Little evidence for reversibility of trimethoprim resistance after a drastic reduction in trimethoprim use

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Objectives: The worldwide rapid increase in antibiotic-resistant bacteria has made efforts to prolong the lifespan of existing antibiotics very important. Antibiotic resistance often confers a fitness cost in the bacterium. Resistance may thus be reversible if antibiotic use is discontinued or reduced. To examine this concept, we performed a 24 month voluntary restriction on the use of trimethoprim-containing drugs in Kronoberg County, Sweden.

Methods: The intervention was performed on a 14 year baseline of monthly data on trimethoprim resistance and consumption. A three-parameter mathematical model was used to analyse the intervention effect. The prerequisites for reversion of resistance (i.e. fitness cost, associated resistance and clonal composition) were studied on subsets of consecutively collected Escherichia coli from urinary tract infections.

Results: The use of trimethoprim-containing drugs decreased by 85% during the intervention. A marginal but statistically significant effect on the increase in trimethoprim resistance was registered. There was no change in the clonal composition of E. coli and there was no measurable fitness cost associated with trimethoprim resistance in clinical isolates. The frequency of associated antibiotic resistances in trimethoprim-resistant isolates was high.

Conclusions: A lack of detectable fitness cost of trimethoprim resistance in vitro together with a strong co-selection of other antibiotics could explain the rather disappointing effect of the intervention. The result emphasizes the low possibility of reverting antibiotic resistance once established and the urgent need for the development of new antibacterial agents.

Keywords: intervention, Escherichia coli, population dynamic

Introduction

The important determinants of the development and maintenance of antibiotic resistance are the volume of antibiotic use1,2 and the biological fitness cost conferred by most resistance mechanisms.3 For several pathogens, the fitness cost measured in vitro has been shown to inversely correlate with the antibiotic resistance rates in clinical settings,4-6 supporting mathematical...
modelling studies that have suggested that fitness cost is a major determinant of resistance rates.7,8 These findings have led to the assumption that resistance is reversible. Outside hospitals and institutions, two nationwide antibiotic policy interventions have been described. In both interventions, antimicrobial resistance rates decreased as predicted. Seppälä et al.9 showed that a reduction in the overall use of macrolide antibiotics in the community was followed by a significant decrease in erythromycin-resistant Streptococcus pyogenes and Kristiessson10 concluded that the decrease seen in Streptococcus pneumoniae resistant to penicillin was caused by the decreased use of antibiotics in children some years earlier. Neither study addressed clonal shifts as a possible cause of the dramatic increase and the following decrease in resistance.9,10 In contrast to these dramatic effects, Enne et al.11 showed in a before/after fashion, based on a limited number of isolates, that a 97% decrease in the consumption of trimethoprim/sulfamethoxazole in the UK between 1991 and 1999 did not result in a reduction in sulfamethoxazole resistance. Additional data from the same area in 2004 showed that sulfamethoxazole and streptomycin resistance in Escherichia coli had remained remarkably stable despite a very low use of these drugs.12,13 The lack of effect on resistance was attributed to a non-existing or very low fitness cost of resistance and the presence of co-selection, where a certain resistance is maintained by its close linkage to another resistance determinant.14–16

Urinary tract infections (UTIs) are the third most common bacterial infection in Swedish primary healthcare. Trimethoprim has for many years been the most commonly used antibiotic for treating these infections, followed by pivmecillinam, fluoroquinolones, nitrofurantoin and oral cephalosporins.17 E. coli is the major UTI pathogen,18 and UTIs caused by trimethoprim-resistant E. coli have been associated with adverse clinical outcomes and an increased workload in general practice.20 Trimethoprim resistance is encoded by dfr genes encoding modified dihydrofolate reductases.21 The dfr genes are considered to be mainly horizontally spread22 and uropathogenic E. coli have only rarely been described as being epidemic.23

We performed a 24 month prospective intervention on the use of trimethoprim-containing antibiotics (trimethoprim and trimethoprim/sulfamethoxazole) in an area where sulphonamides in any other formulation have not been used for >25 years. The intervention was performed on a baseline of 14 years of monthly data on antibiotic prescriptions and antibiotic resistance in consecutive isolates of E. coli. To analyse potential intervention effects, we typed 4275 E. coli isolates, measured the growth rates of clinical isolates with and without trimethoprim resistance, determined associated resistance rates, and constructed a mathematical model to describe the dynamics before and during the intervention.

**Methods**

**Demographics**

The intervention was performed in Kronoberg County, a rural part of Sweden, ~150 x 120 km and with a population of 178000 (mean age 41.3 years). The healthcare system is funded at the county level, and includes two hospitals and 25 primary healthcare centres. The neighbouring Kalmar County has essentially the same demographics as Kronoberg County (Table 1) and was used as a control.

**Antibiotic use**

In Sweden, antibiotics are sold through the National Corporation of Pharmacies (Apoteket AB) and are available only via prescription. No changes in the national or local recommendations for the treatment of UTIs were made during the study period. Monthly outpatient sales data were retrieved from the National Corporation of Pharmacies for Kronoberg and Kalmar counties from 1991 and onwards. From 1991 to 1995, these data were based on random samples (every 25th prescription); from 1996 and onwards, total sales data were collected.

**Intervention**

The intervention was performed over 24 months, starting on 1 October 2004. All 464 physicians in Kronoberg County were asked to substitute trimethoprim-containing drugs with other antibiotics and were given a short pamphlet describing existing alternatives for the treatment of UTIs. In addition, all primary healthcare centres and some selected hospital clinics were informed through personal visits from members of the study group. With two exceptions, surgical prophylaxis and prophylaxis in immunocompromised patients, all indications for trimethoprim and trimethoprim/sulfamethoxazole were included in the intervention. All children with clinical signs of upper UTI were referred to the paediatric department during the intervention. Susceptibility testing to trimethoprim and trimethoprim/sulfamethoxazole was continued during the intervention, but the results were made available only on demand. The study was approved by the regional ethics committee of Linköping University, Sweden (approval no. 03-04).

**Bacterial isolates and susceptibility testing**

Methods for culturing, susceptibility testing and susceptibility interpretation remained unchanged over the period 1991–2008 in both counties. Susceptibility testing was performed using disc diffusion, as previously described.24 All zone diameters were measured with a slide gauge and stored in the laboratory-based computer system. To obtain the most sensitive detection of antibiotic resistance, categorizing isolates as susceptible and non-susceptible/resistant, epidemiological cut-offs were calculated using the NRI (‘normalized resistance interpretation’) method.25 All E. coli isolates were tested for susceptibility to (epidemiological cut-off values in parentheses) ampicillin (11 mm), cefadroxil (13 mm), mecillinam (24 mm),

### Table 1. Demographics and data on microbiological cultures in Kronoberg County (intervention) and Kalmar County (control)

<table>
<thead>
<tr>
<th></th>
<th>Kronoberg county</th>
<th>Kalmar county</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhabitants</td>
<td>178516</td>
<td>238264</td>
</tr>
<tr>
<td>Inhabitants per km²</td>
<td>21.1</td>
<td>21.3</td>
</tr>
<tr>
<td>Age, years (mean)</td>
<td>41.3</td>
<td>42.6</td>
</tr>
<tr>
<td>No. of hospitals</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>No. of primary healthcare centres</td>
<td>25</td>
<td>27</td>
</tr>
<tr>
<td>Physicians per 10000 inhabitants</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>No. of urinary cultures</td>
<td>18510</td>
<td>27263</td>
</tr>
<tr>
<td>No. of urinary cultures per 1000 inhabitants and year</td>
<td>104</td>
<td>114</td>
</tr>
<tr>
<td>Urinary cultures with E. coli (%)</td>
<td>23%</td>
<td>23%</td>
</tr>
</tbody>
</table>
trime thoprim (23 mm), nitrofurantoin (17 mm) and nalidixic acid (20 mm). Nalidixic acid was used as a marker for quinolone resistance.

All consecutive E. coli isolated from urinary specimens at the Departmen ts of Clinical Microbiology, Central Hospital, Växjö and Kalmar County Hospital, Kalmar since 1991 were included in the analysis (71290 and 89837 isolates, respectively). The median number of urinary cultures and the rate of positive cultures were similar in both counties (Table 1). Trimethoprim resistance rates in E. coli were retrieved on a monthly basis from the respective database. Resistance rates to other antibiotics used in UTI treatment (Figure 1a) and for bacteria other than E. coli (Tables 2 and 3) were retrieved only from Kronoberg County. To analyse the influence of co-selection during the intervention, associated resistance frequencies (the proportion of trimethoprim-resistant isolates also displaying resistance to any other antibiotic) were calculated (Figure 2).

Duplicate isolates were excluded using a 30 day algorithm based on the civic registration number given to all Swedes at birth. Trimethoprim and/or trimethoprim/sulfamethoxazole resistance was investigated in several other species during the period 1991–2008, using the same guidelines for susceptibility testing as those used for E. coli. The numbers of isolates were much smaller and no statistical analyses were performed (Tables 2 and 3). All Enterobacteriaceae isolates recovered from urine specimens in Kronoberg County from June 2004 to June 2008 were stored at −70°C.

**Typing of E. coli**

To detect changes in the distribution of E. coli phenotypes during the intervention, consecutive E. coli isolates from the 4 months preceding the intervention (n=1369), the 4 months immediately following the start of the intervention (n=1445) and from the last 4 months of the intervention (n=1461) were analysed using the PhenePlate® (PhP) System.26 Cluster analysis was performed using PHP Win4.2 software. A phenotype (categorized A–X) was defined as ≥1% of the isolates presenting the same (97.5% identical) pattern. Duplicate isolates (i.e. consecutive E. coli isolates from the same patient) were removed within each time period.

**Fitness (growth rate difference) of trimethoprim-resistant and -susceptible E. coli**

The fitness of E. coli susceptible (n=49) and resistant (n=49) to trimethoprim was estimated from growth curves of non-duplicate clinical isolates collected during the study. The susceptible E. coli were susceptible to (i.e. belonged to wild-type distributions of) all tested antimicrobials. To estimate the fitness of each phenotype, the growth rate was determined using BioScreenC (Oy instruments, Helsinki, Finland), a turbidometric reader that continuously monitors bacterial growth. Each isolate was inoculated to a final density of 10^5 cfu/mL in a well with 0.4 mL of Luria Broth. Growth was recorded over 8 h at 37°C as an increase in optical density at 540 nm, as previously described.6 Isolates were run in triplicate in the same run. E. coli ATCC 25922 was run in all experiments as a control.

**Statistics**

**Fitness of trimethoprim-resistant E. coli**

Student’s t-test was used to analyse the differences in bacterial fitness between susceptible and trimethoprim-resistant isolates of E. coli.

**Analysis of intervention effect**

The effect of the intervention on trimethoprim resistance in E. coli was analysed using both segmented regression analysis28,29 and a three-parameter mathematical model taking into account the unobserved competition between resistant and susceptible E. coli in a closed host population.30 The latter compensates for the fact that infectious disease data are auto-correlated in time and that potential effects of an intervention could appear with a lag in time after the intervention was performed. In the three-parameter model, the intestinal tract of each individual has limited space for which resistant and susceptible bacteria compete. The variables in the model are the number of bacteria resistant (r) or susceptible (s) to trimethoprim and the space made available when a bacterium dies (u).

Mathematically, this can be expressed as:

\[
\frac{dr(t)}{dt} = -\kappa r + \lambda r u - \kappa_s s - \kappa_s u
\]

With \(p(t) = r(t)/r(t) + s(t)\), denoting the proportion of resistant isolates, the log odds ratio, \(\theta = \log (p(t)/(1-p(t)))\), of resistant bacteria changes according to:

\[
\frac{ds(t)}{dt} = \theta - \Delta \kappa
\]

where \(\Delta \kappa = \kappa_r - \kappa_s\).

It follows from (1) that the changes in the proportion of resistant isolates can be described by a logistic regression with the link function:

\[
\theta(t) = \beta - \Delta \kappa t + \kappa \alpha(t)
\]

with the intercept \(\beta = \theta(0)\) and the covariates, \(\alpha(t) = \int_0^t \alpha(s)ds\), representing the cumulative antibiotic consumption up to time \(t\), and \(\Delta \kappa\) representing the fitness cost. The parameters in this model were estimated by maximizing the likelihood function using data on trimethoprim consumption in Kronoberg County from 1 January 1991 to the end of 2006. All the statistical analyses were performed using the R language, version 2.6.0.

The model was validated in three steps. First, the Pearson residuals of the model and the corresponding autocorrelation function were calculated (Figure 3). Second, data from the control county were used. After excluding the data from before 1996, where antibiotic consumption is more approximate than for data from 1996 and onwards, the performance of the model was determined by predicting the outcome of the control county. Third, as a further test, the predictive quality of the model was examined by calculating the one-step-ahead predictions.31 Based on the data up to a certain month \(t\), the parameters in the model were estimated and the predicted proportion of resistant isolates for the next month computed. An upper limit for the proportion of resistant isolates was computed based on the quantiles of the binomial distribution. This three-parameter model produced adequate fit, reasonable one-step-ahead predictions (data not shown) and predictions of the outcome in the control county. There was no indication of serious inappropriateness of the model when comparing measured data and model fit.
Figure 1. Consumption of antibiotics in defined daily doses per thousand inhabitants and day (DDD/TID) (left-hand panels) and corresponding resistance rates (%) in *E. coli* (right-hand panels) from 1991 to 2008 in the intervention county (a) and the control county (b). AMP, aminopenicillins/ampicillin; CFR, oral cephalosporins/cefadroxil; FQX, fluoroquinolones/NAL, nalidixic acid; MEC, pivmecillinam/mecillina m; NIT, nitrofurantoin; TMP/SXT, trimethoprim and trimethoprim/sulfamethoxazole.
Results

Consumption of antibiotics

The use of trimethoprim was similar in the intervention county (Kronoberg, Figure 1a) and the control county (Kalmar, Figure 1b) from 1991 to the start of the intervention in 2004. A prompt and sustained decrease of 85% in the total trimethoprim prescription sales was rapidly reached in the intervention county (Figure 1a). The total use of antibiotics was not affected as compared with the 2 years preceding the intervention; pivmecillinam, nitrofurantoin and ciprofloxacin replaced trimethoprim and trimethoprim/sulfamethoxazole (Figure 1a). Ampicillin is not recommended for the treatment of UTIs in Sweden, but consumption of this drug could be important from a co-selection perspective. The consumptions of nitrofurantoin and pivmecillinam, used exclusively for uncomplicated UTIs, had increased by a factor 1.2 and 1.9, respectively, at the end of the intervention period. After 24 months, physicians were informed that the restrictions on the use of trimethoprim and trimethoprim/sulfamethoxazole were lifted. Six and 12 months later, the use of trimethoprim had increased to 70% and 79%, respectively, of the pre-intervention level. In the adjacent county, Kalmar, where no intervention was performed, the use of trimethoprim-containing drugs remained unchanged throughout the intervention period (Figure 1b).
Table 2. Trimethoprim resistance (%) in species other than E. coli, with >100 isolates yearly, in urinary cultures, Kronoberg County 1991–2008 (intervention period 2004–06); all consecutive isolates were analysed for trimethoprim resistance

<table>
<thead>
<tr>
<th>Species</th>
<th>Trimethoprim resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (n=108–250)</td>
<td>3</td>
</tr>
<tr>
<td>Enterococcus faecalis (n=388–953)</td>
<td>4</td>
</tr>
<tr>
<td>Klebsiella pneumoniae (n=276–460)</td>
<td>10</td>
</tr>
<tr>
<td>Klebsiella oxytoca (n=107–161)</td>
<td>10</td>
</tr>
<tr>
<td>Proteus mirabilis (n=210–415)</td>
<td>8</td>
</tr>
</tbody>
</table>

Preliminary (September 2009) rates for 2009 are: S. aureus, 2%; E. faecalis, 38%; K. pneumoniae, 10%; K. oxytoca, 1%; and P. mirabilis, 30%. The range of analysed isolates for each species is given in parentheses.

Table 3. Trimethoprim/sulfamethoxazole resistance (%) in species other than E. coli, with >100 isolates yearly, in blood and wound cultures, Kronoberg County 1991–2008; all consecutive isolates were analysed for trimethoprim/sulfamethoxazole resistance

<table>
<thead>
<tr>
<th>Species</th>
<th>Trimethoprim/sulfamethoxazole resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus (n=150–2507)</td>
<td>0</td>
</tr>
<tr>
<td>Streptococcus pneumoniae (n=240–1101)</td>
<td>ND</td>
</tr>
<tr>
<td>Haemophilus influenzae (n=280–1435)</td>
<td>8</td>
</tr>
</tbody>
</table>

ND, not determined (<100 isolates tested). The range of analysed isolates for each species is given in parentheses.

Frequency of resistance

Rates of resistance to the groups of antibiotics tested on every urinary isolate of E. coli from 1991 to 2008 in Kronoberg County are presented in Figure 1(a) (right-hand panels). As can be seen, there was a continuous and gradual increase in the resistance to ampicillin and fluoroquinolones, whereas resistance to nitrofurantoin, cefadroxil and mecillinam was low and stable before and during the intervention. Because of the close linkage between reduced susceptibility to mecillinam and ampicillin resistance, use of epidemiological cut-offs means that isolates with MICs ranging between 2 and 8 mg/L will be classified as resistant (M. Sundqvist and G. Kahlmeter, unpublished data).

Segmented regression analysis\(^{28,29}\) could not detect any significant trend-break in the trimethoprim resistance rate in E. coli in relation to the intervention. In the mathematical model formulation (2), a positive fitness cost estimate is synonymous with an effect of the intervention on the development of trimethoprim resistance. The model showed a fitness cost parameter estimate \(\hat{D_k} = 0.0034 (SE = 0.0020)\) that was significantly different from zero, resulting in a marginal but significant decrease in the rate of trimethoprim resistance development (Figure 4a). Assuming an average carriage time of trimethoprim-resistant E. coli of 3–6 months, based on previous findings,\(^{20}\) this estimate corresponds to a 1%–2%
fitness cost. No trend-break was seen in the control county (Figure 4b). The estimates of the baseline $\hat{\beta} = -2.46$ (SE = 0.24) and the antibiotic treatment $\hat{\tau} = 0.011$ (SE = 0.0036) were both significant, giving the fundamentals for further predictions. The model predicted that a sustained intervention for 12 years would have reversed trimethoprim resistance back to the rates observed in 1991 (Figure 5) and that the fitness cost required to reach the 1991 level in 24 months would have been 46 times higher than the cost stipulated by the model.

There was no apparent effect of the intervention on resistance rates in seven other species systematically investigated for trimethoprim and/or trimethoprim/sulfamethoxazole susceptibility (Tables 2 and 3).

**Typing of E. coli**

The distribution of phenotypes was investigated over three time periods. This was to detect a possible clonal shift during the intervention. Twenty-two different phenotypes were identified in the material. Sixteen of these corresponded to phenotypes commonly observed in the ECO-SENS study, where phenotypes in UTIs in Europe were characterized.²² No major or systematic changes in the distribution of phenotypes during the intervention...
Figure 5. Predicted trimethoprim resistance development in the case of: (A) no intervention and assuming trimethoprim use at a level corresponding to the 2 years preceding the intervention; and (B) a sustained intervention for 12 years. The light grey line represents observed resistance rates.

were observed and none of the changes could in any way explain the result (Table 4).

Resistance rates of trimethoprim-resistant E. coli (associated resistance)
Trimethoprim-resistant E. coli showed higher resistance to other antibiotics than trimethoprim-susceptible isolates. At the start of the intervention in 2004, the resistance to other antibiotics of trimethoprim-susceptible and -resistant isolates was: ampicillin, 12% and 73%, respectively; fluoroquinolones, 3% and 21%, respectively; mecillinam, 4% and 26%, respectively; cefadroxil, 0.4% and 1%, respectively; and nitrofurantoin, 0.5% and 2%, respectively. The dynamics of ampicillin and fluoroquinolone resistance in the period 1991–2007 of trimethoprim-resistant isolates are presented in Figure 2. The changes in single trimethoprim resistance are shown in the same figure.

Fitness cost (growth rate difference) of resistance
Trimethoprim-resistant E. coli (n=49) did not grow significantly slower than pan-susceptible isolates (n=49). The mean generation time was 16.1 min (SE=1.8) and 16.1 min (SE=1.9), respectively. With a sample size of 49 pan-susceptible isolates, the detectable difference in growth rate, with a significance level of 0.05 and a power of 80%, was 1.0 min (6.5%).

Discussion
To reduce antibiotic resistance development, drastic interventions are needed. One strategy could be to reduce the selective pressure, i.e. decrease antibiotic use, assuming that susceptible bacteria would then outcompete resistant bacteria. However, so far there is no firm scientific support for the usefulness of this approach in the community, as previous studies are retrospective and lacking in experimental detail. Furthermore, theoretical studies of the epidemiology of antibiotic resistance in community settings and experimental estimates of fitness costs and compensatory evolution suggest that reversibility would, at best, be very slow and potentially require many years of reduction in antibiotic use before a decline in resistance would be observed.

To examine the potential of reversibility in a community setting, we performed a highly controlled, prospective study in a community with 178,000 inhabitants and demonstrated that it was possible to voluntarily reduce the use of trimethoprim by 85% over 2 years. The results were otherwise not encouraging: resistance rates in E. coli and other bacteria were not apparently affected and segmented regression analysis could not disclose a trend-break in E. coli trimethoprim resistance. Our results were thus in line with earlier retrospective investigations on E. coli, but in strong contrast to the results on erythromycin-resistant S. pyogenes in Finland and penicillin-resistant S. pneumoniae in Iceland. In these studies, resistance rates were considerably reduced after decreasing the use of what was perceived as the relevant antibiotics. Different serotypes of both S. pyogenes and S. pneumoniae, however, have extensive epidemic capacities, irrespective of the presence of resistance determinants. The clonal distribution and dynamics of the studied pathogens were not examined in these two studies and the observed reduction in resistance rates might thus have been caused by a clonal replacement not only related to the reduction in antibiotic use. This is further supported by the studies showing that in Finland, one phenotype, with single erythromycin resistance, dominated the scene at the advent of the intervention, and it was indeed later found that in Iceland, penicillin resistance in S. pneumoniae was spread clonally, despite the decreased use of β-lactams, indicating other mechanisms of persistence. We consider it most probable that the rapid decreases in resistance observed in these studies were the result of dying epidemics enhanced, rather than caused, by antibiotic interventions. In the present study, such effects were controlled for and the population structure of E. coli was not affected.

Several mechanisms may have contributed to the limited effect of the trimethoprim intervention described. Firstly, the main driving force for reversibility is the fitness cost of resistance. Mutational resistance is often associated with reduced fitness. Although fitness cost has also been reported in plasmid-mediated resistance, several recent studies have shown that this is not always the case. In some cases, plasmids have actually been shown to enhance fitness. We could not observe a fitness cost associated with trimethoprim resistance in vitro. This may either be the result of compensatory evolution or a primary lack of fitness cost associated with the dfr genes, as has been observed for other resistance mechanisms. Our in vitro data on fitness were not stratified to the dfr gene level and fitness cost might thus exist for some dfr genes. On the other hand, the variability (as seen in the standard deviation of measurements) was equal in pan-susceptible and trimethoprim-resistant isolates. Another possibility is that a fitness cost existed but was undetectable with the method used. The three-parameter mathematical model stipulated a small but
significant cost, resulting in a halt in trimethoprim resistance development. This effect could not be detected in any of the distributions of phenotypes with high resistance rates to trimethoprim (Table 4). Neither could an effect be observed on the frequency of isolates harbouring single trimethoprim resistance and, lastly, the distribution of the five most common dfr genes, encoding trimethoprim resistance in E. coli, was stable throughout the intervention (A. Brolund, M. Sundqvist, G. Kahlmeter and M. Grape, unpublished data); all findings together indicating that there was no fitness cost for these genes.

Secondly, it should be emphasized that the associated resistance, in this case resistance to other antibiotics of isolates resistant to trimethoprim, was such that any antibiotic would co-select for trimethoprim resistance and would counteract the potential reversibility caused by a fitness cost of trimethoprim resistance. This has been discussed in earlier publications. 14,43 Since the use of other antibiotics either increased or remained stable during the intervention (Figure 1), co-selection could well explain the result. We did not investigate this at the molecular level, and as co-selection was not included as a parameter in the model, this could mean that the fitness cost parameter was underestimated and could bias the long-term predictions.

Thirdly, an influx of trimethoprim-resistant E. coli from adjacent areas, not part of the intervention, could result in a lack of reversibility. This explanation is unlikely, since trimethoprim-resistant isolates being brought into the community would not have any advantage over the trimethoprim-resistant isolates already present within the community, unless they were more virulent. More virulent phenotypes would most certainly have caused changes in the distributions of phenotypes and would have been recorded as an increase in overall findings of E. coli in urinary cultures. No such changes were observed. The distribution of phenotypes was stable and the presence of specific bacterial phenotypes with high trimethoprim resistance did not increase (Table 4).

An interesting finding is that resistance rates to nitrofurantoin and mecillinam were not affected during the intervention, despite an increase of 85% and 20%, respectively, of these antibiotics. Nitrofurantoin resistance5 and mecillinam resistance44

Table 4. The phenotypes (A–X) of a total of 4275 E. coli isolates, from urinary specimens, presented in rank order (based on the mean frequency (%) from the three periods)

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Frequency</th>
<th>ECO-SENS phenotype (rank)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Pre</th>
<th>Start</th>
<th>End</th>
<th>Trimethoprim</th>
<th>Single trimethoprim</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.7</td>
<td>CT48 (1)</td>
<td>13.0</td>
<td>7.8</td>
<td>17.2</td>
<td>6.3</td>
<td>1.5</td>
</tr>
<tr>
<td>B</td>
<td>4.4</td>
<td>CT08 +29 (9/5)</td>
<td>3.5</td>
<td>2.9</td>
<td>6.8</td>
<td>5.4</td>
<td>3.0</td>
</tr>
<tr>
<td>C</td>
<td>4.0</td>
<td>CT26 +38 (3/--)</td>
<td>5.1</td>
<td>5.7</td>
<td>1.3</td>
<td>6.0</td>
<td>2.0</td>
</tr>
<tr>
<td>D</td>
<td>3.5</td>
<td>CT01(8)</td>
<td>4.1</td>
<td>3.1</td>
<td>3.3</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>E</td>
<td>3.0</td>
<td></td>
<td>2.9</td>
<td>5.6</td>
<td>0.4</td>
<td>6.3</td>
<td>0.9</td>
</tr>
<tr>
<td>F</td>
<td>2.9</td>
<td>CT17 (11)</td>
<td>3.3</td>
<td>2.8</td>
<td>2.6</td>
<td>11.0</td>
<td>0.9</td>
</tr>
<tr>
<td>G</td>
<td>2.5</td>
<td>CT16 (6)</td>
<td>2.9</td>
<td>3.5</td>
<td>1.2</td>
<td>11.7</td>
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aThe frequency rank in parenthesis of the corresponding phenotypes in the ECO-SENS study.32
bThe phenotype frequencies (%) for each of the intervention periods.
cTrimethoprim resistance (%) (total and single) of each phenotype.
Phenotypes with higher than average levels of trimethoprim resistance are highlighted in grey.
have recently been shown to confer a fitness cost in clinical isolates in vitro. It thus seems as if the genes conferring these resistances are so costly to the bacterium that despite a substantial increase in the selection pressure, these resistances are unsuccessful. This implies that these agents could be used more often in the treatment of uncomplicated UTIs. However, during a continued higher usage of nitrofurantoin after the end of the intervention, levels of resistance seem to have increased slightly (Figure 1). Continued vigilance will remain essential.

The period chosen for the intervention (2 years) was discussed thoroughly and chosen partly for practical reasons. Fluctuations in compliance had to be avoided and we feared that an initial success might have been followed by a gradual increase in the use of trimethoprim and/or trimethoprim/sulfamethoxazole if the study dragged on, and that the non-use put pressure on established guidelines and markets. It was also considered that if the intervention should serve as a model to be used for other antibiotics, the intervention period had to be perceived as realistic. Before the intervention an average 472 patients (0.3% of the population) received trimethoprim treatment every month. During the intervention this figure dropped to 16 patients (0.01%). The resistance data are based on urinary samples from ~1000 patients every month, and the demographics of patients cultured during the baseline and intervention periods did not change. We believe that this cohort was representative for those also receiving trimethoprim and thus relevant for measuring the effect of the intervention. During the 2 year follow-up presented in Figure 1, consumption of trimethoprim increased to levels almost as high as in other parts of Sweden. This increase in use was accompanied by a quite dramatic increase in trimethoprim resistance in the intervention county. At the same time, resistance to ampicillin and fluoroquinolones continued to increase. These findings would support the effect of the intervention stipulated by the model, but also shows that the effect that can be expected from such a drastic intervention is limited.

In summary, the apparent lack of effect of the intervention on trimethoprim resistance was explained by a low fitness cost combined with co-selection due to high levels of associated resistance. Even though we cannot quantify the relative contributions of these two forces, our results clearly illustrate that despite a very substantial 2 year reduction in trimethoprim use, the frequency of trimethoprim resistance in E. coli remained practically unaffected. The model showed a significant halt in trimethoprim resistance, but only a slow reversibility in resistance rates given that all other parameters were constant. All together, this indicates that an antibiotic cycling strategy in the community will not be beneficial, unless: (i) the period of non-use is much longer than 24 months; (ii) the cost of resistance is decidedly higher than that seen for trimethoprim resistance in E. coli; and (iii) the drugs chosen for replacement are devoid of associated resistance; so far we have not encountered a drug where pronounced associated resistance is not the rule.

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Transparency declarations

None to declare.

References