Trends in antimicrobial susceptibility of *Escherichia coli* isolates from urology services in The Netherlands (1998–2005)

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**Objectives:** An increase in antibiotic resistance of *Escherichia coli*, the most common pathogen in urinary tract infections (UTIs), is encountered worldwide. Optimal treatment of UTIs will contribute substantially to limit antibiotic use and antimicrobial resistance. This study determined trends in antimicrobial resistance of uropathogenic *E. coli*, which can be of use to optimize UTI guidelines.

**Methods:** During 1998–2005, *E. coli* from urine samples of patients attending urology services were collected in three regions in The Netherlands: north-east (NE, *n* = 1084), west (W, *n* = 1064) and south (S, *n* = 1212). The antibiotic susceptibility was determined using microbroth dilution following CLSI guidelines. *E. coli* ATCC 35218 and ATCC 25922 were used as reference strains.

**Results:** Amoxicillin resistance remained stable over time (37% to 47%), but was higher in the south (44%) compared with the other regions (40%; *P* < 0.02). Resistance to piperacillin increased from 4% (1998) to 32% (2005; *P* < 0.001), and resistance to fluoroquinolones increased from 6% to 13% (*P* < 0.01). Interregional differences were observed for resistance to piperacillin (NE 10%, W 12%, S 14%; *P* < 0.05) and to fluoroquinolones (NE 7%, W 13%, S 8%; *P* < 0.001). Trimethoprim + sulfamethoxazole resistance remained stable (27% to 37%), as did that of nitrofurantoin (4% to 9%). The percentage of strains with multidrug resistance (resistance to three or more groups of antibiotics) for each region increased over time (*P* < 0.05).

**Conclusions:** Antibiotic resistance was fairly constant over time for most agents tested, except for piperacillin and the fluoroquinolones. Regional differences were observed for several compounds. National and regional surveillance of antibiotic resistance is important to keep therapeutic guidelines up-to-date and adequate for the treatment of resistant microorganisms.

Keywords: antimicrobial resistance surveillance, Enterobacteriaceae, multidrug resistance, urinary tract

**Introduction**

An increase in the antimicrobial resistance among pathogens is a problem encountered worldwide. Recent surveillance studies performed in the USA and Canada, and also in Europe, indicate that this observation also applies to *Escherichia coli*, the most prevalent uropathogen. Up to 25% of the isolated *E. coli* from female outpatients are nowadays resistant to trimethoprim + sulfamethoxazole. Therefore, insight into antimicrobial resistance patterns over time and the emergence and development of *de novo* resistance are essential for the development of evidence-based therapeutic guidelines.

In 1998, a national antimicrobial resistance surveillance network was started in cooperation with the medical microbiology laboratories of 14 hospitals throughout The Netherlands. The isolates collected from the urology services for this prospective surveillance study were tested for their antimicrobial susceptibility in one central laboratory to avoid inter-centre deviations in methods and interpretations.

In this study, the antimicrobial resistance trends of *E. coli* isolates from the urology services in the period 1998–2005 are reported for β-lactam antibiotics (and inhibitor combinations), fluoroquinolones and the current agents of first choice in the treatment of an acute uncomplicated urinary tract infection (UTI) in The Netherlands (i.e. nitrofurantoin and trimethoprim + sulfamethoxazole). Furthermore, the antimicrobial resistance data were subdivided by the geographical origin of the isolates (north-east, south, west).
west and south) to be able to assess regional differences within The Netherlands.

**Materials and methods**

**Sample collection**

From January 1998 until December 2005, a total of 3360 consecutive *E. coli* isolates from urine samples from patients attending the urology services were collected. Only the first *E. coli* isolated from a patient was included and duplicate isolates from the same patient were excluded. All isolates were identified to a species level by each laboratory’s existing protocol, stored at −20°C in peptone glycerol (30% w/v) and sent each year to the central laboratory for susceptibility testing. Clinical data of the patients were not available.

Participating medical centres were spread geographically over the country, with four centres in the north-east (1084 strains), five in the west (1064 strains) and five in the south of the Netherlands (1212 strains).

**Susceptibility testing**

Antimicrobial susceptibilities (as MIC values) were determined according to CLSI guidelines using the microbroth dilution method with cation-adjusted Mueller–Hinton broth II (Becton, Dickinson and Company, Sparks, USA), an inoculum of 5 × 10^5 cfu/mL and overnight incubation at 37°C. The MIC plates with freeze-dried antibiotics were provided by MCS Diagnostics BV (MCS Diagnostics BV, Swalmen, The Netherlands), with a guaranteed shelf-life of 1 year. The antimicrobial agents tested were (range, mg/L): amoxicillin, 0.12–64; co-amoxiclav (ratio 4:1), 0.12–64; piperacillin, 0.12–128; piperacillin/tazobactam, 0.12/4–128/4; norfloxacin, 0.12–32; ciprofloxacin, 0.12–32; levofloxacin, 0.12–32; moxifloxacin, 0.12–32; trimethoprim, 0.12–32; trimethoprim/sulfamethoxazole, 0.12–32 and nitrofurantoin, 0.5–64.

*E. coli* ATCC 35218 and ATCC 25922 were used as reference strains. The breakpoints for resistance were: amoxicillin, 32 mg/L; co-amoxiclav (ratio 4:1), 32 mg/L; piperacillin, 128 mg/L; piperacillin/tazobactam, 128 mg/L; norfloxacin, 16 mg/L; ciprofloxacin, 4 mg/L; levofloxacin, 8 mg/L; moxifloxacin, 4 mg/L; trimethoprim, 16 mg/L; trimethoprim/sulfamethoxazole, 4 mg/L and nitrofurantoin, 64 mg/L.

**Statistical analysis**

Data were analysed with SPSS for Windows version 11.0 using a binary logistic regression analysis to determine trends over time and between the three geographical regions, i.e. the north-east, the west and the south of The Netherlands. A P value of less than 0.05 was considered statistically significant.

**Results**

**β-Lactam antibiotics and inhibitor combinations**

Amoxicillin resistance was relatively stable over the time period studied (37% to 47%), with an increase from 38% in 2001 to 45% in 2002 (Figure 1a). The MIC distribution curves before and after 2002 showed a different pattern (Figure 2a). Amoxicillin resistance was significantly higher in the south, compared with both other regions (Table 1; P < 0.02).

Co-amoxiclav resistance remained low and fluctuated within the study period with a peak resistance of 12% in 2000 (Figure 1a). The MIC<sub>90</sub> value, however, remained stable in the time frame tested. The MIC distribution curve was unimodal and showed a peak at 8 mg/L with a steep slope in the resistant subpopulation in 1998 that changed into a platform of resistant clones in 2002–05 (Figure 2b).

Piperacillin resistance percentage increased over time from 4% in 1998 to 11% in 2002 and 32% in 2005 (P < 0.001, Figure 1a). The MIC distribution curves were in concordance with these results: in 1998, the MICs for a small number of isolates were spread over a broad range (8–64 mg/L) in the
resistant part of the curve, and by 2005, the distribution curve demonstrated a high peak at >64 mg/L (Figure 2c). The resistance percentage during the study period was significantly different between the three geographic regions, i.e. they were the highest in the south and the lowest in the north-east of the country (Table 1).

For the combination of piperacillin with a fixed concentration of tazobactam (4 mg/L), no changes in the resistance percentage were found over time (Figure 1a). The resistance percentage in the south was significantly higher than that in the north-east (Table 1; \(P < 0.05\)).

**Fluoroquinolones**

Norfloxacin resistance increased significantly over time from 6% in the late 1990s to 12% in 2001–05 (\(P < 0.01\); Figure 1b), which was reflected in the bimodal MIC distribution curve (Figure 3b), where the peak at 16 mg/L became higher in 2000. The MIC\(_{90}\) of this antimicrobial agent increased from 1 mg/L in 2000 to 16 mg/L or more in the years thereafter (Table 2).

For ciprofloxacin, levofloxacin and moxifloxacin, similar resistance percentages were found for each sampling year with a significant rise in the resistance percentage over time (\(P < 0.01\); Figure 1b). The MIC\(_{90}\) values of these three agents were similar, but lower than those of norfloxacin (Table 2). The distribution curves of these three agents are also shown in Figure 3(a, c and d).

For all quinolones, the resistance percentages over time were significantly different in the three regions, i.e. they were the highest in the western part and the lowest in the south of The Netherlands (Table 1; \(P < 0.001\)).

** Nitrofurantoin resistance remained at a low level throughout the study period (4% to 9%). The MIC\(_{90}\) of this agent remained constant during the 8 years studied. The MIC distribution curves showed one broad peak at 8–16 mg/L, with hardly any changes over time. There were no geographical differences for this agent (Table 1).

Resistance for trimethoprim + sulfamethoxazole remained stable, for the period studied, at 27% to 37% with the highest percentage detected in 2002 (Figure 1c). The distribution curves for both agents were bimodal with a large peak in the sensitive range and a small peak at the resistant end of the curve. Trimethoprim + sulfamethoxazole resistance percentage over time was significantly higher in the south of The Netherlands, compared with both other regions (Table 1; \(P < 0.01\)).

**Multidrug resistance**

In total, 330 of 3360 isolates were resistant for three or more groups of antibiotics tested, one of which from the western part of the country, isolated in 2002, was resistant for all classes tested. The percentage of multidrug-resistant isolates per year for each region (Figure 4) increased over time (\(P < 0.05\)). This percentage was slightly lower in the north-east (12%) than that in the west (16%) and the South (15%) of The Netherlands. The number of combinations to which resistance was found increased, and the number of antibiotics within the combinations increased over the years.
Discussion

In this population of E. coli isolates, from the urology services from 14 hospitals spread over The Netherlands, antimicrobial resistance percentages were rather stable over a time span of 8 years, except for piperacillin and the fluoroquinolones. Also, regional differences were observed.

The most surprising finding was the rather exponential increase in the antimicrobial resistance of E. coli to piperacillin as of the beginning of the 21st century. Indeed, this increase was not only found for E. coli isolated at the urology services, but this trend was also detected in the E. coli isolates from the intensive care units of the same hospitals in The Netherlands during the same period of time.8 In a recent study in Italy (2002–05), even higher piperacillin resistance percentages (26% to 28%) were found for E. coli isolated from urology outpatients. The higher resistance percentages in Italy compared with those in The Netherlands could be explained by the higher use of antibiotics in general (25 versus 10 DDD/1000 inhabitants/day) and the ATC class J01C to which piperacillin belongs in particular (16 versus 4 DDD/1000 inhabitants/day).9,10

In a previous study dealing with E. coli isolated at the urology services between 1995 and 2000, a significant increase in the resistance percentage to fluoroquinolones from 3% to 7% was observed.11 This trend persisted with a further 2-fold increase in fluoroquinolone resistance as of 2001. A concordant increase in the systemic use of fluoroquinolones was observed in hospitals from 4.0 DDD/100 patient-days in 1997 to 7.3 DDD/100 patient-days in 2005.10,12 E. coli isolated from complicated UTI in the urology services were more often exposed to antibiotics and thus probably also to fluoroquinolones. This is in line with the data of other studies that higher resistance percentages in E. coli have been found in countries with a higher use of fluoroquinolones.13–15 Furthermore, Wagenlehner et al.16 found an increase in fluoroquinolone resistance after single-dose prophylaxis with ciprofloxacin and Gagliotti et al.15 found that the chance of finding fluoroquinolone-resistant E. coli decreased with time after the last fluoroquinolone prescription. These findings suggest that resistance to fluoroquinolones rapidly emerges after antibiotic consumption due to the selection of existing resistant subpopulations rather than inducing the development of de novo resistance. To be aware of this mode of rapid selection is very important, as it is recommended not to use these agents empirically in settings where the resistance percentage reaches 10% or higher.4 Our data confirmed that fluoroquinolones should be used judiciously to ensure that E. coli resistance rates do not rise above this threshold for this important group of therapeutic agents.

It must be noted that E. coli isolates in this study originated from male and female patients of all ages, who are more likely
suffering from complicated UTI (among others relapses or recurrences) and abnormal UTIs since they are treated by a urologist. Thus, the observations in this study are not directly relevant to young female outpatients with an acute uncomplicated UTI, but rather more relevant to older patients who are likely to have more often complicated UTIs.

A weakness of this multicentre surveillance study is that no demographic patient data and no clinical data are available. Thus, even though all urinary isolates submitted were found to be uropathogens by the individual laboratories, no distinction can be made regarding age group, gender and upper or lower UTI. The strength of this study is its longitudinal nature and thus describing the changes in the antimicrobial resistance over a period of 8 consecutive years. Also, as several hospitals participated, a large number of *E. coli* were isolated in each region of The Netherlands, making it possible to detect geographical differences in antimicrobial resistance. All data for the different regions are indeed comparable, as all susceptibility tests were performed in one central laboratory.

Another important finding is that even though The Netherlands is not a large country, there are substantial differences in resistance percentages in the north-east, the west and the south of the country. Similar findings have previously been reported for resistance percentages of *E. coli* isolated from acute uncomplicated UTI in the general practice setting and *Staphylococcus aureus* isolated from carriers in the community. These data indicate that both in the community and in the hospital setting, local guidelines of antibiotic prescription (choice and duration) are used.

Even though antimicrobial resistance to most agents tested was fairly stable over time, piperacillin and fluoroquinolones resistance percentages changed remarkably. Furthermore, regional differences in antimicrobial resistance percentages were found within The Netherlands. In conclusion, surveillance studies of antimicrobial resistance on national and regional levels are important to detect the emergence and changes of antimicrobial resistance over time.

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**Table 2.** MIC<sub>90</sub>s (mg/L) of quinolones for the *E. coli* isolated from the urology services for the period 1998–2005

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>NOR</th>
<th>CIP</th>
<th>LVX</th>
<th>MXF</th>
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<tr>
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<td>421</td>
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<td>0.5</td>
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<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2000</td>
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<td>0.25</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>2001</td>
<td>428</td>
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<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>2002</td>
<td>438</td>
<td>16</td>
<td>8</td>
<td>4</td>
<td>8</td>
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<tr>
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<td>411</td>
<td>&gt;16</td>
<td>16</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

NOR, norfloxacin; CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin.
Trends in antimicrobial susceptibility of *E. coli*

![Graphs showing trends in antimicrobial susceptibility of *E. coli* for different regions: North East, West, South, and The Netherlands.](https://academic.oup.com/jac/article-abstract/62/1/126/844178)

**Figure 4.** Trends among multidrug-resistant *E. coli* isolated from the urology services for the period 1998–2005. sulfa-trim, trimethoprim/sulfamethoxazole.

A colour version of this figure is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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**Transparency declarations**

None to declare.

**Supplementary data**

A colour version of Figure 4 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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