High genetic diversity of nitrofurantoin- or mecillinam-resistant *Escherichia coli* indicates low propensity for clonal spread

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Objectives: The empirical treatment with trimethoprim or ciprofloxacin of urinary tract infections (UTIs) is now questioned, partly due to the global expansion of a few resistant clonal groups of *Escherichia coli*.

Methods: In this study we investigated the clonal structure of 34 strains of *E. coli* (collected from non-pregnant women aged 18–65 years with uncomplicated UTIs in Europe and Canada) resistant to either of two other common treatment alternatives for uncomplicated UTIs, mecillinam or nitrofurantoin, using multilocus sequence typing (MLST).

Result: The 34 isolates were, despite high levels of multiresistance, distributed all over the *E. coli* genetic diversity spectrum with little association of antibiotic resistance to specific clonal groups.

Conclusions: The results of this study indicate a low probability of a future clonal spread of resistance to mecillinam and nitrofurantoin.

Keywords: uncomplicated urinary tract infections, successful clones, antimicrobial resistance

Introduction

*Escherichia coli* is a facultative pathogen causing infections such as sepsis, diarrhoea and urinary tract infection (UTI). Using modern techniques for population analyses such as phylogenetic grouping and multilocus sequence typing (MLST), several studies have shown a high genetic diversity among isolates causing extra-intestinal infections. Specific groups of *E. coli* have been associated with specific infections, specific resistance mechanisms and outbreaks with remarkable resistance. Fluctuations of different clonal groups over time have been shown to influence the local prevalence of antibiotic susceptibility.

Uncomplicated UTIs are rarely cultured and treatment is thus given empirically. The empirical treatment guidelines are based on and rely on knowledge of the local susceptibility pattern of uropathogens and a prognosis of future changes in susceptibility patterns. To investigate the antimicrobial susceptibility of *E. coli* from truly uncomplicated UTIs, the ECOSENS study was performed in 1999–2000, comprising non-pregnant women aged 18–65 years from general practice or outpatient clinics from 16 countries in Europe and Canada. A follow-up was performed in 2007–08 when five countries from the first study, representing different parts of Europe, were selected. Comparison of the data showed that the susceptibility patterns in *E. coli* were largely unchanged, with the exception of a higher rate of fluoroquinolone resistance.

Both pivmecillinam and nitrofurantoin are antibiotics used as first-line treatment of uncomplicated UTIs and typically display low resistance rates in *E. coli* (mecillinam 0.9%–1.6% and nitrofurantoin 0.3%–1.4%). Mecillinam resistance is believed to be caused by mutations in the genes affecting the elongation process of the bacteria, while nitrofurantoin resistance is caused by mutations in the *nsfA* and *nsfB* genes. Both these resistances have been shown to impose a substantial fitness cost in vitro to the resistant *E. coli*.

The objective of this study was to investigate whether resistance to mecillinam and nitrofurantoin was associated with specific UTI lineages, indicating a future limitation in the use of these agents. The large number of isolates in the ECOSENS material allowed us to retrospectively analyse the population structure of the subpopulation of *E. coli* resistant to mecillinam and/or nitrofurantoin isolated from uncomplicated UTIs over the first decade of the 21st century using MLST.
**Materials and methods**

All *E. coli* resistant to mecillinam (*n* = 13) or nitrofurantoin (*n* = 21) collected during the ECOSENS studies on uncomplicated UTIs in Europe were retrieved for the present study. This corresponded to 1.0% of all *E. coli* found in both studies (34/3384). Species identification was verified (score value > 2.0) at the retrieval using the MicroflexTM with flexControl software and the Bio-typer 3.0 database (Bruker Daltonics GmbH, Bremen, Germany) using standard parameters. Resistance was verified using EUCAST methodology (www.eucast.org) and MIC determination with Etest (bioMérieux, Solna, Sweden) according to the manufacturer’s instructions. EUCAST breakpoints were used (mecillinam MIC breakpoint ≤ 8/8 mg/L and nitrofurantoin MIC breakpoint ≤ 64 mg/L). Among the 21 isolates with nitrofurantoin resistance when screened with disc diffusion, 4 isolates displayed a borderline MIC of 64 mg/L. These isolates were also included in the study.

The proportion of resistance to other antibiotics separated into two groups. Resistance to two to five or more than five antibiotics was calculated and compared with a similar calculation (H. O. Poulsen and G. Kahlmeter, unpublished data) performed on the ECOSENS II material.6 DNA preparation was performed using an Abbott m2000 with an additional sample preparation system (Abbott Laboratories, IL, USA). The MLST scheme described by Wirth et al.1 was applied according to the protocol at the University College Cork. MLST web site (http://mlst.ucc.ie/mlst/dbs/Ecoli), as described previously.7

The MLST allele designations were determined via the MLST database and novel sequence type (ST) designations were provided by the curator of the database. Concatenated nucleotide data sets of the seven genes were processed using BioNumerics software (version 6.0, Applied Maths), including alignment and calculation of pairwise distances among strains using the Pearson coefficient. Neighbour-joining phylogenetic trees were constructed and goodness of fit of the clustering was determined by cophenetic correlation. To display the genetic diversity of the resistant isolates and the association to phylogenetic groups, reference MLST data of 47 strains of the ECOR collection1,3,4 were used. Strain Z205/ST1251 was used to root the inferred phylogeny.

**Results**

Among the mecillinam- and nitrofurantoin-resistant isolates, 63% displayed resistance to more than five antibiotics, as compared with 9% of the total number of isolates in the ECOSENS II study (P < 0.001).

Interestingly, the mecillinam- and nitrofurantoin-resistant isolates displayed a lower degree of resistance to two to five antibiotics when making the corresponding comparison (P = 0.002; data not shown). Resistance to other antibiotics was more often associated with nitrofurantoin than mecillinam resistance; for example, nalidixic acid resistance was present in 67% of the nitrofurantoin-resistant isolates and in 23% of the mecillinam-resistant isolates (Table 1). The detailed susceptibility patterns for the 34 mecillinam- and nitrofurantoin-resistant *E. coli* to the 14 investigated antibiotics are shown in Table S1 (available as Supplementary data at JAC Online).

Thirty of the 34 isolates belonged to 23 previously known STs, while 4 isolates were designated an ST new to this study: ST2493, ST2494, ST3023 and ST3024.

The isolates resistant to mecillinam and nitrofurantoin showed a distribution within the *E. coli* phylogeny that was similar to the distribution of the ECOR strains (Figure 1) and six STs were represented by one or two or three isolates: ST12 (n = 2), ST73 (n = 4), ST93 (n = 2), ST140 (n = 3), ST167 (n = 3) and ST624 (n = 2). No pattern indicating long-range clonal spread was evident, but there were some geographical associations (Figure 1). For example, ST140 was represented by three nitrofurantoin-resistant isolates, two from ECOSENS and one from ECOSENS II, originating from Portugal (n = 2) and Spain (n = 1).

**Discussion**

During recent years several authors have suggested that local outbreaks of multiresistant successful lineages of *E. coli* are an important contribution to the increasing antibiotic resistance. This study is the first to address the possible clonal relatedness of *E. coli* resistant to mecillinam and nitrofurantoin. These antibiotics are used mainly in the treatment of uncomplicated UTIs, with nitrofurantoin being used worldwide while mecillinam is available in a limited number of countries in northern Europe. These drugs still display low resistance rates,1,3 although increasing mecillinam resistance rates have been observed in Sweden during recent years.9

The collection of isolates analysed here included *E. coli* from uncomplicated UTIs in Europe and Canada collected with a gap of 8 years. It represents a timeline for countries with different levels of antibiotic usage, profiles of use and antibiotic resistance rates. The collection displayed high levels of other resistance traits coupled with the nitrofurantoin or mecillinam resistance (Table 1), but without any tendency to clonal spread. The isolates were scattered all over the *E. coli* population and displayed a diversity resembling the ECOR collection.4 Although intriguing with respect to the multiresistance profiles, this finding is in line with previous findings of both mecillinam and nitrofurantoin resistance being associated with a substantial fitness cost in vitro.5,6

Although the isolates showed a high diversity, some geographical associations were observed. Some STs previously associated with UTIs were observed in the same geographical area in both
Figure 1. Neighbour-joining tree of seven housekeeping genes. Thirty-four mecillinam- or nitrofurantoin-resistant strains from the ECOSENS study year 1999–2000 and the ECOSENS II study year 2007–08 were compared with 47 strains of the reference collection ECOR. Boxes indicate membership in recognized E. coli phylogenetic groups and STs are indicated to the right. The scale bar represents pairwise sequence similarities between strains. A number at a basal tree cluster is a cophenetic correlation coefficient indicating how well a cluster represents the original pairwise similarity data.
collection periods (i.e. ST73 and ST140). These findings could reflect differences in the relative prevalence of STs associated with UTIs in different geographical regions. As the number of isolates was small, these associations are weak and may be coincidental.

Overall this study showed a high diversity of E. coli resistant to mecillinam and nitrofurantoin despite high levels of resistance to other antibiotics. This implies the acquisition of mecillinam or nitrofurantoin resistance to be associated with low epidemiological fitness, or in other words, an evolutionary dead end. From this perspective, an increased use of these agents with a corresponding decrease in the use of antibiotics with a broader spectrum (in line with the current North American and European treatment guidelines for uncomplicated UTIs) seems reasonable from an ecological point of view. However, increased use of these antibiotics calls for continuous monitoring of resistance rates in the coming years.

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Supplementary data
Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

References