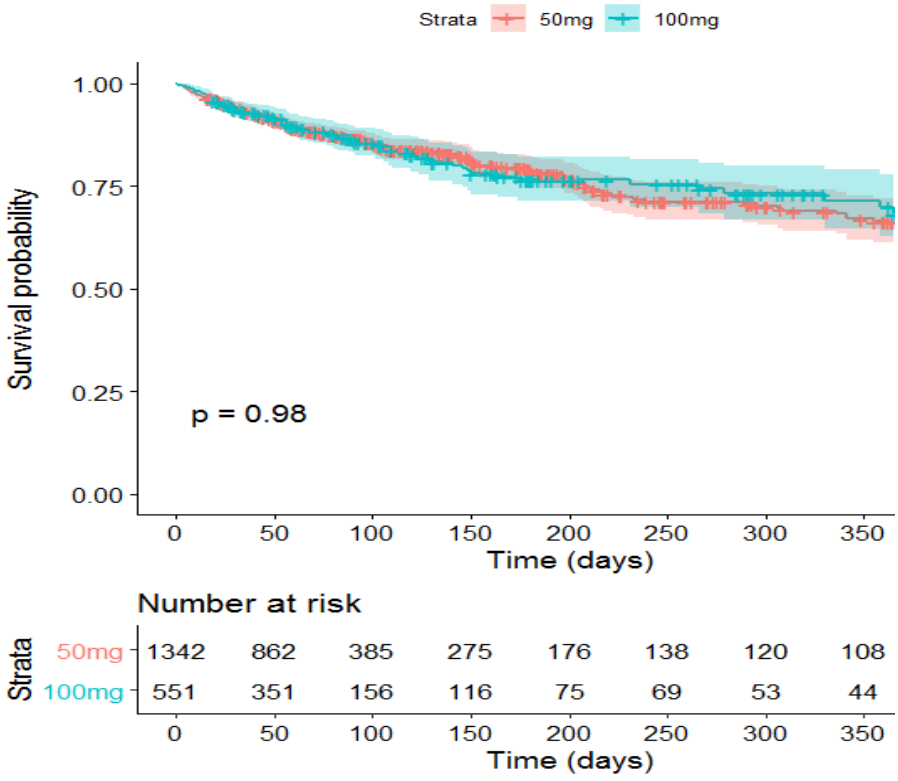
Effectiveness

Figure 2 represents the crude survival plot with the probability on first episode of UTI after initiation of 100 mg and 50 mg nitrofurantoin prophylaxis. After adjustment for confounders, the adjusted hazard ratio (aHR) on first episode of UTI when using 100 mg instead of 50 mg nitrofurantoin was 1.01 (95%CI 0.78-1.30), see Table 1. Factors that were associated with the hazard of UTI were an increased age (aHR annually 1.02, 95%CI 1.01-1.02), having urological or renal comorbidity (aHR 2.15, 95%CI 1.35-3.42), being immunocompromised (aHR 2.64, 95%CI 1.57-4.42) and an increased number of UTI within the prior year (aHR per UTI 1.04, 95%CI 1.02-1.06). The adjusted hazard ratio on the occurrence of pyelonephritis

between 100 mg and 50 mg was 1.37 (95%CI 0.95-1.98). Propensity matched analyses resulted in similar hazard ratios (Table 2).

The sensitivity analysis in which nitrofurantoin prescriptions were included in the endpoint first UTI revealed an adjusted hazard of 100 mg versus 50 mg of 0.94 (95%CI 0.73-1.20). The sensitivity analysis with an interval period of 1 day resulted in an adjusted hazard of 0.83 (95%CI 0.59-1.17) on first UTI and 1.33 (95%CI 0.90-1.98) on first pyelonephritis. The sensitivity analysis with an interval period of 75 days provided an adjusted hazard of 0.88 (95%CI 0.65-1.21) on first UTI and on first pyelonephritis of 1.02 (95%CI 0.65-1.61).

Figure 2. Crude survival plot containing a point-wise 95% confidence interval of time-to- first episode of urinary tract infection after the first prescription of 100 mg or 50 mg nitrofurantoin as continuous daily prophylaxis



Safety

The use of 100 mg instead of 50 mg was associated with an increased hazard on a consultation because of cough (aHR 1.82, 95%CI 1.20-2.74), dyspnoea (aHR 2.68, 95%CI 1.11-6.45) and nausea (aHR 2.43, 95%CI 1.03- 5.74). Figure 3 provides the crude survival plot with the probability of first pulmonary event. Factors that were associated with an increased hazard for first pulmonary event were the number of UTI in the previous 12 months before the episode (aHR per UTI 1.05, 95%CI 1.02-1.08) and pulmonary disease in the medical history or lung-related consultation within 365 days prior to the episode (aHR 2.33, 95%CI 1.68-3.25). The sensitivity analysis for the endpoint first pulmonary event with a fixed duration of follow up of 365 days resulted in a hazard ratio of 1.35 (95%CI 1.06-1.71). Table 2 describes all safety endpoints.

Figure 3. Crude survival plot containing a point-wise 95% confidence interval of time-to- first pulmonary event, defined as any pulmonary related consultation, in the populations that used 100 mg or 50 mg nitrofurantoin as continuous prophylaxis

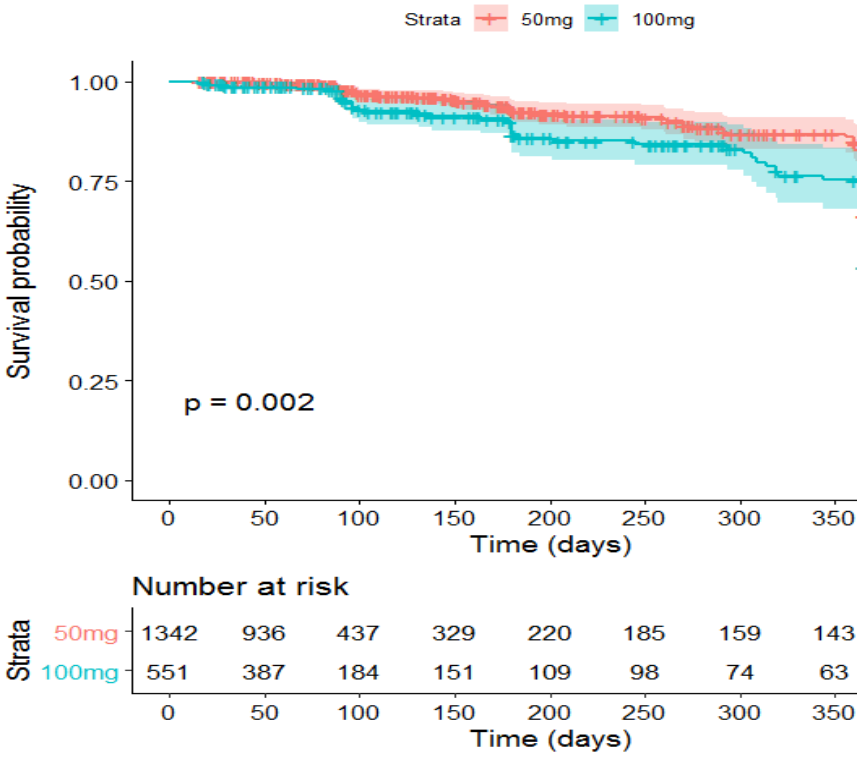


Table 2. All endpoints between the populations that were prescribed nitrofurantoin 100 mg or 50 mg as prophylaxis for urinary tract infections.

	Prophylactic nitrofurantoin		Crude HR	Adjusted HR	Propensity score matching
	100 mg every 24 hours (n=551)	50 mg every 24 hours (n=1,342)	100 mg versus 50 mg (95% CI)	100 mg versus 50 mg (95% CI)	HR 100 mg (n=551) versus 50 mg (n=551) (95% CI)#
Urinary tract infection (%)	82 (15)	199 (15)	1.00 (0.88-1.29)	1.01* (0.78-1.30)	0.98 (0.72-1.33)
Pyelonephritis (%)	46 (8)	81 (6)	1.37 (0.96-1.97)	1.37 * (0.95-1.98)	1.20 (0.78 – 1.85)
Any pulmonary consultation (%)	60 (11)	80 (6)	1.68 (1.20-2.34)	1.75 ** (1.24-2.47)	1.47 ## (0.97-2.23)
Cough (%)	42 (8)	51 (4)	1.84 (1.23-2.78)	1.82 ** (1.20-2.74)	1.56 ## (0.94-2.60)
Dyspnoea (%)	9 (2)	12 (1)	2.99 (1.26-7.09)	2.68 ** (1.11-6.45)	2.59 ## (0.84-8.04)
Unspecified LRTI (%)	17 (3)	24 (2)	1.56 (0.84-2.91)	1.75 ** (0.92-3.31)	1.29 ## (0.61-2.76)
Any gastrointestinal consultation (%)	46 (8)	84 (6)	1.23 (0.86-1.76)	1.26 *** (0.88-1.81)	1.29 ### (0.79-1.81)
Nausea (%)	11 (2)	10 (1)	2.45 (1.04-5.78)	2.43 *** (1.03- 5.74)	2.57 ### (0.82-8.08)
Diarrhoea (%)	7 (1)	20 (1)	0.76 (0.32-1.80)	0.78 *** (0.33-1.85)	1.31 ### (0.42-4.13)
Abdominal pain (%)	25 (5)	44 (3)	1.26 (0.77-2.07)	1.32 *** (0.81-2.16)	1.68 ### (0.88-3.24)
Alopecia (%)	2 (0)	5 (0)	0.89 (0.17-4.59)	1.56 **** (0.03-70.17)	1.71 & (0.16 -18.87)
(Candida) vaginitis (%)	15 (3)	38 (3)	0.90 (0.49-1.63)	0.71 ^ (0.38-1.31)	0.87&& (0.43-1.76)
(Poly)neuropathy (%)	6 (1)	13 (1)	0.99 (0.38-2.65)	0.58 ^^ (0.28-2.04)	1.11 &&& (0.34-3.64)
Hepatotoxicity (%)	3 (1)	5 (0)	1.35 (0.32-5.66)	2.05 ^^^ (0.45-9.40)	2.71 @ (0.28-26.10)
Headache (%)	9 (2)	22 (2)	0.90 (0.41-1.95)	0.97 ^^^^ (0.44-2.13)	0.93 @@ (0.37-2.35)
Allergic reaction NOS (%)	12 (2)	37 (3)	0.72 (0.38-1.38)	0.70 ^^^^^ (0.36-1.34)	0.78@@@ (0.36-1.69)

LRTI = lower respiratory tract infection, HR = Hazard ratio, 95% CI = 95% confidence interval, NOS = not otherwise specified

*adjusted for age, socio-economic status, pregnancy, estimated glomerular filtration rate (eGFR), immunosuppressive state, the use of oral anticonception, urolithiasis, urologic or renal diseases other than urolithiasis, diabetes mellitus, sexually transmitted disease, the number of UTI within 12 months prior to the episode ** adjusted for lung disease in the medical history or lung-related consultations within 365 days prior to the episode, smoking, the number of UTI within 12 months prior to the episode ***adjusted for gastrointestinal disease in the medical history or gastro-intestinal related consultations within 12 months prior to the episode and pregnancy ****adjusted for alopecia in the medical history, age ^adjusted for a consultation because of candida vaginitis within 12 months prior to the episode, pregnancy and age ^^adjusted for a consultation because of polyneuropathy within 12 months prior to the episode, diabetes mellitus and age ^^^adjusted for a consultation because of hepatotoxicity within 12 months prior to the episode, diabetes mellitus and age ^^^^adjusted for a consultation because of headache within 12 months prior to the episode and age ^^^^^adjusted for a consultation because of allergy within 12 months prior to the episode and age

#the populations were matched for age, socio-economic status, pregnancy, immunosuppressive state, diabetes mellitus, the number of UTI within 12 months prior to the episode ##additionally matched for lung disease in the medical history or lung-related consultations within 365 days prior to the episode, smoking ###additionally matched for gastrointestinal disease in the medical history or gastro-intestinal related consultations within 12 months prior to the episode

& additionally matched for alopecia in the medical history && additionally matched for candida vaginitis within 12 months prior to the episode &&& additionally matched for polyneuropathy within 12 months prior to the episode @ additionally matched for hepatotoxicity within 12 months prior to the episode @@ additionally matched for headache within 12 months prior to the episode @@@ additionally matched for allergy within 12 months prior to the episode

DISCUSSION

The results of this study constitute an important indication that daily use of 100 mg compared to 50 mg nitrofurantoin is associated with an increased hazard on pulmonary (adverse) events and nausea, but an equivalent hazard on first UTI or pyelonephritis.

To our knowledge, no head-to-head comparisons have been made between the effectiveness or safety of 100 mg and 50 mg nitrofurantoin as UTI prophylaxis. A meta-analysis of controlled trials that compared nitrofurantoin to placebo made an indirect comparison between 100 mg and 50 mg, which yielded no difference on UTI incidence.[8] A randomized crossover study compared the pharmacokinetic profile of macrocrystalline normal-release nitrofurantoin and found a significantly higher dose-normalized area under the curve in urine after using 50 mg every 6 hours instead of 100 mg every 8 hours, which suggests that the availability of nitrofurantoin into urine is saturable.[18] This could explain why an increased dose would not improve its effectiveness as prophylaxis.

The use of 100 mg over 50 mg nitrofurantoin as continuous prophylaxis was associated with a clinically relevant hazard for pulmonary events, mainly consisting of cough and dyspnea. The number of patients needed to prevent one pulmonary event by using a daily dose of 50 mg instead of 100 mg nitrofurantoin was 20. The nature, severity and prognosis of these events is unfortunately unknown. The high percentage of patients with pulmonary events in our study (7.4%) is not consistent with the low incidence of severe pulmonary toxicity in a meta-analysis (0.001%-0.2%), and we assume that most pulmonary events in our study were mild and self-limiting.[8] Pulmonary events in our study weren't necessarily related to the use of nitrofurantoin but could have rather been the expression of underlying lung disease. In another cohort of 3,400 mainly short-term nitrofurantoin users 641 pulmonary or hepatic adverse events (18.6%) were recorded, using ICD-9 codes, but after manual chart inspection 89% were not considered related to the use of nitrofurantoin.[13] Nevertheless, the comparable populations and the adjusted analysis in our study make it plausible that the observed higher incidence of pulmonary events is attributable to the higher daily dose of nitrofurantoin, through a higher systemic availability and/or an increased cumulative dose.

To monitor and objectify these pulmonary events and to estimate its nature, severity and prognosis we recommend to educate patients about the risk of pulmonary toxicity. A wide variety of pulmonary syndromes has been attributed to the use of nitrofurantoin in literature and pharmacovigilance databases, the most common being acute pulmonary hypersensitivity, which typically arises within one month after start of nitrofurantoin with fever (82%), dyspnea (60%) and cough (43%), and chronic pneumonitis, which is rarer and normally develops after 12 months of use with dyspnea (73%), dry cough (63%), and fatigue (37%). Given our relatively short follow-up period, we may have missed consultations

for chronic pneumonitis. If recognized early with discontinuation of nitrofurantoin, prognosis of both entities is on average good, however, serious irreversible lung damage could also occur and fatalities have also been described.[19,20]

It was already known that macrocrystalline nitrofurantoin prophylaxis is associated with a higher incidence of gastrointestinal adverse events when compared to other antimicrobials, with relative ratios of 2.24 (95% CI 1.77–2.83) and 2.17 (95% CI, 1.34-3.50) in two meta-analyses.[8,9,21] We found that more nausea occurred after using 100 mg instead of 50 mg nitrofurantoin with a low but probably underestimated incidence. No differences were observed between 100 mg and 50 mg regarding other adverse or serious adverse events, e.g. hepatotoxicity, polyneuropathy or headache.

The population that used continuous prophylaxis consisted of mostly post-menopausal women, with a relatively high incidence of diabetes mellitus and urological disorders. Few differences existed in baseline characteristics between the populations that used 100 mg and 50 mg nitrofurantoin, probably as a consequence of the fact that the Dutch guideline does not distinguish between both. Together with the large variation in proportional use of 100 mg to 50 mg that we observed between GP practices, this indicates that the GP preference is based on habitual or logistical factors instead of patient characteristics, which diminishes the risk on confounding bias. Naturally, confounding by indication cannot be excluded. GPs that prescribe 100 mg nitrofurantoin instead of 50 mg may have informed patients better about the risk of pulmonary toxicity, or they could have monitored these patients more intensively. On the other hand, physicians that are aware of this risk of lung toxicity are probably more likely to prescribe 50 mg instead of 100 mg nitrofurantoin. So if there would be confounding by indication it is unclear whether this under- or overestimated the true effect.

Most limitations of this study result from its retrospective nature. Data from the JGPN provides reliable quantitative estimates of demographic data, drug prescriptions (ATC codes), complaints, symptoms and entities (ICPC codes) and laboratory values.[15] Nevertheless, data has not been collected for the purposes of this study, and assumptions needed to be made on the actual presence of UTI episodes, endpoints and confounder estimates. The exact motive to prescribe prophylaxis was unknown, although it is reassuring that the majority of the population suffered from recurrent UTI.[15] The presence of UTI was not confirmed with urine culture or with urinalysis because this data was lacking in the JGPN database, which may have increased the risk of misclassification. This risk is lowered by the fact that Dutch GPs usually confirm the presence of UTI using dipstick before prescribing antimicrobials.[1,15,22] We had no access to prescriptions from hospitals or out of office GP services, as a result of which endpoints may have been underestimated. Only 6% of total antimicrobial prescriptions in primary care occurs out of office hours in the Netherlands,

diminishing this risk.[23] For the estimation of the safety endpoints we relied on ICPC registrations of complaints, symptoms and entities by GPs. As mentioned, the ICPC encoding system does not contain sufficient detail to draw inferences on the incidence, nature or severity of adverse events. Unfortunately, we lacked data and were not able to adjust the calculation of the eGFR on the ethnicity of patients. Only a small minority in the Netherlands are of African descent. Next, the follow-up duration was difficult to estimate. Repeated prescriptions did not always follow prior prescriptions consecutively because these were acquired with delay as a probable consequence of varying treatment adherence. To capture these effects on the effectiveness, we performed two sensitivity analyses with different intervals which revealed similar results. Other limitations resulted from our statistical approach. We used right-censoring if we estimated that prophylaxis was discontinued. This could have occurred for several reasons e.g. because of the intended end of use, because of treatment non-adherence, as a result of unregistered failure or as a result of adverse events. Theoretically, reasons for censoring could have differed between 100 mg and 50 mg, although we expected that this would also have led to differences in the estimated follow-up durations, which we did not observe. The propensity matched analyses pointed towards the same direction as the crude and multivariable analyses. Significance was not reached due to the smaller sample size and insufficient power.

In conclusion, the use of 100 mg instead of 50 mg nitrofurantoin as daily prophylaxis for UTI is associated with an increased hazard on pulmonary events and nausea but an equivalent hazard on clinical failure. The findings in this study emphasize existing concerns about pulmonary toxicity after prophylactic nitrofurantoin use.[8,24] A randomized controlled trial with close and in depth monitoring of pulmonary toxicity would yield an unbiased result in terms of efficacy and safety. Until that time, we would recommend to use 50 mg nitrofurantoin as prophylaxis for recurrent UTI with close monitoring of pulmonary signs and symptoms.

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CHAPTER 5

Carbapenem-alternative strategies for complicated urinary tract infections: a systematic review of randomized controlled trials

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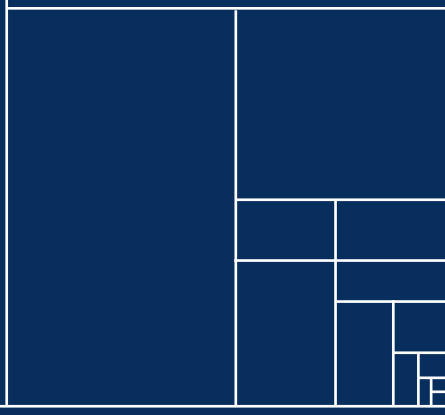


ABSTRACT

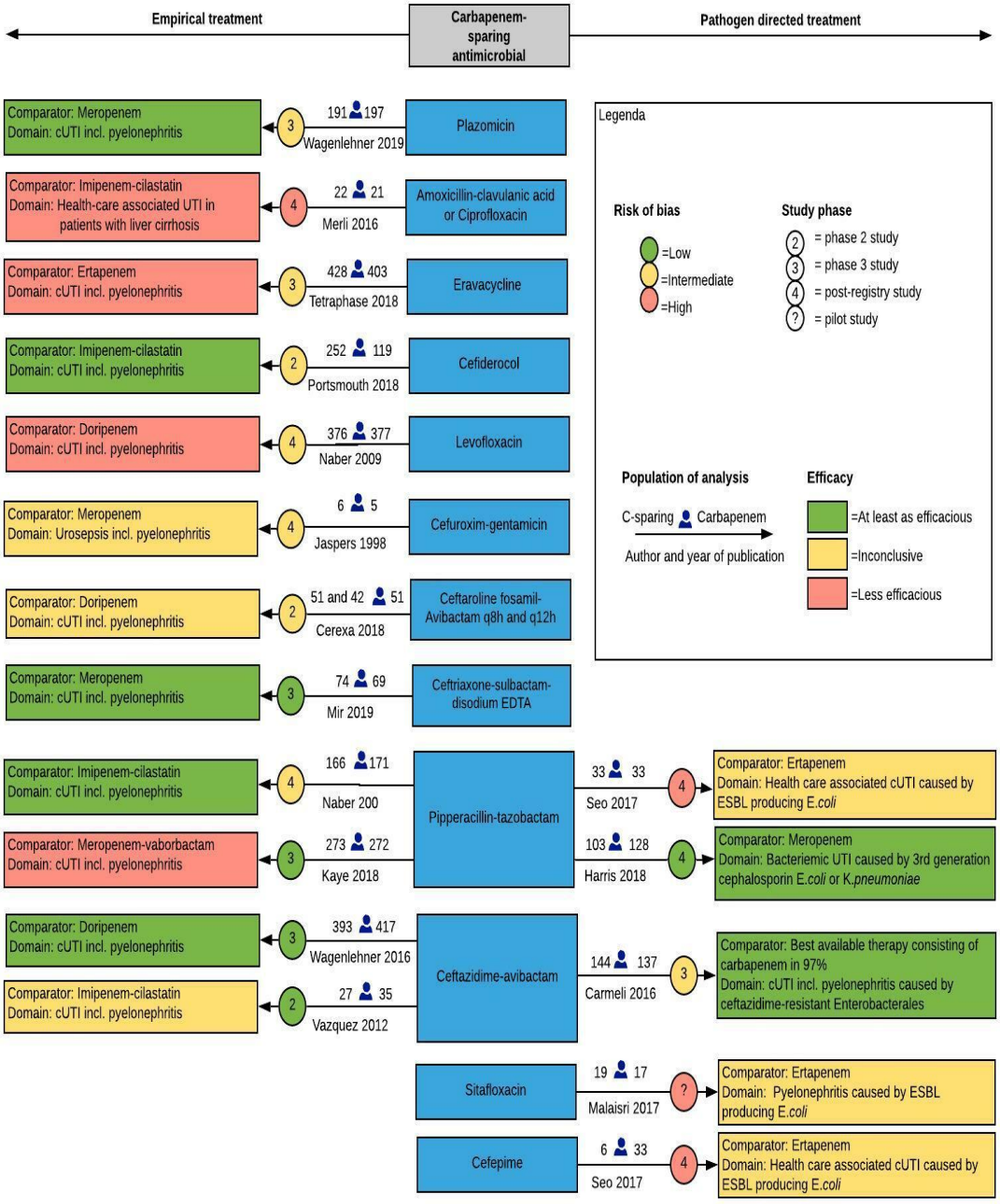
Objectives: We systematically reviewed randomized controlled trials (RCTs) that compare the efficacy and safety of carbapenem-alternatives with *in vitro* activity to ESBL to carbapenem for the treatment of complicated urinary tract infections.

Methods: Medical databases, infectious diseases journals, conference abstracts and registry libraries were systematically searched for relevant RCTs that reported clinical or microbiological efficacy in adult patients. Risk of bias assessment and data extraction was performed in duplicate. The protocol was published at PROSPERO [CRD42017054102].

Results: Of 1,950 identified records, 16 RCTs fulfilled eligibility criteria, of which 5 had low risk of bias. For empirical treatment, ceftazidime-avibactam, plazomicine, cefiderocol and ceftriaxone-sulbactam-Disodium EDTA had equivalent efficacy to carbapenem, of which the first three induced less microbiological failure with risk ratios of respectively 0.78 (95%CI 0.62-0.99), 0.45 (95%CI 0.29-0.70) and 0.62 (95%CI 0.46-0.82). For pathogen-directed treatment, ceftazidime-avibactam had equivalent efficacy to carbapenem with less early microbiological failures (RR:0.50,95%CI 0.33-0.76) in an trial ended prematurely. Inconclusive or inferior results were found for piperacillin-tazobactam, levofloxacin, eravacycline, cefuroxime-gentamicin, amoxicillin-clavulanic acid or ciprofloxacin, ceftaroline fosamil-avibactam (empiric), and cefepime, piperacillin-tazobactam and sitafloxacin (pathogen-directed).

Conclusion: Ceftazidime-avibactam, plazomicine, cefiderocol and ceftriaxone-sulbactam-Disodium EDTA as empirical therapy and ceftazidime-avibactam as pathogen-directed therapy, may be considered as (future) treatment alternatives for carbapenem for cUTI.

GRAPHICAL ABSTRACT



INTRODUCTION

Urinary Tract Infections (UTI) are the most common bacterial infections requiring antibiotic treatment in the western world.[1,2] The clinical spectrum of UTI includes urethritis, cystitis, prostatitis and pyelonephritis. Most UTIs have a rapid and favourable response to antibiotic treatment.[3] Complicated UTI (cUTI) is defined by the presence of systemic symptoms or susceptibility of the host for a complicated course, for example pregnancy or functional deficits of the urinary tract.[4] Most guidelines recommend treating cUTI with a 7-14 day course of antibiotics.[4,5] In cases of febrile-UTI (i.e. pyelonephritis, sepsis or acute prostatitis) empiric intravenous antibiotic therapy is mostly advised, with stepdown to pathogen-directed antibiotics when possible. The empirical antibiotic treatment should cover most prevalent causative pathogens. Appropriate empirical treatment options that cover the unknown causing pathogen are therefore especially critical in severe infections and vulnerable patients.[6] Pathogen-directed adjustments should be made as soon as antibiotic susceptibility patterns are known.[4,5,7] The emergence of *Enterobacterales* carrying Extended-Spectrum β-Lactamase (ESBL) enzymes has limited the antimicrobial arsenal available for both empiric and pathogen-directed treatment of cUTI.[8] Distribution of ESBL-carriage varies greatly worldwide, with a reported prevalence up to 70% in the Asia-Pacific region.[29] Rates of co-resistance to fluoroquinolones, aminoglycosides and trimethoprim-sulfamethoxazole are high, further limiting treatment options.[10]

Until recently, carbapenems were considered the last resort treatment for infections caused by ESBL-producing *Enterobacterales*. Unfortunately, carbapenem use is highly associated with the emergence of Carbapenem Resistant *Enterobacterales* (CRE).[9,11,12] Patients that have cUTI with CRE bacteraemia are at increased risk of receiving inappropriate antimicrobial therapy and are more likely to die from the infection, compared to patients with carbapenem-susceptible *Enterobacterales*.[13]

To find alternative treatment options for - and to reduce the incidence of CRE - carbapenem-alternative antimicrobial strategies for the empirical and pathogen-directed therapy for cUTI have been widely advocated. Several classes of carbapenem-saving antimicrobials have been developed or re-explored, including β-Lactam/β-lactamase inhibitor combinations (BL/BLIs), tigecycline, colistin and fosfomycin.[14–25]

The aim of this systematic review was to identify carbapenem-alternative antimicrobial strategies with comparable efficacy and safety as carbapenems that could be used for the empirical or pathogen-directed treatment of cUTI.

METHODS

Protocol

This systematic review was conducted following the Cochrane handbook.[26] Prior to the search the protocol was published at PROSPERO, the international prospective register of systematic reviews, available from: https://www.crd.york.ac.uk/prospero/display_record.asp?ID=CRD42017054102. Two amendments were made prior to conducting the search. Microbiological cure was added as a co-primary outcome. In the fourth version the third reviewer was added (CW). We deviated from the original protocol by also including randomized controlled trials (RCTs) investigating the empirical treatment if the participant had a complicated urinary tract infection with any pathogen, whereas the protocol was restricted to infections caused by Enterobacterales. Next, the co-primary endpoint 'clinical cure with microbiological success' was included if data for the separate endpoints was not available. The study was reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines.[27] Finally, instead of the Dutch trialregister (https://www.trialregister.nl), the European clinical trial register was searched to find more eligible trials.

Search strategy

MEDLINE, EMBASE, the Cochrane Central Register of Controlled Trials (CENTRAL) and PSYCHINFO were searched on 4 March 2020 combining search terms for carbapenem and UTI and their synonyms. The search was limited to RCTs, using the RCT search filters as recommended by Cochrane.[28] The full search strategy is reported in the online supplementary material S1.

Reference lists of eligible studies and systematic reviews, abstracts from the ECCMID and IDSA conferences from 2016 to 2020, and the top 20 infectious diseases journals in 2017 (online supplementary material S2) were manually searched for eligible studies using the search terms 'randomized controlled trial' and 'urinary tract infection'. Additionally, www.clinicaltrials.gov and www.clinicaltrialsregister.eu were searched using the term 'urinary tract infection' (online supplementary material S2).

Study selection

Titles and abstracts were independently screened by two reviewers (TD and TV), using the Cochrane recommended tool available at http://www.covidence.org. Articles were included for full-text screening if at least one of the researchers deemed a study suitable. Full text-articles were assessed for eligibility independently by the two reviewers. Conflicts were resolved by a third reviewer (CW).

Eligibility criteria

We included RCTs in adult patients (≥18 years, men or women) with a cUTI, including acute pyelonephritis, in which carbapenem-saving antimicrobials with *in vitro* activity against ESBL-producing *Enterobacterales* were compared to carbapenem therapy for either the empirical or pathogen-directed therapy. Both intravenous and oral antimicrobials were allowed, and pre-considered carbapenem-saving antimicrobials included the following: piperacillin-tazobactam, ampicillin-sulbactam, amikacin, plazomicin, gentamicin, tobramycin, ceftazidime–avibactam, ceftolozane-tazobactam, tigecycline, colistin, fosfomycin, levofloxacin, moxifloxacin, ciprofloxacin, sitafloxacin, cefepime, nitrofurantoin, trimethoprim–sulfamethoxazole. RCT's required at least one of the following outcomes: clinical cure, mortality, microbiological cure, length of hospital stay, readmission, recurrence/relapse, Intensive Care admission, and (serious) adverse event. Studies not written in English, Dutch, French, German or Spanish were excluded. A full description of the study selection could be found in online supplementary material S1.

Quality assessment

Risk of bias was assessed independently by TD and TV using the Cochrane Risk of Bias Tool, in which sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessors, incomplete outcome data, selective outcome reporting, and, if applicable, other sources of bias were judged.[26] Pre-considered other sources of bias were: (1) the study was not published in a peer reviewed journal, (2) in case of pharmacy sponsored trials, the pharmaceutical company had a role in the conduct, analysis or reporting of the RCT or this was not well described. Discrepancies were resolved by discussion. Risk of bias was categorized as high, intermediate, or low if ≥4, 2-3, or ≤1 sources of bias were scored as high or unknown.

Data extraction

Data was extracted independently by two reviewers (TD and TV). Discrepancies were resolved by discussion. For each study, information was collected about methodology, design, UTI case definition, population, intervention and comparison characteristics, and the following outcomes were extracted: primary outcomes: clinical cure, microbiological cure and mortality; secondary outcomes: length of hospital stay, readmission rate, recurrence/relapse rate, intensive care unit readmission rate, or (serious) adverse event rate. Results were reported for the intention-to-treat analysis, or, if not available (in order of preference) from the modified intention to treat analysis or the reported primary analysis. Corresponding authors were contacted to retrieve missing data. For studies identified via registration

libraries (marked as completed or recruiting) but not yet published, we contacted the authors for outcome data.

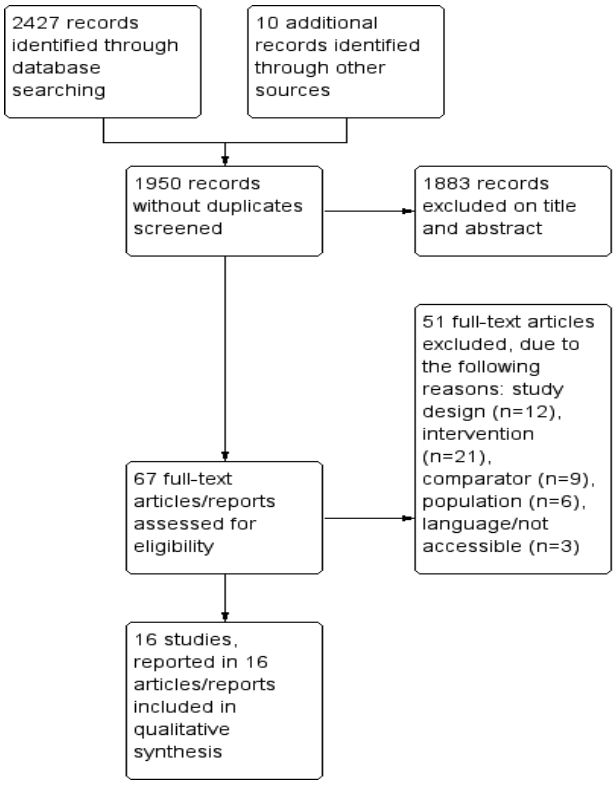
Definitions

In data extraction, a distinction was made between trials investigating empirical and pathogen-directed treatment. Empirical treatment was defined as treatment initiated without knowledge of the causative pathogen. Pathogen-directed treatment was defined as treatment directed against the causative pathogen taking into account the antibiotic susceptibility pattern. Definitions of clinical or microbiological failure used in the original studies were collected and are described. For studies not reporting clinical or microbiological failure, it was calculated as the inverse of clinical or microbiological cure, respectively. We distinguished early clinical or microbiological failure, if measured within 14 days post-end-of-treatment, from late clinical or microbiological failure, if measured between 14 and 60 days post-end-of-treatment.

Analysis

Clinical heterogeneity was assessed by comparing the interventions (antimicrobial, dose), comparators populations, i.e. cUTI, acute pyelonephritis (AP), bacteraemia, community or health-care acquired infection. Meta-analysis was planned if more than one RCT was available for one intervention, provided that the clinical heterogeneity between these trials was small.

Figure 1. Study flow diagram



RESULTS

A total of 1,950 unique records were identified through database screening supplemented with non-database sources. After screening titles and abstracts, 67 references were selected for full-text reading, of which 51 were excluded, leaving 16 studies in 16 articles (Figure 1). Three studies were judged as having high risk of bias (Seo 2017, Malaisri 2017, Merli 2016), eight had intermediate risk of bias (Jaspers 1998, Carmeli 2016, Naber 2002, Naber 2009, Portsmouth 2018, Wagenlehner 2019, Tetraphase 2018, Cerexa 2018) and five studies had low risk of bias (Kaye 2018, Wagenlehner 2016, Vazquez 2012, Harris 2018, Mir 2019). The risk of bias for all studies is described in Table 1, motivation for classification is provided in the online Supplementary material S3. Three were phase 2 studies (Portsmouth 2018, Cerexa 2018, Vazquez 2012), six were phase three studies (Wagenlehner 2019, Tetraphase 2018, Mir 2019, Kaye 2018, Wagenlehner 2016, Carmeli 2016), six were post registry studies (Merli 2016, Naber 2002, Naber 2009, Jaspers 1998, Harris 2018, Seo 2017). One study was a pilot study of a non-registered drug (Malaisri 2017).

Characteristics of studies investigating the empirical or pathogen-directed treatment are described in Tables 2 and 3. Definitions of the primary and secondary outcome measures, follow-up period and analysis population of each study are provided in the online supplementary material S4. High clinical heterogeneity between the studies was found in study populations, interventions, comparators and outcomes. For that reason, a meta-analysis was not conducted.

Table 1. Risk of bias table

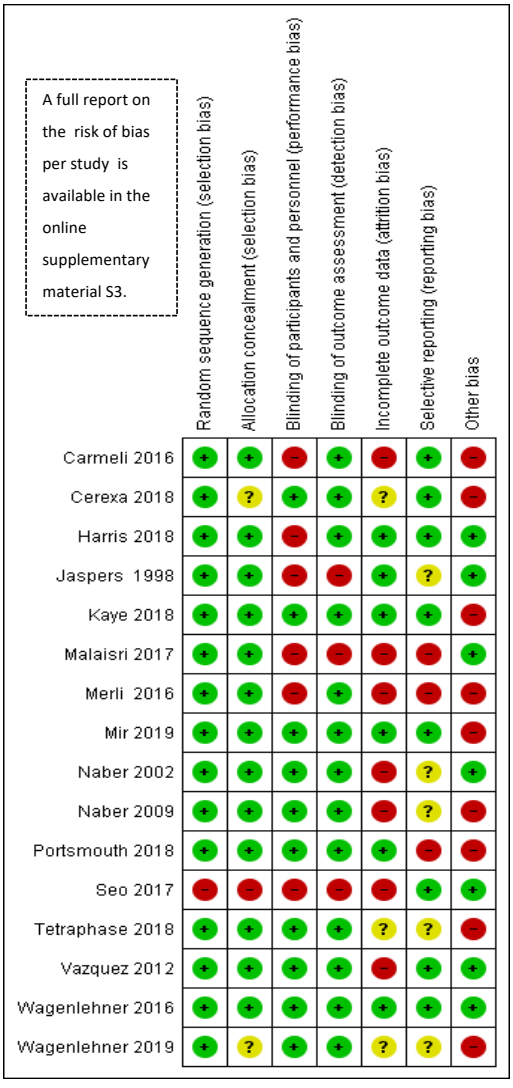


Table 2. Study characteristics of studies evaluating the empirical treatment

Study	UTI type@	Population characteristics	Population comorbidity	Intervention	Comparison	Analysis population size (of which AP)#	Treatment duration (mean/range, days)
Cerexa 2018 [29]	Adults ≥ 18 years with cUTI or AP	60 years (mean) bacteremia 6%	Mean BMI 28.5 kg/m^2	CXL 600mg-600mg ^ every 8 hours, CXL every 12 hours,	Doripenem 500 mg every 8 hours	51 vs. 42 vs. 51	7-10 days (range) 8 days (mean)
Jaspers 1998* [30]	Adults ≥65 years with cUTI or AP with systemic symptoms	76 years (mean) bacteremia 23%	Diabetes Mellitus: 14% Mean APACHE II score: 19	Cefuroxim 1500mg/8 h + gentamicin 4mg/kg body weight /24 h $	Meropenem 1000mg / 8 h $	6 vs. 5	7.5 days (mean)
Kaye 2018 [31]	Adults with cUTI or AP	53 years (mean) 66% female 5% bacteremia	Mean BMI: 26.4 kg/m^2 Diabetes Mellitus: 16% CCI≥3: 52% GFR ≤50 mL/min: 12%	Piperacillin-tazobactam 4000-500mg / 8 h $$	Meropenem-vaborbactam 2000-2000mg/8 h $$	273 vs. 272 (161 vs. 161)	10 days (of which 8 days iv)
Merli 2016* [32]	Adults with liver cirrhosis with health-care associated UTI	58 years (mean)	Diabetes Mellitus: 36% MELD score: 15 Chronic kidney disease: 12%	Amoxicillin/clavulanic acid 2200mg/8 h IV or ciprofloxacin 500 mg/12 h PO	Imipenem/cilastatin 500 mg/6 h IV)	22 vs. 21	Not reported
Mir 2019 [33]	Adults with cUTI or AP	≥65 years: 8% female: 57%	Mean BMI: 23 kg/m^2 Diabetes Mellitus: 13% GFR 30-50 mL/min: 6%	CSE 1000 mg/500 mg/37 mg /12 hours	Meropenem 1000mg /8 h	74 vs. 69 (26 vs. 26)	5-14 days (range) 6.5 days (mean)
Naber 2002 [34]	Adults with cUTI or AP	59 years (mean) female: 43%	Diabetes Mellitus: 29% Cardiopulmonary disease: 33%	Piperacillin - tazobactam 2000-500mg /8 h	Imipenem-cilastatin 500-500mg /8 h	166 vs. 171 (22 vs. 18)	5-14 days (range)
Naber 2009 [35]	Adults with cUTI or AP	51 years (mean) female: 62% bacteremia: 8%	Mean BMI: 26.5 kg/m^2 GFR <50 mL/min: 14%	Levofloxacin IV 250mg /24h $$$	Doripenem 500 mg /8 h $$$	376 vs. 377 (198 vs. 194)	10 days (incl. oral therapy)
Portsmouth 2018 [36]	Adults with cUTI or AP	62 years (mean) female: 57% bacteremia: 7%	Mean BMI: 27.3 kg/m^2 GFR ≤50 mL/min: 21%	Cefiderocol 2000mg/8h &	Imipenem-cilastatin &	252 vs. 119 (130 vs. 64)	9 days (median)
Tetraphase 2018 [37]	Adults with cUTI or AP	NA	NA	Eravacycline 1500 mg/kg/24h &&	Ertapenem 1000mg/24 h &&	428 vs. 403	7-10 days (range, incl. oral)
Vazquez 2012 [38]	Adults with cUTI or AP due to Gram-negative bacteria	47 years (mean) female: 74% bacteremia: 5%	Mean BMI: 27.0 kg/m2	Ceftazidime- avibactam 500- 125mg / 8 h &&&	Imipenem–cilastatin 500-500mg / 6 h &&&	27 vs. 35 (13 vs. 14)	7-14 days (incl. oral)

Wagenlehner 2016 [19]	Adults with cUTI or AP	52 years (mean) female: 70% bacteremia: 8%	Mean BMI: 26.3 kg/m2 GFR <50 mL/min: 10%	Ceftazidime-avibactam 2000-500mg / 8 h	Doripenem 500 mg / 8 h	393 vs. 417 (287 vs. 296)	10 days or 14 days for bacteraemia (incl. oral)
Wagenlehner 2019 [39]	Adults with cUTI or AP	59 years (mean) female: 53% bacteremia: 12%	BMI ≥25 kg/m2: 6% GFR 30-60 mL/min: 34%	Plazomicin 15 mg/kg / 24 h &&&&	Meropenem 1000mg / 8 h &&&&	191 vs. 197 (84 vs. 78)	7-10 days (incl. oral)

NA = Not available, cUTI = complicated urinary tract infection, AP = acute pyelonephritis, CCI = Charlson comorbidity index, BMI = body mass index, IV = intravenous, PO = per Oral, MELD = Model of End-Stage Liver Disease, CXL = Ceftaroline fosamil/Avibactam, CSE = Ceftriaxone, sulbactam, and disodium ethylenediaminetetraacetic acid (EDTA)

@ If not reported health-care related infection, it is considered community-acquired infection.

∗ Population characteristics and comorbidity are only available for the total study population including non-urinary source infections.

$ Adapted dosage in case of renal insufficiency.

$$ If patients met pre-specified criteria for improvement, they could be switched to oral levofloxacin (500 mg/24 h).

$$$ Switch to oral levofloxacin (250 mg administered once daily) if no fever were present for at least 24 h, if signs and symptoms of cUTI were absent or improved from baseline levels, and if at least one follow-up urine culture showed no growth or a colony count of < 10 4 CFU/ml and no subsequent cultures yield an uropathogen at ≥10 4 CFU/ml.

& No oral antibiotic (step-down) therapy was allowed.

&& Eravacycline or ertapenem was given for a minimum of 5 days followed by an optional stepdown treatment to oral levofloxacin (750 mg/24 h).

&&& Switch to oral ciprofloxacin 500 mg/12 h was allowed for the remaining treatment course, or alternative oral therapy if the patient was intolerant to ciprofloxacin or had a ciprofloxacin-resistant pathogen at baseline.

&&&& Switch to optional oral antibiotics after 4 days of empirical treatment (levofloxacin 500 mg/24 h or any other approved oral therapy).

The number of randomized patients (and the size of the safety population) could be higher than the analysis population.

Empirical treatment

For the RCTs that investigated the empirical treatment of cUTI, clinical and microbiological cure are reported in Figure 2 and 3, respectively, and other outcomes in Table 4. A phase II RCT (Vazquez 2012) reported no differences between ceftazidime-avibactam and imipenem-cilastatin regarding clinical failure, microbiological failure or mortality.[38] In a subsequent phase III RCT (Wagenlehner 2016), significantly less early (RR 0.78, 95%CI 0.62-0.99) and late microbiological failures (RR 0.81, 95%CI 0.67-0.98) were reported in patients receiving ceftazidime-avibactam compared to doripenem, with no differences in clinical outcomes.[19,38] A phase III trial (Wagenlehner 2019) compared plazomicin to meropenem for the treatment of cUTI or AP. No differences were found in early clinical failure, whereas significantly less early microbiological failures (RR 0.45, 95%CI 0.29-0.70), less late microbiological failure (RR 0.45, 95%CI 0.31-0.66) and less clinical relapses (RR 0.22, 95%CI 0.07-0.77) occurred in the plazomicin arm.[39] A phase III trial (Mir 2019) compared the efficacy of ceftriaxone-sulbactam-Disodium EDTA to doripenem for the treatment of cUTI or AP and found no differences in clinical or microbiological efficacy.[33] A phase II trial (Cerexa 2018) that compared the efficacy of ceftaroline fosamil- avibactam, administered every 8 or 12 hours, to doripenem found no significant differences in microbiological failure. The results regarding the clinical endpoints were not publically available.[29] A phase II trial (Portsmouth 2018) evaluated the efficacy of cefiderocol vs. high dose imipenem-cilastatin (1000-1000mg every 8 hours) for

cUTI. Cefiderocol resulted in significantly less early (RR 0.62, 95%CI 0.46-0.82) and late microbiological failures (RR 0.76, 95%CI 0.62-0.94) and less late clinical failures (RR 0.60, 95%CI 0.36-0.99). In a phase III trial (Tetraphase 2018) eravacycline, a novel tetracycline, was found inferior to ertapenem for the treatment of cUTI or acute pyelonephritis, with regard to the co-primary endpoint clinical cure and microbiological success at the end of infusion visit (363/428 vs. 382/403, RR 0.89, 95%CI 0.85-0.94), whereas the results at 5 to 10 days post-end of treatment were not significantly different (293/428 vs. 302/403, RR 0.91, 95%CI 0.84-1.00).[37] A RCT (Naber 2002) compared piperacillin-tazobactam to imipenem-cilastatin for cUTI. No differences were observed in clinical failure or microbiological failure.[34] In a phase III registry RCT (Kaye 2018) piperacillin-tazobactam was compared against meropenem-vaborbactam. More late clinical failure was found in the piperacillin-tazobactam arm (RR 1.58, 95%CI 1.01-2.49) with no differences in early clinical or microbiological failure.[31] A pharmacy sponsored RCT (Naber 2009) evaluated low dose intravenous levofloxacin (250mg/24 hours) with doripenem as a comparator and found more early clinical failures in the levofloxacin arm (RR 2.00, 95%CI 1.07-3.74), with no differences in early microbiological or late clinical cure.[35] In a non-pharmacy sponsored RCT (Jaspers 1998), cefuroxime-gentamicin combination therapy was compared to meropenem for the treatment of serious bacterial infections in patients >65 years. Of 79 participants, only 11 suffered urinary tract infection, impeding a valuable comparison.[30] An investigator initiated RCT (Merli 2016) compared a standard antimicrobial strategy, consisting of oral amoxicillin-clavulanic acid or intravenous ciprofloxacin with a broad spectrum strategy consisting of imipenem/cilastatin in patients with hepatic cirrhosis and health-care associated infection. In 43 included patients with cUTI a significant higher mortality rate was found in the 'standard' arm than in the imipenem/cilastin arm (5/22 vs. 0/21, RR 0.23, 95%CI 0.04-0.41), which was the primary outcome.[32] In no other of the RCTs significant differences were reported in mortality for the population with cUTI.

Table 3. Study characteristics of studies evaluating the pathogen-directed treatment

Study	UTI type@	Population characteristics@@	Population comorbidity@@	Intervention	Comparison	Total population size (of which pyelonephritis)#	Treatment duration (mean)
Carmeli 2016 [40]	Adults with cUTI or AP caused by ceftazidime-resistant Enterobacterales or Pseudomonas aeruginosa*	63 years (mean) 45% female 4% bacteremia	Mean BMI: 28.1 kg/m^2 GFR ≤50 mL/min: 22%	Ceftazidime-avibactam 2000-500mg / 8 h $	Imipenem (n=76) meropenem (n=57) ertapenem (n=3) doripenem (n=11) non-carbapenem-class (6)	144 vs. 137 (57 vs. 70)	10 days
Harris 2018 [41]	Adults with 3-GC resistant E.*coli* or K. *pneumonia* bloodstream infection	70 years (mean) 52% female 100% bacteremia	Weight: 67.8kg Renal dysfunction: 20% Diabetes Mellitus: 36% Mean CCI: 2.7	Piperacillin-tazobactam 4500mg /6h$	Meropenem .. 1000mg/8h $	103 vs. 128 (NA)	7 days
Malaisri 2017 [42]	Adults with AP with ESBL- positive Escherichia *coli* **	69 years (mean) 67% female bacteremia NA	Diabetes Mellitus: 28% Chronic kidney disease: 14%	Sitafloxacin 100mg /12 h PO $	Ertapenem 1000mg/ 24 h $	19 vs. 17 (19 vs. 17)	10 days (of which 3 days iv)
Seo (1) 2017 [43]	Adults with healthcare-associated ESBL-positive Escherichia *coli* UTI***	67 years (mean) 85% female 30% bacteremia	Diabetes Mellitus: 41% Mean CCI: 4.6	Piperacillin-tazobactam 4000-500mg / 6 h $	Ertapenem 1000mg / 24 h $	33 vs. 33	14 days
Seo (2) 2017 [43]	Adults with healthcare-associated ESBL-positive Escherichia *coli* UTI***	70 years (mean) 85% female 23% bacteremia	Diabetes Mellitus: 41% Mean CCI: 4.5	Cefepime 2000mg / 12 h $	Ertapenem 1000mg / 24 h $	6 vs. 33	14 days

NA = not available, cUTI = complicated urinary tract infection, AP = acute pyelonephritis, CCI: Charlson comorbidity index, BMI = body mass index, 3-GC = third-generation cephalosporin, IV = intravenous, PO = per Oral

@ If not reported else, the study population consists of community-acquired infection.

@@ For the population with cUTI, unless otherwise stated.

∗ Regardless of previous antibiotic therapy.

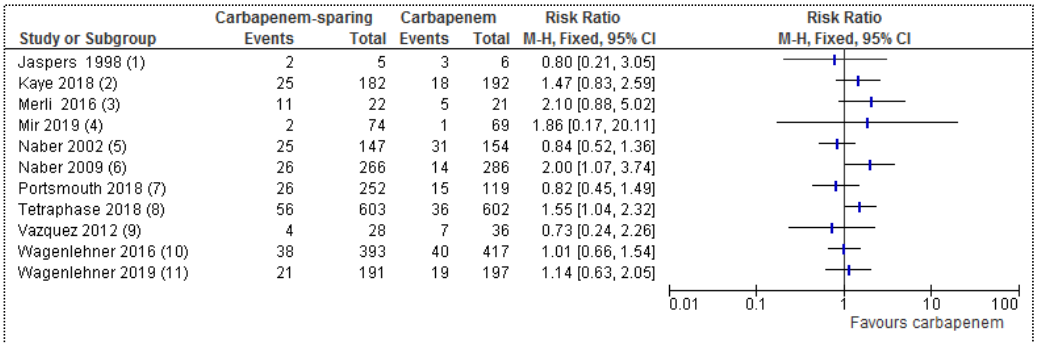
∗∗ Adults needed to use at least 3 days of iv intravenous carbapenems and the results of urine culture needed to be available. Carbapenems included meropenem 1 mg/8 h, imipenem 500 mg/6 h, doripenem 500 mg/8 h, and ertapenem 1 mg/24 h.

∗∗∗ The ESBL-EC needed to be detected and required susceptibility to the study medicines, regardless of the susceptibility to other antibiotics.

$ Adapted dosage in case of renal insufficiency.

The number of randomized patients and the size of the safety population could be higher than the analysis population.

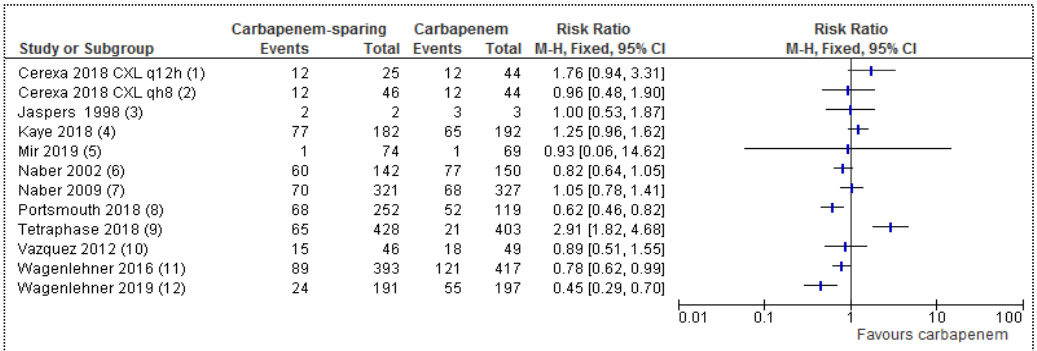
Figure 2. Early clinical failure (empirical treatment)



Study or Subgroup	Carbapenem-sparing Events	Total	Carbapenem Events	Total	Risk Ratio M-H, Fixed, 95% CI
Jaspers 1998 (1)	2	5	3	6	0.80 [0.21, 3.05]
Kaye 2018 (2)	25	182	18	192	1.47 [0.83, 2.59]
Merli 2016 (3)	11	22	5	21	2.10 [0.88, 5.02]
Mir 2019 (4)	2	74	1	69	1.86 [0.17, 20.11]
Naber 2002 (5)	25	147	31	154	0.84 [0.52, 1.36]
Naber 2009 (6)	26	266	14	286	2.00 [1.07, 3.74]
Portsmouth 2018 (7)	26	252	15	119	0.82 [0.45, 1.49]
Tetraphase 2018 (8)	56	603	36	602	1.55 [1.04, 2.32]
Vazquez 2012 (9)	4	28	7	36	0.73 [0.24, 2.26]
Wagenlehner 2016 (10)	38	393	40	417	1.01 [0.66, 1.54]
Wagenlehner 2019 (11)	21	191	19	197	1.14 [0.63, 2.05]

Footnotes
(1) Cefuroxim-gentamicin vs. meropenem (late clinical failure)
(2) Piperacillin-tazobactam vs. meropenem-vaborbactam
(3) Amoxicillin-clavulanic acid IV or ciprofloxacin po vs. imipenem-cilastin
(4) CSE vs. meropenem
(5) Piperacillin-tazobactam vs. imipenem-cilastatin
(6) Levofloxacin IV vs. doripenem
(7) Cefiderocol vs. imipenem-cilastatin
(8) Eravacycline vs. ertapenem
(9) Ceftazidime-avibactam vs. imipenem-cilastin
(10) Ceftazidime-avibactam vs. doripenem
(11) Plazomicin vs. meropenem

Figure 3. Early microbiological failure (empirical treatment)



Study or Subgroup	Carbapenem-sparing Events	Total	Carbapenem Events	Total	Risk Ratio M-H, Fixed, 95% CI
Cerexa 2018 CXL q12h (1)	12	25	12	44	1.76 [0.94, 3.31]
Cerexa 2018 CXL qh8 (2)	12	46	12	44	0.96 [0.48, 1.90]
Jaspers 1998 (3)	2	2	3	3	1.00 [0.53, 1.87]
Kaye 2018 (4)	77	182	65	192	1.25 [0.96, 1.62]
Mir 2019 (5)	1	74	1	69	0.93 [0.06, 14.62]
Naber 2002 (6)	60	142	77	150	0.82 [0.64, 1.05]
Naber 2009 (7)	70	321	68	327	1.05 [0.78, 1.41]
Portsmouth 2018 (8)	68	252	52	119	0.62 [0.46, 0.82]
Tetraphase 2018 (9)	65	428	21	403	2.91 [1.82, 4.68]
Vazquez 2012 (10)	15	46	18	49	0.89 [0.51, 1.55]
Wagenlehner 2016 (11)	89	393	121	417	0.78 [0.62, 0.99]
Wagenlehner 2019 (12)	24	191	55	197	0.45 [0.29, 0.70]

Footnotes
(1) Ceftaroline-fosamil and avibactam every 12 hours vs. doripenem
(2) Ceftaroline fosamil and avibactam every 8 hours vs. doripenem
(3) Cefuroxim-gentamicin vs. meropenem (late microbiological failure)
(4) Piperacillin-tazobactam vs. meropenem-vaborbactam
(5) CSE vs. meropenem
(6) Piperacillin-tazobactam vs. imipenem-cilastatin
(7) Levofloxacin iv vs. doripenem
(8) Cefiderocol vs. imipenem-cilastatin
(9) Eravacyline vs. ertapenem
(10) Ceftazidime-avibactam vs. imipenem-cilastin
(11) Ceftazidime-avibactam vs. doripenem
(12) Plazomicin vs. meropenem

Table 4. Late clinical and microbiological failure, mortality and (serious) adverse events for studies evaluating the empirical treatment

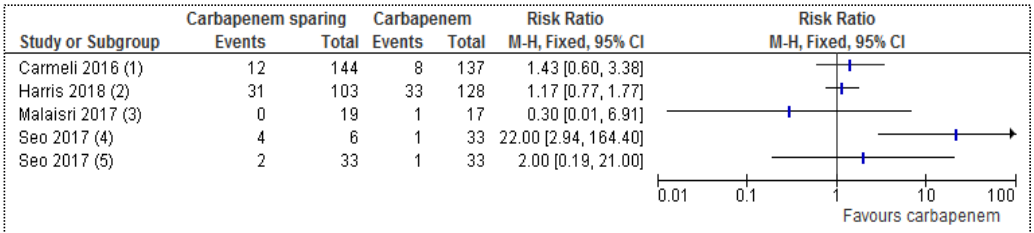
Study	Comparison	Late clinical failure	Late microbiological failure	Mortality	Adverse events	Serious adverse event rate
Cerexa 2018 [29]	CXL every 8 h, CXL every 12 h, Doripenem 500 mg every 8 h	NA	27/34 vs. 22/25 vs. 19/32	1/72 vs. 0/73 vs. 2/73	27/72 vs. 27/73 vs. 29/73	9/145* vs. 3/73
Jaspers 1998 [30]	Cefuroxim-gentamicin vs. Meropenem	2/5 vs. 3/6	2/2 vs. 3/3	NA	NA	NA
Kaye 2018 [31]	Piperacillin-tazobactam vs. meropenem-vaborbactam	39/182 vs. 26/192	NA	2/273 vs. 2/272	97/273 vs. 106/272	13/273 vs. 7/272
Merli 2016 [32]	Amoxicillin/clavulanic acid iv or ciprofloxacin po vs. imipenem/cilastin	NA	NA	5/22 vs. 0/21	NA	NA
Mir 2019 [33]	CSE vs. meropenem	71/74 vs. 62/69	70/72 vs. 61/68	1/117 vs. 0/113	13/117 vs. 14/113	1/117 vs. 0/113
Naber 2002 [34]	Piperacillin –tazobactam vs. Imipenem-cilastatin	40/115 vs. 39/118	72/142 vs. 81/150	2/166 vs. 2/171	28/166 vs. 28/171	2/166 vs. 2/171
Naber 2009 [35]	Levofloxacin iv vs doripenem	11/229 vs. 23/251	NA	0/372 vs. 1/376	222/372 vs. 240/376	15/372 vs. 28/376
Portsmouth 2018 [36]	Cefiderocol vs. imipenem-cilastatin	47/252 vs. 33/119	108/252 vs. 67/119	1/300 vs. 0/148	122/300 vs. 76/148	14/300 vs. 12/148
Tetraphase 2018 [37]	Eravacyline vs. ertapenem	NA	NA	NA	NA	NA
Vazquez 2012 [38]	Ceftazidime-avibactam vs. imipenem-cilastin	7/28 vs. 12/36	23/46 vs. 26/49	0/68 vs. 0/67	46/68 vs. 51/67	6/68 vs. 2/67
Wagenlehner 2016 [19]	Ceftazidime-avibactam vs. Doripenem	58/393 vs. 67/417	125/393 vs. 163/417	0/511 vs. 0/509	185/511 vs. 158/509	21/511 vs. 12/509
Wagenlehner 2019 [39]	Plazomicin vs. meropenem	22/191 vs. 29/197	30/191 vs. 69/197	1/303 vs. 0/301	59/303 vs. 65/301	5/303 vs. 5/301

Results that are statistically different (p<0.05) are highlighted. Outcome definitions and analysis populations for all studies are reported in the online supplementary material S4. Secondary endpoints are reported in the online supplementary material S5, if available.
CXL = Ceftaroline fosamil and avibactam, CSE = Ceftriaxone, sulbactam, and disodium ethylenediaminetetraacetic acid EDTA
*combined groups of CXL^ every 8 h, CXL every 12 h

Pathogen-directed treatment

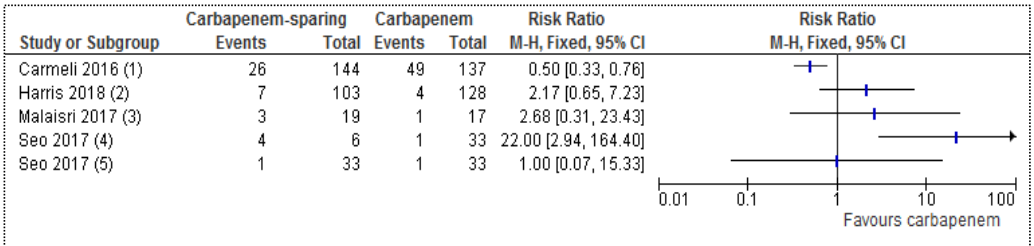
Results for RCTs that investigated the pathogen-directed treatment of cUTI are reported in Figure 4 and 5 and Table 5. In a RCT from 2016 ceftazidime-avibactam was compared to best-available therapy pathogen-directed to ceftazidime-resistant pathogens in patients with cUTI (n=333) or abdominal infection (n=28). Best-available therapy consisted of carbapenem in all except 6 cUTI patients. Lower early and late microbiological failure rates were found in the ceftazidime-avibactam arm, with risk ratios of respectively 0.50 (95%CI 0.33-0.76) and 0.67 (95%CI 0.50 to 0.90), with no differences in clinical failure or mortality.[40] A recent RCT from 2018 compared piperacillin-tazobactam to meropenem for the pathogen-directed treatment of third-generation cephalosporin resistant *K. pneumoniae* or *E. coli* bacteraemia, with mortality as a primary outcome. Microbiological cure 4 days post-end of treatment was defined as sterilization of blood and did not include urine cultures.[41] In an interim analysis after enrolling 378 patients, piperacillin-tazobactam was inferior regarding the endpoint mortality, which led to premature termination of the study. However, in the subpopulation of patients with cUTI, consisting of 231 patients, a non-significant difference was found in mortality, clinical and microbiological failure. In a smaller RCT from 2017 with 66 enrolled patients consisting of three treatment arms, piperacillin-tazobactam, ertapenem and cefepime were evaluated for the pathogen-directed treatment of healthcare-associated UTI, caused by ESBL producing *E. coli*.[43] No differences were found regarding clinical failure, microbiological failure or mortality between piperacillin-tazobactam and ertapenem. The cefepime arm was terminated after only six enrolled patients due to unexpectedly high rates of early clinical (n=4, 67%) and microbiological failure (n=4, 67%).[43] A study from 2017 with 33 enrolled patients compared oral sitafloxacin to ertapenem for acute pyelonephritis caused by ESBL-positive *E. coli.* No differences were found regarding early clinical and microbiological failure.[42] No significant differences in mortality were observed in the populations in which the pathogen-directed treatment of cUTI was evaluated.

Figure 4. Early clinical failure (pathogen-directed treatment



Footnotes
(1) Ceftazidime-avibactam vs. best available therapy
(2) Piperacillin-tazobactam vs. meropenem
(3) Sitafloxacin po vs. ertapenem
(4) Cefepime vs. ertapenem
(5) Piperacillin-tazobactam vs. ertapenem

Figure 5. Early microbiological failure (pathogen-directed treatment))



Footnotes
(1) Ceftazidime-avibactam vs. best available therapy
(2) Piperacillin-tazobactam vs. meropenem (microbiological cure is defined as sterility of blood cultures on day 4 post-randomization)
(3) Sitafloxacin po vs. ertapenem
(4) Cefepime vs. ertapenem
(5) Piperacillin-tazobactam vs. ertapenem

Table 5. Late clinical and microbiological failure, mortality and (serious) adverse events for studies evaluating the pathogen-directed treatment

Study	Comparison	Late clinical failure*	Late microbiological failure	Mortality	Adverse events	Serious adverse event rate
Carmeli 2016 [40]	Ceftazidime-avibactam vs. best available therapy (97% carbapenem)	21/144 vs. 19/137	**45/144 vs. 64/137**	3/164 vs. 3/168	**34/152 vs. 54/153**	4/152 vs. 7/153
Harris 2018 [41]	Piperacillin-tazobactam vs. Meropenem	NA	NA	7/103 vs. 4/128	NA	2/103 vs. 3/128
Malaisri 2017 [42]	Sitafloxacin vs. Ertapenem	NA	NA	0/19 vs. 1/17	1/19 vs. 0/17	0/19 vs. 0/17
Seo 2017 (1) [43]	Piperacillin-tazobactam vs. Ertapenem	NA	NA	2/33 vs. 2/33	NA	NA
Seo 2017 (2) [43]	Cefepime vs. Ertapenem	NA	NA	2/6 vs. 2/33	NA	NA

Results that are statistically different (p<0.05) are highlighted. Outcome definitions and analysis populations for all studies are reported in the online supplementary material S4. Secondary endpoints are reported in the online supplementary material S5, if available

Adverse and serious adverse events

Less adverse events were found when using pathogen-directed ceftazidime-avibactam compared to best available therapy, consisting of 97% carbapenems (RR: 0.63, 95%CI 0.44 to 0.91).[40] In contrary, another study that evaluated its empirical use revealed no difference in adverse events, although there was a trend towards a relevant increase in the ceftazidime-avibactam group, compared to doripenem.[44] Empiric levofloxacin (250mg/24 hours) resulted in less serious adverse events compared to doripenem (RR:0.54, 95%CI 0.29 to 1.00).[35] Less adverse events were reported in the cefiderocol arm versus high dose imipenem-cilastin (RR: 0.79, 95%CI 0.64 to 0.98). In the other studies, no significant differences were reported regarding adverse or serious adverse events between the treatment arms.

Other secondary outcomes

Significantly less relapses were found after using plazomicin, compared to meropenem for empirical treatment of cUTI (RR: 0.22, 95%CI 0.06 to 0.75).[45] The following secondary outcomes were infrequently measured and, if measured, revealed no difference between the carbapenem and non-carbapenem

regimens: hospital stay, readmission, recurrence/relapse and intensive care unit readmission. These are reported in online supplementary material S5.

DISCUSSION

In this systematic review we identified 16 RCTs that evaluated the efficacy and safety of alternatives to carbapenem-class antibiotics for the empirical or pathogen-directed treatment of complicated urinary tract infections, including acute pyelonephritis. In order to provide a comprehensive overview of current and future carbapenem-alternative antimicrobials, we made no restrictions in inclusion based on the completeness of the trial, the sample size, or the phase of the trial. As a consequence, the clinical heterogeneity between the studies was large concerning both the patient populations, interventions, the comparators, and outcomes. This prohibited a meaningful meta-analysis. Conclusions can therefore be drawn on the level of the individual studies and drugs tested. In order to facilitate the interpretation we reported detailed characteristics of each RCT and attempted to retrieve additional data for each study. Analysis populations varied between RCTs; several studies reported no (modified) intention-to-treat analysis or did not specify the analysis population, impeding generalization of the results to clinical practice.

Overall, well conducted trials for alternatives to carbapenems were rare. Eleven out of sixteen studies had an intermediate or high risk of bias. Most studies had small sample sizes as a result of early termination of the trial or from being a phase II trial, with resulting low precision of these studies.[29,30,32,38,42,43] Some of these studies included patients with a wider range of infections,[30,32,41] with the sub-populations of patients with cUTI being too small to provide meaningful interpretation of the results.[30,32] Furthermore, three studies that evaluated the empirical treatment and enrolled patients before 2010 can be considered outdated, as resistance rates to the investigated antibiotics have changed.[30,34,35] Next, in seven out of ten studies that were pharmacy sponsored, the independence of the investigators was not guaranteed: either the sponsor was responsible for the conduct of the study or one or more of the authors were employed by the sponsor, see online supplementary material S3. Last, percentages of participants with bacteraemia were low in most included RCTs (Tables 2 and 3), and results are not automatically generalizable to patients with bacteremic cUTI.

For the empirical treatment of cUTI, ceftazidime-avibactam, plazomicin, cefiderocol and ceftriaxon-sulbactam disodium-EDTA emerged as reasonable alternatives to carbapenem with at least comparable safety and efficacy. All four are FDA and EMA registered for the treatment of cUTI or pyelonephritis. Remarkably, no phase III trial could be found that evaluates the efficacy of cefiderocol. Eravacycline did

not receive FDA approval for the treatment of complicated urinary tract infections, as it did not reach the non-inferiority threshold compared to ertapenem regarding the co-primary endpoint clinical cure and microbiological success. Ceftaroline fosamil-avibactam is not currently registered for the treatment of cUTI. The phase 2 trial results were not published in a peer-reviewed journal and the drug development process seems to be discontinued for unknown reasons. The two studies evaluating the empirical treatment with piperacillin-tazobactam delivered conflicting results, with more late clinical failures compared to meropenem-vaborbactam, but not compared to imipenem-cilastatin. In the meropenem-vaborbactam study, only 3 out of 545 patients had CRE infection, making it unlikely that the vaborbactam was responsible for the difference in efficacy.[34] Levofloxacin proved inferior to doripenem, which is potentially explained by the low dose of 250 mg levofloxacin used in the study. A dose of 750 mg per day may be more appropriate for cUTI.[4,20,35,46] A standard therapy with oral ciprofloxacin or intravenous amoxicillin-clavulanic acid resulted in a higher mortality than when using imipenem-cilastatin for health-care related infections in patients with hepatic cirrhosis, although results should be interpreted with caution, as risk of bias was high.[32]

The generalizability of the RCTs that evaluate the empirical treatment is difficult to establish as susceptibility rates of the causative pathogens against the carbapenem and non-carbapenem antimicrobials in the study populations were not reported. Presumably, the efficacy of empirical treatment regimens depends on the baseline antimicrobial resistance of uropathogen in the population of interest. Based on expert opinion, guidelines proposed a minimal coverage threshold of 90% for the empirical antibiotic treatment of cUTI.[4,5] Of the reviewed carbapenem-alternative options, ceftazidime-avibactam, plazomicin, cefiderocol and ceftriaxone-sulbactam disodium-EDTA reach this threshold throughout most regions and populations and could be used empirically for cUTI, even if an ESBL-producing pathogen is suspected.[47–49] Except for ceftriaxone-sulbactam disodium-EDTA, these antimicrobials possess *in vitro* activity to CRE.[50–52] However, the development of resistance and the sustainability of these carbapenem-alternative drugs remain unknown, as these are currently used to a lesser extent than carbapenems. Susceptibility to piperacillin-tazobactam, cefuroxime-gentamicin, levofloxacin, ciprofloxacin, and amoxicillin-clavulanic acid among uropathogens varies strongly, but is often below 90% among Enterobacterales, with even lower susceptibility rates in case of ESBL-carrying *Enterobacterales*, limiting their applicability as empirical therapy in most regions.[9,53–55]

For the pathogen-directed treatment, ceftazidime-avibactam was found to be as efficacious as carbapenem therapy in one open label, pharmacy driven trial. Interestingly, recruitment in this trial was ended prematurely by the sponsor after inclusion of 278 patients of the pre-planned 400 based on the

amount and variety of cultured species. Since this is the only RCT on ceftazidime-avibactam as pathogen-directed treatment, caution may be warranted before implementation in clinical practice. Piperacillin-tazobactam was evaluated as pathogen-directed treatment in two RCTs. Both studies were underpowered for the treatment of cUTI. The larger, well-conducted open label trial found a significant increase in mortality when piperacillin- tazobactam compared to meropenem was used to treat bacteremia from all sources. The difference in mortality between the two arms was smaller when only looking at urinary-source bacteraemia, possibly because of the overall better prognosis in contrast to non-urinary-source bacteraemia. The other study on pathogen-directed piperacillin-tazobactam only included 66 patients in three arms and had methodological flaws, which severely impedes the interpretability. The third treatment arm consisted of cefepime and was stopped prematurely for safety reasons after 6 participants. For ethical reasons it is not likely that cefepime will be evaluated in future trials for the treatment of cUTI. In a 'pilot' RCT comparing sitafloxacin to ertapenem for the pathogen-directed treatment of cUTI caused by ESBL positive E.*coli* only 36 participants were enrolled and this trial has a high risk of bias. Consequently, no conclusions could be drawn on the efficacy and safety of sitafloxacin, which is also not registered for this indication.

This review restricted to RCTs that directly compared carbapenem-alternative antimicrobials to carbapenems. Three RCTs worth mentioning, were excluded that evaluated two carbapenem-saving antimicrobials: The ASPECT-cUTI trial found that ceftolozane-tazobactam was non-inferior to levofloxacin (750 mg daily) for the empirical treatment of cUTI regarding clinical cure with superiority of ceftolozane-tazobactam regarding microbiological cure, [20] the ZEUS trial found that intravenous fosfomycin was non-inferior to piperacillin-tazobactam for the empirical treatment of cUTI regarding clinical and microbiological cure. [56] The question remains how the efficacy of fosfomycin and ceftolozane-tazobactam compares to that of carbapenem, as the results from this review suggest that levofloxacin and piperacillin-tazobactam are less efficacious than carbapenem.

Various RCTs are currently ongoing or completed but not yet published that evaluate the efficacy of carbapenem-alternatives to carbapenem for the treatment of cUTI, which are listed in online supplementary material S2, e.g. intravenous fosfomycin, temecollin, Tebipenem Pivoxil Hydrobromide, sitafloxacin, polymixin B, cefepime-tazobactam. Some carbapenem-saving antimicrobials with *in vitro* activity to ESBL producing *Enterobacterales* are, to our knowledge, not yet being evaluated in a RCT for the treatment of cUTI; the most important being tigecycline as empirical treatment option for cUTI and oral fosfomycin as stepdown treatment for cUTI caused by ESBL.

Based on this review, ceftazidime-avibactam, plazomicin, cefiderocol and ceftriaxon-sulbactam disodium-EDTA for the empirical treatment and ceftazidime-avibactam for the pathogen-directed treatment for cUTI are potential alternatives to carbapenem. Results for empiric piperacillin-tazobactam, ceftaroline fosamil-avibactam, eravacycline, cefuroxime-gentamicin, amoxicillin-clavulanic acid, ciprofloxacin and low dose levofloxacin and pathogen-directed piperacillin-tazobactam, sitafloxacin and cefepime was either inconclusive or suggested inferiority.

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CHAPTER 6

Fosfomycin Etest for Enterobacteriaceae: Interobserver and Interlaboratory agreement

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ABSTRACT

Objectives: The increasing use of fosfomycin requires reliable susceptibility testing in clinical practice. The reference standard, agar dilution (AD) is rarely used in routine settings. The fosfomycin Etest (Bio Mérieux) is frequently used, though reading of MICs can be hampered by the interpretation of the growth of macrocolonies in the inhibition zone. We investigated the interobserver (IO), interlaboratory (IL), and interobserver-interlaboratory (IOIL) agreement of the fosfomycin Etest and evaluated the agreement to AD.

Methods: Etests were performed for 57 extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae of four bacterial species (*Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Enterobacter cloacae*), in two laboratories. Photographs of fosfomycin Etests were interpreted by four observers following manufacturer's instructions.

Results: Essential Agreement (EA) and categorical agreement (CA) between Etest and AD was 57% and 89% (κ-value 0.68), respectively, with an underestimation of Etest interpretations compared to AD of 0.26 (95% confidence interval [CI] 0.03-0.48) 2-fold dilutions. Between Etest observations, IO-EA and -CA was reached in 82% and 94% of comparisons; IL-EA and -CA in 38% and 85% of comparisons; and IOIL-EA and -CA in 40% and 85% of comparisons, respectively. Agreement of the Etest to AD and between Etests was better for *E.coli* than for other species. Ignoring all macrocolonies and haze during Etest interpretation improved the agreement with AD (CA κ-value 0.80) and between Etests (CA κ-value from 0.68 to 0.81).

Conclusions: In this study on 57 ESBL-producing Enterobacteriaceae, IOIL agreement was low with an EA of 40% and a CA of 85%, affected most by IL agreement and to a lesser extent by IO agreement.

INTRODUCTION

Fosfomycin was discovered as an antibiotic agent in 1969.[1] Its use has gained renewed interest because of increasing resistance against other antibiotics, particularly in Enterobacteriaceae.

Fosfomycin susceptibility testing for Enterobacteriaceae is challenging in the routine setting. The reference standard, agar dilution (AD), is complex and time consuming, making it unsuitable for routine clinical application.[2] Performance of automated broth microdilution methods is not recommended by the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[3]

A potential alternative to determine the Minimum Inhibitory Concentration (MIC) of fosfomycin for Enterobacteriaceae is the Etest (BioMérieux, Durham, USA). Agreement with AD varies and is described to be poor for Enterobacteriaceae other than *Escherichia coli;* this is attributed to difficulties in reading the Etest MIC because of growth of macrocolonies in the inhibition zone.[4,5] The manufacturer instructs to ignore up to five macrocolonies when reading the MIC.

To evaluate the Etest as an alternative fosfomycin testing method for the routine lab, we determined the interobserver (IO), interlaboratory (IL) and interobserver-interlaboratory (IOIL) essential agreement (EA) and categorical agreement (CA).

MATERIALS AND METHODS

Isolates

Isolates originated from a collection of well-defined and sequenced extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae from a multicentre study on transmission in Dutch hospitals.[6,7] The selection of 57 isolates was based on the presence or absence of the FosA gene, the most frequent plasmid-borne fosfomycin resistance gene in Gram-negative bacteria, aiming at a 1:1 ratio.[8] The selection comprised 16 *Escherichia coli*, 16 *Enterobacter cloacae*, 16 *Klebsiella pneumoniae* and 9 *Klebsiella oxytoca* strains.

Microbiological procedures

Agar dilution was performed according to CLSI guidelines on the selection of 57 isolates.[9] The bacteria were recovered from a fresh culture on a blood agar plate that was cultured overnight at 35-37°C. Next, a suspension of 0.5 McFarland of bacteria in 0.45% NaCl (10^8 CFU/mL) was made and diluted to 10^7 CFU/mL in 0.9% NaCl. Bacterial suspensions were pipetted per 12 into a 24- wells plate and replicated. Subsequently, 2 µL bacterial inoculum (±$1x10^4$ CFU/spot of 5-8 mm) from each well was inoculated onto

a Mueller-Hinton II agar plate, containing 25 mg/L glucose-6-phosphate and fosfomycin in concentrations from 0.25 mg/L to 128 mg/L. The agar plates were incubated for 16-20 h at 35-37°C. The highest fosfomycin concentration, at which no visible bacterial growth on the agar plate was observed by the naked eye, was considered the MIC. Single colonies or a weak haze due to the bacterial inoculum were ignored.

Etest susceptibility testing was performed according to the manufacturer's instructions in two Dutch clinical microbiology laboratories. A suspension of 0.5 McFarland of overnight cultured bacteria in 0.85%NaCl was inoculated onto MHA (Oxoid in laboratory A and Becton Dickinson in laboratory B). In both laboratories a sterile swab was used, streaking the agar surface three times rotating the plate 60° each time. Within 15 min after inoculation, Etest strips were applied onto the inoculated MHA. Plates were incubated for 16-20 h at 35-37 ºC. Photographs of the incubated agar plates were made to enable independent reading of inhibition zones (example in online supplementary material).

Etest interpretation

Four clinical microbiology residents interpreted all photographs independently, resulting in 8 separate Etest observations for 57 bacterial isolates. First, observers were instructed to register the number of macrocolonies present in the inhibition ellipse; second, they were to ignore all macrocolonies and haze to determine the MIC at 80% inhibition ($MIC_{80\%}$); and third, to include all macrocolonies to determine the MIC at 100% inhibition ($MIC_{100\%}$). According to manufacturer's instructions, the MIC used for the main analysis (recommended MIC) was $MIC_{80\%}$ if five or less macrocolonies were observed and $MIC_{100\%}$ in case of more than five macrocolonies.

Outcome measurement

Essential agreement (EA) was defined as agreement of Etest MIC values within one MIC dilution step, and Categorical agreement (CA) as MIC values within the same EUCAST susceptibility category, i.e. susceptible (MIC ≤32 mg/L) or resistant (MIC >32 mg/L).[2] Agreement was calculated between Etest and AD, and between the following combinations of Etest observations. Interobserver (IO) agreement was defined as agreement between individual observers within one laboratory; interlaboratory (IL) agreement as agreement between the observations of the same observer for Etests performed in the two laboratories and interobserver-interlaboratory (IOIL) agreement as agreement between combinations of different observers and different laboratories, best reflecting clinical practice. Disagreements were classified as

very major errors (VME) if the Etest resulted in a susceptible and AD in a resistant result and major errors (ME) if the Etest resulted in a resistant and AD in a susceptible result.

Statistical analysis

Cohen's kappa test was used to evaluate CA, as it accounts for the possibility of CA occurring by chance. The κ results are values between 0, which represents no agreement, and 1, representing complete agreement.[10] We also determined the systematic difference between AD and Etest and between Etest observations in laboratory A and laboratory B by calculating the mean difference in 2 fold dilution steps. IBM SPSS Statistics (version 21) was used for statistical analyses.

RESULTS

Etest interpretation

Two of 456 Etest observations were not interpreted because of the low quality of the photographs, leaving 454 Etests for analysis. Growth of macrocolonies within the inhibition zone was reported in 268 of 454 (59%) Etest interpretations (laboratory A 132/228 [58%]; laboratory B 136/226 [60%]). In 71 of 454 (16%) observations, 5 or more macrocolonies were observed, meaning a switch in the recommended MIC from $MIC_{80\%}$ to $MIC_{100\%}$.

Agreement Etest to AD

Overall, EA and CA between the Etest MIC and AD was 57% and 89%, respectively (mean κ-value 0.68, 95%CI 0.42 to 0.95, Table 1). Categorical disagreement resulted in 4% VMEs and 7% MEs. Small differences existed between laboratories and observers. For *E. coli*, CA between Etest and AD was 100%, which is in contrast with the other species (range 77 to 91%). Reading the MIC at 80% inhibition resulted in a higher agreement than the recommended MIC (mean κ-value 0.80, 95%CI 0.54 to 1.07). There was a significant systematic difference between mean AD and Etest of 0.26 (95%CI 0.03 to 0.48) 2-fold dilutions.

Table 1. Comparison between fosfomycin Etest and agar dilution

Antimicrobial susceptibility testing method		No. of comparisons	No. resistant (%)	Essential agreement to agar dilution (%)#	Mean kappa (mean 95%CI)	Categorical agreement to agar dilution (%)	Categorical disagreement of Etest to agar dilution	
							Very major error (%)*	Major error (%)**
Agar dilution		57	11 (19)	N/A	N/A	N/A	N/A	N/A
Etest - recommended MIC	**Total**	454	102 (22)	261 (57)	0.68 (0.42 to 0.95)	406 (89)	17 (4)	31 (7)
	Laboratory A	228	41 (18)	100 (44)	0.70 (0.44 to 0.97)	207 (91)	12 (5)	9 (4)
	Laboratory B	226	61 (27)	161 (71)	0.67 (0.40 to 0.93)	199 (88)	5 (2)	22 (10)
	Observer 1	114	26 (23)	68 (60)	0.68 (0.42 to 0.95)	102 (89)	4 (4)	8 (7)
	Observer 2	114	22 (19)	58 (51)	0.73 (0.46 to 0.99)	104 (91)	5 (4)	5 (4)
	Observer 3	114	25 (22)	65 (57)	0.71 (0.44 to 0.97)	103 (90)	4 (4)	7 (6)
	Observer 4##	112	29 (26)	70 (63)	0.62 (0.36 to 0.89)	97 (87)	4 (4)	11 (10)
	E.coli	128	64 (50)	80 (63)	N/A$	128 (100)	0 (0)	0 (0)
	K. pneumoniae	128	19 (15)	57 (45)	N/A$	99 (77)	13 (10)	16 (13)
	K. oxytoca	71	8 (11)	35 (49)	N/A$	63 (89)	4 (6)	4 (6)
	E. cloacae	127	11 (9)	89 (70)	N/A$	116 (91)	0 (0)	11 (9)
Etest - $MIC_{80\%}$		454	81 (18)	289 (64)	0.80 (0.54 to 1.07)	427 (94)	17 (4)	10 (2)
Etest - $MIC_{100\%}$###		453	225 (50)	221 (49)	0.23 (-0.03 to 0.50)	294 (65)	11 (2)	148 (33)
CLSI breakpoint for Etest and AD (S>64 mg/L)		454	88 (19)	261 (57)	0.62 (0.36 to 0.89)	402 (89)	22 (5)	30 (7)

MIC = Minimal Inhibitory Concentration, No. = Number, AD = Agar Dilution, CSLI = Clinical & Laboratory Standards Institute, CI = Confidence interval

* Etest gives a susceptible result and agar dilution a resistant result.

** Etest gives a resistant result and agar dilution a susceptible result.

Agar dilution measured MICs up to ≥128 mh/L. For these isolates Etest MICs ≥64mg/L were classified as agreement

Observer 4 rated two Etests as not assessable because of low quality of the photographs; these were excluded from all analyses.

Observer 4 left one $MIC_{100\%}$ result empty.

$ Number of isolates for individual species were too low to calculate reliable Kappa values.

Agreement between Etest observations

The overall EA between all Etest observations was 911/1582 (58%) and CA was 1404/1582 (89%) with a κ-value of 0.68 (95%CI 0.63 to 0.73, Table 2). The IO agreement was higher than the IL-agreement. The $MIC_{80\%}$ interpretation resulted in a significantly higher kappa (0.81, 95%CI 0.76 to 0.86) than the recommended MIC. EA and CA between all 8 observations was reached for 8/57 (14%) and 37/57 isolates (65%), respectively. CA was 100% for *E.coli,* and lower for other species. EA was highest for *E.cloacae* (70%). The mean systematic difference between Etest observations in laboratory A and laboratory B was 1.60 (95%CI 1.32 to 1.88) in 2 fold dilution steps.

Table 2. Overall, interobserver, interlaboratory and interlaboratory-interobserver agreement for the reading of fosfomycin Etest, provided by the kappa value, categorical agreement (CA,%) and essential agreement (EA,%)

Variable		No. of comparisons	Essential Agreement (%)	Categorical Agreement (%)	Kappa (95%CI)
Recommended MIC		1582	911 (58)	1404 (89)	0.68 (0.63 to 0.73)
Inter-observer	Laboratory A	342	258 (75)	321 (94)	0.79 (0.68 to 0.90)
	Laboratory B	336	297 (88)	315 (94)	0.84 (0.74 to 0.95)
Inter-laboratory	Observer 1	57	19 (33)	49 (86)	0.60 (0.34 to 0.87)
	Observer 2	57	18 (32)	51 (89)	0.66 (0.40 to 0.93)
	Observer 3	57	22 (39)	48 (84)	0.54 (0.27 to 0.80)
	Observer 4#	55	26 (47)	44 (80)	0.50 (0.23 to 0.76)
Interobserver-interlaboratory		678	271 (40)	576 (85)	0.58 (0.28 to 0.84)*
E.coli		448	287 (64)	448 (100)	1.00 (0.91 to 1.09)
K. pneumoniae		448	186 (42)	359 (80)	0.21 (0.12 to 0.31)
K. oxytoca		245	129 (53)	213 (87)	0.36 (0.24 to 0.49)
E. cloacae		441	309 (70)	384 (87)	0.20 (0.10 to 0.29)
$MIC_{80\%}$		1582	977 (62)	1495 (95)	0.81 (0.76 to 0.86)
$MIC_{100\%}$##		1575	900 (57)	1240 (79)	0.58 (0.53 to 0.63)
CLSI breakpoint		1582	911 (58)	1450 (92)	0.74 (0.69 to 0.79)

MIC = Minimal Inhibitory Concentration, No. = Number, CSLI = Clinical & Laboratory Standards Institute, CI = Confidence Interval
# Observer 4 rated two Etests as not assessable because of low quality of the photographs; these were excluded from all analyses.
Observer 4 left one MIC100 result empty.
*Mean kappa with mean 95% confidence interval

DISCUSSION

In this study on 57 ESBL-producing Enterobacteriaceae strains of four different species, IOIL agreement was low (EA 40%, CA 85%), affected most by IL agreement and to a lesser extent by IO agreement. No previous studies reported the IO or IL agreement for reading the fosfomycin Etest. A systematic difference was found with significantly higher MIC's observed in laboratory A than in laboratory B.

Factors that may have affected IL agreement were the materials used – such as the MHA (a non-synthetic medium that may differ in composition between companies) - and the technician that performed the test. It confirms that there is a significant variation in MIC determination between laboratories, and MIC values obtained should be regarded with caution.[11]

A significant systematic difference was found between AD and Etest leading to an underestimation of the Etest. The low agreement of Etest observations to AD is in line with other studies, as well as the higher agreement for *E. coli* compared to other Enterobacteriaceae.[5,12,13] In contrast to the other species, the presence or absence of the FosA gene in *E. coli* resulted in either very high or very low MICs, respectively. Growth of macrocolonies in the inhibition zone was observed in the majority of Etests. Ignoring macrocolonies and haze from interpretation ($MIC_{80\%}$) improved CA to AD (from 89% to 94%, mean κ-value 0.80) and between Etest observations (from 89% to 95%, κ-value 0.81). Our results suggest that the more

feasible $MIC_{80\%}$ interpretation performs better than the recommended MIC; this observation should be confirmed in larger cohorts.

Our study has several limitations. Firstly, we used a small population of ESBL-producing Enterobacteriaceae isolates from hospitalized patients in the Netherlands. The majority of isolates appeared susceptible to fosfomycin using the current breakpoints. This could affect the generalizability of the results. Otherwise, we aimed to include a large enough number of resistant strains to allow a good estimate of VMEs, as this is can be a problem when using isolates from large surveys with a low resistance frequency. Secondly, we did not interpret the actual Etests, but the photographs, which is not the normal practice.

CONCLUSIONS

In conclusion, the fosfomycin Etest has a low IO-IL agreement and low agreement with AD. The observed variations in the interpretation of the fosfomycin Etest may limit its general use in clinical practice. The better performance of the fosfomycin Etest for *E.coli* isolates compared with other species supports the suggestion to limit its use to *E.coli*.[4,5] Finally, performance and feasibility might improve when ignoring all growth in the inhibition zone.

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CHAPTER 7

In vivo acquisition of fosfomycin resistance in *Escherichia coli* by *fosA* transmission from commensal flora

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Sir,

Fosfomycin is increasingly used to treat infections caused by MDR bacteria.[1] Fosfomycin acts by inhibiting UDP-*N*-acetylglucosamine enolpyruvyl transferase (*murA*), which prevents the formation of *N*-acetylmuramic acid, an essential component of peptidoglycan.[1] Although resistance to fosfomycin is still low in *Escherichia coli,* the acquisition of *fosA* may reduce future activity of fosfomycin to treat infections caused by *E. coli*.[2] FosA is a glutathione transferase that inactivates fosfomycin through catalysing the addition of glutathione. *fosA* genes are often present in the chromosome of *Klebsiella pneumoniae,* but not in the chromosome of E. *coli*.[2,3] *Klebsiella variicola* is closely related and often misidentified as *K. pneumoniae*.[4] While horizontal spread of *fosA* has been demonstrated *in vitro*,[5] we here provide evidence for *in vivo fosA* transmission from *K. variicola* to *E. coli,* resulting in development of fosfomycin resistance. The Medical Research Ethics Committee of the University Medical Center Utrecht confirmed that the Medical Research Involving Human Subjects Act does not apply to this study (reference number WAG/mb/18/027282). We were not able to obtain informed consent because the patient died a few years ago. All information including gender, age, dates and medical history that was not directly clinically relevant has been omitted to protect the privacy of the patient.

An aged patient had a suspicion of chronic endovascular infection of their aortic bifurcation graft, which the patient received after an acute aortic aneurysm 22 years earlier. The patient had suffered from recurrent episodes of sepsis with blood cultures yielding *Propionibacterium spp.*, *K. variicola*, *Citrobacter koseri* and *Pseudomonas aeruginosa*, as determined by MALDI-TOF MS. Positron emission tomography (PET)-CT findings were compatible with prosthetic graft infection. The patient subsequently developed septic shock with *E. coli* bacteraemia without a clear source of infection that was treated successfully with intravenous ceftriaxone. The isolate was resistant to amoxicillin/clavulanic acid and ciprofloxacin that had previously been used to suppress chronic infection, prompting the addition of oral fosfomycin 3 g every 48 h. Seven months later, while still using oral fosfomycin, the patient developed spondylodiscitis. Blood cultures drawn at the time isolated *E. coli* with an identical resistance pattern, except being resistant to fosfomycin. Fosfomycin was discontinued and the patient received a prolonged course of intravenous ceftriaxone.

Fosfomycin susceptibility, determined by agar dilution according to CSLI guidelines,[6] demonstrated a rise in the MIC from 2 mg/L for the initial *E. coli* isolate to >1024 mg/L for the second *E. coli* isolate. WGS revealed five SNP differences between *E. coli* isolates in the core genome, based on core genome MLST (cgMLST) analysis.[7] Yet, the second *E. coli* isolate has a 3573 bp insertion consisting of IS*Ecp1*, a *fosA* gene we named *fosA9* as the next available number according to NCBI, *syrM1* and *lysN2.* The insertion is flanked

by 5 bp DRs (AAAAA) suggesting mobilization of this *fosA*9 gene cluster by IS*Ecp1* (Figure 1).[8] Genes other than *fosA9* responsible for fosfomycin resistance were not found. At the time of the first *E. coli* sepsis episode, six *K. variicola* had been isolated from rectum swabs and blood cultures over a period of 20 months (Table S1, available online as Supplementary data at *JAC* Online). cgMLST analysis revealed a maximum of 16 SNP differences between *K. variicola* isolates.[7] The same cluster as above containing *fosA*9, without the mobile genetic element IS*Ecp1*, was identified in the *K. variicola* isolates, suggesting *K. variicola* to be the source of *fosA9* acquired by *E. coli* (Figure 1). *fosA* genes were not identified in other clinical isolates from this patient. Sequence information of all isolates has been deposited in the European Nucleotide Archive (ENA) under project number PRJEB32329.

fosA transfer from *Klebsiella* spp. to *E. coli*, leading to fosfomycin resistance, has been demonstrated *in vitro*.[3] Based on publicly available genomes, *fosA* and adjacent genes are well conserved in *K. variicola* (minimum 98% identity to *fosA9*) and *K. pneumoniae* (minimum 94% identity to *fosA9*) isolates. According to mlplasmids, PlasmidFinder and contig coverage, *fosA9* was predicted to be located in the chromosome of the second *E. coli* and all *K. variicola* isolates.[9,10] However, based on BLASTn, the contig containing *fosA9* aligns to plasmid sequences. The localization of *fosA9* in *E. coli* can thus only be confirmed by completely assembling its genome using long-read sequencing, as the mobilization of the *fosA9* gene cluster by an IS element might switch its genomic background. We postulate that *fosA9* transfer from *K. variicola* to *E. coli* occurred in the gastrointestinal tract, as *K. variicola* was not co-cultured in the blood at the time of *E. coli* bacteraemia. We hypothesize that fosfomycin pressure played a role in this transfer; however, this has to be confirmed with further experiments *in vitro*. Acquisition of *fosA9* was associated with an 8-fold increase in the MIC for *E. coli* (from 2 to 1024 mg/L) while, despite the presence of *fosA9* in the chromosome of the *K. variicola* isolates, the fosfomycin MICs were below the EUCAST susceptibility breakpoint of ≤32 mg/L (online Table S1).[6] This could suggest either higher dependency of *E. coli* growth on glutathione or a difference in *fosA9* expression or metabolism, i.e. higher expression by the IS*Ecp1* promoter present upstream of the *fosA9* gene cluster.[8]

In conclusion, our case illustrates the potential of long-term use of oral fosfomycin to promote horizontal gene transfer of *fosA9* from commensal gut flora to potential pathogenic microorganisms, such as *E. coli*.

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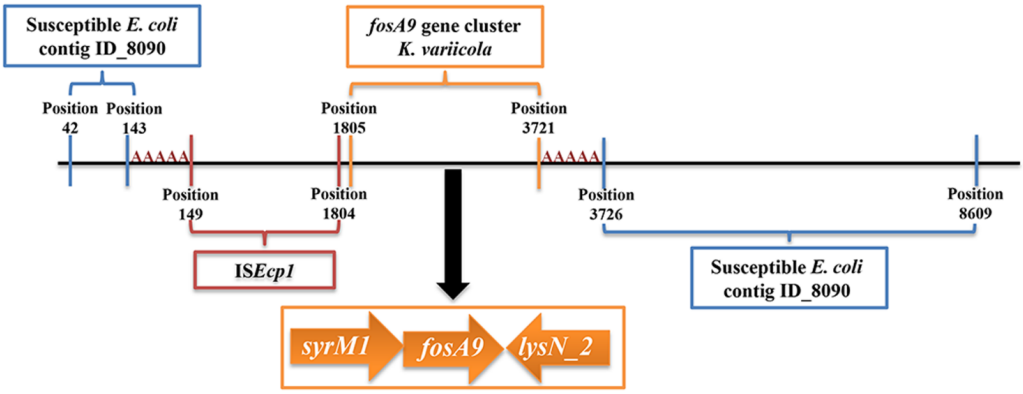
Figure 1. Schematic representation of the contig (ECO-BAB-IMI-103297_P-ACH-BAB-IMI- 103242_1528359160 _131_length_8653_cov_18.1163_ID_8928, 8653 bp) in the fosfomycin-resistant *E. coli* isolate containing a *fosA9* gene duster originating from a *K. variicola* isolate. The IS*Ecp1-syrM1-fosA9-lysN2* region is flanked by 5 bp DRs (AAAAA), suggesting mobilization from *K. variicola* by IS*Ecp1*. Upstream and downstream sequences of the insertion region align to contig ECO-BAB-IMI-103298_P-ACH-BAB-IMI-103242_1528359160_92_length_16411_cov_29.2905_ID_8090 from the first susceptible *E. coli* isolate. Sequence information of complete genomes of all isolates and separate sequences of the relevant contigs (containing *fosA9* in *E. coli* and *K. variicola*, and ECO-BAB-IMI-103298_P-ACH-BAB-IMI-103242_1528359160_92 _length_16411_cov_29.2905_ID_8090 from the susceptible *E. coli*) have been deposited in the ENA under project number PRJEB32329. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.



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CHAPTER 8

Fosfomycin-trometamol for urinary tract infections in kidney transplant recipients

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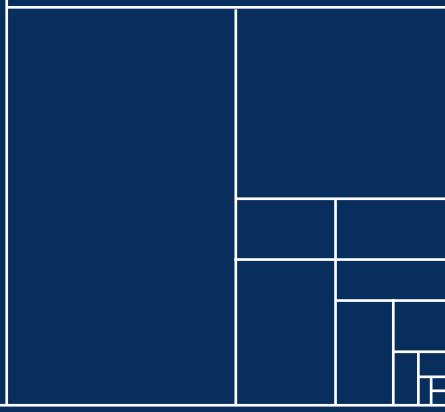
Transplantation, 2019



ABSTRACT

Background: The treatment of urinary tract infections (UTI's) in kidney transplant recipients (KTRs) with oral antibiotics is complicated by increasing resistance to trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid and ciprofloxacin. Fosfomycin-trometamol (FT) could be an alternative, but evidence on clinical effectiveness is scarce. We evaluated the use, effectiveness and safety of FT for UTI in KTRs.

Methods: Data were retrospectively collected in 2 Dutch transplant hospitals from adult KTRs that were treated with FT as initial treatment for loweUTI or asymptomatic bacteriuria (ASB) or as stepdown treatment for upper UTI after initial intravenous antibiotics. Exclusion criteria were *in vitro* resistance to FT or concomitant antibiotic treatment. Endpoints were clinical cure within 14 days and severe clinical failure, microbiological cure, relapse, recurrence, and acquired resistance within 90 days post-end of treatment.

Results: Fifty-three episodes in 40 KTRs were included (ASB, n= 15; lower UTI, n= 33; upper UTI, n= 5). FT was used for a median short duration in a heterogeneous gift interval. FT resulted in microbiological cure in 25%, 28% and 100% of ASB, lower UTI and upper UTI with initial positive culture and follow-up culture performed, respectively. Clinical cure rates were 67% for lower-UTI and 80% for upper-UTI. Relapses or recurrences occurred in 31% and 24% of symptomatic UTI episodes, without severe clinical failure. Acquired resistance to fosfomycin was observed in 6 episodes.

Conclusions: FT has a reasonable effectiveness as last-resort oral treatment for lower UTI and stepdown treatment for upper UTI in KTRs. Randomized controlled trials with optimal dosage regimens are warranted. Use of FT is not recommended for ASB.

INTRODUCTION

Kidney transplantation is the most performed solid organ transplantation and the best therapeutic option for end-stage renal disease.[1] Urinary tract infections (UTIs) occur in an estimated 38% of kidney transplant recipients (KTRs),[2] due to vesical-urethral reflux, underlying urologic diseases, urinary catheters, immunosuppression and comorbidity (eg, Diabetes Mellitus).[2,3] Urinary tract infections account for 30% of the hospitalizations for sepsis within KTRs and sepsis has been identified as a major cause of mortality in KTRs.[3,4] In the first 6 months after kidney transplantation, UTI is associated with a high risk of complicated disease (e.g. pyelonephritis, bacteremia, and relapse), and late UTI has been associated with increased mortality.[5]

The frequent use of antibiotics, for (recurrent) UTI and asymptomatic bacteriuria (ASB) and post transplantation *Pneumocystis jiroveci* prophylaxis, leads to a high prevalence of resistance to first-line oral antibiotics, that is trimethoprim-sulfamethoxazole (STX), amoxicillin-clavulanic acid and ciprofloxacin (CPX), among isolates causing UTI.[3] Frequently, only antibiotics that can be administered intravenously remain for treatment, implying hospitalization or outpatient parenteral antibiotic treatment.[6] Fosfomycin-Trometamol (FT) has raised attention as a last resort oral therapy for UTI in KTRs.[7,8] Fosfomycin is a phosphoenolpyruvate analogue. Oral bio-availability of FT is 30 to 37%, and after uptake, it is excreted non-metabolized in urine, where it reaches high concentrations.[9] In the Netherlands, approximately 1% of outpatient urine *E.coli* isolates are resistant to fosfomycin without clinical evidence for co-resistance to extended spectrum beta-lactamase (ESBL), CPX, STX or cephalosporins.[10,11] FT has little side effects, no reported nephrotoxicity and few interactions, of which no interactions with immunosuppressive medicines.[9] As a consequence, fosfomycin has been used increasingly to treat UTI syndromes in KTRs caused by multiresistant pathogens.[12,13]

Nevertheless, the effectiveness and safety of FT for treating UTI in KTR is largely unknown. This retrospective cohort study aims to provide a detailed description of the use, effectiveness and safety of FT for UTI in KTRs.

METHODS

Study design

Data were retrospectively collected from 2 large Dutch academic kidney transplant hospitals, the University Medical Center Utrecht (UMCU) and the Leiden University Medical Center (LUMC), responsible for respectively 58 and 145 kidney transplantations in 2017. Approval was obtained from the affiliated

ethical board of both hospitals with a waiver for informed consent, because only observational data were collected that was directly anonymized.

Selection of episodes

FT prescriptions in the total populations of KTRs were collected from the electronic medical record (EMR) between January 2010 and September 2017 and checked for eligibility. Episodes were eligible if FT was prescribed as initial treatment for ASB, lower UTI or as stepdown treatment after intravenous treatment of upper UTI in adult (≥18 y) KTRs. Exclusion criteria were: no (presumed) causative pathogen identified within 30 days before the episode, the concomitant start of other antibacterial treatment and known *in vitro* resistance of the causative pathogen to fosfomycin. Susceptibility to fosfomycin was measured with automated panel test (VITEK/PHOENIX), based on the European Committee on Antimicrobial Susceptibility Testing minimal inhibitory concentration (MIC) breakpoint for uncomplicated UTI (S ≤ 32 mg/L).[14]

Asymptomatic bacteriuria was defined as a positive urine culture (≥10^4 CFU/ml) without the report of any local or systemic sign or symptom. In the presence of at least 1 of the following local symptoms an episode was categorized as lower UTI: dysuria, urgency, frequency, suprapubic pain/discomfort, haematuria, new urinary incontinence or worsening of pre-existing incontinence. If at least 1 reported symptom or sign of systemic disease or transplant pyelonephritis was reported, the episode was categorized as upper UTI (i.e. temperature ≥38.0 C° or <36 C°, rigors, delirium or hemodynamic instability as a result of sepsis requiring intravenous fluids, pain or tenderness in the kidney transplant or worsening graft function).

Outcome measurement

Clinical cure was defined as absence of reported local or systemic UTI symptoms and no additional antibiotic therapy for UTI within 14 days posttreatment. Microbiological cure was established for episodes in which the causative pathogen was identified and a control urine culture was obtained after therapy, and was defined as absence of a phenotypically identical isolate (identifiable species with a maximum of 1 different antibiotic resistance pattern to the presumed causative pathogen) without intervening antibiotic therapy in the first control urine culture after treatment collected within 90 days post-end of treatment. Relapse was defined as antibiotic treatment for UTI within 90 days post-end of treatment for a phenotypically identical pathogen; in case of unknown or phenotypically different pathogens, it was classified as a recurrence. Severe clinical failure was defined as ICU-admission or mortality as a consequence of antibiotic failure, as judged by the investigator, within 90 days post-end of treatment.

Hospital (non-ICU) admission as a consequence of FT failure was excluded from the definition, as this is required for the administration of intravenous antibiotics instead of a consequence of severe clinical failure. Acquired resistance was defined as the occurrence of fosfomycin resistance in an isolate within 90 days post-start of treatment that was phenotypically identical to a cultured species 90 days before treatment.

Statistical analysis

We used R-software (version 3.1.1) to process the data. For the subgroup analysis Fishers exact test and student *t* test were used.

RESULTS

Baseline characteristics

Using the EMR, a total of 121 FT prescriptions were found in the KTR population of both hospitals. Of these, 68 episodes did not fulfil the inclusion criteria, because FT was prescribed as prophylaxis for recurrent UTI (n=32), as prophylaxis for a catheter exchange or surgery (n=9), as on demand medication (n=6), as add-on antibiotic treatment (n=2), as treatment for epididymitis (n=1), for unreported reasons (n=15), as no (presumed) causative pathogen was found (n=3). In the end, 53 UTI episodes were included in 40 KTRs, equally distributed between the two study centres (ASB, n=15; lower UTI, n=33; upper UTI, n=5). FT was prescribed 0, 0, 3, 4, 5, 18, 15 and 8 times annually from January 2010 to September 2017. The reason for using FT was known or presumed resistance to first-line oral antibiotics in all episodes, in combination with allergy or intolerance to first-line antibiotics in two episodes.

Of the ASB, lower-UTI and upper-UTI episodes, 15, 27, and 5 had an identified causative pathogen within 7 days before treatment. Of these, follow-up cultures within 30 days were performed after 12, 18, and 5 of episodes, respectively. In the remaining 10 episodes diagnosis was based on clinical symptoms and treatment was targeted to the presumed causative pathogen in urine culture obtained between 7 and 30 days before the episode.

Baseline characteristics of all episodes are reported in Table 1. FT was used for recurrent UTI in middle aged females (81%) after a median of 4.3 years posttransplantation, and 79% were caused by *E.coli*. More than 70% of the isolates were resistant to CPX, STX or amoxicillin-clavulanic acid.

Table 1. Baseline characteristics when using FT for UTI in KTRs

Determinant	Fosfomycin-trometamol (53 episodes)	
Gender female (%)	43 (81)	
Median age (IQR)	62 (49-69)	
Median transplant age (IQR)	4.3 (1.6-7.9)	
Heart-beating donor (%)	42 (79)	
Kidney-pancreas transplantation (%)	5 (9)	
Second kidney transplantation (%)	5 (9)	
Median n of immunosuppressive drugs (IQR)	2 (2-3)	
Cause for renal transplantation		
Diabetic nephropathy (%)	8 (15)	
Polycystic kidney disease (%)	11 (21)	
Hypertension nephropathy (%)	12 (23)	
Urological malformation (%)	5 (9)	
Other (%)	19 (36)	
Median Charlson comorbidity index (IQR)	4 (4-5)	
Diabetes Mellitus (%)	13 (25)	
Mean GFR in mL/min/m² (SD)	42 (18)	
Bacteraemia (%)	0	
Urinary catheter/stent (%)#	8 (15)	
Prophylactic antibiotics (%)	6 (11)	
Trimethoprim-sulfamethoxazole	2 (4)	
Other	4 (8)	
Treatment restriction (%)*	1 (4)	
Prior antibiotic prescriptiosn for UTI ≤ 90 days before the event (%)	35 (66)	
Median n of antibiotic prescriptions (IQR)	2 (1-3)	
≥ 250 leucocytes per uL ** (%)	36/45 (80)	
Pathogen * **	**Causative pathogen (n=43)**	**Presumed causative pathogen (n=10)**
E.coli	36 (84)	6 (60)
K. pneumoniae	5 (12)	2 (20)
P. mirabilis	2 (5)	0
P. auruginosa	0	0
S. agalacteae	1 (2)	1 (10)
Enterobacter species	1 (2)	1 (10)
≥2 pathogens	2 (5)	0
Resistance pattern * **		
ESBL positive	13 (31)	1 (10)
CRE positive	0	0
Amoxicillin	33 (77)	8 (80)
Amoxicillin + clavulanic acid	30 (70)	5 (50)
Ciprofloxacin	34 (79)	6 (60)
Trimethoprim-sulfamethoxazole	30 (70)	5 (50)
Fosfomycin	0	0

IQR = interquartile range, GFR = glomerular filtration rate (ml/min/min2), ESBL = Extended Spectrum Beta-Lactamase, CRE = Carbapenem-Resistant Enterobacteriaceae

double-J nephroureteral stents (n=2), intermittent catheterization (n=5), nephrostomy catheter (n=1) * 'do not resuscitate' with or without additional restrictions, such as no ICU admission ** in 45 episodes for which FT was prescribed a urinary sediment was performed

*** in 43 episodes a culture was obtained and a *causative pathogen* was found within 7 days before treatment. In the remaining 10 episodes diagnosis and treatment was based on the *presumed causative pathogen* that was found between 7 and 30 days before treatment

Dosing

FT for ASB (n=15) was prescribed as a single-gift for all but two episodes, in which 2 gifts with a 4-day interval and 3 gifts with a 3-day interval were used (Table 2). FT was prescribed as a single-gift for 21 of 33 lower-UTI episodes, and in the remaining episodes in 2-day intervals for 6, 6, 14 and 42 days and in 3-day intervals for 6, 6, 9, 9, 9, 12, 15 and 15 days. The patient that received 42 days of FT therapy had a presumed chronic prostatitis. When using FT as stepdown treatment for upper UTI, intravenous antibiotic therapy had consisted of ceftriaxone in 2 and meropenem in 3 episodes with a median duration of intravenous therapy of 7 days (interquartile range [IQR], 6-9).

Effectiveness

Clinical cure was achieved in 67% and 80% of the episodes of lower-UTI and upper-UTI, respectively, and microbiological cure was achieved in 25%, 28% and 100% of episodes of ASB, lower-UTI, and upper UTI after a median of 20 days postend of treatment (IQR, 12-30). Relapses occurred in 47%, 33% and 20%; and recurrences in 13%, 21% and 40% after ASB, lower UTI or upper UTI episodes, respectively, see Table 2. Among patients with lower UTI, clinical cure was associated with less urinary catheter/stent use and single gift use (Table S1, online supplementary material), whereas microbiological cure was associated with glomerular filtration rate (GFR) of 30 mL/min/m^2 or greater. No association was found between *E.coli* as the causative pathogen and clinical and microbiological cure.

Table 2. Clinical and microbiological results after using FT for UTIs in KTRs

	ASB (n=15)	Lower-UTI (n=33)	Upper-UTI (n=5)
Median duration of FT use in days (IQR)	1 (1:1)	1 (1:7)	7 (6:9)
Mean interval in days*	3.5	2.7	2
Microbiological cure (%)**	3/12 (25)	5/18 (28)	5/5 (100)
Clinical cure (%)	N/A	22 (67)	4/5 (80)
Severe clinical failure	0	0	0
Relapse# (%)	7/15 (47)	9/27 (33)	1/5 (20)
Recurrence (%)	2 (13)	7 (21)	2 (40)
Adverse events	0	2 (6)	1 (20)
Acquired resistance	3 (20)	3 (10)	0

ASB = asymptomatic bacteriuria, UTI = urinary tract infection, FT = fosfomycin-trometamol, IQR = interquartile range
*only reported for events in which more than one gift is prescribed ** only reported for events in which the causative pathogen was identified and a control urine culture was obtained # only reported for events in which the causative pathogen was identified

Safety

Severe clinical failures were not reported. One patient had a relapse of *Clostridium difficile* infection after receiving 1 gift of FT as stepdown treatment after 4 days of ceftriaxone. Two patients reported self-limiting diarrhoea after using 3 gifts of FT in a 3 and 2 day's interval for lower UTI.

Acquired resistance within 90 days was reported 6 times, after receiving 1 to 22 gifts of FT, all of these were phenotypically identical to a pathogen cultured in the 90 days before the episode. Of these, four ESBL-producing Enterobacteriaceae were involved (Table 3).

Table 3. Acquired resistance to fosfomycin after fosfomycin-trometamol prescription

Event	Antibiotic (duration, interval in days)	Causative pathogen(s)	ESBL- producing pathogen
ASB	FT (3, 3)	*E.aerogenes*	Yes
ASB	FT (1, N/A)	*K.pneumoniae*	Yes
Lower-UTI	FT (3, 3)	*E.coli*	Yes
Lower-UTI	FT (22, 2)	*K.pneumoniae*	Yes
ASB	FT (1, N/A)	*E.aerogenes*	No
Lower-UTI	FT (4,2)	*K.pneumoniae*	No

ASB= asymptomatic bacteriuria, UTI=urinary tract infection, ESBL= Extended Spectrum Beta-Lactamase, FT=fosfomycin-trometamol

DISCUSSION

In 2 Dutch tertiary care hospitals, FT when used as a last-resort oral therapy for recurrent symptomatic UTI in KTRs caused by multi-resistant pathogens, was associated with clinical cure in 67% of lower UTI and in 4 of 5 episodes of stepdown treatment after intravenous therapy for upper UTI. Most of the 38 UTI and 15 ASB episodes were caused by *E.coli* resistant to CPX and STX.

The microbiological cure rate is low for UTI episodes in which FT is used without initial antibiotic treatment. Microbiological cure was not achieved in 72% of lower-UTI episodes. The overall effectiveness of FT for lower-UTI should be interpreted in the light of the difficult-to treat episodes for which it was used, involving recurrent UTI caused by multiresistant isolates. No patients were admitted to the Intensive Care or died as a consequence of therapy failure, and only few adverse events were reported. Microbiological cure was not achieved in 75% of ASB episodes. Previous studies have not been able to demonstrate benefit of any antibiotic, particularly beyond the earliest post-transplant period.[15–17] Despite, FT is still being used for ASB in KTR involving multiresistant pathogen, in which it carries the risk of inducing resistance to fosfomycin.

Optimizing FT regimen could potentially lead to a higher effectiveness for lower-UTI. Based on the pharmacokinetic profile, FT should probably be prescribed for more than once for the treatment of lower

UTI in KTRs. In healthy volunteers, after a single gift of 3 gram oral fosfomycin, urine concentrations remain above the European Committee on Antimicrobial Susceptibility Testing breakpoint in only 30% over the first 72 hours.[18] The same oral gift leads to low serum peak concentrations of up to 22–32 mg/L.[19] Consequently, FT use should be restricted for non invasive disease.

In clinical practice, FT is used as single or multiple gift therapy with intervals ranging from 1 to 4 days, precluding accurate estimation of its effectiveness.[8,20–22] Increasing the gifts of FT is considered safe. In healthy volunteers, tolerability of using FT every 24 hours was comparable to using it every 48 hours.[23] FT is not (nephro)toxic, has few interactions with other drugs and can be used safely intravenously in gifts up to 24 g/d.[10,24] In our population, a single FT gift for lower UTI, instead of multiple gifts, was associated with clinical cure. This association most likely results from confounding by indi cation as episodes treated with multiple gifts had more comorbidity and more often had an urinary catheter. Few multiple gifts FT regimens were prescribed, impeding further sub analyses. In a previous observational study, no significant difference in clinical cure was observed between single and multiple gifts for cystitis in KTRs, although the dosage for the multi-gift treatment was not reported.[7] Based on existing evidence, it is difficult to establish the optimal FT dose for the treatment of UTI in KTRs, and more clinical dose finding studies are needed.[19] Microbiological cure was associated with a GFR ≥30ml/min/m2. A previous study found no difference in clinical success when treating cystitis in abdominal transplant patients with a creatinine clearance below or above 40 mL/min.[25] To our knowledge, there is no other data on the effectiveness of FT in patients with renal insufficiency. FT is mainly being excreted in urine and renal insufficiency significantly decreases the excretion of fosfomycin in urine. However, urine concentrations remain high, even in severe renal insufficiency.[9]

In vitro data suggests that FT is more effective if the infection is caused by *E.coli,* compared to other Gram-negative bacteria. *E.coli* has low MICs to fosfomycin, around 2-4 mg/L, with high MICs to fosfomycin in most non-*E.coli* isolates (i.e. *K.pneumonia* and *P.mirabilis*).[26] Moreover, fosfomycin susceptibility testing is supposed to be more accurate for *E.coli* than for other isolates.[27]

Acquired resistance to fosfomycin occurred in 6 episodes after FT prescription. If resistance to fosfomycin occurs, a next UTI episode could not be treated with FT, only remaining intravenous antibiotic possibilities. Three out of six acquired resistance events were found in *K.pneumoniae* species. A previous case-report described the *in vivo* acquisition of resistance to fosfomycin in *K.pneumonia* during a long-term intravenous fosfomycin adjunct treatment.[28] Subsequently, an increase in fosfomycin resistance was reported in *K.pneumoniae* in the Netherlands from 22% in 2011 to 31% in 2015.[29]

Three other retrospective studies evaluated the effectiveness of FT for UTI in KTRs. In one study, a relapse, defined as an infection with the same microorganism within 3 months, was found in 7 of 13 episodes (54%) after treating UTI with 3 gram every 48 to 72 hours for 1 to 7 days.[8] In a second study the effectiveness of FT, in a dose of 3-6 gram per day for 7-14 days, was evaluated in 11 KTRs with UTI caused by carbapenem-resistant Enterobacteriaceae and resulted in microbiological and clinical cure in 43% (3 of 7 episodes) and 91% (10 of 11 episodes), respectively.[21] In the third study the effectiveness of FT was determined in 76 cystitis episodes in solid organ recipients, of which 63 KTRs. Clinical success, defined as not having a positive follow-up test leading to an antibiotic prescription after an unknown duration, was achieved in 85.5% of episodes.[7] The results of these studies are difficult to compare to ours, due to differences in patient populations, infections, outcome definitions and treatment.

Limitations of our study include the small size and the retrospective design. All endpoints were derived from the EMR, which resulted in missing data and possibly misclassification. Because of underreporting of signs and symptoms, episodes could have been misclassified as lower UTI instead of upper UTI or ASB instead of lower or upper UTI. In both hospitals, KTRs are explicitly advised to contact their own transplantation nephrologist in case of UTI symptoms or signs, therefore, we are convinced that the risk to misclassify clinical cure, relapse or recurrence is low. A causative pathogen was not identified in 14 out of 56 episodes of lower and upper UTI, as urine samples for microbiological culture had not been obtained. In these patients only clinical effectiveness could be evaluated. Finally, performing a control urine culture after treatment was not standard procedure, making it impossible to establish microbiological cure for all episodes at a fixed time. This selection of episodes for which a reason existed to obtain a control urine culture, could have introduced an underestimation of the microbiological cure. However, this could hardly correct for the observed very low microbiological effectiveness.

In conclusion, FT has a reasonable clinical effectiveness and a low microbiological effectiveness as oral therapy for lower UTI caused by multiresistant pathogen, in case no antibiotic alternatives are available. FT has a high clinical and microbiological effectiveness when used as stepdown treatment after initial intravenous antibiotics for a median of 7 days for 5 upper UTI episodes. Based on our findings, we do not recommend the use of FT for ASB. Currently FT is sometimes used to avoid intravenous antibiotics, and thus hospitalization. Yet, our findings do not lend support to using FT as first-line therapy for symptomatic UTI in KTRs until more evidence exists. Pharmacokinetic/pharmacodynamics studies are needed for defining optimal dosing, and randomized trials to determine the effectiveness of FT for the initial treatment of lower UTI and as a stepdown treatment for upper UTI in KTRs, with monitoring of the

development of acquired resistance. For now, we consider FT as a last-resort oral antibiotic only in KTRs. At this moment, no trials are registered that investigate the efficacy of FT for the treatment of UTI in KTRs. A randomized trial investigates the efficacy of FT compared to CPX for the stepdown treatment of *E.coli* complicated-UTI in non-KTR women (http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=6449).

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CHAPTER 9

Oral fosfomycin versus ciprofloxacin in women with *E.coli* febrile urinary tract infection, a double-blind placebo-controlled randomized controlled non-inferiority trial (FORECAST)

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ABSTRACT

Background: Febrile Urinary Tract Infection (FUTI) is frequently treated initially with intravenous antibiotics, followed by oral antibiotics guided by clinical response and bacterial susceptibility patterns. Due to increasing infection rates with multiresistant Enterobacteriaceae, antibiotic options for stepdown treatment decline and patients more frequently require continued intravenous antibiotic treatment for FUTI. Fosfomycin is an antibiotic with high bactericidal activity against *Escherichia coli* and current resistance rates are low in most countries. Oral Fosfomycin-Trometamol 3000mg (FT) reaches appropriate antibiotic concentrations in urine and blood and is considered safe. As such, it is a potential alternative for stepdown treatment.

Methods: The FORECAST study (Fosfomycin Randomized controlled trial for *E.coli* urinary tract infections as Alternative Stepdown Treatment) is a randomized, double-blind, double-dummy, non-inferiority trial in which 240 patients will be randomly allocated to a stepdown treatment with FT or ciprofloxacin (standard of care) for FUTI, caused by *Escherichia coli* with in vitro susceptibility to both antibiotics. The study population consists of consenting female patients (≥18 years) with community acquired *E. coli* FUTI. After intravenous antibiotic treatment during at least 48 (but less than 120) hours, and if eligibility criteria for iv-oral switch are met, patients receive either FT (3 g every 24 h) or ciprofloxacin (500mg every 12 h) for a total antibiotic duration of 10 days. The primary endpoint is clinical cure (resolution of symptoms) 6-10 days post-treatment. Secondary endpoints are microbiological cure 6-10 days post-treatment, clinical cure, mortality, ICU admittance, relapse, reinfection, readmission, additional antibiotic use for UTI, early study discontinuation, adverse events, days of hospitalization and days of absenteeism within 30-35 days post-treatment. The sample size is based on achieving non-inferiority on the primary endpoint, applying a non-inferiority margin of 10%, a two-sided *p*-value of < 0.05 and a power of 80%.

Discussion: The study aims to demonstrate non-inferiority of oral fosfomycin, compared to oral ciprofloxacin, in the stepdown treatment of *E. coli* FUTI.

Trial registration: Registered at the Nederlands trial register (Dutch trial register) Trialregister.nl on 4-10-2017. Trial registration number: NTR6449. Secondary ID (national authority): NL60186.041.17

BACKGROUND

Urinary Tract Infections (UTI) are the most common bacterial infections requiring antibiotic treatment in the western world.[1] Complicated urinary tract infections (cUTI) are defined upon the presence of systemic symptoms or upon the susceptibility of the host for a complicated course.[2] Systemic symptoms are often fever, febrile UTI (FUTI), or other symptoms, reflecting the presence of tissue infection such as pyelonephritis, prostatitis or the urosepsis syndrome (2). Guidelines recommend to treat FUTI with a 7-14 day course of antibiotics[2,3] and in the majority of cases empiric intravenous antibiotics will be followed by oral stepdown therapy. The choice of stepdown treatment should be targeted to the susceptibility pattern of the causal uropathogen. Unfortunately, antibiotic options for stepdown treatment are becoming limited as the result of increasing antibiotic resistance.[2]

Escherichia coli (*E.coli*) is the causative organism in around 70-75% of FUTI.[4,5] In 2017, resistance rates of *E.coli* isolates of patients admitted to Dutch hospitals were 14% for ciprofloxacin (CPX), 23% for trimethoprim-sulfamethoxazole and 36% for amoxicillin-clavulanic acid with higher resistance rates in patients from urology departments.[6] Though this leaves fluoroquinolones still as one of the oral antibiotics to treat FUTI, one of the goals of antibiotic stewardship is to reduce fluoroquinolone use because of its selective properties for antibiotic resistance.[2] In the Netherlands, about 2-5% of patients hospitalized for community-acquired FUTI cannot be treated with oral antibiotics due to antibiotic resistance of the causal uropathogen.[7] This implies the need of prolonged intravenous antibiotic treatment, prolonged hospitalization, increased healthcare costs and higher risks of forthcoming complications.

Fosfomycin is an alternative antibiotic treatment for the stepdown treatment of FUTI.[8,9] Fosfomycin is a phosphoenolpyruvate analogue and orally available as Fosfomycin-Trometamol (FT). It has been used extensively for uncomplicated cystitis in women, has a good safety profile[10] and possesses a suitable pharmacokinetic profile in healthy volunteers.

In the FORECAST trial (FOsfomycin Randomized controlled trial for *E.coli* urinary tract infections as Alternative Stepdown Treatment), we will investigate the efficacy of FT when used as stepdown therapy of FUTI in women.

METHODS

The objective of this randomized controlled double-blind, double-dummy, non-inferiority, multicentre, investigator-initiated trial is to determine whether oral FT is non-inferior to oral CPX in the step-down treatment of *E.coli* FUTI in women for achieving clinical cure. The study will be performed in 15 Dutch hospitals; 4 academic centers and 11 large teaching hospitals.

Participants

Inclusion criteria

Competent women (≥18 years), that are hospitalized for the presumed diagnosis FUTI and receive appropriate empirical intravenous antibiotics for ≥48 - ≤120 hours, who are judged to be eligible for an intravenous-oral switch by the attending physician, according to the Dutch guideline, that recommends to switch therapy until the patient has been afebrile for 24-48 h and symptoms have improved.[2] Urine ($\geq 10^4$ CFU/ml) and/or blood culture must reveal *E.coli*, susceptible to both CPX and fosfomycin. A patient is not eligible if non-E.*coli*-type Enterobacteriaceae are present in urine culture ($\geq 10^3$ CFU/ml) or blood culture.

FUTI is defined as UTI with at least one forthcoming systemic symptom or sign and one local symptom at presentation. Local symptoms are dysuria, urinary urgency, urinary frequency, suprapubic/pelvic discomfort, macroscopic hematuria, new urinary incontinence or worsening of pre-existing incontinence, lower abdominal pain, low back pain, flank pain, costo-vertebral angle pain or tenderness on physical examination. Systemic symptoms or signs are fever (≥38.0 C°) or low temperature (<36.0 C°), rigors, delirium, hemodynamic instability as a result of sepsis requiring intravenous fluids or an increase in CRP (≥30mg/L) or leucocytes ($\geq 12*10^9$/L). Local symptoms are not required in case the urine and blood culture yield are positive for a phenotypically identical matched *E.coli* and UTI is the presumed source of infection according to the treating physician.

Adequate empirical intravenous treatment may consist of amoxicillin+/-clavulanic acid, 2nd or 3rd generation cephalosporin, aminoglycoside, carbapenem, fluoroquinolones, trimethoprim-sulfamethoxazole or a combination and *in vitro* susceptibility of the causative *E.coli* to at least one of the used agents.

Exclusion criteria

Pregnancy or breastfeeding, glomerular filtration rate below 30ml/min/1,73 m3 or renal replacement therapy, concomitant systemic antibacterial treatment, ascertained or presumptive hypersensitivity to (compounds of) the study products, participation in any trial with an investigational product involved in the 30 days before the screening visit, specific patient groups: patients with renal transplant, polycystic kidney disease, neutropenia (<500 /μL), paraplegia, suspicion/presence of renal abscess, suspicion of septic metastatic foci/endocarditis, urostomy, ileal loops, long-term urinary catheter (placed ≥24 hours before admission), e.g., double-J catheter, nephrostomy catheter, suprapubic catheter, specific contra-indications for CPX or fosfomycin: concurrent use of tizanidin, clozapin or theophylline, a history of

tendon disease/disorder related to quinolone treatment, patients with known risk factors for prolongation of the QT interval, glucose-6-phosphate dehydrogenase deficiency, inadequate understanding of the study risks or its requirements or unwilling to plan a follow-up visit and every other laboratory result, clinical condition, disease or treatment that, in investigator's opinion, make the subject non suitable for the study. Patients with partial obstruction of the urinary tract, ureteral stones or intermittent catheterization will not be excluded.

Setting

Potential subjects for inclusion will be identified through daily screening for *E.coli* in urine and blood cultures in the clinical microbiology laboratories of participating centres. The study investigator will assess potential subjects for eligibility with consultation of the attending physician. After identification, eligible patients will be informed and, after obtaining consent, will be randomized.

Randomization

The data manager of the University Medical Centre Utrecht constructed a randomization list, that connects the unique medication code with the active substance (1:1). Only the data manager and the pharmacist on duty, responsible for the deblinding process in case of emergency, have access to the randomization code. Constrained randomization will take place with permuted blocks, that contain a pre-specified number of treatment assignments in a random order. As we expect to include more than 200 participants in a two-arm trial, the probability of a significant imbalance is negligible.[11] To prevent imbalances between both study arms stratification will be performed per study centre, as each study centre handles their own local guideline for the empirical treatment of FUTI.

Treatment

Participants will be randomized for an intravenous-oral antibiotic switch to either FT 3000 mg every 24 h or CPX 500 mg every 12 h. CPX is chosen for the control group, because most evidence exists for CPX as an oral treatment of FUTI [12,13]. A double-dummy design is chosen as it is impossible to equalize fosfomycin granules to any form of CPX. An identical placebo will be used for both active substances. All medicines will be manufactured according to Good Manufacturing Principles by the pharmaceutical company Basic Pharma BV, the Netherlands. The duration of total antibiotic therapy is 10 days for all participants, of which 2-5 days empirical intravenous antibiotics and the remaining 5-8 days stepdown therapy with FT or CPX.

FT will be dosed 3 g every 24 h, which is based on a review of the scientific data and expert opinion on the treatment of FUTI and the pharmacokinetics of FT that was available at the moment the study was designed (May 2017). The use of FT every 24 h for more than a single dose was tolerated well in retrospective studies and according to personal off-label use [14]. We do not expect negative effects of accumulation as fosfomycin is registered for intravenous use in dosages as high as 24 g per 24 h [15]. A medication diary needs to be filled by the participant in order to improve medication adherence. All patients receive oral and written information on the trial, including possible risks of participating. Additionally, patients receive a medication folder with instructions for use, contra-indications, interactions and adverse events of both FT and CPX (probable, possible and seldom adverse events). For questions or concerns, participants could contact the local or principle investigator or an independent expert physician.

Data collection

In each hospital, authorized and qualified clinical investigators and research nurses will obtain informed consent for participation. Demographic, clinical and microbiological data will be collected and stored for at least 15 years. Data will be collected from questionnaires and supplemented with data from the electronic medical record, see Table 1.

All participating microbiological and clinical chemistry laboratories are accredited by the Dutch foundation for the promotion of the quality and the accreditation of laboratories in health care (ISO). Susceptibility to fosfomycin and CPX is tested using validated local susceptibility methods, following the European Committee on Antimicrobial Susceptibility (EUCAST) recommendations. The clinical (*E. coli*) isolates will be stored for additional susceptibility measurements, i.e. agar dilution for fosfomycin susceptibility, and molecular analysis.

The primary and secondary endpoints will be collected 6-10 days post-end-of-treatment during a visit and 30-35 days post-end-of-treatment by telephone. Data will be entered into the Case Report Form (CRF) pseudo-anonymously in each participating centre using an unique participant code. The data will be processed by the data management program 'ResearchOnline'. All steps of the data process will be stored to be able to check validity and plausibility.

Table 1. Enrolment, interventions and assessments in the FORECAST study

	Patient identification	Before randomization	Study treatment (5-8 days)	6-10 days post-end of treatment	30-35 days post-end of treatment
	Enrolment				
Screening for eligibility	x				
Entry criteria		x			
Informed consent		x			
	Interventions				
Venapunction*		x			
Urine/blood culture	x			x (urine)	
Study treatment			x		
	Assessments				
Electronic patient file		x		x	x
Patient questionnaire		x		x	x
Hand in study diary and residual study medicines				x	

*in case of doubt about the following exclusion criteria: pregnancy, neutropenia or renal insufficiency

Endpoints

The primary endpoint is clinical cure (resolution of symptoms) 6-10 days post end-of-treatment. Definitions and criteria of primary and secondary endpoints are described in Table 2.

Ethics

This study has been set up in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. The medical ethics committee of the University Medical Centre Utrecht approved the study protocol, followed by the Institutional Scientific Boards of each participating centre. Written informed consent is obtained prior to randomization. Structural protocol modifications, including amendments, will be communicated as soon as possible to the medical ethics committee, the trial register, and all involved investigators and participants.

Statistical analysis

Sample size calculation

We determined the sample size based on an assumed cure rate of 92.5% for both groups and a margin of inferiority of 10%, consistent with assumptions in similar UTI trials.[5,12,13,16] Assuming that a difference of

10.0 percentage points or less is irrelevant and with the two-sided alpha set at 0.05, the sample size needed in the two groups is 109. The study has a 80% power to reject the null hypothesis that the clinical cure rate for the study arm (FT) is 10% lower than the cure rate for the standard of care (CPX). Equivalently, the likelihood is 80% that the two-sided 95.0% confidence interval for the difference in clinical cure rates will exclude a 10% difference in favour of standard care. In order to evaluate 109 patients in both treatment arms, taking into account 10% of lost participants for various reasons, 240 subjects will be enrolled in total.

Intention to treat and per-protocol analysis

Participants are evaluable if they are randomized and received at least one dose of the study drug, and all will be included in the intention to treat analysis. Analysis of all endpoints will be performed according to the intention-to-treat principle, on which data non-inferiority is based. A per-protocol analysis will be performed for the primary endpoint and the secondary endpoint 'microbiological cure' for patients that completed at least 80% of study medicines.

Statistical methods

For the primary endpoint, the difference between the study arm and standard of care ($p<0.05$) will be calculated with a two-sided Z-score for proportions. Secondary outcomes will be analysed using the following tests: two-tailed Z-score for proportions ($p<0.05$), t-test, chi-square, logistic regression, when appropriate. Linear and logistic regression will be used to identify associations between patient, disease and treatment characteristics) with both study arms with regard to the primary and secondary endpoints. Clinical cure 6-10 and 30-35 days post-treatment in specific subgroups will be investigated in exploratory analyses, based on host or disease characteristics, e.g. age, BMI, Charlson index, use of immunosuppressive drugs, presence of host factors, presence of a short term indwelling catheter, creatinine clearance, diabetes mellitus, days of empirical intravenous antibiotics, class of empirical intravenous antibiotics, and concomitant bacteraemia.

Missing data

Attempts will be made to complete the data from all enrolled participants. Missing data will be tracked or retrieved by the coordinating team after consultation with the local team. Missing information will be extracted directly from the electronic patient file without disclosing patient identifiers. Multiple imputation will be used for missing data.

Safety

Our entry criteria are designated to reflect daily practice with regard to the safety of the participants. Candidates should be diagnosed with FUTI as the primary reason for hospitalization. All identified uropathogens must be *E.coli* susceptible to both fosfomycin and CPX. Patients should be treated with appropriate intravenous antibiotics for at least 48 h. The attending physician determines the eligibility for the intravenous-to-oral switch. Patients that require more than 5 days of intravenous antibiotics will be excluded as this is considered a complicated course. Our goal is to investigate a representative population by including as many patients as possible, and only exclude extremely vulnerable patients (e.g. non-competent patients, renal transplant recipients, pregnant women), patients that require an alternate antibiotic strategy (e.g. renal abscess, endocarditis) or patients for which other endpoints should be used to estimate efficacy (e.g. men).

Adverse events should be actively reported in the medication diary by participants in order to measure tolerability. Furthermore, these will be checked actively with questionnaires at 6-10 days and 30-35 days post-end of treatment. De-blinding is possible 24/7 h in case severe harm could be potentially prevented, and after consultation of the coordinating investigator. Serious adverse events and Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported during follow-up. All participants are automatically insured by a subject and liability insurance.

The study will be monitored by qualified monitors from the University Medical Centre Utrecht. For each participating centre the following procedures will be checked after the first 5 inclusions and thereupon annually: at least 10% of signed Informed Consent forms, in- and exclusion criteria, defined variables including the primary endpoint, source data verification, missed SAE's, or SUSAR's.

An interim analysis will be performed by the Data and Safety Monitoring Board (DSMB) after inclusion of 50 and 100 participants. De-blinded data will be delivered by an independent statistician to the DSMB, which will function as an advisory board, i.e. it will provide non-binding advice to the principal investigator. After inclusion of 50 participants an interim analysis will be performed regarding adverse events, medication adherence and early study medicine discontinuation as a result of adverse events/intolerance. Based on these results, the DSMB could recommend to adjust the dosage of FT. After inclusion of 100 participants, an interim analysis will be performed regarding Serious Adverse Events (SAE's), early study withdrawal and the primary endpoint. Based on these results, the DSMB could recommend to stop the study, in case the interim analysis shows a probability of finding non-inferiority at the final analysis of less than 5% based on the conditional power (futility)[17] or an unexpected high rate of possible related SAEs or early study withdrawals, in particular in the FT arm (safety).

Table 2. Endpoints, provided with definitions and a time frame

Endpoint	Definition	Time frame
Clinical cure	Alive with reduction of all initial local and systemic FUTI related symptoms and without additional systemic antibiotic therapy for UTI (except antibiotic prophylaxis)	6-10 days post-end of-treatment (PET) + 30-35 days PET
Microbiological cure	Negative urine culture for E.coli ($<10^3$ CFU/ml), phenotypically identifiable to the initial culture (assessment by microbiologist) *	6-10 days PET
Acquired resistance	Resistance to ciprofloxacin, fosfomycin or new ESBL-producing bacteria in phenotypically identical strain	6-10 days PET
Mortality	-Mortality for any reason -Mortality related to UTI or study medicines	Within 30-35 days PET
ICU admission	-ICU admission for any reason -ICU admission related to UTI or study medicines	Within 30-35 days PET
Readmission	-Readmission for any reason -Readmission related to UTI or study medicines	Within 30-35 days PET
Relapse	Development of new symptoms of UTI after previous clinical and microbiological cure with a phenotypically identical strain as isolated during the initial blood or urine ($\geq 10^3$) cultures.	Within 30-35 days PET
Reinfection	Same definition as relapse, but with phenotypically different strains isolated in cultures (urine, $\geq 10^3$)	Within 30-35 days PET
Additional antibiotic use	Additional systemic antibiotic therapy for UTI (except antibiotic prophylaxis)	Within 30-35 days PET
Length of hospital stay	-Total days of hospital stay -Total days of ICU stay	Within 30-35 days PET
Days of absenteeism	Converted to full work days: -Paid work -Voluntary work	Within 30-35 PET
Adverse events	Possible or probable related to study protocol	Within 30-35 days PET
Early study medicine discontinuation	Early study medicine discontinuation: -because of intolerance/adverse events -because of clinical failure -because of resolution of symptoms	-

*Other strains in urine culture will be reported, but do not fall within this definition

DISCUSSION

As a result of emerging antibiotic resistance among Enterobacteriaceae the options for oral antibiotic stepdown treatment of FUTI are becoming limited. Whereas new intravenous antibiotics are being developed and registered, little interest has been put in the development of oral applications in the past decade.[4,16,18] As a consequence, patients with FUTI caused by (or carrying) a multiresistant Enterobacteriaceae usually require full intravenous antibiotic regimens with a higher risk on subsequent

complications, psychological burden and increased health care costs, partly due to the forthcoming extended length of hospital stay.[19]

This has led to renewed interest in FT, a relatively infrequent used antibiotic, invented in 1969.[20] FT could be a suitable choice for targeted stepdown treatment of *E.coli* FUTI. FT was non-inferior in comparison to carbapenems in observational studies considering cUTIs.[8,9] This investigator-initiated randomized controlled trial aims to evaluate FT as stepdown treatment for *E.coli* FUTI in women.

The eligibility criteria of this study were defined such that results will be generalizable to the population being treated for FUTI in clinical practice. Consequently, patients with FUTI with a positive urine or blood culture revealing *E.coli* will be included if both local and systemic UTI signs or symptoms are present and the patient is treated with adequate intravenous antibiotics with the presumptive focus of infection being the urinary tract. The presence of fever is not required for enrolment, as we aimed to include certain vulnerable patient populations that sometimes do not develop fever (i.e. elderly, immunocompromised patients) during sepsis. In order to better fit our domain, minor changes were made in eligibility criteria after we recruited 15 participants: (1) As a systemic sign, we included an increase in CRP (≥30mg/L) or leucocytes (≥12*109/L). (2) Next, local symptoms are not required in case the urine and blood culture are positive for a phenotypically matched *E.coli* and UTI is the presumed source of infection according to the treating physician. (3) Short term urinary catheters, if placed at least within 24 hours before admission, will be allowed, e.g. double-J catheter, nephrostomy catheter, suprapubic catheter. (4) Finally, a patient is not eligible if any non-*E.coli*-type Enterobacteriaceae are present in urine (≥10^3 CFU/ml) or blood culture.

As primary endpoint, clinical cure 6 to 10 days post-end of treatment was chosen, with, next to safety endpoints, microbiological cure at 6-10 days and clinical cure at 30-35 days post-end of treatment as secondary endpoints. For future perspectives, cost-effectiveness variables will be collected, i.e. length of hospital and intensive care stay and days of absenteeism, in order to perform a model-based cost-effectiveness analysis between oral FT as stepdown treatment versus a full intravenous antibiotic course (e.g. with carbapenems) for the treatment of multiresistant *E.coli* FUTI.

There were several aspects related to the spectrum of FT that guided the study design. First, it is not rational to use FT for the empirical treatment of FUTI. The pharmacokinetic-pharmacodynamics profile is presumably not suitable to treat FUTI empirically. One gift of 3 gram (oral) FT results in a peak fosfomycin urinary concentration of 1.600 mg/L and concentrations above the Minimal Inhibitory Concentration (MIC) of most *E.coli* isolates (below 2-4 mg/L including Extended Spectrum Beta-Lactamase-ESBL) for about 48 hours.[21–23] The same gift leads to a peak serum concentration of around 20 mg/L, declining below

4 mg/L within 8-12 hours.[21,24] Second, in our opinion, FT is investigated for its use as targeted stepdown treatment of *E.coli* pathogens only, as *Klebsiella* and *Proteus* spp. are less susceptible to fosfomycin with proportions of resistance to fosfomycin of 31% and 16%, respectively, in the Netherlands.[25] The prevalence of resistance to fosfomycin among *E.coli* isolates from urine samples has been stable for years (+-1%) in the Netherlands,[25] and is even low among ESBL-producing Enterobacteriaceae.[26] Finally, determination of fosfomycin susceptibility in daily practice seems more reliable for *E.coli* than for non-*E.coli* Enterobacteriaceae.[27] Third, although FT has been used in men to treat prostatitis and FUTI, we decided not to include men as they require longer antibiotic treatment and because treatment failure expresses itself as relapses for an extended period of time.[12] Stratification for gender has been considered, but would, within the limits of perceived feasibility, reduce statistical power to draw meaningful conclusions about non-inferiority.

Finally, a study that was published recently evaluated the tolerability and pharmacokinetics of using FT every 48 hours for three doses versus every 24 hours for 7 doses.[24] The 'every 24 hours' arm was associated with significantly less days of being free of diarrhoea, however no subject discontinued FT due to adverse events. The study was not placebo-controlled, and was performed in healthy volunteers, therefore it is unknown how this reflects on the participants in this study. For safety reasons, adverse events and adherence will be evaluated during the interim analyses after 50 participants. Currently, the FOREST study (www.clinicaltrial.gov, NCT02142751) compares the efficacy of intravenous fosfomycin to meropenem for bacteraemic ESBL-*E.coli* UTI. In this study, intravenous fosfomycin is followed by stepdown therapy with 3 gram FT every 48 hours.[18]

In summary, the FORECAST study will determine the efficacy of fosfomycin-trometamol, compared to CPX, in the stepdown treatment of *E.coli* FUTI in women.

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CHAPTER 10

Fosfomycin versus ciprofloxacin as oral stepdown treatment for *Escherichia coli* febrile urinary tract infection in women: a randomized, placebo-controlled, double blind, multicenter trial

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ABSTRACT

Background: We aimed to determine the non-inferiority of fosfomycin, compared to ciprofloxacin, as oral stepdown treatment for *E.coli* febrile urinary tract infections (fUTIs) in women.

Methods: This was a double-blind, randomised controlled trial in 15 Dutch hospitals. Adult women receiving 2-5 days of empirical intravenous antimicrobials for *E.coli* fUTI, were assigned to stepdown treatment with once-daily 3 gr fosfomycin or twice-daily 0.5 gr ciprofloxacin, for 10 days of total antibiotic treatment. For the primary endpoint clinical cure at day 6-10 post-end-of-treatment a non-inferiority margin of 10% was chosen. The trial was registered on Trialregister.nl (NTR6449).

Results: After enrolment of 97 patients between 2017-2020, the trial ended prematurely because of the Covid-19 pandemic. The primary endpoint was met in 36/48 patients (75.0%) assigned to fosfomycin and 30/46 patients (65.2%) assigned to ciprofloxacin (Risk Difference: 9.6%, 95%-Confidence-Interval: -8.8% to 28.0%). In patients assigned to fosfomycin and ciprofloxacin, microbiological cure at day 6-10 post-end-of-treatment occurred in 29/37 (78.4%) and 33/35 (94.3%; RD: -16.2%, 95%CI -32.7 to -0.0%), and clinical cure at day 30-35 post-end-of-treatment occurred in 35/47 (75.6%) and 33/44 (75.0%; RD: 0.4%, 95%CI -17.6% to 18.4%), respectively. Any adverse event was reported in 35/48 (72.9%) and 32/46 (69.6%) patients (RD: 3.3%, 95%CI -15.0% to 21.6%%), and any gastro-intestinal adverse event in 25/48 (52.1%) and 14/46 (30.4%) patients (RD: 20.8%, 95%CI 1.6% to 40.0%), respectively.

Conclusions: Fosfomycin is non-inferior to ciprofloxacin as oral stepdown treatment for fUTI caused by *E.coli* in women. Fosfomycin use is associated with more gastro-intestinal events.

Funding: University Medical Center Utrecht (investigator initiated study).

BACKGROUND

Febrile Urinary Tract Infections (fUTIs), defined as urinary tract infections with systemic symptoms, frequently occur in women and are predominantly caused by *Escherichia coli* (*E.coli*).[1,2] Guidelines recommend to treat severe cases of fUTI requiring hospitalization with a 7-14 day course of antibiotics consisting of initial usually empiric intravenous treatment preferably followed by an oral stepdown treatment that is targeted to the susceptibility pattern of the causal uropathogen.[3,4] Optimal treatment of fUTIs is hampered by the increase of multiresistant Gram-negative bacteria.[5] Where new antibiotics are being developed for the intravenous treatment of fUTI, the arsenal of oral antibiotics has remained stable for decades.[1,6,7]

In 2019, among *E. coli* urine isolates from patients admitted to Dutch hospitals, resistance rates were 14% for ciprofloxacin, 22% for trimethoprim-sulfamethoxazole and 36% for amoxicillin-clavulanic acid, with even higher resistance among patients from urology departments.[8] In the Netherlands, based on antimicrobial resistance, 2% to 5% of patients hospitalized for community-acquired fUTI cannot be treated with oral antibiotics,[9] implying the need of prolonged intravenous antibiotic therapy and extended hospitalization.[10–12]

Fosfomycin is a phosphoenolpyruvate analogue, which is orally available as fosfomycin-trometamol. It has been used extensively for uncomplicated cystitis in women, has a good safety profile,[13] and has high *in vitro* activity against *E.coli*.[14] Despite the increased use of fosfomycin, persisting low resistance rates are observed against fosfomycin.[14] In retrospective studies fosfomycin appeared effective as stepdown treatment for fUTI.[15,16] The objective of this randomised controlled trial was to determine if fosfomycin is non-inferior to ciprofloxacin for the oral step-down treatment of fUTI caused by *E.coli* in women.

METHODS

Study design

A randomised controlled double-blind, double dummy, multicentre, investigator-initiated trial was conducted to assess whether oral fosfomycin is non-inferior to oral ciprofloxacin for achieving clinical cure in the step-down treatment of *E.coli* fUTI in women. The manuscript was written according to the CONSORT checklist.[17] The protocol was published,[18] and the trial was registered at www.trialregister.nl (NL6275). The institutional review board of the University Medical Center Utrecht provided ethical approval.

The study was performed in 15 Dutch hospitals; 4 academic centers and 11 large teaching hospitals. All respective institutional review boards approved the study. The conduction complied with the Helsinki

principles and the International Conference on Harmonization-Good Clinical Practice guidelines. All patients were automatically insured by a subject and liability insurance. The study was monitored by qualified monitors from the University Medical Centre Utrecht.

Participants

Eligible patients were competent women ≥18 years of age, who were hospitalized with a diagnosis of fUTI, with at least one urinary tract symptom and forthcoming systemic symptoms or signs, and evidence of *E. coli* as the causative pathogen. If blood and urine culture both revealed *E.coli,* local symptoms were not required. To be eligible, patients should have been treated with appropriate empirical intravenous antibiotics for 2 to 5 days, consisting of 2nd or 3rd generation cephalosporin, amoxicillin+/-clavulanic acid, an aminoglycoside, carbapenem, fluoroquinolones, trimethoprim-sulfamethoxazole or a combination of these with *in vitro* susceptibility of the causative *E.coli*, according to European Committee on Antimicrobial Susceptibility (EUCAST) criteria, to at least one of the used agents.[19] Patients were judged to be eligible for an intravenous-oral switch by the attending physician, according to the Dutch guideline, that recommends to switch therapy if the patient has been afebrile for 24-48 hours.[20] Urine ($\geq 10^4$ CFU/ml) and/or blood culture had to reveal *E.coli*, susceptible to both ciprofloxacin (Minimal Inhibitory Concentration, MIC ≤ 0.25 mg/L) and fosfomycin (MIC ≤ 32 mg/L) according to EUCAST criteria, as measured with automated panel tests (PHOENIX© or VITEK©), disc diffusion or Etest.[21] A patient was not eligible if non-*E.coli* Enterobacterales were present in urine culture ($\geq 10^3$ CFU/ml) or blood culture. Other exclusion criteria are listed in the protocol as online supplementary material S1.

Randomisation and masking

Constrained randomization was performed by the data manager of the UMCU with permuted blocks that contained a pre-specified number of treatment assignments in a random order. Randomization was stratified per hospital, so that each hospital contained a blinded allocation list, as empirical antimicrobial treatment for fUTI differed between hospitals. Patients, physicians, local pharmacists and investigators were blinded for treatment allocation. In case of emergency, the pharmacist in the UMCU could be reached 24/7, who had access to the randomization code.

Procedures

Eligible patients were recruited and enrolled by authorized study staff. Patients were randomly assigned (1:1) to an intravenous-oral antibiotic switch to fosfomycin-trometamol every 24 hours as powder for solution, equivalent to 3 gr fosfomycin, or ciprofloxacin 0.5 gr every 12 hours as capsules. Patients received an identical placebo for both active substances to ensure blinding (double-dummy). The duration of antimicrobial treatment was set at 10 days, consisting of 2 to 5 days empirical intravenous treatment and 5 to 8 days of the assigned oral study treatment.

Patients were asked to register the intake of study medication and the occurrence of adverse events. A physical appointment was planned 6 to 10 days after finishing study treatment to assess early endpoints and a telephone appointment at 30 to 35 days to assess late endpoints. At the first meeting a urine sample was handed in for culture. At inclusion and during both follow-up meetings, structured questionnaires were obtained regarding urinary tract and systemic symptoms, antimicrobial use, health status and healthcare consumption.

Outcomes

The primary endpoint was clinical cure at day 6-10 post-end-of-treatment. Secondary endpoints included microbiological cure at day 6-10 post-end-of-treatment, clinical cure at day 30-35 post-end-of-treatment and adverse events. Clinical cure was defined as being alive with reduction of all initial local and systemic fUTI related symptoms, without the requirement of additional antibiotic therapy for UTI (except for antibiotic prophylaxis). In case of an indwelling catheter, local symptoms were not counted. According to the definition of clinical cure, patients that did not meet the criteria for early clinical cure could do so for late clinical cure and vice versa. Microbiological cure was defined as a negative urine culture for *E. coli* ($<10^3$ CFU/ml) with an identical antibiotic resistance profile as the initially cultured *E. coli*. Microbiological cure was only established in patients that did not use additional antibiotic treatment. Definitions and criteria of all secondary endpoints are specified in the protocol (Supplemental material S1).

Statistical analysis

The planned sample size of 240 subjects, including 10% loss to follow up, was based on an assumed cure rate of 92.5% and using a non-inferiority margin of 10% difference in clinical cure, with a power of 80% and a two-sided 95% confidence interval. Analysis of all endpoints was performed according to the intention-to-treat principle. The intention-to treat population contained patients that received at least one dose of the oral study drug. A per-protocol analysis was planned for the primary endpoint and the

secondary endpoint 'microbiological cure' for patients that completed at least 80% of study medication. Risk differences between study arms ($p<0.05$) were calculated with a two-sided Z-score for proportions. A Mann-Whitney U test was used to compare means. Two interim analyses were performed by the Data and Safety Monitoring Board (DSMB) to assess the safety of the study after inclusion of 50 patients and to assess the safety and futility of the study after inclusion of 97 patients.

Role of the funding source

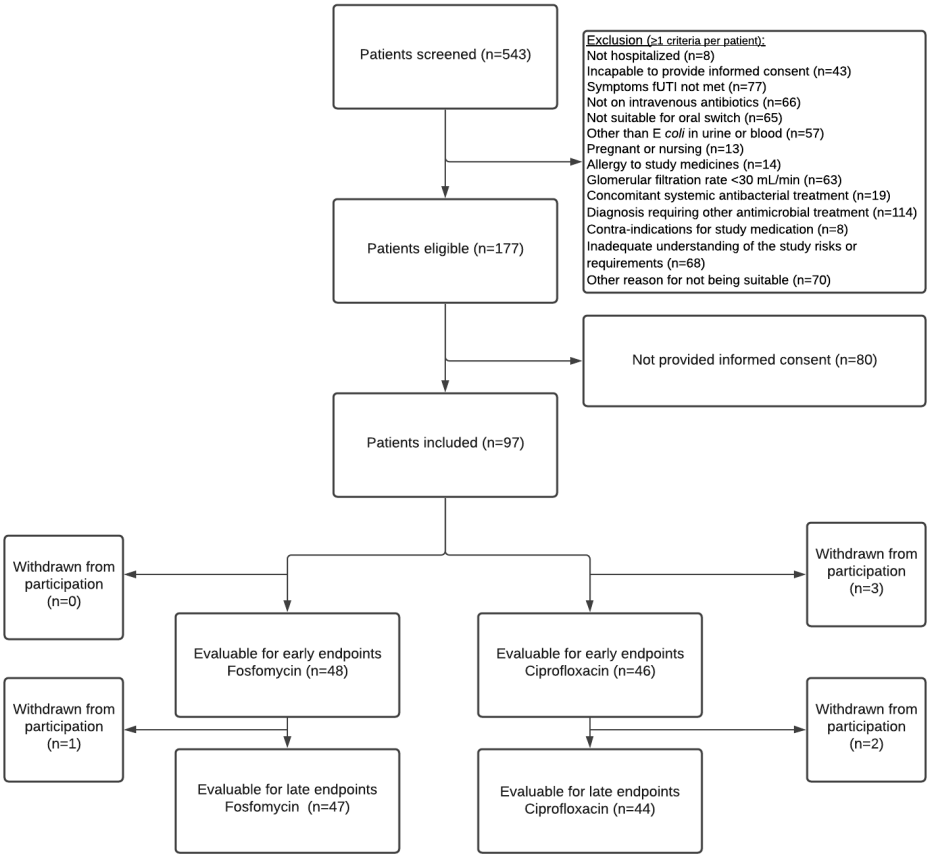
There was no external funding source for this study.

RESULTS

The trial was halted on July 1st 2020 as a consequence of low enrolment during the covid-19 pandemic, and discontinued on October 26th 2020 because of lack of resources for achieving the pursued enrolment number during the ongoing pandemic. Based on the results of the DSMB interim analyses on October 13th with 97 randomised patients, there was no reason to stop the study prematurely for safety reasons or futility.

Between Nov 11, 2017 and June 24, 2020, 543 patients were screened for participation of which 177 were eligible and 97 provided informed consent. Of these, 48 patients were assigned to fosfomycin and 49 to ciprofloxacin(Figure 1). Of these, three were not evaluable for early endpoints as they were withdrawn from the study directly on the day of initiation, because of a renal abscess that required intravenous antibiotic therapy (n=1), failure to perform study procedures in a nursing home (n=1) and withdrawal of consent (n=1). Three patients were not evaluable for late endpoints, due to loss to follow-up. Yet, safety of these 6 patients could be assessed and – at discontinuation of the study – all were alive without hospital readmissions.

Figure 1. Trial profile



At admission, the mean age of enrolled patients was 59.4 years (sd 20.2), the mean Charlson comorbidity index (CCI) was 7.3 (sd 4.6), 9 patients (9.3%) had treatment restrictions and 50 (51.6%) had *E. coli* bacteraemia (Table 1). Of note, patients that declined participation (n= 80) had a mean age of 60.3 years (sd 22.2), a mean CCI of 5.9 (sd 5.5), treatment restrictions in 2 (out of 58 with non-missing data, 3.4%) and 30 (37.5%) had *E. coli* bacteraemia. Empirical antimicrobial treatment was given for a mean duration of 3.3 days (sd 1.1), leaving a mean of 6.7 days (sd 1.1) of oral study medication. Empirical antimicrobial treatment consisted of a 2nd generation cephalosporin (n= 35), a 3rd generation cephalosporin (n=33), a 2nd generation cephalosporin with an aminoglycoside (n=15), a carbapenem (n=2) or another regimen

(n=12). At the time of randomization, the presumptive diagnosis according to the treating physician was urosepsis in 48 patients (49.5%), acute pyelonephritis in 35 (36.1%) and unspecified fUTI in 14 (14.4%). In twenty-seven (27.8%) patients an indwelling catheter was placed at some point during admission. At the moment oral study medication started, patients were afebrile for a median of 2 days (IQR: 1-3). The causative *E. coli* isolate was resistant against amoxicillin-clavulanic acid in 28/97 patients (28.9%), against sulfamethoxazole-trimethoprim in 21/97 patients (21.6%) and was ESBL-producing in 6/97 patients (6.2%).

Table 1. Characteristics of enrolled patients

		Fosfomycin (n=48)	Ciprofloxacin (n=49)
General characteristics			
Age (mean, sd)		58.9 (18.8)	59.9 (21.7)
Charlson comorbidity index (age adjusted), mean (sd)		7.4 (4.7)	7.2 (4.5)
History with diabetes mellitus (%)		17 (35.4)	7 (14.6)
History with anatomic abnormalities of the urine tract (%)		1 (2.1)	1 (2.1)
History with nephrolithiasis (%)		2 (4.2)	6 (12.5)
Characteristics at admission			
Days of urinary tract infection symptoms/signs (median, IQR)		3.0 [1.0 to 5.3]	3.00 [1.0 to 5.0]
UTI symptoms/signs*	Fever (%)	33 (68.8)	40 (81.6)
	Rigors (%)	39 (81.3)	32 (65.3)
	Confusion (%)	16 (33.3)	18 (37.5)
	Hallucinations	9 (18.7)	7 (14.2)
	Flank pain	26 (53.0)	32 (66.7)
Vital signs**	Temperature, mean (sd)	39.0 (1.0)	39.1 (1.0)
	Pulse, mean (sd)	105.7 (17.1)	104.7 (19.1)
	Blood pressure, mean (sd)	79.4 (15.3)	82.0 (15.8)
Hemodynamic instability requiring intravenous fluids***		13 (27.7)	13 (27.7)
Laboratory values**	C-reactive protein in mg/L, mean (sd)	167.7 (137.4)	169.2 (111.8)
	White blood count 10^9/mL, mean (sd)	14.2 (6.7)	13.8 (5.3)
	eGFR (ml/mL), mean (sd)	83.2 (29.0)	77.5 (35.2)
	Leucocyte esterase in urine (>25 µl) (%)	46 (97.9)	43 (91.5)
Blood culture positive for *E coli* (%)		25 (52.1)	25 (51.0)
Urine culture positive for *E* coli (%)		44 (91.7)	47 (95.9)
Hospital department	Urology (%)	10 (20.8)	14 (28.6)
	Internal medicine (%)	34 (70.8)	32 (65.3)
	Other (%)	4 (8.3)	3 (6.1)

Characteristics of empirical treatment			
Antibiotic class	2nd generation cephalosporin (%)	18 (37.5)	17 (34.7)
	3rd generation cephalosporin (%)	16 (33.3)	17 (34.7)
	2nd generation cephalosporin with aminoglycoside (%)	6 (12.5)	9 (18.4)
	Carbapenem (%)	1 (2.1)	1 (2.0)
	Other (%)	6 (12.5)	5 (10.2)
Hours from presentation till antibiotic injection, mean (sd)		3.0 (4.8)	2.8 (4.4)
Days of intravenous therapy, mean (sd)		3.4 (1.1)	3.2 (1.1)
Characteristics at randomization			
Presumptive diagnosis@	Urosepsis (%)	24 (50.0)	24 (49.0)
	Acute Pyelonephritis (%)	18 (37.5)	17 (34.7)
	Unspecified (%)	6 (12.5)	8 (16.3)
Treatment restrictions		5 (10.4)	4 (8.2)
Intensive care requirement (%) &		2 (4.2)	0
Indwelling catheter (%) &		12 (25.0)	15 (30.6)
Vital signs&&	Temperature, mean (sd)	37.2 (0.6)	37.0 (0.5)
	Pulse, mean (sd)	79.1 (12.3)	78.1 (14.2)
	Blood pressure, mean (sd)	94.6 (12.2)	98.0 (15.4)
Laboratory values&&	C-reactive protein in mg/L, mean (sd)	121.7 (86.0)	118.3 (66.0)
	White blood count 10^9/mL, mean (sd)	11.1 (4.8)	10.4 (5.9)
	Creatinine in µmol/L, mean (sd)	95.6 (22.9)	90.9 (27.2)

*reported by the patient
**measured at admission
***within 24 hours before or after admission
@ as decided by the attending physician
& on any moment during admission
&& if measured within 24 before or after randomization

Sixty-six patients (70.2%) met the criteria for clinical cure; 36/48 (75.0%) assigned to fosfomycin and 30/46 (65.2%) patients assigned to ciprofloxacin, yielding an absolute risk difference for clinical cure of 9.6% (95% confidence interval -8.8% to 28.0%; point estimate in favour of fosfomycin). The lower bound of -8.8% is within the predefined non-inferiority margin of 10% (Figure 2). In the per-protocol analysis, 74/81 (79.0%) met the criteria for clinical cure, 28/38 (73.7%) in the ciprofloxacin arm and 36/43 (83.7%) in the fosfomycin arm, resulting in an absolute risk difference of 10.2% (95 % confidence interval -8.0 to 28.4). In a post-hoc analysis of patients with *E. coli* bacteraemia, early clinical cure was found in 18/25 (72.0%) patients assigned to fosfomycin and 15/22 (68.2%) patients assigned to ciprofloxacin (RD: 3.9%, 95% CI: -22.2 to 30.0).

Follow-up urine cultures could be obtained from 77 patients, of which 67 (87.0%) met the criteria for microbiological cure; 29/37 (78.4%) assigned to fosfomycin and 33/35 (94.3%) assigned to ciprofloxacin (RD: -16.2%, 95%CI -32.7% to 0.0%) (Table 2). In one patient, assigned to ciprofloxacin, a follow-up urine culture yielded *E. coli* resistant to ciprofloxacin but with the same susceptibility profile as the initial *E. coli* isolate. Isolates detected are listed in online supplementary material Table S2. Other secondary endpoints listed in Table 2.

Sixty-seven of 94 (71.3%) patients reported one or more adverse event; 35 of 48 patients (72.9%) assigned to fosfomycin and 32 of 46 (69.5%) assigned to ciprofloxacin (RD: 3.3%, 95%CI -15.0% to 21.6%). Probably-related adverse events occurred in 25 of 48 (52.1%) patients assigned to fosfomycin and 20 of 46 (43.5%) assigned to ciprofloxacin (RD: 8.3%, 95%CI -11.6% to 28.1%). The nature, relatedness, duration and severity of adverse events are reported in Table 3. Most notably, gastro-intestinal AEs were reported by 25 of 48 (52.1%) patients assigned to fosfomycin and 14 of 46 (30.4%) assigned to ciprofloxacin (RD: 20.8%, 95%CI 1.6% to 40.0%). Seven patients discontinued study medication prematurely as a consequence of adverse events; 3 of 48 (6.3%) assigned to fosfomycin and 4 of 46 (8.7%) assigned to ciprofloxacin (RD: -2.8%, 95%CI -15.1% to 9.5%).

Figure 2. Non-inferiority margin for the risk difference on early clinical cure between fosfomycin and ciprofloxacin. In the blue area the risk difference is in favour of fosfomycin. In the yellow area the risk difference is in favour of ciprofloxacin with a margin up to 10%. In the red area the risk difference is in favour of ciprofloxacin with a margin beyond 10%. The 95% confidence interval remains within the blue and yellow area, indicating that fosfomycin is non-inferior to ciprofloxacin with a margin of 10%.

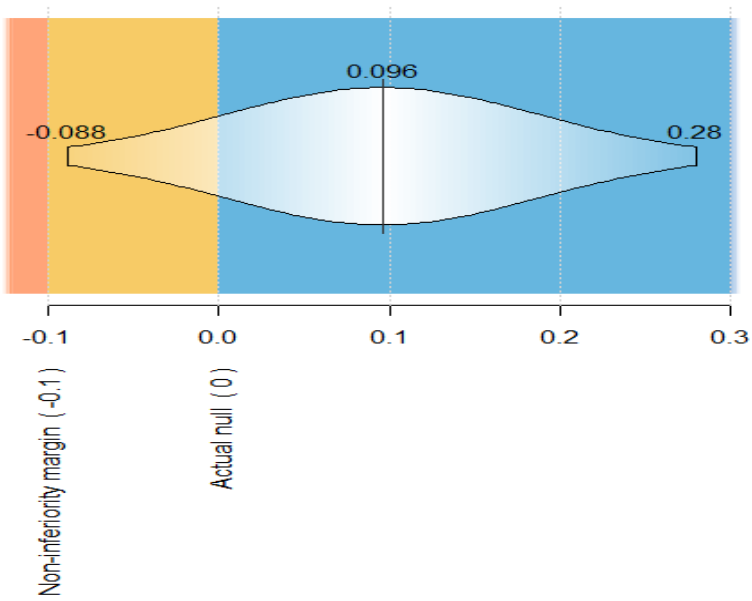


Table 2. Secondary endpoints

	Fosfomycin n=48 (%)	Ciprofloxacin n=49 (%)	Risk Difference (95%CI / p-value)
	6-10 days post-end of therapy		
Microbiological cure	29/37 (78.4)	33/35 (94.3)	-16.2% (-32.7% to -0.0%)
	30-35 days post-end of therapy		
Clinical cure	35/47 (74.5)	33/44 (75.0)	0.4% (-18.4% to 17.6%)
Reinfection	4/47 (8.5)	7/44 (15.9)	-7.8% (-22.3% to 6.6%)
Relapse	2/47 (4.3)	0/44 -	5.2% (-4.0% to 14.3%)
Additional antibiotic therapy for presumed UTI	6/47 (12.8)	7/44 (15.9)	-3.4% (-18.6% to 11.9%)
Length of hospital stay, mean (SD)	4.4 (1.2)	5.4 (2.5)	P= 0.91**
Hospital readmission (any cause)	3/48 (6.3)	1/49 (2.0)	5.0% (-5.3% to 15.2%)
Absenteeism days*, mean (SD)	3.0 (6.7)	2.5 (7.0)	P=0.55**
Mortality (any cause)	2/48 (4.2)	0/49	5.4% (-3.3% to 14.0%)
Mortality (probably related)	0/48	0/49	NA

*from paid or voluntary work
** calculated with a Mann-Whitney-U test

There were eight serious adverse events reported, of which 6 in patients assigned to fosfomycin and 2 in patients assigned to ciprofloxacin. Of these, 4 were considered to be probably related to study medication, 3 after use of fosfomycin and 1 after use of ciprofloxacin. One patient felt shortness of breath immediately after taking fosfomycin. She injected herself with an epinephrine auto-injector to counteract an anaphylactic reaction, which she carried for presumed allergy to penicillin. She did not seek medical help and the complaints improved gradually. The next day, she contacted the investigator, after which she was prescribed trimethoprim-sulfamethoxazole. Signs of anaphylaxis were not observed, and the treating physician considered a panic attack more likely. Another patient developed fever on day 5 after start of fosfomycin with diarrhoea and declined consciousness. She was hospitalized with presumed relapse pyelonephritis and received intravenous amoxicillin targeted to the *E. coli* isolate previously detected, after which she recovered fully. The third patient developed fever (40 °C), chills and debilitation after initial recovery with oral fosfomycin. CRP was 50 mg/L (which had declined from 220 mg/L during prior admission). Study medication was stopped and, without deblinding, ciprofloxacin was started by the physician on duty. Within one day hereafter the patient recovered without sequelae. One patient

developed fever on day 5 of oral therapy with ciprofloxacin. She was diagnosed with hydronephrosis. Intravenous antibiotics were started, a double J catheter was placed, and she required prolonged hospitalization. The hydronephrosis appeared to result from disseminated malignancy. Two patients assigned to fosfomycin died during the follow-up period, which were considered a consequences of underlying diseases and not related to (failure of) study medication. A more detailed description of all serious adverse events is provided in the online supplementary material S2.

Table 3. List of adverse events

		Fosfomycin (n=48)	Ciprofloxacin (n=46)
Total number of adverse events		83	79
Mild symptoms* (score 1-5)		33	35
Severe symptoms* (score 6-10)		27	20
Duration in days, median (IQR)		3 (1 to 6)	2 (1 to 4)
Related		44	39
Gastro-intestinal		42	19
	Diarrhea	22	4
	Nausea	9	6
	Abdominal cramping	7	2
Skin		1	5
Increased vaginal discharge		1	4
Neurological/mental		11	8
Thoracic		0	2
Other		13	20
	Change of smell or taste	0	5
Patients without adverse events		13 (27.1%)	12 (30.4%)

*Severity is scored by the patient on a scale of 1 to 10 (not to be confused with a serious adverse event)

DISCUSSION

In this randomised controlled double-blind trial oral step-down treatment with fosfomycin after initial intravenous antibiotic treatment in women with *E.coli* fUTI was non-inferior to ciprofloxacin in achieving clinical cure 6-10 days after the end of treatment. The risk difference for clinical cure was 9.6% in favour of fosfomycin with a lower 95% confidence interval boundary of -8.8%, which was within the predefined 10% non-inferiority margin. These results indicate that fosfomycin can be used for the stepdown treatment of *E. coli* fUTI in women, reducing the need of prolonged intravenous antibiotic regimens and hospitalisation for patients with an *E. coli* resistant to other oral antibiotic options.[10–12]

We have chosen a stringent definition for our primary endpoint, i.e. the reduction of initial urinary tract and systemic symptoms without additional systemic antibiotic therapy for UTI, reflecting the clinical goal of stepdown treatment. The proportion of patients that met this definition was 70%, which was considerably lower than assumed (92.5%) in the sample size calculation. Yet, this high clinical cure rate was based on trials that evaluated empiric intravenous instead of oral stepdown treatment in less ill patients, as bacteremia in these studies was demonstrated in 7.7% to 27% of the patients [7,22,23], compared to 51% in the current study. Moreover, the endpoint 'recurrences requiring additional antibiotic treatment', as used in these studies, occurs less frequently than a primary endpoint that also includes clinical criteria. In fact, the secondary endpoint of our study 'no additional antibiotic therapy for presumed UTI' at days 30-35 after end-of-treatment was met in around 85% of the patients. For this secondary endpoint the risk difference between fosfomycin and ciprofloxacin was 3.4% (95% CI: -11.9 to 18.6%).

Microbiological cure 6-10 days after the end of treatment was lower for patients assigned to fosfomycin. Although only two of ten patients with microbiological failure, both assigned to fosfomycin, had symptoms requiring antimicrobial treatment. Possibly, the higher microbiological failure may have been related to a higher proportion of patients with diabetes mellitus assigned to fosfomycin. Diabetes mellitus is associated with a two to threefold higher prevalence of asymptomatic bacteriuria. Four out of ten patients with microbiological failure had diabetes mellitus, all of them assigned to fosfomycin.[24]

Fosfomycin was more frequently associated with gastro-intestinal adverse events, most notably diarrhoea. Yet, this did not result in more frequent discontinuation of fosfomycin (RD -2.8%, 95%CI -15.1% to 9.5%). In the current study fosfomycin was dosed every 24 hours. In a previous study, healthy subjects less frequently experienced diarrhoea when fosfomycin (3 gr) was dosed every 48 h instead of every 24 h, while the mean 24 h urine fosfomycin concentration and urinary antibacterial activity was comparable in the two populations.[25] It is unclear how the levels of fosfomycin in the blood compare between these two regimens. Therefore, it remains to be determined if fosfomycin every 48 hours is also efficacious for this indication.[6]

Strengths of this study are the double-blind design with the use of a double dummy placebo, which diminishes the risk of information bias. Second, the high percentage of patients with bacteraemia illustrates that patients were seriously ill with the evident need for intravenous and oral stepdown antimicrobial treatment. Third, adverse events were queried with a diary, which provided a complete picture of the safety and tolerability of multi-dose fosfomycin. Last, the research was conducted in hospitals of various sizes, both academic and regional hospitals and the variety of patients and empirical antibiotic regimens was large, which benefits the generalizability.

This study also has some limitations. First, the study was terminated early as a consequence of the covid-19 pandemic and lack of resources. Yet, there was sufficient data to meet the non-inferiority margin for the primary endpoint analysis. A larger study population, though, would have provided more precision and more power for secondary endpoints. Second, the current study was performed in settings with low levels of antibiotic resistance. Eligibility was conditional on susceptibility to both fosfomycin and ciprofloxacin, and among these the prevalence of ESBL-production was 6.2%. Yet, although resistance to other antibiotics may be more prevalent in many other countries, we consider our findings – non-inferiority of fosfomycin to ciprofloxacin as oral step-down treatment – valid in such settings for fosfomycin susceptible isolates. Last, for feasibility and safety reasons, we used a total treatment duration of 10 days for all patients, even though 7 days of ciprofloxacin has been demonstrated to be sufficient for treatment of acute pyelonephritis.[22,23,26,27]

Implementation of fosfomycin use for step-down treatment requires reliable susceptibility testing. The minimal inhibitory concentration (MIC) of *E.coli* to fosfomycin, as measured with automated panel tests, may correlate poorly with clinical and microbiological efficacy of fosfomycin for empirical treatment of cystitis.[28] High-level resistant (HLR) subpopulations within *E.coli* colonies, that are not represented in the MIC, may not be killed by fosfomycin, and these subpopulations could be identified by measuring the Mutant Prevention Concentration (MPC).[29] Improvements in routine fosfomycin susceptibility testing possibly affect the domain for the targeted use of fosfomycin, although theoretically it would lead to an improvement in fosfomycin efficacy.

In conclusion, this trial demonstrates that fosfomycin 3gr every 24 hours as a targeted step-down treatment for *E. coli* fUTI in women is non-inferior to ciprofloxacin with regard to clinical cure. Fosfomycin is an additional oral antibiotic option for this indication, especially in case of resistance, intolerance or allergies to the existing options. In patients with cardiovascular risk factors fosfomycin may be preferable over ciprofloxacin, given the association between fluoroquinolone use and the development of aneurysms or heart valve lesions.[30,31]

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CHAPTER 11

Persistence of rectal carriage with highly resistant Enterobacterales in the hospital

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ABSTRACT

Background: Highly resistant Enterobacterales (HRE) possess ESBL (ESBL-E), are carbapenem-resistant and/or are resistant to both fluoroquinolones and aminoglycosides. To prevent HRE transmission in Dutch hospitals, inpatient infection control measures are taken with obtainment of surveillance cultures in previously HRE colonized patients. We used these rectal surveillance cultures to estimate the duration of HRE carriage.

Methods: Data was collected from two Dutch hospitals from 01-01-2012 to 06-06-2018. Patients were included if a (first) HRE was isolated from any specimen with at least one rectal surveillance culture in the subsequent year. Survival analysis was used to calculate the moment of HRE decolonization, taking the sensitivity of the culture and censoring into account.

Results: In total 1,484 patients were included, with a median of 2 (IQR: 1 to 5) rectal surveillance cultures in the subsequent year. The median duration of HRE colonization was 175 days overall (IQR: 21 to >365), 140 days (IQR 14 to 364) in the University Medical Center Utrecht and 364 days (IQR: 63 to >365) in the Diakonessenhuis. This difference in duration was attributed to long survival of ESBL-E in the Diakonessenhuis (>365 days, IQR: 72 to >365) compared to the UMCU (138 days, IQR: 20 to 365). HRE carriage persists for 5 years in around 20% of patients.

Conclusion: The long median duration of HRE carriage in patients underlines the importance of obtaining inpatient rectal surveillance cultures after colonization with HRE. Given the high variation between patients, infection control measures could probably be allocated more efficiently.

INTRODUCTION

The emergence of Highly Resistant Enterobacterales (HRE) hampers successful antimicrobial treatment of Gram negative infections.[1–3] In the Netherlands, HRE are defined as ESBL-producing (ESBL-E), Carbapenem-Resistant (CRE) and/or Multi-Drug Resistant (MDRE) if resistant to both fluoroquinolones and aminoglycosides.[4] In 2019 in the Netherlands, 8% of all inpatient *Escherichia coli* and 10% of *Klebsiella pneumonia* isolates were HRE.[27]

Infection control measures (ICM) are used to reduce the risk of in-hospital transmission in Dutch hospitals. ICM pose a burden for both patients and healthcare workers, and require resources in terms of money, materials and beds for contact precautions. An important aspect of these measures is the identification – at the time of admission - of patients with documented HRE colonization in the prior year, as this is considered the best predictor for current HRE carrier status.[4–9] According to the Dutch policy, hospitalized patients are treated with contact precautions if they had a culture yielding HRE in the last year, although no evidence underlies this period of one year.[14] Better understanding of the duration of HRE carriage may assist in finding a better balance between benefits and "costs" to improve the ICM policy for HRE. Besides, it could help clinicians in deciding the empiric treatment of Gram-negative infections.

Most Dutch hospitals have a surveillance program based on regularly obtained rectal cultures following documented HRE colonization.[10] We have used these rectal surveillance culture results from two Dutch hospitals to estimate the duration of HRE colonization, and to explore determinants for prolonged carriage.

METHODS

Data collection

Microbiological data was retrospectively collected from two hospitals situated in the city of Utrecht, the Netherlands, the University Medical Centre Utrecht (UMCU), an university hospital with 1,042 beds and the Diakonessenhuis Utrecht, a teaching hospital with 536 beds. Data was obtained from the period of 01-01-2012 to 06-06-2018.

Surveillance programs

The surveillance programs for HRE were slightly different between both hospitals. In patients in the UMCU, rectal surveillance cultures were obtained at each hospital admission during at least one year after documented HRE carriage. If ESBL-E and MDRE were not detected during this year, ICM and surveillance were discontinued automatically. For patients carrying CRE, discontinuation of ICM required at least one

additional negative rectal culture result beyond one year after the last culture yielding CRE. To cease contact precautions after finding HRE in patients from the Diakonessenhuis, three negative rectal surveillance cultures were required with a minimum interval of 24 hours between each culture, of which at least one culture should have been obtained beyond one year of the last found HRE in that patient. Under the authority of the medical microbiologist, less than 3 negative cultures could suffice to cease someone from ICM, with at least one obtained beyond a year after the last found HRE. In both hospitals, surveillance cultures were not obtained during antimicrobial treatment, as this could reduce sensitivity of identifying HRE. Rectal surveillance cultures were supplemented with surveillance cultures from wounds, (catheter) urine or other lesion or insertions if HRE had previously been detected in these specimens and if available at that moment. The surveillance programs did not change during the study period.

Microbiological procedures

Laboratory methods to detect HRE in both laboratories are described in detail in online supplementary material S1. For clinical specimen, antimicrobial susceptibility testing was performed using automated platforms for susceptibility testing (UMCU: PHOENIX®, BD, Franklin Lakes, USA; Diakonessenhuis: VITEK® BioMérieux, Durham, USA). Disc diffusion was used to detect ESBL-E in both hospitals, although the panel of antibiotics tested with disc diffusion was slightly different between both hospitals. In both hospitals, the presence of CRE was identified by (intermediate) resistance to meropenem or imipenem, and the resistance was confirmed with a meropenem and imipenem E-test, respectively. The production of carbapenemase was confirmed both with the Carbapenem Inactivation Method (CIM) as with a PCR for CRE, respectively. In the UMCU, MDRE was determined on the presence of Enterobacterales with (intermediate) resistance to one of the following fluoroquinolones: ciprofloxacin, norfloxacin, levofloxacin, ofloxacin or moxifloxacin, together with resistance to one of the following aminoglycosides: gentamicin, tobramycin or amikacin. In the Diakonessenhuis, MDRE was determined on the presence of Enterobacterales with (intermediate) resistance to ciprofloxacin or norfloxacin, together with resistance to gentamicin or tobramycin.

For surveillance of ESBL-E in rectal cultures, selective media were used; ChromID ESBL-E agar® (BioMérieux, Durham, USA) in the Diakonessenhuis, and Brilliance ESBL-E agar® (Fischer, Landsmeer, The Netherlands) in the UMCU. In both hospitals, detection of CRE from rectal surveillance after prior CRE colonization cultures was performed with a CRE selective agar that contained a modified carbapenem. Detection of MDRE from rectal surveillance cultures after prior MDRE colonization was performed with

selective agars, containing gentamicin and tobramycin in the UMCU and ciprofloxacin and gentamicin in the Diakonessenhuis, followed by the identification of species using MALDI-TOF MS and susceptibility testing with PHOENIX® in the UMCU and VITEK® in the Diakonessenhuis. After previous MDRE colonization, rectal surveillance cultures were also plated on ESBL selective plates after which ESBL-E was confirmed according to the aforementioned procedure.

Eligibility criteria

Eligibility criteria for episodes to be included for analysis were the presence of (1) a first HRE (index HRE) cultured from any clinical or surveillance specimen and (2) at least one follow-up rectal surveillance culture obtained in the subsequent year. From the UMCU, follow up rectal surveillance cultures were not included if obtained for research purposes.

The surveillance program in the Diakonessenhuis allowed the development of a model to estimate the duration of HRE carriage beyond the period of one year, which included follow-up rectal surveillance culture data beyond one year after the last culture yielding HRE.

Statistical analysis

Survival analysis was used to predict the duration of HRE carriage on the basis of follow-up rectal surveillance cultures. Surveillance cultures from other specimens were disregarded for the model, as these were obtained selectively and optionally with a low sensitivity to find HRE. Time varied between index and rectal surveillance cultures in patients and it was impossible to determine the exact duration until clearance upon readmission, resulting in interval-censored data.[24] Maximum likelihood analysis was used to simultaneously calculate the HRE-survival curve and the sensitivity of the surveillance culture, taken censoring into account. The day of obtaining an HRE negative surveillance culture would not be deemed as the day of clearance, as with the Kaplan-Meier method, but rather a day in the interval between the last positive and the first negative culture. Moreover, decolonization could have occurred after the HRE negative surveillance culture if the sensitivity of the model to find HRE within a rectal culture was estimated to be less than one.[25] This resulted in survival plots with a 95% confidence interval. Separate analyses were conducted to estimate the duration of carriage in the following stratified populations; patients from the UMCU, the Diakonessenhuis, patients with HRE index species containing either Escherichia *coli*, Klebsiella *species* or other Enterobacterales (index species), and patients with their first HRE isolated from rectal or from non-rectal specimen (index specimen). Moreover, the duration of ESBL-E, CRE or MDRE carriage was estimated separately, using the same statistical analysis.

RESULTS

Population

HRE carriage was identified in 3,483 patients, of which 2,552 in the UMCU and 931 in the Diakonessenhuis. In 1,484 out of 3,483 patients (43%) at least one rectal surveillance culture was obtained within the first year; 1,083 in the UMCU (42%) and 401 in the Diakonessenhuis (43%). The baseline characteristics of these populations are summarized in Table 1. On the index day, multiple specimen could yield distinct HRE isolates belonging to multiple species, possessing both ESBL-E, CRE and/or MDRE. On the day of the index culture, patients in the UMCU were younger than in the Diakonessenhuis (median 58 vs. 71 years of age), the index HRE less often consisted of *E. coli* (69% vs. 80%) and the index HRE more often produced ESBL (88% vs. 68%), respectively.

The median number of rectal surveillance cultures per patient within the first year was 2 (Inter Quartile Range: 1-5) in the total population, 2 (IQR: 1-5) in the UMCU and 3 (IQR: 1-6) in the Diakonessenhuis. The median number of days from the index HRE to the first rectal surveillance culture was 49 (IQR: 14-142) overall, 43 (IQR: 14-133) in the UMCU and 63 (IQR: 14-165) in the Diakonessenhuis.

For the model that estimated carriage duration beyond the first year in the Diakonessenhuis, 490 patients were included with 2,050 rectum cultures with 3 rectal surveillance cultures per patient at median (IQR: 2-6) and a median number of days from the index HRE to the first rectal surveillance culture of 159 (IQR: 31-489).

Of HRE that were isolated from a rectal surveillance culture, 64.3% (n=3,180/4,944) were not identified from non-rectal cultures in the same period (+-7 days). In contrast, in 13.2% (n=483/3,663) of negative rectum surveillance cultures, HRE was identified from other specimen obtained in the same period (+- 7 days). Of these 483 negative rectum surveillance cultures, HRE was initially isolated from the rectum (or feces) in 146 cases (30%), urine (midstream or catheter) in 198 cases (41%) and other body sites in 139 cases (29%).

Table 1. Baseline characteristics of patients on the day the first HRE was found

Determinants from the day of the index HRE		UMCU		Diakonessenhuis	
		All patients with HRE carriage (n=2,552)	Patients with ≥1 rectal surveillance culture within the first year (n=1,083)	All patients with HRE carriage (n=931)	Patients with ≥1 rectal surveillance culture within the first year (n=401)
Patient characteristics	Age (median, IQR)	59 (36-70)	58 (34-70)	72 (59-83)	71 (60-81)
	Men (%)	1,436 (56)	610 (56)	446 (48)	235 (48)
Species*	Escherichia coli (%)	1,696 (66)	751 (69)	677 (73)	319 (80)
	Klebsiella species (%)	481 (19)	230 (21)	150 (16)	75 (19)
	Other Enterobacterales (%)	460 (18)	249 (23)	133 (14)	60 (15)
Specimen*	Rectal culture (%)	1,147 (45)	604 (56)	317 (34)	203 (51)
	Other (%)	1,471 (58)	624 (58)	643 (69)	251 (63)
Resistant type*	ESBL-E (%)	1,912 (75)	948 (88)	652 (70)	311 (68)
	CRE (%)	46 (2)	27 (2)	9 (1)	8 (2)
	MDRE (%)	1,210 (47)	522 (48)	534 (57)	254 (56)

IQR = interquartile range, ESBL-E = Extended Spectrum Beta-Lactamase producing Enterobacterales, CRE = Carbapenem Resistant Enterobacterales, MDRE = Multi-Drug Resistant Enterobacterales with resistance to both fluoroquinolones and aminoglycosides. *On the day of the index HRE, multiple specimen could be obtained, specimen could carry multiple HRE species, and species could carry ESBL-E, CRE and/or MDRE.

Persistence of HRE carriage

Overall, the median duration of HRE carriage was 175 days (IQR: 21 to >365). After one year, 62% of patients had lost HRE carriage (95% CI: 53%-80%). The median duration of HRE carriage was 140 days (IQR 14 to 364) in patients from the UMCU and 364 days (IQR 63 to >365) in patients from the Diakonessenhuis (Figure 1). Table 2 provides the median duration of HRE carriage and the -by the model- estimated sensitivity of the test for the total population, for the distinct hospitals, index species and index specimens. Online supplementary material 2 provides the survival plots for these populations.

The model that evaluates survival beyond the first year in the Diakonessenhuis estimated a median duration of HRE carriage of 364 days (IQR: 63 to 1407), see Figure 2 for the survival plot.

Table 2. The median duration of HRE carriage and the sensitivity of the test for different populations.

Determinant		Number of patients	Median duration of HRE carriage (Inter Quartile Range)	Sensitivity of the test (95% confidence interval)
Hospital	Overall	1,484	175 (21 to >365)	0.70 (0.68 to 0.72)
	UMCU	1,083	140 (14 to 364)	0.68 (0.65 to 0.70)
	Diakonessenhuis	401	364 (63 to >365)	0.74 (0.71 to 0.78)
	Diakonessenhuis (>365 days)	490	364 (163 to 1407)	0.75 (0.72 to 0.78)
Index species	Escherichia *coli*	1,024	217 (21 to >365)	0.73 (0.71 to 0.75)
	Klebsiella *species*	356	182 (21 to >365)	0.70 (0.66 to 0.73)
	Other**	324	112 (7 to >365)	0.65 (0.60 to 0.69)
Index specimen*	Rectal specimen	1,020	231 (21 to >365)	0.73 (0.71 to 0.75)
	Other specimen	922	175 (14 to >365)	0.66 (0.63 to 0.69)

UMCU = University Medical Center, HRE = Highly resistant Enterobacterales, defined as Enterobacterales that are ESBL-E-producing, carbapenem-resistant and/or multi-drug resistant (MDRE) to both fluoroquinolones and aminoglycosides

* Specimen from which the index HRE was cultured

***Enterobacter species* (44%, 634/1,439), *Proteus species* (25%, 356/1,439), *Citrobacter species (19%, 275/1,439), Morganella morganii* (7%, 96/1,439) and others (5%, 78/1,439)

In the Diakonessenhuis, from 68 HRE index patients a rectal surveillance was obtained beyond a year without HRE being found within the first year in a surveillance or clinical culture. These 68 rectal surveillance cultures were obtained after a median of 556 days (IQR: 423 to 788), and would not have been obtained according to the surveillance program of the UMCU. HRE was found in 14.7% (10/68) of these, which corresponds, when taken into account an estimated sensitivity of 0.75, in an HRE prevalence of 19.6%.

Figure 1. Survival of HRE carriage at hospital admission within one year after finding HRE in the total population, and stratified for the UMCU and the Diakonessenhuis

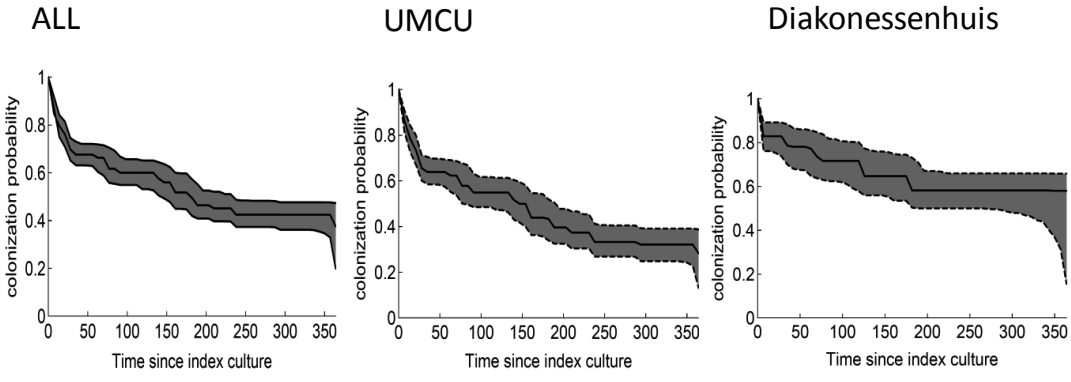
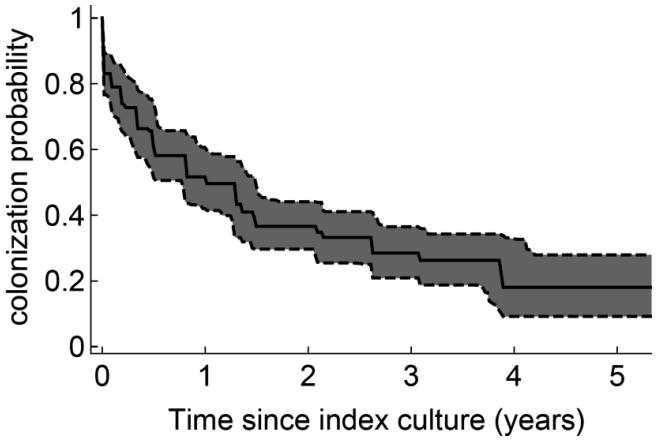


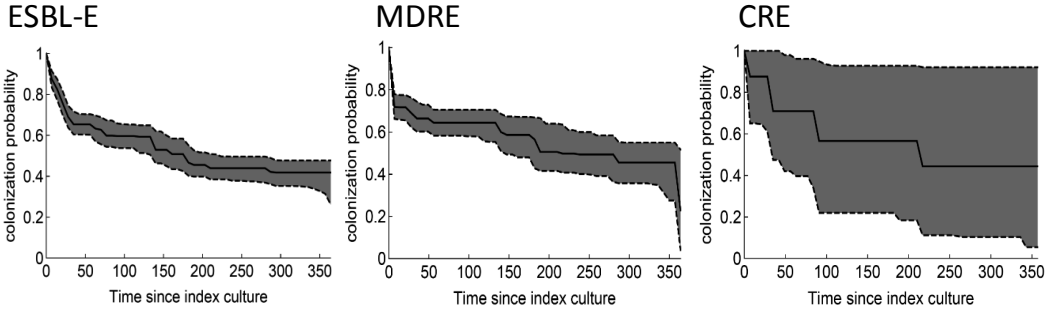
Figure 2. Survival of HRE in the Diakonessenhuis beyond the period of one year



Persistence of ESBL-E, CRE and MDRE carriage.

After one year, 58% (95% CI: 52 to 74) of patients had lost ESBL-E carriage, 56% (95% CI: 18 to 100) of patients had lost CRE carriage, and 77% (95% CI: 48 to 96) of patients had lost MDRE carriage. Table 3 provides the median duration of ESBL-E, CRE or MDRE carriage with the estimated sensitivity, also specified per hospital. Figure 3 represents the survival plots of ESBL-E, CRE and MDRE carriage.

Figure 3. Duration of ESBL-E, CRE or MDRE carriage within one year after its finding in the University Medical Center and the



ESBL-E =Extended Spectrum Beta-Lactamase, MDRE = Multi-Drug Resistant Enterobacterales (MDRE) with resistance to both fluoroquinolones and aminoglycosides, CRE = Carbapenem Resistant Enterobacterales,

Table 3. The number of patients and duration of ESBL, CRE or MDRE carriage in the different populations with the models' estimated test sensitivity.

Determinant	Number of patients	Median duration of carriage (Inter Quartile Range)	Sensitivity of the test (95% confidence interval)	Determinant
ESBL	Overall	1,181	175	0.72
			(21 to >365)	(0.70 to 0.74)
	UMCU	888	138	0.68
			(20 to 365)	(0.66 to 0.70)
	Diakonessenhuis	293	>365	0.79
			(72 to >365)	(0.75 to 0.83)
CRE	Overall	44	210	0.30
			(28 to >365)	(0.18 to 0.43)
	UMCU	37	210	0.70
			(30 to 365)	(0.68 to 0.72)
	Diakonessenhuis	7	>365	0.21
			(>365 to >365)	(0.5 to 0.56)
MDRE	Overall	778	210	0.53
			(7 to >365)	(0.49 to 0.56)
	UMCU	527	358	0.41
			(5 to >365)	(0.37 to 0.46)
	Diakonessenhuis	251	185	0.67
			(24 to 339)	(0.62 to 0.72)

UMCU = University Medical Center, ESBL-E = Extended Spectrum Beta-Lactamase, CRE = Carbapenem Resistant Enterobacterales, MDRE = Multi-Drug Resistant Enterobacterales (MDRE) with resistance to both fluoroquinolones and aminoglycosides

DISCUSSION

The Dutch guideline recommends to take ICM in hospitalized patients that were colonized with HRE in the previous year.[4–6] The results of this study suggest that this period of one year is inaccurate, as 62% of patients lost HRE carriage within one year, while HRE carriage persisted for more than 5 years in another 20% of patients. These patients respectively received -or were ceased- falsely from ICM. Meanwhile, the long median duration of HRE carriage (175 days) underlines the importance of obtaining rectal surveillance cultures after previous HRE colonization, because 64.3% of HRE's isolated from rectal surveillance cultures would not have been identified otherwise, representing an unknown source of transmission.

Our study was not designed to disentangle the mechanism behind persisting HRE carriage. Previous studies found that in-patient and in-household persistence contribute to the duration of ESBL-E carriage after hospital discharge.[12] The risk of in-hospital HRE acquisition under the use of ICM in the Netherlands was found negligible, so this probably does not play a role.[3,13] One year after returning from international travelling only 11% persisted to be a ESBL-E carrier, which was remarkably lower than the estimated 42% of patients in our study. This suggests that patient factors increase the duration of ESBL-E carriage, e.g. illness, comorbidity, residency, health care exposure and antibiotic use.[10]

A remarkable finding was the shorter median duration of ESBL-E carriage found in the UMCU (138 days) than in the Diakonessenhuis (364 days). Unfortunately, our data provide insufficient insight into the reason for this difference. The hospitals differ in many respects, because the UMCU provides tertiary care, while the Diakonessenhuis has a regional function. What could contribute is that a higher percentage of ESBL-E index isolates belonged to other than *E.coli* or Klebsiella *species* in the UMCU (14%) than in the Diakonessenhuis (10%), as these HRE seem to survive shorter. Causes could also be found in differences in baseline ESBL-E prevalence and antimicrobial use. The percentage of first urinary E. *coli* and K. *pneumonia* isolates that possess ESBL-E was higher in the UMCU (6.9% and 11.8%, resp.) than in the Diakonessenhuis (5.4% and 7.2%, resp.).[3,26] And the percentage of study patients that used carbapenem between the identification of HRE and the next rectal culture was much higher in the UMCU than in the Diakonessenhuis (43% vs. 27%), while the percentage of cephalosporin use was higher in the Diakonessenhuis than in the UMCU (70% vs. 40%). Hypothetically, as a result of their antimicrobial spectrum, administration of carbapenem may shorten ESBL-E survival, while cephalosporin's may only select for the survival of ESBL-E. We do not expect that the observed differences result from differences in surveillance programs or microbiological methods between both hospitals. Both microbiological laboratories worked according to EUCAST standards and followed the Netherlands Society for Medical Microbiology guideline.[22,23] Moreover, differences between hospitals in the interval of rectal cultures or the sensitivity of rectal cultures were taken into account in the model.

One other study investigated the duration of HRE carriage, although in patients discharged from European intensive care departments where patients received universal chlorhexidine body-washing and personnel used hand hygiene improvement measures.[8] The median duration of HRE colonization was remarkably lower (66 days) than in our study (175 days), possibly as a consequence of these intensified hygiene measures and the high antibiotic consumption on the intensive care. Results of other studies that estimated the duration of ESBL-E or CRE carriage after hospital discharge correspond better to the results of this study. A large French retrospective study found that ESBL-E colonization lasted for a median of 200 days, compared to 175 days in our study.[16] Three small prospective studies with structural obtainment of rectal surveillance cultures after hospital discharge found an ESBL-E positivity percentage after one year of 50% in the Netherlands (11/22),[12] 43% in Sweden (26/61),[17] and 50% in France (7/14),[18] compared to 42% in this study. Studies found a median duration of CRE carriage of 295 days (Israel) and 270 days (USA), compared to 210 days, with large uncertainty, in this study.[19,20] To our knowledge, no prior studies estimated the duration of MDRE carriage.

This study has limitations, the most important being the non-structural obtainment of rectal surveillance cultures that pended on the local surveillance guidelines. The model took interval censoring into account, although a structured follow-up would have been preferable. Second, because only patients with a follow-up surveillance culture were included, the study is not generalizable to HRE carriers without a subsequent rectal surveillance culture. However, patients without a subsequent surveillance culture are of less interest, as these were not re-admitted to the hospital and were no source of in-hospital transmission. Besides, prospective studies with structural obtainment of rectal cultures after hospital discharge revealed a comparable survival. Third, since surveillance cultures after the period of one year were not included in the models (except for one) the probability of carriage is estimated erroneously lower at the end of follow-up with wide skewed confidence intervals. Fourth, the population carrying CRE was small which resulted in uncertain estimates. Fifth, the relatively low baseline prevalence of HRE in the Netherlands, the cautious use of broad spectrum antibiotics, and the use of inpatient ICM probably influences the survival of HRE carriage. Results of this study may therefore not apply to areas with much higher HRE rates.

In conclusion, the long duration of HRE carriage endorses the importance of obtaining inpatient rectal surveillance cultures in HRE colonized patients. Given the high variation in the duration of HRE carriage between patients, the current policy of using a fixed year of ICM after colonization with HRE is inaccurate. The use of ICM could possibly be allocated more efficiently if HRE carriage can be predicted individually.

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CHAPTER 12

GENERAL DISCUSSION

This thesis addresses aspects in the treatment of urinary tract infection (UTI) and the etiology, diagnosis and prognosis of antibiotic resistance among Gram-negative bacteria. A distinction is made between studies that concern the treatment of cystitis in general practice **(chapters 2 to 4)**, and studies regarding resistant Gram-negative pathogens in the treatment of UTI in the hospital **(chapter 5 to 11)**.

The distinction arises from existing differences in the incidence, complexity and disease burden of UTI between both settings. In the Netherlands, patients with UTI symptoms are initially assessed in general practice and are referred to the hospital if specific care is needed. The complexity and disease burden of UTI managed in primary care is lower than of episodes that require management in the hospital setting. Yet, the incidence of UTI managed in primary care is much higher, especially that of cystitis. The general practice guideline for UTI is more pragmatic with a limited diagnostic and therapeutic arsenal; urine cultures are not routinely obtained in the initial approach of cystitis and pathogen-directed treatments are not standard practice. In contrast, UTI care in the hospital is comprehensive, with multiple diagnostic and therapeutic options. Urine cultures are being obtained routinely, which creates the opportunity to switch from empirical to pathogen-directed antimicrobial treatment.[1] A substantial part of the treatment guideline for UTI in the hospital covers the management of antibiotic resistance among Gram-negative bacteria.[1–4]

Above differences imply that different challenges exist in each setting. To make a valuable contribution for clinical practice these require a modified scientific approach. In the next two paragraphs, these challenges and approaches are further discussed. Moreover, the paragraphs provide a summary of findings from studies described in this thesis, recommendations for clinical practice and research perspectives.

Treatment of cystitis in general practice

Rationale behind studies

Managing cystitis is a daily activity in general practice. Given its high incidence, scientific findings that result in nuanced differences in effectiveness can have important consequences on a population level. The common aim of the observational studies in **chapter 2 to 4** was to address issues in the treatment of cystitis for which insufficient evidence exists and for which RCTs were not available neither expected soon. Interestingly, existing RCTs focused on the comparison between two distinct antimicrobials for cystitis, with inclusion of a homogeneous population without risk factors for a complicated course.[5,6] How these antimicrobials should be used for an optimal efficacy has been underexposed in these RCTs. Hardly any RCT compared multiple uses of the same antimicrobial. The consequence of this is that the optimal

duration and dose of different antimicrobials is largely unknown, resulting in a large variety in duration and dose in international guidelines.[7] Moreover, there is no consensus about the optimal treatment of cystitis in high risk patients such as men and pregnant women as RCT results are not automatically generalizable to these high risk populations.[5,6] Guideline recommendations for these populations are therefore insufficiently substantiated.

The epidemiological approach

In the absence of RCTs, only observational data can be used to estimate treatment effectiveness. The availability of large quantities of patient data makes cystitis an attractive domain for epidemiological studies. Nevertheless, observational data are prone to a high risk of bias and the main challenge in chapter 2 to 4 was to overcome this.

The risk of selection bias was diminished as the Julius GP Network (JGPN) database can be considered as a representative sample of the Dutch general practice population. It contains routinely registered longitudinally gathered data from almost half a million patients that are under care at more than 75 Dutch general practices.[8] GPs that participate to the JGPN database have undergone coding training sessions, and, together with the mandatory registration of ICPC codes and ATC codes, this improved the reliability of the dataset, which diminishes the risk of misclassification.[8] Research demonstrated that Dutch GPs better adhered to guideline recommendations for treating cystitis than their colleagues in other European countries.[9] Other factors improve the statistical inference of epidemiological findings. Treatment of cystitis is not subject to many determinants, as it mainly consists of an antimicrobial prescription, apart from motivating adherence and promoting behaviour to prevent recurrent urinary tract infection (UTI).[3] The large majority of antimicrobials for UTI are prescribed within the general practice itself and there is no such thing as de-escalation therapy for cystitis.[10] This makes it plausible that a second antibiotic prescription in the same patient indicates either clinical failure or intolerance.[3,11,12] A limited arsenal of antimicrobial regimens is recommended for the treatment of cystitis, consisting of nitrofurantoin, fosfomycin and trimethoprim, and no other indications exist for these antimicrobials.[11,13] Therefore, the effectiveness of cystitis treatment could be estimated quite adequately by a second antimicrobial prescription for UTI accompanied by a registration of a cystitis episode. The biggest analytical challenge remained to diminish confounding. In **chapter 2**, confounding by indication complicated the comparison between the effectiveness between nitrofurantoin, fosfomycin and trimethoprim, because the treatment guideline creates a hierarchy in their recommendation, being first, second and third choice, respectively. In an effort to encounter this, we only selected first episodes per patient. The Dutch treatment guideline

does not distinguishes between different options for treatment an prophylaxis of UTI. In **chapter 3**, we compared four times daily 50 mg and twice daily 100 mg slow-release nitrofurantoin for the treatment of cystitis and **in chapter 4** we compared nitrofurantoin 50 mg and 100 mg as daily prophylaxis for UTI.[3,14] This in itself, and the observed equivalence in baseline characteristics between the compared populations, reduced the risk of confounding. In **chapter 3** next to multivariable regression analysis, instrumental variable analysis was used to adjust for residual confounders, with GP practice as instrumental variable.[15,16]

Summary of findings and clinical recommendations

The pathophysiological rationale for the studies in **chapters 2 to 4** is that the effectiveness of cystitis treatment (or prophylaxis) depends on the activity of an antimicrobial against common uropathogens and on the availability that it reaches in urine.[17–19] The urine availability is subject to the dosage schedule and on patient characteristics that can influence the pharmacokinetic profile of the antimicrobial, such as the renal function or being pregnant.

In **chapter 2**, we found that a decrease in renal function (eGFR) is linearly related to an increased risk on clinical failure when using nitrofurantoin for cystitis, but not when using fosfomycin-trometamol (FT) or trimethoprim, and that, in patients with a eGFR below 60 mL/min, FT was associated with significantly less clinical failures than nitrofurantoin. Noteworthy, any residual confounding by indication would have underestimated the observed effect. In **chapter 3** we observed that, in pregnant women with cystitis, the use of slow-release nitrofurantoin (Furabid®) was associated with less clinical failures than the use of normal release nitrofurantoin (Furadantine®). In **chapter 4** we found that the use of 50 mg nitrofurantoin as continuous daily prophylaxis has an equivalent risk of clinical failure as the use of 100 mg nitrofurantoin, while being accompanied with fewer consultations for cough, dyspnea or nausea. Until unbiased results are obtained with RCTs, the results of these observational studies recommend making the following adjustments to the treatment guideline:

- In patients with eGFR below 60 mL/min, fosfomycin is preferred over nitrofurantoin for treatment of cystitis.
- In pregnant women, slow-release nitrofurantoin is preferred over normal release nitrofurantoin for treatment of cystitis.
- For continuous prophylaxis for UTI with nitrofurantoin, a daily dose of 50 mg is preferred over 100 mg.

Research perspectives

Fosfomycin, nitrofurantoin and trimethoprim originate from more than 50 years ago.[20–23] Although new agents for cystitis are welcome, many research questions remain to be answered to optimize cystitis treatment with use of the existing antimicrobials, e.g. the optimal treatment duration of nitrofurantoin, fosfomycin and trimethoprim, the optimal antimicrobial choice in certain populations, such as men. Ideally these should be answered with RCTs as these provide the highest quality of evidence for treatment questions. But setting up a RCT requires a lot of work, time and money.[24] Funding often depends from scarce public resources,[5] as little commercial interest exists in researching existing treatments nor in the development of new antimicrobials for cystitis. To improve the efficiency and to widen the domain, RCTs investigating cystitis treatment could be designed smarter with simultaneous testing of multiple hypotheses and inclusion of more heterogeneous populations.[25,26] In my experience, in particular the set-up of a RCT is time-consuming and expensive, making an ongoing trial wherein multiple treatment arms can be compared simultaneously more efficient. Likely, the high incidence of cystitis and the streamlined care provide the opportunity to answer multiple questions in a relatively short time. An example of such a trial is the EXFOCY trial that is currently being set-up, which is an acronym for 'EXtended use of FOsfomycin for the treatment of CYstitis in primary care'. The rationale for this trial is the uncertainty about the optimal dose of FT for the treatment of uncomplicated cystitis. This trial corresponds to the content of this thesis. It investigates whether the efficacy of FT can be increased by addition of an extra dose on day three. A single dose of FT in healthy female volunteers results in high inter-individual variability of urinary fosfomycin concentrations, being below the EUCAST breakpoint in two-thirds of participants after 72 hours.[27] A cluster or sequential randomized trial with randomization of treatments to GP practices could also be a suitable design to further optimize cystitis treatment, given the high incidence of cystitis in a large amount of GP practices.

Although no matter how smart RCTs are designed, they cannot provide an answer to all research questions.[24] It is therefore important to facilitate the conduct of observational studies by generating reliable data by means of a registry, such as the Julius GP network. A disadvantage of the used Julius GP Network (JGPN) database is that microbiological results and hospital data are missing in the JGPN database. Although UTI-related hospitalization is rare after antimicrobial treatment for cystitis, it greatly affects patients' health. Linking patient datasets from general practice to laboratory and hospital databases would allow new research possibilities.

Urinary tract infections and antibiotic resistance among Gram-negative bacteria in the hospital

A limited arsenal of antimicrobials exists for the treatment of febrile UTI, compared to for cystitis.[28,29] These antimicrobials should possess *in vitro* activity to the full spectrum of causative uropathogens and should reach sufficient concentrations in both urine as in infected tissue, e.g. kidney, prostate and/or bloodstream.[1,3,13,30] Antimicrobial options are further declined as a result of acquired antimicrobial resistance to cephalosporins, aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole.[1,13] In case of resistance to these antimicrobials, patients with UTI often rely on the use of carbapenems.[1] Fortunately, resistance rates of common uropathogens to carbapenems is less than 0.1% in the Netherlands, but these rates are much higher in other countries.[13,29,31]

A comprehensive approach is required to preserve the current treatment of UTI in the hospital. Table 2 provides an overview of components of such an approach, to which the chapters in this theme contribute. Within this approach, investigating antimicrobial options for the treatment of UTI should go hand in hand with the prevention of acquisition and transmission of resistant Enterobacterales.

Table 2. Components of an approach to preserve the future treatment of UTI in the hospital.

Aim	Actions	Comments/examples
Broaden the arsenal of antimicrobials for UTI	Investigate non-antibiotic options for UTI prophylaxis	• Cranberries[3] • Increase daily water intake[77] • Hormone replacement for postmenopausal women[3] • Vaccine development against uropathogens[78] • Antibody development against uropathogens[79] • Gastrointestinal Decolonization of Multiresistant Bacteria[80]
	Develop new antimicrobials	• For the empirical and stepdown treatment (**chapter 7**) • As prophylaxis
	Expand the spectrum of existing antibiotics	• Fosfomycin for UTI in kidney transplant recipients (**chapter 8**) • Fosfomycin as stepdown treatment for E.*coli* febrile UTI in women (**chapter 9 and 10**) • Intravesical administration of gentamicin[81]
Optimize the current treatment of UTI	Update and coordinate guideline recommendations	• The Dutch guidelines for UTI in general practice and the hospital are regularly updated and closely coordinated[1–4]
	Target antimicrobials to the causative uropathogen	• Direct susceptibility measuring on urine samples[82] • Optimize susceptibility testing (**chapter 6**)

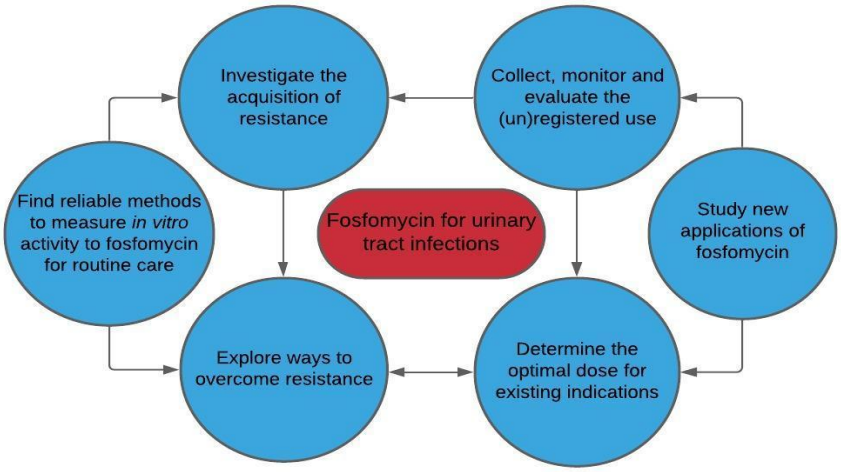
	Optimize the duration and dose of antimicrobial regimens	• Investigate the optimal duration and dose of UTI treatment[83,84]
	Prevent the treatment of asymptomatic bacteriuria	• Prevent unnecessary urine cultures[88,89] • Do not treat asymptomatic bacteriuria, except during pregnancy, before urological surgery or in the first month after kidney transplantation[85]
Regulate the use of antibiotics (antibiotic stewardship)	Surveillance and monitoring of antimicrobial use for UTI	• National[13] and hospital wide antibiotic stewardship
	Regulate the consumption of antimicrobials	• Reduce veterinary antimicrobial use[86] • Prevent the spill of antimicrobials in the environment • Release antibiotics solely on doctor's prescription • Consult an infectious disease specialist or medical microbiologist in the treatment of complex patients (**chapter 8**)
	Avoid unnecessary use of antimicrobials	• Make a list of last-resort antibiotic options[87] • Predict antimicrobial resistance in gram negative infections[76]
Infection control measures	Monitor carriage of highly resistant Enterobacterales*	• Obtain follow-up surveillance cultures in known carriers of highly resistant Enterobacterales **(Chapter 11)**
	Take infection control measures to prevent transmission	• Use contact precautions in case of carriage of highly resistant Enterobacterales[90] • Predict persisting carriage of HRE to individualize the use of infection prevention measures
	Prevent health care related UTI	• Encourage early removal of urinary catheters[91]

Enlarging the antimicrobial arsenal for UTI can be accomplished by the development of new antimicrobials and by widening the spectrum of existing antimicrobials. In **chapter 5** we systematically reviewed RCTs that compared alternatives to carbapenems with *in vitro* activity to ESBL for the treatment of complicated UTI (cUTI). We identified four newly developed antimicrobials for the empirical treatment of cUTI that had a comparable efficacy as carbapenems; these were ceftazidime-avibactam, ceftriaxone-sulbactam with disodium-EDTA (both beta lactamase inhibitors), plazomicine (an aminoglycoside) and cefiderocol (a siderophores). These antimicrobials, except for ceftriaxone-sulbactam disodium-EDTA, also possess *in vitro* activity to CRE. These antimicrobials belong to several antibiotic classes, which diminishes the risk of

cross-resistance.[32–34] Ceftazidime-avibactam was found the only antimicrobial equivalent to carbapenems for the pathogen-directed therapy of cUTI.

Yet, none of these empirical or pathogen-directed antimicrobials, nor carbapenems, can be administered orally. This implies that a substantial proportion of patients depends on intravenous antimicrobials for the treatment of febrile UTI. In the Netherlands, 6% of inpatient *E.coli* and 5% of inpatient *K.pneumoniae* isolates were resistant to all oral administrable antimicrobials (in 2019), i.e. ciprofloxacin, trimethoprim-sulfamethoxazole and amoxicillin-clavulanic acid.[13] Several chapters within this thesis address the use of fosfomycin-trometamol (FT), a potential oral administrable alternative for the treatment of febrile UTI. In recent years, new applications for FT have been investigated, e.g. as prophylaxis for transurethral resection of the prostate, as continuous prophylaxis for UTI, for treatment of cystitis during pregnancy.[35–42] Fosfomycin has become available for intravenous use as Fomicyt® for the last resort treatment of infections caused by multi-resistant bacteria.[43] The efficacy and safety of Fomicyt for the empirical treatment of complicated UTI against piperacillin-tazobactam has been established in a RCT.[44] Though, the efficacy of piperacillin-tazobactam for this indication against carbapenem has been questioned in chapter 5.[44] Fomicyt® is used in much higher concentrations with a distinct treatment spectrum than FT, putting it beyond the scope of this thesis.[45] Before addressing new clinical indications of FT, it is important to consider potential issues and challenges that are accompanied with its use, see Figure 3.

Figure 3. Issues and challenges accompanied with the use of fosfomycin for urinary tract infections



One of these issues has been addressed in the first theme, as the EXFOCY trial evaluates whether increasing the dose leads to better efficacy for cystitis. Second, there are doubts about the reliability of routine susceptibility methods against fosfomycin in Enterobacterales, since a poor agreement was found between *in vitro* and *in vivo* activity of FT for the treatment of uncomplicated cystitis.[46] For the pathogen directed treatment of UTI it is of vital importance to reliably determine the susceptibility to an antimicrobial. Several methods are being used to measure susceptibility to fosfomycin in routine care, i.e. Etest, MIC test strip, Vitek2, Phoenix and disc diffusion. The reference standard, agar dilution (AD) is too time consuming and technically complex for routine testing.[47] In **chapter 6** we found a low agreement between fosfomycin Etest results that were performed in two distinct laboratories and/or interpreted by different observers. Moreover, agreement to AD was low. Another study concluded that none of the routine methods in clinical practice had sufficient agreement to AD to be used for routine testing.[48] In both studies, a higher agreement was observed for *E. coli* compared to other Enterobacterales. Although, according to a RCT on the use of fosfomycin for *E.coli* uncomplicated cystitis, the correlation of the *in vitro* activity of fosfomycin (based on measurement of the Minimum Inhibitory Concentration -MIC) with the clinical and *in vivo* microbiological efficacy seems to be poor.[46] An possible explanation for this is provided by a small cohort study that found that *E.coli* sometimes possess high-level resistant subpopulations that have the ability to regrow under fosfomycin pressure, which may lead to recurrent infections. These subpopulations would not be picked up with classical MIC measurement, but by testing of the MPC (Mutant Prevention Concentration).[19] These results need clinical confirmation. To do so, it is important to correlate and adjust the measurement of *in vitro* activity, as measured with MIC and MPC, with the activity *in vivo*. This could be done in the upcoming EXFOCY trial.[41] If this would be confirmed, it could have major implications for the pathogen-directed deployment of FT.

Third, concerns exist about the development of resistance to fosfomycin among Enterobacterales, which emergence would not only withhold its use for new applications, but would also put pressure on existing indications, such as uncomplicated cystitis. In a similar way, the use of trimethoprim for uncomplicated cystitis has fallen into disrepair.[49] Fosfomycin works by inhibiting the first step in the biosynthesis of the cell wall, where it prevents the formation of peptidoglycan by binding to the enzyme MurA.[50] Resistance to fosfomycin could be induced by lowering the bacterial permeability to fosfomycin, by decreasing the bacterial affinity to fosfomycin, by reducing the intracellular level of cyclic adenosine monophosphate and by inactivating it through a modification of its structure.[51–59] Resistance to fosfomycin could be mediated through chromosomal mechanisms and through transmission of plasmids. On a population level, associations have been found between high consumption of FT and increased resistance to fosfomycin,

although other studies contradict this.[60] And acquisition of resistance as a direct result of intravenous fosfomycin administration has been observed in three patients with recurrent *K. pneumonia* bacteremia.[61] In **chapter 7** we described *in vivo* acquisition of fosfomycin resistance in *E.coli* through inactivation of fosfomycin by the *fosA* gene, which was probably transmitted from *Klebsiella variicola* in the patient's gut microbiome. *Klebsiella spp.* live as commensals in the gut and 99% of these bacteria intrinsically carry fosA. This case-report therefore illustrates a plausible mechanism of acquiring resistance to fosfomycin.[62] Next, the case suggested that fosfomycin pressure may have contributed to this transmission. The patient received 3 gram oral fosfomycin every 48 hours for an unregistered indication, i.e. a chronic endovascular infection of the aortic bifurcation graft. FT is being used for numerous unregistered applications in varying doses, carrying potential risks of acquisition of resistance.[6,35,63] An interesting new development is the discovery of FosA inhibitors, which, like beta-lactamase inhibitors, expand the *in vitro* activity of fosfomycin to FosA expressing Enterobacterales such as *Klebsiella varricola*. On the same way, it could restore activity of fosfomycin to bacteria that acquired FosA, such as in our case.[64] FosA inhibitors have not been evaluated *in vivo* and are not yet available for clinical use.

Patients that would benefit from an additional oral option for UTI are kidney transplant recipients (KTR). KTRs are prone for the development of multidrug resistant UTI as they suffered high antimicrobial pressure from frequent infections and antimicrobial prophylaxis.[65] FT is used regularly to treat UTI in KTRs to avert intravenous antimicrobial therapy and forthcoming hospitalization. In **chapter 8** we observed that FT resulted in clinical cure in 67% (22/33) cystitis episodes in KTRs. No patients were admitted to the ICU or died as a consequence of therapy failure. FT resulted in microbiological cure in only 28% (5/18) cystitis episodes and 25% (3/12) of asymptomatic bacteriuria episodes. Other studies found comparable clinical cure rates.[66–68] A more recent study found clinical and microbiological cure rates of 84% (120/143)) and 70% (59/84), respectively, although in these studies FT was used as first-line treatment (83.2%) instead of as salvage treatment in our study (66.0%) and for a longer duration (median 7 days vs. 1 day in our study).[69] Also within each study, variations in treatment duration and interval were large. Finding the optimal dose of fosfomycin in this population, ideally with the conduction of a RCT, is recommended.

Chapter 9 and 10 contain, respectively, the study protocol and results of the FORECAST RCT, which aimed to provide an additional option for stepdown treatment of febrile UTI in women that were caused by multi-resistant *E.coli*. Despite its early termination, results support the use of oral fosfomycin as stepdown treatment of *E.coli* febrile UTI in women. Therewith it reduces the need of prolonged intravenous

antibiotic regimens and hospitalisation in these patients.[70–72] With the pharmacokinetics / pharmacodynamics of oral fosfomycin in mind, but also because there was hardly any clinical evidence for the efficacy of fosfomycin in systemic infections, we deliberately chose not to investigate the empirical treatment of febrile UTI.[50,73] The positive results of this trial pave the way for further exploration of the efficacy of oral fosfomycin for systemic infections. Compared to ciprofloxacin, the use of fosfomycin more often resulted in diarrhoea, although patients did not stop taking fosfomycin more often as a result of adverse events. Follow-up studies are needed to find out if the treatment with fosfomycin can be shorter and/or if fosfomycin can be dosed every 48 h to have the same effect, instead of 24 h as in our trial. Occurrence of diarrhoea in healthy subjects was lower when fosfomycin (3 gr) was dosed every 48 h instead of every 24 h.[74]

The misuse and overuse of antimicrobials is associated with increased antimicrobial resistance.[75] In the empirical treatment of febrile UTI, the challenge is to treat as narrow as possible without compromising efficacy.[76] The Dutch guideline recommends to base empirical treatment on documented colonization with Highly Resistant Enterobacterales (HRE) in the previous year, consisting of Enterobacterales that are ESBL-E-producing (ESBL-E), Carbapenem-Resistant (CRE) and/or Multi-Drug Resistant (MDRE) to both fluoroquinolones and aminoglycosides. This duration of one year is not substantiated. **Chapter 11** provides insight into the duration of carriage with HRE. The results suggest that this period of one year is inaccurate, as the majority of patients lost HRE carriage within one year, while HRE carriage persisted for more than 5 years in another 20%. As a next step we aim to predict the probability of HRE persistence at admission after previous colonization. This could hopefully support in the decision making of empirical antimicrobial treatment of UTI in this population, and also in the allocation of infection control measures for HRE during hospital admission.

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APPENDICES

Nederlandstalige samenvatting/ Dutch summary

Dit proefschrift heeft als doel om de behandeling van urineweginfecties te verbeteren. De hoofdstukken zijn onderverdeeld in twee thema's. Het eerste thema gaat over de behandeling van blaasontsteking in de huisartsenpraktijk en het tweede thema over de behandeling van urineweginfecties in het ziekenhuis met speciale aandacht voor het voorkomen van bacteriële resistentie tegen antibiotica. Deze thema's zullen apart worden besproken.

De behandeling van blaasontstekingen in de huisartsenpraktijk

Binnen dit thema onderzoek ik de behandeling van blaasontsteking in de huisartsenpraktijk. Om de hoofdstukken makkelijker te begrijpen beschrijf ik eerst de etiologie en behandeling van een blaasontsteking. Vervolgens leg ik de epidemiologische principes uit die gebruikt zijn om de behandeling te onderzoeken. Ten slotte beschrijf ik de bevindingen in de hoofdstukken en de implicaties voor de klinische praktijk.

Een blaasontsteking ontstaat wanneer bacteriën uit de darm via het perineum de urinewegen en de blaas binnendringen. Door een infectie met een bacterie ontstaat een ontsteking van de bekleding van de blaas en urinewegen, wat kan leiden tot een pijnlijk gevoel bij het plassen, het vaker kleine hoeveelheden plassen en loze aandrang. Blaasontstekingen komen vaak voor, vooral bij vrouwen. Met name bacteriën uit de familie Enterobacterales veroorzaken urineweginfecties, de belangrijkste verwekker is de bacterie *Escherichia coli*. De behandeling van urineweginfecties vormt een dagelijkse bezigheid voor huisartsen en praktijkassistenten.

Wanneer een bacterie vanuit de blaas doordringt in de nieren, de bloedbaan of bij mannen naar de prostaat dan kan dit leiden tot een 'opstijgende urineweginfectie' met ernstige ziekteverschijnselen als koorts, koude rillingen en bij ouderen tot verwardheid. Het risico dat dit gebeurt vanuit een blaasontsteking is gering, maar dit is verhoogd bij bepaalde risicogroepen, zoals mannen, zwangere vrouwen, patiënten met diabetes mellitus (suikerziekte), een verlaagde weerstand of anatomische of functionele problemen van de urinewegen. Er wordt in de richtlijn daarom onderscheid gemaakt tussen een blaasontsteking bij vrouwen zonder risicofactoren ("ongecompliceerde blaasontsteking") en een blaasontsteking bij risicogroepen ("gecompliceerde blaasontsteking"). Hoewel een blaasontsteking in de meeste gevallen spontaan geneest, wordt er doorgaans toch met antibiotica behandeld. Dit is zowel voor de vermindering van hinderlijke klachten als ter voorkoming van een ernstige infectie of sepsis. Antibiotica doden of remmen de groei van bacteriën waarmee ze het lichaam ondersteunen om infecties te bestrijden. Voor een goede werkzaamheid van een antibiotica bij een blaasontsteking moet na orale inname een voldoende hoge antibiotica-concentratie worden bereikt in de urine en moet het werkzaam

zijn tegen de veroorzakende bacteriën. De antibiotica die in Nederland worden aanbevolen voor de behandeling van een blaasontsteking zijn nitrofurantoïne, fosfomycine en trimethoprim.

In **hoofdstuk 2, 3 en 4** hebben we geprobeerd om een oorzakelijk verband te vinden tussen een determinant (bijv. de dosering van een antibioticum of de nierfunctie) en de werkzaamheid van de antibiotische behandeling voor een blaastonsteking. Dit hebben we gedaan door reguliere zorggegevens te analyseren die door huisartsen zijn verzameld (observationeel onderzoek). Omdat de zorggegevens al verzameld waren voordat de analyse werd verricht (retrospectief) was het niet mogelijk om een determinant te manipuleren om daarmee een effect op de uitkomst te bewerkstelligen, zoals bij een experiment gebeurt. Bij observationeel onderzoek is het daarom moeilijk om te onderscheiden of er sprake is van een oorzakelijk verband of van een associatie. Het vinden van een oorzakelijk verband is belangrijk omdat dit veronderstelt dat je de determinant (bijv. de dosering van de antibiotica) kunt manipuleren om hiermee een betere uitkomst te bewerkstelligen. Bij een associatie zonder oorzakelijk verband zal het manipuleren van de determinant niets veranderen aan de uitkomst. Het daadwerkelijk vaststellen van een oorzakelijk verband is niet mogelijk met observationeel onderzoek doordat er altijd een mate van vertekening bestaat. Wél kan worden geprobeerd om een oorzakelijk verband aannemelijk te maken door zo goed mogelijk te corrigeren voor vertekenende factoren (“bias”). Hiervoor worden epidemiologische methoden gebruikt.

Voor de interpretatie van de resultaten is het belangrijk om de invloed van de drie vormen van bias op de resultaten te begrijpen; dit zijn selectiebias, informatiebias en confounding.

Selectiebias ontstaat als een niet representatieve selectie van onderzoeksgegevens leidt tot een vertekening van het gemeten effect. Dit kan gebeuren als een onderzoekspopulatie wordt geselecteerd op basis van de te onderzoeken determinant of op basis van de uitkomst. Als je wilt onderzoeken of geslacht een risicofactor is voor het krijgen van blaasontsteking, krijg je een vertekend effect als je alleen mannen met prostaatklachten selecteert en die vergelijkt met gezonde vrouwen, omdat hierdoor ten onrechte de suggestie wordt gewekt dat het mannelijk geslacht inderdaad een risicofactor is voor het optreden van blaasontsteking. Een ander probleem ontstaat als je ná de uitkomst van een behandeling toestemming vraagt om gegevens te verzamelen. Patiënten bij wie een behandeling werkzaam was, geven mogelijk eerder toestemming dan patiënten bij wie een behandeling faalde, bijvoorbeeld omdat ze teleurgesteld waren of te ziek. Hierdoor selecteer je patiënten uit bij wie de behandeling werkzaam was, waardoor de mate van falen van een behandeling wordt onderschat. Hierdoor ontstaat een vertekend effect. Omdat voor onze onderzoeken data van nagenoeg alle patiënten van meer dan 74

huisartsenpraktijken zijn verzameld, kan dit worden beschouwd als een representatieve steekproef van de Nederlandse huisartsenpopulatie. De gegevens zijn onafhankelijk van de uitkomst van de behandeling of onderzochte determinanten verzameld. Het effect dat we proberen aan te tonen wordt daarom niet beïnvloed van selectiebias.

Informatiebias ontstaat als ontbrekende of verkeerd gemeten informatie leidt tot vertekening van het gemeten effect. Reguliere zorggegevens worden niet verzameld voor onderzoeksdoeleinden, waardoor het risico op ontbrekende of verkeerd geclassificeerde informatie groter is. Als een patiënt een bepaalde klacht ervaart, maar zich niet bij de huisarts meldt of als de huisarts dit niet registreert, dan ontbreekt deze informatie. En als een huisarts een klacht onleesbaar of met veel typefouten registreert, dan kan dit verkeerd worden geclassificeerd. De huisartsen, die de reguliere zorggegevens voor deze onderzoeken registreren, worden getraind om zorggegevens zo nauwkeurig en eenduidig mogelijk te registreren, waardoor het risico op informatiebias afneemt. Maar het risico op informatiebias zou nog veel kleiner zijn als gegevens direct en specifiek voor de onderzoeksdoeleinden worden nagevraagd bij deelnemers, zoals mogelijk is bij een prospectief onderzoek. Informatiebias is nog een groter probleem als informatie structureel verschilt tussen de te vergelijken behandelingen. Als de werkzaamheid door structurele meetfouten bij de ene behandeling wordt onderschat en bij de andere behandeling niet, dan vertekent dit de vergelijking (zogeheten differentiële informatiebias). We verwachten dat hiervan geen sprake is in ons onderzoek, maar we kunnen dit niet controleren.

Confounding ontstaat als er een factor aanwezig is die verschilt tussen de behandelgroepen die vergeleken worden én die invloed heeft op de werkzaamheid van de behandeling. Een voorbeeld hiervan is dat nitrofurantoïne als behandeling van blaasontstekingen met name wordt gebruikt bij jonge vrouwen, terwijl fosfomycine wordt gebruikt bij oudere vrouwen. Het is bekend dat behandelingen voor blaasontstekingen, onafhankelijk van de behandeling die gegeven is, vaker falen bij oudere dan bij jongere vrouwen. Een gevonden hogere werkzaamheid van nitrofurantoïne in vergelijking met fosfomycine kan dus zowel worden verklaard door de 'betere' behandeling als ook vertekend worden door de jongere leeftijd van de populatie die nitrofurantoïne gebruikt. Leeftijd is hiermee een confounder die gemeten kan worden en waarvoor in een analyse kan worden gecorrigeerd. In de hoofdstukken hebben we voor meerdere confounders gecorrigeerd, zoals leeftijd, diabetes mellitus en geslacht. Een ingewikkelde situatie ontstaat als een confounder niet gemeten kan worden ("ongemeten confounder"). Bijvoorbeeld als eigengereide patiënten een voorkeur zouden hebben voor fosfomycine boven nitrofurantoïne en eigengereidheid samenhangt met een hogere kans op falen, bijvoorbeeld door het minder goed opvolgen

van adviezen. Eigengereidheid laat zich als factor niet meten en hiervoor kan dus niet worden gecorrigeerd.

Nu zullen de bevindingen worden besproken van de hoofdstukken in dit thema. **Hoofdstuk 2** onderzoekt de invloed van een verminderde nierfunctie op de werkzaamheid van nitrofurantoïne, fosfomycine en trimethoprim. Het is bekend dat een verminderde nierfunctie leidt tot lagere concentraties van antibiotica in de urine, maar dit effect kan verschillen per antibioticum. We vonden in de behandeling met nitrofurantoïne, na correctie voor confounders, dat hoe slechter de nierfunctie was, hoe méér patiënten faalden. Dit effect werd niet geconstateerd bij de behandeling met fosfomycine of trimethoprim. Vrouwen met een eerste episode van een ongecompliceerde blaasontsteking en een verminderde nierfunctie faalden daarom vaker na het geven van nitrofurantoïne dan vrouwen die fosfomycine kregen. Wij hebben hiermee aannemelijk gemaakt dat fosfomycine beter werkt dan nitrofurantoïne bij patiënten met een verminderde nierfunctie, terwijl fosfomycine bij patiënten met een normale nierfunctie juist slechter lijkt te werken dan nitrofurantoïne. De aanbeveling van het artikel is dat de nierfunctie moet worden meegenomen in de antibioticakeuze voor een blaasontsteking.

In **hoofdstuk 3** onderzoeken we de werkzaamheid van twee formuleringen van nitrofurantoïne voor de behandeling van een blaasontsteking. Nitrofurantoïne kan worden gebruikt in een dosering van viermaal daags 50 mg als een normale afgifte formulering (Furadantine®), maar ook tweemaal daags 100 mg als een vertraagde afgifte formulering (Furabid®). Cumulatief wordt dezelfde dosis nitrofurantoïne voorgeschreven, maar uit onderzoek blijkt dat na gebruik van Furabid® een relatief hogere fractie nitrofurantoïne in de urine wordt gemeten dan na gebruik van Furadantine®. Bovendien wordt aangenomen dat de therapietrouw van een tweemaal daagse kuur beter is dan die van een viermaal daagse kuur. We zagen dat de populaties die 50 mg en 100 mg gebruikten nagenoeg gelijk waren in gemeten factoren (bijv. leeftijd, comorbiditeit), waarschijnlijk als gevolg van het feit dat de behandelrichtlijn geen onderscheid maakt tussen beiden. Dit vermindert het risico op confounding. We zagen dat Furabid® een betere werkzaamheid heeft dan Furadantine® bij zwangeren met een blaasontsteking, maar zagen geen verschil in werkzaamheid bij patiënten met een gecompliceerde of ongecompliceerde blaasontsteking.

In **hoofdstuk 4** hebben we de werkzaamheid van twee doseringen nitrofurantoïne als profylaxe voor urineweginfecties onderzocht. Het dagelijks gebruik van nitrofurantoïne is effectief in het voorkomen van

urineweginfecties. In de Nederlandse behandelrichtlijn wordt geen onderscheid gemaakt tussen het dagelijks gebruik van 50 mg en 100 mg nitrofurantoïne. Ook hierbij geldt dat de vergeleken populaties grotendeels dezelfde karakteristieken hadden, omdat de richtlijn geen onderscheid maakt tussen beide doseringen, waardoor de kans op confounding klein is. In het geval van 100 mg wordt een twee keer zo hoge cumulatieve dosis nitrofurantoïne gegeven. Dit is opvallend, omdat het cumulatieve gebruik van nitrofurantoïne in verband wordt gebracht met schadelijke bijwerkingen, met name bepaalde vormen van longschade. We zagen dat het dagelijks gebruik van 100 mg nitrofurantoïne in plaats van 50 mg niet effectiever is in het voorkomen van een urineweginfectie, terwijl er wel meer longklachten worden gemeld na dit gebruik. De aanbeveling uit dit onderzoek is dan ook om 50 mg nitrofurantoïne voor te schrijven als dagelijkse profylaxe voor urineweginfecties.

De conclusies uit **hoofdstuk 2 t/m 4** leiden tot concrete aanbevelingen voor belangrijke openstaande klinische vragen. Gerandomiseerd experimenteel onderzoek kan nog meer bewijs genereren voor deze onderzoeksvragen, omdat hiermee minder vertekening optreedt. Het verrichten van gerandomiseerd onderzoek is echter kostbaar en het is de vraag of deze in de toekomst worden opgezet voor deze onderzoeksvragen.

Urineweginfecties en antibioticaresistentie in het ziekenhuis

Antibioticaresistentie treedt op als bacteriën eigenschappen bezitten of zich toe-eigenen die de effecten van antibiotica tegengaan. Bacteriën kunnen antibiotica buitensluiten, uitstoten of inactiveren. Antibioticaresistentie kan worden toegeëigend als respons op antibioticagebruik, het kan worden uitgeselecteerd in de darm, het kan uitgewisseld worden tussen bacteriën en het kan overspringen tussen mensen. Antibioticaresistentie vormt een obstakel in de behandeling van urineweginfecties en met name in die van opstijgende urineweginfecties in het ziekenhuis. Slechts een beperkt antibiotisch arsenaal is hiervoor beschikbaar, omdat voldoende hoge concentraties moeten worden bereikt in zowel de urine, het bloed en het geïnfecteerde weefsel. Daarnaast moet het antibioticum werkzaam zijn tegen de veroorzakende bacterie. De behandeling van ernstige opstijgende urineweginfecties vindt meestal plaats in het ziekenhuis. Hierbij wordt er doorgaans gestart met empirische antibiotica via het infuus met werkzaamheid tegen de meest voorkomende verwekkers, waarna er wordt geswitcht naar een oraal antibioticum gericht tegen de gevonden verwekker. Het arsenaal aan antibiotica dat via het infuus kan worden toegediend is groter dan het arsenaal aan orale opties, welke bestaat uit ciprofloxacine, amoxicilline (met clavulaanzuur) en cotrimoxazol.

Een relatief veel voorkomende vorm van antibioticaresistentie die de behandeling van urineweginfecties compliceert is de productie van het enzym 'Extended Spectrum Beta-Lactamase' (ESBL) door Enterobacterales. Hierdoor worden belangrijke groepen antibiotica afgebroken, namelijk de penicillinen en cefalosporinen, waardoor hun werkzaamheid verloren gaat. Omdat ESBL producerende Enterobacterales vaak tevens resistent zijn tegen andere antibiotica als ciprofloxacine of cotrimoxazol, is alleen een gering arsenaal aan reserve antibiotica nog werkzaam. Het meest bekende reserve antibioticum is carbapenem. Het (verkeerd) gebruik van carbapenem gaat automatisch gepaard met een toename van antibioticaresistentie tegen carbapenem, met name door selectie van carbapenem-resistente Enterobacterales of het induceren van resistentie. Als respons op de toenemende antibioticaresistentie tegen carbapenem zijn er de afgelopen decennia alternatieve antibiotica ontwikkeld die werkzaam zijn tegen ESBL-producerende en carbapenem resistente Enterobacterales. In **hoofdstuk 5** hebben we een systematisch overzicht gemaakt van de effectiviteit van deze alternatieve antibiotica voor de behandeling van urineweginfecties. Voor de empirische behandeling vonden wij vier antibiotica met een gelijkwaardige effectiviteit en veiligheid als carbapenem, dat waren ceftazidim-avibactam, ceftriaxon-sulbactam met disodium-EDTA, plazomicine en cefiderocol. Ceftazidim-avibactam werd als enig antibioticum gelijkwaardig bevonden voor de gerichte behandeling van urineweginfecties. Deze antibiotica bieden nieuwe behandelmogelijkheden voor de toekomst, maar monitoring van het gebruik ervan en surveillance van resistentie ertegen zijn vereist om de werkzaamheid te behouden in de toekomst.

Naast het ontwikkelen van nieuwe antibiotica is het nodig dat het spectrum van reeds bestaande antibiotica wordt vergroot. Een voorbeeld van zo'n antibioticum is fosfomycine, dat is ontdekt in 1969 maar tot voor kort alleen is toegepast voor de behandeling van ongecompliceerde blaasontsteking. Onderzoek bij vrijwilligers laat zien dat fosfomycine behalve in de urine ook doordringt in andere weefsels, zoals de bloedbaan en de prostaat. Bovendien wordt er amper antibioticaresistentie tegen fosfomycine gezien bij de bacterie *Escherichia coli*. Dit heeft nieuwe toepassingen gecreëerd voor oraal fosfomycine. Fosfomycine is tevens beschikbaar voor infusie voor de behandeling van infecties veroorzaakt door multiresistente bacteriën. Er zijn een aantal aspecten die de toepassing van fosfomycine onzeker maken. Zo vonden we in **hoofdstuk 6** dat als je bij urineweg-bacteriën in verschillende laboratoria met een reguliere gebruikte test (Etest) bepaalt of ze resistent zijn tegen fosfomycine en je laat deze testen beoordelen door verschillende personen dit tot verschillende uitslagen leidt. Bovendien kwamen de

uitslagen niet overeen met de referentiestandaard voor deze bepaling, genaamd agar dilutie. Een ander onderzoek liet bovendien een slechte overeenkomst zien tussen die van andere reguliere testen en agar dilutie. De betrouwbaarheid van reguliere testen voor de bepaling van resistentie tegen fosfomycine wordt hiermee in twijfel getrokken. Dit heeft consequenties voor de gerichte toepassing van fosfomycine. De betrouwbaarheid van de reguliere testen was overigens veel beter voor *Escherichia coli* dan voor andere urinewegbacteriën.

Een andere bron van zorg is het ontstaan van resistentie tegen fosfomycine. Eerder onderzoek liet zien dat tijdens gebruik van fosfomycine resistentie kan ontstaan tegen fosfomycine en ook dat een stijging in het gebruik van fosfomycine op populatieniveau gepaard kan gaan met stijgende resistentiecijfers. In **hoofdstuk 7** hebben we met behulp van een illustratieve casus weergegeven hoe resistentie tegen fosfomycine kan ontstaan. De betreffende patiënt gebruikte oraal fosfomycine vanwege een geïnfecteerde vaatprothese, veroorzaakt door *Escherichia coli*. Gedurende behandeling met fosfomycine ontwikkelde deze bacterie resistentie tegen fosfomycine, doordat een gen genaamd FosA werd toegeëigend. Kweken van het rectum lieten zien dat dit gen hoogstwaarschijnlijk was overgesprongen vanuit een andere darmbacterie, genaamd *Klebsiella variicola.* Via dit mechanisme zal in de praktijk hoogstwaarschijnlijk vaker resistentie ontstaan tegen fosfomycine. *Klebsiella variicola* bacteriën leven ten slotte als commensalen in de darm, en bezitten in 99% van de gevallen FosA.

Urineweginfecties komen frequent voor in risicogroepen, wat bij hen leidt tot overmatig antibioticagebruik, waardoor hoge resistentiepercentages ontstaan tegen veel gebruikte antibiotica. Een van de risicogroepen zijn niertransplantatiepatiënten. Omdat orale antibiotische opties ontbreken moet bij hen vaak worden uitgeweken naar antibiotica via het infuus, ook voor niet ernstige urineweginfecties. De ziekenhuisopname die meestal nodig is voor antibiotica via het infuus vormt een belasting voor deze patiënten, die toch al frequent in het ziekenhuis moeten zijn voor reguliere controles. Oraal fosfomycine werd in de praktijk al regelmatig gebruikt als alternatief, terwijl het niet geregistreerd was voor deze toepassing en er weinig bewijs bestond voor dit gebruik. Dit was de aanleiding om in **hoofdstuk 8** retrospectief de effectiviteit en veiligheid van oraal fosfomycine voor urineweginfecties bij niertransplantatiepatiënten te onderzoeken. We vonden dat het gebruik van fosfomycine er in 67% van de gevallen toe leidt dat geen nieuw voorschrift nodig is, waarmee een ziekenhuisopname of antibiotica via het infuus dus voorkomen wordt. Het lukt met fosfomycine echter maar in 25% van de gevallen om de bacterie uit de urine te klaren. Een belangrijke bevinding was dat het gebruik van oraal fosfomycine niet

resulteert in ernstig behandel-falen met als gevolg een opname op de intensive care of het overlijden van een patiënt.

Het arsenaal aan orale antibiotica voor opstijgende urineweginfecties is beperkt en de resistentiepercentages van *Escherichia coli* tegen ciprofloxacine, amoxicilline met clavulaanzuur en cotrimoxazol in ziekenhuizen zijn hoog, respectievelijk 14%, 36% en 22% in 2019. Het resistentiepercentage van *Escherichia coli* tegen fosfomycine is slechts 1 tot 2%, waarmee fosfomycine een geschikt alternatief kan zijn voor de orale uitbehandeling van urineweginfecties. Dit kan voor patiënten een langdurige antibiotische behandeling via het infuus en ziekenhuisopname voorkomen. Dit was voor ons de reden om de FORECAST studie op te zetten, een gerandomiseerde, dubbelblinde non-inferioriteitsstudie, waarin fosfomycine wordt vergeleken met ciprofloxacine voor de gerichte uitbehandeling van opstijgende urineweginfecties bij vrouwen, veroorzaakt door *Escherichia coli*. **Hoofdstuk 9** en **hoofdstuk 10** bevatten respectievelijk het studieprotocol en de resultaten van de FORECAST studie. Wij vonden dat fosfomycine ten minste zo goed werkt als ciprofloxacine op het verbeteren van klachten, wat het primaire doel was van de studie. Maar dat het waarschijnlijk minder goed werkt op het klaren van bacteriën uit de urine. In vergelijking met ciprofloxacine resulteerde het gebruik van fosfomycine vaker in diarree, hoewel dit er niet toe leidde dat patiënten eerder stopten met het innemen van fosfomycine als gevolg van bijwerkingen. Vervolgonderzoeken zullen moeten uitwijzen of de behandeling met fosfomycine (1) korter kan en of (2) fosfomycine elke 48 uur kan worden gedoseerd om hetzelfde effect te bewerkstelligen, in plaats van elke 24 uur zoals in ons onderzoek. Het vergroten van het doseringsinterval leidt in een andere studie namelijk tot minder voorkomen van diarree.

De term multiresistente Enterobacterales is gedefinieerd als Enterobacterales die ESBL produceren, resistentie bezitten zijn tegen carbapenem of resistent zijn tegen tenminste twee antibiotica-klassen, namelijk de fluoroquinolonen (met onder andere ciprofloxacine) én aminoglycosiden. Dragerschap van multiresistente Enterobacterales vindt meestal plaats in de darm. Als patiënten drager zijn van multiresistente Enterobacterales, dan worden er in het opeenvolgende jaar contactmaatregelen genomen om verdere verspreiding tijdens ziekenhuisopnames te voorkomen. En bij elke opname wordt een rectumkweek afgenomen om te zien of iemand nog drager is. Met behulp van deze rectumkweken hebben we in **hoofdstuk 11** de tijd tot het verliezen van dragerschap berekend. De mediane duur van dragerschap van multiresistente Enterobacterales in twee ziekenhuizen is een half jaar. Na een jaar is 38% nog drager en na 5 jaar is naar schatting 20% nog drager. Wij constateerden hiermee dat een vol jaar van

het nemen van contactmaatregelen onnodig lang is voor de meeste dragers terwijl het voor sommige juist veel te kort is. Momenteel zijn we bezig met de ontwikkeling van een model dat bij ziekenhuisopname de kans voorspelt op dragerschap van een multiresistente Enterobacterales. Indien deze voorspelling goed is dan kan dit in de toekomst worden gebruikt om contactmaatregelen beter in te zetten. En wellicht ook om te bepalen welke empirische antibiotische behandeling moet worden gebruikt op de spoedeisende hulp. Via deze route kan dit hopelijk leiden tot het voorkomen van onnodig gebruik van reserve antibiotica en tot het voorkomen van antibioticaresistentie.

Het waarborgen van de behandeling van urineweginfecties vereist een strategie bestaande uit (1) het ontwikkelen en onderzoeken van nieuwe antibiotica, (2) het juist gebruik van antibiotica en (3) de monitoring van resistentie. **Hoofdstuk 5 t/m 11** dragen direct of indirect bij aan deze strategie. Als gevolg van het succesvolle beleid in Nederland is het percentage antibioticaresistentie onder Enterobacterales min of meer stabiel gebleven en kunnen bijna alle patiënten met een urineweginfectie antibiotisch worden behandeld.

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Nu de Covid-19 pandemie in Nederland op zijn retour lijkt, wordt de impact steeds beter zichtbaar. In Nederland overleden bijna 18.000 mensen als een direct gevolg van Covid-19. Daarnaast heeft de pandemie onmeetbare gevolgen gehad op het welzijn en de welvaart van mensen. De huidige afname van Covid-19 ziekenhuisopnames is een direct gevolg van de stijgende vaccinatiegraad. Ik ben als zorgverlener in januari gevaccineerd, mijn ouders ontvingen in juni hun eerste vaccin en onlangs zijn mijn broers en vrienden gevaccineerd. We zijn deze crisis doorgekomen dankzij de tomeloze inzet van zorgverleners en we wisten de omvang te beperken door wetenschappelijke doorbraken. Dit heeft veel indruk gemaakt. Mijn proefschrift draag ik op aan hen die zich hebben ingespannen om de pandemie te bestrijden.

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Onno, twee jaar geleden stond jij hier. Sinds we elkaar 10 jaar geleden leerden kennen tijdens ons debuut als arts-assistent, bewandelen we min of meer dezelfde weg. We ervaren daarbij dezelfde uitdagingen en de gesprekken hierover waardeer ik zeer. Ik ben blij dat je vandaag naast me staat als paranimf.

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Mijn Lise en Pepijn. Zo klein nog, maar me zo dierbaar. Mijn leven draait om jullie. Beleef je mooiste dromen en voel je eindeloos vrij.

About the author

Thijs ten Doesschate was born in Nijmegen on August 20th 1986. After graduating from the Athenaeum at the Nijmeegse Scholengemeenschap Groenewoud, he started medical school in the Radboud University in Nijmegen and finished this with a scientific internship in Ecuador on the emotional well-being of left-behind-children of economic migrants. His career was continued in Utrecht in 2012, where he worked as a resident Internal Medicine at the St. Antonius hospital Nieuwegein and started his official training to become an internal medicine specialist under supervision of Dr. A.B.M. (Tom) Geers. In 2015, he continued his training at the University Medical Center Utrecht under supervision of Prof. K.A.H. (Karin) Kaasjager. He combined his training as a member and later as the chair of the board of the junior Dutch internists association, where he, among other things, campaigned against unemployment, and championed medical leadership among residents internal medicine. In 2018 he was given the opportunity to start a PhD under the supervision of Prof. M.J.M. (Marc) Bonten and Dr. C.H. (Henri) van Werkhoven. As an integral part of this PhD trajectory he graduated as a Postgraduate master Epidemiology at the University of Utrecht. Thijs is currently training as an infectious disease specialist, supervised by Dr. J.J. (Jan-Jelrik) Oosterheert. He is married to Evelien and together they have a daughter (Lise, 2016) and a son (Pepijn, 2019).