

Evolution of bacterial susceptibility pattern of *Escherichia coli* in uncomplicated urinary tract infections in a country with high antibiotic consumption: a comparison of two surveys with a 10 year interval

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Objectives: For the empirical treatment of cystitis, clinicians are often guided by susceptibility data taken from urinary samples that sent to regional microbiological laboratories, which are not representatives for uncomplicated urinary tract infections (UTIs). To offer adequate recommendations, the distribution and susceptibility pattern of uropathogens in uncomplicated UTIs in women were compared with those obtained 10 years ago in our uropathogen surveillance in a primary healthcare setting.

Methods: Sixty-six general practitioners in the region of the city of Ghent were asked to inoculate a dipslide with midstream urine from every adult female patient with complaints suggestive for cystitis, during a period of 1 year. The dipslides were further processed in a central microbiological laboratory, where counting, identification and susceptibility testing were performed.

Results: Three hundred specimens were collected, of which 187 (62.3%) yielded a positive culture of 10⁵ cfu/mL. In the age group of 18–54 years, *Escherichia coli* was the most frequently isolated uropathogen (77.5%), followed by *Staphylococcus saprophyticus* (13.5%) and *Proteus* spp. (2.7%). There were no statistically significant differences when compared with the data from 1996. In 2006, susceptibility of *E. coli* to nitrofurantoin was 100%, to quinolones 100%, to ampicillin 62.8% and to co-trimoxazole 86%, compared with 99.3%, 99.3%, 73.2% and 83.3%, respectively, in 1996 (no statistically significant differences).

Conclusions: Over a period of 10 years, a systematic surveillance of uropathogens in female patients with uncomplicated UTI in general practice could not demonstrate a significant change in species distribution or antimicrobial susceptibility.

Keywords: cystitis, uropathogens, resistance

Introduction

Uncomplicated urinary tract infections (UTIs) in healthy women are common in general practice, with an incidence of 50/1000/year.¹ The diagnosis of cystitis is usually based on the typical history of dysuria, frequency and urgency, often in combination with urinary sediment or dipstick testing.² The empirical choice

of antimicrobial treatment is generally guided by susceptibility data provided by regional microbiological laboratories. However, since samples of uncomplicated UTIs are rarely sent for culture,³ these data are mainly derived from complicated UTIs. Data on resistance rate and uropathogen distribution from laboratories do not reflect the situation in uncomplicated UTIs, occurring mostly in young and otherwise healthy women.⁴

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To deal with this problem and to offer general practitioners' (GPs) recommendations based on data from uncomplicated UTIs, our department performed a systematic surveillance of uropathogens in each woman complaining of dysuria in the region of Ghent, Belgium, in 1996.⁴ The data from this study were used in the development of the Belgian recommendations for the diagnosis and treatment of cystitis in general practice.⁵

Ten years later, it was important to evaluate possible changes in the uropathogen resistance or distribution pattern, especially because the prescription rate of antibiotics in Belgium is among the highest in Europe, as illustrated in Figure 1.⁶ Outpatient quinolone use is also very high, both for cystitis and for respiratory infections.⁷

Therefore, we repeated the systematic uropathogen surveillance in the same region in Belgium in order to compare the present distribution and susceptibility pattern of uropathogens in uncomplicated UTIs in women with the results obtained 10 years ago.

Materials and methods

Participating GPs and patients

Sixty-four practices (97 GPs) in the Ghent region were invited to participate. For recruitment, all practices serving as training places for Ghent University and situated in the Ghent region were contacted by letter. The contacted practices were the same as in 1996. Non-responders received a new invitation by phone and if declined were asked for the reason. Twenty-eight practices (66 GPs) accepted. In group practices every GP participated. Characteristics of both surveys are given in Table 1.

The geographical area and recruitment method were the same as in the 1996 surveillance.

GPs were asked to include all female patients of 18 years or older presenting with symptoms of dysuria, urgency, frequency or a combination. Exclusion criteria were symptoms of or predisposing factors for complicated UTIs (pregnancy, symptoms lasting longer than 7 days, temperature of 38°C, known urological or nephrological problems, diabetes mellitus and other immunocompromising diseases)

Table 1. Characteristics of the surveys from 1996 and 2006 (subgroup comparable to 1996)

	1996	2006
Study duration	19 months	17 months
Number of samples	279	166
Percentage of positive samples (%)	59	65
Proportion of <i>E. coli</i> (%)	78.5	77.5

and obvious gynaecological complaints (abnormal discharge, labial irritation, intermittent vaginal blood loss and vaginal itch).

The period of inclusion was between November 2004 and March 2006.

Urine collection and processing

The urine specimens were taken at the surgery after instructing the patient on the midstream technique. A dipslide (Uriline, bioMérieux, Plainview, NY, USA) was immediately inoculated using the manufacturer's instructions and sent to the Laboratory of Bacteriology and Virology at the University Hospital, University Ghent, for incubation and further analysis. GPs completed a questionnaire on the patient's antibiotic use and UTIs in the previous 12 months.

Informed consent from all participating women was obtained after giving oral information on the study and a written information sheet.

The study was approved by the Ethics Committee of the University Hospital, Ghent, under the approval number OG017.

Dipslide processing

The dipslides were immersed in the freshly voided urine, incubated at 35°C overnight and finally discarded after another 24 h of incubation. Positive dipslides were sent to the laboratory for culture and identification of the microorganisms. For identification, standard laboratory

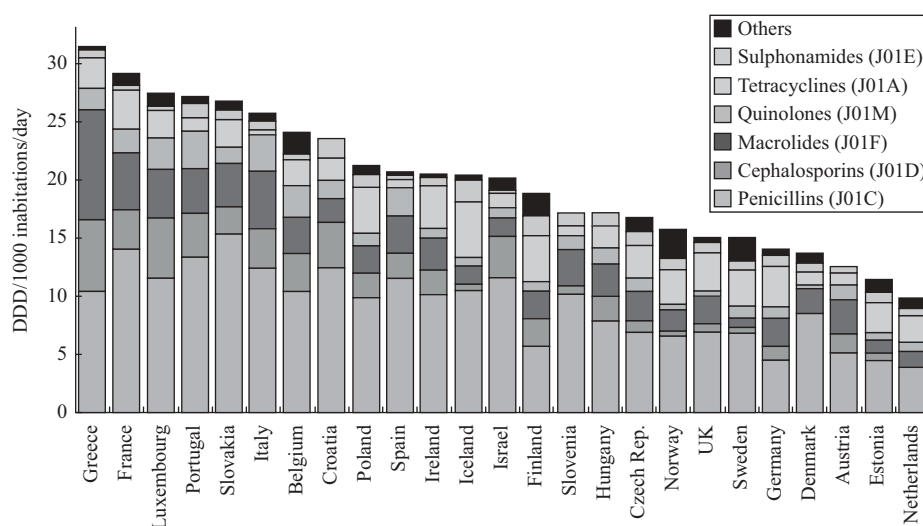


Figure 1. Total outpatient antibiotic use in 25 European countries in 2003. Cephalosporins include monobactams and carbapenems; macrolides include lincosamides and streptogramins; sulphonamides include trimethoprim; and others include J01B, J01G, J01R and J01X. For Iceland, total data are used, and for Poland, 2002 data are used.⁶ A colour version of this figure is available as Supplementary data at JAC Online (<http://jac.oxfordjournals.org/>).

techniques⁸ and analytical profile index (API) strips for Gram-negative bacilli (bioMérieux, France) were used. If two species were isolated, the relative number of each species was reported semi-quantitatively. Antibiotic susceptibility was tested with the Kirby–Bauer disc diffusion method, according to the CLSI guidelines.^{9,10} For Gram-negative bacilli, ampicillin, trimethoprim/sulfamethoxazole, cefuroxime, gentamicin, temocillin, ofloxacin, nitrofurantoin, fosfomicin, amoxicillin-clavulanic acid and trimethoprim were tested, and for *Pseudomonas aeruginosa*, ceftazidime, gentamicin, tobramycin, amikacin, ciprofloxacin, piperacillin-tazobactam and meropenem were tested. For staphylococci, nitrofurantoin, ampicillin, cefoxitin, vancomycin, ofloxacin, gentamicin, rifampicin, erythromycin and clindamycin were tested, and for enterococci and streptococci, ampicillin, nitrofurantoin, vancomycin, tetracycline, ofloxacin, erythromycin and gentamicin were tested. Vancomycin resistance in staphylococci, enterococci and streptococci was also assessed by a vancomycin screen test.⁹

For significant bacteriuria, the Kass criterion was used, with 10^5 cfu/mL urine as the cut-off value. When *Staphylococcus saprophyticus* was involved, every pure culture was considered positive.¹¹

Intermediate-resistant strains were considered resistant.

Statistics

The statistical program SPSS 12.0 for Windows was used for all statistical analyses. A χ^2 test was performed to detect differences in uropathogen susceptibility and distribution between 1996 and 2006. Where a χ^2 test was not suitable, a Fisher exact probability test was used. A *P* value of ≤ 0.05 was considered significant. Confidence intervals for the difference were calculated.

Results

Distribution of uropathogens and susceptibility

Three hundred specimens were collected. Eight samples contained more than one species.

Sixty-two percent of the cultures were positive (10^5 cfu/mL or more). *Escherichia coli* was the most frequently isolated uropathogen, followed by *S. saprophyticus* and *Proteus* spp.

The mean age of the women was 39 years (range 18–84). Data on age were missing in 33 patients.

When divided into two age groups with a cut-off of 50 years, a shift in distribution of uropathogens was noticeable. In the older group, *S. saprophyticus* was less frequently isolated, whereas the prevalence of *Klebsiella pneumoniae* and *Proteus* spp. was higher (Table 2).

E. coli ($n = 148$) showed near-total susceptibility to ofloxacin, nitrofurantoin and fosfomicin (99.5%, 99.5% and 98.5%, respectively) and lower levels of susceptibility to trimethoprim and co-trimoxazole (85.5% and 86%). Susceptibility to ampicillin was the lowest at 62%.

Susceptibility of *S. saprophyticus* ($n = 20$) to ofloxacin, nitrofurantoin and ampicillin was 100%, 95% and 60%, respectively.

Using a cut-off of 10^3 cfu/mL had no impact on the antibiotic susceptibility of *E. coli* or on the distribution of uropathogens.

Comparison of 1996–2006 data

One hundred and sixty-six patients answered to the same inclusion and exclusion criteria as the 1996 population, namely age under 55 years, no UTIs in the past 3 months and no

Table 2. Distribution of uropathogens in positive cultures in 2006

	All ages ($n = 193$)		Age 18–49 ($n = 127$)		Age 50–84 ($n = 49$)	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
<i>E. coli</i>	148	76.7	98	77.2	36	73.5
<i>S. saprophyticus</i>	20	10.4	15	11.8	3	6.1
<i>Proteus</i> spp.	8	4.1	2	1.6*	4	8.2*
<i>Klebsiella pneumoniae</i>	7	3.6	1	0.8**	5	10.2**
Other Gram-negatives	6	3.1	6	4.7	0	0.0
Other Gram-positives	4	2.1	3	2.4	1	2.0

*Significant difference ($P = 0.05$) between younger and older group.

**Significant difference ($P \leq 0.01$) between younger and older group.

recurrent UTIs in the history (3 or more in the past year). In this subgroup, 108 or 65% of the cultures were positive compared with 164 out of 279 or 59% in 1996. The distribution of uropathogens in these two groups was very similar (Table 3) and there had been no changes in the susceptibility to four frequently used antimicrobial agents (Table 4).

In 1996, co-amoxiclav, trimethoprim and fosfomicin were not tested.

Discussion

In two surveys performed in the same region in very similar populations, the *E. coli* and overall susceptibility in uncomplicated UTIs for co-trimoxazole, quinolones and nitrofurantoin stayed very high and virtually unchanged over a period of 10 years.

This finding is remarkable when considering the fact that Belgium has a very high prescribing rate for antibiotics, in general, and quinolones, in particular, even for uncomplicated UTIs.

Our findings are surprising, given the known epidemiological association between antimicrobial use and resistance.¹² Malhotra-Kumar *et al.*¹³ even demonstrated a causal relationship between antibiotic exposure and resistance in the oral

Table 3. Distribution of uropathogens isolated in 1996 and 2006 (subgroup comparable to 1996)

	1996 ($n = 176$)		2006 ($n = 111$)	
	<i>n</i>	%	<i>n</i>	%
<i>E. coli</i>	138	78.4	86	77.5
<i>S. saprophyticus</i>	16	9.1	15	13.5
<i>Proteus</i> spp.	7	4.0	3	2.7
<i>Klebsiella pneumoniae</i>	0	0.0	1	0.9
Other Gram-negatives	5	2.8	4	3.6
Other Gram-positives	8	4.5	2	1.8

No significant differences, $P > 0.05$.

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Table 4. Susceptibility of *E. coli* and all uropathogens in 1996 and 2006 (subgroup comparable to 1996)

	<i>E. coli</i>					All uropathogens						
	1996 (n = 138)		2006 (n = 86)		difference (95% CI)	P	1996 (n = 176)		2006 (n = 108)		difference	P
	n	%	n	%			n	%	n	%		
Nitrofurantoin	137	99.3	86	100	0.5% (-3.5; 4)	0.6 NS	164	93.2	103	95.4	2.5% (-4; 7.5)	0.5 NS
Co-trimoxazole	115	83.3	74	86.0	2.5% (-7.5; 12)	0.6 NS	NT	NT	NT	NT		
Ofloxacin	137	99.3	86	100	0.5% (-3.5; 4)	0.6 NS	174	99	108	100	1% (-2.5; 4)	0.4 NS
Ampicillin	101	73.2	54	62.8	-10.5% (-23; 1.9)	0.1 NS	123	69.9	66	61.1	-9% (-20; 2.5)	0.1 NS

NS, no statistically significant difference; NT, not tested.

streptococcal flora. On the specific subject of uropathogens, a number of alarming papers concerning rising resistance rates have been published,^{14–16} and a recent case–control study by Hillier *et al.*¹⁷ provides evidence that exposure to antibiotics is a strong risk factor for a resistant *E. coli* UTI.

Several explanations for this absence of a susceptibility change in our results can be given. First, short-term treatments used in cystitis may not induce much resistance. Second, otherwise healthy women might be less frequently exposed to antibiotics, with a consequent lower risk of selection of resistant species. This is reflected by the comparison of the susceptibility data: although there was no statistical significant change, the ampicillin data showed a trend towards significance with an increase in resistance of 10%. This is not surprising, as ampicillin is widely used in upper airway infections in Belgium. Whatever the explanation for the status quo in resistance, it shows the need for local systematic surveillances that can be used to give recommendations to GPs. This is also illustrated by comparing our results with those of Mangin *et al.*¹⁸ In their study on uncomplicated UTIs in Christchurch, New Zealand, they found a clear increase over a 2 year period of resistance of *E. coli* to trimethoprim, the local recommended antibiotic for the treatment of uncomplicated UTIs.

In accordance with other studies, the predominant uropathogen was *E. coli*, followed by *S. saprophyticus*. The *S. saprophyticus* percentage was higher than in some comparable surveillances,^{19–21} which can be explained by the low mean age (39 years) in our study, with 83% younger than 55 years. *S. saprophyticus* is a leading cause of uncomplicated UTIs in young, healthy, sexually active women.²² Its important share in the uropathogen distribution therefore indicates that our study dealt with the expected population of young women prone to uncomplicated UTIs.

The antibiotic susceptibility of *E. coli* to ampicillin, trimethoprim, ciprofloxacin and nitrofurantoin in our surveillance was very similar to the susceptibility found in other countries.^{19,20,23}

In our study, a cut-off value of 10⁵ cfu/mL was used. We are aware that there is an ongoing discussion about the most appropriate cut-off value. Several values have been proposed or used in different guidelines and articles.^{24,25} For this surveillance, we chose to use the 10⁵ cfu/mL criterion for reasons of comparability with the previous surveillance.

To our knowledge, this is the first study that compares in a prospective way the results of bacteriological cultures from patients with uncomplicated cystitis with an interval of 10 years.

Data were gathered from urine specimens that normally would not have been cultured in a GP setting. The study was conducted in two similar populations, in the same geographical region and, for a large part, in the same practices. This lends the comparison great validity and is surely one of its strengths. However, the fact that the study took place in a geographically confined area could jeopardize the generalizability.

The relatively low number of patients included can be considered a second shortcoming. In the course of the 1 year study, it became apparent that the number of samples was smaller than could be expected on epidemiological grounds. Therefore, we investigated the reason for non-inclusion. The main reason for non-inclusion mentioned by the GPs was time pressure. However, given the fact that the study did not require any additional effort from the patient, apart from delivering a urine sample, there was no interference with the patient's management. Selection on the basis of patient characteristics seems therefore very unlikely. Moreover, the very strict selection criteria left no room for extra selection.

Extra workload was also a reason for invited GPs for not participating in the study and explains the GP response rate of 68%.

Nitrofurantoin and trimethoprim remain the first-line agents for the treatment of uncomplicated UTIs. Although trimethoprim susceptibility was not tested for *S. saprophyticus*, we used the data for *E. coli* as an indicator for the empirical use of trimethoprim.

Conclusions

Despite abundant use of antibiotics in Belgium, there was no change in the antibiotic susceptibility of *E. coli* from female patients with complaints of uncomplicated UTIs over a 10 year period. There was consequently no need for a change of the local recommendations for the treatment of UTI, with nitrofurantoin and trimethoprim remaining the agents of first choice.

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Transparency declarations

None to declare.

Supplementary data

A colour version of Figure 1 is available as Supplementary data at *JAC* Online (<http://jac.oxfordjournals.org/>).

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