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Effect of point-of-care susceptibility testing in general practice on appropriate prescription of antibiotics for patients with uncomplicated urinary tract infection: a diagnostic randomized controlled trial

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TITLE PAGE

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Effect of point-of-care susceptibility testing in general practice on appropriate prescription of antibiotics for patients with uncomplicated urinary tract infection: a diagnostic randomized controlled trial

AUTHORS

Anne Holm, AH, PhD fellow (1)

Gloria Cordoba, GC, PhD fellow (1)

Tina Møller Sørensen, TMS, PhD fellow (2)

Lisbeth Rem Jessen, LRJ, associate professor (2)

Niels Frimodt-Møller, professor (3)

Volkert Siersma, statistician (1)

Lars Bjerrum, professor (1)

1) University of Copenhagen, Research Unit for General Practice and Department of General Practice, Øster Farimagsgade 5, PO box 2099, 1014 Copenhagen
2) University of Copenhagen, Department of Veterinary Clinical and Animal Sciences, Dyrlægevej 16, 1870 Frederiksberg C
3) Clinical Microbiological Department, Rigshospitalet, Blegdamsvej 3, 2100 København Ø

CORRESPONDING AUTHOR

Anne Holm, MD, PhD fellow

University of Copenhagen, Department of General Practice, Øster Farimagsgade 5, PO box 2099, 1014 Copenhagen

TEL +45 35 32 79 60

DIR +45 35 33 72 44

MOB +45 61 67 81 81

anneholm@sund.ku.dk

WORD COUNT

3171

ABSTRACT

OBJECTIVES

To investigate the effect of adding point-of care (POC) susceptibility testing to POC culture on appropriate use of antibiotics as well as clinical and microbiological cure for patients with suspected uncomplicated urinary tract infection (UTI) in general practice.

DESIGN

Open-label randomized controlled trial (RCT)

SETTING

General practice in the Copenhagen area, Denmark

PARTICIPANTS

Female patients with suspected uncomplicated UTI, including elderly patients, patients with recurrent UTI and patients with orally treated diabetes without complications. 851 patients were screened for eligibility, 376 patients agreed to participate, and 363 were included in analysis.

INTERVENTIONS

Flexicult™ SSI-Urinary Kit was used for POC culture and susceptibility testing in the intervention arm and ID Flexicult™ was used for POC culture-only in the control arm.

MAIN OUTCOME MEASURES

Primary: Appropriate antibiotic prescribing the day after consultation

Secondary: Clinical cure on day 5 and microbiological cure on day 14.

RESULTS

Patients randomized to culture-only received significantly more appropriate treatment than those randomized to culture and susceptibility testing (OR (95% CI): 1.44 (1.03-1.99), p=0.03). There was no significant difference in clinical or microbiological cure. Sub-group analysis showed that culture-only was the superior test for appropriate treatment, both for young patients without comorbidities and for patients who were elderly, who had diabetes or who had recurrent UTI. However, the difference was only significant for young patients without comorbidities.

CONCLUSIONS

Adding POC susceptibility testing to POC culture did not improve antibiotic prescribing for patients with suspected uncomplicated UTI in general practice. Susceptibility testing should be reserved for patients at high risk of resistant bacteria and complications.

TRIAL REGISTRATION

ClinicalTrials.gov NCT02323087.

KEY WORDS

"Urinary Tract Infections", “Microbiological diagnosis”, “Culture media”, “Point-of-Care Testing“, "General Practice"; “Antibiotics”

ARTICLE SUMMARY - STRENGTHS AND LIMITATIONS OF THIS STUDY

- Performing point-of-care (POC) susceptibility testing prior to treatment may improve antibiotic prescribing
- The effect of adding POC susceptibility testing to POC culture has never been tested in a randomized design
- There is no benefit of adding POC susceptibility testing to POC culture for patients with uncomplicated UTI.
- Patients in this trial had lower use of antibiotics than previously described for other diagnostic strategies, which suggests that POC urine culture is a good diagnostic tool to reduce inappropriate antibiotic prescribing.
- POC susceptibility testing should be reserved for patients with high risk of resistance.

INTRODUCTION

Urinary tract infection (UTI) is a common condition in general practice and the second leading cause for the prescribing of antibiotics (1). Resistance rates for the most common uropathogen; *E. coli*, are rising, and the inappropriate prescription of antibiotics in primary care is known to lead to antibiotic resistance (2–4). Resistant strains of bacteria can cause treatment failure and prolonged symptoms (5–7). Many countries recommend diagnosing UTI based on symptoms and urine dipstick, but combinations of symptoms and dipstick have proven inaccurate in ruling UTI in or out (8,9). In Denmark there is no national guideline for diagnosing UTI and doctors have varying strategies based on urine dipstick, microscopy, point-of-care (POC) culture and POC culture and susceptibility testing (10,11). Urine culture gives a definite answer for UTI in the symptomatic patient (12). However, sending urine to the microbiological laboratory for culture and susceptibility testing can delay treatment for several days. Point-of-care (POC) tests for urine culture and urine culture and susceptibility testing are commercially available. They can provide a result within 24 hours, a delay to treatment which the majority of patients would accept (13). The Flexicult™ SSI-Urinary Kit is commonly used in general practice due to its ease of use and the fact that both culture and susceptibility testing can be performed on the same plate (14). Similar chromogenic agars for culture-only exist, but are less commonly used and have not been validated in general practice. The most commonly used antibiotics in Denmark for treatment of acute UTI are pivmecillinam and sulfamethizole. Resistance rates in *E. coli* isolates in urine from primary care in Denmark are approximately 30% for sulfamethizole and 5-10% for pivmecillinam (15). Since other uropathogens can be inherently resistant to pivmecillinam, overall resistance would be expected to be 15-20% for this drug. We hypothesised that performing a susceptibility test prior to initiation of treatment could target treatment to the individual patient, potentially reducing inappropriate antibiotic prescribing and leading to faster clinical recovery. This study aimed to investigate the effect of POC culture and susceptibility testing against POC culture-only on the appropriate use of antibiotics and clinical and microbiological cure for patients with suspected uncomplicated UTI in general practice.

MATERIAL AND METHODS

The design is described in detail in the protocol (16).

RECRUITMENT OF PRACTICES

An invitation letter was mailed to 200 randomly selected general practices in the Copenhagen area with the aim of recruiting 50 general practitioners (GPs) with experience in using POC culture. The recruited GPs participated in a pre-study instruction course on handling and reading both POC tests, and had to pass an online test measuring ability to diagnose UTI based on photographs of urine cultures prior to the inclusion of patients.

RECRUITMENT OF PATIENTS

The inclusion criteria were: women, 18 years or older, presenting to their GP with dysuria, frequency or urgency, for seven days or less, and for which the GP suspected uncomplicated UTI, including elderly patients, patients with recurrent UTI and patients with orally treated diabetes without complications. The broader definition of uncomplicated UTI was chosen to ensure applicability to a larger group of patients in general practice. The exclusion criteria were: negative dipstick analysis on both leucocytes and nitrites, serious comorbidities, former participation in the study and patients presenting on a Friday (since POC culture is read the following day). All patients had to consent to wait until the next day to receive the result of the POC test before commencing possible treatment. After informed consent, patients were randomized to either POC culture or POC culture and susceptibility testing. A urine sample from the same portion of urine was sent to the local microbiological laboratory for culture and susceptibility testing. The GP filled out a case-report form and the patient was asked to fill out a seven-day symptom diary and return to the GP after 14 days for a control urine sample. Validation of the symptom diary has previously been published (17). Patients were reminded by text messages and telephone calls to return the diary and bring the control urine sample. Each practice kept an anonymous screening log of patients who fulfilled the inclusion criteria but who were not included in the study. GPs received no treatment protocol concerning choice of antibiotics, but could decide freely on treatment.

PATIENT INVOLVEMENT.

One of the secondary outcome measures was clinical cure. This was measured using a content validated symptom diary, where items were generated through cognitive interviews with patients (17). Patients could state on their consent form whether they wished to be informed about the results of the study. This will be done using a text message with a short summary and a link after publication. Patients were not involved in the design of the study. All recruiting practices received a poster displaying information about the trial to hang in the waiting room, so patients could enquire about participation in case they were not approached regarding this.

POINT-OF-CARE TESTS

Culture-only group: The ID Flexicult™ (SSI DIagnostica, Denmark) is a chromogenic agar allowing identification and quantification of: 1) *E. coli*, 2) other Enterobacteriaceae (Gram-negative rods), 3) enterococci, 4) Proteus spp., 5) *S. saprophyticus* and 6) *P. aeruginosa.* The plate is inoculated with freshly voided urine using a 10µL loop-needle and incubated at 35°C overnight. It is read the following day, but negative culture can only be determined after 24 hours. Significant growth was prespecified as $\geq 10^3$ colony-forming units per millilitre (cfu/mL) for *E. coli* and *S. saprophyticus* and $\geq 10^4$ cfu/mL for other typical uropathogens in accordance with European guidelines (12).

Culture and susceptibility testing group: the Flexicult™ SSI-Urinary Kit (SSI Diagnostica, Denmark) is an agar dish consisting of a large compartment containing the same agar material as in the ID Flexicult™ and five small compartments, each containing agar with a specific antibiotic: 1) trimethoprim, 2) sulfamethizole, 3) ampicillin, 4) nitrofurantoin, and 5) mecillinam. The agar plate is flooded with freshly voided urine for 3-5 seconds. Any excess urine is discarded. The plate is incubated and handled in the same way as the ID Flexicult™. Significant growth was prespecified (advised by manufacturer) to $\geq 10^3$ cfu/mL for any uropathogen.

REFERENCE CULTURE IN THE MICROBIOLOGICAL LABORATORY

Urine samples were sent by special delivery service to the reference microbiological laboratories at the Department of Clinical Microbiology, Copenhagen University Hospital, Herlev, Denmark or the Department of Clinical Microbiology, Copenhagen University Hospital, Hvidovre, Denmark. Urine samples were analysed on Inoqul A™ Bi-plate (CHROMagar and blood agar) with 10 µL on each half of the agar. The susceptibility pattern was determined on Mueller Hinton agars with disks containing antibiotics, including mecillinam, trimethoprim, nitrofurantoin, sulfamethizole, ampicillin

and ciprofloxacin. All samples were quantified. Significant growth was defined as growth of $\geq 10^3$ cfu/mL for *E. coli* and *S. saprophyticus*, $\geq 10^4$ cfu/mL for other typical uropathogens and $\geq 10^5$ cfu/ml for possible uropathogens. Plates with growth of more than two uropathogens were labelled as mixed cultures and classified in the analysis as negatives.

Randomization and concealment of allocation

The randomization code was produced by an online random number generator as permuted block randomization in blocks of 10 by the investigators. The allocation of each included patient was placed in a sealed envelope, which was opened after inclusion of the patient.

Outcomes

Primary outcome: appropriate treatment was defined as either 1) If the patient had UTI in the reference: to prescribe a first-line antibiotic to which the infecting pathogen was susceptible. 2) If the patient had UTI but was allergic to the antibiotic or the pathogen was resistant to all first-line antibiotics: to prescribe a second-line antibiotic. 3) If the patient did not have UTI in the reference: to not prescribe an antibiotic. Secondary outcomes: Clinical cure was defined as the patient reporting herself as symptom free in the symptom diary on day 5 (four days after initiation of treatment). Microbiological cure was defined as no significant growth in the control urine sample after 14 days.

Statistical analysis

The distributions of baseline presentation characteristics were compared between the randomization groups using chi-squared tests. Investigated variables were: age, number of days with symptoms, key symptoms (dysuria, frequency and urge), complicating factors and reference culture and susceptibility test. Primary and secondary outcomes were analysed in logistic regression models; clustering within practices was adjusted for by generalised estimating equations (GEE). Patient factors (age, number of days with symptoms, key symptoms, and complicating factors) and practice factors (number of GPs and organisation of practice) were investigated for effect modification on the primary outcome. All analyses were performed as intention-to-treat (ITT) analyses. The significance level was 5%. Statistical analysis was performed with SAS version 9.4 for Windows 7, SAS Institute Inc.

RESULTS

Twenty general practices with a total of 45 GPs were recruited from the Copenhagen area and screened 851 patients for eligibility between 1st March 2015 and 1st May 2016. Of these, 376 patients agreed to participate: 199 were randomized to culture and susceptibility testing, and 177 were randomized to culture-only. 13 patients were excluded from the analysis, leaving a total of 363 patients with data on at least one of the outcomes to be included in the analysis. An overview of the inclusion and exclusion of patients can be seen in Figure 1.

Patient characteristics and distribution between groups can be seen in Table 1. Most of the baseline variables did not differ significantly between groups, but the proportion of patients who were over 65 years or who had recurrent UTI or diabetes (complicated cases of uncomplicated UTI) differed significantly. The prevalence of confirmed UTI (significant growth of uropathogens in the reference standard) and susceptibility pattern in the reference standard did not differ between groups.

Three quarters (75%) of the patients were appropriately treated in the culture-only group and two thirds (67%) were appropriately treated in the culture and susceptibility testing group. This difference was significant both in the unadjusted analysis and when controlled for baseline characteristics. Sub-group analyses on young patients without co-morbidity and patients who were elderly, or who had diabetes or recurrent UTI showed that young patients with no comorbidities were significantly more appropriately treated in the culture-only group compared to the culture and susceptibility group. The difference was not significant for patients who were elderly, or who had diabetes or recurrent UTI, although culture-only was still superior to culture and susceptibility testing (Table 2).

Table 3 shows the distribution of patients and the reasons why they were labelled as appropriately or inappropriately treated. Overtreatment of patients without UTI was the major reason for inappropriate treatment and was almost equally distributed between groups. Undertreatment was slightly higher in the culture and susceptibility group. Surprisingly, treatment with an antibiotic to which the infecting pathogen was resistant was higher in the culture and susceptibility group. None of the individual differences was significant.

308 patients (85%) had data for the secondary outcome, clinical cure on day 5. Cure rates were equal between groups and there was no significant difference between the proportions of patients cured on day 5. See Table 2 and Figure 2. 144 patients (40%) delivered a control urine sample after 14 days. There was no significant difference in microbiological cure rate between groups.

In accordance with the protocol, we investigated whether practice or patient factors could modify the primary outcome (effect modification). Neither practice factors (size and organisation of participating practices), nor patient factors (any complicating factor, age, diabetes, number of UTIs and number of key symptoms at inclusion) modified the effect of the intervention significantly.

Six patients in the culture-only group had the wrong test performed (culture and susceptibility testing). Per protocol analysis essentially reproduced our findings with culture-only still leading to 75% appropriate treatment and culture and susceptibility testing to 67% appropriate treatment (P= 0.05 unadjusted and 0.02 adjusted).

DISCUSSION

Patients in the POC culture group received significantly more appropriate prescribing than patients in the POC culture and susceptibility group. There was no difference in clinical recovery, despite the difference in appropriate prescribing. This may be partly due to the fact that pivmecillinam has been shown to have a clinical and microbiological effect despite the infecting pathogen being resistant *in vitro* (5).

We aimed at investigating the effect of adding susceptibility testing to POC culture on the appropriate use of antibiotics so the randomized controlled trial was the most appropriate design (18–20). We succeeded in enrolling a sample of GPs with experience in POC culture. These GPs recruited a sample of patients with symptoms of uncomplicated UTI, which was sufficient to detect a small but significant difference between appropriate prescribing based on two different POC culture tests. The inclusion criteria broadened the usual strict definition of uncomplicated UTI, which ensures applicability of our findings to a much broader group of patients in general practice. It may be controversial to include patients with diabetes and recurrent UTI in a sample of patients with uncomplicated UTI, but since these conditions are very common among patients with suspected UTI in general practice and they could be safely included, we decided to include these conditions and investigate whether they modified the effect of the intervention on the outcome. Both the sub-group analysis and the investigation of effect modification indicated that these patients' disease was not more complicated than that of young women with no co-morbidity. We did not recruit our initially planned sample, but the difference between groups turned out to be larger than originally expected when sample size was calculated. A type I error in determining the superiority of the ID Flexicult^{TM} is possible, since the significance level was not overwhelming, but a type II error in failing to detect the expected superiority of the Flexicult^{TM} SSI-Urinary Kit is unlikely. Subgroup analysis could easily be subjected to both type I and type II errors and should be interpreted with caution.

Bias in the interpretation of the test was low as described previously (21). GPs were blinded to the result of the reference at the time of deciding on treatment; POC test and reference were performed on the same portion of urine; the reference was adequate for ruling disease in or out; and all data were included in the analysis. Allocation was concealed using sealed envelopes. It is very unlikely that GPs introduced any selection bias due to strong beliefs of the effect of one of the tests. Applicability of the results was also high, since patients, GPs and tests were very similar to

those which would be relevant in daily practice. Patients with negative dipstick results were excluded. Spectrum bias should therefore be considered if the tests are applied to all patients regardless of their dipstick result.

The study was subjected to clinical review bias in the interpretation process, since the interpreter of the POC tests was not blinded to clinical history. The two groups did not differ in terms of number of symptoms or number of days with symptoms, and patient factors did not seem to have different effects on the two groups, so the difference in this bias between groups was probably minimal. Confirmation bias in the interpretation process could also be present, since treatment had to be initiated based on the result of the test and only patients with suspected UTI were included. GPs were slightly more compliant with regard to the familiar test (culture and susceptibility testing) than with the new test. However, since overtreatment was similar in the two groups, it does not seem to have had a major effect (see suppl. 1). Our trial was open-label and it is possible that ascertainment bias was present if GPs had a stronger belief in one of the tests. Six patients had the wrong test performed, but per-protocol analysis reproduced the ITT findings, suggesting that this was unintentional. The reference, sending urine in boric acid for culture at the microbiological laboratory, has its flaws as previously described (21). However, these flaws should have a similar effect on the two groups, since the distribution of growth and the resistance pattern did not differ significantly between groups.

There are no previous diagnostic RCTs comparing the use of POC culture versus POC culture and susceptibility testing in general practice. A study from 2010 investigated five different management strategies and found differences in antibiotic use (more antibiotics were used when treatment was based only on symptoms), but no difference in patient recovery (22). They found the lowest antibiotic use in the group in which antibiotics were delayed (77%). In comparison, total antibiotic use was 76% for culture-only and 73% for culture and susceptibility testing in this study.

The significant overall difference in appropriate prescribing between the groups was driven by three factors (none of them individually significant): firstly, undertreatment; secondly, treatment with an antibiotic to which the infecting pathogen was resistant; and thirdly, inappropriate choice of a second-line antibiotic. The first factor, undertreatment, could be partly due to a slightly lower sensitivity of the Flexicult^{TM} SSI-Urinary Kit (21) and partly to GPs being generally more compliant with a negative result in this group (see suppl. 1). The second factor, treatment with an antibiotic to which the infecting pathogen was resistant, was surprising and could be partly due to the fact that susceptibility testing in general practice is not always accurate (11), and partly due to ID Flexicult^{TM} possibly being a

better test to identify pathogens, thereby identifying the inherent susceptibility pattern. Correct identification of pathogens is essential for determining the inherent susceptibility pattern, since the inherent susceptibility pattern does not necessarily show on the culture plate (23). The GPs in our study may have relied too much on their susceptibility test and only looked up the inherent susceptibility when they were forced to do so. The study on the accuracy of the two tests investigated in this study showed that the GPs identified pathogens correctly in about 60% of the positive cultures (21). A post-hoc analysis showed that the ID Flexicult™ was actually significantly better at identifying uropathogens than the Flexicult SSI Urinary kit™. However, the most common uropathogen, *E. coli*, does not have inherent resistance to first-line antibiotics, so this second factor may just be a random finding. The third factor, inappropriate choice of a second-line antibiotic, happened in a few cases and none of them had an obvious reason, such as identification of resistance on the practice susceptibility test or patient allergies.

The findings of this study support current recommendations that uncomplicated UTI should not have susceptibility testing performed prior to initiation of treatment. Women generally accepted delaying treatment for one day to await the POC culture result and inappropriate treatment was low in both groups. If all patients had been treated with first-line antibiotics based on clinical history and positive dipstick finding, then about 45% of patients would have been inappropriately treated compared to 29% in this study (data not shown). Also, total antibiotic use was lower than previously described in a similar setting (22). Based on these results, performing POC culture prior to treatment for patients with uncomplicated UTI seems rational, but adding POC susceptibility testing should be reserved for those patients at high risk of a resistant infection or complications.

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ETHICAL APPROVAL

All procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983. The study was approved by the Ethical Committee for the Capital Region of Denmark (ref.no: H-3-2014-107). All patients gave written informed consent prior to participating in the study.

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DATA ACCESS

All authors had access to and can take responsibility for data and analysis. The authors commit to making the relevant anonymised patient level data available on reasonable request

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AUTHORSHIP

All authors have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. The corresponding author drafted the manuscript and all other authors revised it critically for important intellectual content. All authors have approved the final version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONTRIBUTORSHIP

All authors took part in the design and planning of the study. AH conducted the study supported by all other authors. AH drafted the first manuscript and all other authors revised the entire manuscript critically and approved the final version for publication. VS has mainly supervised statistics and NFM has mainly supervised technical issues regarding the POC tests and microbiological culture. AH is guarantor for the study.

CONFLICTS OF INTEREST

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; LRJ and TMS declares non-financial support from SSI Diagnostica since SSI Diagnostica is supplying Flexicult (R) VET POC culture plates for an ongoing veterinary multi-center RCT study being co-ordinated by LRJ at the University of Copenhagen**.** None of the other authors has other relationships or activities that could appear to have influenced the submitted work**.**

TRANSPARENCY DECLARATION

The lead author* (AH) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned and registered have been explained.

*The manuscript's guarantor.

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LEGENDS

Figure 1: Inclusion flow chart

Table 1: Distribution of baseline data between the two groups. Numbers are total numbers with proportions in brackets unless otherwise stated. NS=Not significant

Table 2: Comparison of primary and secondary outcomes between the two groups. OR: Odds for having a positive outcome if randomized to culture-only (ID Flexicult™) compared to culture and susceptibility testing (Flexicult™ SSI-Urinary Kit). NS=Not significant

Table 3: Reasons for appropriate and inappropriate prescribing and distribution of patients between groups

Figure 2: Cure rates for the two groups. The level of the coloured lines indicates the proportion of patients still having symptoms. Day 0 is the evening of the day of the consultation. The first vertical grey line indicates initiation of treatment (the morning after the consultation), the second vertical grey line indicates the data used for calculation of the secondary outcome: clinical cure on day 5 (four days after consultation).

TABLES AND FIGURES

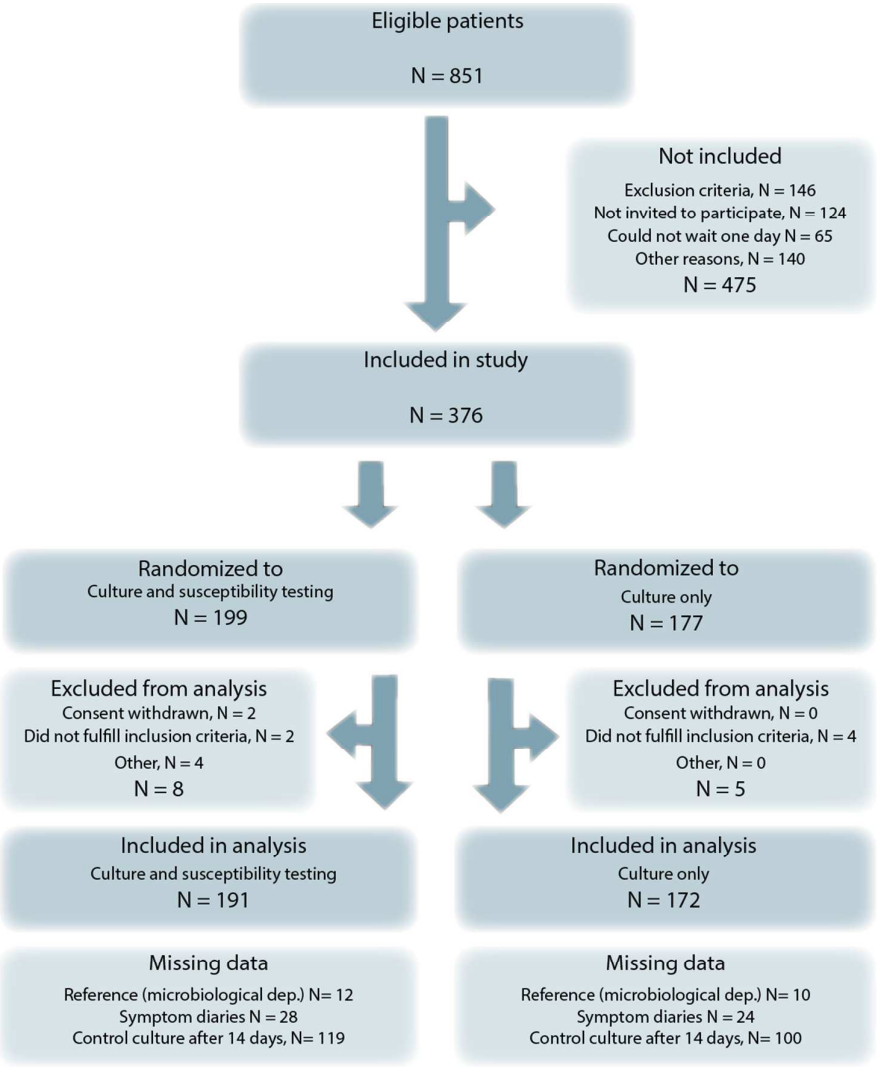


Figure 1: Inclusion flow chart

	Culture and susceptibility, n (%)	Culture only, n (%)	P
Age groups			
Age below 50 years	105 (55%)	83 (48%)	NS
Age 50 years or above	86 (45%)	89 (52%)	NS
Number of days with symptoms			
Symptoms for less than 3 days	77 (41%)	67 (40%)	NS
Symptoms 3 days or more	109 (59%)	101 (60%)	NS
Key symptoms (dysuria, frequency, urge)			
One or two key symptoms	75 (40%)	67 (40%)	NS
Three key symptoms	111 (60%)	100 (60%)	NS
Complicating factors			
Any complicating factor	43 (26%)	62 (38%)	0.0209
Elderly above 65 years	34 (20%)	50 (29%)	0.0496
Recurrent UTI (>3 past year)	11 (6%)	6 (4%)	NS
Uncomplicated diabetes	11 (6%)	17 (10%)	NS
Reference culture and susceptibility test			
Significant growth of uropathogens (UTI)	100 (62%)	104 (64%)	NS
Trimethroprim resistance	27 (26%)	21 (20%)	NS
Sulfamethizole resistance	29 (29%)	24 (24%)	NS
Nitrofurantoine resistance	3 (3%)	3 (4%)	NS
Mecillinam resistance (pivmecillinam)	15 (14%)	9 (9%)	NS

Table 1: Distribution of baseline data between the randomization groups. Numbers are total numbers with percentages in brackets. NS = Not significant

	n	OR (95% CI)	p
Unadjusted analysis			
Odds for appropriate prescribing if culture-only	341	1.44 (1.03-1.99)	0.0311
Odds for being symptom-free on day 5 if culture-only	308	0.91 (0.56-1.49)	NS
Odds for no significant bacteriuria on day 14 if culture-only	144	1.15 (0.62-2.13)	NS
Adjusted for complicating factors			
Odds for appropriate prescribing if culture-only	324	1.65 (1.12-2.42)	0.0112
Odds for being symptom-free on day 5 if culture-only	293	0.89 (0.55-1.44)	NS
Odds for no significant bacteriuria on day 14 if culture-only	140	1.23 (0.64-2.38)	NS
Sub-group analysis (unadjusted)			
Odds for appropriate prescribing for young patients without comorbidities if culture-only	222	1.79 (1.06-3.02)	0.0300
Odds for appropriate prescribing for patients that were elderly, had diabetes or had recurrent UTI if culture-only	102	1.37 (0.78-2.41)	NS

Table 2: Comparison of primary and secondary outcomes between the randomization groups. OR: Odds for having a positive outcome if randomized to culture only (ID Flexicult™) compared to culture and susceptibility testing (Flexicult™ SSI-Urinary Kit). NS = Not significant

		Culture and susceptibility, n (%)	Culture only, n (%)
Appropriate choice of treatment		120 (67%)	121 (75%)
1	UTI and first-line antibiotic and pathogen susceptible	85 (47%)	90 (56%)
2	UTI, second-line antibiotic and pathogen susceptible and first-line antibiotic impossible due to allergies or resistance	0 (0%)	0 (0%)
3	No UTI and no antibiotic	35 (20%)	31 (19%)
Inappropriate choice of treatment		59 (33%)	41 (25%)
1	UTI and no antibiotic	13 (7%)	7 (4%)
2	UTI and antibiotic but uropathogen not susceptible to antibiotic	10 (6%)	7 (4%)
3	UTI and inappropriate second-line antibiotic	3 (2%)	0 (0%)
4	No UTI and antibiotic	33 (18%)	27 (17%)

Table 3: Reasons for appropriate and inappropriate choice of treatment and distribution of patients between groups. The overall difference was significant as shown in Table 2, but none of the individual differences was significant.

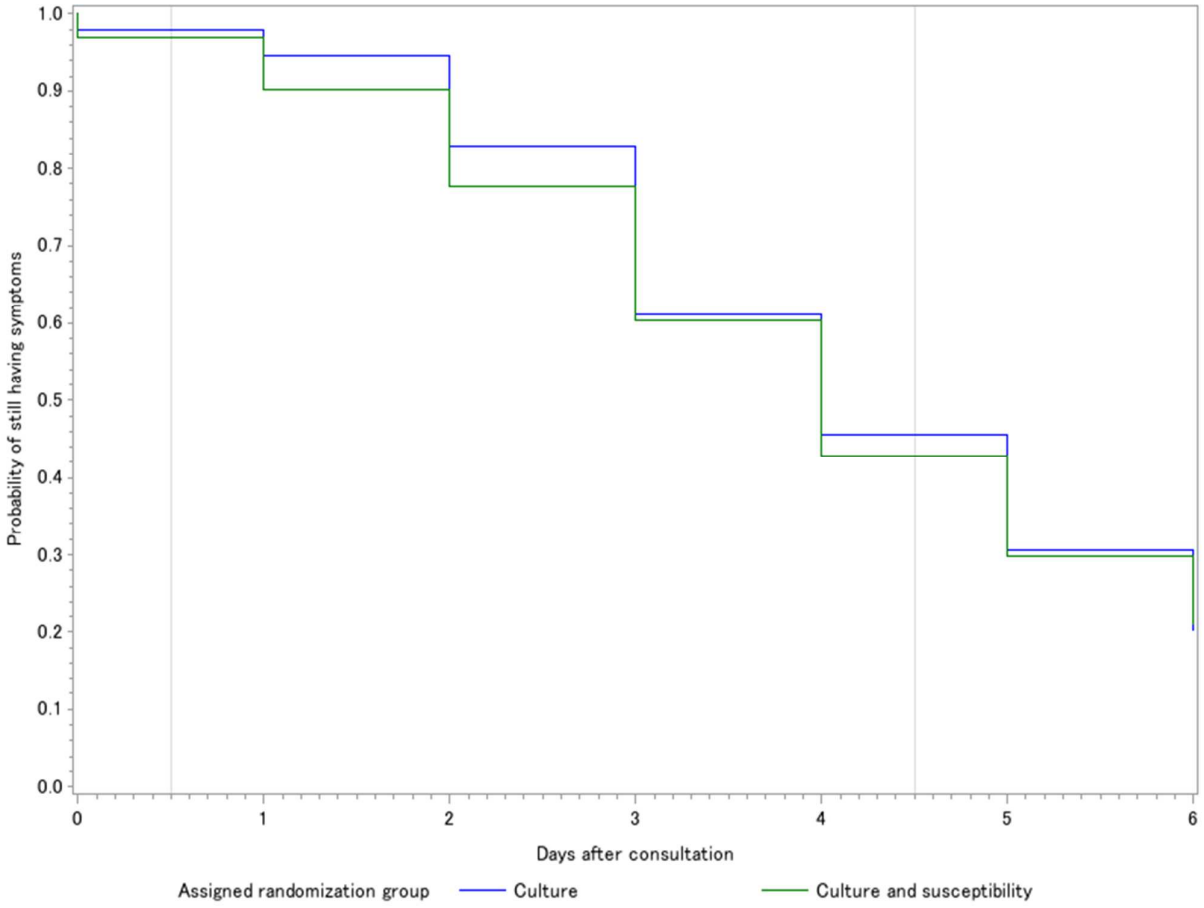


Figure 2: Cure rates for the two groups. The level of the coloured lines indicates the proportion of patients still having symptoms. Day 0 is the evening of the day of the consultation. The first vertical grey line indicates initiation of treatment (the morning after the consultation), the second vertical grey line indicates the data used for calculation of the secondary outcome: clinical cure on day 5 (four days after consultation)

Appendix 1: Data on 341 patients, where the results of both index and reference as well as treatment were available. The red boxes contain cases where the GP was not compliant with the test result. The red numbers are cases where the treatment was inappropriate for any of the reasons stated in Table 3 in the main manuscript.

Appendix 2: Statistical code

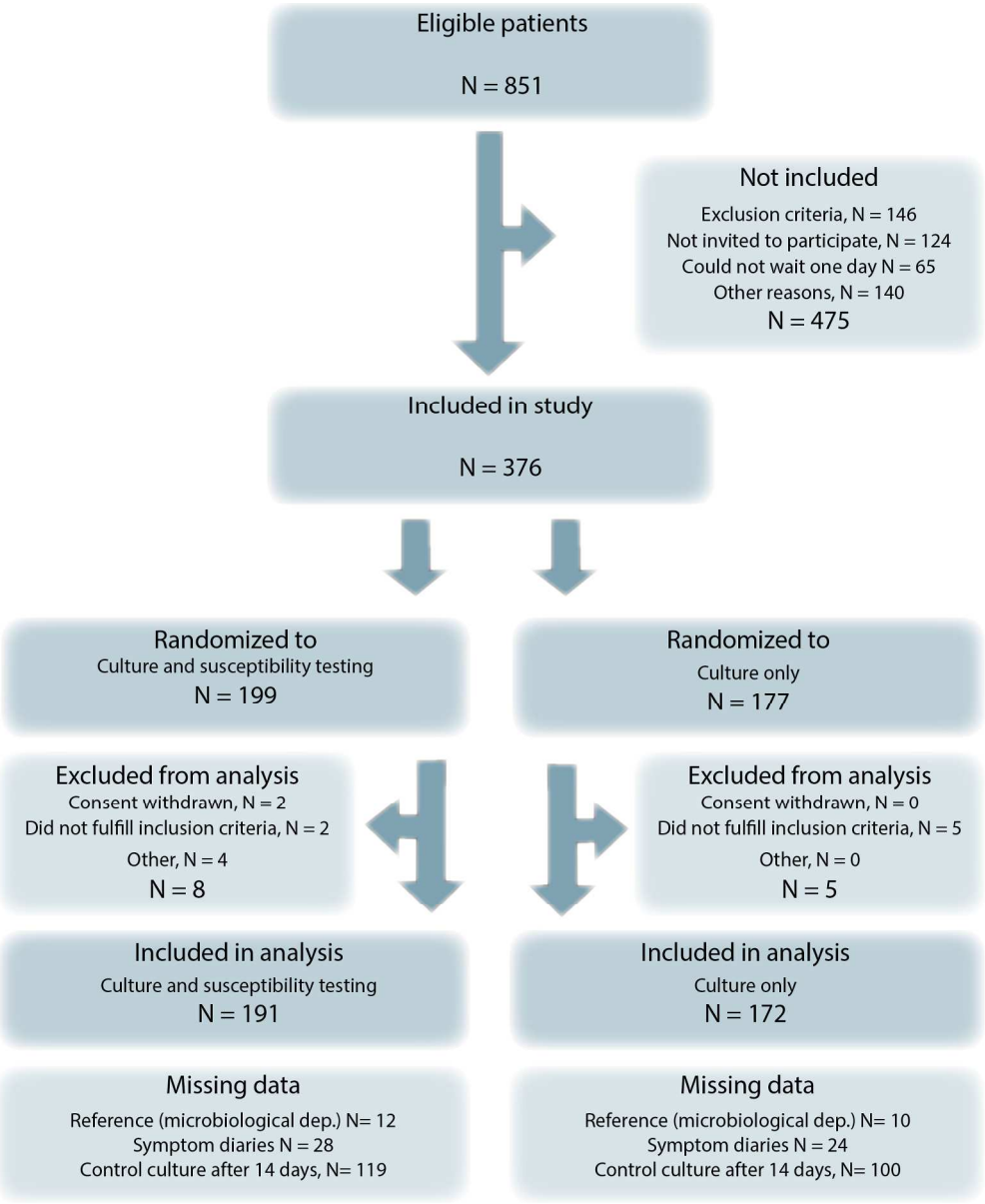
```
LABEL

Age = "Age"

Diabetes = "Diabetes"

Elderly = "Elderly above 65"

Allergy1 = "Intolerance to first-choice antibiotics 1"

Allergy2 = "Intolerance to first-choice antibiotics 2"

Number_UTI = "No of UTIs past year"

Many_UTI = "More than 3 UTIs past year"

Symp_days = "Number of days with symptoms prior to consultation"

keysymp = "Number of key symptoms"

Group= "Assigned randomization group"

Growth = "Growth of uropathogen bacteria in practice culture"

AB_treat ="Treated with antibiotics"

AB = "Choice of antibiotics"

Choice = "Choice of treatment, 1 = first choice AB, 2 = other AB, 0 = No AB"

day_cured = "Dage til helbredelse fra konsultationsdagen, AB p·dag 1"

Days = "Days after consultation"

day1_cured= "Cured on day 1"

day3_cured= "Cured on day 3 or before"

day5_cured= "Cured on day 5 or before"

day7_cured="Cured on day 7 or before"

AB_pt ="Antibiotic treatment according to patient"

Pain_kill = "Taken painkillers"

UTI = "Patient had UTI"

Coli = "E. coli in reference culture"

UTI1_AB0 = "UTI and no antibiotic"

UTI0_AB1 = "No UTI and antibiotic"
```

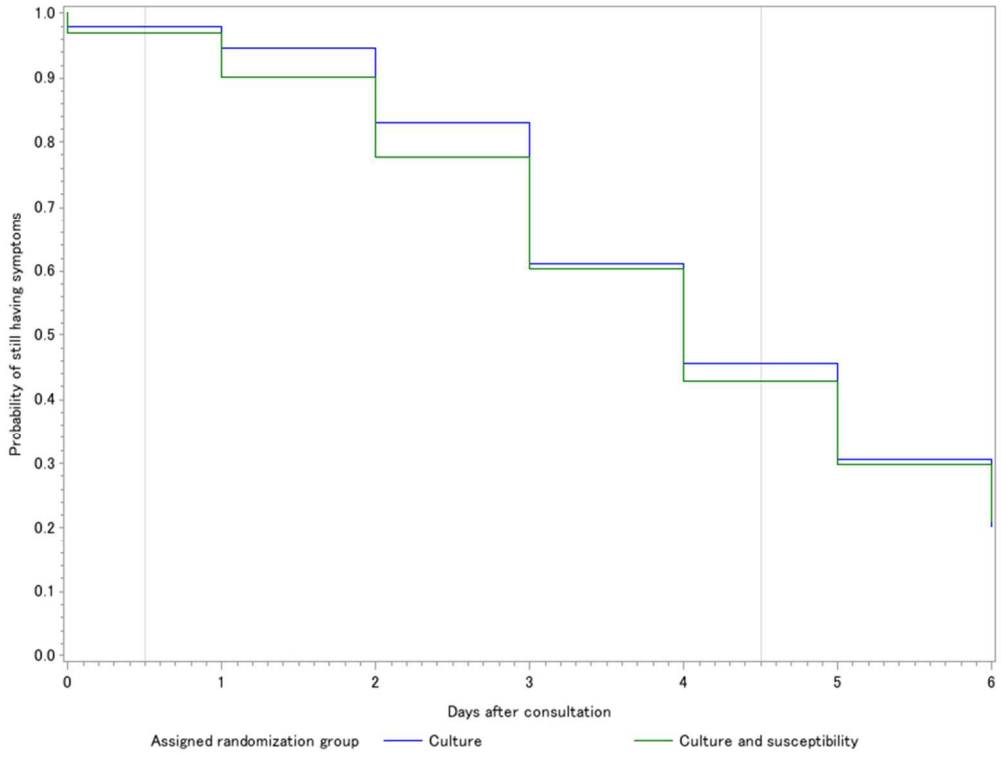
```
UTI0_AB0 = "No UTI and no antibiotic"
DTT = "Decision to treat"
UTI1_AB1_susc ="UTI and first-line antibiotic and pathogen susceptible"
first_impossible = "First-line antibiotic impossible due to resistance or allergy"
UTI1_AB2_susc_first_impossible ="UTI, second-line antibiotic and pathogen susceptible and first-line impossible"
UTI1_AB2_susc1_first_impossible0="UTI and inappropriate second-line antibiotic"
Susc = "Pathogen susceptible to chosen antibiotic"
UTI1_DTT1_susc0="UTI and antibiotic but uropathogen not susceptible to antibiotic"
App = "Appropriate choice of treatment"
inapp = "Inappropriate choice of treatment"
UTI14 = "Patient had UTI 14 days after consultation"
noUTI14 = "No significant bacteriuria 14 days after consultation"
testtreat= "Treated in accordance with index test result"
;

run;

/* Primary Outcome */
/* unadjusted*/
proc genmod data=flexi1 descending ;
class group app clinic_no;
model app=group/dist=bin link=logit type3 lrci;
repeated subject=clinic_no/type=exch;
run;

/* adjusted for complicating factors*/
proc genmod data=flexi1 descending ;
```

```
class group app compl clinic_no;
model app=group compl /dist=bin link=logit type3 lrci;
repeated subject=clinic_no/type=exch;
run;

/* Secondary Outcome */

/* Clinical cure */
/* unadjusted*/
proc genmod data=flexi1 descending;
class group day5_cured clinic_no;
model day5_cured=group/dist=bin link=logit type3 lrci;
repeated subject=clinic_no/type=exch;
run;

/* adjusted for complicating factors*/
proc genmod data=flexi1 descending;
class group day5_cured compl clinic_no;
model day5_cured=group compl/dist=bin link=logit type3 lrci;
repeated subject=clinic_no/type=exch;
run;

/* unadjusted*/
/* Microbiological cure 14 days */
proc genmod data=flexi1 descending ;
class group noUTI14 clinic_no;
model noUTI14=group/dist=bin link=logit type3 lrci;
repeated subject=clinic_no/type=exch;
run;
/* adjusted for complicating factors*/
```

```
proc genmod data=flexi1 descending ;
class group noUTI14 compl clinic_no;
model noUTI14=group compl/dist=bin link=logit type3 lrci;
repeated subject=clinic_no/type=exch;
run;



/* Effect modification example*/


/* adjusted for complicating factors*/
proc genmod data=flexi1 descending ;
class group app compl clinic_no ;
model app=group doctors compl group*doctors/dist=bin link=logit type3 lrci;
repeated subject=clinic_no/type=exch;
run;


/* unadjusted*/
proc genmod data=flexi1 descending ;
class group app  clinic_no ;
model app=group doctors  group*doctors/dist=bin link=logit type3 lrci;
repeated subject=clinic_no/type=exch;
run;



/* Kaplan Meier */
goptions reset=all cback=white border htitle=12pt htext=10pt;
axis1  order= 0 to 6 by 1 label=("Days after consultation");
axis2    order=0  to  1  by  0.1  label=(angle=90  "Probability  of  still  having
symptoms") ;
proc phreg data = flexi1 NOPRINT;
```

```
where censuring<>.;

model Days*censuring(1)=;

strata group;

baseline

out=km

survival=kmcurves

LOWER=LowerBound UPPER=UpperBound

/METHOD=PL CLTYPE=LOGLOG;

Run;

Proc GPLOT DATA=km;

PLOT kmcurves*days=group

/ HAXIS=AXIS1 HREF=0.5 4.5 LHREF=1 CHREF=GRAYDD

  VAXIS=AXIS2 LEGEND=LEGEND1;

SYMBOL1 C=Blue V=None I=STEPLJ;

SYMBOL2 C=Green V=NONE I=STEPLJ;

      ;

run;
```


Eligible patients
N = 851
Not included
Exclusion criteria, N = 146
Not invited to participate, N = 124
Could not wait one day N = 65
Other reasons, N = 140
N = 475
Included in study
N = 376
Randomized to
Culture and susceptibility testing
N = 199
Randomized to
Culture only
N = 177
Excluded from analysis
Consent withdrawn, N = 2
Did not fulfill inclusion criteria, N = 2
Other, N = 4
N = 8
Excluded from analysis
Consent withdrawn, N = 0
Did not fulfill inclusion criteria, N = 5
Other, N = 0
N = 5
Included in analysis
Culture and susceptibility testing
N = 191
Included in analysis
Culture only
N = 172
Missing data
Reference (microbiological dep.) N= 12
Symptom diaries N = 28
Control culture after 14 days, N= 119
Missing data
Reference (microbiological dep.) N= 10
Symptom diaries N = 24
Control culture after 14 days, N= 100



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BMC Family Practice

STUDY PROTOCOL

Open Access

Point of care susceptibility testing in primary care - does it lead to a more appropriate prescription of antibiotics in patients with uncomplicated urinary tract infections? Protocol for a randomized controlled trial

Anne Holm[1*], Gloria Cordoba[1], Tina Møller Sørensen[2], Lisbeth Rem Jessen[2], Volkert Siersma[1] and Lars Bjerrum[1]

Abstract

Background: Urinary tract infection (UTI) is a common infection in primary care and is the second leading reason for prescription of antibiotics in Denmark. The diagnosis is often based on symptoms and urine dip-stick, which has limited validity, causing the risk of unnecessary antibiotic prescription. Additionally, with increasing antibiotic resistance, the risk of choosing an antibiotic to which an infecting pathogen is resistant is rising. Combined point-of-care-tests (POCT) for urine culture and susceptibility testing have been developed and validated for primary care, and performing such a test in all patients with suspected UTI in primary care seems rational in order to reduce the use of inappropriate antibiotics. However, the clinical effect of the culture and susceptibility test has not yet been investigated. This study aims to investigate whether POCT urine culture and susceptibility testing decreases the inappropriate use of antibiotics and leads to faster patient recovery.

Methods/design: Randomized controlled open label trial of two diagnostic approaches. 750 patients with symptoms of uncomplicated UTI, consecutively contacting their general practitioner (GP), randomized to either POCT urine culture and susceptibility testing and targeted treatment or POCT urine culture without susceptibility testing and empirical treatment. Treatment is started when the POCT is read. The two groups are compared with regard to appropriate choice of antibiotics, clinical remission, and microbiological cure rates.

Discussion: The results of this study may provide important evidence to recommend POCT culture and susceptibility testing in all patients with suspected uncomplicated UTI. This could become an additional strategy to fight antibiotic resistance.

Trial registration: ClinicalTrials.gov NCT02323087.

Background

Antibiotic resistance is rapidly spreading, making it one of the most serious threats to human health. The World Health Organization has stated that a post-antibiotic era is a very real possibility and that urgent actions are needed in order to maintain the effect of antibiotics [1].

Primary health care in Denmark is responsible for about 90 % of all redeemed prescriptions of antibiotics, and it is known that a high out-patient consumption of antibiotics leads to high levels of resistance [2, 3]. Thus, a cornerstone in the efforts to reduce antibiotic resistance is to reduce and improve prescription of antibiotics in primary health care.

In 2008, 1.8 % of all patients consulting their GP in Denmark were diagnosed with a UTI [4]. Resistant strains of *E. Coli*, which is the causative organism in 70–80 % of all UTIs, are spreading world-wide [5, 6]. In Denmark,

* Correspondence: anneholm@sund.ku.dk
[1]Department of Public Health, The Research Unit for General Practice and Section of General Practice, University of Copenhagen, Øster Farimagsgade 5 opg. Q, PO box 20991014 Copenhagen K, Denmark
Full list of author information is available at the end of the article

33–40 % of *E. Coli* isolated from urine samples from primary care are resistant to sulfamethizole and 6–10 % to pivmecillinam, which account for about 80 % of all antibiotic treatments of adults with UTI in primary care in Denmark [3, 7]. It is, therefore, critical that a UTI is treated only when clinically indicated and using an appropriate antibiotic, i.e., one to which the infecting pathogen is susceptible, taking into account the use of first-line agents over second-line agents.

Urine culture is necessary to accurately determine if a patient has a UTI since other tests have limited predictive values in primary care and treating based on symptoms can cause up to 50 % being inappropriately treated [8, 9]. Susceptibility testing adds the advantage to predict whether a first-line antibiotic can be expected to eliminate the infecting pathogen. However, delaying treatment for several days while waiting for the results of the susceptibility test cannot be justified as symptoms are painful and affect quality of life [10, 11]. Point of care test (POCT) culture and susceptibility testing provides the result within 24 h, and can, therefore, be used to target individual therapy without compromising patient welfare. Inappropriate antibiotic prescribing can be partly avoided by performing a POCT culture since this will assumedly eliminate treatment of patients without bacteriuria. However taking into account the above-mentioned resistance rates in *E. Coli* and for example enterococci being inherently resistant to both antibiotics, this could result in about 20–30 % inappropriate antibiotic prescriptions for UTI. Adding susceptibility testing to the POCT should raise the appropriate antibiotic prescriptions above 90 %.

This study aims to answer the questions: 1) Does POCT urine culture and susceptibility testing decrease the use of inappropriate antibiotics, and 2) Does targeted therapy improve clinical outcomes in patients with suspected uncomplicated UTI in general practice when compared to POCT urine culture without susceptibility testing? We hypothesize that the use of POCT susceptibility testing improves the following outcomes: Appropriate choice of antibiotic, clinical remission, and microbiological cure rate.

Methods

Study design

Randomized controlled open label trial of two diagnostic approaches in a primary care setting.

Recruitment process

General practitioners (GPs) 200 general practices in the Copenhagen area will be contacted by letter with the aim of recruiting 50 GPs. All GPs will receive relevant training in the use of POCT culture and susceptibility testing, and their skills will be validated using an online test on how to read the POCT.

Patients

Patients presenting with symptoms of UTI will be recruited at the general practice during consultation. To ensure interpretation of POCT within 24 h, only patients contacting practice from Monday to Thursday will be included. Each GP will recruit and randomize 15 patients.

Inclusion criteria

Female adult patients, 18 years or older, presenting at their GP with dysuria, frequency or urgency, which has been present for 7 days or less, and for which the GP suspects uncomplicated UTI (including recurrent UTI, uncomplicated diabetes mellitus defined as orally treated, well regulated and without secondary complications, and elderly patients). Patients should be able to deliver a mid-stream urine sample, to provide informed consent, and should be willing and able to fill out a symptom diary.

Exclusion criteria

- Negative dip-stick analysis on leucocytes and nitrites (to reduce the number of negative cultures)
- Complicated UTI
  - Known pregnancy
  - Severe systemic symptoms, high fever, flank pain
  - Recent bladder surgery (within past 4 weeks)
  - Urinary tract abnormalities
- Serious systemic disease
  - Life-threatening cancer
  - Insulin-dependent diabetes
  - Long-term corticosteroid treatment
  - Other conditions with compromised immunity
- Former participation in the study
- Patients presenting on a Friday (since POCT is read after 24 h)

Randomization and groups

The patients are block randomized in blocks of 10 to ensure approximately equal sizing of the groups. The randomization group for each patient is placed in a sealed envelope which is opened either during or after consultation.

- For the intervention group, POCT culture and susceptibility testing is performed. Treatment is based on the result of the susceptibility test and clinical guidelines.
- For the control group, POCT culture without susceptibility testing is performed, and treatment is based on clinical guidelines.

Informed consent

All patients receive oral and written information before signing informed consent forms.

Screening logs

All participating general practitioners, secretaries, and nurses will be asked to maintain an anonymous screening log of all patients fulfilling the inclusion criteria in the inclusion period. This will be used to assess selection and its effect on the study results and for the attrition flow chart.

Data collection

Case-report form

After oral and written information about the project and written consent to enrollment, the GP will take a structured history and fill out a case report form. Data from day 1 consist of:

- Name and social-security number
- Drug allergies
- Diabetes
- Number of UTIs within past year
- Symptoms of UTI
  - Dysuria
  - Frequency
  - Urgency
- Duration of symptoms
- Randomization group

The patients are asked to contact the GP the next morning by telephone or e-mail for treatment. The patients are also asked to contact the GP if symptoms persist after 4–5 days. The GP can advise on painkillers if necessary.

The next day, the GP will read the plate and inform the patient about the result and potential treatment with antibiotics. The GP will complete the case report form including the following data:

- Reading of culture plate
  - No significant growth of uro-pathogens
  - Significant growth of at least one uro-pathogen
  - Inconclusive
- For identified uro-pathogen(s):
  - Species
  - Amount in cfu/mL
  - Resistance pattern towards trimetroprim, sulfamethizol, ampicillin, nitrofurantoin, and pivmecillinam (intervention group)
- Treatment:
  - Name, dose, and duration of antibiotic

Symptom diary

The patients are asked to compile and return a paper symptom diary. Through personalized text messages, they are reminded on day 3 to fill out the diary and on day 7 to send it to the Section of General Practice, University of Copenhagen. If they have not sent the diary on day 10 or do not have a cell phone, they receive a phone call. The diary has been face- and content validated through focus groups and personal interviews. The scales for symptom severity, bothersomeness and impact on daily activities are currently under psychometrical validation using the partial credit Rasch model for polytomous items. The secondary outcome of clinical cure is measured using a single item where the patient by the end of each day answers if her symptoms of urinary tract infection are completely gone. The scales for symptom burden are not a part of the secondary outcome but serves to improve the patient's evaluation of her own cure.

The diary measures:

- Employment status, job and number of employees
- Use of medication other than antibiotics and painkillers
- Symptom severity on day 1–7
- Symptom bothersomeness on day 1
- Impact on daily activities on day 1
- Use and possible change of antibiotics on day 1–7
- Use of painkillers on day 1–7
- Re-consultation with their GP/out-of-hour service on day 1–7
- Sick-leave on day 1–7
- Day of becoming symptom-free

Urine samples

A mid-stream urine sample from the day of consultation will be divided in two. One part is sent to the local microbiological department, and the other part will be examined at the general practice using the POCT. On day 14 another urine sample will be sent to the local microbiological laboratory for culture.

Microbiological analyses performed at the microbiological laboratory– Gold standard

A mid-stream urine sample from day 0 to day 14 are analyzed at the local microbiological laboratory. The sample from day 0 serves as a quality control of the culture and susceptibility testing performed in general practice. The sample from day 14 is the microbiological outcome measure. The samples are transported to the microbiological laboratory in Urine-Monovette® (Sarstedt) containing boric acid to stabilize the bacterial count.

At the microbiological laboratory urine sample are dispersed on Inoqul A™ Bi-plate (CHROMagar and blood agar) with 10 μL on each half of the agar. The susceptibility pattern is determined on Mueller Hinton agars with disks containing mecillinam, cefpodoxim, cefuroxim, gentamicin, piperacillin + tazobactam, meropenem, ampicillin, nalidixic acid, trimethoprim, nitrofurantoin, sulfamethizol, and vancomycin. All samples are quantified. If the bacterial

count on the two agars on Inoqul A™ differs with more than a factor 10, the procedure is repeated.

Significant growth is defined as growth of $\geq 10^3$ colony forming units per millilitre (cfu/mL) for *E. coli* and *S. saprofyticus*, $\geq 10^4$ cfu/mL for other typical uro-pathogens and $\geq 10^5$ cfu/ml for possible uro-pathogens following current consensus [12]. All pathogens with significant growth are identified and susceptibility pattern determined. Any pathogen growing at least 10^3 cfu/ml, unless the above mentioned criteria are fulfilled, is classified as contamination, and in these cases the susceptibility pattern is not determined. Insignificant growth is defined as $\leq 10^2$ cfu/mL or less. Susceptibility pattern is determined according to EUCAST and NordicAST recommendations. The internal quality control is performed measuring inhibition zones on chosen reference strains from American Type Culture Collection (ATCC) and National Collection of Type Cultures (NCTC).

Microbiological analyses performed on-site at the general practice

Culture (control group) Point-of-care culture will be performed using ID Flexicult™ (SSI DIagnostica, Denmark) which is a chromogenic agar plate for identification and quantitation of urinary tract pathogens. The sample is seeded with a 10 μL inoculation needle, the lid is applied, and the agar plate incubated upside down at 35 °C overnight. The plate is read the next day. If it is positive, no further incubation is needed, if it is negative, incubation is continued until 24 h after inoculation. The bacterial identification is based on colony color and size. The agar plate can be seen on the right side of Fig. 2.

Culture and susceptibility testing (intervention group) Urine culture and susceptibility testing will be performed on the intervention group by means of a POCT, the FLEXICULT™ SSI-Urinary Kit (SSI Diagnostica, Denmark). The kit is a chromogen agar in an ordinary Petri dish, but with higher sides. The Petri dish is divided into 6 compartments: 1 large compartment for quantitative analysis and 5 smaller compartments for susceptibility testing. The agar in each of the smaller compartments contains 1 of 5 antimicrobials: trimethoprim, sulfamethoxazole, ampicillin, nitrofurantoin, and mecillinam. The agar plate can be seen on the left side of Fig. 2. The agar plate is flooded with the urine specimen for a couple of seconds and then incubated at 35 °C over night. The plate is read on the following day.

As the concentrations of the antimicrobials in the 5 smaller compartments are adjusted in accordance with breakpoints, growth on these compartments indicates resistance of the pathogen in question and hence a potential risk of treatment failure.

Figure 1 illustrates the data collection process and Fig. 2 explains the study design and the difference between the intervention and control arm.

Definition of outcomes

Primary outcome

– The proportion of patients receiving appropriate antibiotic treatment on the day after consultation. Data obtained from case-report form.

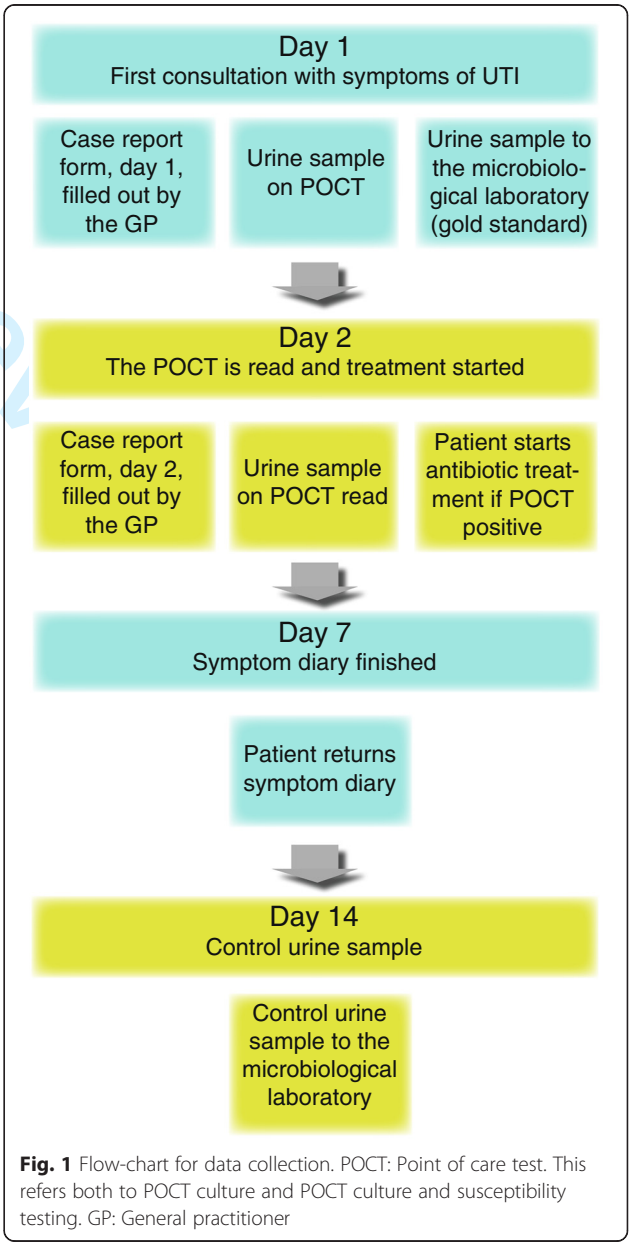

Fig. 1 Flow-chart for data collection. POCT: Point of care test. This refers both to POCT culture and POCT culture and susceptibility testing. GP: General practitioner

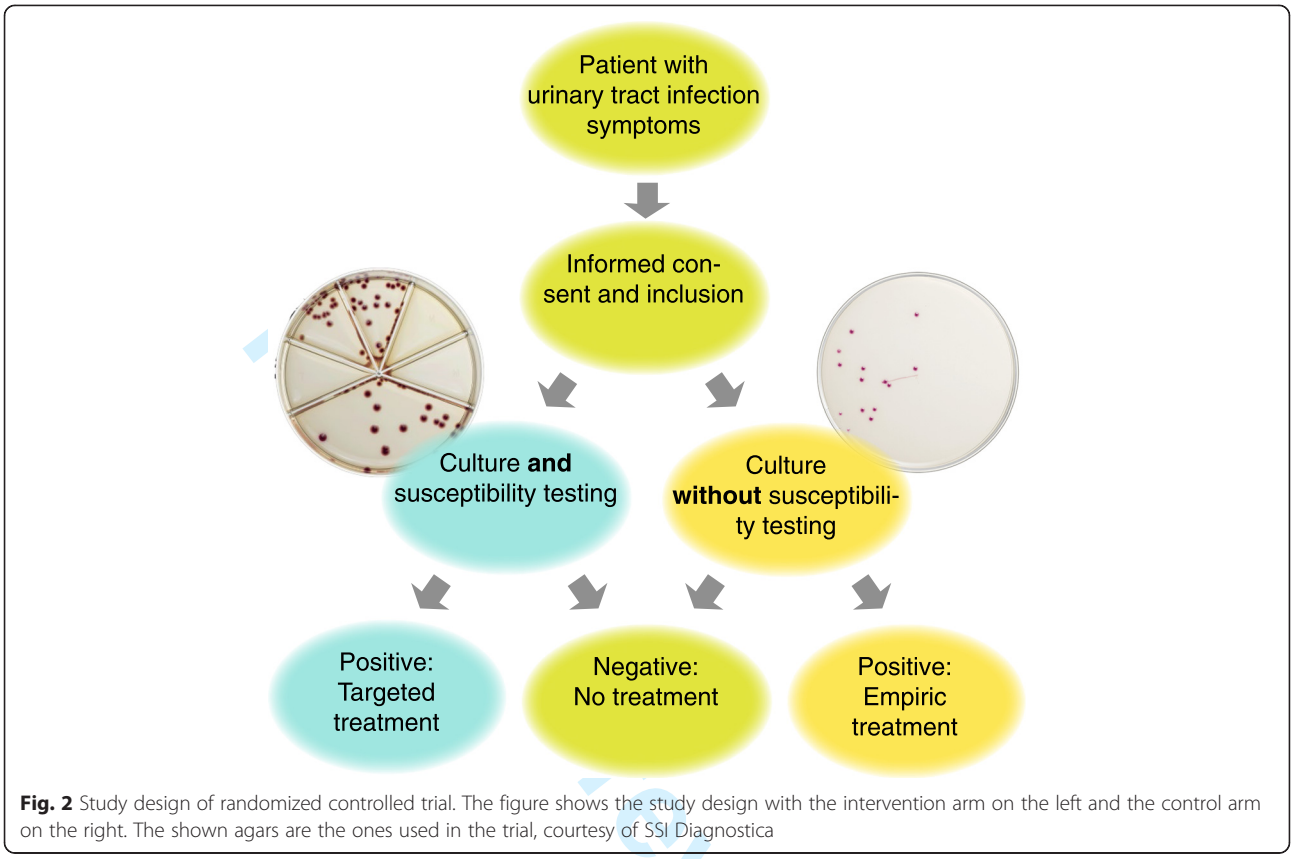

Fig. 2 Study design of randomized controlled trial. The figure shows the study design with the intervention arm on the left and the control arm on the right. The shown agars are the ones used in the trial, courtesy of SSI Diagnostica

Appropriate antibiotic treatment is defined as receiving a first-line antibiotic to which the infecting organism is susceptible, if there is significant growth in the gold standard or receiving any antibiotic to which the infecting organism is susceptible if there is significant growth in the gold standard if the patient is allergic or the infecting organism is resistant to all first-line antibiotics or not receiving an antibiotic if there is no significant growth in the gold standard. The definition is illustrated in Fig. 3.

Secondary outcomes

- The proportion of patients who are asymptomatic on the fourth day of treatment (clinical cure) defined as the patient stating, her symptoms are completely gone regardless of symptom score. Data obtained from symptom diaries
- The proportion of patients with no significant bacteriuria on day 14 (bacteriological cure). Data obtained from control urine sample

Ethical aspects and patient safety

The study has been approved by the Ethical Committees for the Capital Region of Denmark and reported to the Danish Data Protection Agency. All patients entering this study receive a higher level of diagnostics and treatment than standard care at the moment. The improved diagnostics and, thereby, the reduction of overtreatment will benefit the individual patient more than the disadvantage of delaying treatment. All data are kept under the same security as other sensitive data at a GP office. In case of any adverse event that could be attributed to participation in the trial (eg. worsening of symptoms due to delay of treatment), the GP in charge of care of the participant will follow a flow-chart to determine if the trial-responsible investigator should be notified and how fast. If the event is considered harmless or unlikely to be related to the trial, it is registered on the case-report form. If it is considered serious and likely related to the trial, the trial coordinator is contacted by telephone within 24 h. At least two members of the trial team evaluate serious events related to the trial and decide if the trial team should be gathered. All adverse events that could be attributed to participation in the trial are recorded and analyzed biannually by the coordination team. All results, positive, negative, and inconclusive, will be published.

Analysis

Sample size calculation

Primary outcome The proportion of appropriately treated patients in the control group is assumed to be 70–80 %

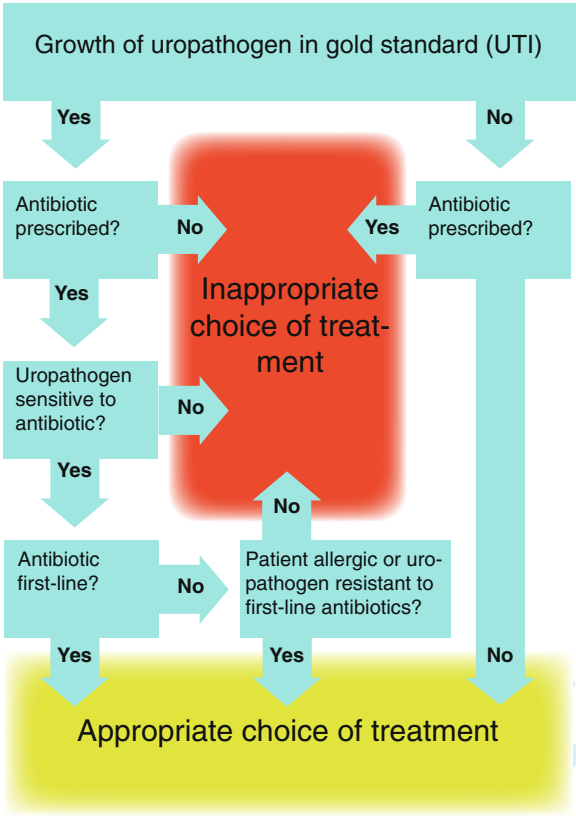

Fig. 3 Short title of figure: Flow-chart for primary outcome and definition of appropriate and inappropriate choice of treatment

based on an assumption that POCT culture will be precise in determining UTI, but current local resistance rates in *E. Coli* (70–80 % of infections) of about 6–10 % to pivmecillinam (50 % of patients with UTI) and 30–40 % to sulfamethizole (30 % of patients with UTI) will result in inappropriate treatments as defined in Fig. 3 [3, 7]. To detect a statistically significant ($\alpha = 0.05$) 10 percentage-point difference between the two groups with 80 % probability, assuming an intra-class correlation of 0.2 between patients in the same practice, a sample of 600 patients is needed. In order to take possible drop-outs and sub-analyses into account, the study aims to enroll 750 patients.

Secondary outcomes

Clinical remission

McNulty and Ferry reported clinical cure rates of 69 % on day 5 after targeted treatment with trimethoprim and 44 % on day 5 empiric treatment with pivmecillinam respectively in patients with uncomplicated UTI [13, 14]. Assuming a cure rate of 60–70 % on the fourth day of treatment (day 5) in the intervention group, a difference of at least 15 percentage points could be detected with the chosen sample while accounting for a 25 % drop-out on clinical follow-up.

Bacteriological cure rate

Since bacteriological cure with empiric antibiotics on day 8–10 is about 90 % [14] as reported in a Swedish study, we are not expecting to see a significant difference between the groups regarding this outcome.

Statistical analysis

Comparison of the two randomization groups for both the primary and the secondary outcomes will be done by means of an odds ratio (OR) from a logistic regression model; clustering within practices is adjusted for by generalized estimating equations (GEE). Analyses will be performed intention-to-treat, i.e., the patients are analyzed in the groups they are randomized to regardless of the treatment they actually received. Effect modification – whether the effect of the intervention differs between subgroups in the data – will be investigated for GP factors (organization of practice, performance in reading the POCT), patient factors (age, concurrent illness, socio-demographic data, initial symptom score) and microbiological factors (amount and species). If a sufficient sample is obtained, sub-group analysis will be performed for patients with diabetes, elderly patients and patients with recurrent UTI since these groups are expected to benefit the most from the intervention. In an additional analysis of the primary outcome, the group inappropriately treated will be divided into under-treated and over-treated and analyzed in multinomial logistic regression models. Comparison of cure-rates will be done with Kaplan-Meier curves and log-rank tests. A P-value of 0.05 will be considered significant. Analyses will be performed with SAS v9.4.

Discussion

In Denmark, POCT combined culture and susceptibility testing has been in use for decades, and the use has increased since introduction of the FLEXICULT™ SSI-Urinary Kit. Despite this popularity no clinical trials have yet validated its impact in clinical practice. This study will investigate the effect of POCT susceptibility testing on appropriate choice of antibiotics and on clinical and microbiological cure in patients with uncomplicated UTI in primary care in Denmark.

The clinical effect and cost-effectiveness of POCT culture and susceptibility testing in UTI is currently being investigated by another research group [15]. Although both studies aim at investigating the effect of the Flexicult on the appropriateness of antibiotic use and the impact on patient outcomes, there are at least three important differences. Firstly, in the study by Bates et al., the effect of combined culture and susceptibility testing is compared to

various forms of standard care in a four-country multicenter setting. The focus of the present study is narrower, specifically aiming at determining the value of the susceptibility component compared to culture alone in a single region in Denmark. Secondly, all GPs in this study are experienced users of POCT susceptibility testing, and their skills are validated before enrollment of patients as described under the recruitment process, thus inter-practice variation is minimized. Thirdly, in this study, both groups will have treatment delayed until a positive culture is obtained, thereby minimizing the number of culture-negative patients receiving inappropriate antibiotic treatment.

We have chosen to include patients with diabetes, recurrent UTI and elderly patients when they are otherwise healthy and can be safely included. In the analysis, they are investigated for effect modification and, if the sample allows it, they are analyzed separately, since they could be expected to benefit more from the intervention than other groups.

A challenge of this study is the similarity between the intervention and control groups. The potential difference between the groups in this study will mainly be driven by those patients in the control group receiving an antibiotic to which, the infecting pathogen is resistant. Since *in vitro* resistance rates in Denmark against the most commonly used antibiotics for UTI are 15–40 %, the effect could turn out minor at present [3]. If the study detects no additional benefit of susceptibility testing over culture alone, this will provide important information for the Danish national health care system. However, the results may not be directly applicable to countries outside Scandinavia. On the other hand, if susceptibility testing proves superior to culture alone, the impact of such a finding will likely be much higher in countries where resistance rates are higher. In conclusion, the present study will test the hypothesis that POCT susceptibility testing for uncomplicated UTI and individually targeted therapy will decrease the use of inappropriate antibiotics and positively influence clinical cure rates. If this proves true, the results of the study may provide important evidence to recommend POCT susceptibility testing for patients with suspected UTI. This could become one of many strategies to fight antibiotic resistance.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
AH is the primary investigator, responsible for the design and conduction of the trial. LB has conceived the study and taken part in the design. GC has taken part in the design and conduction of the study. LRJ and TS have taken part in the design of the study. AH and TS have proposed the statistical analysis, and VS has revised this section. AH has drafted the manuscript. All authors have critically revised the manuscript and approved the final version.

Authors' information
Anne Holm: Medical doctor and PhD student at The Research Unit for General Practice and Section of General Practice, Department of Public Health, University of Copenhagen, Denmark
Gloria Cordoba: Medical doctor and PhD student at The Research Unit for General Practice and Section of General Practice, Department of Public Health, University of Copenhagen, Denmark
Tina Møller Sørensen: Doctor of Veterinary Medicine and PhD student at University Hospital for Companion Animals, Dept. of Clinical Veterinary and Animal Sciences, University of Copenhagen
Lisbeth Rem Jessen, Doctor of Veterinary Medicine and assistant professor in internal medicine at the University Hospital for Companion Animals, Dept. of Clinical Veterinary and Animal Sciences, University of Copenhagen
Volkert Siersma: Statistician employed at the The Research Unit for General Practice and Section of General Practice, Department of Public Health, University of Copenhagen, Denmark
Lars Bjerrum: General practitioner and professor in general practice at The Research Unit for General Practice and Section of General Practice, Department of Public Health, University of Copenhagen, Denmark

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Author details
[1]Department of Public Health, The Research Unit for General Practice and Section of General Practice, University of Copenhagen, Øster Farimagsgade 5 opg. Q, PO box 20991014 Copenhagen K, Denmark. [2]Department of Veterinary Clinical and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Dyrlægevej 16, 1870 Frederiksberg C, Denmark.

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Health and Quality of Life Outcomes

RESEARCH

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Development and validation of a condition-specific diary to measure severity, bothersomeness and impact on daily activities for patients with acute urinary tract infection in primary care

Anne Holm*, Gloria Cordoba, Volkert Siersma and John Brodersen

Abstract

Background: Urinary tract infection (UTI) is a common condition in primary care. Patient-reported outcome measures (PROMs) are crucial in the evaluation of interventions to improve diagnosis, treatment and prognosis of UTI. The aim of this study was to identify an existing condition-specific PROM to measure symptom severity, bothersomeness and impact on daily activities for adult patients with suspected urinary tract infection in primary care; or, in the absence of such a PROM, to test items identified from existing PROMs for coverage and relevance in single and group interviews and to psychometrically validate the resulting PROM.

Methods: The literature was searched for existing PROMs covering the three domains. Items from the identified PROMs were tested in single and group interviews. The resulting symptom diary was psychometrically validated using the partial credit Rasch model for polytomous items in a cohort of 451 women participating in two studies regarding UTI.

Results: No existing PROM fulfilled the inclusion criteria. Content validation resulted in one domain concerning symptom severity (18 items), one concerning bothersomeness (18 items), and one concerning impact on daily activities (7 items). Psychometrical validation resulted in four dimensions in each of the first two domains and one dimension in the third domain.

Conclusions: Domains were not unidimensional, which meant that we identified dimensions of patient-experienced UTI that differed substantially from those previously found. We recommend that future studies on UTI, in which PROMs are to be used, should ensure high content validity of their outcome measures and unidimensionality of the included dimensions.

Keywords: Urinary tract infections, Cystitis, Validation studies, Psychometrics, Item-response theory, Rasch analysis, Patient-reported outcomes, Patient-reported outcome measures, PROM, Primary care

* Correspondence: anneholm@sund.ku.dk
Research Unit for General Practice and Section of General Practice, Department of Public Health, University of Copenhagen, Øster Farimagsgade 5, PO box 2099, 1014 Copenhagen, Denmark

Background

Urinary tract infection (UTI) is a common condition and accounts for about 2% of consultations in general practice in Denmark [1]. It mainly affects women, one in every two women experiences a UTI at least once in her life-time [2]. Symptoms of UTI are known to be painful and bothersome, impacting quality of life [3–6]. In addition to the symptoms experienced by the patient, laboratory confirmation of a significant amount of bacteria in the urine is required for the diagnosis of a clinically relevant UTI [7]. Patient-reported outcome measures (PROMs) are important both for the evaluation of the extent to which an intervention can improve the diagnosis and treatment of UTI, and for following the patient's experience of symptoms and recovery. A PROM should be face and content validated to ensure that its items are relevant and covering for the construct that is to be measured. Moreover, items and instructions in the PROM should be clear and understandable for the target population [8–10]. If the PROM encompasses domains of items, then these should be psychometrically validated in a larger sample of the target population using item response theory (IRT) models to ensure unidimensionality of the domains allowing for sum scores [11]. When items in a domain fit IRT Rasch models, invariant measurement is achieved [12–16]. A number of PROMs exist, but to our knowledge none of them have been tested for both content validity and unidimensionality of domains using IRT models [6, 17–19].

The aims of this study were to 1) Perform a literature search to identify an existing condition-specific PROM to measure symptom severity, bothersomeness and impact on daily activities over time for adult patients with uncomplicated and complicated UTI in primary care; or 2) in the absence of such a PROM, to test items identified from existing PROMs for relevance in single and group interviews with patients who had experienced UTI; and 3) to psychometrically validate the resulting PROM using Rasch models.

Methods

Aim 1: literature search for existing PROMs

We searched Medline and Embase for development and validation studies published before September 2014 in English, Swedish, Danish or Norwegian. Combinations of the words "urinary tract infection", "cystitis", "patient-reported outcome measure", "psychometrics", "PROM", "instrument", "validation" and "scale" were used.

Inclusion criteria

PROM development and validation studies performed in primary care or a comparable setting investigating adult patients with symptoms of UTI including the three domains: Symptom severity, symptoms bothersomeness and impact on daily activities and reporting a sufficient content validation involving either single or groups interviews to ensure coverage and relevance of items and a sufficient content validation using IRT models and analysis of differential item functioning (DIF).

Aim 2: face and content validity

Overview of content validation procedure

The process of content validation involved two primary elements: 1) Item generation and construction of a draft PROM, 2) single and group interviews with members of the target population.

Item generation

Items relevant to the three domains were selected from existing PROMs identified in the literature search. To narrow down the initial pool of items, the items that had proved most predictive of confirmed UTI in previous research were selected and some items were modified based on clinical experience [20, 21]. Double-barreled items (For example "pain or burning when passing urine") were split into two individual items. The resulting draft version of the PROM was converted into a 7-day symptom diary, one of several types of PROMs.

Group interviews

Group interviews were aimed at expanding our knowledge on symptoms experienced by patients with UTI, their bothersomeness and impact on daily activities. The method of group interviews was chosen to ensure a dynamic generation of new items and an open discussion about the content and layout of the diary [22]. The participants were encouraged to talk about their experience of having UTI using open-ended questions. When they had no more new symptoms or activities to add, the draft version of the diary was presented. The participants completed the draft version of the diary and it was corrected according to their suggestions. Participants were recruited from a general practice, an elderly activity center and the researchers' network. The group interviews took about two hours and were audio recorded; the recordings were used to analyze the interviews and change the draft version of the diary.

Single interviews

The purpose of single interviews was to ensure relevance, coverage and understandability of the diary. The participant firstly told about his or her experience of the UTI. Afterwards, the participant was told to complete the diary and comment on relevance, coverage and understandability. Female participants were recruited from the researchers' network and male participants were recruited at a urological department. Single interviews lasted about 30 min.

Aim 3: psychometric validation

Patient recruitment

Patients with symptoms of UTI participating in two ongoing studies [23, 24] were asked to complete the diary after seeing their general practitioner. The diary was handed out in the consultation and the participant returned it by post using a prepaid reply envelope. We used text-message reminders and telephone calls to remind patients to complete and return the diary.

Statistical analysis: Rasch analysis

The responses were analyzed using the partial credit Rasch model for polytomous items [25, 26]. If an item shows misfit to a Rasch model, it indicates that the item does not belong to the same theoretical dimension. We tested the three domains for unidimensionality. If items showed misfit we tested alternative configurations of items based on clinical, empirical or theoretical relations between symptoms rather than results from analyses. The resulting dimensions were tested for DIF. DIF indicates that other factors, such as age, affect the responses to a specific item, causing the scale to behave differently in the different subgroups [27, 28]. Finally, we tested for local dependency (LD) to evaluate whether individual items within the resulting dimensions were so closely linked that they, to some extent, were measuring the same nuances of the construct. If two items have high local dependency, they nearly correspond to a single item. Since individual symptoms are known to have poor predictive value for confirmed UTI, we did not test for discriminative ability of the identified dimensions [20, 21]. If an item did not fit any dimensions it was kept in the final questionnaire if it had high content validity.

Data management and statistics

The psychometric properties of the involved scales were tested for unidimensionality, homogeneity and DIF in relation to age, study group, and confirmed UTI, by using likelihood ratio tests on appropriately conditioned Rasch models [29]. Confirmed UTI was defined as having significant growth of uropathogens in a reference culture. The patient was not aware of the result at the time of completing the diary. The reliability of the dimensions was examined using Cronbach's alpha (Table 2). Statistical analyses were performed in DIGRAM [30]. To adjust for multiple testing the false discovery rate was fixed at 5% for each set of analyses using the Benjamini-Hochberg method [31].

Results

Aim 1: literature search for existing PROMs

No PROMs were identified measuring all three domains: symptom severity, bothersomeness and impact on daily activities. We identified four development and validation studies for patients with symptoms of UTI [6, 17–19]. None of these studies described the use of single or group interviews to ensure content validity. All of them were statistically validated, but only one study tested for unidimensionality using IRT but not for DIF [17]. The identified studies are listed in Additional file 1.

Aim 2: face and content validity

Item generation

The first draft version of the diary contained eight items regarding symptom severity, eight items regarding symptom bothersomeness and five items regarding daily activities (Fig. 1). Four response categories to these 21 items were drafted: 0 (none), 1 (a little), 2 (some) and 3 (a lot). Before interviewing men, six items regarding complicated UTI were added.

Single and group interviews

Two group interviews were conducted: one with four women aged 29–63 (six invited) and one with seven women from 70 to 89 (seven invited). The first group was in the latter part of the interview presented to a draft questionnaire including 21 items (Fig. 1). In the first group interview twelve new items (four on symptom severity, four on symptom bothersomeness and four on activities) were generated (Fig. 1). None of the symptoms in the draft version was considered irrelevant, but two items regarding activities were discarded (ability to concentrate and spare time activities). In the second group interview with elderly women, almost all of the same symptoms were repeated but two new items were generated: Severity and bothersomeness of feeling unwell. The elderly women had more problems identifying individual activities that were impacted when they had UTI but did not find the activities from the draft version irrelevant. They also found the diary quite long and repetitive to complete. They could, however, accept completing the items in all three scales in a single day and just the items in one scale on the following days. Both groups found the response categories sufficient and used all four options when completing the diary. We had planned to perform a group interview with men as well, but recruitment proved so difficult that we decided to conduct single interviews with men.

Six single interviews with women were performed – two after the changes following the first group interview and four after the second group interview. The result of these single interviews was minor corrections to phrasing and layout. No new items were generated in the single interviews with women.

Three men were interviewed; one interview was performed in person and two were conducted over the telephone after the diary had been sent in the post. All

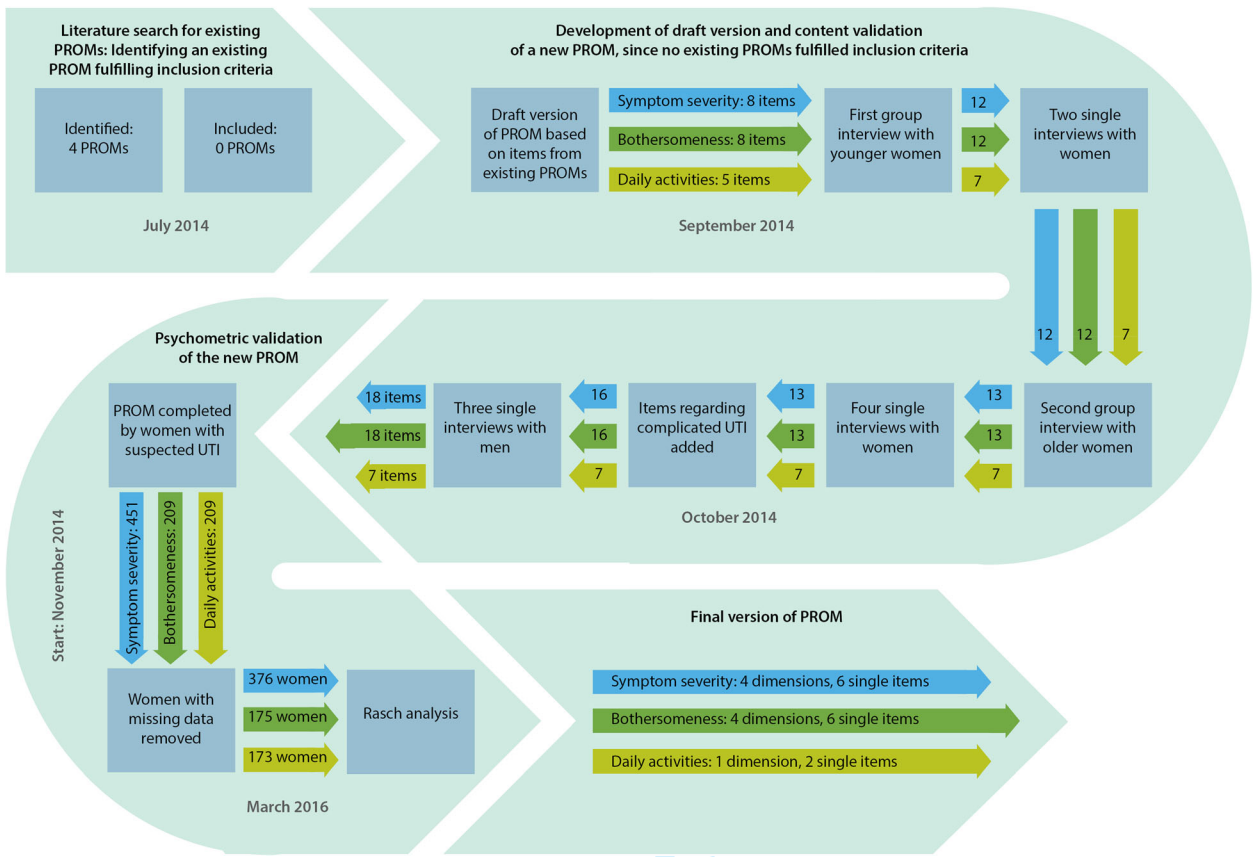

Fig. 1 Content and psychometric validation process and inclusion of items. Legend: UTI = Urinary tract infection

three men had experienced having both cystitis and pyelonephritis. Their vocabulary for describing their symptoms was different, but they found the vocabulary in the diary understandable and the items relevant. The first interview resulted in four new items related to complicated UTI. No additional items were generated in either of the other two interviews.

After four single interviews with women and two with men without any new items, we concluded that data saturation had been reached.

The result of the qualitative evaluation was three domains: one for symptom severity containing 18 items, one for symptom bothersomeness containing 18 items and one for impact on daily activities containing seven items. The process and result of the content validation can be seen in Fig. 1.

Aim 3: psychometric validation

Recruitment of patients

We included data on 451 female patients consulting their general practitioner with at least one UTI symptom. 209 of the women completed the full questionnaire regarding symptom severity, bothersomeness and daily activities. The remaining 242 only completed the items regarding symptom severity. Response rates in the two studies were 86 and 78%. We included age, job status, if the patient had confirmed UTI and which study she participated in as covariates. Inclusion of patients can be seen in Fig. 1 and characteristics of included patients in Table 1.

Rasch analysis

None of the three domains revealed unidimensionality in the initial Rasch analyses. Subsequent empirical analysis of the three domains revealed nine new dimensions covering symptom severity, symptom bothersomeness and impact on daily activities. 14 single items did not fit the dimensions, but could not be excluded from the diary without compromising content validity. The overall fit of the nine dimensions can be seen in Table 2 and the fit of individual items in Table 3.

Symptom severity (Domain S)

We suggested domain S to have a dimension regarding frequency and a dimension regarding pain (dysuria). Four symptom-items fitted the Rasch model in the

Table 1 Characteristics of patients included in psychometric validation

	Study 1 (all domains) n (%)	Study 2 (symptom severity only) n (%)
Age group		
0–24 years	17 (9.8)	20 (9.9)
25–34 years	29 (16.7)	23 (11.4)
35–54 years	50 (28.7)	73 (36.1)
55–69 years	48 (27.6)	50 (24.8)
70 years or more	30 (17.2)	36 (17.8)
Job status		
Employed	90 (51.7)	No data
Under education	21 (12.1)	No data
Job seeking	7 (4.0)	No data
Retired or otherwise not job seeking	56 (32.2)	No data
Confirmed UTI (growth in urine culture)		
Confirmed UTI	133 (76.4)	118 (58.4)
No UTI	41 (23.6)	84 (41.6)

Numbers in this table refer to the 376 women used to analyze domain S (symptom severity). Domain B (bothersomeenss, $n = 175$) and D (daily activities, $n = 173$)) were validated with data from study 1

frequency-dimension: Frequent urination – daytime, Increased urge for urination, Having to hurry to the toilet and Incontinence. Four combinations of items showed LD: Frequency and Urge, Frequency and Incontinence, Incontinence and Urge, and Incontinence and Having to hurry to the toilet. Three items fitted the Rasch model in the pain-dimension (Pain on urination, Difficult to empty bladder and Uncomfortable pressure around the bladder). One combination – Pain around the bladder and Difficult to empty bladder – showed LD. We suggested a dimension regarding symptoms from the lower back, and two items fitted this with no LD. Finally, we tested a dimension regarding general symptoms, which encompassed three items and had a high fit to the model and no LD. None of the final four dimensions showed DIF. Six items regarding symptom severity did not fit a dimension.

Symptom bothersomeness (Domain B)

Since the bothersomeness domain contained the same items as the symptom domain, but asked about bothersomeness instead of severity, we tested the dimensions identified in the analysis of domain S. All four dimensions fitted the model and showed no DIF. There were only two combinations of symptoms in the frequency dimension showing LD: Frequency and Urge, and Incontinence and Having to hurry to the toilet.

Impact on daily activities (Domain D)

The daily activities domain showed unidimensionality and no DIF if two items (Sleep and Sex) were removed. These two items were removed because they were related to nighttime, which the other items were not. These two items did not compose a separate dimension together. The item "Cycling" showed a low fit to the dimension, but removing it did not improve overall fit, so we decided to keep it in the dimension. The final dimension showed high levels of LD; only three combinations did not have LD: Work and Exercise, Social activities and Exercise, and Social activities and Domestic duties.

Discussion

This study resulted in a substantially new symptom diary for patients with symptoms of UTI with high content validity and adequate psychometric properties, comprising four dimensions of symptom severity and bothersomeness – dysuria, frequency, lower back symptoms and general symptoms – as well as one dimension of impact on daily activities. This is to our knowledge the first symptom diary regarding UTI to have been both content and psychometrically validated.

Table 2 Initial three domains and overall fit statistics

	Dimension (n items)	CLR χ^2	DF	*P*	Chronbach alpha
Symptoms	Dysuria (3)	12.1	8	0.146	0.554
	Frequency (4)	17.2	11	0.102	0.823
	Lower back (2)	8.5	5	0.132	0.938
	General (3)	8.4	8	0.392	0.735
Bothersomeness	Dysuria (3)	6.2	8	0.629	0.574
	Frequency (4)	21.1	11	0.032	0.839
	Lower back (2)	0.3	5	0.998	0.930
	General (3)	4.2	8	0.840	0.716
Daily Activities	Daily activities (5)	22.6	14	0.067	0.888

Initial three domains and overall fit statistics A NON-significant *P*-value for CLR χ^2 suggests a good fit to the unidimensional model. A high Chronbach alpha suggests the dimension has internal consistency. *CLR* χ^2 = conditional likelihood chi-square, *DF* degrees of freedom

Table 3 Fit statistics of individual items

Final dimension	Item number	Item	Item rest-score		SD	*P*
			observed	expected		
Symptom severity						
Dysuria	1	Pain on urination	0.380	0.367	0.046	0.765
	2	Difficult to empty bladder	0.370	0.375	0.047	0.928
	3	Uncomfortable pressure around the bladder	0.375	0.365	0.046	0.831
Frequency	4	Frequent urination - daytime	0.704	0.679	0.034	0.474
	5	Increased urge for urination	0.682	0.682	0.034	0.999
	6	Has to hurry to the toilet	0.737	0.690	0.030	0.118
	7	Incontinence	0.672	0.704	0.032	0.314
Lower back	8	Pain in lower back	0.957	0.957	0.011	0.993
	9	Uncomfortable pressure in lower back	0.957	0.957	0.011	0.993
General	10	Feeling unwell	0.663	0.651	0.041	0.770
	11	Fever	0.705	0.669	0.044	0.422
	12	Shivering	0.629	0.672	0.043	0.327
Single items	13	Burning sensation on urination	–	–	–	–
	14	Smelly urine	–	–	–	–
	15	Urine changed appearance	–	–	–	–
	16	Blood in urine	–	–	–	–
	17	Frequent urination - nighttime	–	–	–	–
	18	Pain around the bladder	–	–	–	–
Symptom bothersomeness						
Dysuria	19	Pain on urination	0.396	0.365	0.067	0.650
	20	Difficulty emptying bladder	0.385	0.403	0.067	0.790
	21	Uncomfortable pressure around the bladder	0.397	0.390	0.066	0.917
Frequency	22	Frequent urination - daytime	0.663	0.690	0.045	0.551
	23	Increased urge to urinate	0.699	0.693	0.046	0.888
	24	Has to hurry to the toilet	0.758	0.704	0.043	0.217
	25	Incontinence	0.724	0.727	0.046	0.946
Lower back	26	Pain in lower back	0.966	0.967	0.014	0.987
	27	Uncomfortable pressure in lower back	0.966	0.967	0.014	0.987
General	28	Feeling unwell	0.692	0.707	0.056	0.792
	29	Fever	0.766	0.694	0.059	0.223
	30	Shivering	0.671	0.714	0.059	0.457
Single items	31	Burning sensation on urination	–	–	–	–
	32	Smelly urine	–	–	–	–
	33	Urine changed appearance	–	–	–	–
	34	Blood in urine	–	–	–	–
	35	Frequent urination - nighttime	–	–	–	–
	36	Pain around the bladder	–	–	–	–
Daily activities						
Daily activities	37	Work	0.861	0.762	0.039	0.010*
	38	Social activities	0.811	0.768	0.038	0.251
	39	Exercise	0.758	0.775	0.036	0.652

Table 3 Fit statistics of individual items *(Continued)*

	40	Cycling	0.620	0.777	0.042	0.000**
	41	Tasks in the home	0.792	0.767	0.040	0.538
Single items	42	Sleep				
	43	Sex				

A non-significant *P*-value suggests a good fit to the unidimensional model of the individual items. Critical levels adjusted by the Benjamini-Hochberg procedure: * < 5% FDR, ** < 1% FDR

Strengths and limitations of this study

The diary was developed through interviews with patients attending general practice, thus yielding high content validity for patients in this setting. The domains were psychometrically analyzed using a large cohort obtained through two different studies. The psychometric validation ensured unidimensionality of the scales within the three domains and no DIF. We found corresponding scales in the symptom severity and symptom bothersomeness domains, suggesting the scales to be a solid finding.

It is a limitation in this study that we were unable to recruit men for a group interview or for the psychometrical validation. However, single interviews with men showed good relevance and coverage of the identified items and the items generated in the interviews with men were not gender-specific, but related to complicated UTI. In the psychometrical validation, we did not have sufficient sociodemographic data to include covariates, such as job status and education, in all analyses. This does not compromise the identified domains, but we do not have data to confirm whether any of the items possessed DIF in relation to sociodemographics. Another weakness is the high level of LD in the scale regarding daily activities. However, this finding corresponds with data from our second group interview, where participants stated that all activities were equally affected when they had UTI. This PROM is for research purposes and the fit-statistics indicate it should not be used for individual patients.

Findings in relation to other studies

Previous instruments regarding symptoms of UTI have also covered aspects such as frequency and dysuria [18]. However, our content validation process showed that patients do not see frequency as a uniform aspect that can be scored in a single item, but as a group of symptoms and experiences of having to hurry to the toilet, having to void often in both the daytime and the nighttime and having incontinence problems. The psychometric validation showed that most of these items – but not all – were part of the same construct. Urgency, which is usually investigated separately, turned out to be part of the frequency scale. The term dysuria was even more differently perceived by patients than by us, the clinicians. The content validation resulted in several new items dealing with different aspects of pain, since the term "pain" turned out to be too broad a concept. In the psychometric validation we found a three-item dimension comprising "pain on urination", "difficulties emptying the bladder" and "uncomfortable pressure around the bladder"; but the items "a burning sensation on urination" and "pain around the bladder" were not part of this dimension and the patients must have perceived these as fundamentally different symptoms.

Unanswered questions and future research

This study demonstrates that patient-experienced symptoms differ from the ways in which professionals perceive them as has been previously shown [4]. It indicates that patient interviews with the target population should always be conducted before introducing a new instrument. The study has identified new dimensions of patient-experienced UTI that differ, in terms of content, from those previously been found. The symptom diary is a robust instrument when used in studies investigating women with UTI symptoms in general practice, but we do not have sufficient data to determine whether it could be used in a male population. Before using it in a study on male patients, we would suggest performing a psychometric validation on men. We recommend that future studies on UTI, in which PROMs are to be used, should ensure high content validity of their outcome measures and unidimensionality of the included dimensions.

Conclusions

Several instruments have been validated to measure symptoms in patients with suspected UTI. Items and dimensions are usually generated by the researcher and statistical validation does not test for unidimensionality, but assumes, rather, that each item represents a different feature of the same construct. This study has content and psychometrically validated a new symptom diary for UTI, identifying nine unidimensional scales measuring different constructs of symptom severity, bothersomeness and impact on daily activities in patients with UTI. These scales differ substantially from those previously described in the scientific literature.

Additional file

Additional file 1: Identified PROMs. (DOCX 27 kb)

Abbreviations

DIF: Differential item functioning; IRT: Item response theory; LD: Local dependency; PROM: Patient-reported outcome measure; UTI: Urinary tract infection

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Availability of data and materials

The datasets generated and analyzed during the current study are not publicly available due to privacy issues but are available in anonymized form from the corresponding author on reasonable request.

Authors' contributions

All authors have made substantial contributions to the scientific work and the manuscript and have approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable

Ethics approval and consent to participate

Participation in the single and group interviews was voluntary and anonymous. The two trials received approval from the Ethical Committees for the Capital Region of Denmark (approval no H-3-2014-107 and H-4-2014-097). All patients gave written informed consent to participate in the study. There were no safety issues regarding this study. The projects were presented to the Danish Data Protection Agency, but did not require additional approval since approval was obtained from the Ethical Committees.

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TITLE PAGE

Clinical Accuracy of Point-of-care Urine Culture in General Practice

AUTHORS

Anne Holm (1)

Gloria Cordoba (1)

Tina Møller Sørensen (2)

Lisbeth Rem Jessen (2)

Niels Frimodt-Møller (3)

Volkert Siersma (1)

Lars Bjerrum (1)

1) Research Unit for General practice and Department of General Practice, University of Copenhagen
2) Department of Veterinary Clinical and Animal Sciences, University of Copenhagen
3) Department of Clinical Microbiology, Rigshospitalet

CORRESPONDING AUTHOR

Anne Holm, MD, phD-fellow

University of Copenhagen, Department of General Practice, Øster Farimagsgade 5, PO box 2099, 1014 Copenhagen

TEL +45 35 32 79 60

DIR +45 35 33 72 44

MOB +45 61 67 81 81

anneholm@sund.ku.dk

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RUNNING TITLE

Accuracy of Point-of-care Urine Culture

KEY POINTS

- Accurate diagnosis is important before starting antibiotic treatment for patients with suspected urinary tract infection.
- Point-of-care culture performed in general practice can identify patients with UTI within 24 hours
- The sensitivity of point-of-care culture performed in general practice is acceptable, but specificity is low

ABSTRACT

OBJECTIVE

To assess the clinical accuracy (sensitivity, specificity, positive predictive value and negative predictive value) of two point-of-care (POC) urine culture tests for the identification of urinary tract infection (UTI) in general practice.

DESIGN

Prospective diagnostic accuracy study comparing two index tests (Flexicult™ SSI-Urinary Kit or ID Flexicult™) with a reference standard (urine culture performed in the microbiological department).

SETTING

General practice in the Copenhagen area

PATIENTS

Adult female patients consulting their general practitioner with suspected uncomplicated, symptomatic UTI.

MAIN OUTCOME MEASURES

1) Overall accuracy of POC urine culture in general practice. 2) Individual accuracy of each of the two POC tests in this study. 3) Accuracy of POC urine culture in general practice with enterococci excluded, since enterococci are known to multiply in boric acid used for transportation for the reference standard. 4) Accuracy based on expert-reading of photographs of POC urine cultures performed in general practice. Standard culture performed in the microbiological department was used as reference standard for all four measures.

RESULTS

Twenty general practices recruited 341 patients with suspected uncomplicated UTI. The overall agreement between index test and reference was 0.76 (CI: 0.71-0.80), sensitivity 0.88 (CI: 0.83-0.92) and

specificity 0.55 (CI: 0.46-0.64). The two POC tests produced similar results individually. Overall agreement with enterococci excluded was 0.82 (0.77-0.86) and agreement between expert-readings of photographs and reference results was 0.81 (CI: 0.76-0.85).

CONCLUSION

POC culture used in general practice has high sensitivity but low specificity. Low specificity could be due to both misinterpretation in general practice and an imperfect reference standard.

KEY WORDS

"Urinary Tract Infections", “Microbiological diagnosis”, “Culture media”, “Point-of-Care Testing“, "General Practice"

INTRODUCTION

Urinary tract infection (UTI) is common in general practice and is the second leading reason for antibiotic prescriptions [1]. Patients with suspected UTI are usually treated with antibiotics, since it is a painful and bothersome condition and antibiotic treatment shortens the duration of the symptoms [2–4]. However, overtreatment can result in unnecessary side effects for the patient and increasing bacterial resistance [5–8]. Accurate diagnosis is essential for correct treatment, and combinations of symptoms and urine dipstick tests have proved inadequate for establishing or ruling out UTI [9–11]. This has led to the use of point-of-care (POC) urine culture in general practice in Scandinavia for both complicated and uncomplicated UTI. Most guidelines recommend treating uncomplicated UTI based on symptoms and urine dipstick findings [12,13]. However, in a recent study conducted in an outpatient-setting in Norway, patients with suspected uncomplicated UTI were treated based on dipstick and symptoms, which lead to antibiotic treatment of almost all patients although 43 % did not have confirmed UTI [14]. Performing additional urine tests to increase accuracy could potentially decrease overtreatment of both uncomplicated and complicated UTI. POC urine culture can usually be performed by practice staff and has the advantage of providing a definite result within 24 hours if handled correctly, while sending urine to the microbiological department usually involves a delay of several days [15–17]. The FlexicultTM SSI-Urinary Kit test and ID FlexicultTM (SSI Diagnostica, Denmark) are available in general practice in Denmark and have proven accurate in several laboratory studies and one validation study, but remain to be tested in the daily practice setting [17,18].

The aim of this study was to determine the accuracy of chromogenic agar-based POC culture in identifying significant bacteriuria in women with symptoms of UTI and a positive dipstick finding (leucocytes or nitrites) in general practice. A secondary analysis of the results excluding enterococci was performed in order to take into account the potential multiplication of entercocci during transport to the reference laboratories in boric acid tubes [19–21]. A separate analysis was performed based on expert-readings of photographs of POC culture plates from the study in order to investigate whether accuracy could be improved if the plates were read by an expert.

MATERIAL AND METHODS

This study is based on data from a randomized controlled trial, in which the design is described thoroughly in the protocol [22].

Recruitment of practices

An invitation letter was mailed to 200 randomly selected general practices in the Copenhagen area with the aim of recruiting 50 GPs with experience in using POC culture. The GPs who were recruited participated in a pre-study instruction course on handling and reading both POC tests, and had to pass an online test prior to the inclusion of patients.

Recruitment of patients

Female adult patients, 18 years or older, presenting to their GP between 1st March 2015 and 1st May 2016 with dysuria, frequency or urgency, of 7 days duration or less, and for whom the GP suspected uncomplicated UTI, were included in the study. Exclusion criteria were; negative dipstick analysis on leucocytes and nitrites, complicated UTI (except uncomplicated diabetes, elderly patients and recurrent UTI), previous participation in the study and patients presenting on a Friday (since the POC is read the following day).

Urine sampling and transportation

Having given informed consent, patients were randomized to one of the two POC tests and instructed to deliver a midstream urine sample without prior cleaning in accordance with Danish recommendations [23]. Part of the urine sample was inoculated immediately on the POC test and the remaining urine sample was sent to the microbiological department in a standardized boric acid container (Urine-Monovette®, Sarstedt).

POC tests (Index test)

The ID Flexicult™ (SSI DIagnostica, Denmark) is a chromogenic agar allowing identification and quantification of: 1) *E. coli*, 2) Other Enterobacteriaceae (Gram-negative rods), 3) Enterococci, 4) Proteus Spp., 5) *S. saprophyticus* and 6) *P. aeruginosa.* The plate is inoculated with freshly voided urine using a 10µL loop-needle and incubated at 35°C overnight. It is read the following day, but negative culture can only be determined after 24 hours. Significant growth was prespecified as $\geq 10^3$ colony-forming units per

milliliter (cfu/mL) for *E. coli* and *S. saprophyticus*, ≥10^4 cfu/mL for other typical uro-pathogens in accordance with European consensus [24].

The Flexicult™ SSI-Urinary Kit (SSI Diagnostica, Denmark) is an agar dish consisting of one big well containing the same agar material as in the ID Flexicult™ and five small wells containing agar with one of five antibiotics: 1) trimethoprim, 2) sulfamethizole, 3) ampicillin, 4) nitrofurantoin, and 5) mecillinam. The plate is inoculated by flooding with urine for 3-5 seconds and hereafter discarding superfluous urine. The plate is incubated and handled as the ID Flexicult™. Significant growth was prespecified (advised by manufacturer) to ≥10^3 cfu/mL for any uropathogen.

The GPs registered the index test as "significant growth of uropathogens", "No significant growth of uropathogens" or "inconclusive". A positive result of the index test was defined as having "significant growth of uropathogens", while "No significant growth of uropathogens" or "inconclusive" were labeled as negative.

PHOTOGRAPHS OF INDEX TESTS

All index tests were photographed using a digital camera. The primary investigator (AH) interpreted photographs, and a separate analysis was performed with the result of the photograph reading by AH as the index test to investigate whether accuracy could be improved if plates were read by an expert unaffected by the patient history.

REFERENCE TEST & LABORATORIES

Urine samples were sent by a specialized delivery service to the reference microbiological laboratories at the Department of Clinical Microbiology, Copenhagen University Hospital, Herlev, Denmark or the Department of Clinical Microbiology, Copenhagen University Hospital, Hvidovre, Denmark. Urine samples were analyzed on Inoqul A™ Bi-plate (CHROMagar and blood agar) with 10 µL on each half of the agar. All samples were quantified. Significant growth was defined as growth of ≥10^3 cfu/mL for *E. coli* and *S. saprophyticus*, ≥10^4 cfu/mL for other typical uropathogens and ≥10^5 cfu/ml for possible uropathogens in accordance with European consensus [24]. Plates with growth of more than two uropathogens were labeled as mixed cultures. A positive result was defined as having significant growth of uropathogens, while all other results including mixed flora were labeled as negative.

Data collection and management

Information regarding symptoms, interpretation of culture (positive/negative/inconclusive) and identification, quantification and susceptibility pattern of possible uropathogens were recorded in case report forms by the GPs or their staff. The data was double-typed. Results from the microbiological department were obtained from the hospital laboratory system and linked with the case-report forms from general practice using social security numbers.

Blinding

The interpreter of the POC index test in general practice was blinded to the result of the reference test, as far as the result of the reference test was not available before 2-3 days and the result of the index test was consistently recorded 24 hours after the consultation. The interpreter of the reference test was likewise blinded to the result of the index test. AH was blinded to both the interpretation from general practice and the microbiological department when evaluating the photographs.

Statistical analysis

Sensitivity (SEN), specificity (SPE), positive predictive value (PPV), negative predictive value (NPV) and agreement (ACC, true positives + true negatives/all) were calculated. 95% confidence intervals (95% CI) for this collection of proportions were calculated with the exact method. Statistical analysis was performed with SAS version 9.4 for Windows 7, SAS Institute Inc.

RESULTS

BASELINE DATA

Twenty general practices with a total of 45 GPs were recruited from the Copenhagen area. Only three were solo practices. The 20 practices recruited 341 female, non-pregnant patients with symptoms of UTI (mean age 48.5 years). Data collection can be seen in the attrition flow chart (Figure 1). The prevalence of UTI was 72 % according to the two index tests and 63% according to the reference standard. The most prevalent uropathogen in both general practice and the microbiological department was *E. coli*. In general practice the second most frequent single uropathogen was enterococci, however, this uropathogen was not identified on reference cultures. See Table I for details.

ACCURACY

Table II shows the measures of test accuracy for the various analyses. Overall agreement of POC urine culture with the reference was 0.76 (95% CI: 0.71-0.80). Sensitivity was 0.88 (95% CI: 0.83-0.92) and specificity was 0.55 (95% CI: 0.46-0.64). The two tests produced similar results. Since estimation of enterococcal growth after transportation in boric acid was expected to pose a challenge, a subgroup analysis was performed without enterococci monocultures identified in general practice. This improved overall specificity from 0.55 to 0.71 without lowering sensitivity. Expert photograph reading by AH (including enterococci) increased specificity to 0.71, and agreement to 0.81 but did not change sensitivity.

PHOTOGRAPH EVALUATION OF DISCREPANCIES BETWEEN INDEX TESTS AND REFERENCE

83 index test results differed from the reference. 75 of these had a photograph acceptable for evaluation. When evaluating these photographs, 31 (41%) of discrepancies could be explained by incorrect interpretation of the culture plate, since the photograph reading corresponded to the reference while the interpretation in general practice did not. For enterococci identified in general practice, but with a negative reference culture, 13 out of 28 (46%) were overdiagnosed due to incorrect interpretation of the culture plate. In one case, the photograph was missing. Figure 2 shows six examples of culture plates with discrepancies between the index test and the reference.

IDENTIFICATION OF UROPATHOGENS

Table III shows the identification of uropathogens and the agreement between results from general practice and the microbiological department. *E. coli* identified in general practice was also identified by the microbiological department in 114 out of 128 cases (89%). The total number of monoculture *E. coli* identified by the microbiological department was 176 and general practice identified 114 of these (65%). The 62 remaining cultures were reported as two uropathogens in 27 cases from general practice. 19 of these were *E. coli* and enterococci and this combination was reported as monoculture *E. coli* by the microbiological department according to their guideline. When this discrepancy in identification is taken into consideration, general practice identified 76% of the *E. coli* identified by the microbiological department.

DISCUSSION

PRINCIPAL FINDINGS

This study on 341 symptomatic, female patients from general practice found that GPs can identify those with significant bacteriuria with an agreement of 0.76 using chromogenic agars as POC test. We found that enterococci posed a certain challenge since they were often identified in general practice (13% of cases) but not at all in the microbiological department. This study cannot accurately determine whether enterococci were overdiagnosed in general practice or underdiagnosed in the microbiological department, but the photograph readings suggest that both could be the case.

STRENGTHS AND WEAKNESSES OF THE STUDY

The interpreters of the index test and reference test were both sufficiently blinded, the tests were performed on the same sample of urine and all patients included in analysis were investigated with both the index test and the reference test. 22 patients in the trial did not have the reference performed. This should not affect accuracy measures as, according to the participating GPs, it was due to forgetfulness. Since all patients were symptomatic and the interpretation of the reference standard corresponded to current consensus, a positive reference corresponds to the definition of having UTI. Verification and interpretation procedures, therefore, had low bias. However, the GPs had access to clinical information, which the interpreter of the reference test and the photographs did not. This could partly be the cause of the low specificity, since GPs were instructed only to include patients where UTI was suspected, leading to overestimation of UTI in general practice. All results were included in the analysis. We handled ambiguous results as negative in both the index test and in the reference test.

The study was conducted in the daily practice setting and GPs were obliged and motivated to screen all patients for eligibility. We had decided to include elderly patients, patients with uncomplicated diabetes and recurrent UTI to improve the applicability of our results. However, the inclusion period was quite long and practices were not active in recruiting at all times, which compromised obtaining a consecutive sample. Because our data came from a randomized controlled trial, the design was quite time-consuming; patients had to wait one day for the POC culture result before treatment could be initiated, causing some to refuse to participate. We do not know if they differed from included patients, but the

mean age of patients refusing to participate was similar to those who did participate (52.0 vs. 48.5 years) We only included patients with symptoms and a positive dipstick result, since most patients with a negative dipstick result do not have UTI; however, we do not know anything about the performance of the tests for the group with a negative dipstick but strong symptoms, where urine culture could still be indicated. This could introduce spectrum bias if our results were applied to a population who were not screened with urine dipstick and therefore possibly had a lower prevalence of UTI [25]. The index and reference tests were performed as in daily clinical practice and threshold values were predefined. However, the reference has been shown to have limitations. The perfect reference test would have involved quantifying the bacteria of urine by means of serial dilution for every sample included in the study in general practice [26]. However, this is not feasible, and sending urine to the microbiological department is the "gold standard" in daily practice. Also, since prevalence of UTI in this study was intermediate, reference standard misclassification would probably be low making our findings valid despite an imperfect reference standard [27]. All practices in the study had prior experience with performing POC culture and most were already using the FlexicultTM SSI-Urinary Kit on a daily basis. None of them had experience using the ID FlexicultTM. However, The ID FlexicultTM did not exhibit a lower agreement than the FlexicultTM SSI-Urinary Kit, which suggests that our results could be applied to GPs with little prior experience in using any of the tests.

The photographs proved particularly advantageous in investigating the causes of low agreement. Without access to the photographs, all the wrong diagnoses would have been attributed to incorrect interpretation of the test results in general practice, but only 41% could be explained this way according to the photographs.

FINDINGS IN RELATION TO OTHER STUDIES

Agreement in this study was higher than most studies investigating urine dipstick in symptomatic patients, but comparable to a recent study diagnosing UTI with a combination of dipstick and microscopy [11,28]. The field trial validation study of FlexicultTM SSI-Urinary Kit [17] does not report overall agreement, but reports discrepancies in quantification between index and reference as 16 % before adjustment for various factors. That study does not report ambiguous results or the overall prevalence of UTI, and its results are therefore difficult to compare with our results; however, problems

with their reference samples being transported in boric acid were also reported. One study on Uricult® and Sensicult® dipslides have shown higher sensitivity and specificity for symptomatic patients than we found [16]. The Uricult® study results were obtained from a single health center, which would be expected to yield better results than our multi-center study. They also reported data from a multi-center study, but ambiguous cultures were excluded from analysis. Another study on dipslide found a lower sensitivity and a higher specificity than this study [15]. The study included symptomatic patients and the prevalence of UTI was comparable to its prevalence in our study. However, they used a dipslide identical to the index test sent to the laboratory as reference. This would partly explain the higher specificity, but not the lower sensitivity. They suggest themselves that not all their samples were incubated at 37□C. Previous research has shown that enterococci are at risk of multiplying when transported in boric acid containers [19,20]. This contradicts our finding as enterococci were often diagnosed in general practice but not by the microbiological department. Enterococci, identified in general practice and on the photograph, could possibly have been eliminated in the boric acid due to too little urine in the container resulting in too high a concentration of boric acid.

MEANING OF THE STUDY AND IMPLICATIONS FOR PRACTICE

This study is one of the first to investigate the accuracy of chromogenic agars in general practice. We found sensitivity to be acceptable, but specificity was too low, since studies on dipstick have produced similar specificities, and a combination of symptoms and dipstick has even greater specificity [11]. However, overall agreement was higher than in most studies evaluating urine dipstick. Previous studies have shown that training can raise GPs' accuracy in evaluating microbiological tests, and evaluation of the photographs suggests that this could also apply here [29]. It remains to be investigated whether improved microbiological diagnosis using chromogenic agars increases appropriate antibiotic prescribing. Two ongoing studies are investigating this with slightly different designs [22,30]. With the accuracy identified in this study, GPs would be expected to improve their antibiotic prescribing for UTI by performing a POC culture in addition to dipstick analysis before starting antibiotic treatment.

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ETHICAL APPROVAL

All procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983. The study was approved by the Ethical Committee for the Capital Region of Denmark (ref.no: H-3-2014-107). All patients gave written informed consent prior to participating in the study.

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CONFLICTS OF INTEREST

None

REGISTRATION NUMBER

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LEGENDS

Figure 1: Attrition flow-chart

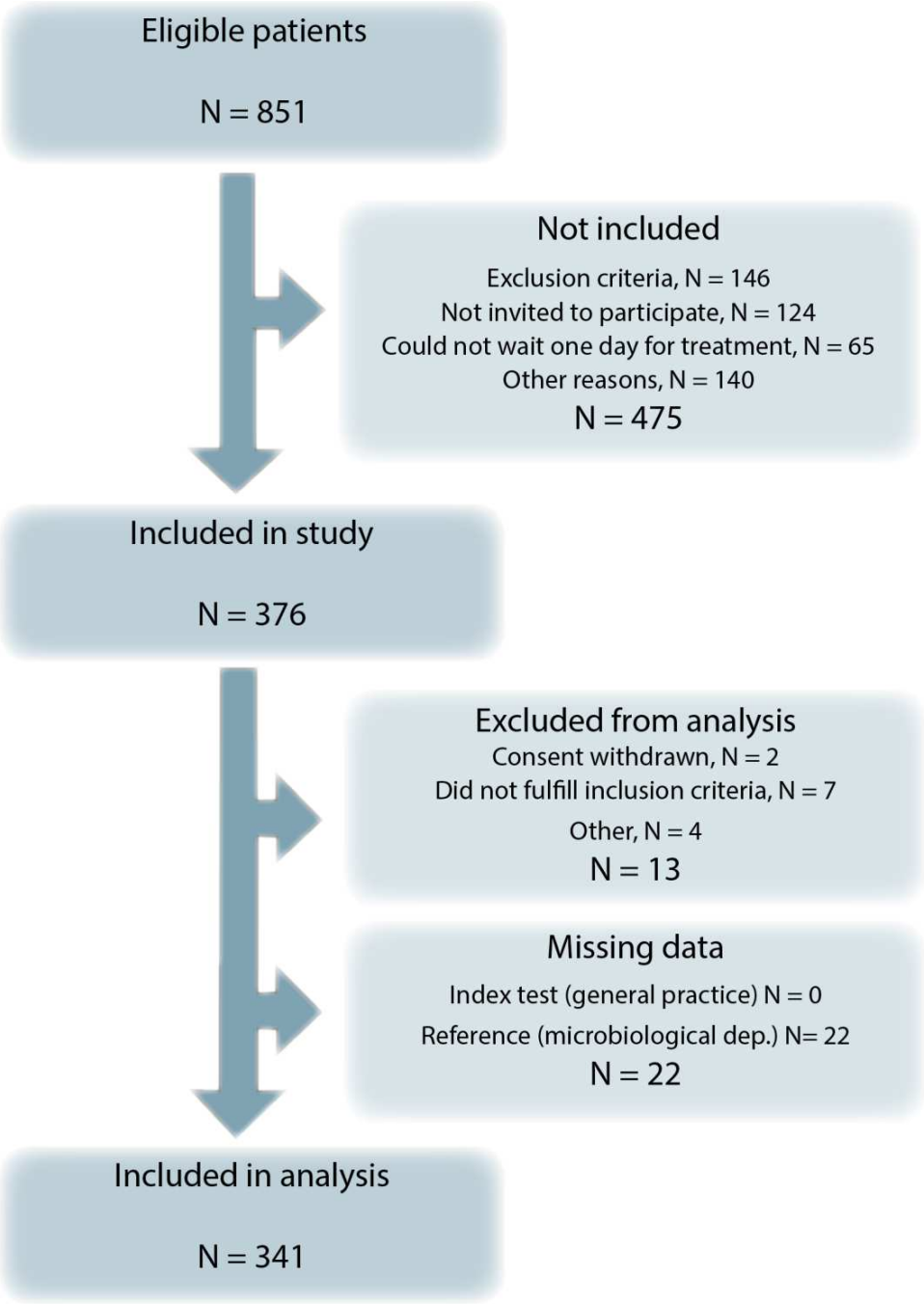
Figure 2: Examples of cultures diagnosed incorrectly in general practice according to the reference standard. A and D: Correctly answered as negative in general practice according to the photograph and as *S. saprofyticus* 10^4cfu/ml and *C. koseri* 10^4cfu/ml in the microbiological department. B and E: Correctly answered as *E. coli* 10^3cfu/ml and *E. faecalis* 10^5cfu/ml in general practice but as negative in the microbiological department. C and F: Incorrectly answered as significant growth in general practice, and as negative and mixed flora in the microbiological department.

Table I. Characteristics of samples from 341 patients: test results and distribution of uropathogens from general practice and the microbiological department. percentages in bracKets

Table II: Accuracy of point-of-care culture in relation to culture at the reference laboratories. 95% confidence intervals in brackets. PPV = Positive predictive value, NPV = Negative predictive value, SEN = Sensitivity, SPE = Specificity, ACC = Accuracy (True positive+true negatives / all)

Table III: Identification of uropathogens in general practice compared to the microbiological department.

TABLES AND FIGURES



393

394 **FIGURE 3: ATTRITION FLOW-CHART**

	Index tests	Reference
Both index tests, n = 341		
Significant growth	246 (72 %)	215 (63 %)
E. coli	128 (38 %)	176 (51 %)
Enterococci	40 (12 %)	0 (0 %)
S. saprophyticus	15 (4 %)	8 (2 %)
Other single uropathogen	19 (6 %)	25 (7 %)
Two uropathogens	44 (13 %)	6 (2 %)
Index test 1: ID Flexicult, n = 158		
Significant growth	116 (74 %)	103 (65 %)
E. coli	71 (45 %)	89 (56 %)
Enterococci	16 (11 %)	0 (0 %)
S. saprophyticus	7 (4 %)	2 (1 %)
Other single uropathogen	10 (6 %)	10 (6 %)
Two uropathogens	12 (8 %)	2 (1 %)
Index test 2: SSI-Urinary Kit n= 183		
Significant growth	129 (70 %)	112 (61 %)
E. coli	57 (31 %)	87 (48 %)
Enterococci	23 (13 %)	0 (0 %)
S. saprophyticus	8 (4 %)	6 (3 %)
Other single uropathogen	9 (5 %)	15 (8 %)
Two uropathogens	32 (17 %)	4 (2 %)

TABLE I. CHARACTERISTICS OF SAMPLES FROM 341 PATIENTS: TEST RESULTS AND DISTRIBUTION OF UROPATHOGENS FROM GENERAL PRACTICE AND THE MICROBIOLOGICAL DEPARTMENT. PERCENTAGES IN BRACKETS

	N	PPV	NPV	SEN	SPE	ACC
All cultures	341	0.77 (0.71-0.82)	0.73 (0.63-0.81)	0.88 (0.83-0.92)	0.55 (0.46-0.64)	0.76 (0.71-0.80)
Enterococci in practice culture excluded	301	0.86 (0.81-0.91)	0.73 (0.63-0.81)	0.87 (0.82-0.92)	0.71 (0.61-0.80)	0.82 (0.77-0.86)
ID Flexicult	158	0.79 (0.71-0.86)	0.76 (0.60-0.88)	0.90 (0.83-0.95)	0.56 (0.42-0.70)	0.78 (0.71-0.85)
SSI-Urinary Kit	183	0.74 (0.66-0.82)	0.70 (0.56-0.82)	0.86 (0.78-0.92)	0.54 (0.41-0.65)	0.73 (0.66-0.79)
All cultures – photo readings	309	0.84 (0.79-0.89)	0.75 (0.66-0.83)	0.87 (0.81-0.91)	0.71 (0.62-0.79)	0.81 (0.76-0.85)

TABLE II: ACCURACY OF POINT-OF-CARE CULTURE IN RELATION TO CULTURE AT THE REFERENCE LABORATORIES. 95% CONFIDENCE INTERVALS IN BRACKETS. PPV = POSITIVE PREDICTIVE VALUE, NPV = NEGATIVE PREDICTIVE VALUE, SEN = SENSITIVITY, SPE = SPECIFICITY, AGR = AGREEMENT (TRUE POSITIVE+TRUE NEGATIVES / ALL)

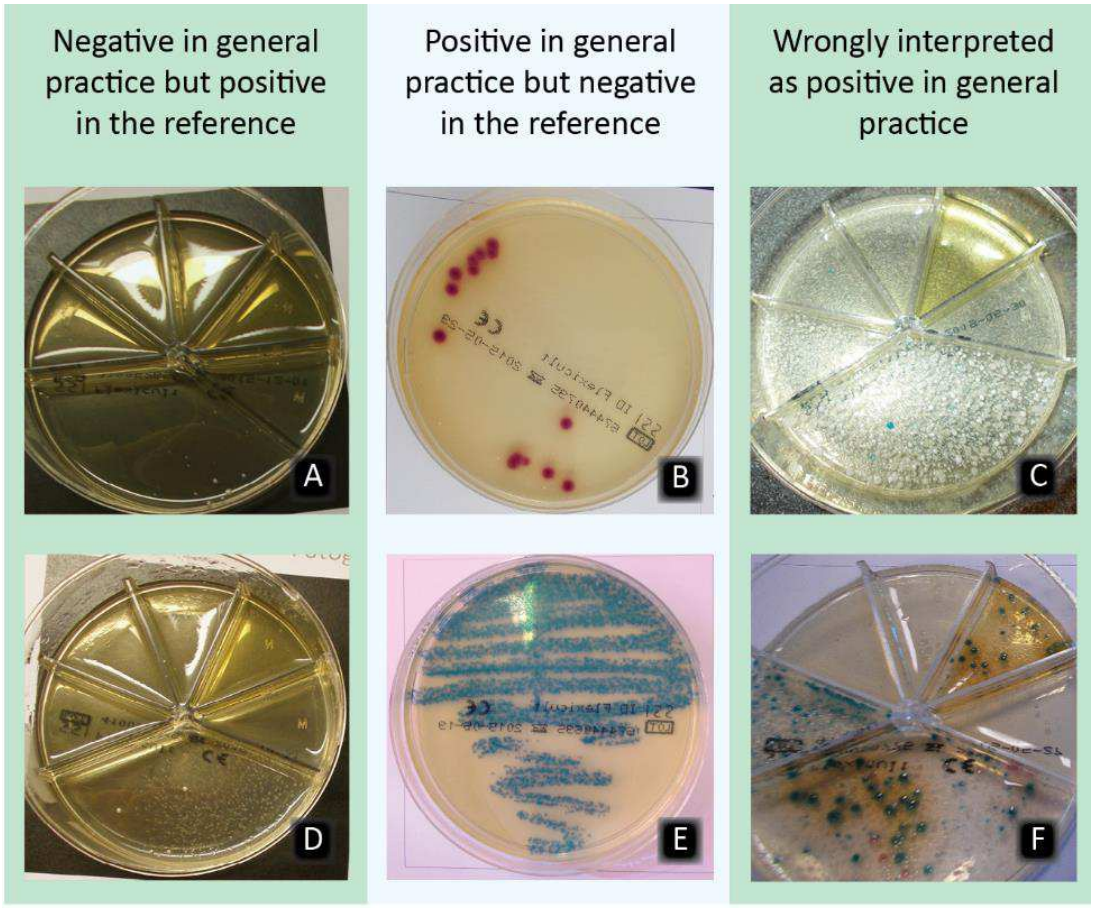


FIGURE 4: EXAMPLES OF CULTURES DIAGNOSED INCORRECTLY IN GENERAL PRACTICE ACCORDING TO THE REFERENCE STANDARD. A AND D: CORRECTLY ANSWERED AS NEGATIVE IN GENERAL PRACTICE ACCORDING TO THE PHOTOGRAPH AND AS *S. SAPROFYTICUS* 10^4CFU/ML AND *C. KOSERI* 10^4CFU/ML IN THE MICROBIOLOGICAL DEPARTMENT. B AND E: CORRECTLY ANSWERED AS *E. COLI* 10^3CFU/ML AND *E. FAECALIS* 10^5CFU/ML IN GENERAL PRACTICE BUT AS NEGATIVE IN THE MICROBIOLOGICAL DEPARTMENT. C AND F: INCORRECTLY ANSWERED AS SIGNIFICANT GROWTH IN GENERAL PRACTICE, AND AS NEGATIVE AND MIXED FLORA IN THE MICROBIOLOGICAL DEPARTMENT.

General practice	Reference (microbiological department)						
	E. Coli	Entero-cocci	*S. Sapro-phyticus*	Other single pathogen	Two pathogens	No pathogens	Total
E. Coli	114	0	3	1	2	8	128
Enterococci	7	0	2	2	0	29	40
S. Saprophyticus	3	0	11	0	1	4	19
Other single pathogen	12	0	0	1	0	2	15
Two pathogens	27	0	2	0	1	14	44
No pathogens	13	0	7	4	2	69	95
Total	176	0	25	8	6	126	341

TABLE III: IDENTIFICATION OF UROPATHOGENS IN GENERAL PRACTICE COMPARED TO THE MICROBIOLOGICAL DEPARTMENT.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3-4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	Protocol
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	-

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	7+11
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	9+figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	9+figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	9+figure 1
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1 page 22
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Table 2 page 23
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Table 2 page 23
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	10-table 3
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	11
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	11
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	6
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	14

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Effect of point-of-care susceptibility testing in general practice on appropriate prescription of antibiotics for patients with uncomplicated urinary tract infection: a diagnostic randomized controlled trial

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TITLE PAGE

TITLE

Effect of point-of-care susceptibility testing in general practice on appropriate prescription of antibiotics for patients with uncomplicated urinary tract infection: a diagnostic randomized controlled trial

AUTHORS

Anne Holm, AH, PhD fellow (1)

Gloria Cordoba, GC, PhD fellow (1)

Tina Møller Sørensen, TMS, PhD fellow (2)

Lisbeth Rem Jessen, LRJ, associate professor (2)

Niels Frimodt-Møller, professor (3)

Volkert Siersma, statistician (1)

Lars Bjerrum, professor (1)

1) University of Copenhagen, Research Unit for General Practice and Department of General Practice, Øster Farimagsgade 5, PO box 2099, 1014 Copenhagen
2) University of Copenhagen, Department of Veterinary Clinical and Animal Sciences, Dyrlægevej 16, 1870 Frederiksberg C
3) Clinical Microbiological Department, Rigshospitalet, Blegdamsvej 3, 2100 København Ø

CORRESPONDING AUTHOR

Anne Holm, MD, PhD fellow

University of Copenhagen, Department of General Practice, Øster Farimagsgade 5, PO box 2099, 1014 Copenhagen

TEL +45 35 32 79 60

DIR +45 35 33 72 44

MOB +45 61 67 81 81

anneholm@sund.ku.dk

WORD COUNT

3466

ABSTRACT

OBJECTIVES

To investigate the effect of adding point-of care (POC) susceptibility testing to POC culture on appropriate use of antibiotics as well as clinical and microbiological cure for patients with suspected uncomplicated urinary tract infection (UTI) in general practice.

DESIGN

Open, individually randomized controlled trial (RCT).

SETTING

General practice

PARTICIPANTS

Women with suspected uncomplicated UTI, including elderly patients above 65, patients with recurrent UTI and patients with diabetes. The sample size calculation predicted, 600 patients were needed.

INTERVENTIONS

Flexicult™ SSI-Urinary Kit was used for POC culture and susceptibility testing and ID Flexicult™ was used for POC culture-only.

MAIN OUTCOME MEASURES

Primary outcome: Appropriate antibiotic prescribing on the day after consultation defined as either 1) patient with UTI: to prescribe a first-line antibiotic to which the infecting pathogen was susceptible or a second-line if a first-line could not be used or 2) patient without UTI: not to prescribe an antibiotic . UTI was defined by typical symptoms and significant growth in a reference urine culture performed at one of two external laboratories.

Secondary outcomes: Clinical cure on day 5 according to a 7-day symptom-diary and microbiological cure on day 14. Logistic regression models taking into account clustering within practices were used for analysis.

Results

Twenty general practices recruited 191 patients for culture and susceptibility testing and 172 for culture-only. 63% of the patients had UTI and 12% of these were resistant to the most commonly used antibiotic, pivmecillinam. Patients randomized to culture-only received significantly more appropriate treatment (OR: 1.44 (95% CI: 1.03-1.99), p=0.03). There was no significant difference in clinical or microbiological cure.

Conclusions

Adding POC susceptibility testing to POC culture did not improve antibiotic prescribing for patients with suspected uncomplicated UTI in general practice. Susceptibility testing should be reserved for patients at high risk of resistanceand complications.

Trial registration

ClinicalTrials.gov NCT02323087.

Key words

"Urinary Tract Infections", “Microbiological diagnosis”, “Culture media”, “Point-of-Care Testing“, "General Practice"; “Antibiotics”

Article summary - Strengths and limitations of this study

- This study was a diagnostic RCT allowing for evaluation of patient-relevant outcomes.
- Bias in the interpretation process was low and allocation concealment was sufficient.
- The study was not blinded.
- The study took place in general practice, which enhances applicability of our findings to other primary care settings.

- Inclusion criteria were quite broad and our findings may be applied to the majority of patients in general practice with suspected UTI.
- We did not succeed to recruit ourinitially planned sample of patients, but enough patients were recruited to detect a significant difference between the groups.

INTRODUCTION

Urinary tract infection (UTI) is a common condition in general practice and the second leading cause for the prescribing of antibiotics (1). Resistance rates for the most common uropathogen; *E. coli*, are rising, and the inappropriate prescription of antibiotics in primary care is known to lead to antibiotic resistance (2–4). Resistant strains of bacteria can cause treatment failure and prolonged symptoms (5–7). Many countries recommend diagnosing UTI based on symptoms and urine dipstick, but combinations of symptoms and dipstick have proven inaccurate in ruling UTI in or out (8,9). In Denmark, there is no national guideline for diagnosing UTI and doctors have varying strategies based on urine dipstick, microscopy, point-of-care (POC) culture and POC culture and susceptibility testing (10,11). Urine culture gives a definite answer for UTI in the symptomatic patient (12). However, sending urine to the microbiological laboratory for culture and susceptibility testing can delay treatment for several days. Point-of-care (POC) tests for urine culture and urine culture and susceptibility testing are commercially available. They can provide a result within 24 hours, a delay to treatment which the majority of patients would accept (13). The Flexicult™ SSI-Urinary Kit is commonly used in general practice due to its ease of use and the fact that both culture and susceptibility testing can be performed on the same plate (14). Similar chromogenic agars for culture-only exist, but are less commonly used and have not been validated in general practice. The most commonly used antibiotics in Denmark for treatment of acute UTI are pivmecillinam and sulfamethizole. Resistance rates in *E. coli* isolates in urine from primary care in Denmark are approximately 30% for sulfamethizole and 5-10% for pivmecillinam (15). Since other uropathogens can be inherently resistant to pivmecillinam, overall resistance would be expected to be 15-20% for this drug. We hypothesised that performing a susceptibility test prior to initiation of treatment could target treatment to the individual patient, potentially reducing inappropriate antibiotic prescribing and leading to faster clinical recovery. This study aimed to investigate the effect of POC culture and susceptibility testing against POC

culture-only on the appropriate use of antibiotics and clinical and microbiological cure for patients with suspected uncomplicated UTI in general practice.

MATERIAL AND METHODS

DESIGN

This study was an open, randomized controlled trial (RCT). Patients were individually randomized to having either POC culture and susceptibility testing or POC culture-only performed. The design is described in detail in the published protocol (16).

RECRUITMENT OF PRACTICES

An invitation letter was mailed to 200 randomly selected general practices in the Copenhagen area with the aim of recruiting 50 general practitioners (GPs) with experience in using POC culture. The recruited GPs participated in a pre-study instruction course on handling and reading both POC tests, and had to pass an online test measuring ability to diagnose UTI based on photographs of urine cultures prior to the inclusion of patients.

RECRUITMENT OF PATIENTS

The inclusion criteria were: women, 18 years or older, presenting to their GP with dysuria, frequency or urgency, for seven days or less, and for which the GP suspected uncomplicated UTI, including elderly patients above 65, patients with recurrent UTI and patients with orally treated diabetes without complications. The broader definition of uncomplicated UTI was chosen to ensure applicability to a larger group of patients in general practice. The exclusion criteria were: negative dipstick analysis on both leucocytes and nitrites, serious comorbidities, former participation in the study and patients presenting on a Friday (since POC culture is read the following day). All patients had to consent to wait until the next day to receive the result of the POC test before commencing possible treatment. After informed consent, patients were randomized to either POC culture or POC culture and susceptibility testing. A urine sample from the same portion of urine was sent to the local microbiological laboratory for culture and susceptibility testing. The GP filled out a case-report form and the patient was asked to fill out a seven-day symptom diary and return to the GP after 14 days for a control urine sample. Validation of the symptom diary has previously been published (17). Patients were reminded by text messages and telephone calls to return the diary and bring the control urine sample. Each practice kept an anonymous screening log of patients who fulfilled the inclusion criteria but who were not

included in the study. GPs received no treatment protocol concerning choice of antibiotics, but could decide freely on treatment.

PATIENT INVOLVEMENT.

One of the secondary outcome measures was clinical cure. This was measured using a content validated symptom diary, where items were generated through cognitive interviews with patients (17). Patients could state on their consent form whether they wished to be informed about the results of the study. This will be done using a text message with a short summary and a link after publication. Patients were not involved in the design of the study. All recruiting practices received a poster displaying information about the trial to hang in the waiting room, so patients could enquire about participation in case they were not approached regarding this.

POINT-OF-CARE TESTS

Culture-only group: The ID Flexicult™ (SSI DIagnostica, Denmark) is a chromogenic agar allowing identification and quantification of: 1) *E. coli*, 2) other Enterobacteriaceae (Gram-negative rods), 3) enterococci, 4) Proteus spp., 5) *S. saprophyticus* and 6) *P. aeruginosa.* The plate is inoculated with freshly voided urine using a 10µL loop-needle and incubated at 35°C overnight. It is read the following day, but negative culture can only be determined after 24 hours. Significant growth was prespecified as $\geq 10^3$ colony-forming units per millilitre (cfu/mL) for *E. coli* and *S. saprophyticus* and $\geq 10^4$ cfu/mL for other typical uropathogens in accordance with European guidelines (12).

Culture and susceptibility testing group: the Flexicult™ SSI-Urinary Kit (SSI Diagnostica, Denmark) is an agar dish consisting of a large compartment containing the same agar material as in the ID Flexicult™ and five small compartments, each containing agar with a specific antibiotic: 1) trimethoprim, 2) sulfamethizole, 3) ampicillin, 4) nitrofurantoin, and 5) mecillinam. The agar plate is flooded with freshly voided urine for 3-5 seconds. Any excess urine is discarded. The plate is incubated and handled in the same way as the ID Flexicult™. Significant growth was prespecified (advised by manufacturer) to $\geq 10^3$ cfu/mL for any uropathogen.

REFERENCE CULTURE IN THE MICROBIOLOGICAL LABORATORY

Urine samples were sent by special delivery service to the reference microbiological laboratories at the Department of Clinical Microbiology, Copenhagen University Hospital, Herlev, Denmark or the Department of Clinical Microbiology, Copenhagen University Hospital, Hvidovre, Denmark. Urine samples were analysed on Inoqul A™ Bi-plate (CHROMagar and blood agar) with 10 µL on each half of the agar. The susceptibility pattern was determined on Mueller Hinton agars with disks containing antibiotics, including mecillinam, trimethoprim, nitrofurantoin, sulfamethizole, ampicillin and ciprofloxacin. All samples were quantified. Significant growth was defined as growth of $\geq 10^3$ cfu/mL for *E. coli* and *S. saprophyticus*, $\geq 10^4$ cfu/mL for other typical uropathogens and $\geq 10^5$ cfu/ml for possible uropathogens. Plates with growth of more than two uropathogens were labelled as mixed cultures and classified in the analysis as negatives.

Randomization and concealment of allocation

The randomization code was produced by an online random number generator as permuted block randomization in blocks of 10 by the investigators. The allocation of each included patient was placed in an opaque, sequentially numbered, sealed envelope, which was opened in general practice after inclusion of the patient.

Outcomes

Primary outcome: appropriate treatment was defined as either 1) If the patient had UTI in the reference: to prescribe a first-line antibiotic to which the infecting pathogen was susceptible. 2) If the patient had UTI but was allergic to the antibiotic or the pathogen was resistant to all first-line antibiotics: to prescribe a second-line antibiotic. 3) If the patient did not have UTI in the reference: not to prescribe an antibiotic. Secondary outcomes: Clinical cure was defined as the patient reporting herself as symptom free in the symptom diary on day 5 (four days after initiation of treatment). Microbiological cure was defined as no significant growth in the control urine sample after 14 days.

Statistical analysis

Sample size calculation was performed assuming the proportion of appropriately treated patients in the control group would be 70–80 %, since POC culture had proven quite accurate and local resistance rated to pivmecillinam and sulfamethizole was 6-10 % and 30-40% respectively (14,18). To detect a statistically significant ($\alpha = 0.05$) 10 percentage-point difference between the two groups with 80% probability, assuming an intra-class correlation of 0.2

between patients in the same practice, a sample of 600 patients was needed. In order to take possible drop-outs and sub-analyses into account, the study originally aimed at enrolling 750 patients.

The distributions of baseline presentation characteristics were compared between the randomization groups using chi-squared tests. Investigated variables were: age, number of days with symptoms, key symptoms (dysuria, frequency and urge), complicating factors and reference culture and susceptibility test. Primary and secondary outcomes were analysed in logistic regression models; clustering within practices was adjusted for by generalised estimating equations (GEE). Patient factors (age, number of days with symptoms, key symptoms, and complicating factors) and practice factors (number of GPs and organisation of practice) were investigated for effect modification on the primary outcome. All analyses were performed as intention-to-treat (ITT) analyses. The significance level was 5%. Statistical analysis was performed with SAS version 9.4 for Windows 7, SAS Institute Inc.

RESULTS

Twenty general practices with a total of 45 GPs were recruited from the Copenhagen area and they screened 851 patients for eligibility between 1st March 2015 and 1st May 2016. Of these, 376 patients agreed to participate: 199 were randomized to culture and susceptibility testing, and 177 were randomized to culture-only. 13 patients were excluded from the analysis, leaving a total of 363 patients with data on at least one of the outcomes to be included in the analysis. An overview of the inclusion and exclusion of patients can be seen in Figure 1.

Patient characteristics and distribution between groups can be seen in Table 1. Most of the baseline variables did not differ significantly between groups, but the proportion of patients who were over 65 years or who had recurrent UTI or diabetes (complicated cases of uncomplicated UTI) differed significantly. The prevalence of confirmed UTI (significant growth of uropathogens in the reference standard) and susceptibility pattern in the reference standard did not differ between groups.

Three quarters (75%) of the patients were appropriately treated in the culture-only group and two thirds (67%) were appropriately treated in the culture and susceptibility testing group. This difference was significant both in the unadjusted analysis and when controlled for baseline characteristics. Sub-group analyses on young patients without

co-morbidity and patients who were elderly, or who had diabetes or recurrent UTI showed that young patients with no comorbidities were significantly more appropriately treated in the culture-only group compared to the culture and susceptibility group. The difference was not significant for patients who were elderly, or who had diabetes or recurrent UTI, although culture-only was still superior to culture and susceptibility testing (Table 2).

Table 3 shows the distribution of patients and the reasons why they were labelled as appropriately or inappropriately treated. Overtreatment of patients without UTI was the major reason for inappropriate treatment and was almost equally distributed between groups. Undertreatment was slightly higher in the culture and susceptibility group. Surprisingly, treatment with an antibiotic to which the infecting pathogen was resistant was higher in the culture and susceptibility group. None of the individual differences was significant.

308 patients (85%) had data for the secondary outcome, clinical cure on day 5. Cure rates were equal between groups and there was no significant difference between the proportions of patients cured on day 5. See Table 2 and Figure 2. 144 patients (40%) delivered a control urine sample after 14 days. There was no significant difference in microbiological cure rate between groups.

In accordance with the protocol, we investigated whether practice or patient factors could modify the primary outcome (effect modification). Neither practice factors (size and organisation of participating practices), nor patient factors (any complicating factor, age, diabetes, number of UTIs and number of key symptoms at inclusion) modified the effect of the intervention significantly.

Six patients in the culture-only group had the wrong test performed (culture and susceptibility testing). Per protocol analysis essentially reproduced our findings with culture-only still leading to 75% appropriate treatment and culture and susceptibility testing to 67% appropriate treatment (P= 0.05 unadjusted and 0.02 adjusted).

DISCUSSION

Patients in the POC culture group received significantly more appropriate prescribing than patients in the POC culture and susceptibility group. There was no difference in clinical recovery, despite the difference in appropriate prescribing. This may be partly due to the fact that pivmecillinam has been shown to have a clinical and microbiological effect despite the infecting pathogen being resistant *in vitro* (5).

We aimed at investigating the effect of adding susceptibility testing to POC culture on the appropriate use of antibiotics so the randomized controlled trial was the most appropriate design (19–21). We succeeded in enrolling a sample of GPs with experience in POC culture. These GPs recruited a sample of patients with symptoms of uncomplicated UTI, which was sufficient to detect a small but significant difference between appropriate prescribing based on two different POC culture tests. The inclusion criteria broadened the usual strict definition of uncomplicated UTI, which ensures applicability of our findings to a much broader group of patients in general practice. It may be controversial to include patients with diabetes and recurrent UTI in a sample of patients with uncomplicated UTI, but since these conditions are very common among patients with suspected UTI in general practice and they could be safely included, we decided to include these conditions and investigate whether they modified the effect of the intervention on the outcome. Both the sub-group analysis and the investigation of effect modification indicated that these patients' disease was not more complicated than that of young women with no co-morbidity. We did not recruit our initially planned sample, but the difference between groups turned out to be larger than originally expected when sample size was calculated. A type I error in determining the superiority of the ID Flexicult^{TM} is possible, since the significance level was not overwhelming, but a type II error in failing to detect the expected superiority of the Flexicult^{TM} SSI-Urinary Kit is unlikely. Subgroup analysis could easily be subjected to both type I and type II errors and should be interpreted with caution.

Bias in the interpretation of the test was low as described previously (22). GPs were blinded to the result of the reference at the time of deciding on treatment; POC test and reference were performed on the same portion of urine; the reference was adequate for ruling disease in or out; and all data were included in the analysis. Allocation was concealed using sealed envelopes. It is very unlikely that GPs introduced any selection bias due to strong beliefs of the effect of one of the tests. Applicability of the results was also high, since patients, GPs and tests were very similar to

those which would be relevant in daily practice. Patients with negative dipstick results were excluded. Spectrum bias should therefore be considered if the tests are applied to all patients regardless of their dipstick result.

The study was subjected to clinical review bias in the interpretation process, since the interpreter of the POC tests was not blinded to clinical history. The two groups did not differ in terms of number of symptoms or number of days with symptoms, and patient factors did not seem to have different effects on the two groups, so the difference in this bias between groups was probably minimal. Confirmation bias in the interpretation process could also be present, since treatment had to be initiated based on the result of the test and only patients with suspected UTI were included. GPs were slightly more compliant with regard to the familiar test (culture and susceptibility testing) than with the new test. However, since overtreatment was similar in the two groups, it does not seem to have had a major effect (see appendix 1). The number of patients recruited in the two groups was not the same, but if allocation concealment was insufficient leading GPs to avoid recruiting patients when the patient was intended to receive culture without susceptibility testing, we would have expected more patients with any complicating factor in the culture and susceptibility groups, but the opposite was the case. The unequal distribution of patients between the groups was more likely random due to the GPs not recruiting to number. Our trial was open-label and it is possible that ascertainment bias was present if GPs had a stronger belief in one of the tests. Six patients had the wrong test performed, but per-protocol analysis reproduced the ITT findings, suggesting that this was unintentional. The reference, sending urine in boric acid for culture at the microbiological laboratory, has its flaws as previously described (22). However, these flaws should have a similar effect on the two groups, since the distribution of growth and the resistance pattern did not differ significantly between groups.

There are no previous diagnostic RCTs comparing the use of POC culture versus POC culture and susceptibility testing in general practice. A study from 2010 investigated five different management strategies and found differences in antibiotic use (more antibiotics were used when treatment was based only on symptoms), but no difference in patient recovery (23). They found the lowest antibiotic use in the group in which antibiotics were delayed (77%). In comparison, total antibiotic use was 76% for culture-only and 73% for culture and susceptibility testing in this study.

The significant overall difference in appropriate prescribing between the groups was driven by three factors (none of them individually significant): firstly, undertreatment; secondly, treatment with an antibiotic to which the infecting

pathogen was resistant; and thirdly, inappropriate choice of a second-line antibiotic. The first factor, undertreatment, could be partly due to a slightly lower sensitivity of the Flexicult™ SSI-Urinary Kit (22) and partly to GPs being generally more compliant with a negative result in this group (see appendix 1). The second factor, treatment with an antibiotic to which the infecting pathogen was resistant, was surprising and could be partly due to the fact that susceptibility testing in general practice is not always accurate (11), and partly due to ID Flexicult™ possibly being a better test to identify pathogens, thereby identifying the inherent susceptibility pattern. Correct identification of pathogens is essential for determining the inherent susceptibility pattern, since the inherent susceptibility pattern does not necessarily show on the culture plate (24). The GPs in our study may have relied too much on their susceptibility test and only looked up the inherent susceptibility when they were forced to do so. The study on the accuracy of the two tests investigated in this study showed that the GPs identified pathogens correctly in about 60% of the positive cultures (22). A post-hoc analysis showed that the ID Flexicult™ was actually significantly better at identifying uropathogens than the Flexicult SSI Urinary kit™. However, the most common uropathogen, *E. coli*, does not have inherent resistance to first-line antibiotics, so this second factor may just be a random finding. The third factor, inappropriate choice of a second-line antibiotic, happened in a few cases and none of them had an obvious reason, such as identification of resistance on the practice susceptibility test or patient allergies. The factor expected to drive the difference between the groups: choice of an antibiotic to which the infecting pathogen was resistant, happened in few cases with no difference between the groups. Resistance levels in Denmark are low, and in countries with high resistance rates, the results would probably be different. It remains to be investigated if adding POC susceptibility testing in a high-resistance setting improves prescribing.

The findings of this study support current recommendations that uncomplicated UTI should not have susceptibility testing performed prior to initiation of treatment. Women generally accepted delaying treatment for one day to await the POC culture result and inappropriate treatment was low in both groups. If all patients had been treated with first-line antibiotics based on clinical history and positive dipstick finding, then about 45% of patients would have been inappropriately treated compared to 29% in this study (data not shown). Also, total antibiotic use was lower than previously described in a similar setting (23). Based on these results, performing POC culture prior to treatment for patients with uncomplicated UTI seems rational, but adding POC susceptibility testing should be reserved for those patients at high risk of a resistant infection or complications or for geographical areas with high levels of resistance.

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ETHICAL APPROVAL

All procedures followed were in accordance with the Helsinki Declaration of 1975, as revised in 1983. The study was approved by the Ethical Committee for the Capital Region of Denmark (ref.no: H-3-2014-107). All patients gave written informed consent prior to participating in the study.

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DATA ACCESS

All authors had access to and can take responsibility for data and analysis. The authors commit to making the relevant anonymised patient level data available on reasonable request

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AUTHORSHIP

All authors have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. The corresponding author drafted the manuscript and all other authors revised it critically for important intellectual content. All authors have approved the final version to be published. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CONTRIBUTORSHIP

All authors took part in the design and planning of the study. AH conducted the study supported by all other authors. AH drafted the first manuscript and all other authors revised the entire manuscript critically and approved the final version for publication. VS has mainly supervised statistics and NFM has mainly supervised technical issues regarding the POC tests and microbiological culture. AH is guarantor for the study.

CONFLICTS OF INTEREST

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; LRJ and TMS declares non-financial support from SSI Diagnostica since SSI Diagnostica is supplying Flexicult (R) VET POC culture plates for an ongoing veterinary multi-center RCT study being co-ordinated by LRJ at the University of Copenhagen**.** None of the other authors has other relationships or activities that could appear to have influenced the submitted work**.**

TRANSPARENCY DECLARATION

The lead author* (AH) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned and registered have been explained.

*The manuscript's guarantor.

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LEGENDS

Figure 1: Inclusion flow chart

Table 1: Distribution of baseline data between the two groups. Numbers are total numbers with proportions in brackets unless otherwise stated. NS=Not significant

Table 2: Comparison of primary and secondary outcomes between the two groups. OR: Odds for having a positive outcome if randomized to culture-only (ID Flexicult™) compared to culture and susceptibility testing (Flexicult™ SSI-Urinary Kit). NS=Not significant

Table 3: Reasons for appropriate and inappropriate prescribing and distribution of patients between groups

Figure 2: Cure rates for the two groups. The level of the coloured lines indicates the proportion of patients still having symptoms. Day 0 is the evening of the day of the consultation. The first vertical grey line indicates initiation of treatment (the morning after the consultation), the second vertical grey line indicates the data used for calculation of the secondary outcome: clinical cure on day 5 (four days after consultation).

TABLES AND FIGURES

	Culture and susceptibility, n (%)	Culture-only, n (%)	P
Age groups			
Age below 50 years	105 (55%)	83 (48%)	NS
Age 50 years or above	86 (45%)	89 (52%)	NS
Number of days with symptoms			
Symptoms for less than 3 days	77 (41%)	67 (40%)	NS
Symptoms 3 days or more	109 (59%)	101 (60%)	NS
Key symptoms (dysuria, frequency, urge)			
One or two key symptoms	75 (40%)	67 (40%)	NS
Three key symptoms	111 (60%)	100 (60%)	NS
Complicating factors			
Any complicating factor	43 (26%)	62 (38%)	0.0209
Elderly above 65 years	34 (20%)	50 (29%)	0.0496
Recurrent UTI (>3 past year)	11 (6%)	6 (4%)	NS
Uncomplicated diabetes	11 (6%)	17 (10%)	NS
Reference culture and susceptibility test			
Significant growth of uropathogens (UTI)	100 (62%)	104 (64%)	NS
Trimethroprim resistance	27 (26%)	21 (20%)	NS
Sulfamethizole resistance	29 (29%)	24 (24%)	NS
Nitrofurantoine resistance	3 (3%)	3 (4%)	NS
Mecillinam resistance (pivmecillinam)	15 (14%)	9 (9%)	NS

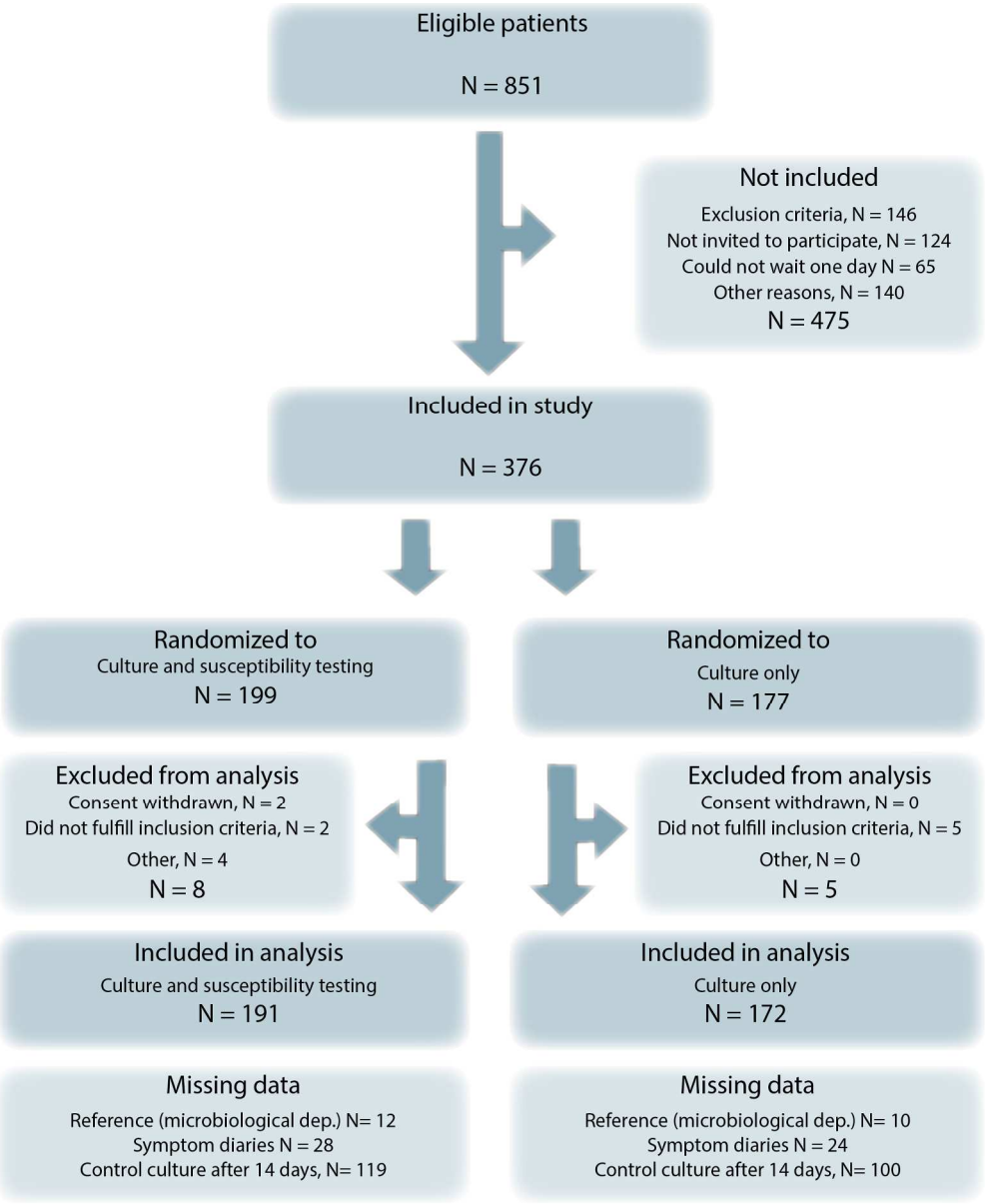
Table 1: Distribution of baseline data between the randomization groups. Numbers are total numbers with percentages in brackets. NS = Not significant

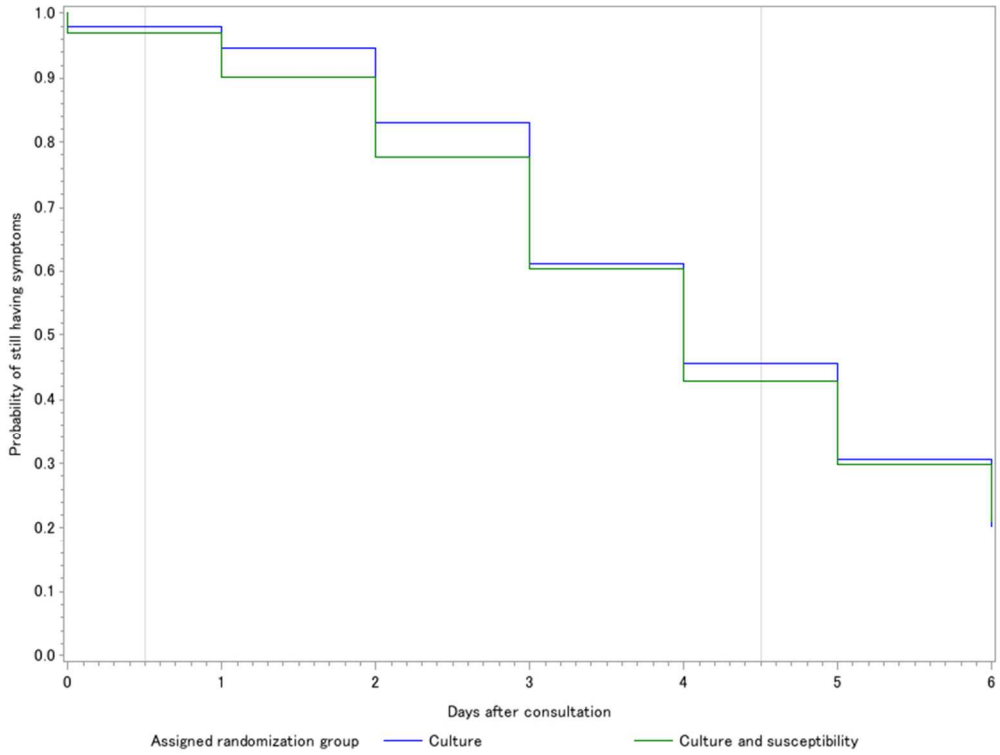
	n	OR (95% CI)	p
Unadjusted analysis			
Odds for appropriate prescribing if culture-only	341	1.44 (1.03-1.99)	0.0311
Odds for being symptom-free on day 5 if culture-only	308	0.91 (0.56-1.49)	NS
Odds for no significant bacteriuria on day 14 if culture-only	144	1.15 (0.62-2.13)	NS
Adjusted for complicating factors			
Odds for appropriate prescribing if culture-only	324	1.65 (1.12-2.42)	0.0112
Odds for being symptom-free on day 5 if culture-only	293	0.89 (0.55-1.44)	NS
Odds for no significant bacteriuria on day 14 if culture-only	140	1.23 (0.64-2.38)	NS
Sub-group analysis (unadjusted)			
Odds for appropriate prescribing for young patients without comorbidities if culture-only	222	1.79 (1.06-3.02)	0.0300
Odds for appropriate prescribing for patients that were elderly, had diabetes or had recurrent UTI if culture-only	102	1.37 (0.78-2.41)	NS

Table 2: Comparison of primary and secondary outcomes between the randomization groups. OR: Odds for having a positive outcome if randomized to culture-only (ID Flexicult™) compared to culture and susceptibility testing (Flexicult™ SSI-Urinary Kit). NS = Not significant

		Culture and susceptibility, n (%)	Culture-only, n (%)
Appropriate choice of treatment		120 (67%)	121 (75%)
1	UTI and first-line antibiotic and pathogen susceptible	85 (47%)	90 (56%)
2	UTI, second-line antibiotic and pathogen susceptible and first-line antibiotic impossible due to allergies or resistance	0 (0%)	0 (0%)
3	No UTI and no antibiotic	35 (20%)	31 (19%)
Inappropriate choice of treatment		59 (33%)	41 (25%)
1	UTI and no antibiotic	13 (7%)	7 (4%)
2	UTI and antibiotic but uropathogen not susceptible to antibiotic	10 (6%)	7 (4%)
3	UTI and inappropriate second-line antibiotic	3 (2%)	0 (0%)
4	No UTI and antibiotic	33 (18%)	27 (17%)

Table 3: Reasons for appropriate and inappropriate choice of treatment and distribution of patients between groups. The overall difference was significant as shown in Table 2, but none of the individual differences was significant.


Eligible patients
N = 851
Not included
Exclusion criteria, N = 146
Not invited to participate, N = 124
Could not wait one day N = 65
Other reasons, N = 140
N = 475
Included in study
N = 376
Randomized to
Culture and susceptibility testing
N = 199
Randomized to
Culture only
N = 177
Excluded from analysis
Consent withdrawn, N = 2
Did not fulfill inclusion criteria, N = 2
Other, N = 4
N = 8
Excluded from analysis
Consent withdrawn, N = 0
Did not fulfill inclusion criteria, N = 5
Other, N = 0
N = 5
Included in analysis
Culture and susceptibility testing
N = 191
Included in analysis
Culture only
N = 172
Missing data
Reference (microbiological dep.) N= 12
Symptom diaries N = 28
Control culture after 14 days, N= 119
Missing data
Reference (microbiological dep.) N= 10
Symptom diaries N = 24
Control culture after 14 days, N= 100



Appendix 1: Data on 341 patients, where the results of both index and reference as well as treatment were available. The red boxes contain cases where the GP was not compliant with the test result. The red numbers are cases where the treatment was inappropriate for any of the reasons stated in Table 3 in the main manuscript.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3-4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	5
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	6
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	Protocol
	7b	When applicable, explanation of any interim analyses and stopping guidelines	-
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	8
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	-

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	7+11
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	9+figure 1
	13b	For each group, losses and exclusions after randomisation, together with reasons	9+figure 1
Recruitment	14a	Dates defining the periods of recruitment and follow-up	9+figure 1
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	Table 1 page 22
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	Table 2 page 23
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	Table 2 page 23
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	10-table 3
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	-
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	11
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	11
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	12
Other information			
Registration	23	Registration number and name of trial registry	4
Protocol	24	Where the full trial protocol can be accessed, if available	6
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	14

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.