Clinical Microbiology and Infection 23 (2017) 647–652

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Original article

Women with symptoms of a urinary tract infection but a negative urine culture: PCR-based quantification of *Escherichia coli* suggests infection in most cases

S. Heytens [1,*], A. De Sutter [1], L. Coorevits [2], P. Cools [2], J. Boelens [2], L. Van Simaey [2], T. Christiaens [1], M. Vaneechoutte [2], G. Claeys [2]

[1] *Department of Family Medicine and Primary Health Care, Ghent University, Gent, Belgium*
[2] *Department of Clinical Chemistry, Microbiology and Immunology, Ghent University Hospital, Gent, Belgium*

ARTICLE INFO

Article history:
Received 9 January 2017
Received in revised form
30 March 2017
Accepted 5 April 2017
Available online 28 April 2017

Editor: Professor L. Leibovici

Keywords:
Escherichia coli
Primary care
Quantitative PCR
Standard urine culture
Urethral syndrome
Urinary symptoms
Urinary tract infections
Urinary tract infections in women

ABSTRACT

Objectives: Our objective was to examine whether or not women with symptoms of a urinary tract infection but with a negative culture (20%–30%) do have an infection.
Methods: We performed quantitative PCR (qPCR) for *Escherichia coli* and *Staphylococcus saprophyticus*, on top of a standard culture, in urine samples from 220 women with dysuria and/or frequency and/or urgency and from 86 women without symptoms. For symptomatic women, qPCR was also carried out for four sexually transmitted agents.
Results: In the symptomatic group, 80.9% (178/220) of the urine cultures were positive for any uropathogen and 95.9% (211/220) were *E. coli* qPCR-positive. For the control group, cultures for *E. coli* and *E. coli* qPCR were positive in, respectively, 10.5% (9/86) and 11.6% (10/86). In the symptomatic group, qPCR yielded 19 positive samples for *S. saprophyticus* qPCR, one positive sample for *Mycoplasma genitalium* and one for *Trichomonas vaginalis*.
Conclusions: These findings suggest that almost all women with typical urinary complaints and a negative culture still have an infection with *E. coli*. **S. Heytens, Clin Microbiol Infect 2017;23:647**

Introduction

Dysuria in women represents 2%–5% of the reasons for encounter in general practice [1].

In female patients, 60%–80% of these dysuric episodes are related to significant bacteriuria [2–4]. Diagnosis is generally based on clinical signs and urine dipstick testing for leucocyte esterase and nitrite. As typical urinary symptoms, e.g. dysuria, frequency and urgency, are highly predictive of a urinary tract infection (UTI) in female patients [5,6], therapy can be empirically started without performing a culture in women with symptoms of an uncomplicated UTI [2,7–9]. However, 25%–30% of these symptomatic women will have a negative urine culture, according to the bacterial count threshold in a midstream urine sample that was used [2–4].

In the 1980s, Stamm *et al.* questioned the threshold of 10^5 CFU/mL [10], arguing that it was never meant for diagnosing uncomplicated UTI and proposed cut-off rates as low as 10^2 CFU/mL for symptomatic women [11]. Recently Hooton *et al.* confirmed that the presence of low counts of *Escherichia coli* in midstream urine is highly predictive for its presence in the bladder and is not caused by contamination [12]. Although lowering the cut-off rate can explain at least partially the problem of 'negative culture', results in symptomatic women, applying a cut-off rate of 10^3 CFU/mL for *E. coli*, according to the European Guidelines for Urinalysis, still results in nearly 20% negative cultures [4,13]. This specific group of patients was historically referred to as having the 'urethral syndrome', a condition that has been heavily debated since the 1960s [14,15].

In 1998, Baerheim *et al.* found no difference in symptomatic outcome after antibacterial treatment whether the patient was classified as having an acute cystitis or acute urethral syndrome [16]. In a small randomized controlled trial, Richards *et al.* found

* Corresponding author. S. Heytens, Department of Family Medicine and Primary Health Care, Ghent University, De Pintelaan 185, B-9000 Gent, Belgium.
E-mail address: stefan.heytens@ugent.be (S. Heytens).

http://dx.doi.org/10.1016/j.cmi.2017.04.004
1198-743X/

that adult women with symptoms of a UTI but a negative dipstick urine test result benefit from a treatment with trimethoprim compared with a placebo [17].

To explore whether women with complaints but a negative culture may still have an infection, we performed a quantitative PCR (qPCR) for *E. coli* in urine samples of symptomatic women and compared the results with those of healthy volunteers without symptoms. We also examined the possibility of sexually transmitted pathogens being responsible for our patients' symptoms, using commercially available, validated PCR kits.

Materials and methods

Study design and population

This study is nested within an observational survey on uropathogens and their susceptibility pattern in 256 adult women with symptoms of uncomplicated UTI [4].

Between May 2014 and January 2016, 256 women in the Ghent region (Belgium), presenting with complaints of dysuria, urgency and/or frequency were included by their general practitioner (GP) into the study. Women were excluded if they had symptoms suggestive of complicated UTI at presentation (symptoms >7 days, axillary temperature >38°C), had predisposing factors (pregnancy, known urological/nephrological problems, diabetes mellitus other immunocompromising diseases), received antibiotics during the past 4 weeks or had obvious gynaecological complaints.

Patients with a history of recurrent uncomplicated UTI (more than three UTI episodes during the past year) were included.

This group was compared with a control group of 86 women without symptoms of a UTI that participated in a study examining the resident microflora of midstream urine by means of an extended urine culture protocol (August to October 2015) [18]. These women were recruited from among healthy volunteers of the Ghent University campus and so were not recruited during a GP visit. The exclusion criteria were the same as in the symptomatic group.

Assessments of symptomatic group

Collection of urine samples

After collecting a midstream urine sample at the GP's office, a dipslide with McConkey Agar and CLED agar (Uricult®, Orion Diagnostica, Espoo, Finland) was immediately inoculated, according to the manufacturer's instructions, and sent to the Laboratory of Bacteriology at the Ghent University Hospital for incubation and microbiological analysis. The GP also collected urine in Abbott multi-collect tubes (Abbott Laboratories, Abbott Park, IL, USA) for qPCR.

Urine culture

At the laboratory, the dipslides were incubated at 35°C overnight and for another 24 h. All colony types were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonics, Bremen, Germany). Quantitative data ($<10^3$, 10^3, 10^4, 10^5, $\geq 10^6$ CFU/mL) were obtained by matching the colony density on the dipslide agars with the model charts in the package insert, according to the manufacturer's instructions [19].

Quantitative PCR

To detect *E. coli*, a specific qPCR was carried out in urine DNA extracts, using primers targeting the *E. coli* β-glucuronidase-encoding gene *uidA* as previously described [20]. For the *S. saprophyticus* qPCR, primers targeting the *trk* region were used [21].

Quantitative PCR for *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Trichomonas vaginalis* was carried out by means of commercial kits—Abbott Real-Time CT/NG assay (Abbott Laboratories) and Diagenode S-DiaMGTV (Diagenode Diagnostics, Seraing, Belgium)—and analysed on an Abbott m2000sp/rt.

Assessments of control group

Collection of urine samples

The participants were asked to deliver a midstream urine sample, which was immediately transported to the laboratory.

Urine culture

For the standard urine culture a uricult dipslide with CLED and McConkey agar (Mediphos, Renkum, the Netherlands) was immersed and incubated in ambient atmosphere at 35°C for 24 h. The colony count density on the agar surfaces of the dipslides was compared with the colony density chart provided to obtain a semi-quantitative colony count in CFU/mL of urine.

Next, a trypticase soy agar with 5% sheep blood and Schaedler agar (Becton Dickinson Co., Franklin Lakes, NJ, USA) were inoculated with 10 μL of urine and incubated in ambient atmosphere and anaerobically at 35°C for 7 days (extended urine culture). Lastly, 2 mL of urine was aspirated in a Urine Monovette tube (Sarstedt, Nümbrecht Germany) for urinalysis on UF-1000i (Sysmex Corporation, Kobe, Japan). The urines were then transferred in an Abbott multi-Collect Specimen medium (Abbott Laboratories).

For extended urine culture, a consensus colony count was made of the two agars and all CFUs with distinct morphological appearance were identified using MALDI-TOF mass spectrometry using the MALDI Biotyper 3.0 Real-time Classification software (Bruker Daltonics, Billerica, MA, USA).

A single measurement was performed once for each isolate. A score between 2000 and 3000 allowed for species-level identification; a score between 1700 and 1999 for genus-level identification and a score <1700 was considered unreliable.

Quantitative PCR

In the control group, the qPCR was carried out only for *E. coli*.

Comparability of both groups

Eight of the women in the symptomatic group were excluded because no qPCR data were available.

As the control group did not comprise women >65 years of age, another 28 women in the symptomatic group >65 years were excluded, resulting in a total of 220 eligible symptomatic women (Table 1). To compare both groups correctly, further analysis is made for these remaining 220 women in the symptomatic group.

Statistical analysis

The statistical program SPSS 22 for Windows was used for all statistical analyses.

As the women's ages did not follow a normal distribution, both groups were compared with a Mann–Witney *U* test. A p value <0.05 was considered significant.

For the power calculation, the aim was to detect, with a 90% probability, a statistically significant difference at $\alpha = 0.05$ for a one-sided test, between the *E. coli* qPCR test in symptomatic women (with an estimated detection rate of at least 90%) and the same test in the control group (with a detection rate of 20%). The

Table 1
Characteristics of women with symptoms of urinary tract infection (UTI) and control group

Characteristic	Control group	Women with symptoms of UTI
Number of women	86	220
Age, years, mean (range)[a]	37.2 (23–65)	38.5 (17–65)
Recruitment	University of Ghent campus volunteers	Patients consulting their GP's practice
Culture technique	Standard urine culture EQUC[b]	Dipslide

[a] No statistically significant difference between the two groups. Student's *t* test: p 0.440.
[b] Extended Quantitative Urine Culture (EQUC); explained in Material and methods [18].

calculated sample size was 12. This was the minimum size of a group. As we did not expect the qPCR detection to be lower than the culture result we used a one-sided test.

A chi-squared test was performed to detect differences between qPCR results for *E. coli* and standard bacteriological culture procedures on samples in symptomatic women. When sample size in one cell was <5, a Fisher's exact probability test was used.

A p-value <0.05 was considered significant.

Ethical approval

Study approval was obtained by the ethical committee of the Ghent University Hospital, under approval number B670201318317 for the observational survey in symptomatic women and under approval number B670201525210 for the control group study in women without symptoms.

Results

Characteristics

A total of 256 women from 17 to 91 years (mean age 42.6 years, SD 17.35) with symptoms of a UTI were enrolled over a 20-month period. Thirty-six were excluded, of whom 28 were aged over 65 years, resulting in 220 symptomatic women with a mean age of 38.5 years (SD 13.84) (Table 1). Eighty-six women between 23 and 65 years old (mean age of 37.2, SD 11.5) without symptoms of a UTI were recruited over a 2-month period (Table 1). The mean ages of both the control group and the remaining 220 women in the symptomatic group were not significantly different. Of the 28 women >65 years that were excluded from the symptomatic group 24 had a positive *E. coli* qPCR and *E. coli* culture.

Symptomatic group

Urine culture

A uropathogen (primary—*E. coli* and *S. saprophyticus*—or secondary—mainly other Gram-negative rods and *Enterococcus* spp.—as defined by the European Guidelines for Urinalysis [13]) was isolated in 80.9% of the urine cultures of symptomatic women ($\geq 10^2$ for *E. coli*; $\geq 10^4$ for other uropathogens), with *E. coli* being the most frequently isolated uropathogen (83.2% of the positive cultures) (Table 2, Fig. 1).

Quantitative E. coli PCR

The *E. coli* qPCR was positive in 95.9 % ($n = 211$) of the 220 urine samples of symptomatic women (Table 2). Except for one, all *E. coli* culture-positive samples were also *E. coli* qPCR-positive.

Table 2
Symptomatic group ($n = 220$): *Escherichia coli* quantitative PCR (qPCR) result related to conventional culture result (from dipslide)

Culture ($n = 220$)		qPCR *E. coli*		qPCR *Staphylococcus saprophyticus*
Culture result	Number	Positive $n = 211$ (95.9%)	Negative $n = 9$	
Culture positive (80.9%)	178	173	5	19
Escherichia coli (67.7% of 220 samples)	149	148	1	6
Staphylococcus saprophyticus[a]	15	14	1	12
Klebsiella pneumoniae[a]	5	5	—	—
Enterococcus faecalis[a]	3	3	—	1
Other Gram-positive bacteria[a]	3	1	2	—
Other Gram-negative bacteria[a]	3	2	1	—
Culture negative[b]	42	38	4	—

Other Gram-positive bacteria: *Streptococcus agalactiae*: two; *Staphylococcus aureus* (10^5): one.
Other Gram-negative bacteria: *Pseudomonas* spp.: one; *Enterobacter* spp.: two.
[a] All $\geq 10^4$ CFU/mL.
[b] Not positive using the European Guidelines for Urinalysis (2001) criteria and in *E. coli* isolates considering 10^2 CFU/mL as a positive sample.

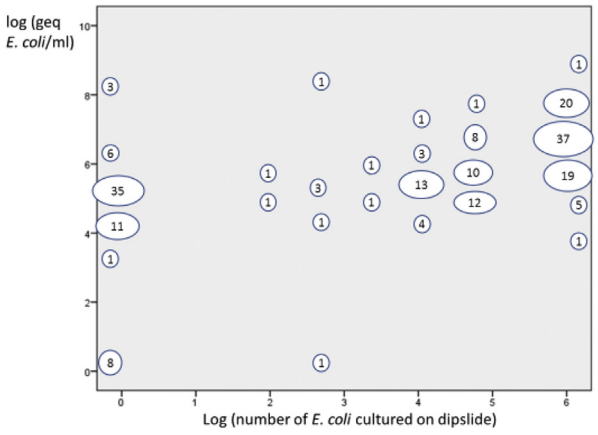

Fig. 1. Correlation between *Escherichia coli* quantitative PCR and culture for *E. coli* (on dipslide) in symptomatic women. Figures shown in circles represent the number of samples.

Fig. 1 shows that for positive cultures the bacterial counts are positively correlated with the *E. coli* qPCR counts (Fig. 1). However, in 63/211 (29.9%) *E. coli* qPCR-positive samples the culture was negative for *E. coli* (Table 2, Fig. 1). Another predominant uropathogen was isolated in 25/211 (11.8%) of these samples.

Nine specimens were negative for *E. coli* qPCR, of which four also had a negative culture result for *E. coli* and five were culture-positive.

Besides the 211 *E. coli* qPCR-positive samples, an additional five samples were culture-positive for a uropathogen, resulting in 216 (98.2%) positive samples in 220 symptomatic women (Tables 2 and 3).

Table 3
Overall number of positive samples in 220 women with symptoms of urinary tract infection according to the used method of detection

Method of detection	Positive urine samples	
	n	%
Culture of *Escherichia coli*	149	67.7
Culture of any uropathogen	178	80.9
Quantitative PCR for *E. coli*	211	95.9
Culture and quantitative PCR	216	98.2

Quantitative S. saprophyticus PCR

The qPCR for *S. saprophyticus* was positive in 19 samples with large bacterial counts of this uropathogen ($\geq 10^4$ genomic equivalents (geq)/mL). Of the 15 *S. saprophyticus* positive cultures 12 were qPCR-positive for *S. saprophyticus* and 14 were also *E. coli* qPCR-positive.

PCR detection of sexually transmitted agents

PCR for *C. trachomatis* and *N. gonorrhoeae* was negative in all samples and positive for *M. genitalium* and *T. vaginalis* each time in one sample.

Control group

Urine culture

A uropathogen could be cultured from ten of the 86 women (11.6%) in the control group ($\geq 10^2$ for *E. coli*; $\geq 10^4$ for other uropathogens) (Table 4). *Escherichia coli* was cultured from nine samples, of which five in low numbers and *Klebsiella pneumoniae* was found in the other positive sample.

Quantitative E. coli PCR

Only ten out of the 86 participants in the control group were *E. coli* qPCR-positive (Table 4).

Five qPCR-positive samples had counts of $\geq 10^5$ geq/mL, of which four had also a high count *E. coli* culture result ($\geq 0^5$ CFU/mL). The five other qPCR-positive samples had a count of 10^4 geq/mL and *E. coli* could be cultured from only one of these (10^4 CFU/mL). *Escherichia coli* was also cultured from three other samples that were qPCR-negative. The control group showed a good quantitative correlation between culture and *E. coli* qPCR (Fig. 2).

Table 4
Control group ($n = 86$): culture (Extended Quantitative Urine Culture) and quantitative PCR (qPCR) results

Culture ($n = 86$)		qPCR *Escherichia coli*		qPCR *Staphylococcus saprophyticus*
Culture results	Number	Positive $n = 10$	Negative $n = 76$	
Escherichia coli				
10^2	2	1	1	—
$\geq 10^3$	7	5	2	—
Klebsiella pneumoniae				
$\geq 10^4$ CFU/mL	1	—	—	—
Culture negative[a]	76	4	73	4

[a] Negative: containing species not-considered uropathogens, or possible uropathogens, with numbers $<10^4$ CFU/mL. *E. coli* isolates in low numbers are counted as positive to compare with *E. coli* qPCR.

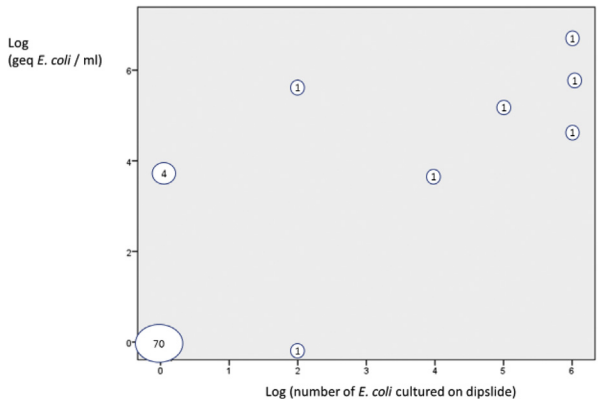

Fig. 2. Correlation between *Escherichia coli* quantitative PCR and culture of *E. coli* (on dipslide and by extended quantitative urine culture) in control group. Figures shown in circles represent the number of samples.

Comparison of the qPCR results in culture-negative women

Forty-two women in the symptomatic group and 76 in the control group had a negative culture. The qPCR was positive in 90.5% (38/42) of these negative samples in the symptomatic group and in only 5.3% (4/76) of the negative samples in the control group, a difference that was statistically significant (Fisher's exact test; $p < 0.0001$).

Discussion

Main findings

We found 90.5% of the urine samples in 42 culture-negative symptomatic women to be qPCR-positive for *E. coli.* Overall, 95.9% of the urine samples of 220 symptomatic women were qPCR-positive for *E. coli* in contrast to *E. coli* or another uropathogen cultured in 80.9% of the samples. In the control group *E. coli* qPCR remained negative in 90% of the samples, overlapping almost completely with the culture result, indicating that our findings in the symptomatic group are not caused by the detection of contamination due to the higher sensitivity of the qPCR (Table 5).

The rate of positive results in the control group reflects the expected rate of asymptomatic bacteriuria (defined as a bacterial count $\geq 10^5$ CFU/mL—in the absence of symptoms) (1%–8%) in the general female population [22,23].

Although three of the 15 samples that were strongly culture-positive for *S. saprophyticus* unexpectedly yielded a negative qPCR result for *S. saprophyticus*, the overall correspondence between culture and qPCR for *S. saprophyticus* was high, with 15 culture positives and 19 qPCR positives of which 12 in correspondence.

We found no sexually transmitted agents to be responsible for the cystitis symptoms in culture-negative women.

The detection of *E. coli* DNA in 25 samples in which a uropathogen other than *E. coli* was found by culture is intriguing. Moreover, the qPCR test for *S. saprophyticus* was positive in six women in whom *E. coli* had been cultured and in 14 women for whom *E. coli* qPCR was positive. This could suggest a co-infection. But our data do not allow us to confirm this.

One possibility is that inflammation by another uropathogen provides an econiche that is better suited for *E. coli* colonization, or vice versa, the presence of *E. coli* could facilitate infection of another pathogen. The exact significance of this finding needs further research.

Limitations

A possible limitation could be the fact that all participants in the control group were recruited within the campus of the Ghent University whereas the symptomatic group was recruited in the family practices and was therefore probably more heterogeneous with regard to socio-economic status.

Also a larger control group would have been desirable. However, in our purpose to compare with women without symptoms, we

Table 5
Comparison of culture and quantitative PCR (qPCR) results for *Escherichia coli* between women with symptoms of a urinary tract infection (UTI) and control group of women

	Positives urine samples	
	PCR	Culture[a]
Women with symptoms of UTI ($n = 220$)	211 (95.9%)	178 (80.9%)
Control group ($n = 86$)	10 (11.6%)	9 (10.5%)

[a] Culture details: see Materials and methods.

found nearly 96% of the symptomatic women had a positive *E. coli* qPCR result compared with only 11.6% of the samples in the control group. Taking into account the unambiguous results, we are confident that this would not have changed the outcome.

The mean lag time between collection of the urine sample and delivery to the laboratory is 2.87 days (median 2 days). Concerning the dipslide, and hence the culture, it can be assumed that this will not have been a problem, but for the PCR urine recipient, the qPCR counts may have been affected by the duration of the transport. Published reports on this particular problem are sparse, probably because until now the qPCR approach has been used only in non-ambulatory settings, where lag-time problems are less of an issue. However, when comparing the rate of qPCR-positive samples between short (0–3 days) and long ($\geq$ 4 days) lag times, we found no significant difference (Fisher's exact; p 0.042).

The PCR recipient used in the study is intended for detection of *C. trachomatis* and *N. gonorrhoeae* in routine clinical molecular testing. The manufacturers' specifications of this product mention that collection, transport and storage conditions at 2°C to 30°C ensure DNA stability for up to 14 days.

Ingersoll *et al*. found that DNA of *T. vaginalis* remained stable in urine samples for up to 30 days when stored at 20°C–22°C with the use of a Becton Dickinson urine preservative transport kit [24]. The recipients used in our study contain guanidine thiocyanate in Tris buffer, which stabilizes DNA.

Also Van Dommelen *et al*. investigated differences in the influence of temperature, medium and storage duration on *C. trachomatis* concentration in urine samples as determined with qPCR. They found no difference in *C. trachomatis* concentrations in urine samples for different storage durations [25].

The comparable qPCR positivity rate for different storage/transport durations, the manufacturers' specifications of the used recipient and the findings in the studies of Ingersoll and Van Dommelen and colleagues provide arguments against a possible influence on qPCR quantification by the differences in lag time of processing urine samples between symptomatic women and asymptomatic women.

Although the qPCR remained negative in the control group, a positive result of this test in symptomatic women may not be seen as absolute proof of causation, as in some samples it may reflect colonization.

Literature

Sixty years of debating the 'urethral syndrome' has yielded a number of possible explanations. Many articles focus on the bacterial count threshold, questioning the Kass criterion. Consecutive studies provide evidence to consider a 'low-count bacteriuria' as a positive result [11,12,14,15]. Yet, even at a lower threshold, one-quarter to one-third of the urine cultures remained negative [3,4], which made some authors question the presence of an infectious agent all together and even propose a psychosomatic explanation for the complaints [15]. This could not explain why even women with a negative culture seemed to benefit from antimicrobial therapy [16,17].

Failure to detect the causative pathogen with standard microbiology protocols could be a reason for negative culture results. Fastidious uropathogens such as *Gardnerella vaginalis*, and recently advocated uropathogens, i.e. *Aerococcus urinae*, *Corynebacterium urealyticum* and *Actinobaculum schaalii*, are only detected using rich media, and extended incubation times [18,26–28]. However, the clinical relevance of these organisms is controversial because they can also be found in asymptomatic patients with expanded culture techniques [18].

Another possible explanation can be found in the capacity of some *E. coli* strains to persist as intracellular 'biofilms', causing inflammation and symptoms, but remaining undiscovered by conventional culture [29,30]. This may be the reason why these strains are only detected by qPCR.

Clinical implications

Our findings support the management proposed by Knottnerus *et al*. [6], namely, if non-pregnant women consult their GP with typical symptoms of an uncomplicated UTI and without vaginal irritation or arguments for a sexually transmitted infection, the diagnosis of a UTI can be accepted without further investigations and can be treated empirically, according to the guidelines [2,7–9].

Instead of spending time and energy to prove an uncomplicated UTI, physicians can rely on the typical symptoms and focus on acknowledging and managing the patient's complaints.

Acknowledgements

We thank the participating women, their GPs and the healthy volunteers for their collaboration. Our gratitude also goes to the laboratory technicians who not only supported the study with their executive skills but were so kind to involve us in their work. Especially, we want to thank Thérèse Lippens who did a large part of the administrative work.

Funding

This study was funded by the Belgian Society for Infectiology and Clinical Microbiology (BVIKM) (grant number: KW/1392/LBV/001/013) and the Belgian Antibiotic Policy Coordination Committee (BAPCOC) (grant number: KW/1392/LBV/001/013/023).

Transparency declaration

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.cmi.2017.04.004.

References

[1] Verheij RA, Van Dijk CE, Abrahamse H, Davids R, Vanden Hoogen H, Braspenning J, et al. Landelijk informatienetwerk Huisartsenzorg. Feiten en cijfers over huisartsenzorg in Nederland. Utrecht/Nijmegen: NIVEL/IQ healthcare; 2009.

[2] Naber K, Schito G, Botto H, Palou J, Mazzei T. Surveillance study in Europe and Brazil on clinical aspects and microbial resistance epidemiology in females with cystitis (ARESC): implications for empiric therapy. Eur Urol 2008;54:1164–78.

[3] De Backer D, Christiaens T, Heytens S, De Sutter A, Stobberingh E, Verschraegen G. Evolution of bacterial susceptibility pattern of *E. coli* in uncomplicated urinary tract infections in a country with high antibiotic consumption: a comparison of two surveys with a 10 year interval. J Antimicrob Chemother 2008;62:364–8.

[4] Heytens S, Boelens J, Claeys G, De Sutter A, Christiaens T. Uropathogen distribution and antimicrobial susceptibility in uncomplicated cystitis in Belgium, a high antibiotics prescribing country: 20-year surveillance. Eur J Clin Microbiol Infect Dis 2016. http://dx.doi.org/10.1007/s10096-016-2776-8.

[5] Giesen LG, Cousins G, Dimitrov BD, van de Laar FA, Fahey T. Predicting acute uncomplicated urinary tract infection in women: a systematic review of the diagnostic accuracy of symptoms and signs. BMC Fam Pract 2010;11:78.

[6] Knottnerus B, Geerlings E, Moll van Charante E, ter Riet G. Towards a simple diagnostic index for acute uncomplicated urinary tract infections. Ann Fam Med 2013;11:442–50.
[7] Little P, Moore MV, Turner S, Rumsby K, Warner G, Lowes JA, et al. Effectiveness of five different approaches in management of urinary tract infection: randomised controlled trial. BMJ 2010;340:c199.
[8] SIGN 88. Management of suspected bacterial urinary tract infection in adults. A national clinical guideline. Updated July 2012. Available at: www.sign.ac.uk.
[9] Gupta K, Hooton T, Naber K, Wult B, Colgan R, Miller L, et al. International Clinical Practice Guideline for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis 2011;52:e103–20.
[10] Kass E, Finland M. Asymptomatic infections of the urinary tract. Trans Am Physiol 1956;69:56–64.
[11] Stamm WE, Counts GW, Running KR, Fihn S, Turck M, Holmes KK. Diagnosis of coliform infection in acutely dysuric women. N Engl J Med 1982;307:463–8.
[12] Hooton T, Roberts P, Cox M, Stapleton A. Voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med 2013;369:1883–91.
[13] Aspevall O, Hallander H, Gant V, Kouri T. European guidelines for urinalysis: a collaborative document produced by European clinical microbiologists and clinical chemists under ECLM in Collaboration with ESCMID. Clin Microbiol Infect 2001;7:173–8.
[14] Stamm WE, Wagner KF, Amsel R, Alexander ER, Turck M, Counts GW, et al. Causes of the acute urethral syndrome in women. N Engl J Med 1980;303:409–15.
[15] Nazareth I, King M. The urethral syndrome: a controlled evaluation. J Psychosom Res 1993;37:737–43.
[16] Baerheim A, Digranes A, Hunskaar S. Equal symptomatic outcome after antibacterial treatment of acute lower urinary tract infection and the acute urethral syndrome in adult women. Scand J Prim Health Care 1999;17:170–3.
[17] Richards D, Toop L, Chambers S, Fletcher L. Response to antibiotics of women with symptoms of urinary tract infection but negative dipstick urine test results: double blind randomised controlled trial. BMJ 2005;331(7509):443.
[18] Coorevits L, Heytens S, Boelens J, Claeys G. The resident microflora of voided midstream urine of healthy controls: standard versus expanded urine culture protocols. Eur J Clin Microbiol Infect Dis 2017;36:635–9.
[19] Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-assisted laser desorption ionization-time of flight mass spectrometry: a fundamental shift in the routine practice of clinical microbiology. Clin Microbiol Rev 2013;26:547–603.
[20] Cools P, Jespers V, Hardy L, Crucitti T, Delany-Moretlwe S, Mwaura M, et al. A multi-country cross-sectional study of vaginal carriage of group B streptococci (GBS) and *Escherichia coli* in resource-poor settings: prevalences and risk factors. PLoS ONE 2016;11:e0148052.
[21] Enderle JL, Miller AL, Pyles RB. Quantification of bacterial uropathogens in preclinical samples using real-time PCR assays. Curr Microbiol 2014;68:220–6.
[22] Nicolle LE, Bradley S, Colgan R, Rice JC, Schaeffer A, Hooton TM. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. Clin Infect Dis 2005;40:643–54.
[23] Wagenlehner F, Naber K. Asymptomatic bacteriuria—shift of paradigm. Clin Infect Dis 2012;55:778–80.
[24] Ingersoll J, Bythwood T, Abdul-Ali D, Wingood G, Diclemente R, Caliendo A. Stability of *trichomonas vaginalis* DNA in urine specimens. J Clin Microbiol 2008:1628–30.
[25] Van Dommelen L, Wolffs P, van Tiel F, Dukers N, Herngreen S, Bruggeman C, et al. Influence of temperature, medium and storage duration on *Chlamydia trachomatis* DNA detection by PCR. J Clin Microbiol 2013;51:990–2.
[26] Cattoir V. *Actinobaculum schaalii*: review of an emerging uropathogen. J Infect 2012;64:260–7.
[27] Hilt E, McKinley K, Pearce M, Rosenfeld A, Zilliox M, Mueller E, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. J Clin Microbiol 2014;52:871–6.
[28] Pearce M, Hilt E, Rosenfeld A, Zilliox M, Thomas-White K, Fok C, et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. mBio 2014;5:e01283–14.
[29] Rosen DA, Hooton TM, Stamm WE, Humphrey PA, Hultgren SJ. Detection of intracellular bacterial communities in human urinary tract infection. PLoS Med 2007;4:e329.
[30] Hunstad DA, Justice SS. Intracellular lifestyles and immune evasion strategies of uropathogenic *Escherichia coli*. Annu Rev Microbiol 2010;64:203–21.