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Title	Antimicrobial prescribing and resistance in Irish general practice
Author(s)	Vellinga, Akke
Publication Date	2011-04-20
Item record	http://hdl.handle.net/10379/2062

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Antimicrobial prescribing and resistance in Irish general practice

Akke Vellinga, BSc, Master in Science (Biology), Master in Epidemiology

A thesis submitted for the degree of Doctor of Philosophy

Supervisors of research:

Professor Andrew W Murphy

Professor Martin Cormican

Discipline of General Practice, Discipline of Bacteriology

School of Medicine

National University of Ireland

Galway

April 2011

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List of Abbreviations

AM	Antimicrobial
AMC	Co-amoxyclav or β -lactam antimicrobials in general
AMP	Ampicillin
CEF	Cefpodoxime
cfu	Colony forming units
CI	Confidence Interval
CIP	Ciprofloxacin
CLSI	Clinical and Laboratory Standards Institute
CrI	Credible Interval
DIC	Deviance Information Criterion
<i>E.coli</i>	<i>Escherichia coli</i>
ESAC	European Surveillance of Antimicrobial Consumption
ESBL	Extended Spectrum Beta Lactamase
GMS	General Medical Services
GP	General Practitioner
GUH	Galway University Hospitals
HRT	Hormone Replacement Therapy
HSE-PCRS	Health Service Executive - Primary Care Reimbursement Service
ICC	Intraclass Correlation Coefficient
IDSA	Infectious Disease Society of America
MCMC	Markov Chain Monte Carlo
MIC	Minimal Inhibitory Concentration
mOR	median Odds Ratio
MSU	Mid-Stream Urine
NIT	Nitrofurantoin
NPV	Negative Predictive Value
OC	Oral Contraception
OR	Odds Ratio
PBP	Penicillin Binding Proteins
PCV	Proportional Change in Variance
PFGE	Pulse Field Gel Electrophoresis

PI	Prediction Interval
PPV	Positive Predictive Value
Quin	Quinolones
REC	Research Ethics Committee
SAHRU	Small Area Health Research Unit
SARI	Strategy for the control of Antimicrobial Resistance in Ireland
SD	Standard Deviation
SES	Socio-Economic Status
<i>spp.</i>	species
SSRI	Selective Serotonin Reuptake Inhibitor
TRI	Trimethoprim
UTI	Urinary Tract Infection
vs	versus

Declaration

This work is submitted to fulfil the requirements of the degree of Doctor of Philosophy at the National University of Ireland, Galway. No part of this thesis has been previously submitted at this or at any other university. Apart from due acknowledgements, it is entirely my own work.

Signed: _____

Akke Vellinga

Date: _____

Acknowledgements

This PhD has been a wonderful journey during which I met many people to whom I wish to express my sincere thanks; to Andrew W Murphy for the opportunity to do this research and for being such a fixer; to Martin Cormican for his persistence in correcting text after text, any language, and to both of you for the learning experience that has been so enjoyable; to Kathleen Bennett for helping me understand; to Belinda Hanahoe and Breda Kelleher, for their friendship and practical support and without whom this thesis would not exist; to Brian Buckley, for helping out with our ethical approval but above all, for the off the record chats; to Una St John, Deidre Goggin and Clare Robinson for their help, to Colin Bradley, Pater Davey and Chris Butler for being on the steering group; to Peter Cantillon, to Hans Pieper, to all in Distillery Road, to the laboratory staff, to all the participating practices, to all the patients, for making this research happen; to my family, to all my friends, who, without knowing, kept me sane during this process; to Donal, Kian, Oran and Luka Anna, this work is dedicated to you, for a better future!

Abstract

The emerging problem of antimicrobial resistance in bacterial pathogens is complex and the result of individual and population factors. Antimicrobial agents are unique therapeutics in that their impact goes beyond the individual; antimicrobials also affect the microbial population of the host (including the pathogen population) and thereby society. The practical application of quantifying direct, individual level antimicrobial effects is to assess the short-term risk of infection with a resistant organism to an individual about to initiate antimicrobial treatment. The long-term population effect, also known as the collateral effect, involves a chain of low probability events which result in a population risk of an infection with a resistant organism which affects the individual in turn.

Standard statistical analytic approaches make the assumption that outcomes in different subjects are independent, but for antimicrobial prescribing and resistance this assumption of independence is violated as the group level prevalence of antimicrobial resistance is likely to affect the individual's risk. For this reason, studies into antimicrobial resistance need to combine information from individual and group level antimicrobial use and resistance and analyse intra as well as inter level variation.

Urinary tract infections (UTIs) are common infections and treatment of UTI in daily practice is largely empirically based. The easy availability of urine samples from patients with a suspected UTI, the established empiric treatment with antimicrobials, standard methods for diagnosis, and high antimicrobial use, make urinary tract infections an ideal subject to study antimicrobial resistance in the community.

The thesis is set up in two distinct parts, each divided into chapters representing discrete research areas (published/submitted papers) within each part. The first part used retrospective data to address the multilevel structure in the analysis of antimicrobial resistance of uropathogenic *E.coli* in the individual and prescribing at the general practice level. Data on practice antimicrobial prescribing were obtained from the prescriptions of medical

card patients (patients with free medical care and free medication) and aggregated at the practice level. Data on antimicrobial resistance of uropathogenic *E.coli* from individuals were obtained from the laboratory and consisted of more than 14,000 positive urine culture results from general practices in the West of Ireland. The results from this analysis confirmed a significant association between practice level prescribing and individual risk of a resistant *E.coli* for trimethoprim and ciprofloxacin. The odds ratio for trimethoprim was 1.02 (95% CI 1.01-1.04) and for ciprofloxacin 1.08 (95% CI 1.04-1.11) for every additional prescription of trimethoprim or ciprofloxacin respectively per 1000 patients per month. Additionally, a theoretical risk for the practice was quantified as a median odds ratio (mOR); 1.10 (95% Credible Interval (CrI) 1.03-1.16) for trimethoprim and 1.37 (95% CrI 1.22-2.59) for ciprofloxacin. The mOR can be interpreted as the increase in risk of being diagnosed with a resistant *E.coli* in the imaginary event of a patient moving from a practice with low to a practice with high resistance.

Another detailed retrospective analysis which studied patients with repeated urinary tract infection, of whom only details on the resistance pattern of the *E.coli* were available, showed the persistence of resistance against trimethoprim as well as ciprofloxacin in repeated *E.coli* UTIs. The probability that an *E.coli* isolated from urine from a patient was still resistant up to three months after the previous isolate was found to be resistant, was 78% for trimethoprim and 84% for ciprofloxacin. For nitrofurantoin, the probability that a subsequent *E.coli* infection was resistant after resistance against this antimicrobial was detected in the *E.coli* from a previous infection, was 20%. Knowing the antimicrobial test results from previous episodes of UTI may help general practitioners in their choice of empiric antimicrobial treatment for the current episode.

The second part of the study was a prospective study in which 22 practices co-operated. All patients with a suspected UTI were requested to submit a urine sample. Patients were informed of the study and included in the study by means of an opt-out methodology. Participation of 86% was achieved. Patient

data were obtained from the practice records and merged with the (antimicrobial susceptibility) results from their urine sample.

The analysis from this study resulted in two papers. Firstly, management of UTI in general practice showed important differences between practices. Overall, of the 866 patients, an organism was identified in the urine sample of 21%, while 56% received an antimicrobial. Comparing the laboratory report on the urine sample with the treatment received, treatment was interpreted as appropriate for 55% of the patients. National guidelines on antimicrobial prescribing were not always implemented, which raises concern when general practices showed preferences for antimicrobials which should be used prudently. In the second paper analysis of previous individual antimicrobial prescribing and practice resistance levels showed both have an important impact on the risk of a UTI with a resistant *E.coli*. The odds of a trimethoprim resistant *E.coli* UTI increased by 1.4 (95% CI 0.8-2.2) for one, 4.7 (95% CI 1.9-12.4) for two and 6.4 (95% CI 2.0-25.4) for three or more prescriptions of trimethoprim in the previous year, and for ciprofloxacin resistance by 2.7 (95% CI 1.2-5.6) for one and 6.5 (95% CI 2.9-14.8) for two or more prescriptions of ciprofloxacin in the previous year. Similar to the retrospective study, a mOR was calculated as 1.17 (95% CrI 1.03-1.46) for trimethoprim and 1.33 (95% CrI 1.03-1.9) for ciprofloxacin.

The thesis' discussion links the papers together, resulting in some practical suggestions for setting up interventions to curtail antimicrobial resistance.

Chapter 1: Introduction

1.1 Rationale

1.1.1 Antimicrobial resistance

The use of antimicrobials combined with improvements in sanitation, housing, and nutrition, and the introduction of widespread immunisation programmes, has caused a dramatic decline in the often fatal diseases that were previously untreatable. These gains are today seriously jeopardised by another development: the emergence and spread of microbes that are resistant to these, once described, ‘wonder drugs’ (World Health Organization 2011). The emergence and spread of antimicrobial resistance is a complex problem involving antimicrobial agents, bacterial species, resistant genes and various mechanisms of resistance (Guardabassi and Courvalin 2006). Antimicrobial resistance is a relative term and in its clinical definition a strain is defined resistant when it survives antimicrobial therapy. This resistance can be intrinsic, due to a structural or functional trait which diminishes the effect of a particular drug by all members of a bacterial species (tolerance). Acquired resistance, on the other hand, is a major threat to health because it is the source of the emergence and spread of resistance in normally susceptible bacterial populations and consequently may lead to therapeutic failure (Guardabassi and Courvalin 2006). An antimicrobial resistance problem will not emerge in the absence of the selective pressure of the antimicrobial agent. Antimicrobial use is influenced by the knowledge and expectations of patients and their interactions with prescribers, by economic incentives, and by the regulatory environment (D’Agata, Dupont-Rouzeyrol *et al.* 2008). Antimicrobial agents are unique therapeutics because they treat more than just the individual; antimicrobials also affect the pathogen population and thereby the host population or society (Levy and Marshall 2004). Optimising treatment success for the individual can lead to population level effects which can substantially differ in magnitude or even be opposite in direction (Lipsitch and Samore 2002).

Antimicrobial use affects resistance through direct individual effects and indirect population effects. The aim of quantifying direct, individual level antimicrobial effects is to assess the short-term risk of infection with a resistant organism for a person about to initiate antimicrobial treatment. The underlying processes that contribute to the long-term problem involve a chain of low probability events, like mutation, genetic linkage or intra and inter species transfers (Magee, Heginbothom *et al.* 2005). Standard statistical analytic approaches make the assumption that outcomes in different subjects are independent, but in the case of antimicrobial resistance this assumption is violated (Halloran and Struchiner 1991). Additionally, observational studies with antimicrobial prescribing data for a population and aggregated individual surveillance data on antimicrobial susceptibility are prone to ecological fallacy, the wrong conclusions due to the aggregation of individual exposures to a higher group level. Understanding the mechanisms by which antimicrobial use selects for antimicrobial resistance in treated patients and in the population requires methods that take into account the direct individual effects as well as the indirect effects of population level selection. Few studies have adequately addressed the issues of individual and group level interactions and until the complexities of the spread of antimicrobial resistance are better understood, the design of effective interventions will be difficult.

1.1.2 Uropathogens as a model for studying community resistance

Urinary tract infections (UTIs) are common although infrequently serious. Treatment of UTI in daily practice is largely empirically based. The identity of the causative organisms is generally predictable and clinical practice guidelines state that cultures are not necessary (Hooton and Stamm 1997; Gupta, Hooton *et al.* 2011). Antimicrobial prescribing for UTIs represents 12% of overall antimicrobial prescribing, in fourth place after lower respiratory tract infections (18%), sore throat (16%) and upper respiratory tract infections (14%) (Petersen and Hayward 2007). The diagnosis of UTI is suggested by the presentation of classical symptoms such as frequency and dysuria and by the presence of white blood cells and nitrates in the urine. While empiric therapy for UTIs is a rational and cost-effective approach for individual treatment, it may contribute to inappropriate antimicrobial use (Fenwick, Briggs *et al.* 2000; DeAlleaume, Tweed *et al.* 2006). Antimicrobials may be given to patients who do not have an infection, while patients with an infection may be prescribed a drug that is inappropriate for the causative organism due to resistance. Prudent and appropriate antimicrobial use should be informed by changing trends in resistance in the community.

The ready availability of urine samples due to the high incidence of UTIs, the importance of adequate empiric antimicrobial therapy, standard methods for diagnosing UTIs, the general acceptance of a consistent approach to laboratory diagnosis, and the high levels of antimicrobial use, make urinary tract infections a suitable condition to study antimicrobial resistance in the community.

An additional issue with respect to UTIs is recurrent infections which are more likely to be associated with resistant organisms due to exposure to antimicrobial agents in the treatment of the previous episode. Culture and susceptibility test results and antimicrobial treatment of previous episodes of UTI may also be able to guide empiric therapy in subsequent episodes.

1.2 Research objectives

1.2.1 Primary research objectives

1. To retrospectively determine the relationship between antimicrobial prescribing and antimicrobial resistance with a multilevel analysis of existing databases using individual resistance data and aggregated prescribing data.
2. To prospectively determine the direct and indirect relationship between antimicrobial prescribing and antimicrobial resistance at the individual and population level.

1.2.2 Secondary research objectives

3. To describe of the occurrence and management of UTI in Irish general practice.
4. To assess the applicability of susceptibility test results from a prior episode of UTI in the selection of antimicrobial treatment for a subsequent episode.

1.3 Outline of the thesis

The introductory literature review on antimicrobial resistance (*Chapter 2*) covers literature, background ideas and principles necessary to understand and link together the different chapters. The study itself is set up in two distinct parts, each divided into chapters representing discrete research areas within each part.

The first part uses retrospective data from various sources to analyse the association between antimicrobial resistance and antimicrobial prescribing using data (*Chapter 3*). Data on antimicrobial prescribing within the general practice were obtained from the Health Service Executive - Primary Care Reimbursement Service (HSE-PCRS) database, which records all the prescriptions of medical card patients (free medical care and prescriptions). The individual antimicrobial resistance data provided by the laboratory of the Galway University Hospitals (GUH) consisted of the antimicrobial susceptibility results and organism identification for significant bacteriuria from practices in the West of Ireland. Multilevel modelling was used to analyse the combined databases.

Additionally, retrospective data from the laboratory of the UHG were further analysed to describe the antimicrobial resistance patterns of *E.coli* from significant recurrent episodes of bacteriuria in the community (*Chapter 4*). Findings and limitations of the retrospective study guided the second part of the study. A selection of 22 practices participated in this part, in which information on previous antimicrobial use taken from patients' charts was combined with the susceptibility test results of isolates from their urine samples.

The first chapter in this part of the thesis represents the set-up of the prospective study including obtaining ethical approval for using an opt-out methodology (*Chapter 5*). This is followed by a description of the management of UTI in general practice in Ireland (*Chapter 6*). The analysis of antimicrobial

prescribing and resistance at the individual level, taking the overall area resistance level into consideration, is then described in detail (*Chapter 7*). The final chapter discusses the conclusions as well as their implications for interventions (*Chapter 8*).

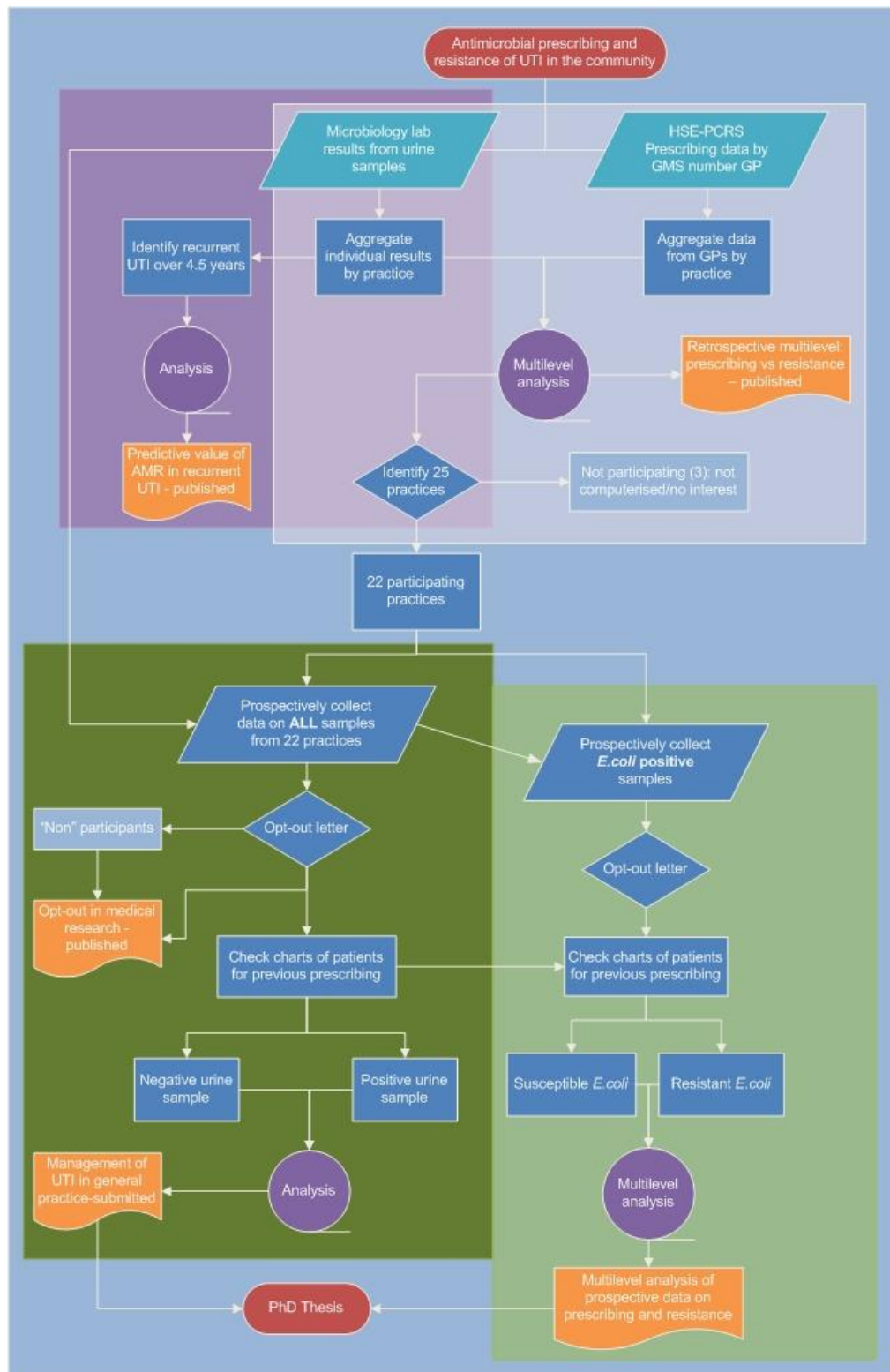


Figure 1.1: Overview of the PhD thesis

Overview of the project with the data, analyses and papers which combined result in the PhD thesis. Papers are shown in orange boxes, analyses in purple circles. The purple background shows the retrospective part of the study and the green background the prospective part.

Chapter 2: Antimicrobial resistance

'Hit hard and hit early', Ehrlich's advice on treatment of infections, 1913

2.1 Introduction

2.1.1 Antimicrobial resistance

Since their discovery, penicillin and its successors have completely transformed humanity's approach to infectious disease. Antimicrobial therapy has been the main medical intervention against infectious diseases caused by bacterial pathogens. However, with the increasing availability and use of antimicrobial agents, a continuing decline in therapeutic effectiveness due to increased resistance has occurred. Alexander Fleming already referred to the ease with which resistance can occur in his Nobel lecture on penicillin in 1945 (Fleming 1964). Since then, many reviews have been devoted to the subject of increasing antimicrobial resistance but our knowledge of the processes contributing to this problem remains sketchy (Magee, Heginbotham *et al.* 2005).

Antimicrobial activity is due to the inhibition of biochemical pathways that are involved in the biosynthesis of essential components of the bacterial cell. The three main bacterial targets of antimicrobial agents are cell wall, protein, and nucleic acid biosynthesis. Various mechanisms neutralising the action of antimicrobial agents have developed in bacteria. The most widespread antimicrobial resistance mechanisms are enzymatic drug inactivation, modification or replacement of the drug target, active drug

Text box 1: Antimicrobial resistance mechanisms:

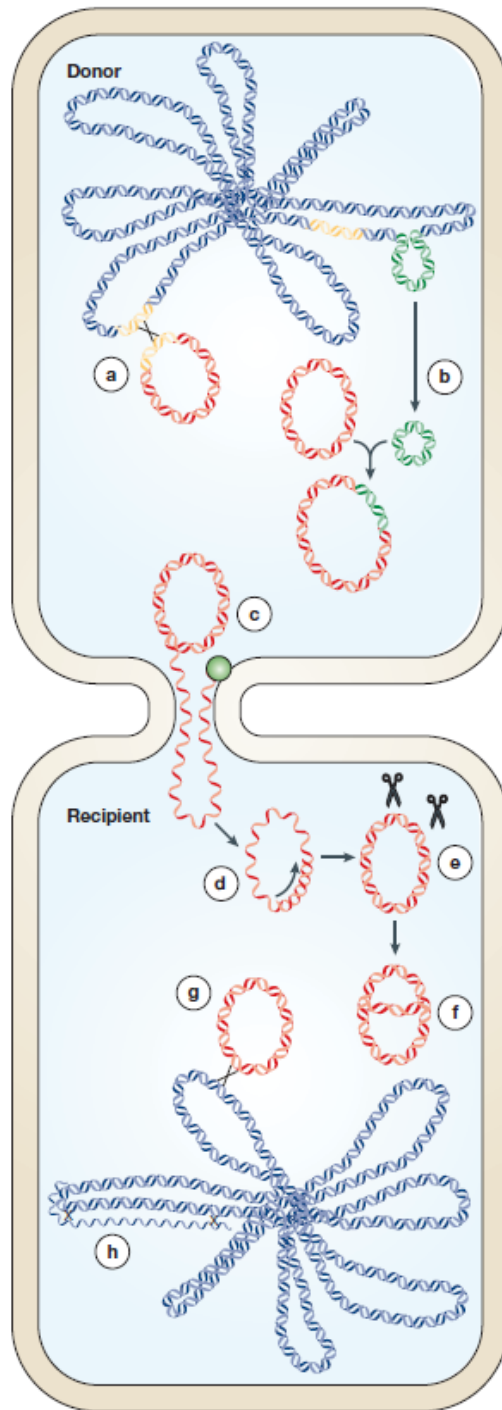
- Alter the bacterial cell wall permeability (influx/efflux systems)
- Produce a mutation at the target site
- Degrade the antibiotic via enzymatic action
- Develop alternative metabolic pathways to block the antimicrobial action

efflux, and reduced drug uptake (seeText box 1, page 8) (Austin, Kristinsson *et al.* 1999; Doyne, Paterson *et al.* 2006; Guardabassi and Courvalin 2006; Jayaraman 2009). Bacterial resistance was present before antimicrobials were used. This intrinsic resistance is the innate ability of a bacterial species to resist the activity of a particular antimicrobial agent through its inherent structural or functional characteristics. Acquired bacteria antimicrobial resistance is a result of a genetic change, which occurs in the presence or absence of the antimicrobial (Hart 1998; Guardabassi and Courvalin 2006). This genetic change can be the result of a mutation or horizontal exchange of genetic material (transformation, transduction and conjugation). Whereas transformation and transduction are processes limited to closely related bacteria belonging to the same species or genus, conjugation is not restricted like this and is therefore likely to play a much larger role in the spread of antimicrobial resistance. Conjugation is a mechanism of horizontal genetic material transfer, most often with plasmids or transposons, due to which resistance can be passed on to other species (Figure 2.1).

These genetic events occur in the presence or absence of antimicrobials. However, antimicrobial therapy exerts a selective effect and a subsequent competitive effect which, when followed by a bacterial genetic transfer, contributes to antimicrobial resistance (Furuya and Lowy 2006):

1. The selective effect takes place during antimicrobial administration and is due to the survival advantage of the resistant organisms when the susceptible organisms are killed.
2. The subsequent competitive effect is due to the enhanced colonisation of the individual with the resistant organism due to the eradication of benign commensals from the normal microbiotic environment by the treatment.
3. With bacterial genetic transfer the antimicrobial resistance traits persist in bacteria.

For a resistant pathogen to be 'successful' the fitness cost of its resistance mechanism must be low enough to be sustained even in the absence of antimicrobial selective pressure (Furuya and Lowy 2006).



In the donor, a: integration of the plasmid into the chromosome by recombination; b: movement of a transposable element through a circular intermediate from the chromosome to the plasmids; c: initiation of rolling-circle replication at the mating-pair apparatus. In the recipient cell, d: re-circularization; e: attack by restriction endo-nucleases (scissors); f: replication; g: integration; h: recombination.

Figure 2.1: Overview of plasmids and conjugative transfer in the horizontal spread of genes (Thomas and Nielsen 2005) Copyright obtained from Nature Reviews/Microbiology .

2.1.2 The spread of antimicrobial resistance in the community

To understand the complexity of the occurrence and spread of antimicrobial resistance, biochemical and genetic resistance mechanisms have to be interpreted at the population level. Whereas *in vitro* studies provide detailed and comprehensive information on resistance mechanisms, clinical observations of individual infections on how these mechanisms behave in natural conditions are less abundant.

Antimicrobial resistance in the community is a multifactorial problem in which key factors are acquisition of genetic resistance elements, antimicrobial selective pressure and clonal dissemination (Patrick and Hutchinson 2009). Antimicrobial selective pressure refers to the impact of

antimicrobial use on a population of organisms in which organisms that are resistant to the antimicrobial gain a survival advantage over those susceptible. This bacterial population includes both potential pathogens and commensal flora. Antimicrobial resistant pathogens gain an advantage due to this selective survival and the elimination of the antimicrobial susceptible commensal flora which creates a niche into which resistant pathogens can spread (Furuya and Lowy 2006).

Clonal dissemination refers to the spread of strains that carry antimicrobial resistance genes under conditions of antimicrobial selective pressure (Furuya and Lowy 2006). The direct effect of antimicrobial treatment in promoting antimicrobial resistance can be seen as the mechanism which plays at the individual level, while the population level mechanism is how antimicrobial treatment promotes the spread of resistant organisms (Lipsitch and Samore 2002).

Colonisation with resistant bacteria is not necessarily associated with antimicrobial treatment. Acquisition of drug resistant bacteria can happen through individual contact, in the community or at home, travel or food.

2.2 Urinary tract infections

2.2.1 Overview

The majority of urinary tract infections (UTIs) develop in the normal urinary tract and are therefore termed ‘uncomplicated’; they can affect the lower or upper urinary tract. Symptoms of urinary tract infection include frequency, painful urgency and haematuria (Bishop 2004). UTIs are the second most common bacterial infections in primary care (Bishop 2004; Car 2006).

The Infectious Diseases Society of America guidelines (Warren, Abrutyn *et al.* 1999; Gupta, Hooton *et al.* 2011) categorise UTIs as follows:

- Acute uncomplicated (lower) UTI in women
- Acute uncomplicated pyelonephritis
- Complicated UTI and UTI in men
- Asymptomatic bacteriuria
- Recurrent UTI.

A distinction between UTI in males and females is made due to the difference in prevalence as well as associated risk factors. Acute uncomplicated cystitis or pyelonephritis in healthy adult males is uncommon but is generally caused by the same spectrum of uropathogens with the same antimicrobial susceptibility profile as that seen in women (Hooton and Stamm 1997). For men the risk factors include intercourse with an infected female partner and homosexuality, whilst circumcision is a protective factor (Hooton and Stamm 1997; Hooton 2000). Risk factors associated with uncomplicated UTIs in women are sexual intercourse, spermicide use, a history of recurrent UTI, and recent antimicrobial chemotherapy (Hooton 2000; Nicolle 2001). Acute uncomplicated UTIs are usually caused by single bacterial species of which *Escherichia coli* is the most common (Kahlmeter 2003). Other aetiological agents include: *Proteus mirabilis* which is common in males and is associated with renal tract abnormalities, particularly calculi; and *Staphylococcus saprophyticus* which is responsible for about 20% of UTIs in sexually active and

otherwise healthy young women. Other coagulase-negative staphylococci are often considered as urinary contaminants as they are part of the normal perineal flora. However, they may cause complicated infections in patients with structural or functional abnormalities of the urinary tract, prostatic calculi or predisposing underlying disease (Health Protection Agency 2009). Streptococci rarely cause uncomplicated UTI, although *Streptococcus agalactiae* may cause infection in some women. Enterococci may occasionally cause uncomplicated UTIs (Health Protection Agency 2009).

2.2.2 Diagnosis of UTI

Diagnosis of UTI requires clinical evaluation supplemented where appropriate by laboratory analysis. Microscopy is used to identify the presence of white blood cells, red blood cells, casts, squamous epithelial cells, bacteria and other cellular components in the urine. Microscopy (or an automated alternative) is often performed for symptomatic patients, to assist in the interpretation of culture results and the diagnosis of UTI.

Significant pyuria defined as the occurrence of ≥ 10 leucocytes/ml in urine, is a widely used term; but much higher counts are often found in symptomatic infection (Graham and Galloway 2001; Health Protection Agency 2009). Pyuria alone is not a reliable indicator of urinary tract infection as it may be present as a result of other conditions such as genital tract infection, catheterisation, calculi (stones) or bladder neoplasm. Sterile pyuria (i.e. pyuria without any growth on routine culture media) may be due to prior treatment with antimicrobial agents, extreme frequency, in asymptomatic bacteriuria (e.g. in pregnancy), or sexually transmitted diseases. Sterile pyuria may also be due to UTI caused by *Proteus* species, as the urease produced by the bacteria results in alkaline urine.

UTI without symptoms is known as covert or asymptomatic bacteriuria.

Because urine must pass through the distal urethra, and in women over the perineum, contamination by the normal flora of these regions can occur.

Isolation of more than one bacterial species suggests such contamination, but

even when a single species is isolated, quantitative culture is required to determine whether it indicates true bacteriuria. The bacterial count is calculated from the number of colony forming units (cfu) on the plate after overnight incubation and with a urine quantity of 1 µl, one colony represents 10^3 organisms/ml. Significant bacteriuria is generally defined as $\geq 10^5$ cfu/ml of a single colony type (Graham and Galloway 2001). However, a lower threshold of 10^4 or 10^3 bacteria/ml of urine is applied in various clinical studies or for specific bacteria or risk groups (Morgan and McKenzie 1993; European Confederation of Laboratory Medicine 2000; McNulty, Richards *et al.* 2006).

2.2.3 Antimicrobial susceptibility testing

Significant isolates are tested against a range of antimicrobials. In the laboratory of the UHG antimicrobial susceptibility testing is performed by disk diffusion; antimicrobial-impregnated paper disks are placed on the surface of an agar plate which has been seeded with the isolate being tested. If the organism is susceptible to the antimicrobial tested its growth will be inhibited and a zone of inhibition will result around the antimicrobial disk. The diameter of the zone of inhibition of growth is proportional to the minimal inhibitory concentration (MIC). Disk content and zone size interpretation are according to recommendations from standardised methodology (Andrews 2009; Clinical and Laboratory Standards Institute 2010).

The initial panel of six antimicrobials used for sensitivity testing in the laboratory of the UHG are as follows (abbreviation): Co-Amoxyclav (AMC), Ampicillin (AMP), Trimethoprim (TRI), Nitrofurantoin (NIT), Ciprofloxacin (CIP) and Cefpodoxime (CEF).

2.2.4 Antimicrobial treatment in the management of UTI

According to the recommendations of the Strategy for the control of Antimicrobial Resistance in Ireland, first line antimicrobial treatment for uncomplicated UTI (no fever or flank pain) is trimethoprim (200mg BD for three days) or nitrofurantoin (50-100 mg QDS for seven days). Second line,

depending on the susceptibility of the organism isolated, is amoxicillin, cefadrine or co-amoxyclav (Strategy for the control of Antimicrobial Resistance in Ireland (SARI) 2008). Updated IDSA guidelines (Gupta, Hooton *et al.* 2011) give similar recommendations with nitrofurantoin and trimethoprim advised as good first line empiric treatment of UTI and additionally fosfomycin and pivmecillinam with lower efficacy (see overview Figure 2.2).

Fluoroquinolones should be reserved for important uses other than acute cystitis and thus should be considered alternative antimicrobials for acute cystitis. The β -lactams generally have inferior efficacy and more adverse effects compared with other UTI antimicrobials, and therefore β -lactams other than pivmecillinam should be used with caution for uncomplicated cystitis. Amoxicillin or ampicillin should not be used for empirical treatment given their relatively poor efficacy.

An intervention Cochrane review comparing different classes of antimicrobials for acute uncomplicated UTI in women found no differences between trimethoprim, fluoroquinolones, β -lactam/ β -lactamase antibiotics and nitrofurantoin for the symptomatic cure of acute uncomplicated UTI (Zalmanovici Trestioreanu, Green *et al.* 2010).

The treatment of a resistant infection with the antimicrobial to which it is resistant would be expected to be similar to one in which antimicrobials were withheld (Little, Merriman *et al.* 2010). The IDSA recommends that when the prevalence of trimethoprim resistance in the community exceeds 10-20%, empirical treatment of UTIs with this agent should be switched to another antimicrobial (Warren 2001). These recommendations were based on cost analyses showing that the treatment cost with trimethoprim becomes unacceptably high over this threshold level when compared to the cost of switching treatment to fluoroquinolones. This 10-20% threshold (Naber 2000; Miller and Tang 2004) was suggested to be lowered to 10% in an economic analysis incorporating a new once daily formulation of ciprofloxacin (Perfetto, Keating *et al.* 2004). None of these economic analyses considers the cost of resistance to society (Foster and Grundmann 2006).

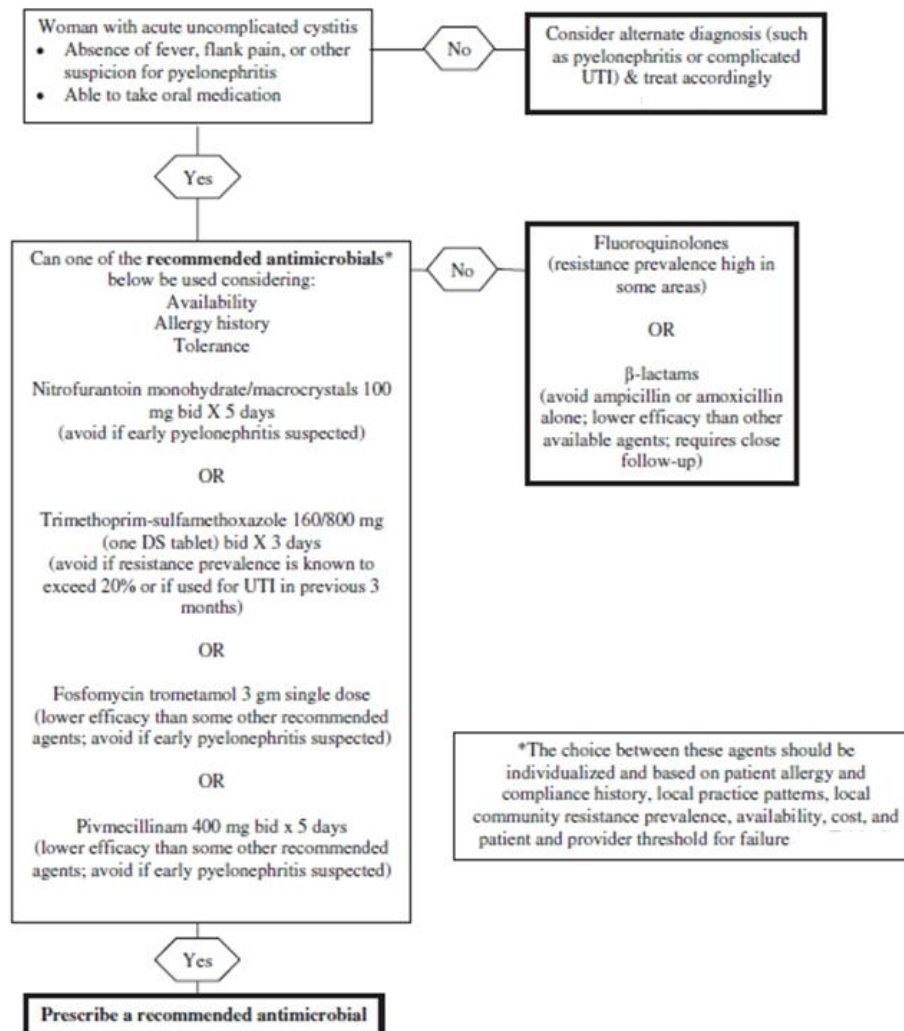


Figure 2.2: Approach to choosing an optimal antimicrobial agent for empirical treatment of acute uncomplicated cystitis. (DS, double-strength) (Gupta, Hooton *et al.* 2011). Copyright obtained from Clinical Infectious Diseases as well as from the first author.

The routine treatment of UTI with antimicrobials has recently received more attention with the publication of a trial that showed that treatment with ibuprofen resulted in equivalent outcomes as treatment with ciprofloxacin (Bleidorn, Gagyor *et al.* 2010). Symptomatic treatment might be a therapeutic alternative for women with symptoms of uncomplicated UTI. Other trials have found improved symptoms after three days without treatment or symptom relief (Christiaens, De Meyere *et al.* 2002) but also slightly poorer results for placebo or delayed antimicrobial treatment (Ferry, Holm *et al.* 2004; Little, Turner *et al.* 2009). A commentary on urinary tract infections in otherwise healthy women acknowledges treatment guidelines should include symptom relief for women who show no evidence of bacterial infection by urinary dipstick (Del Mar 2010).

2.3 *Escherichia coli* and antimicrobial resistance

E.coli is the leading cause of urinary tract infections in the Western world. (White, Alekshun *et al.* 2005). The mechanisms of antimicrobial resistance can be classified into four groups (target site mutation, enzymatic inactivation/degradation of the antimicrobial, reduced accumulation mediated by decreased cellular permeability or active efflux, and metabolic bypass) and *E.coli* can exhibit examples of all these mechanisms concomitantly (Fluit, Schmitz *et al.* 2001; White, Alekshun *et al.* 2005).

Co-resistance is the situation when resistance to one antimicrobial is associated with resistance to another antimicrobial due to the co-existence of genes or mutations in the same strain. Acquisition of multiresistance plasmids that code for combined resistance to ampicillin and trimethoprim is common in *E.coli* (Amyes 1989). Clear associations between resistance and usage have been demonstrated for ampicillin and trimethoprim (Howard, Magee *et al.* 2001; Steinke, Seaton *et al.* 2001).

Cross-over effects, in which the antimicrobial is prescribed for an infection different to the *E.coli* infection, are also described. Ampicillin/amoxicillin is mostly prescribed for respiratory tract infections and this usage has been shown to be associated with ampicillin/amoxicillin resistance of urinary *E.coli* isolates (Howard, Magee *et al.* 2001; Priest, Yudkin *et al.* 2001).

2.4 Overview of (mechanisms of) resistance to main classes of antimicrobials in the treatment of UTI

2.4.1 Trimethoprim

Trimethoprim is a synthetic broad-spectrum antimicrobial agent. Although no naturally occurring enzymes to inactivate trimethoprim were known, resistant bacterial strains rapidly developed after its introduction in 1962, which was partly due to its extensive use in both human and veterinary medicine (Huovinen, Sundstrom *et al.* 1995). Trimethoprim is an analogue of dihydrofolic acid, an essential component in the synthesis of aminoacid and nucleotides that inhibits the enzyme dihydrofolate reductase (DHFR) (Fluit, Visser *et al.* 2001). Folate is needed by rapidly dividing cells to make thymine and this effect is used to therapeutic advantage.

Resistance can be caused by various mechanisms including the overproduction of DHFR, mutations in the structural gene (*dfr*) resulting in altered DHFR enzyme that is less susceptible to inhibition by trimethoprim and by the acquisition of a novel *dfr* gene less susceptible to trimethoprim inhibition (Fluit, Visser *et al.* 2001). The presence of plasmid-encoded trimethoprim resistant *dfr* genes is the most common mechanism responsible for trimethoprim resistance (Huovinen 2001; Skold 2001). The *dfr* genes are considered to be mainly horizontally spread (the exchange of genetic material between bacteria which is not by descent) (Blahna, Zalewski *et al.* 2006). Previous research has shown associations between trimethoprim and amoxicillin prescribing and resistance (co-resistance) and this is thought to be plasmid related (Amyes 1989).

Trimethoprim was launched in 1969 in a combination of trimethoprim and sulfamethoxazole, in the ratio of 1 to 5. This combination, known as Trimethoprim/sulfamethoxazole or Co-trimoxazole, has a sulphonamide group which inhibits an earlier step in the folate synthesis pathway. The claimed benefit of the combination was not seen in clinical use (Bean, Livermore *et al.* 2005) and after media attention regarding the safety of this combination

(Williams, Kelly *et al.* 2000), it is no longer routinely prescribed for the treatment of uncomplicated UTIs in Ireland.

Trimethoprim as monotherapy is marketed as Monotrim. Co-trimoxazole, under trade names like Bactrim, remains indicated for some infections.

2.4.2 Quinolones

Nalidixic acid, the first (generation) quinolone, was discovered in 1962 and introduced for clinical use in the treatment of urinary tract infections in humans in 1967 (Emmerson and Jones 2003). Modification of the chemical structure with a fluorogroup improved and expanded the antibacterial efficacy of quinolones (Andersson and MacGowan 2003). Fluoroquinolones became available for use in the mid-1980s.

Quinolones are purely synthetic broad-spectrum antimicrobials that exert their antibacterial effect by inhibiting certain bacterial topoisomerase enzymes (Fluit, Visser *et al.* 2001; Jayaraman 2009). Topoisomerases are enzymes that unwind and wind DNA in the DNA replication process.

Resistance to quinolones is mainly due to two mechanisms: target alterations (mutations in the code of the topoisomerases) and decreased accumulation inside the bacteria due to impermeability of the membrane or due to increased efflux (Fluit, Visser *et al.* 2001; Ruiz 2003). Furthermore, plasmids conferring quinolone resistance have been described and were an important finding in the explanation of the rapid emergence of quinolone resistance (Strahilevitz, Jacoby *et al.* 2009). The strong association between resistance to quinolones and resistance to other agents (Giske, Monnet *et al.* 2008) also suggest the importance of plasmids in this process. Today, several plasmid-mediated quinolone resistance (PMQR) genes have been discovered (Cattoir and Nordmann 2009; Strahilevitz, Jacoby *et al.* 2009).

Ciprofloxacin is one of the most used fluoroquinolones, but ofloxacin and levofloxacin are also prescribed in general practice

2.4.3 Nitrofurans

Nitrofurans are synthetic antimicrobials used to treat UTI. Nitrofurans have a bacteriostatic effect mediated through the inhibition of enzyme synthesis and a bactericidal effect which causes lesions in the DNA for which the normal enzymatic repair is also inhibited by nitrofurans. Nitrofurans' bactericidal effect is activated by its rapid reduction inside the bacterial cell (Herrlich and Schweiger 1976). Although the specific mode of action of nitrofurantoin is still not fully understood, studies of *E.coli* extracts have shown that strains resistant and susceptible to nitrofurans differ in their ability to reduce the compounds, suggesting that nitrofurans need to be activated by reduction to exert their antimicrobial effect.

Resistance to nitrofurans occurs by step-wise mutations where increased resistance is accompanied by a decrease in the activity of their reductive capacity (Sandegren, Lindqvist *et al.* 2008). Resistance is caused by loss-of-function mutations and plasmid-mediated resistance has been described (Garau 2008). Resistance to nitrofurans is suspected to be mainly due to poor compliance to treatment (Sandegren, Lindqvist *et al.* 2008). Nitrofurans do not share cross-resistance with other commonly prescribed antimicrobial agents. Resistance to nitrofurans is uncommon, probably due to its narrow spectrum of activity, limited indication, narrow tissue distribution and limited contact with bacteria outside the urinary tract (Sheehan and Chew 2005). Nitrofurans became available in 1953 as nitrofurantoin, furazolidone and nitrofurazone. Nitrofurantoin is taken orally and prescribed for use in treatment of uncomplicated UTIs (Conklin 1978; Gupta, Hooton *et al.* 2007; Gupta, Hooton *et al.* 2011).

2.4.4 β -lactam/ β -lactamase Inhibitor combinations

β -lactam antimicrobials are among the most commonly prescribed drugs worldwide (Pitout, Sanders *et al.* 1997) and include penicillins, narrow-and extended-spectrum cephalosporins, monobactams and carbapenems (Drawz and Bonomo 2010). This group of antimicrobials share a structural feature, the

β -lactam ring. β -lactam antimicrobials exert their effect by interfering with cell wall synthesis through binding to the penicillin binding proteins (PBPs) in the cytoplasmic membrane of the bacterium (Georgopapadakou 1993).

Covalent binding to PBPs interferes with synthesis of the cell wall and ultimately leads to cell death. β -lactam antimicrobials have an especially lethal effect on Gram-positive bacteria and less so for Gram-negative bacteria as their cell wall has an outer membrane.

Resistance to β -lactam antimicrobials arises through one or more of the following mechanisms: (1) PBP modifications (mutations or the acquisition of supplementary foreign genes encoding new PBPs), (2) decreased permeability due to alterations in the porins, (3) the production of β -lactamases inactivating the antimicrobial. The most prevalent mechanism of resistance to β -lactams in Gram-negative bacilli is the production of a diverse and numerous range of β -lactamases, enzymes which destroy the β -lactams ((Sanders and Sanders 1992). To increase the utility of β -lactam antimicrobials, this antimicrobial was combined with β -lactam inhibitors. The β -lactam inhibitor is designed to overwhelm all β -lactamases and bind irreversibly to them, allowing the β -lactam antimicrobial to work. Clavulanic acid was the first β -lactam inhibitor introduced in clinical medicine in 1970, and later sulbactam and tazobactam were developed as synthetic compounds (Drawz and Bonomo 2010). Mechanisms of resistance to the combination β -lactam/ β -lactam inhibitors in *E.coli* are through the production of β -lactamases not susceptible to the inhibitors or enzyme hyperproduction (Pitout, Sanders *et al.* 1997; Drawz and Bonomo 2010).

Penicillin is a β -lactam antimicrobial agent. Co-amoxyclav contains the combination of amoxicillin and potassium clavunate. This combination has an increased spectrum of action including Gram-negative bacteria.

2.5 Antimicrobial prescribing and resistance

Colonisation with antimicrobial resistant bacteria may occur independently of antimicrobial exposure by acquisition of resistant bacteria or through spontaneous mutations in sensitive bacteria (Steinke and Davey 2001).

Exposure to an antimicrobial can occur through individual contact, at home or in the community, travel or food. Evidence from genotyping studies showed that food can be a reservoir for extra-intestinal *E.coli* and can result in community-acquired UTIs (Manges, Johnson *et al.* 2001; Vincent, Boerlin *et al.* 2010). However, prescribing of antimicrobials

'It would be naïve to assume that prescription of an antimicrobial is either necessary or sufficient for exposure to an antimicrobial'
(Steinke and Davey, 2001)

at population and individual level is associated with resistance in bacteria even though prescribing can only explain part of the variation in the occurrence of resistance. An overview of studies linking antimicrobial prescribing with resistance is shown in table 2.1. Differences between studies and their findings result from the type of study and the source of the data (individual or aggregated level data on prescribing and/or resistance), which also influences the type of statistical analysis.

Ecological studies (also known as correlation studies) link data at an aggregated level, for instance aggregated individual resistance data and overall prescribing at country, area or GP practice level. Individual linkage studies allow for direct associations within the individual: previous antimicrobial prescribing to the patient and subsequent antimicrobial resistance of the organism causing the UTI. Multilevel studies will combine the methods used in these studies, allowing associations between aggregated level data and individual data as well as the interaction between these levels. Ecological studies, measuring both antimicrobial resistance and antimicrobial use at the population level, demonstrate that the spatial and temporal distribution of resistance is strongly associated with the use of specific classes

of antimicrobials in human populations, even though not all studies find the same strength of association (Patrick and Hutchinson 2009). A European-wide cross-national database study (ESAC, European Surveillance of Antimicrobial Consumption) showed higher levels of antimicrobial resistance in high consuming countries (Goossens, Ferech *et al.* 2005). Livermore *et al.* reported weak correlations between regional variations in ampicillin and trimethoprim resistance in *E.coli* and consumption of the corresponding antimicrobials in England (Livermore, Stephens *et al.* 2000). A cross sectional study plotted resistance data from practices against the prescribing rate (number of prescriptions per 1000 patients per year) of ampicillin, amoxicillin and trimethoprim and calculated Spearman's rank correlation coefficients (Priest, Yudkin *et al.* 2001). Even though they found significant correlations, the size of the correlations was modest and only 16% of the variation in resistance was explained by prescribing. The correlations were stronger at the primary care group level, probably due to the geographically more coherent population for which the prevalence of resistance was calculated. Kahlmeter *et al.* found no statistically significant associations between prescribing and resistance of a range of antimicrobials in an ecological analysis of *E.coli* isolates from community-acquired UTI and country level consumption (Kahlmeter, Munday *et al.* 2003). An association between consumption of fluoroquinolone and resistance of *E.coli* to fluoroquinolones was observed in an ecological study from the Netherlands (Goettsch, van Pelt *et al.* 2000). A Finnish study on the association between consumption and resistance in *E.coli* studied a number of antimicrobials: ampicillin, co-amoxyclov, trimethoprim, cephalosporins, fluoroquinolones and nitrofurantoin. Statistically significant associations were found for nitrofurantoin use and resistance but not for the other antimicrobial agents (Bergman, Nyberg *et al.* 2009).

One of the differences between the findings of these ecological studies was the type of antimicrobial studied. The prescription of nitrofurantoin for instance is for UTI only, which allows for a direct correlation of prescribing for the condition with the occurrence of resistance. In contrast, quinolones or β -

lactam antimicrobials are prescribed for a wider variety of conditions. Another factor in finding associations in ecological studies was the intersection and size of the area of the antimicrobial prescribing and the area for which resistance data are available. This was the case in the English study, where stronger correlations were found at the primary care level group (Priest, Yudkin *et al.* 2001). Interestingly, the study of Donnan *et al.* (Donnan, Wei *et al.* 2004) combined the two methods; a first analysis of prescribing and resistance data aggregated at practice level showed no association, but the individual level data did show an association.

Individual level studies have shown more consistent results. Trimethoprim resistance in community-acquired urinary infections was independently associated with exposure to trimethoprim and to antimicrobials other than trimethoprim (odds ratios of 4.35 (95% CI 3.03-5.73) and 1.32 (95% CI 1.10-1.60) respectively) (Steinke, Seaton *et al.* 2001). Similar associations were found for prescribing in the previous year and subsequent resistant infections for both ampicillin (odds ratio 1.70 (95% CI 1.24-2.32)) and trimethoprim (odds ratio 2.39 (95% CI 1.62-3.53)) by Hillier (Hillier, Roberts *et al.* 2007). In this study the proportion of variation explained in the logistic model was also calculated; 6% of the variation in ampicillin resistance was explained by prescribing of amoxicillin in the previous year and 19% of trimethoprim resistance was explained by trimethoprim prescribing. The number of studied antimicrobial exposures was expanded in an American veteran retrospective case-control study, predominantly male and of older age, which linked individual antimicrobial exposure data (six months) to the laboratory database (Metlay, Strom *et al.* 2003). They found a strong association of antimicrobial exposure within six months (including trimethoprim, quinolones, and ampicillin) with trimethoprim resistance with an odds ratio of 4.1 (95% CI 2.2-7.5). A study enrolling asymptomatic patients found no evidence of an association between ampicillin and trimethoprim resistance in *E.coli* isolates and exposure to any antimicrobial in the previous 12 months (Hay, Thomas *et al.* 2005). However, secondary analysis revealed greater resistance in patients

exposed to antimicrobials within two months, as well as a dose-response relationship to increasing exposure to trimethoprim in the previous 12 months. A causal link has also been suggested by further analysis of the timing of exposure and dose-response relationship in several studies. Individual data showed significant associations between trimethoprim prescribing and subsequent trimethoprim resistance with an overall odds ratio of 1.2 (95% CI 1.2-1.3), ranging from 9.2 for trimethoprim use in the two weeks prior to the identification of a trimethoprim resistant UTI to 1.5 for 4-6 months prior, but no association was found for exposures more than six months prior (Donnan, Wei *et al.* 2004). A dose-response relationship (Hay, Thomas *et al.* 2005) was identified for an increased number of doses of trimethoprim in the previous year with an increased odds of a subsequent trimethoprim resistance UTI. This was not so for amoxicillin, but an analysis of MICs against prescription of the antimicrobial did show increased resistance for amoxicillin only. Even though these were conflicting results they do show the most efficient use of exposure and outcome data as continuous variables.

A recent systematic review and meta-analysis of the effect of antimicrobial prescribing in primary care on resistance in individual patients included eight studies involving patients with UTI (Costelloe, Metcalfe *et al.* 2010), five of which were included in the meta analysis. Particular reference was made to the period at which antimicrobial exposure occurred and effects were detectable up to 12 months. The pooled odds ratios were calculated on results from trimethoprim and amoxicillin studies as well as 'any antimicrobial'. The pooled odds ratio decreased from 4.4 (95% CI 3.8-5.1) for exposure between 0-1 month, 2.5 (95% CI 2.1-3.0) for 0-3 months, 2.2 (95% CI 1.6-3.0) for 0-6 months to 1.3 (95% CI 1.2-1.5) for 0-12 months for the comparison against those who were not exposed to these antimicrobials.

From these studies it seems clear that there is strong evidence of an association between prescribing and resistance at the individual patient level for trimethoprim and ampicillin/amoxicillin. The number of studies on other antimicrobial agents is limited. A case-control study of risk factors for

quinolone resistant *E.coli* found an odds ratio of 20.6 (95% CI 2.3-179.2) for ciprofloxacin use in the previous year for patients with quinolone resistant *E.coli* compared to patients with an *E.coli* infection not resistant to quinolones (Colodner, Kometiani *et al.* 2008).

If a link between antimicrobial prescribing and antimicrobial resistance is shown, to what extent will limiting prescribing reduce resistance? The exposure to trimethoprim, as with any other antimicrobial was predictive of trimethoprim resistance in a record linkage study and the authors concluded that a reduction in trimethoprim prescribing alone may not reduce the prevalence of trimethoprim resistance (Steinke, Seaton *et al.* 2001). An intervention over a two year period that drastically reduced trimethoprim use in one area in Sweden showed no effect on the resistance rates of *E.coli* or other bacteria (Sundqvist, Geli *et al.* 2010). Similarly, after the widespread withdrawal of sulphonamide, sulphonamide resistance in *E.coli* remained unchanged and sulphonamide resistant genes were still present 10 years after the withdrawal (Enne, Livermore *et al.* 2001; Bean, Livermore *et al.* 2005). More encouraging were the reduced local antimicrobial resistance levels of ampicillin and trimethoprim over a seven year period during which prescribing of these agents declined (and others were introduced) (Butler, Dunstan *et al.* 2007). Conversely, but supporting this theory, was the observation of an immediate decrease in resistance to quinolones after a nationwide restriction of all quinolones in Israel (Gottesman, Carmeli *et al.* 2009). The success of prescribing restriction policies depends on three key determinants: the clonal structure of the resistant bacterial population, co-selection of resistant organisms by other antimicrobials and the fitness cost of resistance (Enne 2010). Additional problems exist as boundaries between intervention and non-intervention groups are not strict and potential exchange of bacteria from animals is difficult to take into account. The resistant bacterial phenotypes which are most easy to eliminate will be those composed of relatively clonal populations that bear a fitness cost of resistance and are not significantly subjected to co-selection by other antimicrobials.

Table 2.1 Chronological overview of studies on antimicrobial prescribing and resistance in UTI.

Antimicrobial	Organism	Place, Design, N	Data prescribing	Data resistance	analysis	Resistance/prescribing	Conclusion
AMP, TRI (Pedersen, Schonheyder <i>et al.</i> 1999)	<i>E.coli</i>	Denmark, Record linkage, N=575	Population based database, <i>E.coli</i> linkage on personal number individual	Community acquired, blood cultures	Multiple logistic regression	AMP 26%, TRI 10% Prescriptions AMP/TRI 31% within previous 3 months	OR 2.8(1.8-4.5) for AMP, OR 14.3 (6.3-32.4) for TRI, with 'any antimicrobial' OR 2.3 (1.4-4.0) for AMP, OR 2.7 (1.3-5.9) for TRI
Quin (Goettsch, van Pelt <i>et al.</i> 2000)	<i>E.coli</i>	Netherlands, Ecological, >90,000	Healthcare insurer, regional	Lab from 1989-1998, regional	Multivariate regression, correlation	Resistance 1.3% in 1998 to 5.8% in 1998	Increased resistance associated with increased prescribing of fluoroquinolones
AMP and TRI (Livermore, Stephens <i>et al.</i> 2000)	<i>E.coli</i>	Ecological correlation from 200 hospitals England	Prescription authority and overall IMS health medical data index, regional	Public Health Laboratory Service (PHLS), resistance 1990-1997, regional	X ² (trend), correlation coefficient	AMP 53 - 56%, TRI 18% - 28%	Correlations were weak, some trend for AMP
AMP, AMX, TRI (Priest, Yudkin <i>et al.</i> 2001)	<i>Coliform</i>	Cross sectional correlation, 405 GP practices in England	Pharmacy database, practice level	Laboratory data, practice level proportion resistant isolates	Spearman's rank and linear regression	AMP/AMX 44%, TRI 25%, prescriptions 251 AMP/1000pts/yr, 371 TRI/1000pts/year	16% of resistance explained by practice
TRI (Steinke, Seaton <i>et al.</i> 2001)	<i>Gram-negative organisms</i>	Scotland, Nested case-control, N=3435	Population based database on personal number, individual	Lab cultures, individual	Multiple logistic regression	TRI 24%, 8% prescribed previous 6 months	OR 3.8 (2.9-5.0)
AMC, TRI, CIP,	<i>E.coli</i>	14 European	IMS Health,	Isolates	Pearson	Depending on country,	No significant

Antimicrobial	Organism	Place, Design, N	Data prescribing	Data resistance	analysis	Resistance/prescribing	Conclusion
NIT, fosfomycin and others (Kahlmeter, Menday <i>et al.</i> 2003)		countries, ecological correlation	European	Ecosens project, European	regression with Bonferroni correction	AMP 16-54%, TRI 6-27%, CIP 0-15%, NIT 0-6%	correlations
TRI-SMX (Metlay, Strom <i>et al.</i> 2003)	Gram-negative organisms	USA, Retrospective case-control, 559 male veterans	Linked pharmacy database, individual	Laboratory urine cultures, individual	Multiple logistic regression	TRI 13%, 33% prescribing overall in past 6 months	OR 2.9 (1.4-6.2) for TRI, OR 4.4 (2.3-8.5) for any antimicrobial
TRI (Donnan, Wei <i>et al.</i> 2004)	Gram-negative bacteria	Scotland, 28 GP Practices, record linkage, N=8833	Overall prescription database, practice level and individual	Urine samples, individual and practice level aggregation	Multiple logistic regression and multilevel regression (outcome prevalence of resistance)	Variation between practices 26-50% TRI resistance, 67-357 prescriptions/100 practice pts	OR 1.22 (1.16-1.28) for antimicrobial up to 6 months.
AMP, TRI or both (Hay, Thomas <i>et al.</i> 2005)	<i>E.coli</i>	12 general practices, South West England, Prospective general community sample, N=618 isolates	GP records, individual	Urine samples, individual	Multivariate logistic regression model individual	36% AMP, 7% TRI, either or both 39% AM Prescribing in past 12 months 27%, TRI 5%, β -lactams 19%	No significant association for past year. but OR 1.95 (1.1-3.5) for any antimicrobials within 2 months
AMP, TRI (Hillier, Roberts <i>et al.</i> 2007)	<i>E.coli</i>	10 GP in UK, Case-control, N=903	Medical records, individual	Urine samples, individual	Multivariate logistic regression	40% AMP, 19% TRI Prescribing 24% AMP, 23% TRI	OR for antimicrobial in previous year 1.7 (1.2-2.3) for AMP, OR 2.4 (1.6-3.5) for TRI
TRI-SMX (Colgan, Johnson <i>et al.</i> 2008)	<i>E.coli</i>	Primary care practice in Baltimore (USA),	Questionnaire, individual	Urine samples, individual	Multivariate logistic regression	14.6% TMP-SMX, 15% prescribed TMP in past 3 months	No association with TMP-SMX or other antimicrobial in past

Antimicrobial	Organism	Place, Design, N	Data prescribing	Data resistance	analysis	Resistance/prescribing	Conclusion
		Prospective study, N=165					3 months
Quin (Colodner, Kometiani <i>et al.</i> 2008)	<i>E.coli</i>	Israel, Prospective case-control, N=300	Demographic and clinical data, individual	Individual urine samples	Logistic regression	10% resistance, estimate 30% prescribing	Ciprofloxacin OR 20.6 (2.4-179.2) Ofloxacin OR 7.6 (2.9-19.5) for prescribing in previous 6 months
AMP, AMC, TRI, Quin, NIT, (Bergman, Nyberg <i>et al.</i> 2009)	<i>E.coli</i>	Finland, Ecological, N=754,293	National agency for medicines Regional,	Regional Lab,	Linear mixed model for repeated measures		Only association for NIT consumption and resistance

AMP: ampicillin, AMC: co-amoxycylav, TRI: trimethoprim, TRI-SMX: trimethoprim- sulfamethoxazole, CIP: ciprofloxacin, Quin: (fluoro)quinolones, NIT: nitrofurantoin. Odds ratios (OR) are given with their 95% CI between brackets. pts : patients.

2.6 Bacterial fitness cost of resistance

The concept of reducing antimicrobial prescribing to reduce resistance is based on the underlying rationale that antimicrobial resistance exerts a fitness cost in the absence of antimicrobial selection (Enne 2010). Resistance, according to this concept, would disappear (or at least decrease to the frequency of regeneration) in the absence of selection pressure (Lipsitch 2001; Johnsen, Townsend *et al.* 2009). To study potential decline in resistance when removing the selection pressure, the time scale has to be taken into account as the time to emergence of resistance under selective pressure is much shorter than the time after cessation or decline in the volume of antimicrobial use (Austin, Kristinsson *et al.* 1999; Harbarth 2007).

Persistence of sulphonamide resistance was described after a progressive switch from co-trimoxazole to trimethoprim, resulting in a huge decrease in sulphonamide use in the UK between 1991 and 1999. No dramatic decrease in resistance was shown after this time probably due to genetic linkage of the index resistance to other resistance determinants (Enne, Livermore *et al.* 2001). The genes responsible for sulphonamide resistance (located on large plasmids) were still present in 45% of the *E.coli* nine years after withdrawal from prescribing (Bean, Livermore *et al.* 2005).

In *E.coli* and other Gram-negative bacterial species, mechanisms of resistance are mainly plasmid born and frequently genetically linked to resistance determinants of other antimicrobial classes. Plasmid-conferred resistance was most likely the difference between the unsuccessful reduction in trimethoprim and sulphonamide resistance (Enne, Livermore *et al.* 2001; Sundqvist, Geli *et al.* 2010) and the decline in quinolone resistance (Gottesman, Carmeli *et al.* 2009) after prescribing reductions. Most clinically significant quinolone resistance in *E.coli* is still due to chromosomal mutations and less so plasmid-mediated. Plasmid-encoded resistance appears to be the most difficult to reduce as fitness costs have been shown to be relatively low or even non-existent depending on the host (Enne 2010). Many plasmids are maintained by

co-selection and plasmids are self transmissible (Enne 2010). For nitrofurans, resistance confers a reduction in fitness in *E.coli* in the absence of an antimicrobial. In the presence of therapeutic levels of nitrofurantoin, even resistant mutants are so disturbed that they are probably unable to grow and establish an infection (Sandegren, Lindqvist *et al.* 2008).

2.7 Antimicrobial resistance and clinical outcomes

Some antimicrobial resistant strains seem to be less virulent than sensitive strains, as shown in a pneumococcal pneumonia case-control study in which resistance was associated with milder clinical presentations albeit longer hospital stays (Einarsson, Kristjansson *et al.* 1998). Other reports have shown that infections caused by resistant pathogens had a higher occurrence of morbidity and mortality compared to infections caused by susceptible pathogens, in particular when measured by the length of hospital stay (Holmberg, Solomon *et al.* 1987; Vandijck, Blot *et al.* 2008). In the community, resistant *E.coli* strains were found to be associated with longer duration of symptoms, even if treated with an appropriate antimicrobial (Butler, Hillier *et al.* 2006), as well as worse clinical outcomes (McNulty, Richards *et al.* 2006). A study on the natural course of (severe) symptoms and the role of antimicrobial prescribing and resistance found that resistance and withholding antimicrobials were associated with a greater than 50% increase in the duration of more severe symptoms of UTI (Little, Merriman *et al.* 2010). Overall, these results suggest that resistant *E.coli* UTIs have poorer clinical outcomes compared to susceptible *E.coli* UTIs. However, poorer clinical outcomes might be related to delayed start of effective antimicrobial prescribing rather than the virulence of the pathogen (Lautenbach, Metlay *et al.* 2005).

2.8 Population and individual benefits of antimicrobial therapy

Antimicrobial treatment focused on the most efficient cure for the patient may exert population level effects that in the long run will substantially differ in magnitude or even become opposite. Whereas the clinician focuses primarily on the most effective cure for a specific individual, an epidemiologist focuses on the effect of cumulative prescribing on resistance at the population level (Schwaber, De-Medina *et al.* 2004). This dilemma is an example of the ‘tragedy of the commons’, described in an influential article from 1968 with an example on land tenure in which individuals acting locally to benefit themselves inadvertently contributed to catastrophe at the ecological level (Hardin 1968). The example in the ‘tragedy of the commons’ refers to common land to which many people have rights. The herdsman knows that by putting too many cows on a field, the field will eventually be destroyed by overgrazing. However, when fields are shared commons the benefit of adding a cow goes entirely to the owner even though all herders share the cost. Crucial in Hardin’s discussion is that he identifies that a tragedy of the commons lacks a technical solution, which he defines as ‘one that requires a change only in the techniques of the natural sciences, demanding little or nothing in the way of change in human values or ideas of morality’. Current campaigns to limit the use of antimicrobials and the quest to find new antimicrobials can be seen as such technical solutions. Hardin’s metaphor emphasises the paradox that exists between antimicrobial therapy prescribed to maximise individual patient care on one hand and population benefits on the other. To get this balance right requires a clear understanding of the individual and the population benefits of antimicrobial use, the cost of

‘Our collective prescriptions constitute an ecological problem that may reduce the success of future therapy. (..) ‘

‘We may well ask how we may reduce our “resistance footprint” without causing harm by withholding antimicrobials from those that need them.’

(Patrick and Hutchinson 2009)

resistant pathogens, in particular for the population, and the potential for investments into new antimicrobials and infection control to limit these costs (Foster and Grundmann 2006). The proposed solutions in the ‘tragedy of the commons’ framework were ‘mutual coercion mutually agreed upon’ and privatisation. Potential solutions for countries can be the introduction of regulatory policies to restrict the prescription of antimicrobial agents and/or professional and public strategies to encourage appropriate prescribing of antimicrobials (Goossens, Ferech *et al.* 2005; Goossens, Guillemot *et al.* 2006). However, it has been argued that restricting regulations jeopardise the medical freedom of prescribing (Sagar, Daemmrich *et al.* 2000). To protect public goods, like antimicrobial therapy, from overuse, a context in which overprescription is damaging for the reputation of doctors has been suggested as an alternative (Milinski, Semmann *et al.* 2002; Baquero and Campos 2003). However, this would only work if supported by education of patients about societal benefits, and these benefits would be most powerful on a local scale (Davey, Pagliari *et al.* 2002; Metlay, Shea *et al.* 2002; Finch, Metlay *et al.* 2004). Strong regional effects of differences in antimicrobial use and resistance suggest that local benefits are realistic (Foster and Grundmann 2006).

2.9 Bias

Epidemiological studies, in particular cross sectional studies, are prone to bias and confounding. Bias, or systematic error, is defined as any trend in the collection, analysis, interpretation, publication or review of data that can lead to conclusions that show a systematic error (Last 2003; Porta and Last 2008). A selection bias can occur when there is a systematic difference between the characteristics of individuals who do and who do not participate in a study, which is particularly so when the factor or disease under investigation itself is associated with the reason for participating. A bias can arise from factors determining whether or not a patient consults a doctor, whether the doctor obtains a sample and how a sample is processed.

Previous studies have shown that GPs differ in their prescribing patterns and appropriate requests for laboratory tests (Kelsey, Kouloumas *et al.* 1996; Steffensen, Schonheyder *et al.* 1997). However, this potential bias was not of importance when studied by Magee as no association was detected between the number of trimethoprim prescriptions per 1000 practice population and the number of

Text box 2: Example of bias from fixed number sampling:

A laboratory is asked to submit 30 consecutive isolates of E.coli during each year of surveillance.

In the first year, 30 isolates are collected over a 10 day period, 20 isolates are susceptible to antimicrobial 'A'. This results in an average susceptible to resistant each day of 2 to 1.

*During the second year, antimicrobial 'A' is introduced into the hospital, reducing the recovery of susceptible isolates by 50% but without any effect on the recovery of resistant isolates. The average number of susceptible isolates each day is reduced to 1. In year 2 the 30 isolates take 15 days to collect and the average susceptible to resistant is 1 to 1. The proportion of resistant isolates increased from 33% to 50% after introduction of the antimicrobial 'A' when in fact the average number of resistant isolates per day (burden of resistance) did not change (Schwaber, De-Medina *et al.* 2004).*

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urine samples submitted to the laboratory (Magee, Pritchard *et al.* 1999). Surveillance of resistance is generally through the routine collection of diagnostic susceptibility results. Selection bias may arise when a large proportion of patients with infections are treated without the causative agent being identified through laboratory culture. Bias may arise if patients from whom bacterial samples are obtained are different from the study population, for instance patients with recurrent infections. Surveillance of the prevalence of resistance will not accurately reflect the actual prevalence of resistance. Studies in which spotter and sentinel practices were compared to other general practices, have shown that the differences in the occurrence of resistance were small, and that data derived from routine urine samples received by the laboratory provide reliable information for formulating empirical prescribing guidelines (Richards 2002; Ludlam, Sule *et al.* 2004). Selection bias can also arise when there is a difference in the clinical outcome of infections caused by resistant isolates compared to susceptible isolates (Steinke and Davey 2001). Some studies have suggested that resistant strains have different clinical outcomes compared to susceptible strains (Butler, Hillier *et al.* 2006; McNulty, Richards *et al.* 2006). However, neither bias would cause a deviation of the association between antimicrobial prescribing and antimicrobial resistance as neither interferes with the pathway between exposure and response (see also section 2.10). A potential selection bias could arise if there was an association between the occurrence of a resistant infection and other factors that influence treatment, like co-morbidities (confounding) which can be adjusted for in the statistical analysis. Another methodological issue when monitoring changes in the proportions of resistant isolates arises from the perspective taken: clinical or population. Whereas proportions are helpful for the clinician to prescribe empirical therapy, the same proportion based laboratory data can yield biased estimates when analysing a potential association between antimicrobial use and resistance (Schwaber, De-Medina *et al.* 2004). This can lead to finding a significant trend when there is no change, as proportions are dependent on

the susceptible population and many factors can influence this without actually affecting the absolute numbers. The use of proportions for analysing the relationship between antimicrobial use and resistance poses a particular problem (see Text box 2, page 37) as the use of an antimicrobial will result in a decline in the absolute number of susceptible isolates, resulting in an increase in the proportion of resistant isolates (Schwaber, De-Medina *et al.* 2004). Paradoxically, this will in turn change the prescribing behaviour of the clinician and increase the patient's chance of receiving effective therapy. The more effective an antimicrobial is against a susceptible strain of a pathogen, the greater the proportion of resistant isolates will be after its introduction. Understanding why resistance proportions are useful in a clinical setting but not for epidemiological interpretation is important when setting up and analysing interventions. A related concern that duplicate and screening isolates would result in an overestimate of regional resistance and thereby affect the prescribed empiric treatment, was shown to be untrue (Magee 2004).

Studies of antimicrobial use and resistance at the population level are prone to ecological fallacy: the bias that may occur because an association observed between variables on an aggregate level that does not necessarily represent the association that exists at an individual level (Last 2003; Porta and Last 2008). A special case of ecological fallacy is the Simpson's paradox, in which a correlation (trend) present in different groups is reversed when the groups are combined (Reintjes, de Boer *et al.* 2000). Different conclusions according to the analysis of individual or aggregated data (Harbarth, Harris *et al.* 2001) can be avoided by the use of adequate statistical modelling allowing individual and group level factors to interact.

Additionally, measuring and monitoring changes in antimicrobial resistance at population level can more appropriately be done through burden of disease studies, in our case burden of resistance, as it is a function of the resistant population alone and does not depend on the susceptible population. The burden of resistance is a the absolute number of resistant isolates in a

population over time (Schwaber, De-Medina *et al.* 2004). Few studies have been published using true rates (examples are (Harbarth, Martin *et al.* 2000; Sundqvist, Geli *et al.* 2010)) even though many studies wrongly report changes in rates of resistance when in fact they are reporting changes in proportions of resistant isolates (for example (Goossens, Ferech *et al.* 2005; Kurtaran, Candevir *et al.* 2010; Oteo, Bautista *et al.* 2010)). The effect of intervention studies at population level is best analysed using rates.

2.10 Exploring causation

Causation is an essential concept in epidemiology, but there is no clear definition (Parascandola and Weed 2001). The importance of understanding cause is that when a cause can be identified, interventions can be designed to manipulate these factors with the aim of improving the level of health (Martin 2008). A useful scientific definition should be specific enough to distinguish causation from correlation, but not so narrow that only obvious direct causation is taken into consideration (Parascandola and Weed 2001).

In the probabilistic causation concept, cause is not a dichotomous but a continuous factor ranging between the extremes of 0 and 1. A cause in this context is any factor that significantly alters the risk of disease (Parascandola and Weed 2001). However, the lack of an implied causal model in the probabilistic causation model is not in line with the component cause model. This model of causation describes causes in terms of sufficient causes and their component causes. A sufficient cause is a set of minimal conditions that are necessary to produce disease, while a component cause is part of this sufficient cause. This means that a disease can be caused by more than one causal mechanism involving a number of component causes. The biological synergy between components can produce an effect which is larger than its components (Rothman and Greenland 2005). Understanding the component cause model for a disease gives the basic biologic features of a disease and its indirect factors and these can be of great value in setting up interventions (Martin 2008). The importance of the complex interaction of individual and population level factors is the reason that strategies for decreasing antimicrobial prescribing to reduce existing antimicrobial resistance have shown contrasting results (Enne 2010). Statistically significant associations are not necessarily true causes of the hazard, and seemingly plausible associations may lead to management actions that can be counterproductive (Cox and Ricci 2005). A multidisciplinary approach is necessary to understand causal factors interacting at more than one level (Dohoo 2008).

Any epidemiologic investigation attempting to describe the relationship between an exposure and outcome must consider potential confounders. Confounding factors are variables that 1) are associated with the outcome as well as the exposure, and 2) are not variables in the causal pathway. If confounding exists, an association may appear to be present when this is not true, or no association is found when a true association does exist (Greenland and Morgenstern 2001). An omitted confounding variable can distort or even reverse the apparent relation between exposure and response inferred from a statistical risk model and bias the effects estimated from variables (Cox and Ricci 2005). Additionally, including intermediate variables (events in the causal pathway between the exposure and outcome) as confounders can also lead to false results (Schwaber and Carmeli 2006).

Once confounders are identified they can be controlled for during the design phase through restriction, matching or randomisation, or during analysis via stratification, or multivariable analysis (Fleischer and Diez Roux 2008).

Chapter 3: A multilevel analysis of trimethoprim and ciprofloxacin prescribing and resistance of uropathogenic *E.coli* in general practice.

This chapter was published as a paper in the Journal of Antimicrobial Chemotherapy, May 2010 (Appendix 12).

'Essentially, all models are wrong, but some are useful', George Box.[‡]

3.1 Introduction

The association between antimicrobial prescribing and resistance has been studied at individual as well as at population level. An international effort to correlate prescribing and resistance data at country level showed north-south patterns (Goossens, Ferech *et al.* 2005). Ecological studies of the geographical differences in outpatient antimicrobial use show a correlation with geographic variation of resistance in Europe suggesting that variation of resistance can be explained by differential selection pressure (Ferech, Coenen *et al.* 2006). However, the ecological study design does not imply causality. Various other studies also identified a correlation with resistance at ecological level (Magee, Pritchard *et al.* 1999; Priest, Yudkin *et al.* 2001; Gottesman, Carmeli *et al.* 2009). At individual level the link is clearer and studies have shown that previous use of an antimicrobial will increase the risk of resistance against this agent. As resistance epidemiology is a complex issue with individual level and population level factors interacting, the importance of linking individual and group level data is apparent. A number of multilevel studies have been published. Donnan *et al.* applied a multilevel regression with individual data on trimethoprim prescribing and resistance and practice level confounders (Donnan, Wei *et al.* 2004). Butler *et al.* used a multilevel linear model in which changes in antimicrobial resistance at practice level were found to be

[‡] DeGroot, M. H. (1987). "A Conversation with George Box." *Statistical Science* 2(3): 239-58.

associated with antimicrobial prescribing (Butler, Dunstan *et al.* 2007). Even though these studies used multilevel modelling, none of the studies included the possible effect of prescribing on the risk of resistance at individual as well as practice level.

This multilevel analysis will analyse the effect of overall practice prescribing on individual resistance of uropathogenic *E.coli*. Both individual and practice risk, as well as their interrelation, will be assessed.

3.2 Methods

3.2.1 Data

Data were collected from various sources. The two main databases were the anonymised database from the laboratory of the Galway University Hospitals (GUH) and the Health Service Executive - Primary Care Reimbursement Service (HSE-PCRS). In addition to this the HSE Primary Care Unit supplied data on the practices (not the GPs) comprising the number of GPs in the practice, number of medical card patients registered with the practice, number of female GPs, mean age of the GPs, rural allowance, and practice nurse. To assess the relative affluence of each area, the Small Area Health Research Unit (SAHRU) index was used (Kelly 2009). This index has been compiled by the Department of Community Health and General Practice in Trinity College, Dublin and is based geographically on district electoral divisions. It rates indices of relative poverty on a scale from 1 to 5, where 1 is the most affluent and 5 the most deprived. Deprivation is rated in terms specific to the particular socio-demographic conditions in Ireland.

Microbiology

All urine samples from GP practices are sent to the laboratory of GUH where diagnosis of UTI by microscopy and semi-quantitative culture of a urine sample is performed. Specimens with less than 20 white blood cells/ μ l were considered negative and were not processed further. Pyuria was defined as greater than 20 white blood cells/ μ l and 10^5 cfu/ml of causative organisms was considered a pure or predominant growth. Antimicrobial susceptibility testing was performed on isolates to guide selection of an appropriate antimicrobial to treat the infection. Protocols for the performance and interpretation of these results are based on guidelines from the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute 2010).

Health Service Executive-Primary Care Reimbursement Service (HSE-PCRS)

The Health Service Executive- Primary Care Reimbursement Service (HSE-PCRS) (HSE-PCRS 2010) supports the delivery of primary healthcare by providing reimbursement services to primary care contractors including general practitioners, pharmacists, dentists and optometrists / ophthalmologists , for the provision of health services to members of the public in their own community.

Persons who are unable without undue hardship to arrange general practitioner, medical and surgical services for themselves and their dependants, and all persons aged 70 years and over, receive a free general medical service (GMS) card. Drugs, medicines and appliances supplied under the scheme are provided through retail pharmacies. In most cases the GP gives a completed prescription form to a person, who takes it to any pharmacy that has an agreement with the Health Service Executive to dispense GMS prescription forms. In rural areas the GP may dispense to those persons who opt to have their medicines dispensed by him/her. All GMS claims are processed and paid by the PCRS.

3.2.2 Data linking

To link the database from microbiology with the prescription database of the HSE-PCRS, data were aggregated by practice. All individual resistance data received a practice code. This code corresponded with the practice code in the HSE-PCRS database, in which prescribing of antimicrobials was recorded as well as hormone replacement therapy (HRT), oral contraception (OC), selective serotonin reuptake inhibitor (SSRI), benzodiazepines and overall prescribing combined for each practice from 2004 to 2008. The inclusion of the prescriptions for HRT as well as OC was to allow for higher prescribing due to a higher number of women (older or younger age). Antidepressant use (SSRI) and insomnia medication (benzodiazepines) were included to allow corrections for confounding for (increased) GP visits due to the tolerance to and dependence on these medications. The overall prescribing of the practice was a measure to indicate high and low prescribing practices.

3.2.3 Selection of the antimicrobials

Data analysis was performed with data on trimethoprim and ciprofloxacin prescribing and resistance. Trimethoprim was chosen to compare the results with results from other studies on prescribing and resistance. Additionally, trimethoprim would represent an established antimicrobial as it was introduced in clinical practice in 1975. Trimethoprim resistance levels in *E.coli* have remained stable since 2002, at around 30% (Chulain, Murray *et al.* 2005). Ciprofloxacin was introduced into clinical practice more recently and resistance levels in *E.coli* were low at 2.5% in 2003 but are increasing (Emmerson and Jones 2003). Ciprofloxacin is particularly interesting when studying patterns of the spread of resistance as it is a relatively ‘new’ antimicrobial.

3.2.4 Multilevel logistic regression

In every population various hierarchical structures can be identified. This structure can affect the measure of interest. Multilevel analysis was first developed for educational research by the ‘Goldstein group’ (Goldstein 1987) at the University of Bristol. When analysing the performance of students they realised that the observations of students in the same class were not independent. Standard statistical tools assume independence between observations and were not really appropriate to use in the analysis of the performance of students. The general idea of multilevel analysis is that the hierarchy of students clustered in classes is taken into account, thereby correcting the analysis for the dependency between observations. Multilevel data structures also arise in longitudinal studies where an individual’s responses over time are correlated with each other.

Many kinds of data, including observational data collected in the human and biological sciences, have a hierarchical or clustered structure. For example, children from the same parents will be more alike in their physical and mental characteristics than individuals chosen at random from the population at large.

Additional explanation of multilevel modelling

When data are statistically analysed the aim is to find out something about a group that cannot be deduced from the individual. An individual has many traits. If an individual has a condition, the occurrence of this condition may be related to one or more of these traits. Grouping many individuals with a certain condition and comparing them with a group without this condition, will show which individual traits are more often present in those with the condition compared to individuals without the condition. Epidemiology aims to find these patterns by comparing groups of individuals.

One of the techniques to analyse data is *regression analysis*, a method in which information on a dependent or outcome variable y is explained by one

or more independent variables x_1, x_2, x_3, \dots . Regression analysis will describe y (the outcome) as a function of the x 's (traits), or in other words, predict y from the x 's. The simplest expression of a regression analysis is illustrated in the graph below. Each individual is represented by a point where the value of the variable y is plotted against the value of variable x . When this is done for a group of individuals, a diagonal line can be drawn through all the points. The point where the line intersects with the y axis is the *intercept* a , the angle of the line is the *slope* b (Figure 3.1).

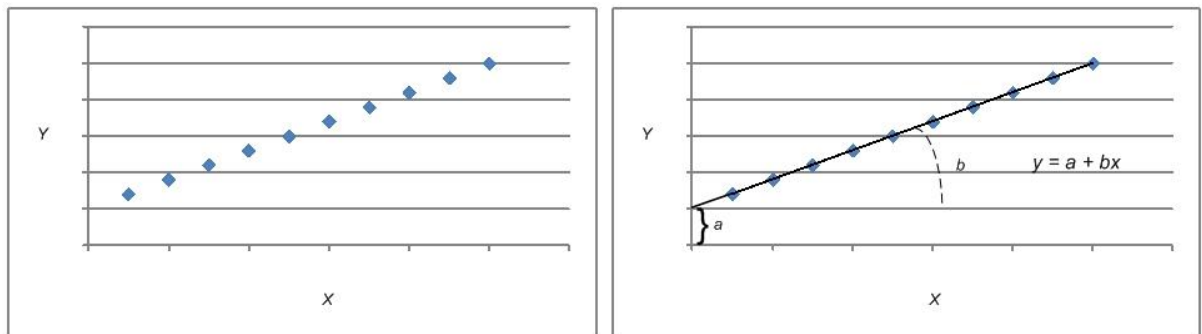


Figure 3.1: The simplest expression of a linear regression analysis.

The first graph shows a scatter plot of points. The line calculated through these points in the second graph is the linear regression line. The formula of this line is $y=a+bx$, with a the intercept (where the line crosses the Y axis) and b the slope of the line. With this formula, the value of y can be predicted from every value of x .

In reality, not every point lies as neatly on the diagonal line. Points are usually scattered forming a cloud (Figure 3.2, first graph). Linear regression is a method to find the line that best describes the scattered points. To determine the best fitting line, a *least square method* is used. This method will start with a line and determine the vertical distance of each of each point to this line (*residual*). The residual can be positive if the point lies above the line, or negative, if the point lies below the line. To eliminate the negative values, the residuals are squared. The sum of these squared residuals (*sum of squares*) measures how well the line fits the cloud of points (Figure 3.2, second graph). A sum of squares can be calculated for various lines and the one with the smallest sum of squares is the best fitting line.

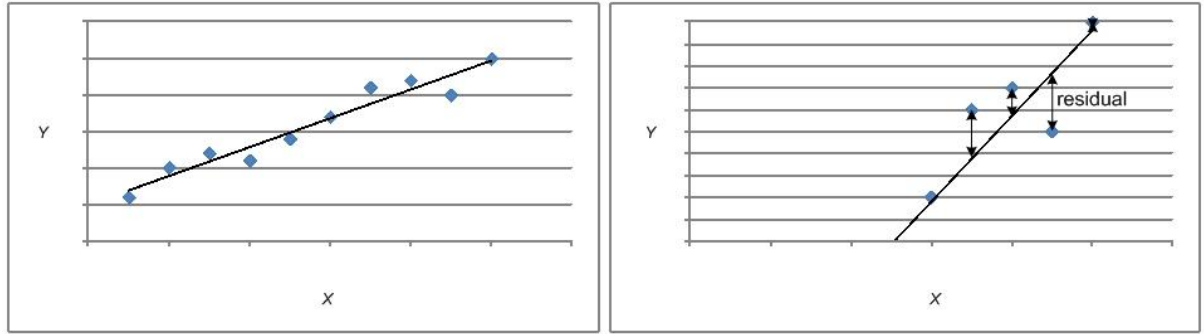


Figure 3.2: Scatterplot and residuals in linear regression analysis.

In reality the points do not lie neatly on a line but usually form a cloud. The linear regression line is the line that has the lowest sum of squares of the residuals. The sum of squares is the sum of the squared residuals.

The sum of squares is therefore the variation between the individuals and an indication of the individuals' deviation from the regression model.

An example of a regression analysis could be the exam results (y) of all students from one year plotted against their study time (x). The expectation would be that the more the student studied, the higher their exam score, indicating a positive correlation between study time and exam score.

However, other variables might also influence the outcome, for instance, gender. When gender is also included in the regression analysis, a line for girls and a line for boys can be calculated (Figure 3.3). The graph can be interpreted in this context as the more a student studies, the better their exam results and that girls generally perform better than boys on exams; the original association is the same for girls and boys, but the intercept for girls is bigger. The difference between girls and boys is the difference in intercept. This concept can be expanded to more variables; the calculations become more complex, but the interpretation remains the same.

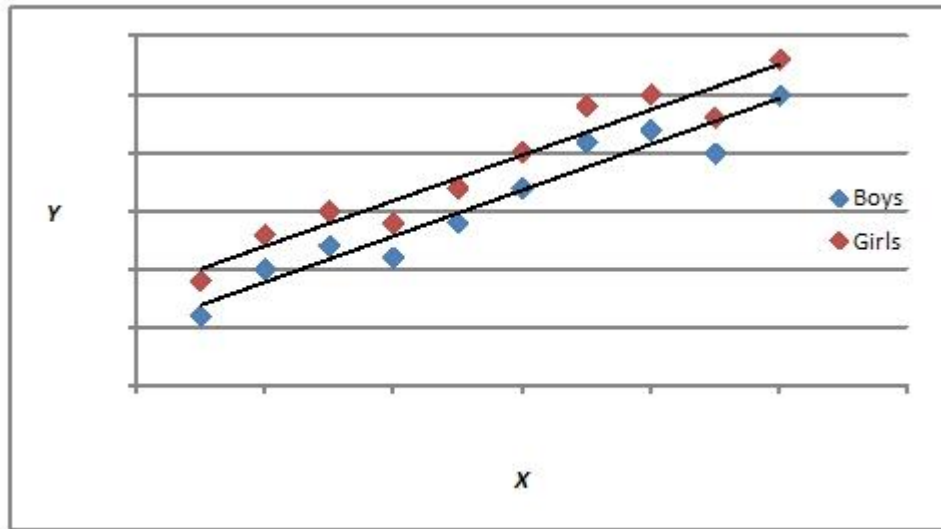


Figure 3.3: Different regression lines according to the variable gender.

A linear regression line can be calculated for different variables. In this case, the correlation between outcome and variable of interest is the same (they have the same slope) but girls do better than boys (different intercept).

When, however, the association is not the same for boys as it is for girls, there is *interaction*. An extreme case would be if girls do better when they study less and boys do better when they study more. The difference between girls and boys in this case is not only the difference in intercept, but now the lines also have a different (even opposite) slope (Figure 3.4). If this effect were real and the interaction is not taken into account in the regression analysis, it is very possible that gender will not be taken into account as a significant variable in predicting the outcome, as the boys' effect of better results with increased study time is fully or partially 'cancelled out' by the girls' effect.

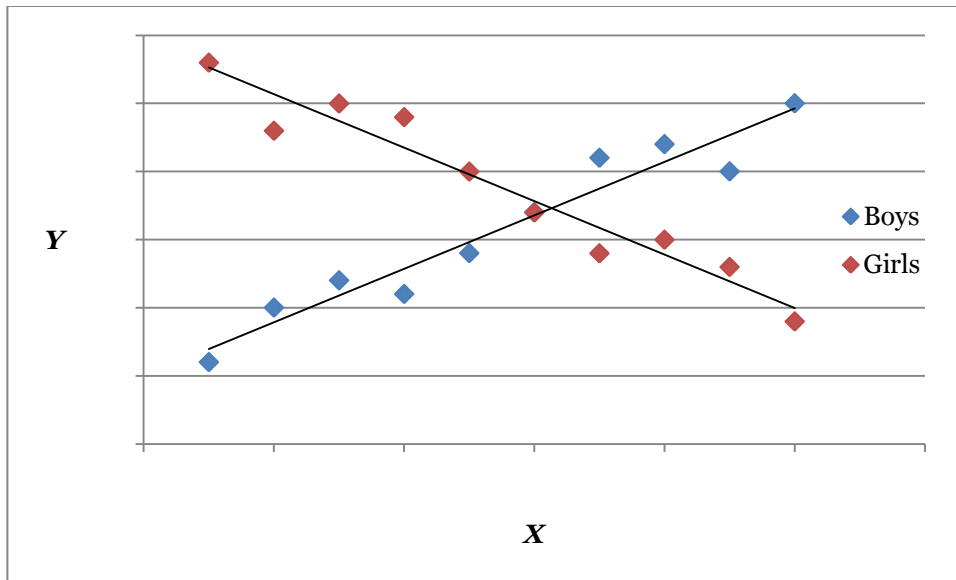


Figure 3.4: Interaction in regression analysis.

Interaction of the outcome with gender (or any other variable) occurs if the effect of one variable is different according to gender. With interaction, the slope between outcome and explanatory variable is different.

Up to now, students' exam results have been compared taking individual factors like study time and gender into account. However, the school the students attend can also have an influence. Some schools have better overall results compared to others. A crude way to compare schools would be to take the mean of the exam results of all the students in the school and compare that with the 'study time' allocated within the curriculum of the school. This is a so-called *ecological* study, where individual exam results are *aggregated* (mean exam results) at a higher level, the school, and compared to a school variable, 'allocated study time in the curriculum'. Intuitively it can be understood that by aggregating individual results at a higher level, interesting details can be lost. For instance, Asian students often perform better in exams. This factor can be included in the individual regression analysis in the same way as gender was included (Asian vs non-Asian student). When comparing the overall results obtained in schools, an overall school factor for 'Asian' (for instance percentage Asian students in the school) can be included

in the analysis. This percentage Asian students in a school might not convey the same information as the individual detail.

Similarly, individuals in the school share factors typical for the school. For instance, students going to a school in an affluent area might overall do better than students going to a school in a deprived area. The individual exam result is influenced by an overall group effect related to the school, or in other words, a poor student in an affluent school will generally do better compared to the same poor student in a deprived school. This is called *dependency* between students within a school. The phenomenon in general is called dependency of data. This means that the individual outcome is more than the sum of the individual traits. This additional effect is the group (for instance the school or area) level effect.

In a multilevel analysis all the detailed, individual information is included and modelled. In our example this would mean that a regression analysis will be done for each school based on the individuals in the school and resulting in a regression line for each school (Figure 3.5, first graph). The difference between schools in this graphical example is a difference in intercepts, as the slope of each school is the same which means that the effect of the variables is the same in each school. A school regression analysis can be performed with the outcome of these school regression analyses by the calculation of a similar sum of squares from the school residuals, the distance of each school to the overall regression line. The overall regression line gives shows the overall effect of study time on exam result, taking school and student differences into account (Figure 3.5, second graph, red line is combined effect).

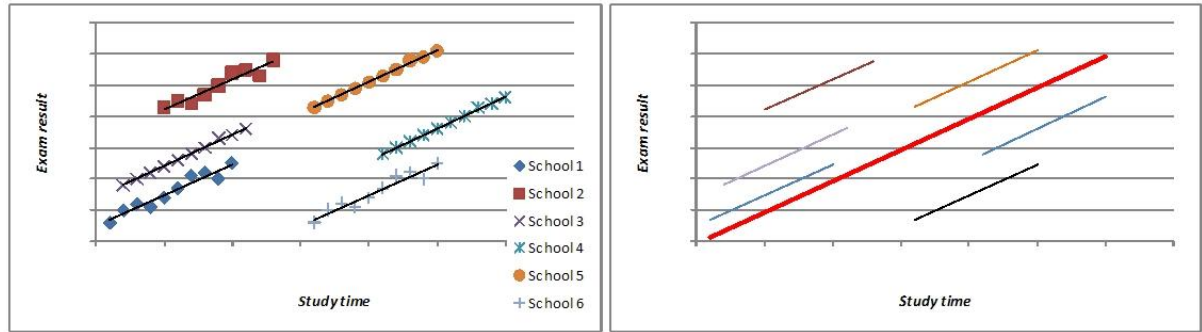


Figure 3.5: A multilevel model.

The first graph shows how multilevel models are calculated. For each cluster (in this case school) a separate regression analysis is performed. With the outcome of these different cluster regression analyses, an overall regression analysis is performed (the thick red line in the second graph).

In this multilevel model, a distinction can be made between the variation explained by the differences between students and the variation explained by the differences between schools. The overall variation in outcome is ‘partitioned’ into a between-school component (the variance of the school-level residuals) and a within-school component (the variance of the student-level residuals). The student residuals, or the individual effect, can be explained by individual characteristics like the time they studied, gender, ethnicity, etc. The school residuals, or school effects, represent school characteristics that affect student outcomes, like affluence of the area where the school is situated.

In a multilevel regression analysis modelling starts with an ‘empty’ model, a model without any explaining variables. The empty model will quantify and partition the variation between individual and school effects. Subsequent further modelling will include individual and school variables which will explain part or all of the variation in individual or school effects.

These effects can be fixed or random; a fixed effect is a variable with different intercepts depending on the school, while a random effect also has a different slope depending on the school. So, if the students’ study time is a fixed effect between schools, the effect of study time on exam result is the same for a student in an affluent school and in a deprived school and increased study time increases the exam score by the same amount. If

students' study time is a random effect between schools, a student with a particular study time obtains better than expected results in an affluent school compared to the same student in a deprived school.

The previous explanation refers to linear regression, i.e. the outcome variable is a continuous variable. Another form of regression analysis is *logistic regression* the outcome variable is a dichotomous variable: the presence or absence of a condition (for instance, the presence or absence of a condition). The underlying principles for logistic regression are the same as for linear regression but rather than predicting the value of a variable y from predictor/explanatory variables x , a logistic regression predicts the *probability* P of y occurring, given known values of x . This probability is a continuum between 0 and 1, or a probability between 0% and 100%. This continuum in probability can be compared to the continuum in the example of the exam results in linear regression. The outcome of the linear regression remain the same, but a conversion is used to apply it to dichotomous outcomes. In its simplest form with only one predictor variable x , the equation will be given by $P(y) = 1 / 1 + e^{-z}$ with z being the linear regression equation $z = a + \beta x$. As a result, the value from the equation is a probability value that varies between 0 and 1. In linear regression, the value b represented the change in the outcome resulting from a unit change in the predictor variable. In logistic regression, this value β represents the change in the logit (natural logarithm) of the outcome variable associated with a one-unit change in the predictor variable, and calculating the exponent of β (e^{β}) gives the *odds* of y occurring.

3.2.5 Data analysis

Average practice prescription rates were calculated per 1000 patients (panel size) per month. For easier interpretation, socio-economic status (SES) was categorised into high and low SES and age was categorised into five age groups. The resistance of *E.coli* to trimethoprim and ciprofloxacin was compared to the pooled prescriptions of these antimicrobials within the

practice. The association was studied with a multilevel logistic model with trimethoprim and ciprofloxacin resistance as binary outcomes (yes/no). This approach allows the separation of practice and patient level factors. A model was built starting with an empty model and introducing the main factor of interest, antimicrobial prescribing, patient variables (age and gender) and practice level variables at each step.

Prescription rates were specified as random effects (varying intercept for each practice) which allows rates to be specific for each practice. Prescription rates were also tested for random slopes to see whether the effect of prescribing differs between practices, but this did not improve the models. An alternative model in which 'cohort' (year) was included as a level (individuals within cohorts within practices) was not significantly better than the two-level model. Models were tested for interactions, but these did not show any statistical significance.

The adjusted odds ratios (OR) with 95% confidence intervals (95% CI) gives the increase in the probability of having a resistant *E.coli* for every additional prescription of the antimicrobial (per 1000 patients per month) or, for the other variables, for one category compared to the reference category.

In order to quantify the variability between practices in antimicrobial resistance level, a median odds ratio (mOR) was calculated using Larsen's mOR (Larsen, Petersen *et al.* 2000; Merlo, Chaix *et al.* 2006). The mOR can be interpreted as the increase in risk in the imaginary event of a patient moving from a practice with low resistance to a practice with high resistance. A mOR equal to one means no differences between practices in the probability of resistance. For the mOR a Bayesian credible interval (CrI) was calculated based on the distribution of the mOR to distinguish it from a fixed effects odds ratio confidence interval.

The multilevel logistic regression models were estimated with the Markov Chain Monte Carlo (MCMC) method using MLwiN software (version 1.2) developed by the Goldstein research group (Browne 2003; Rasbash 2003). The deviance information criterion (DIC), which combines the deviance with

information about the number of parameters in the model, was used to compare different models.

3.3 Results

Our study sample consisted of 14,181 *E.coli* isolates from 72 practices. The number of samples per practice ranged from 44 to 567 (median 175) over the 4.5 year study period. The panel size (number of GMS patients per practice) ranged from 71 to 3195 with a median of 1066 per practice.

Table 3.1 gives an overview of the characteristics of the individual and practice level variables. An overview of the final models for trimethoprim and ciprofloxacin resistant *E.coli* for individual and practice level variables is shown in table 3.2. The specific correlation for each practice (each represented by a thin line) and the overall correlation (thick red line) between prescribing and predicted resistance are shown in Figure 3.6 for trimethoprim and Figure 3.7 for ciprofloxacin.

Table 3.1: Basic characteristics of individual and practice level variables

Individual level variables (N=14,181)	% or mean	median	SD	Min-max
Male/Female	7.9% / 88.9%			
Age (years)	46.3	45	25.0	0-102
<18	12.3%			
18-<40	31.4%			
40-<60	21.5%			
60-<80	24.1%			
≥80	10.7%			
Practice level variables N = 72				
Number of samples	197	175		44-567
Panel size (patients)	1,081	1,066	660	71-3,195
Age GPs (years)	52.3	51.7	7.0	38.9-68.7
Number of GPs	1.6	1	0.9	1-5
Socio-economic status (SES) of area	6.8	8	3.3	1-10
Distance to hospital (kilometres)	25.0	21.6	25.2	0-85
Practice nurse	55.6%			
Dispensing	13.9%			
Rural allowance	20.8%			
Single handed	63.3%			
Predictor variables - Prescription				
Trimethoprim prescribing per month	4.7	4.3	3.2	0-18.1
Ciprofloxacin prescribing per month	4.6	4.1	3.4	0-17.9
Antimicrobials per year	1,037	1,058	461	4-2,412
Overall medication per year	20,604	21,869	8,976	4-44,576
Outcome variables				
Overall trimethoprim resistance	25.9%		5.0	15.0-40.0
Overall ciprofloxacin resistance	5.5%		3.5	0.8-18.6

Overview of individual and practice characteristics. All categorical variables shown as percentages (%) and continuous variables aggregated as in mean, median, standard deviation (SD), minimum and maximum value. For all the individuals in the database: gender (%male vs %female, for 3.2% gender unknown) and age distribution (mean and % of each age group). For practice characteristics: number of samples included, panel size (number of medical card patients registered with this practice), age and number of GPs in practice, area socio-economic status (according to SAHRU), distance of practice from hospital/laboratory, indication of rurality of the practice is presented as % of practices that dispense medication, receive a rural allowance and are single handed. Predictor variables: Number of prescriptions of trimethoprim, ciprofloxacin, antimicrobials and total number of medical prescriptions per month. Outcome variables: percentage practice resistance to trimethoprim and ciprofloxacin.

Table 3.2: Overview of the final multilevel models for trimethoprim and ciprofloxacin resistant *E.coli* in urinary isolates.

		Trimethoprim		Ciprofloxacin	
		OR	95% CI	OR	95% CI
Prescribing per 1000 patients per month		1.02	1.01-1.04	1.08	1.04-1.11
Gender	Male	Reference		Reference	
	Female	0.96	0.96-1.10	0.50	0.40-0.63
	Unknown	1.17	0.92-1.50	0.80	0.48-1.27
Age	<18	Reference		Reference	
	18-40	0.92	0.81-1.06	1.38	0.94-2.05
	40-60	1.04	0.91-1.20	2.14	1.47-3.18
	60-80	1.43	1.25-1.64	4.69	3.31-6.80
	≥80	2.25	1.94-2.62	11.52	3.00-16.61
Year	2004	Reference		Reference	
	2005	0.99	0.87-1.12	1.05	0.78-1.41
	2006	1.09	0.96-1.23	1.70	1.29-2.28
	2007	1.09	0.96-1.23	1.89	1.42-2.52
	2008	1.19	1.04-1.36	2.18	1.62-2.98
SES area	High	Reference		Reference	
	Low	1.05	0.94-1.17	1.04	1.01-1.08
Overall prescribing					
	low	Reference		Reference	
	medium	1.01	0.90-1.14	0.78	0.59-1.02
	high	0.82	0.71-0.96	0.77	0.51-1.14
Practice mOR		1.10	1.03-1.16	1.37	1.22-2.59

Overview of the result of the multilevel model with outcome trimethoprim and ciprofloxacin resistance respectively. Odds ratio (OR) for each predictor and confounding variable shown with 95% confidence interval (CI). If the 95% CI does not include 1, the odds ratio is significant. The reference category has an OR of 1 for the comparison of the categorical variables. For every prescription per 1000 patients per month of trimethoprim, the odds of a trimethoprim resistant *E.coli* UTI increased with 1.02. For ciprofloxacin this was 1.08. The other variables that were significant in predicting individual chance of resistant *E.coli* UTI were being male for ciprofloxacin (shown in the significant lower odds ratio for females), visiting a practice in a more affluent area for ciprofloxacin (borderline), the chance increased over the years for both antimicrobials and the odds decreased for trimethoprim when visiting a high prescribing compared to a low prescribing practice. The median Odds Ratio (mOR) showed the variation between practices and indicates the median value of the odds ratio between the practice at highest risk and the practice at lowest risk when randomly picking out two practices.

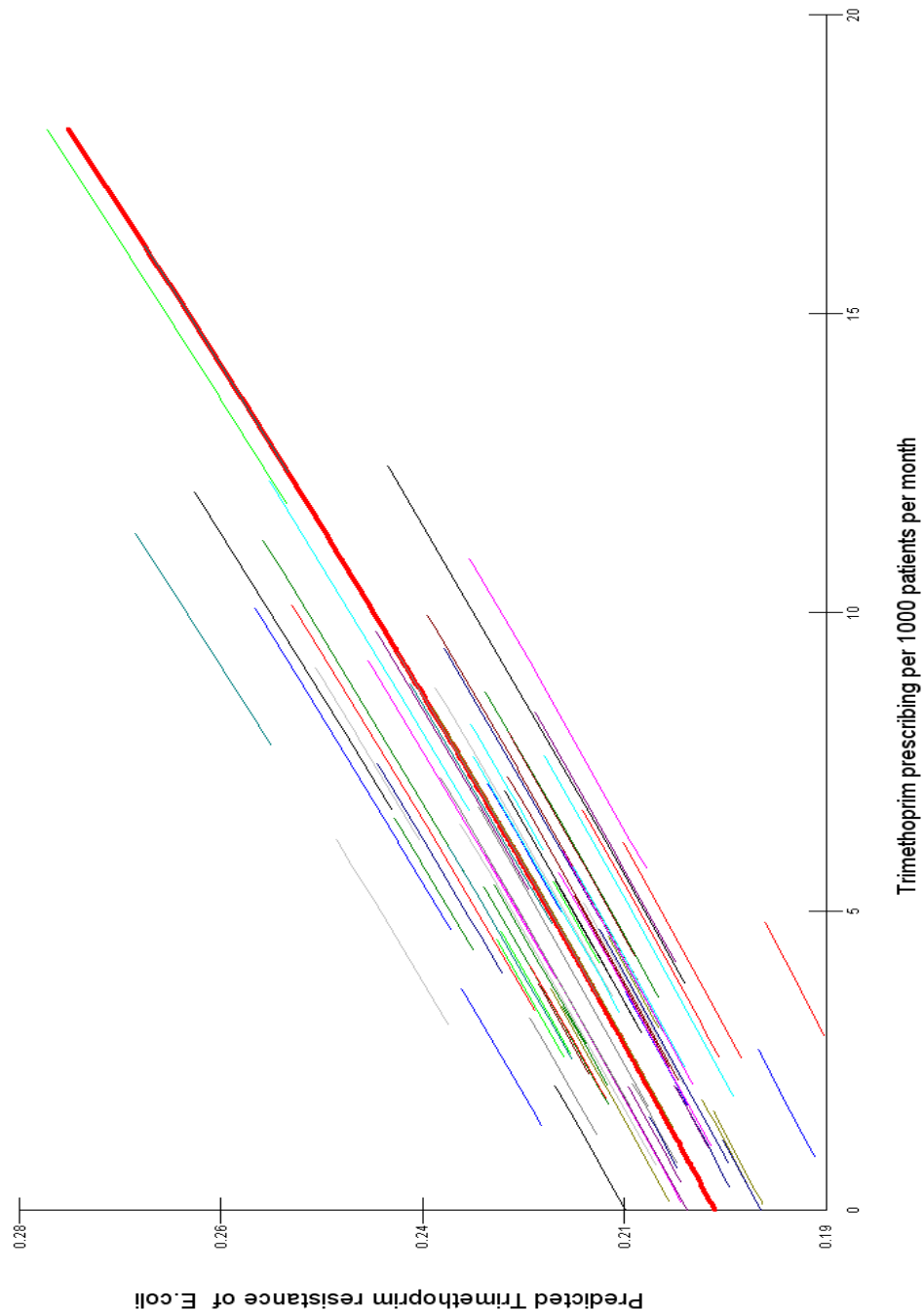


Figure 3.6: Predicted practice and overall (thick red line) correlation between trimethoprim prescribing and resistance.

The graph shows the association between the number of trimethoprim prescriptions per 1000 patients and the predicted resistance according to the multilevel model. Each line represents the range of prescribing for one practice over time. The scale of the predicted resistance is shown between 19% and 28% (0.19 and 0.28) for each practice. The overall prediction, thick red line (resulting from combining all the practice predictions), shows the increasing predicted probability of trimethoprim resistant *E.coli* UTI with increasing number of trimethoprim prescriptions per 1000 patients per month.

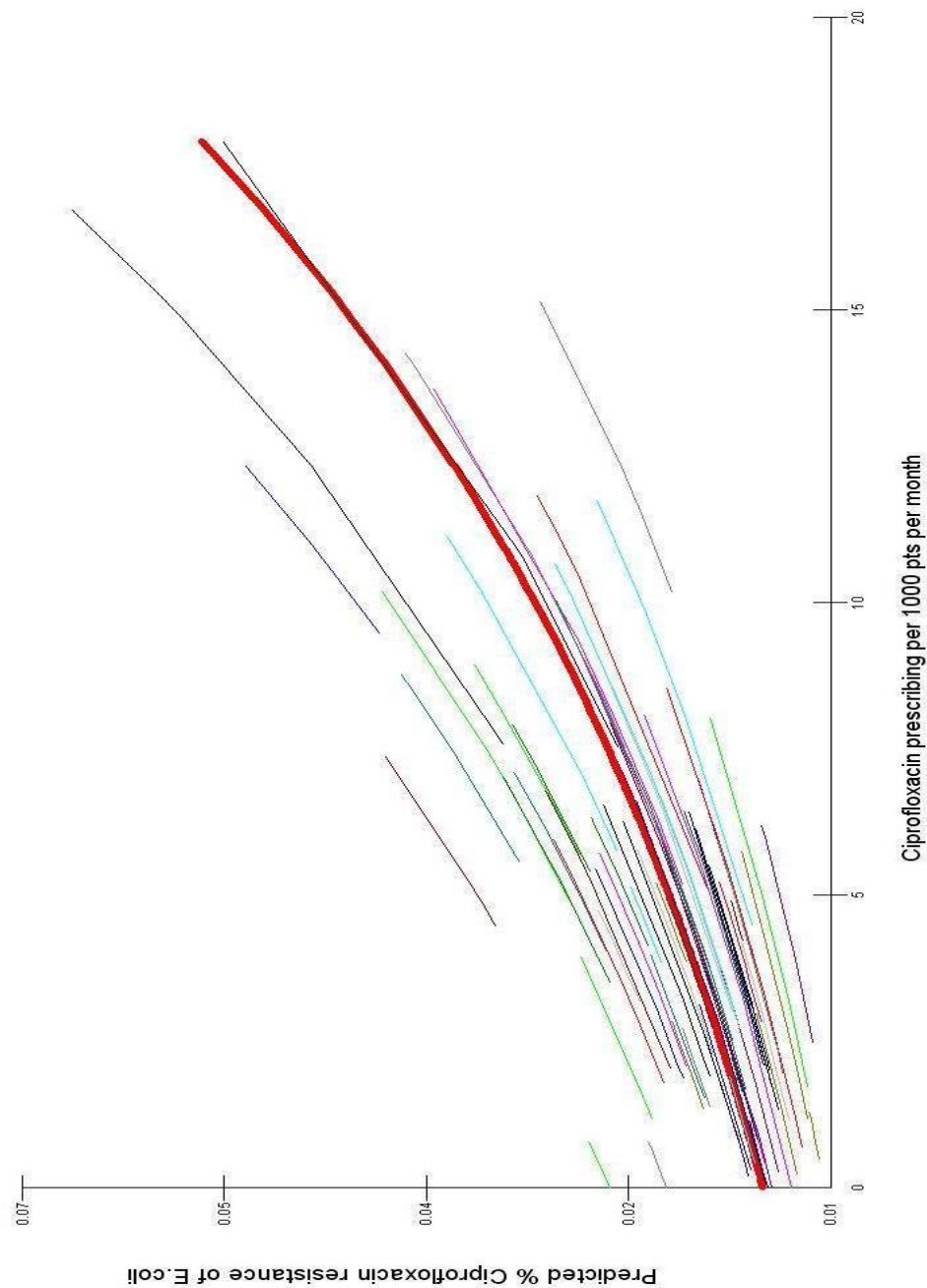


Figure 3.7: Predicted practice and overall (thick red line) correlation between ciprofloxacin prescribing and resistance.

The graph shows the association between the number of ciprofloxacin prescriptions per 1000 patients and the predicted resistance according to the multilevel model. Each line represents the range of prescribing for one practice over time. The scale of the predicted resistance is shown between 1% and 7% (0.01 and 0.07) for each practice. The overall prediction, thick red line (resulting from combining all the practice predictions), shows the increasing predicted probability of ciprofloxacin resistant *E.coli* UTI with increasing number of ciprofloxacin prescriptions per 1000 patients per month.

Trimethoprim prescribing is significantly correlated with trimethoprim resistance of *E.coli* in urinary isolates with an increase of 1.02 (95% CI 1.01-1.04) for every additional prescription of trimethoprim per 1000 patients per month (table 3.2). Increasing age increased the odds of resistance for trimethoprim, but this is only significant for ages over 60. No differences were observed between males and females within practices. No yearly changes were observed in trimethoprim resistance at practice level, except for a small increase for 2008 compared to the reference year 2004 (OR 1.19 (95% CI 1.04-1.36)). No practice level variables were found to be significantly associated with trimethoprim resistance. Overall prescribing of medication at the practice level was kept in the model but did not show a significant effect. Ciprofloxacin prescribing is significantly associated with ciprofloxacin resistance of *E.coli* in urinary isolates with an increase of 1.08 (95% CI 1.04-1.11) for every additional prescription of ciprofloxacin per 1000 patients in the practice. The odds ratio for year (cohort) showed an increase in ciprofloxacin resistance with increasing year. The odds ratio of resistance for an individual in 2008 is double the odds of 2004 (OR 2.18, 95% CI 1.62-2.98). The linear trend for year was statistically significant ($p < 0.01$, results not shown). Age was the most important individual level factor; compared to a person under 18 years old, the odds of a ciprofloxacin resistant isolate for a person over 40 doubles and increases to 11.5 (95% CI 3.0-16.6) for over 80 years of age. Females were significantly less likely to have a ciprofloxacin resistant *E.coli* infection compared to males. Socio-economic area was found to be borderline significant with lower socio economic class increasing the odds of resistant isolates.

Differences in resistance between practices were smaller for trimethoprim than for ciprofloxacin (mOR 1.10 compared to 1.43) and the credible interval for the trimethoprim model is narrower than is the case for ciprofloxacin. A caterpillar plot (Figure 3.8) showing the variance of the practice level residuals for each practice compared to the overall resistance (horizontal line) in increasing order of this difference from the left to right. The practice level

residuals are greater for ciprofloxacin, showing a higher variability in antimicrobial resistance between practices for ciprofloxacin compared to trimethoprim.

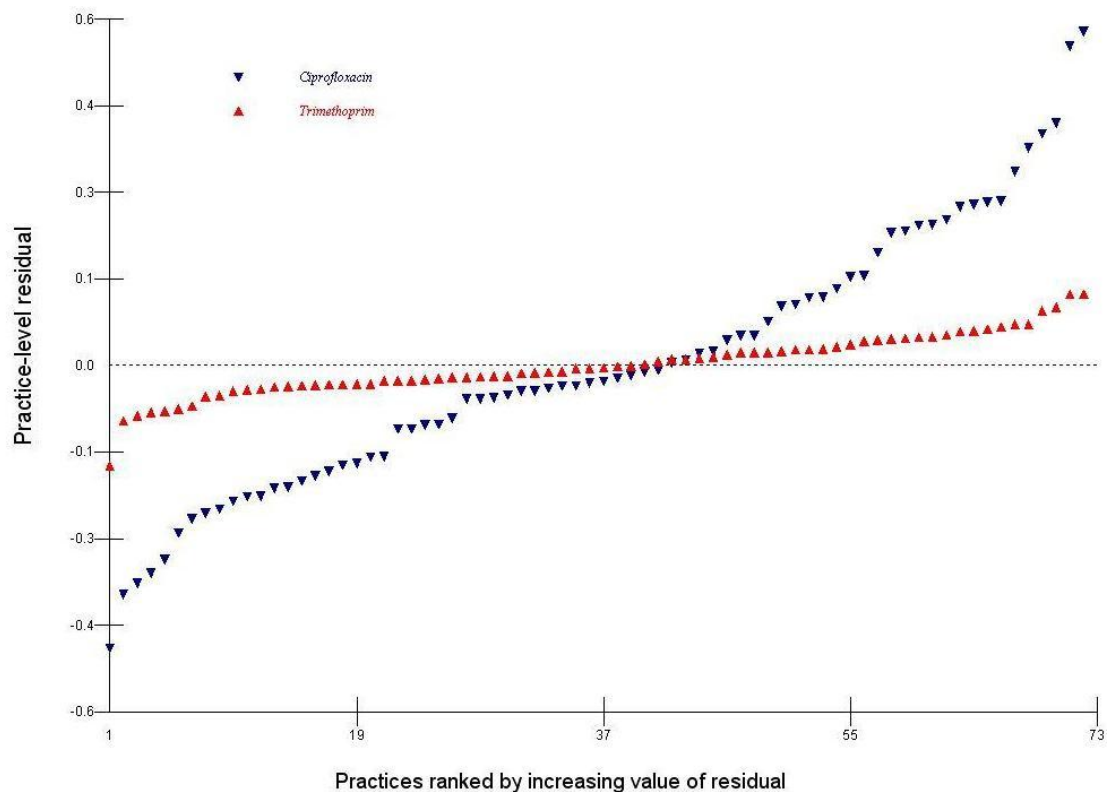


Figure 3.8: Practice level residuals for trimethoprim and ciprofloxacin resistance

Caterpillar plot of the residuals (the difference between the observed values and predicted values according to the calculated model) of each practice. The further the residual is from the horizontal line at zero, the more extreme variation in resistance levels between the practices. If there was no practice effect, all points would lie on a horizontal line. Practice residuals were ranked from low to high to produce this plot.

A prediction of the overall level of resistance was made with varying prescribing per month. For each practice the same prediction was made varying the prescribing within the practice limits. Predictions (and 95% confidence interval) were calculated based on the final models, with all variables set at their mean value and practice prescribing ranging from 0 to 20 prescriptions per 1000 patients per month.

Figure 3.9 shows the predicted probability of having a resistant *E.coli* for the 'mean' patient in the 'mean' practice for every increase in prescription of the antimicrobial agent per 1000 patients per month. The probability of a 'mean' patient having a resistant *E.coli* in a 'mean' practice with one trimethoprim prescription per 1000 patients per month was 23.9% (95% CI 22.6-25.2%), for 10 prescriptions per 1000 patients per month 27.5% (95% CI 26.0-29.2) and for 20 prescriptions per 1000 patients per month 32.0% (95% CI 27.7-36.5%). Similarly, for ciprofloxacin the probability was respectively 3% (95% CI 2.5-3.5%) for one prescription, 5.5% (95% CI 4.5-6.7%) for 10 prescription and 10.7% (95% CI 6.4-16.4%) for 20 prescriptions.

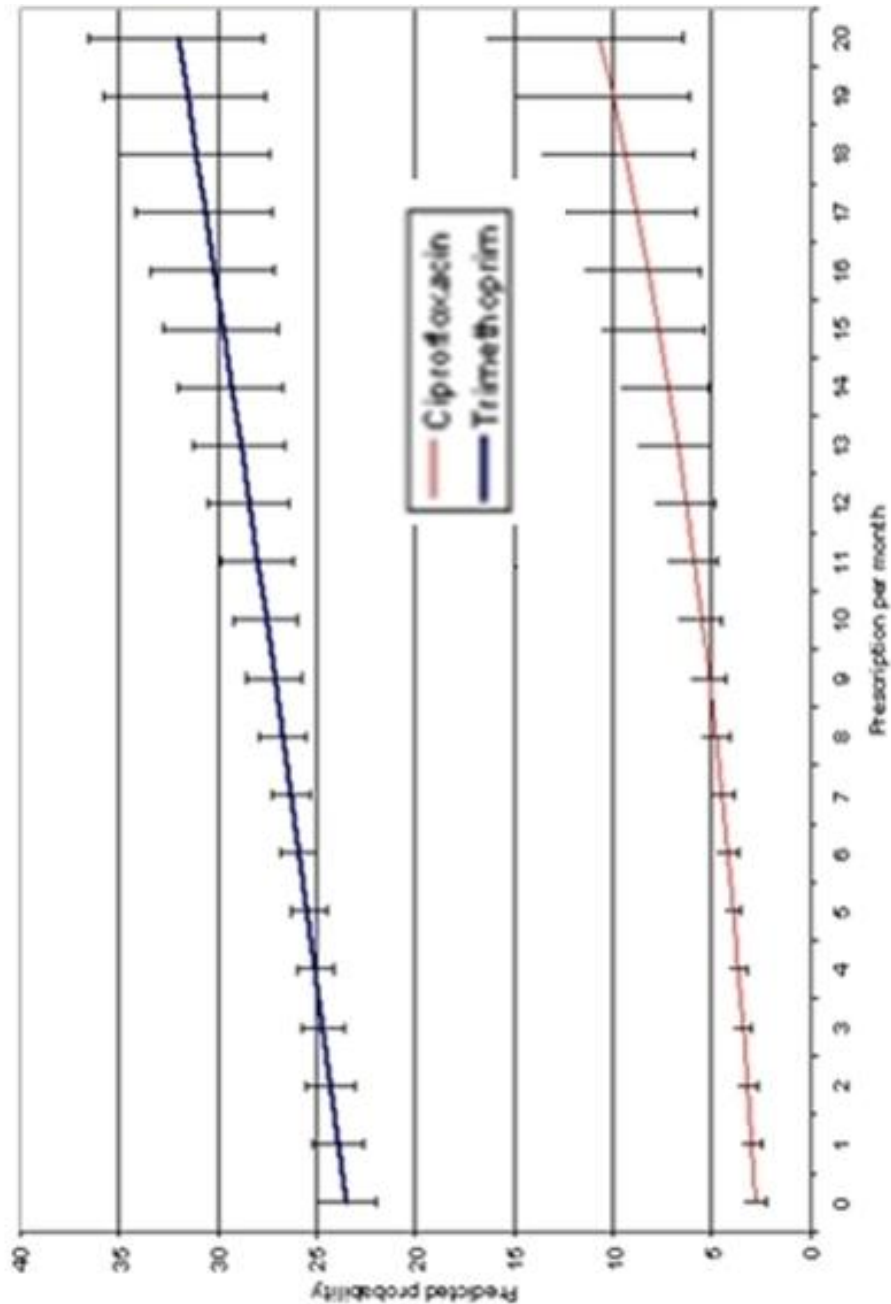


Figure 3.9: Predicted probability of resistance for every increase in prescription per 1000 patients per month (based on the final model for 'mean' patient and 'mean' practice)

The predicted probability of a resistant *E.coli* infection for every increase in the number of prescriptions per 1000 patients per month. This graph was made by calculating the probability of resistance at each increase of prescription while keeping all other variables at their mean value in the final model. A prediction interval (95% PI) was also calculated around this prediction. The prediction interval increases in size with an increasing number of prescriptions away from the observed number of prescriptions. For instance, an increase from 4 to 15 prescription of trimethoprim per 1000 patients per month in a practice increases the predicted probability from 25% to 30%. For ciprofloxacin, the predicted probability increases from 5% to 10% when increasing the number of prescriptions of ciprofloxacin per 1000 patients per month from 9 to 19.

3.4 Discussion

Our results demonstrate that increased prescribing of both trimethoprim and ciprofloxacin within a practice is associated with an increased probability of a resistant *E.coli* for the patient, independent of other risk factors. This means that a patient consulting a practice with a high antimicrobial prescribing pattern will have a higher probability of infection with antimicrobial resistant *E.coli* than a patient visiting a low prescribing practice.

The additional information obtained from the use of a multilevel logistic regression showed that the variation between practices was higher for ciprofloxacin than for trimethoprim. None of the practice level variation was explained by any of the practice variables included in the model which were mainly related to structure of care, like the presence of a practice nurse or the number of GPs associated with the practice.

The association between antimicrobial use and antimicrobial resistance in the community is well described internationally, in correlation as well as individual level studies. Goossens *et al.* reported in an international ecological study a high correlation between resistance levels and prescribing patterns in 26 European countries (Goossens, Ferech *et al.* 2005). Whereas the strength of this evidence was relatively weak due to the study design, the consistent patterns were striking. Various other studies also identified a correlation with resistance at ecological level (Magee, Pritchard *et al.* 1999; Priest, Yudkin *et al.* 2001; Gottesman, Carmeli *et al.* 2009) and with the use of multilevel studies for β -lactam/ β -lactamase antibiotics and trimethoprim (Donnan, Wei *et al.* 2004; Butler, Dunstan *et al.* 2007) in bacteriuria. Donnan's multilevel study showed an increase in odds ratio of 1.22 (95% CI 1.16-1.28) for every prescription of trimethoprim at patients level, after adjusting for practice level variables (Donnan, Wei *et al.* 2004). Butler *et al.* used a multilevel linear model in which changes in antimicrobial resistance at practice level were found to be associated with prescribing (Butler, Dunstan *et al.* 2007). Our study combined the approaches in these studies as it showed an association of

practice level prescribing and individual level resistance for trimethoprim, with the addition that this also applied to fluoroquinolone (ciprofloxacin) resistance. The opportunity to compare the predictions from the two models demonstrated that the scale of the impact was relatively more influential for ciprofloxacin compared to trimethoprim. A practice with 1 prescription per 1000 patients per month compared to one with 10 prescriptions per 1000 patients per month showed a difference in predicted resistance of 3.6% for trimethoprim and 2.5% for ciprofloxacin. For ciprofloxacin this represented an almost two-fold increase in the predicted probability of resistance.

Geographical variation between the proportions of resistance was shown for β -lactams and macrolides resistance for *S. pneumoniae* (McCormick, Whitney *et al.* 2003). Goossens *et al.* (Goossens, Ferech *et al.* 2005) supported the hypothesis that selection pressure was the main factor in the geographical variation in resistance patterns and not clonal dissemination. A paper from Garcia- Rey, however, showed a reverse association between fluoroquinolone consumption and resistance in both *S. pneumoniae* and *E.coli* between provinces (Garcia-Rey, Martin-Herrero *et al.* 2006). They performed a linear regression analysis in which an inverse relationship was found between consumption and resistance after removal of outliers. The lower geographical level (practice) used in our multilevel analysis might be the reason why our results show evidence supporting the overall hypothesis that increasing antimicrobial use in practices is associated with increased resistance in isolates of patients attending that practice.

It is biologically plausible that differences in practice prescribing of trimethoprim and ciprofloxacin have a different impact on the frequency of resistance in *E.coli* to these antimicrobials. Trimethoprim was introduced in clinical practice in 1975 and resistance levels in *E.coli* have remained stable at around 30% for some years (Chulain, Murray *et al.* 2005). Trimethoprim resistance in *E.coli* is associated with plasmid-encoded resistance determinants capable of horizontal transmission. As the plasmids may encode for resistance to one or more agents other than trimethoprim, resistance may

be selected for and maintained by antimicrobial agents other than trimethoprim. Ciprofloxacin was introduced into clinical practice more recently and resistance levels in *E.coli* were low at 2.5% in 2003 but are increasing (Emmerson and Jones 2003). Although plasmid encoded low-level ciprofloxacin resistance is well described, high level resistance to ciprofloxacin in *E.coli* is generally associated with point mutations in the chromosomal housekeeping genes *gyrA* (Strahilevitz, Jacoby *et al.* 2009). Therefore, the ‘opportunities’ for trimethoprim resistant genes to spread and maintain themselves may be greater than those of ciprofloxacin. Irrespective of how resistance emerges, the time scale of emergence of resistance under constant selective pressure was found to be much shorter than the decay time after cessation or decline in the level of prescribing (Austin, Kristinsson *et al.* 1999). Eight years after a major decrease in sulphonamide prescribing in the UK, no effect on the prevalence of resistance to this antimicrobial in *E.coli* was found (Enne, Livermore *et al.* 2001). The prevalence of sulphonamide resistant *E.coli* in this study was very high (46%) and prescribing was established. Nasrin looked at the effect of β -lactam antibiotic use in children on pneumococcal resistance (14%) to penicillin in a cohort study and showed that reduction in antimicrobial use could result in a reduction in the prevalence of resistance within six months (Nasrin, Collignon *et al.* 2002). A recent study from Sundqvist *et al.* (Sundqvist, Geli *et al.* 2010) did not show a decrease in trimethoprim resistance in *E.coli* after a 24 month intervention which decreased trimethoprim prescribing by 85%. In contrast, Gottesman showed in a retrospective ecological study an immediate effect of quinolone restriction on the susceptibility of *E.coli* in community urine cultures. The quinolone resistance level of *E.coli* decreased from 12% before to 9% during the intervention restricting ciprofloxacin. Our study may explain the differences in outcome between these interventions as it suggests that antimicrobials with less established and disseminated resistance levels, i.e. more variation in resistance levels between practices,

might be more likely to show an impact of changing prescribing and vice versa.

Our data support the message to general practitioners with respect to antimicrobial prescribing: Not only is prudent use of antimicrobial agents of general value to the community as a whole in limiting the emergence and dissemination of infection, but conservative antimicrobial prescribing is of specific benefit to patients within a practice by reducing the likelihood of infection with antimicrobial resistant bacteria. Also, interventions aimed at reducing resistance against antimicrobials should take its potential impact into account as this could be different for more or less established antimicrobials.

3.4.1 Limitations of the study

The main limitation of the study is the use of the HSE-PCRS database for the prescribing data. Ireland does not have a comprehensive national prescribing database; the HSR-PCRS database covers 70% of all primary care prescriptions. The missing 30% is largely prescribing to middle-aged and relatively affluent patients. Possible biases resulting from this exclusion are difficult to identify and for this reason our conclusions need to be interpreted with some caution. A second limitation of the study refers to the possible impact of routine laboratory sampling on the results. Overall, urine sample submission did not show any discernible changes over time in a stable population and it appears reasonable to suggest that no change in sampling behaviour has occurred. A prospective individual based study is currently being conducted in 22 practices in the West of Ireland (the laboratory results from these practices were also part of this analysis) (www.antibiotics.nuigalway.ie). The 22 practices were requested to send in urine samples of all patients with suspected UTI. Preliminary results from the first six months of this study showed no increase in the number of urine samples submitted to the laboratory, suggesting that generally all patients with suspected urinary tract infections have a sample submitted for culture.

Furthermore, the statistical modelling used in his paper takes ‘time’ and ‘number of samples’ into account as independent variables and only the variable time remained a significant factor in the model.

From these results it is expected that urine samples are routinely submitted from all suspected UTI patients and no differences in resistance levels are likely to be due to changes in submissions.

3.5 Conclusion

This multilevel analysis with patient and practice level data showed that an increase in trimethoprim and ciprofloxacin prescribing within a practice is associated with an increase in the probability of diagnosis of an uropathogenic *E.coli* resistant to these antimicrobials for the patients. Other practice variables appeared to have little impact on the prevalence of resistance. The variation between practices was higher for ciprofloxacin than for trimethoprim, which suggests that before resistance to an antimicrobial agent becomes widely disseminated in the community, variations in prescribing behaviour may have a greater impact on selection for resistance.

3.6 Publication process

The paper was initially rejected for publication. We requested a reconsideration of this decision by addressing specific issues but simultaneously took the opportunity to add some new evidence to the paper. No further comments came back and our paper was published after being resubmitted with these arguments.

Our initial abstract conclusion read '*A higher level of antimicrobial prescribing in a practice is associated with a higher probability of a resistant E.coli for the patient. The significant variation between practices was higher for ciprofloxacin than for trimethoprim. This suggests that before resistance to an antimicrobial agent becomes widely disseminated in the community, variations in prescribing behaviour have a greater impact on selection for resistance*'. The reviewers of the paper considered this too speculative and suggested taking it out. The hypothesis was that trimethoprim, being well established showed less variation between practices, while antimicrobials with less established and disseminated resistance levels (ciprofloxacin), i.e. more variation in resistance levels between practices, would be more likely to show an impact of changing prescribing. Two papers published around the same time were added to the discussion after rejection of the paper which supported our hypothesis. A paper from Sunqvist *et al.* (Sundqvist, Geli *et al.* 2010) suggested that trimethoprim restriction did not impact on resistance levels while Gottesman *et al.* (Gottesman, Carmeli *et al.* 2009) found that limiting quinolone prescribing did impact on resistance levels. The results from our study help to explain why such a difference may exist.

Shortly after the publication of our paper, a discussion paper on 'Reducing antimicrobial resistance in the community by restricting prescribing: can it be done?' was published (Enne 2010). The paper concluded that while reducing prescribing is one of the steps in controlling the emergence and spread of new resistant bacteria, it is not sufficient. It also stated that more needs to be known about the mechanisms of spread, in particular the clonal composition

of the bacterium, the genetic linkage of resistance determinants and the fitness cost of resistance. In this paper the different mechanisms and their spread were discussed in relation to the emergence of resistance against ciprofloxacin. Overall, Enne's discussion followed a similar line of thinking to that proposed in our discussion.

Chapter 4: Predictive value of antimicrobial susceptibility from previous urinary tract infection in the treatment of re-infection

This chapter was published as a paper in the British Journal of General Practice, July 2010 (Appendix 13)

4.1 Introduction

Urinary tract infection (UTI) is considered to be one of the most common bacterial infections (Hooton 2000). Approximately 5% of young adults have bacteriuria at any one time (Bishop 2004) with up to 50% of adult women reporting a UTI at some time in their life (Hooton 2000). The incidence of UTI increases with age at the rate of 1-2% per decade (Bishop 2004). Recurrence happens frequently, in 27-48% of healthy women (Hooton 2001), after spontaneous clearance as well as after antimicrobial treatment (Foxman, Gillespie *et al.* 2000; Bishop 2004). Prospective studies have shown that the vast majority of recurrent UTIs are re-infections by a previously identified strain (Hooton 2001).

Guidelines on empirical treatment of acute uncomplicated urinary tract infection suggest that agents may not be suitable for empirical use when the community prevalence of resistance to the antimicrobial in *E.coli* exceeds 10-20% (Warren, Abrutyn *et al.* 1999). Empiric antimicrobial therapy requires a balance between the need to achieve effective therapy as well as limit the use of broad spectrum antimicrobial agents. Antimicrobial susceptibility test results from previous episodes of UTI may guide the decision making process in the selection of empiric therapy in a subsequent episode of UTI, and indicate the use of antimicrobials with high community resistance levels.

There are limited data to confirm or quantify the predictive value of the antimicrobial resistance pattern of previous isolates. This analysis assesses the value of the antimicrobial susceptibility of a previously isolated *E.coli* on predicting the susceptibility of a subsequent isolate of *E.coli* from a urinary tract infection in routine clinical practice.

4.2 Methods

The laboratory of the Galway University Hospitals is the main regional laboratory for over 200,000 patients (of a total Irish population of c. 4 million) and provides a microbiology service to general practitioners in the West of Ireland as well as to the hospital.

All records from general practices of patients with more than one sample of significant bacteriuria ($>10^5$ cfu pure culture/ml), i.e. recurrent infections were extracted from the database over a 4.5 year period (April 2004-September 2008). The first isolate during this period was identified for each patient and the time to each subsequent isolate was calculated. A recurrence was defined as a re-infection if it was caused by the same species and if it occurred more than two weeks after the original UTI (Hooton 2001). Relapse isolates, defined as recurrence with the same species within two weeks of the previous sample, were excluded from this analysis (Hooton 2001). If more than one sample was given during a three month period, only the first of these samples was considered.

The positive predictive value (PPV) was calculated as the proportion of patients with an *E.coli* resistant to an antimicrobial at first isolate who remain infected with an isolate resistant to this antimicrobial at the subsequent episode. Similarly, the negative predictive value (NPV) gives the proportion of patients with an *E.coli* infection susceptible to an antimicrobial at the first isolate, who show the same susceptibility in a subsequent isolate. As the PPV and NPV are directly proportional to the prevalence of resistance in the population, a correction (Bayes theorem) is applied (Leeflang, Bossuyt *et al.* 2009) with a correction for the variability introduced by the prevalence according to Zou (Zou 2004).

The PPV is calculated as $P(\text{disease} | +\text{test}) = (\text{sensitivity} \times \text{prevalence}) / [(\text{sensitivity} \times \text{prevalence}) + ((1 - \text{specificity})(1 - \text{prevalence}))]$.

PPV and NPV and their 95% CIs were calculated using WINPEPI(Abramson 2004). The prevalence was obtained from the full database (all general practice samples over the 4.5 year period). PPV and NPV are only presented for re-infection within 3 months and between 9 and 12 months for simplicity.

4.3 Results

Over the 4.5 year period 147,306 urines were analysed; in 21.3% an organism was identified and in 14.4% an *E.coli*. A total of 3,413 patients provided at least 2 *E.coli* positive samples over the study period. The mean age of the prospective cohort was 51.7 years (SD 25.7) and median 56.0 years. The study population consisted of 90.2% females and 11.0% were under 18 years of age. No changes in age or gender were observed over the time period.

A total of 1,092 of patients had a re-infection within 3 months, 693 patients had a re-infection between 3 and 6 months after the first sample, 543 between 6 and 9 months, and 450 between 9 and 12 months. Little difference was found between age and gender when comparing the full database from the 4.5 year period to the re-infection database (table 4.1).

Table 4.1: Comparison of full and re-infection database.

	Full database	Re-infection database
N	14,495	3,413
Mean age in years	46.8	51.7
Gender		
Female	86.4%	90.2%
Male	9.1%	8.2%
Unknown	4.5%	1.6%

An overview of the number, age and gender of individuals in the full database (data from the 4.5 year period) and in the database of individuals with a re-infection over this period.

Table 4.2 gives an overview of the positive and negative predictive values of the first sample when the subsequent sample is within 3 months and between 9 and 12 months. The PPV and NPV for the periods in between (3-6 months and 6-9 months) declined gradually between these periods.

There is an 84.6% probability that a patient with an ampicillin resistant *E.coli* in a previous sample will still have an ampicillin resistant *E.coli* in a subsequent episode of bacteriuria within 3 months. PPVs are obtained for

ciprofloxacin (83.8%) and trimethoprim (78.3%). The probability of a nitrofurantoin resistant re-infection within 3 months if the previous isolate is resistant is particularly low (20.2%). The probability that a re-infection between 9 months and a year remains resistant is high at 75.6% for ampicillin and 59.5% for trimethoprim. In contrast, the probability that a re-infection within 3 months and up to a year is susceptible if the initial *E.coli* was susceptible is nearly 100% for ciprofloxacin and nitrofurantoin and 86% for trimethoprim.

Table 4.2: Overview of prevalence of resistance, positive predictive value (PPV) and negative predictive value (NPV) and 95% confidence interval (95% CI) for each antimicrobial for a re-infection within 3 months and after 9-12.

	Within 3 months					9 - 12 months			
	Resistance (%)	PPV	95% CI	NPV	95% CI	PPV	95% CI	NPV	95% CI
Co-amoxyclav	23.9	54.5	49.8-59.1	87.3	85.7-88.7	43.1	35.9-50.4	82.4	80.0-84.7
Ampicillin	60.7	84.9	82.2-87.2	77.6	74.2-80.7	75.9	71.4-80.0	60.1	54.7-65.2
Ciprofloxacin	5.7	83.8	71.7-90.7	98.3	97.8-98.7	43.4	30.1-56.9	96.8	96.0-97.5
Nitrofurantoin	2.6	20.2	12.3-31.3	98.0	97.6-98.3	5.7	1.5-26.3	97.5	97.1-97.9
Trimethoprim	26.4	78.3	73.1-82.5	91.3	89.9-92.5	59.2	51.9-66.0	86.3	83.6-88.6

The prevalence of resistance was obtained from the overall database. The positive predictive value gives the probability that a re-infection is still resistant after 3 and after 9-12 months if the previous sample was resistant. The negative predictive value gives the probability that a re-infection is still susceptible after 3 and after 9-12 months if the previous sample was susceptible.

4.3.1 Limitations

The use of routine laboratory urine samples as the basis for this analysis may influence the results due to varying request behaviour or changes in laboratory procedures. However, the number of urine samples submitted did not change over time (data available from authors). Also, the GPs follow up of patients with a laboratory confirmed UTI, as well as its potential effect on the data, is unknown. More in-depth research into patients presenting with another positive *E.coli* UTI, in particular within 3 months, would be of interest to further improve prescribing practice. An ongoing prospective study will address these concerns (www.antibiotics.nuigalway.ie).

4.4 Conclusion

These results may help general practitioners to conserve broad-spectrum agents by using antimicrobial test results from previous episodes of UTI to prescribe more narrow-spectrum agents such as trimethoprim even when community resistance levels are high. The high positive predictive value of previous ampicillin, trimethoprim and ciprofloxacin resistance warrants against the re-prescription of these agents within three months while the high negative predictive values indicates prescription of these antimicrobials if susceptibility for these antimicrobials was shown in a previous sample of the patient. The low prevalence of resistance and high negative predictive value of nitrofurantoin at both three and twelve months promotes nitrofurantoin as a beneficial first line agent for initial and repeat presentations.

4.5 After publication

The publication of this paper in the British Journal of General Practice gave way to some other initiatives.

- A newsletter (Appendix 15) to the practices participating in the prospective study, which was ongoing at that time, included a section on this analysis as well as guidelines on the prescription of nitrofurantoin. This newsletter was sent at the end of data collection and can be viewed as an intervention as it suggested guidelines for first line prescribing of antimicrobials for UTI in general practice.
- An editorial was written (Hay 2010) in which the role of mid-stream urines (MSUs) in clinical practice were described. Hay pointed out that routine MSUs are beneficial to the society for the surveillance of antimicrobial resistance in UTI. The benefit for the patient was that previous results can guide subsequent treatment and to identify patients with symptoms but without a UTI. He added that understanding the individual circumstances of the patient to determine the best treatment for them is facilitated by a urine culture, a clinical point of view.
- The paper on the predictive value of antimicrobial susceptibility from previous urinary tract infection in the treatment of re-infection, has been shortlisted by the Royal College of General Practitioners for research paper of the Year award 2010. Each year, the College selects a winning paper and on some occasions, a highly commended paper, to give recognition to a group of researchers, or an individual researcher, who have/has undertaken and published an exceptional piece of research relating to general practice or primary care. The results will be known after the panel decision meeting in late April 2011.
- The British Medical Journal invited Prof. Martin Cormican to write an article on '*Asymptomatic bacteriuria (or pyuria)*' for their 'Rational testing series', a series of short articles to update GPs and hospital doctors on the best use of laboratory tests. The requested format was not that of a full

reviews but a succinct yet evidence-based encapsulation of the initial diagnostic steps only. Martin Cormican invited Prof Andrew W Murphy and Akke Vellinga to be co-authors. The manuscript is accepted for publication (May 2011).

Chapter 5: Prospective study of antimicrobial prescribing and resistance in general practice: study design and methodology

5.1 Introduction

The association between antimicrobial prescribing and resistance should not be analysed independently for individuals and for populations/practices as this will not give a complete picture. The ‘event’ of having a UTI caused by a resistant *E.coli* depends on the number of other individuals in the group already affected, or in other words, incidence depends on the prevalence, a characteristic of many infectious diseases (Halloran and Struchiner 1991; Diez Roux and Aiello 2005). For the study of antimicrobial resistance this means that not only individual prescribing has an impact on the chance of a resistant *E.coli* but also the occurrence of resistance in the group wherein the individual lives. The retrospective analysis (*Chapter 3*) showed the importance of practice level factors in the probability of a UTI with a resistant *E.coli*. The multilevel model applied accounted for dependencies between outcomes for individuals within groups.

The limitations of the multilevel analysis in the retrospective part of the study (no individual prescribing information, temporality of prescribing and UTI, biological gradient) can be addressed in a prospective study. Additionally, as data will be analysed within a multilevel model, the relative impact of individual and group level factors, can be further explored.

5.2 Ethical approval process

5.2.1 Introduction

Health services research is particularly vulnerable to the current ethical climate which prioritises explicit individual consent above all other goods (Cassell and Young 2002). This explicit individual consent often results in non-representative study populations due to response bias, also known as consent, authorisation or volunteer bias; a systematic error in creating patient groups that differ with respect to study outcome (Junghans and Jones 2007).

However, if equality of access to quality health and social services is aimed for, this should be reflected in its research (Cassell and Young 2002) and community consent should be considered.

The idea of informed consent is the fundamental principle to provide options for people and helping them to make the optimum choice in the light of their circumstances, needs and values. If it can be assumed that most people would probably be happy to take part in a study, then an opt-out arrangement is the most efficient method (for participants and researchers) and does not undermine the principle of providing choice (Clark, Jamieson *et al.* 2004; Hewison and Haines 2006; Singleton and Wadsworth 2006).

In the set-up of the prospective study it was envisaged that an opt-out methodology would be most appropriate and efficient for patient recruitment.

5.2.2 Approval

Ethical approval for the study was sought from the Irish College of General Practitioners. The initial decision from the committee rejected the request for an opt-out arrangement (Appendix 1). The committee suggested 'should there be a pilot study to prove that response was affected by the opt-in method, the decision of the Committee could be reconsidered'. This decision was challenged by the research team.

- A letter was written outlining the concerns of the research team (Appendix 2).

- A literature overview of the context of opt-out methodology to highlight its issues and consequences was performed (Appendix 3). This review highlighted that in order to adhere to the patients' right to information to obtain full consent as well as respect of their personal choice while still serving the community, an opt-out strategy can be appropriate. The principle strategy should be to take all reasonable measures to inform those whose personal data were requested and make opt-out as easy as possible. This strategy aims to fulfil the ethical imperative of informed consent as well as to optimise participation.
- As part of a focus group study (Inform study) performed by Dr. Brian Buckley on patients' attitudes (Buckley, Murphy *et al.* 2011), a scenario on the use of identifiable data to contact patients with urinary tract infections was used (Appendix 4). The participants were overall happy to consider the UTI scenario and would hypothetically not have any difficulty their information being passed on to researchers in the light of the 'greater good' effect. The participants additionally emphasised the importance of assurances around confidentiality and security of data.

The approval of the opt-out method of recruitment was received on the 19 of February 2009 (Appendix 5).

5.2.3 Addressing issues

A number of issues arose from the literature (Singleton and Wadsworth 2006) and focus group concerning the use of opt-out in research. A point by point response to these issues is given below.

1. Information to patients

- ✓ A letter was drafted informing the patient of the study (Appendix 7). This letter included information on the aims of the work and intended benefits of the research for health and health care in general. The letter contained information on how their details were obtained, the type of information needed from the patient, how the researcher would

access this information, where to get more information and how to opt-out.

- ✓ Notices were placed in the waiting rooms of all participating practices (Appendix 8). These notices described the proposed study with particular emphasis on the ethical aspects of the opt-out recruitment strategy. Contact details for the lead researcher, were provided
- ✓ A website was set up with supporting and background information to optimise the concept of informed consent (Appendix 9). The website also allowed for feedback of the results of the study by regular updates on published work.
- ✓ A feedback 'button', contact names and telephone numbers were all available on the website. Opt-out was possible by phone call, attached letter and freepost envelope or a special opt-out 'button' on the home page (index page).

2. Data confidentiality and security

- a) A secure folder on the university secure server was set up to transfer patient data from the laboratory. The folder was only visible to named researchers of the project and password protected. Additionally, all data on the server were encrypted with additional passwords with restricted use.
- b) For data collection (patient information) a number of additional security measures were set up to keep personal and collected information separate.
 - a. A data entry programme was written (Epidata (Lauritsen 2000-2008)) using the sample number as identifier
 - b. A notebook computer was acquired only containing the data entry programme using the sample number.
 - c. Data collected at each practice visit were transferred to the secure server immediately after collection in the practice using remote access by mobile broadband connection to the secure server.

- d. A list of patients was printed before each visit and confidentially destroyed immediately after data collection.
- e. All paper with individual patient details were destroyed by a confidential shredding company
- c) Laptop computer used for linking the databases and the notebook computer for data collection were kept locked in a filing cabinet in the alarmed offices of the discipline of general practice.
- d) The patient information file and the file with personal information were at all times encrypted with different passwords and only available on the secure server.
- e) After data collection the patient information and microbial data were linked using the sample number and personal information. The final data file was encrypted and stripped of all personal information.
- f) All data on the server, except the final encoded datafile, were shredded and deleted to secure indefinite deletion.
- g) Data encryption was done using AxCrypt (Seleborg). Coding of the personal information was done with an irreversible encryption add-in for Excel (Buchan 2006).

3. Researcher's confidentiality

The researcher, who conducted the study, collected and linked the data, signed confidentiality agreements with each practice. For the duration of the study the researcher became part of the GP practice and adhered to the normal procedures governing patient confidentiality (Appendix 10). An additional similar agreement was set up with the laboratory of the UHG (signed by Prof Martin Cormican) by which the researcher became part of their staff and agreed to normal confidentiality procedures (Appendix 11).

5.2.4 Opt out as an acceptable method of obtaining consent in medical research: results and conclusions

This part was published as a short paper in BMC Health Research Methodology, April 2011 (Appendix 14)

Results

A total of 1362 urine samples were submitted by the 22 participating practices during the study period. The samples were from 1178 adult patients. The 22 practices sent in between 15 and 115 samples.

In total 193 patients actively responded to the letter: 142 opted out by letter, 15 through the website, 2 by phone and 12 sent the letter back without indication, making a total of 171 patients (14.5%) who opted out; the remaining 22 patients (1.9%) explicitly opted in (Figure 1). The letters of 24 patients had a wrong address and were returned.

Two patients expressed concerns regarding the use of the opt-out method. Both questioned the way their address was obtained and whether this interfered with the confidentiality of their patient data. An individual response to these concerns was sent to their GP with a request to forward this to the patient. No further concerns were expressed.

Patients consisted of 941 women (79.9%) and 237 men (20.1%). Their mean age was 50.9 years (SD 20.8) and the median age was 47 years. Patients who opted out were slightly older (52.8 vs 50.4 years) and the percentage of females was slightly higher (83% vs 79.5%) but these differences were not statistically significant. Patients who opted out through the website were significantly younger than those who used the letter (non-parametric, 53.5 vs 38.7 years, $p < 0.05$).

A significant isolate (pure culture at greater than 10^5 cfu/ml) was identified from the urine sample of 402 (34.1%) patients. Patients with a positive culture were no more likely to opt out compared to those with a negative culture.

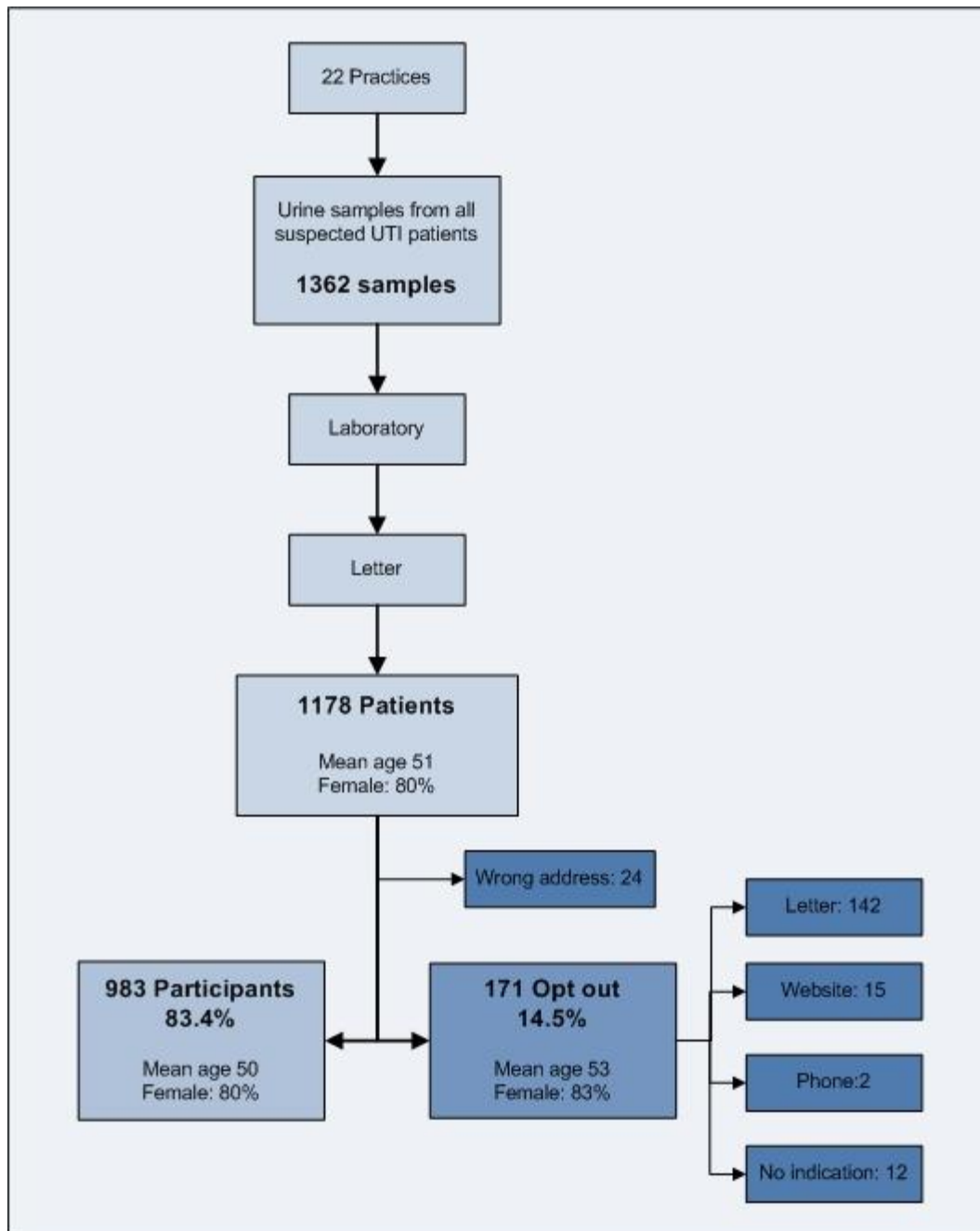


Figure 5.1: Flow chart of participation of patients in the study.

Flow chart of enrolment of patients from the 22 participating practices in the prospective study. A total of 1178 patients were sent a letter explaining the study and opt-out. Opt-out was facilitated by an enclosed opt-out letter and free post envelope, by phone, by logging onto the website and clicking the opt-out button. 12 'opt-out' letters were returned without an indication of opt-out.

Conclusions

Overall the opt-out method was well received by both general practitioner and patients and achieved a high level of participation in the study at 83.4%. The low number of complaints indicates that this is a generally acceptable method of patient recruitment. The 14.5% opt-out of patients shows that the process effectively empowered patients to decline participation. The high similarity between patients opting out and the participating patients with respect to age, gender and isolation of a positive culture is reassuring for extrapolation of the results of the study. However, as no other potentially important variables were available about the patients who opted out, it cannot be ruled out that other factors were of importance for participation in the study. Similarly, even though every effort was made to inform patients of the study, it cannot be guaranteed that all patients received this information through the different media offered by us.

Our findings are in line with other studies which have shown that an opt-out methodology is generally well accepted and will result in high participation rates (Inskip, Godfrey *et al.* 2006; Junghans and Jones 2007; Nathan, Thacker *et al.* 2008). A recent Cochrane review looked at ways to increase recruitment into clinical studies and also identified opt-out as a possible method (Treweek, Pitkethly *et al.* 2010). The lack of further involvement in the study by participants and general practitioners, acknowledged in the ethical approval given to the study, favours this type of recruitment which might be less applicable for studies with more involvement or risk. For non-interventional, low-risk studies in which rigorous measures to inform patients and protect patient confidentiality are in place, recruitment by opt-out is an easy and acceptable methodology for patients, GPs and researchers. As earlier stated by Junghans *et al.* (Junghans, Feder *et al.* 2005), the opt-out approach should be the default recruitment strategy for studies with low risk to participants.

5.2.5 Additional considerations

- One associated GP expressed concerns at the researcher's visit to the practice. Reassurance was obtained after additional explanations concerning the precautions taken to safeguard patient confidentiality.
- Two patients did express serious concerns about the method by which their results and address was obtained. They were unaware of the procedures by which urine samples were analysed and results were reported. A personal letter giving additional information about confidentiality and addressing the particular issues was sent to the GP practice. A telephone call to the GP explaining the concerns was made with the request to forward the letter to their patient. No further feedback was obtained from these patients.
- Blinding of the research was established by not linking the databases until all data were collected. The sequence of antimicrobial prescribing and then urine samples was chosen in order to eliminate a possible bias due to knowing the organism and/or resistance pattern. Similarly, the sheet with the patient name and date of birth did not contain information on the urine sample result.
- The visit to the GP practice attempted to make as little contact with the GP as courteously possible in order not to affect the GPs prescribing.

5.3 Sample size

The aim of the sample size calculation was to ensure the case-control study (second part) would have sufficient power to establish accurate estimates of the odds ratio of resistance after previous prescribing. The initial calculations were based on trimethoprim prescribing and resistance.

With 80% power, a two- sided significance of 5%, the sample size was calculated (Dupont and Plummer 1990) based on odds ratios from the literature of which 1.32 was the most conservative estimate (Steinke and Davey 2001). This generated an estimate of 688 cases for an odds ratio of 1.32 with two controls per case assuming that 30% of the controls would have had trimethoprim previously prescribed. Alternatively, if a difference between exposure in cases versus controls of 10 percentage points was considered important (30% exposure in cases and 20% in controls), with a total of 200 cases, we would be able to detect an odds ratio of 2 with 80% power, 5% two sided α and with two controls per case. Considering an inter-cluster coefficient of 1%, a design effect of 1.29 for a mean cluster size of 30 was calculated (Eldridge, Ashby *et al.* 2006). Based on these scenarios we aimed to include 250 cases.

A list was compiled based on the 2007 laboratory urine sample submissions, ranking the general practices by the number of urines submitted and the number of positive isolates. The mean number of *E.coli*'s submitted in a practice per month was calculated. To maximise the efficiency of the study, the top 25 practices, with at least a mean of five cases per month, were requested to participate.

5.4 Recruitment of the practices

The selected practices were invited to participate by a letter explaining the study (Appendix 6) and a follow-up phone call was made within two weeks. A further explanation of the study was given over the phone and 22 out of the 25 approached practices agreed to participate. Two practices did not have computerised records and it was considered too time-consuming to include these. One practice declined participation on the basis that they never participated in research studies.

The start of the study was set at 14th of September and ran until the 9th of November 2009 (first part of the study), after which only patients with a positive sample were invited to participate (second part of the study). Data collection ceased on the 30th of May 2010.

A first practice visit was set up four weeks after the start and all practices had a visit from the researcher within eight weeks from the start of the study. A phone call was made to set a time and a date for the researcher to have access to a practice computer. An agent nomination form was signed by a GP and a confidentiality statement was signed in the presence of a practice employee. Patients were identified in the patient management system and data on prescribing were recorded in a computerised data entry programme. At the end of each visit the recorded data were transferred to the secure server of the university.

Follow-up visits were organised once patient numbers were high enough. Each practice received a newsletter with results from the study and a practice profile of antimicrobial resistance (Appendix 15), as well as a nominal fee for participation.

5.5 Enrolment of the patients

A weekly search of the laboratory system of the GUH was done, identifying patients from the participating practices for whom a urine sample was sent in. Patients' first name, last name and address were extracted. A mail merge was set up to personalise the letter sent to each patient and print out an address label (Appendix 7).

5.6 Patients' medical records search

The following information was recorded for each patient:

1. ID number (lab) urine sample
2. Date of GP visit
3. GMS (medical card or GP visit card holder: yes/no)
4. Eligible
 - a. Reason for non-eligible:
 - i. Pregnancy
 - ii. Catheter
 - iii. Other
5. Number of visits in previous year (more or less than 10)
6. Antimicrobial prescribed (going back one year), up to 14
 - a. Group of antimicrobial
 - i. Penicillins
 - ii. β -lactam/ β -lactamase combinations
 - iii. Trimethoprim/Sulfamethoxazole
 - iv. Quinolones
 - v. Cephalosporins
 - vi. Tetracycline
 - vii. Nitrofurantoin
 - viii. Macrolides
 - ix. Other
 - x. Missing/unknown
 - b. Date of prescription
 - c. Prescription for UTI: yes/no
7. MSUs (including this one), up to 10
 - a. Date of submission of sample to lab
 - b. Laboratory number
8. Other conditions

List of possible other conditions based on notes and prescriptions

- a. Diabetes: according to notes or prescription of medication
 - b. High blood pressure/Cardiovascular disease: according to notes or medication
 - c. Medication for depression, insomnia, anxiety, etc.
 - d. Epilepsy; according to notes or medication
 - e. Thyroid: according to notes or medication
 - f. Prostate problems: according to notes
 - g. Inhalers: according to prescription
 - h. Other conditions not mentioned above for which regular visits would be necessary
9. Hospital visits (yes/no) in the past year
10. Hormonal therapy (yes/no): hormone replacement therapy or contraception

5.7 Microbiological analysis

Microbiological analysis was performed at the Department of Medical Microbiology (GUH) which is an accredited diagnostic laboratory (ISO 15189). All urine samples from GP practices are sent to the laboratory where diagnosis of UTI by microscopy and semi quantitative culture of a urine sample is performed. Specimens with less than 20 white blood cells/ μl were considered negative and were not processed further. Pyuria was defined as greater than 20 white blood cells/ μl and 10^5 cfu/ml of causative organisms was considered a pure or predominant growth. Antimicrobial susceptibility testing was performed on isolates to guide selection of an appropriate antimicrobial to treat the infection. Protocols for the performance and interpretation of these results are based on the guidelines from the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute 2010).

5.8 Data preparation

Univariate analysis was performed at individual as well as practice level. For each patient the first sample during the study period was used as the index visit. The antimicrobial therapy prescribed in a period of seven days around and closest to the date of the sample was identified as the medication given for this episode.

Chapter 6: Management of urinary tract infection in general practice

6.1 Introduction

Despite its frequency of diagnosis, the management and treatment of this condition is often inconsistent in clinical practice (Hummers-Pradier and Kochen 2002; Mehnert-Kay 2005). During the first two months of the prospective study, all samples from patients with suspected UTI were included in the study. The files of all participating patients were reviewed in the general practice and information on prescribing was recorded. The aim of our study was to describe current management and treatment of UTI in general practice. An overview of the variation in treatment practice between GP practices was carried out. The appropriateness of the antimicrobial treatment was evaluated and different empiric prescription scenarios were compared. Additionally the impact of socio-economic status, measured by free medical care, on treatment was assessed.

Treatment of UTI in daily practice is largely empirically based and diagnosis of UTI generally requires clinical evaluation in addition to laboratory results. Empiric treatment recommendation for first line antimicrobial treatment of simple uncomplicated UTI is trimethoprim or nitrofurantoin (Strategy for the control of Antimicrobial Resistance in Ireland (SARI) 2008; Zalmanovici Trestioreanu, Green *et al.* 2010; Gupta, Hooton *et al.* 2011).

6.2 Comparison of the participating practices

The 22 participating practices were selected based on the number of urine samples and positive samples in 2007. At that time these practices sent in the most samples, but circumstances in some practices changed which changed their relative rank in 2009. A comparison of the patients (with suspected UTI) from the participating practices and all practices in 2009 is shown in table 6.1. The overall mean or percentage shown is based on the practice mean or percentage. The differences between the participating practices and the other practices are due to the low number of (positive *E.coli*) samples from some practices.

For this comparison, only the first sample per patient in 2009 was taken into account. The percentage female, age, percentage *E.coli* positive samples and percentage resistance for each antimicrobial showed no significant differences between participating and non-participating practices (t-test, comparing means). As the selection of practices was done after ranking the practices according to the number of samples sent in for analysis, participating practices will have a significantly higher number of samples compared to all other practices.

Table 6.1: Comparison of characteristics of patients for whom a urine sample was sent in (first part) and practice percentage resistance (second part) from participating practices and non-participating practices.

	Participating practices (N=22)		Other Practices (N=166)	
	Mean	SD	Mean	SD
Age	44.0	±7.1	50.9	±20.3
Percentage female	84.4	±4.5	79.0	±28.5
Number of samples	125.3	±59.5	27.7	±38.0
Percentage <i>E.coli</i>	43.3	±7.3	37.9	±29.3

Percentage resistance of <i>E.coli</i> positive urine samples only				
	Mean	SD	Mean	SD
AMP	63.6	±9.4	62.3	±28.3
AMC	17.5	±4.7	21.6	±25.2
CIP	6.6	±5.0	8.6	±14.0
TRI	31.0	±9.9	30.3	±26.1
NIT	2.2	±3.0	2.1	±5.7
CEF	2.9	±1.8	3.2	±8.9

An overview of gender and age distribution as well as the total number of samples submitted by the practices and the percentage of these samples with an *E.coli* isolate for the participating practices and the non-participating practices for the year 2009 (only the first sample of each individual was included). Similarly, resistance of the *E.coli* isolates for the participating and non-participating practices is shown. Differences were compared with a t-test and no difference was found to be statistically significant (except for the number of samples submitted as the selection of practices was based on the rank of the number of samples).

An overview of the resistance levels in the participating practices is shown in Figure 6.1. The scale of the percentage resistance for the top three graphs runs from 0-100%, while the bottom three are scaled at 0-50%. This comparison was made for the *E.coli* samples only, and only the first positive *E.coli* sample was included.

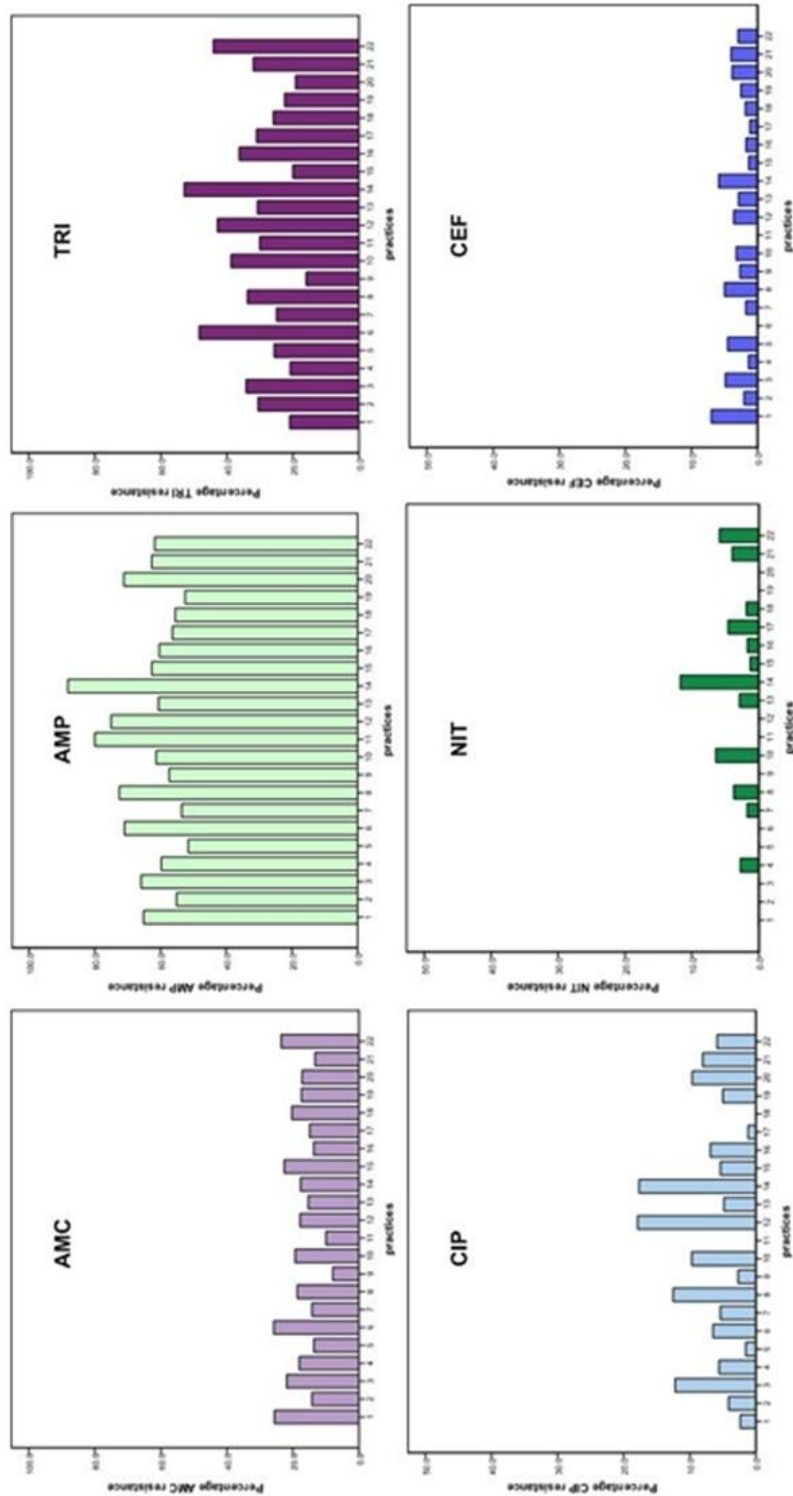


Figure 6.1: Overview of the resistance levels (%) for each antimicrobial for the participating practices (N=22) in the year prior to the study period.

The resistance levels for co-amoxycylav, ampicillin, trimethoprim, ciprofloxacin, nitrofurantoin and cefpodoxime for each practice (N=22, x-axis) of *E.coli* samples only for the year prior to the study period. The top three graphs are scaled (Y-axis) from 0-100% resistance, the bottom three graphs are scaled from 0-50% resistance.

To compare the participating practices a similar overview of graphs was made in which the percentage resistance in the participating practices is shown for the study period (with the exclusion of opt-out and non eligible subjects) and the year prior to the study period (Figure 6.2). The differences between the data are mainly due to the exclusion of pregnant women (whose *E.coli* are less likely to be resistant) and the exclusion of catheter patients (whose *E.coli* are more likely to be resistant). Additionally, one practice in particular, practice 12, showed peaks in resistance levels during the study period. This was most likely due to a transition period during which different locum GPs helped out in this practice during a period of about a year previous to and overlapping for a time with the study.

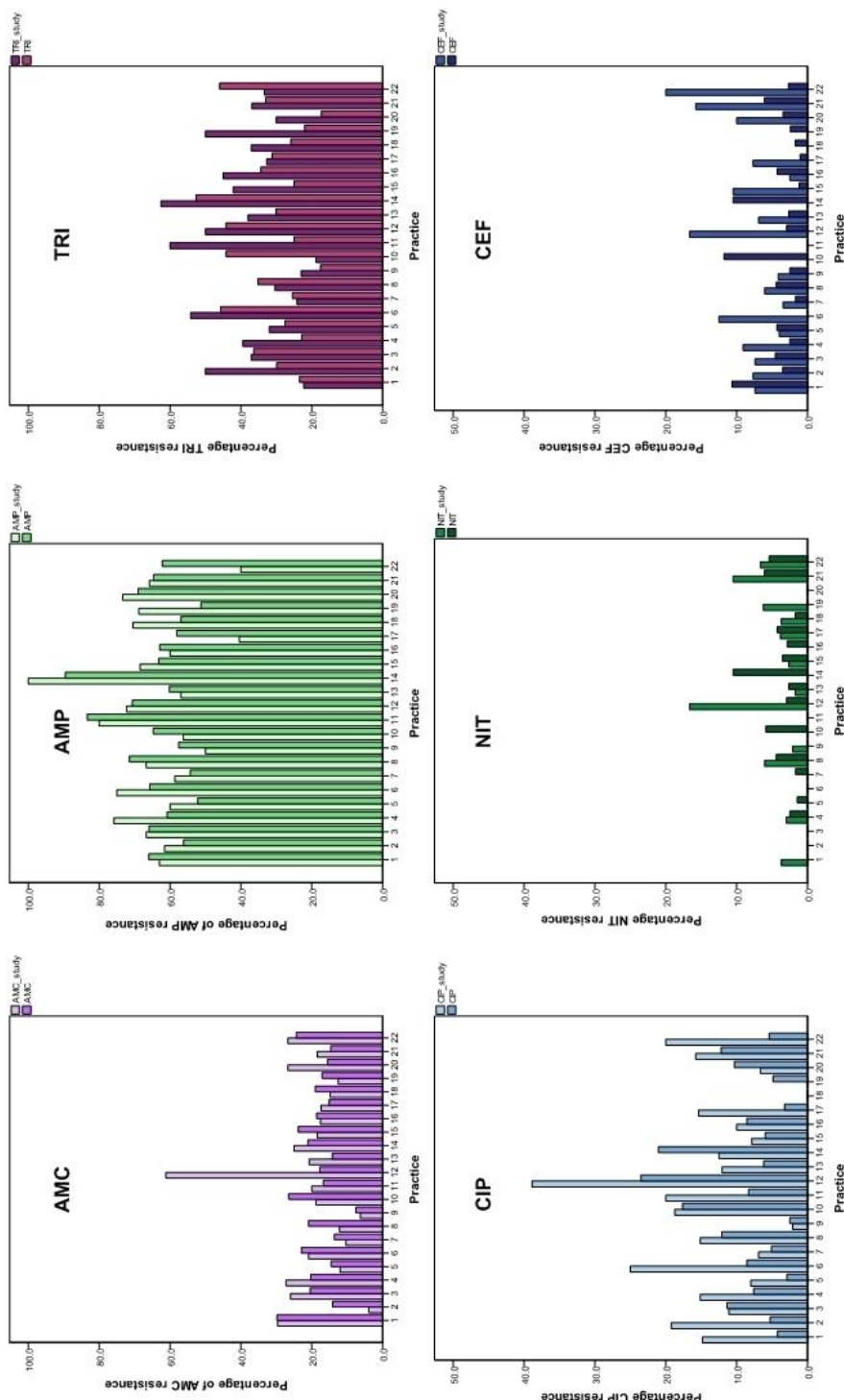


Figure 6.2: Comparison of percentage resistance in the participating practices during the study and in the year previous.

Comparison of the resistance levels during the study period with the year previous to the start of the study. The top three graphs are scaled (Y-axis) from 0-100% resistance, the bottom three graphs are scaled from 0-50% resistance. For instance, practice 12 shows peaks for co-amoxycylav, ciprofloxacin and nitrofurantoin during the study (which were mainly due to a change of GP during the study period).

6.3 Overview of the study sample

A total of 1362 urine samples were submitted from 1178 patients of whom 145 (12.3%) opted out of the study, 152 (12.9%) patients did not meet the eligibility criteria (pregnant or catheter) and the letter of 15 patients was returned because of a wrong address or unknown name*. Of the 866 eligible patients the mean age was 52.4 years (95% CI 51.0-53.8, ranging from 18-100) and 77.9% were females. Women were generally younger (50.7 years, SD 20.7 compared to males 58.3 years, SD 19.7, t-test p-value <0.01). A flow chart of the study sample is shown in Figure 6.3.

* The difference in numbers here from those reported in the opt-out paper resulted from the inclusion of additional patients after resending the returned letters to corrected addresses, or a patients contacted for a subsequent sample returned an 'opt-out' letter with an indication of willingness to participate (N=11).

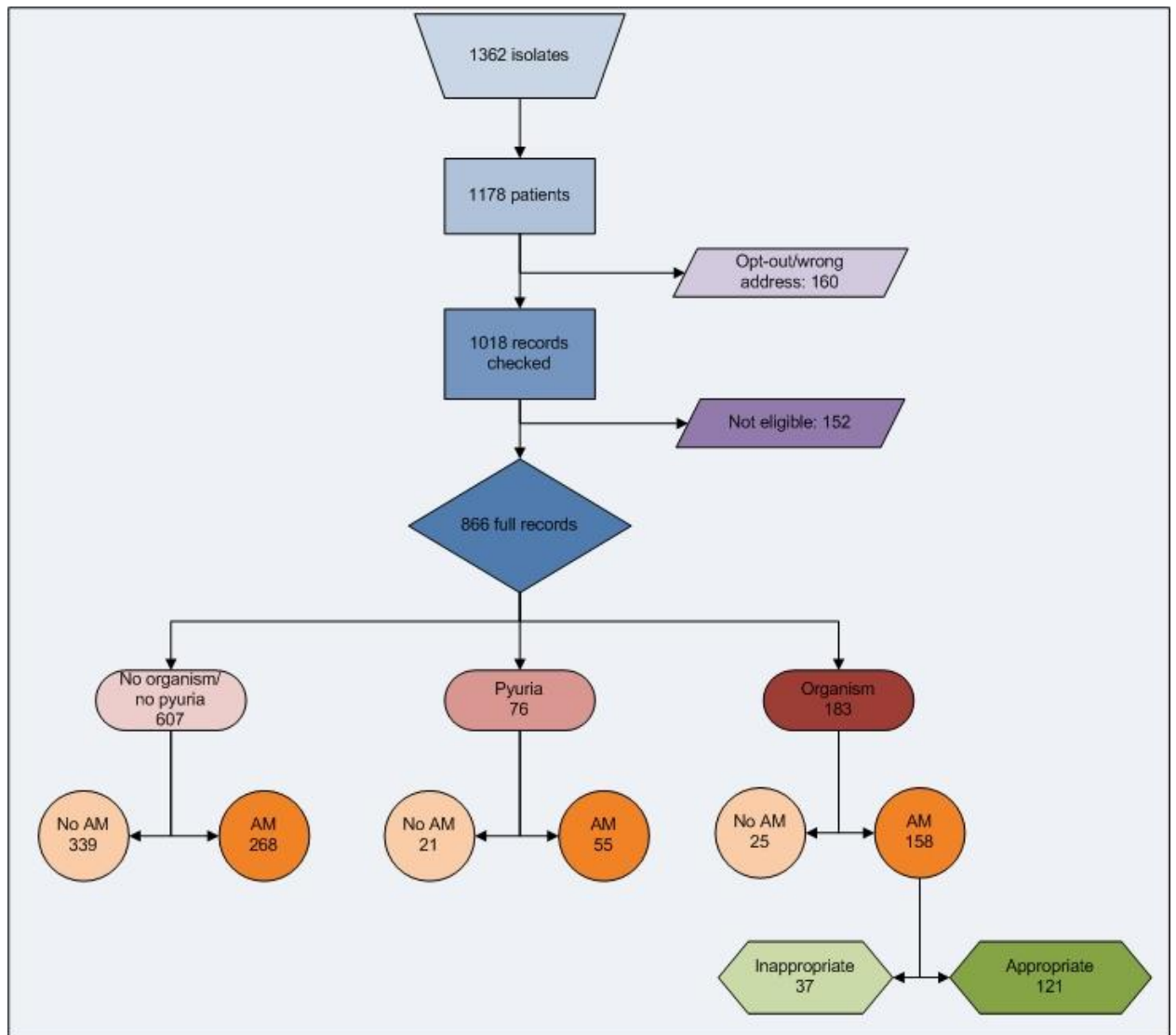


Figure 6.3: Flow chart of study population, management of UTI in general practice.

Flow chart of the study population from the 22 participating practices in the prospective study. A total of 1178 patients were sent a letter explaining the study and opt-out. The charts of 1018 of these patients were reviewed in the practice. The chart information was merged with the outcome of the microbiological analysis of the urine samples. Antimicrobial prescribing was assessed for its appropriateness according to the outcome of the microbiological analysis and antimicrobial susceptibility testing. AM: antimicrobial treatment.

For 183 of the 866 patients an organism was identified (21%), mainly *E.coli* (147 or 80.3%). Other organisms identified were *Proteus* spp. (9), other Enterobacteriaceae (coliforms, 8) *Staphylococcus saprophyticus* (6), *Enterococcus* spp. (5) and other organisms (8) (table 6.2). Pyuria in the absence of bacteriuria was detected in 76 patients (8.8%) and 607 (70.1%) patients showed no laboratory evidence of UTI.

Table 6.2: Overview of bacteria isolated from the urine samples included in the study.

	N	%
<i>Escherichia coli</i>	147	80.3
Coliform	8	4.4
<i>Proteus</i> spp.	9	4.9
<i>Staphylococcus saprophrophiticus</i>	6	3.3
<i>Enterococcus</i> spp.	5	2.7
Coagulase Negative <i>Staphylococcus</i>	2	1.1
Group B Streptococcus	2	1.1
<i>Klebsiella pneumoniae</i>	2	1.1
<i>Providencia stuartii</i>	1	0.5
<i>Pseudomonas aeruginosa</i>	1	0.5

Number and percentage of the pathogens isolated from urine samples of patients included in the study.

An antimicrobial was prescribed to 56% of the patients (481). An overview of antimicrobial prescribing is shown in table 6.3.

Table 6.3: Overview of antimicrobial prescription (any or none) at the index visit according to the microbiological analysis of the urine sample.

		No organism, no pyuria	Pyuria	Organism	Total
No antimicrobial medication	N	339	21	25	385
	Column %	55.8%	27.6%	13.7%	
	% of total	39.1%	2.4%	2.9%	45.5%
Any antimicrobial medication	N	268	55	158	481
	Column %	44.2%	72.4%	86.3%	
	% of total	30.9%	6.4%	18.2%	55.5%
Total	N	607	76	183	866
	% of total	70.1%	8.8%	21.1%	100.0%

Antimicrobial medication prescribed to patients according to the result of the urine sample analysis. Of the patients who did not receive a prescription, 339 (55.8%) had no organism and no pyuria, 21 had pyuria and 25 had an organism identified in their urine sample. Of the patients without an organism identified in their urine sample and without pyuria (607), 55.8% did not receive an antimicrobial and 44.2% did. Of all the patients included in the study (866), 45.5% did not receive an antimicrobial prescription and 2.9% did not receive an antimicrobial but had an organism identified in their urine sample.

Co-amoxyclav (33.1%) and trimethoprim (26.0%) were most often prescribed, fluoroquinolones represented 17% of the prescriptions and nitrofurantoin nearly 12% (table 6.4). Details of two antimicrobials were not fully recorded (an antimicrobial was prescribed but the detail of the specific group was not recorded).

More than half of the antimicrobials prescribed (55.7%) were for patients without pyuria or significant bacteriuria and 11% were for patients with pyuria only (in the absence of significant bacteriuria) (table 6.4). In total 179 patients (or 37% of all prescriptions) received a recommended first line antimicrobial.

Table 6.4: Details of antimicrobial therapy prescribed at the index visit for patients who received an antimicrobial according to the outcome of the urine analysis.

		No organism, no pyuria	Pyuria	Organism	Total
Co-amoxycylav	N	87	21	51	159
	Column %	32.5%	38.2%	32.3%	
	% of total	18.1%	4.4%	10.6%	33.1%
Ampicillin	N	18	1	8	27
	Column %	6.7%	1.8%	5.1%	
	% of total	3.7%	0.2	1.7%	5.6%
Trimethoprim	N	60	15	50	125
	Column %	22.4%	27.3%	31.6%	
	% of total	12.5%	3.1%	10.4%	26.0%
Quinolone	N	54	8	20	82
	Column %	20.1%	14.5%	12.7%	
	% of total	11.2%	1.7%	4.2%	17.0%
Nitrofurantoin	N	29	6	22	57
	Column %	10.8%	10.9%	13.9%	
	% of total	6.0%	1.2%	4.6%	11.9%
Other	N	20	4	7	31
	Column %	7.5%	7.3%	4.4%	
	% of total	3.7%	0.4%	1.5%	5.7%
Total	N	268	55	158	481
	Column %	100.0%	100.0%	100.0%	100.0%
	% of total	55.7%	11.4%	32.8%	100.0%

Overview of the outcome of the urine sample analysis according to the type of antimicrobial prescribed. Of the 481 patients who received an antimicrobial (55.5% of the total study population), 268 (55.7%) had no organism identified in their urine and had no pyuria, 55 (11.4%) had pyuria and in the urine sample of 158 patients (32.8%) an organism was identified. Of the 159 patients (33.1%) who received co-amoxycylav, 51 had an organism identified. Of the 481 patients 10.6% had an organism identified in their urine and received co-amoxycylav.

Of the 158 patients for whom a significant isolate was identified, the antimicrobial susceptibility was known and compared with the antimicrobial therapy prescribed. Co-amoxycylav was prescribed 51 times but for 10 of those patients the isolated organisms were resistant (20%), for ampicillin 6 out of 8 prescriptions (75%) were for resistant organisms, for trimethoprim 18 out of 50 (36%), fluoroquinolone resistance was found for 2 isolates out of the 20 prescriptions (10%), and 2 out of 22 prescriptions (9%) for nitrofurantoin were prescribed for a resistant organism. In total 37 out of 158 prescriptions (23%) were for an agent to which the isolate cultured was resistant. When taking all the records into account, the antimicrobial treatment prescribed was for 121 out of 866 records (14.0%) for a patient with a confirmed UTI and for an organism susceptible to this antimicrobial.

6.4 Practice prescribing

The 22 practices co-operating in the study varied in size and set-up. All practices were computerised fully or at least for the prescription of medication.

The practices sent in on average 62 samples (between 15 and 115 per practice). After exclusion of duplicates, opt-outs and ineligible patients, an average of 39 primary samples (between 9 and 72) per practice were sent in over the study period. Between 25% and 88% of the UTI patients per practice had a medical card (mean 57%) and between 18% and 75% of the patients in each practice had more than 10 visits in the past year.

Two practices had relatively more male patients in their practice. Both practices were actively implementing the 'healthy man screening' programme, a screening programme often provided by occupational health insurance. Two practices had particularly high numbers of nursing home patients (31% and 12%); one of these practices was solely responsible for one nursing home, but the high number for the other practice is unclear. The practices with relatively fewer medical card patients were those with generally younger patients and relatively fewer patients over 70 or residing in a nursing home.

An overview by practice is presented in table 6.5. There were no significant differences in the mean age, percentage females or nursing home patients with and without opt-out patients included (paired t-test, $p < 0.000$).

Table 6.5: Overview of characteristics of the participating 22 practices.

	Mean or %	Min-max
Isolates received in lab	62	15-115
Patients (total)	53	13-95
Patients included (excluding opt-out)	46	10-87
Patients eligible	39	9-72
<i>E.coli</i> isolates	7	1-14
Medical card patients	57.0%	25.0-87.5
More than 10 visits	49.4%	18.2-75.0
Nursing home patients	4.5%	0-30.8
Age patients	52.7 years	39.1-70.8
Females	78.7%	57.1-88.9
Antimicrobial prescribed on visit	55.5%	38.9-77.8
If so		
Co-amoxyclov	30.2%	0-59.4
Ampicillin	5.5%	0-18.2
Trimethoprim	28.3%	0-83.3
Quinolones	16.5%	0-47.8
Nitrofurantoin	11.9%	0-45.5
Other	7.6%	0-30.0

Overview of the overall mean, minimum and maximum number of isolates, patients and E.coli isolates per practice. A breakdown is given of the total number of patients, the number of patients included (excluding the patients who opted out) and the number of patients eligible per practice. Of the eligible patients, the % with a medical card, the % with more than 10 visits in the previous year, the % residing in a nursing home, the % females and their mean age. For the 55.5% of eligible patients who received an antimicrobial, the mean percentage between practices of each type of antimicrobial is also shown.

Practices showed preferences for certain antimicrobials and prescribing differed considerably between practices. An overview of practice prescribing is shown in Figure 6.4. The percentage of patients receiving any antimicrobial therapy ranged from 39% to 78% between practices.

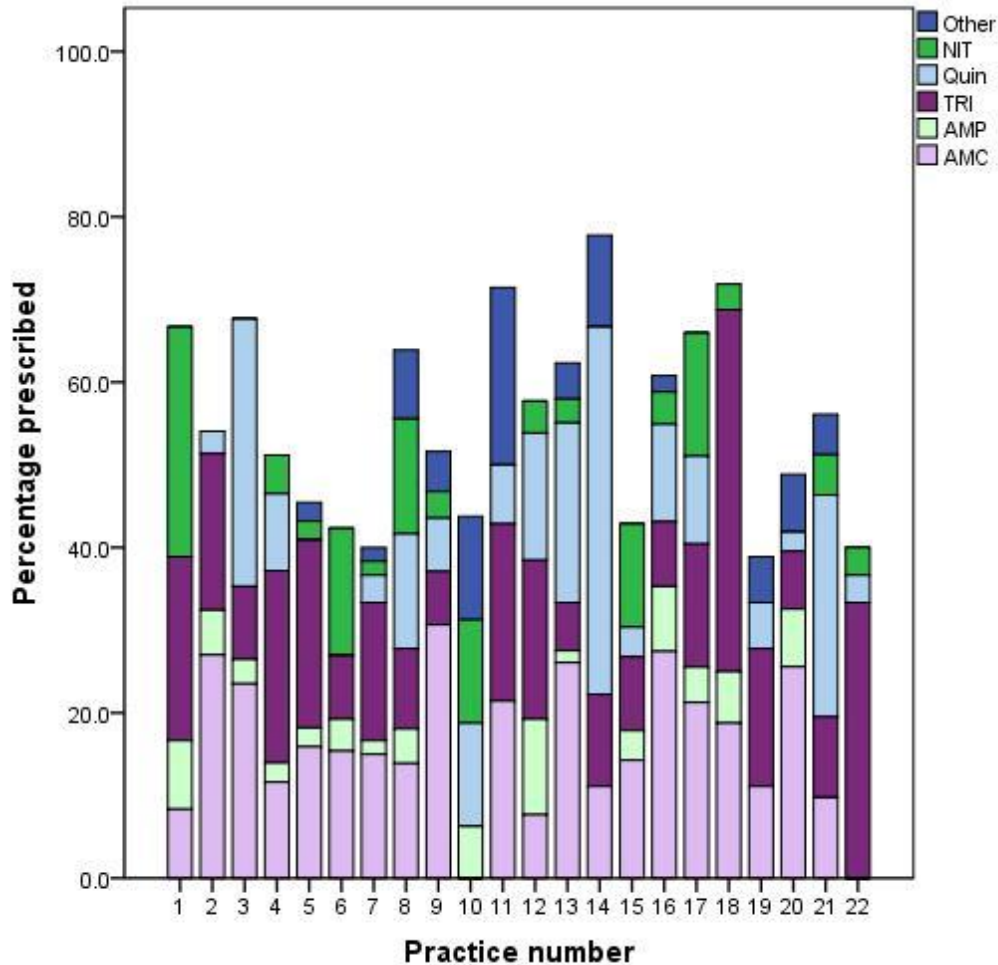


Figure 6.4: Total and specific prescribing by practice.

Total and specific percentage prescribing of each type of antimicrobial by practice. Depending on the practice, between 61% (practice 19) and 22% (practice 14) of patients did not receive an antimicrobial (white area). The group of antimicrobials the patients received is shown in different colours. For instance, no quinolones were prescribed in practice 1 and mainly trimethoprim was prescribed in practices 18 and 22.

Some practices mainly prescribe trimethoprim and nitrofurantoin, according to the recommended first line treatment of UTI, while other practices predominantly prescribe fluoroquinolones.

The variation in prescriptions of ampicillin was limited; overall around 6% of the prescriptions were for ampicillin (or about 4% of the visits). All practices had relatively high prescribing of co-amoxycylav and trimethoprim, with the exception of practice 10 where neither was prescribed, and practice 22 where no co-amoxycylav was prescribed. Some practices showed very high prescribing

of fluoroquinolones: practices 3 and 21, and in particular practice 14 (44% of their prescriptions were for quinolones). Nitrofurantoin showed extremes with practice 1 (high nitrofurantoin but no fluoroquinolone prescribing) and practices 2, 3, 11, 14, 19 and 20 (no nitrofurantoin was prescribed at all).

6.5 Prescribing and HSE-PCRS

In the retrospective part of the study (Chapter 3), the antimicrobial prescribing for each practice was calculated using the HSE-PCRS and was used as a proxy of total practice antimicrobial prescribing. The percentage antimicrobial prescribing by practice according to the results of the study and overall practice prescribing according to the HSE-PCRS were compared. The HSE-PCRS records all the prescriptions of medical card patients by GP and the data from all the practice GPs were summed for the practices' total antimicrobial prescribing. Data were difficult to compare as the ratio of medical card patient to private patient was unknown, nor was it known for what type of infection the prescriptions were given. The percentage of each antimicrobial group (co-amoxycylav, ampicillin, trimethoprim, quinolones and nitrofurantoin) was compared between practice prescribing according to the study and according to the HSE-PCRS and the correlation was calculated (table 6.6). This correlation was significant for all antimicrobials except ampicillin; higher practice prescribing according to the HSE-PCRS is correlated with higher practice prescribing as found in the study.

Table 6.6: Correlation of practice prescribing between the study and HSE-PCRS.

Antimicrobial	Correlation	p-value
Co-amoxycylav	56.1%	<0.01
Ampicillin	11.0%	>0.05
Trimethoprim	42.6%	<0.05
Quinolones	62.9%	<0.01
Nitrofurantoin	51.7%	<0.01

The percentage of each antimicrobial group prescribed within each practice was calculated according to the study and according to the HSE-PCRS (medical card patients prescribing database). The correlation between these percentages was compared and the corresponding p-value is shown. A p-value smaller than 0.05 is considered significant.

6.6 Antimicrobial prescribing according to age and gender

When an antimicrobial was prescribed at the index visit, the type of antimicrobial was compared between males and females and according to age. Females received trimethoprim relatively more often than males (29% vs 9%), while males received a prescription for quinolones significantly more often than females (35% vs 14%). None of the other antimicrobial prescriptions showed significant differences between males and females (table 6.7).

Table 6.7: Comparison of type of antimicrobial prescription at the index visit according to gender.

	Females		Males		p
	N	%	N	%	
Co-amoxycylav	129	32.0	30	38.5	ns
Ampicillin	23	5.7	4	5.1	ns
Trimethoprim	118	29.3	7	9.0	<0.01
Quinolones	55	13.6	27	34.6	<0.01
Nitrofurantoin	51	12.7	6	7.7	ns
Total	403	83.8	78	16.2	

Comparison of the type of antimicrobial prescribed according to the gender of the patient. P-value from the χ^2 test for comparison between those who received a prescription for the antimicrobial and those who did not. Males more often receive a prescription for quinolones while females receive a prescription for trimethoprim more often.

No significant differences were found between the type of antimicrobial and the age of the patient (ANOVA, $p > 0.05$). Figure 6.5 shows the boxplot of this comparison. More detailed comparison of the age distribution for each antimicrobial compared to the rest of the group (e.g. the age distribution for patients who received trimethoprim compared to all the other patients) showed that patients who received co-amoxycylav were slightly younger compared to the rest of the group. None of these comparisons were significant (t-test with a correction for multiple comparisons (Bonferroni)).

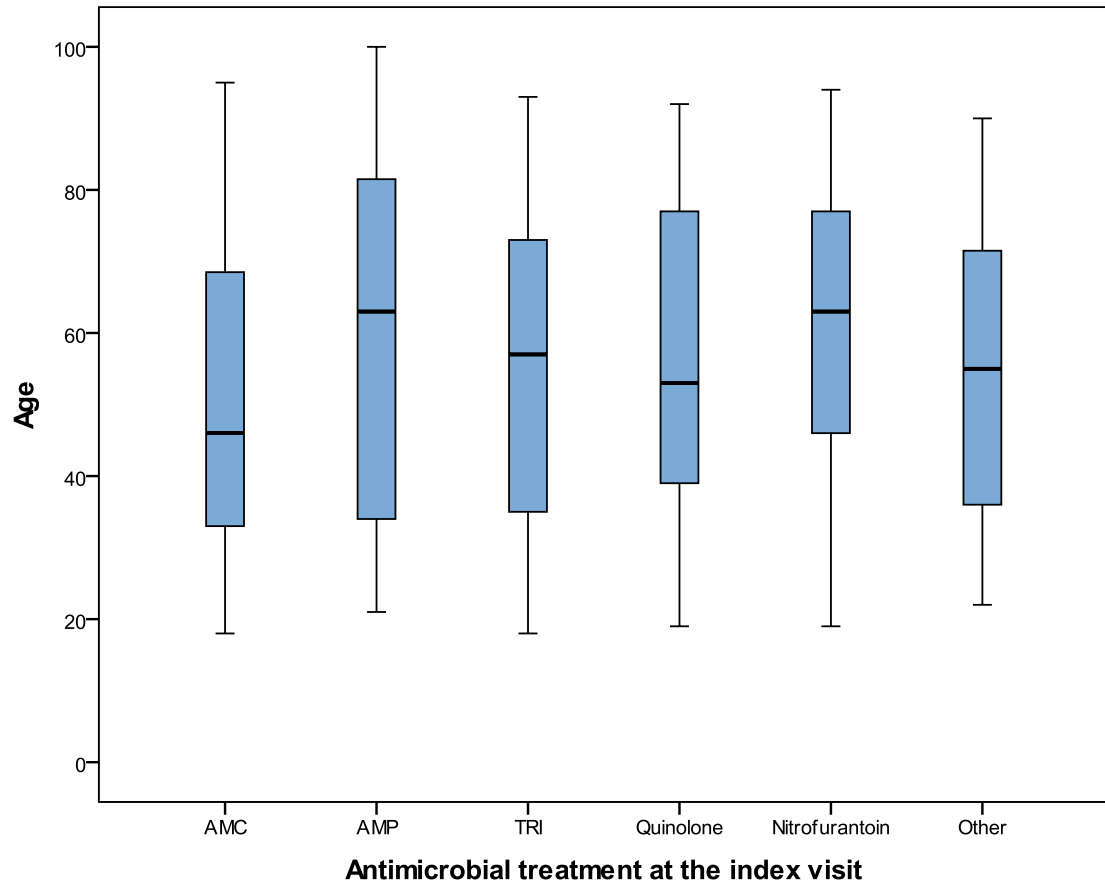


Figure 6.5: Boxplot of the distribution of age according to the type of antimicrobial prescribed at the index visit.

The boxplot of the distribution of age according to the type of antimicrobial prescribed shows the 'box', which represents the middle 50% of values. The line in the box is the median. If the median is in the middle of the box, mean and median are the same and the data are not skewed. The whiskers show the minimum and maximum of the data range. The overlap between the six boxplots indicates that there are no significant differences in age according to the type of antimicrobial prescribed.

6.7 Medical card and antimicrobial prescribing and resistance

The total number of medical card patients was 495 (57.2%) and the percentage varied by practice from 25% to 88%. An overview of factors compared between medical card and private patients is given in table 6.8.

Table 6.8: Overview of differences in patient characteristics between medical card and private patients.

	Medical card		Private		
Total	495		371		
	N	%	N	%	p
Female	387	78.2	288	77.6	ns
> 10 visits in previous year	357	72.1	66	17.8	<0.01
Positive index sample	121	24.4	62	16.7	<0.01
Any antimicrobial prescribed on index visit	299	60.4	182	49.1	<0.01
	Mean (SD)	Min-max	Mean (SD)	Min-max	p
Age	59.3 (±21.6)	18-100	43.1 (±15.2)	18-88	<0.01
Nr antimicrobials prescribed in previous year	2.0 (±2.5) median 1	0-13	0.7 (±1.2) median 0	0-7	<0.01
Nr samples in previous year	0.8 (±1.3) median 0	0-8	0.4 (±0.9) median 0	0-7	<0.01
Nr positive samples in previous year *	0.5 (±0.9) median 0	0-5	0.2 (±0.5) median 0	0-6	<0.01

An overview of the number and percentage in each category according to their medical card status. The p-value for the χ^2 test is presented for the categorical variables (first part). For the continuous variables (second part) mean, standard deviation (SD), minimum and maximum are given. For the skewed data the median is included and analysis is done with non-parametric tests.

* The number of positives is based on a link of the laboratory urine sample number in the file of the patients with the laboratory results. If no data were available, the sample was assumed negative.

Males and females are equally represented in the medical card and private patient groups. As all individuals over 65 years of age automatically qualify for a medical card, medical card patients are significantly older compared to private patients.

Medical card holders were significantly more likely to have more than 10 visits and to have a positive sample in the previous year but they also had more samples in the previous year compared to private patients (Figure 6.6).

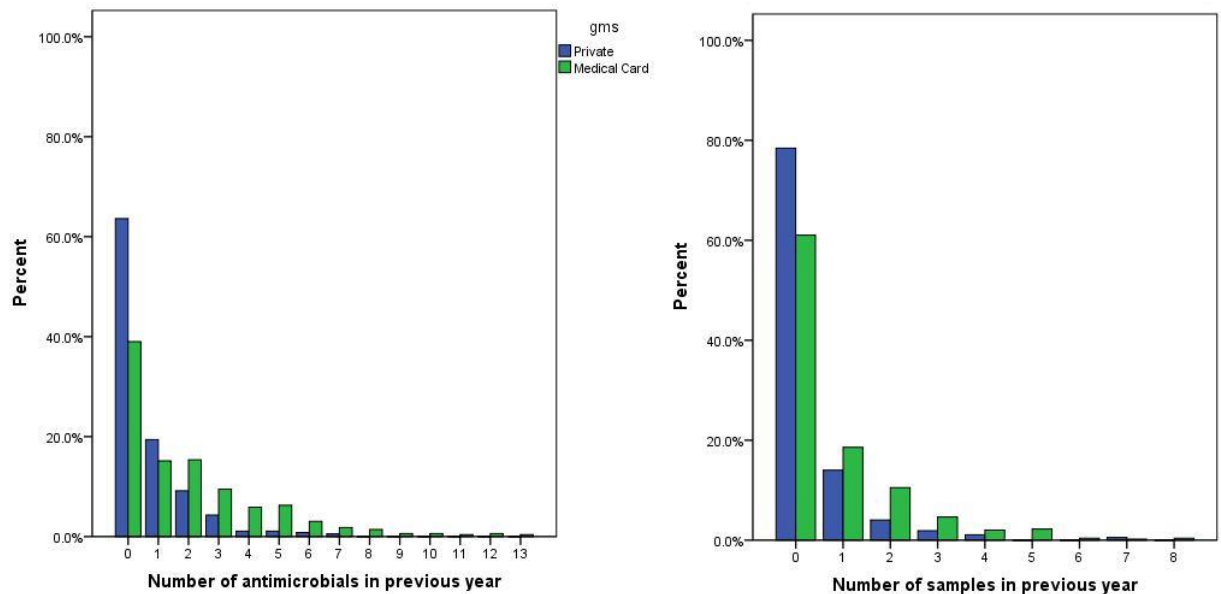


Figure 6.6: Comparison of number of antimicrobials and number of urine samples in the previous year in medical card and private patients.

The number of antimicrobials prescribed to medical card patients (green bars) and to private patients (blue bars). Private patients have a higher percent in the group without any prescriptions for antimicrobials in the previous year while medical card patients have a higher percentage of patients with two or more prescriptions in the previous year. The second graph shows more private patients without any urine samples submitted in the previous year and a higher percentage of medical card patients with one or more urine samples submitted in the previous year.

Private patients are less likely to get antimicrobials prescribed on the index visit to the GP for a suspected UTI, they have fewer antimicrobials prescribed, fewer urine samples in the previous year, and these samples are less often positive compared to medical card patients. However, if an antimicrobial was prescribed, the choice differs between medical card holders and private

patients; private patients get quinolones prescribed significantly more often and medical card patients get nitrofurantoin significantly more often (table 6.9). No significant differences were found for the other types of antimicrobials.

Table 6.9: Comparison between medical card and private patients of type of antimicrobial prescribed.

	Medical Card		Private		
Total N	299		182		
	N	%	N	%	p
AMC	94	31.4	65	35.7	ns
AMP	19	6.4	8	4.4	ns
TRI	79	26.4	46	25.3	ns
Quinolone	38	12.7	44	24.2	<0.01
NIT	49	16.4	8	4.4	<0.01
Other	20	6.7	11	6.0	

The number and % of each type of antimicrobial prescribed according to medical card status (medical card or private patient) are compared. Significance of the difference is stated as the p-value, ns: not significant.

Comparing the prescription of any antimicrobial between medical card and private patients, stratified for more or less than 10 visits per year, showed that antimicrobials were prescribed significantly more often to medical card patients when they have more than 10 visits per year (OR 2.3, 95% CI 1.1-3.7) but not so when the number of visits to the GP is less than 10 in the previous year (OR 1.4, 95% CI 0.9-2.3). No differences were found between private and medical card patients with an *E.coli* infection resistant to any or specific antimicrobials, overall or stratified by the number of visits in the previous year.

6.8 Appropriateness of treatment

Appropriateness of different treatment approaches was assessed by comparing the treatment prescribed by the GP with the antimicrobial susceptibility of the organism cultured. Overall, not treating a culture-confirmed UTI with antimicrobials was considered inappropriate as well as treatment with an antimicrobial to which the organism was resistant. Antimicrobial treatment for patients without a positive culture was also considered inappropriate. Not treating patients with a negative culture and treating patients with culture-confirmed UTI with an antimicrobial to which the pathogen is susceptible were considered appropriate. A simulation of different treatment options, i.e. if all patients who received an antimicrobial were treated with nitrofurantoin, with trimethoprim or with ciprofloxacin, is also presented. Based on the relative numbers of *E.coli* in each treatment group, an additional analysis was performed to assess at what trimethoprim resistance level the trimethoprim scenario would reach similar levels of appropriate treatment as nitrofurantoin and fluoroquinolones. The price of treatment is calculated from the average price of all the prescriptions of these practices for this antimicrobial group. Prices were according to the manufacturing cost of the medicine as recorded in the HSE-PCRS.

An antimicrobial treatment was prescribed to 481 (56%) of the 866 patients included in this study; the two unknown antimicrobial therapies were interpreted as appropriate. The appropriateness of treatment was compared with the laboratory report on the urine sample and interpreted as appropriate for 55% of patients (table 6.10). Additionally, the appropriateness of three specific scenarios was assessed; all antimicrobial prescriptions were nitrofurantoin (scenario 1), trimethoprim (scenario 2) or fluoroquinolone (scenario 3).

Table 6.10: Overview of appropriateness of different treatment approaches.

Treatment option	Appropriately treated patients N (%)	Inappropriately treated patients N (%)	Total Cost (€) for 481 treatments
As prescribed by GP	478 (55.2)	388 (44.8)	€ 3,685*
Empiric treatment with:			
Nitrofurantoin only	498 (57.5)	368 (42.5)	€ 2,222
Trimethoprim only	455 (52.5)	411 (47.5)	€ 2,001
Fluoroquinolones only	499 (57.6)	367 (42.4)	€ 7,691

*The treatment prescribed by the GP was appropriate if patients with an organism identified received treatment to which the organism was susceptible, or not treating patients with a negative culture. Alternative scenarios suggested were if only nitrofurantoin, only trimethoprim or only fluoroquinolones were prescribed when the GP prescribed an antimicrobial. The total cost (according to manufacturing cost) of each treatment was calculated for each scenario. (*2 unknown treatments not included in cost)*

Analyses of these scenarios showed that treatment as prescribed by the GP and nitrofurantoin only treatment reached similar levels of appropriately treated patients but at a much lower cost for nitrofurantoin. Additional use of urine dipstick to exclude prescribing of nitrofurantoin for patients with alkaline urine (often resulting from an infection with *Proteus spp.* for which nitrofurantoin would be considered inappropriate treatment) could further increase the appropriateness of treatment of UTI with nitrofurantoin (Simerville, Maxted *et al.* 2005). Comparable levels of appropriateness of treatment were observed for treatment with ciprofloxacin only, but at a higher cost. Empirical treatment of all patients with trimethoprim only was less often appropriate due to higher resistance levels. The additional analysis of different trimethoprim resistance levels showed that appropriate treatment would reach 57.4% at resistance levels for trimethoprim of 10% (Figure 6.7).

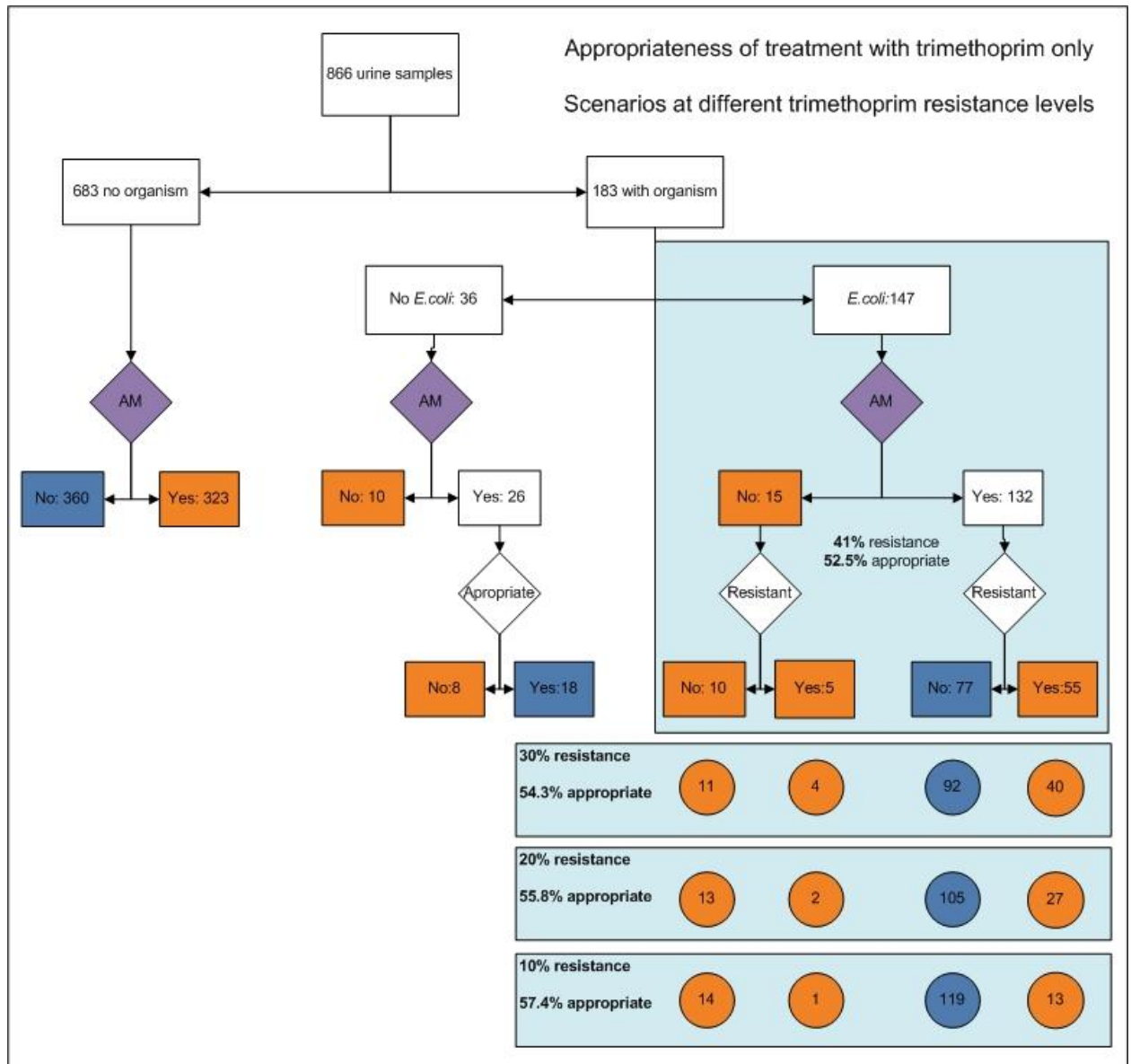


Figure 6.7: Appropriateness of treatment with trimethoprim only and scenarios at different trimethoprim resistance levels.

A flow chart of the microbiological analysis of the urine samples and the assessment of the appropriateness of therapy if the GP were to prescribe trimethoprim only. Dark blue boxes indicate appropriate treatment; orange boxes indicate inappropriate treatment. An additional analysis of the treatment of *E.coli* UTI according to different resistance levels in the community is shown (light blue back ground). Treatment with trimethoprim is more often appropriate with less resistance, as the likelihood that the *E.coli* is susceptible is higher.

6.9 Discussion

The high level of participation of patients due to the opt-out method of recruitment, allows generalisation of the results of this study. Overall, only one in five urine samples of patients with suspected UTI have significant bacteriuria according to the definition used in this study. A high proportion of patients (481 or 56%) are treated with antimicrobials. Of these patients, 56% (268) had no laboratory evidence of UTI and a further 11% (55) of these patients have pyuria but no significant bacteriuria. A number of these prescriptions were not appropriate, because the organism was resistant or the treatment was not appropriate for the infection with the organism identified in the urine culture, which resulted in 14% effective prescribing.

Recommended first line treatment is only prescribed for 38% of patients (trimethoprim or nitrofurantoin); 33% of the prescriptions are co-amoxycylav, 26% trimethoprim and only 12% nitrofurantoin. Treatment of UTI in general practice shows important variation between practices and clear practice preferences for certain antimicrobials. This was most striking for quinolones, which is the prescription of first choice in some practices, and which was prescribed to 17% of all patients in the study. Additionally, quinolones were more often prescribed to private patients and nitrofurantoin more often to medical card patients. Generally however, medical card patients did not receive more antimicrobials compared to private patients when stratified for the number of visits. Differences in prescribing did not (yet) affect resistance levels according to medical card status as there was no significant difference in the resistance patterns of the isolates. Also, quinolones were more often prescribed to males than females and trimethoprim was more often prescribed to females than males. UTIs are often considered to be more serious in males, especially with the difficulty of excluding prostatitis in men (Lipsky 2000; Hummers-Pradier, Ohse *et al.* 2004) and this is probably the explanation why a gender difference in prescribing was found.

Finally, 55% of patients received treatment by the GP that was classified as appropriate, defined as no treatment when this was not necessary or appropriate treatment in the case of a laboratory-confirmed UTI. If all antimicrobial prescriptions by the GP were for nitrofurantoin, 57% of the treatments would have been classified as appropriate with a total cost that would be lower than the total cost of the antimicrobial treatments actually prescribed by the GP. While there are patients for whom nitrofurantoin is not appropriate (Zalmanovici Trestioreanu, Green *et al.* 2010; Gupta, Hooton *et al.* 2011), this finding suggests that nitrofurantoin may be the preferred agent for empiric therapy in the absence of a specific contraindication. Even though quinolones also reach this higher level of appropriateness, the price of treatment with quinolones is nearly 3.5 times higher than nitrofurantoin treatment. In addition, there are specific concerns related to increasing levels of resistance to fluoroquinolone agents (Woodford and Livermore 2009; Gupta, Hooton *et al.* 2011) as well as concerns that patients with fluoroquinolone resistant *E.coli* bacteraemia were found to be at higher risk of mortality, most likely due to delayed adequate antimicrobial therapy (Lautenbach, Metlay *et al.* 2005; Ortega, Marco *et al.* 2009). As empirical therapy is initiated before the causative pathogen is identified, the prescription of antimicrobial treatment should aim to be active against the most likely pathogens, taking into account local resistance profiles while not increasing the potential impact on resistance levels (Kollef 2008).

6.10 How this compares

The high number of participants in this study shows the success of the opt-out methodology. A paper from Germany on management of UTI in female patients (Hummerts-Pradier, Ohse *et al.* 2005), enrolled 585 patients with suspected UTI from 36 practices over a period of four months while Fahy *et al.* (Fahey, Webb *et al.* 2003) enrolled 160 patients from eight practices in Bristol over a four month period. An observational study recruited 288 patients from 9 practices and required the GP to enrol patients and the patients to fill out a questionnaire (O'Brien, Hillier *et al.* 2007). They obtained 60% response and 39% subsequent participation. A prospective study with two recruitment arms obtained 66% participation for patients approached within the healthcare facility and 41% participation in a random sample of non attending patients (Hay, Thomas *et al.* 2005). In a spotter practice model, clinicians from three general practices were asked to submit MSUs from all patients presenting with symptoms suggestive of UTI (Richards 2002). The percentage of patients with significant bacteriuria was 26%. Additional information from a sentinel practice group showed significant growth in 28% of the urine samples received (Ludlam, Sule *et al.* 2004). Our results with a high inclusion rate (866 patients over two months) and 21% positive urine samples compares favourably. The high inclusion is partially due to the fact that the GPs in our study were not requested to enrol the patients, and neither the patient nor GP were requested to provide additional information. This additional information is available from the other studies, which can be seen as the trade off between more detailed patient information and a more representative population. It is clear from our results that decisions on empiric prescriptions of antimicrobials often show discrepancies with the subsequent outcome of the laboratory analysis of urine by culture and microscopy. Additionally, the recommended first line empirical treatment is trimethoprim or nitrofurantoin (Strategy for the control of Antimicrobial Resistance in Ireland (SARI) 2008), which were prescribed to only 37% of patients. These guidelines do not include

the limitation included in other recommendations (Gupta, Hooton *et al.* 2011) to avoid trimethoprim when trimethoprim resistance levels exceed 20% or when trimethoprim was prescribed in the previous three months.

Some practices showed clear preferences for recommended first line agents while others preferred fluoroquinolones. More effective approaches are needed to influence antimicrobial prescribing in general practices and specifically to limit the use of fluoroquinolones. Levels of resistance to ciprofloxacin in *E.coli* increased from 5.3% in 2002 to 8.3% in 2009 (Chulain, Murray *et al.* 2005). Similar tendencies have been observed in other countries (Gupta, Hooton *et al.* 2001; van de Sande-Bruinsma, Grundmann *et al.* 2008; Reynolds 2009). On a bigger scale, an analysis of antimicrobial use in four UK administrations also revealed important practice variations (Davey, Ferech *et al.* 2008). The relevance of practice variation in resistance levels has previously been described by us in a multilevel analysis of retrospective data from 72 practices over a 4.5 year period. In this analysis it was shown that the variation in levels of resistance (in uropathogenic *E.coli*) between practices was higher for ciprofloxacin than it was for trimethoprim and that both were associated with overall practice prescribing of the antimicrobial (Vellinga, Murphy *et al.* 2010). It has been suggested that it is likely that limitation of fluoroquinolone prescribing will curtail resistance levels (Gottesman, Carmeli *et al.* 2009) while this seems to be less likely for the more disseminated trimethoprim (Enne 2010; Sundqvist, Geli *et al.* 2010).

Empiric antimicrobial treatment was prescribed to 56% of the patients and 37% of patients received a prescription for an antimicrobial without laboratory evidence of UTI. A surveillance study showed that more than 80% of patients presenting with a suspected UTI in English general practice received an antimicrobial (Petersen and Hayward 2007). A comparable study from Germany, in which similar inclusion criteria to ours were used, also found 56% prescribing overall and 22% of the prescribing was for patients without any evidence of urinary tract infection (Hummers-Pradier, Ohse *et al.* 2005). It is clear that there remains scope for reductions in antimicrobial prescribing in

general practice and symptomatic treatment of patients with suspected UTI might be an option. A Belgian study has shown that half of the patients were free of symptoms after three days of placebo (Christiaens, De Meyere *et al.* 2002) and a recent trial showed no difference between symptomatic treatment with ibuprofen or ciprofloxacin for uncomplicated UTI (Bleidorn, Gagyor *et al.* 2010). A study comparing different antimicrobial strategies with placebo in a large UTI trial showed slightly poorer results for the placebo group (Ferry, Holm *et al.* 2004). These results suggest that UTI is often a self-limiting disorder and symptomatic treatment of uncomplicated UTI deserves further research. However, when empiric treatment is preferred, preference should be given to nitrofurantoin in the absence of any specific contraindication. A recent review comparing different classes of antimicrobials for treatment of acute uncomplicated UTI in women found no differences between trimethoprim, fluoroquinolones, β -lactam/ β -lactamase antibiotics and nitrofurantoin for the symptomatic cure of acute uncomplicated UTI (Zalmanovici Trestioreanu, Green *et al.* 2010). Nitrofurantoin is an appropriate agent because of low resistance levels in the community and relatively low cost. Additionally, a recent analysis of recurrent infections showed that resistance to nitrofurantoin was generally low and, once detected, decays relatively quickly (Vellinga, Cormican *et al.* 2010). There are theoretical reasons to believe that it may be less likely to select for antimicrobial resistance as it is concentrated in urine whereas other agents are distributed extensively in all body compartments including the gastrointestinal tract (Roe 2008; Sandegren, Lindqvist *et al.* 2008). Interestingly, socio-economic differences were also identified in the type of antimicrobials prescribed. These differences did not show increased antimicrobial use in lower socio-economic classes, but rather increased consultations. However, when corrected for increased visits, it was shown that ciprofloxacin was more often prescribed to private patients while medical card patients were more likely to get nitrofurantoin. The socio-economic status of the patient as a factor in the prescribing behaviour of the physician,

irrespective of medical reasons, has previously been described in various studies (Scott, Shiell *et al.* 1996; Ohlsson, Chaix *et al.* 2009). However, it is not always clear whether it is the socio-economic status of the patient, the type of practice, or the GP that influences the prescribing of medication. A Canadian study in which province level prescribing was analysed showed associations between fluoroquinolone prescribing and socio-economic variables; fluoroquinolones were more likely to be used in advantaged populations (Glass, Pearl *et al.* 2010). A Swedish multilevel study showed that patients visiting a private health-care practice had a four times higher probability of being prescribed a new, more expensive medicine (but with limited evidence on its safety), compared to patients visiting public health care practices. This variation between practices was, according to a prior study from the same research group, for 50% due to variations among GPs (Hjerpe, Ohlsson *et al.* 2010) while a New Zealand study showed 10% to be explained by practitioners variation (Davis, Gribben *et al.* 2002). Socio-economic status can be described in various ways, from income to education level, but particularly private health insurance has been shown to be an important factor in the decision to prescribe, follow up or test (Scott, Shiell *et al.* 1996). In our retrospective study, socio-economic status of the area where the practice was situated was found to be (border line significantly) associated with the outcome of the probability of a resistant *E.coli* infection. The information from the prospective study showed that GPs seem to prescribe differently for private patients compared to medical card patients. Further multilevel analysis of this particular association will be pursued.

Chapter 7: Linking antimicrobial prescribing and resistance: a prospective case-control study.

7.1 Introduction

This analysis was part of the prospective study on antimicrobial resistance and prescribing for UTI in general practice. This case-control study was set up in 22 participating practices in the West of Ireland and ran over a period of nine months. All adult patients visiting a participating practice with a suspected UTI were requested to supply a urine sample. For this part of the study data were collected on *E.coli* positive urine samples only. Cases were patients with a resistant *E.coli* UTI, controls were patients with a susceptible *E.coli* UTI.

7.2 Methods

7.2.1 Microbiology

All urine samples with laboratory confirmed *E.coli* isolates (colony count $>10^5$ cfu/ml) were included. Only the first *E.coli* confirmed isolate during the study period was included in this analysis. Antimicrobial susceptibility testing was performed by disk diffusion methods according the Clinical and Laboratory Standards Institute (CLSI) methods (Clinical and Laboratory Standards Institute 2010). Susceptibility testing was performed and analysed for ampicillin, co-amoxyclov, trimethoprim, ciprofloxacin and nitrofurantoin. For further statistical analysis, *E.coli* isolates classified as ‘intermediate’ were categorised as resistant.

7.2.2 Statistical analysis

Individual level variables

At the patient level, data were collected on previous antimicrobial prescriptions up to a year prior to the first sample submitted during the study period. Any antimicrobial prescribed during this period was recorded according to the major antimicrobial group: penicillins, β -lactam/ β -lactamase inhibitor combinations (co-amoxyclov mainly), trimethoprim, fluoroquinolones, cephalosporins and other antimicrobials. The fluoroquinolones recorded were mainly ciprofloxacin and occasionally ofloxacin. For univariate analysis, prescribing in the previous year was dichotomised (yes/no). For multilevel analysis, the number of prescriptions of antimicrobials in the previous year was included as a categorical variable: no prescriptions, one prescription, two prescriptions and three or more prescriptions. For ciprofloxacin the highest two categories were collapsed into two or more prescriptions in the previous year. From the laboratory system, age, gender (female the reference category) and nursing home residence of the patients were available. Additional data were collected from the individual charts on previous urine samples, medical card status, number of

GP visits in the previous year (more or less than 10), number of urine samples in the previous year, and other prescriptions categorised as medication for asthma, diabetes, thyroid, cardiovascular or circulatory medication, mood altering and sleep medication or any other medication. Age was centred around the general mean age. Medical card eligibility depends on income and age and can be interpreted as a proxy measure of socio-economic status. A medical card gives the individual access to free medical care and medication. Hospitalisation in the previous year was recorded from the GP charts and checked with the hospital patient administration system of the main hospital for the region.

Group level variables

The study included data on 22 practices of various sizes. The number of urine samples included for each practice over the study period was included as a variable. For each practice prescription rates were calculated based on (1) data obtained from the Health Service Executive-Primary Care Reimbursement Service (HSE-PCRS) which records all prescriptions for medical card patients and (2) data collected during the first part of this study (Chapter 6). A practice resistance level was calculated from retrospective laboratory data for the year prior to the start of the prospective study (September 2008 to August 2009) as a proxy for exposure to practice level resistance.

Statistical analysis

Univariate analysis (PASW version 18.0) was performed to compare demographic variables and antimicrobial prescribing between individuals with a susceptible isolate and individuals with a resistant isolate. Continuous variables were compared using non-parametric tests. Antimicrobial prescribing in the previous year was dichotomised (yes/no) to be able to generate odds ratios. Unadjusted associations were calculated and variables significant at a p-value <0.1 were included in subsequent multilevel analysis.

A multilevel logistic regression analysis was used to estimate the odds of patients being diagnosed with a resistant *E.coli* UTI, allowing for the hierarchical structure of the data (patients nested within practices). The model building used a forward stepwise selection process. The ‘empty’ model only including the random parameter was calculated, and individual and practice level variables were introduced. Cross-level and intra-level interactions were checked for significance. Models were compared using the deviance information criterion (DIC) which combines the deviance with information about the number of parameters in the model (a lower DIC implies a better model). To avoid collinearity, variables that were closely correlated were separately introduced in each model and only the most significant variable was retained (for instance, hospitalisation in the previous year and emergency department admission; medical card status and number of visits in the previous year; medical card status and prescriptions for other illnesses). The percentage of proportional change in variance (PCV) was calculated, representing the percentage of variation explained by the variables in the model compared to the empty model.

The adjusted odds ratios (OR) with 95% confidence intervals (95% CI) for resistance to the antimicrobial agents were calculated for the fixed effects. In order to quantify the variability between practices in antimicrobial resistance level, median odds ratios (mOR) were calculated using Larsen’s mOR (Larsen, Petersen *et al.* 2000; Merlo, Chaix *et al.* 2006). A mOR equal to one signifies no differences between practices in the probability of an antimicrobial resistant *E.coli*. For the mOR a Bayesian credible interval (CrI) was calculated based on the distribution of the mOR, to distinguish it from a fixed effects odds ratio confidence interval.

To optimise comparison, a final model was built for the outcome resistance to the particular antimicrobial using the same variables, irrespective of their significance. Parameters were estimated by Markov Chain Monte Carlo (MCMC) methods in the MLwiN version 2.21 software (Browne 2003; Rasbash 2003). A p-value <0.05 was considered significant.

7.3 Results

7.3.1 Practices and participants

The 22 practices submitted between 6 and 58 *E.coli* positive urines. The number of antimicrobial prescriptions per practice ranged from 28 to 171, with a mean of 129. The mean practice resistance for ciprofloxacin was 8% and 31% for trimethoprim (table 7.1).

Over the nine month period, 633 patients with a laboratory-confirmed *E.coli* UTI and a full record for all variables were included (table 7.2). Of the *E.coli* isolates, 36% were resistant to trimethoprim and 12% to ciprofloxacin. Overall 66.1% of patients had at least one antimicrobial prescribed in the year prior to this episode of UTI: 14.7% had at least one prescription for ciprofloxacin and 20.5% for trimethoprim.

Table 7.1: Overview of practice level characteristics.

Practice level	Mean	Median	Min-max	IQR
N patients	28.8	27	6-58	18-38
N of antimicrobial prescriptions/month	129	131	28-171	116-150
% /month				
Quinolones	5.3	5.5	2.0-8.0	4.2-6.1
Trimethoprim	6.7	7.0	2.2-11.3	4.2-8.7
Resistance 2008-2009 (%)				
Ciprofloxacin	8.4	6.9	0-23.5	4.7-11.5
Trimethoprim	31.5	30.0	17.2-52.6	24.6-38.3

Overview of the mean, median, minimum, maximum and interquartile range (IQR) for the number of patients, the prescriptions of antimicrobials per month and the percentage of quinolone and trimethoprim prescribing of the practices. Additionally, the percentages of ciprofloxacin and trimethoprim resistance in the year previous to the study are given.

7.3.2 Univariate analysis

Patients with a medical card represented 61% of this group, and the proportion of UTI patients with a resistant *E.coli* was significantly higher in medical card patients compared to private patients for both antimicrobials. Similarly, increasing age, increasing number of antimicrobial prescriptions, nursing home residence , prescription for other conditions (in particular patients receiving cardiovascular medication), and number of visits in the previous year were associated with a higher chance of a resistant *E.coli* compared to susceptible *E.coli* UTI patients. Male gender and hospitalisation in the previous year had a significantly higher odds ratio for patients with a ciprofloxacin resistant *E.coli* compared to susceptible *E.coli* UTI patients. An overview of the patient and practice characteristics and univariate analysis is presented in table 7.2.

Table 7.2: Overview of patient level characteristics and univariate associations (*: p<0.05).

Characteristics	%	Trimethoprim		Ciprofloxacin	
		Susceptible	Resistant	Susceptible	Resistant
N		403	230	555	78
%		63.7	36.3	87.7	12.3
Patient level categorised variables and odds ratios (95% CI)					
		Susceptible	Resistant	Susceptible	Resistant
Male gender	7.7	31	18	38	11
		1.0 (0.6-1.9)		2.2 (1.1-4.6)*	
Nursing home residence	6.3	16	23	22	17
		2.7 (1.4-5.2)*		6.8 (3.4-13.4)*	
Hospitalisation	16.4	59	44	81	22
		1.4 (0.9-2.1)		2.3 (1.3-4.0)*	
Medical Card	60.9	230	155	320	65
		1.5 (1.1-2.2)*		3.7 (2.0-6.8)*	
Other condition	63.1	237	162	335	64
		1.7 (1.2-2.4)*		3.0 (1.6-5.5)*	
CardioVascular	33.0	118	90	168	40
		1.6 (1.1-2.2)*		2.4 (1.5-3.9)*	
> 10 visits in previous year	48.7	176	132	251	57
		1.7 (1.3-2.4)*		3.3 (1.9-5.6)*	
Prescribing in previous year and odds ratios (95% CI)					
TRI	20.5	61	69	101	29
		2.4 (1.6-3.6)*		2.7 (1.6-4.4)*	
CIP	14.7	47	45	63	29
		1.8 (1.2-2.9)*		4.6 (2.7-7.8)*	
Continuous variables (mean)					
Age	56.4	53.8	60.8*	54.5	69.4*
Number of antimicrobial prescriptions					
	1.98	1.7	2.5	1.8	3.5

Overview and univariate analysis of patient level characteristics. The second column shows the % of the patients with this risk factor. The number and % of patients with an E.coli susceptible for trimethoprim/ciprofloxacin are given in the next columns. For the categorised variables an odds ratio

*was calculated for the risk factor (compared to the risk factor not present). For instance, 6.3% of patients were nursing home residents, 16 patients in nursing homes had a trimethoprim susceptible E.coli infection and 23 a resistant, the odds of a patient in a nursing home being diagnosed with a trimethoprim resistant E.coli UTI was 2.7 times higher than a patient in the community. Odds ratios were significant when their confidence interval did not include 1. For continuous variables the overall mean age was 56.4 years, patients with a trimethoprim resistant E.coli UTI were on average 53.8 years old and those with a resistant E.coli UTI 60.8 years, and this difference was significant (indicated by *). The number of antimicrobial prescriptions in the previous year was a skewed variable and the comparison was analysed with non-parametric tests. This difference was not significant for either antimicrobial.*

7.3.3 Multilevel modeling

The final model was generated including age, male gender, nursing home residence, hospitalisation in the previous year, medical card eligibility, prescriptions for other conditions, and antimicrobial prescribing in the previous year at the patient level (table 7.3). At practice level the percentage antimicrobial prescribing of the respective agents per month was included even though this model was only slightly better than the model with practice resistance levels and neither practice level variable obtained significance.

Table 7.3: Overview of multilevel analysis.

	Trimethoprim		Ciprofloxacin	
Patient level	OR	95% CI	OR	95% CI
Prescribing previous year				
1 prescription	1.40	0.83-2.19	2.67	1.24-5.60
2 prescriptions	4.72	1.91-12.44	6.47	2.86-14.78
3/> prescriptions	6.40	1.99-25.41		
Age	1.01	1.00-1.02	1.02	1.00-1.04
Male gender	1.00	0.52-1.94	1.60	0.66-3.70
Nursing home residence	1.77	0.85-3.71	3.45	1.50-7.86
Hospitalisation in previous year	1.12	0.70-1.83	1.55	0.82-2.90
Medical card	1.05	0.70-1.56	1.97	0.94-4.01
Practice level				
Practice prescribing	1.04	0.94-1.13	0.95	0.79-1.14
Measures of variation				
σ ² (SD)				
Empty model	0.03 (0.04)		0.16 (0.18)	
Final model	0.03 (0.05)		0.09 (0.13)	
ICC	1%		5%	
PCV	4%		43%	
mOR				
Empty model	1.17	1.03-1.39	1.46	1.03-2.15
Final model	1.17	1.03-1.46	1.33	1.03-1.90

Overview of the odds ratios and 95% confidence interval (CI) for all the variables entered in the multilevel analysis. Odds ratios are significant when their confidence interval does not include 1. The odds ratio is for the risk factor present compared to the risk factor absent. For previous prescribing for instance, the odds ratio was 1.4 times higher for patients who had one prescription of trimethoprim in the previous year compared to patients who did not have a previous prescription of trimethoprim, 4.72 times higher for a patient with 2 previous prescriptions of trimethoprim compared to a patient with no prescriptions, and so on. σ^2 is the variance at practice level and SD the standard deviation. The ICC is the intraclass correlation coefficient, PCV Proportional Change in Variance, mOR is the median Odds Ratio. For more background see 'Statistical Analysis' part of methods.

The odds of an infection with a resistant *E.coli* increased with an increasing number of prescriptions over the previous year: for ciprofloxacin the odds ratio was 2.7 (95% CI 1.2-5.6) for one and 6.5 (95% CI 2.9-14.8) for two or more ciprofloxacin prescriptions in the previous year compared to no prescriptions. For trimethoprim this effect only became significant after at least two trimethoprim prescriptions in the previous year: OR 4.7 (95% CI 1.9-12.4) and up to 6.4 (95% CI 2.0-25.4) for three or more trimethoprim prescriptions per year. For both antimicrobials the trend (increased chance of a resistant *E.coli* with increased previous prescribing), tested significant. Figure 7.1 shows a graph of the predicted probability (and prediction interval) of a resistant *E.coli* UTI with increasing number of prescriptions. The predicted probability is calculated based on the final model in which all other variables were set at their mean value. The predicted probability of a UTI with a trimethoprim resistant *E.coli* was 32.6% (95% prediction interval 28.1-37.2) if the individual had no previous prescriptions of trimethoprim, 39.9% (95% PI 30.0-50.8) with one previous prescription, 68.4% (95% PI 47.5-85.0) with two previous prescriptions and 73.7% (95% PI 46.7-91.8) with three or more prescriptions of trimethoprim in the previous year. For ciprofloxacin the predicted probability of a UTI with a ciprofloxacin resistant *E.coli* was 7.4% (95% PI 5.3-10.2) if the individual had no previous prescriptions of ciprofloxacin, 17.9% (95% PI 9.2-30.4) with one previous prescription and 33.7% (95% PI 18.8-51.4) with two or more previous prescriptions of ciprofloxacin in the previous year.

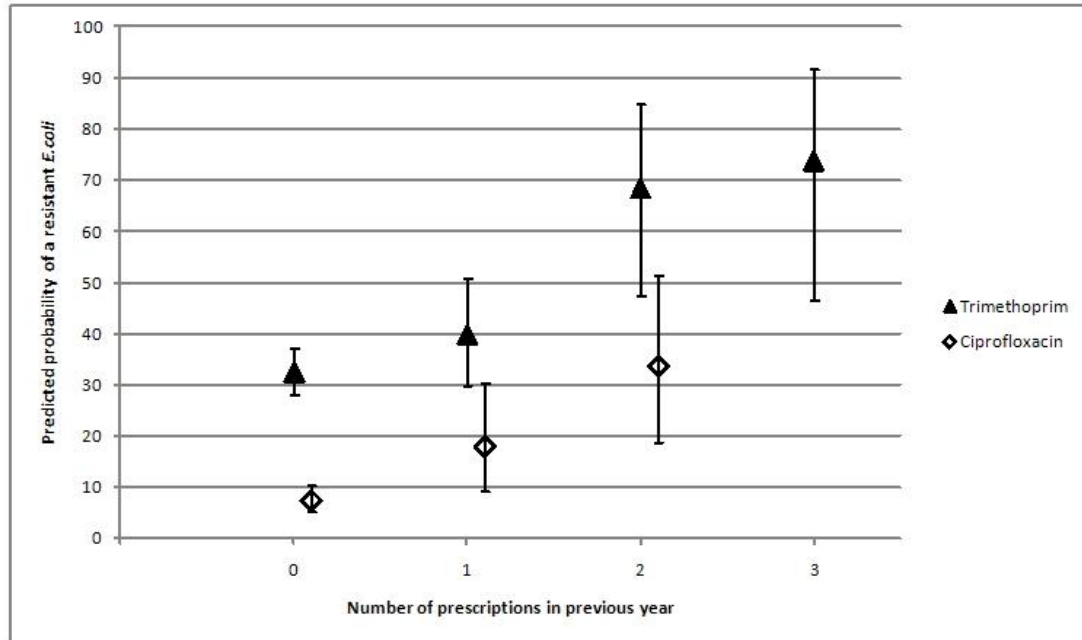


Figure 7.1: Predicted probability and prediction interval of a resistant *E.coli* with increasing number of prescriptions for ciprofloxacin and trimethoprim prescribing in the previous year.

The predicted probability of a resistant *E.coli* with an increasing number of trimethoprim/ciprofloxacin prescriptions was based on the final model in which the other variables were set at their mean value. The error bars are the 95% prediction interval. This means that for the 'mean' patient with a UTI the probability of a trimethoprim resistant *E.coli* increased from just over 30% (prevalence) to nearly 40% for one previous prescription, nearly 70% with two previous prescriptions and more than 70% for three or more prescriptions of trimethoprim in the previous year. For ciprofloxacin the probabilities were 7%, nearly 18% and 34% for no, one and two or more prescriptions respectively.

Age is a significant factor in the odds of a resistant *E.coli*. Male gender, hospitalisation and medical card status are of no significance for either antimicrobial in the odds of a resistant *E.coli*, after correction for the other factors. Nursing home residence is a significant factor for ciprofloxacin resistance (Figure 7.2), with a threefold increase in odds ratio but this was not found for trimethoprim.

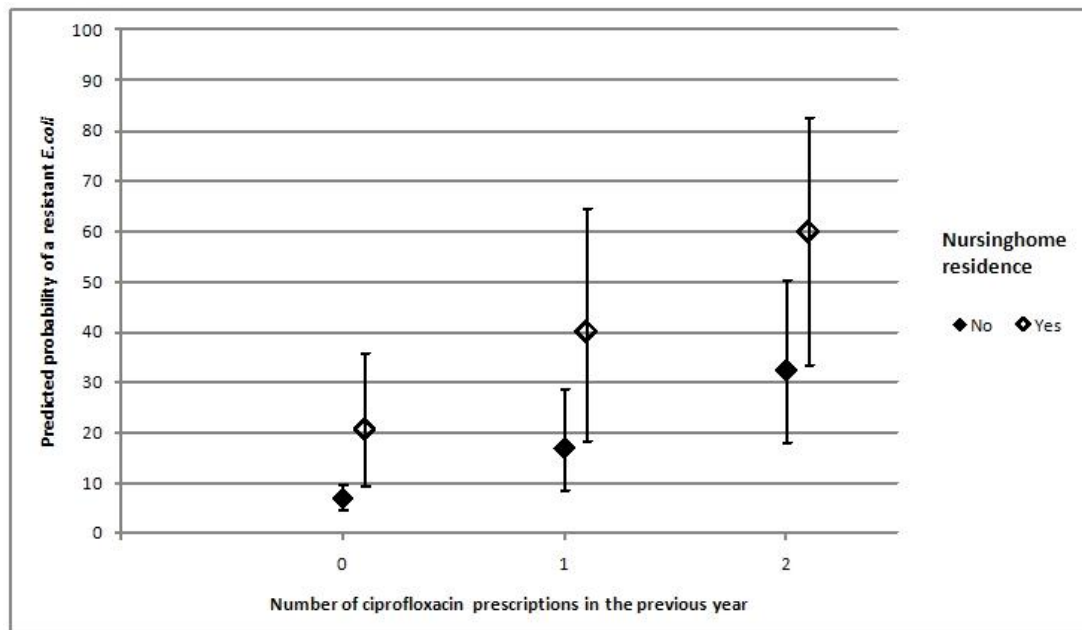


Figure 7.2: Predicted probability of a ciprofloxacin resistant *E.coli* for patients in the community or in a nursing home.

*The predicted probability (and prediction interval (error bars)) of a ciprofloxacin resistant *E.coli* with an increasing number of ciprofloxacin prescriptions in the previous year and according to nursing home status was calculated based on the final model in which the other variables were set at their mean value. For a patient with UTI and two or more previous prescriptions of ciprofloxacin, the predicted probability in the community is just over 30% and double this (60%) in a nursing home.*

The cluster effect calculated in the ICC was relatively small for trimethoprim and larger for ciprofloxacin which means that the intercluster effect of ciprofloxacin was relatively more important while the individual factors were more important for explaining the variation in resistance to trimethoprim. A caterpillar plot showing the estimated residuals for all the practices in rank order was made based on the final model (Figure 7.3). The horizontal line at zero is the average resistance at practice level. The practice level residuals

are greater for ciprofloxacin, which showed a higher variability in antimicrobial resistance between practices for ciprofloxacin compared to trimethoprim.

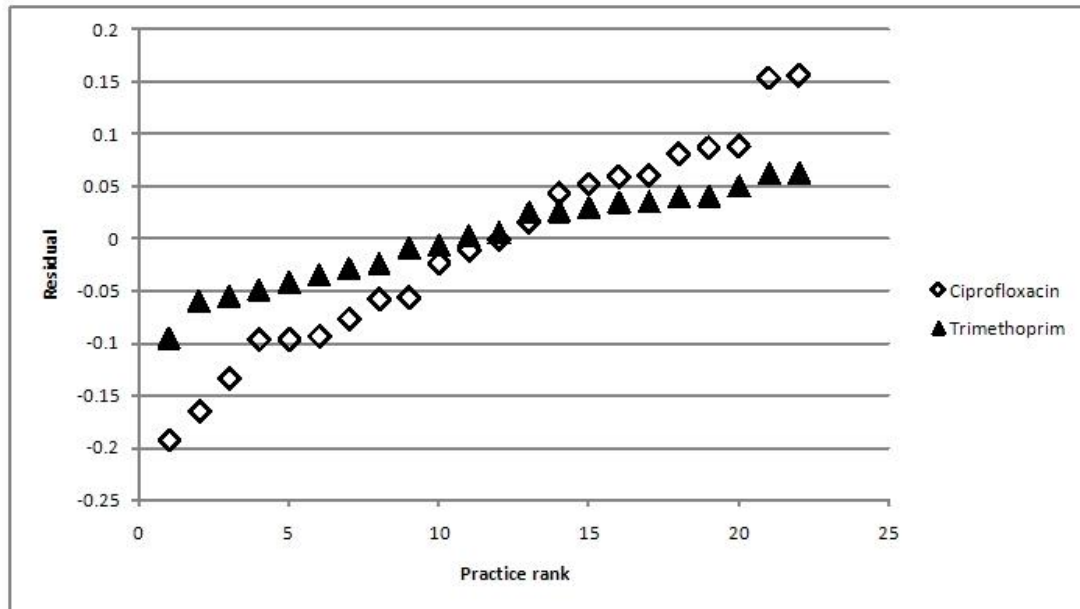


Figure 7.3 Practice level residuals for trimethoprim and ciprofloxacin resistance.

Caterpillar plot of the residuals (the difference between the observed values and predicted values according to the calculated model) of each practice. The further the residual is from the horizontal line at zero, the more extreme variation in resistance levels between the practices. If there was no practice effect, all points would lie on a horizontal line. Practice residuals were ranked from low to high to produce this plot.

The cluster effect or practice level variance is also reflected in the mOR; the higher the mOR, the more important the difference between practices is. The mOR for trimethoprim is 1.17 (CrI 1.03-1.46) and 1.33 (CrI 1.03-1.90) for ciprofloxacin. This can be interpreted as an increased risk of 17% for trimethoprim or 33% for ciprofloxacin of a UTI with a resistant *E.coli*, had the patient consulted in another practice with higher risk. Comparing the relevance of variables for understanding variations in the odds of a resistant *E.coli*, the impact of the person's previous prescriptions was of highest relevance but the between practices variation was similarly important (mORs with similar magnitude). The decrease of mOR from the empty to the full

model for ciprofloxacin suggests that the practice variance is due to practice level variables and not due to a different individual composition of the practice. For trimethoprim no difference between the empty model and the final model was observed indicating that the (small) variance observed between practices was not due to differences in the introduced individual or practice level variables.

A number of additional analyses were performed. Firstly, excluding the extended-spectrum beta-lactamase⁵ (ESBL's) *E.coli* from the multilevel analysis resulted in the non-significance of nursing home residence for ciprofloxacin. Trimethoprim resistance was not significantly affected by nursing home residence in either model. Secondly, a trimethoprim resistance model was run replacing trimethoprim prescribing with the combination of ampicillin and trimethoprim prescription in the previous year but this did not improve the model. Thirdly, differences in hospitalisation (with and without emergency admission and outpatient visits) were compared as well as the period of admission (one year or six months). No important differences were observed.

7.3.4 Power

To assess the power of the study, a model with dichotomised prescribing in the previous year was fitted (yes/no previous prescribing). The odds ratio for the prescription of trimethoprim in the previous year was 2.1 (95% CI 1.4-3.0) and for ciprofloxacin 3.9 (95% CI 2.2-7.1). The design effect, a correction for the cluster effect, was calculated as $\rho(m-1)+1$ where ρ is the intraclass correlation coefficient and m is the average number of patients per cluster (Eldridge, Ashby *et al.* 2006). The design effect for trimethoprim was 1.29 and for ciprofloxacin 2.4. The corresponding calculated power adjusted for clustering was 92% for the model with trimethoprim and 86% for ciprofloxacin.

⁵ Extended-spectrum-beta-lactamases (ESBL) are enzymes that can be produced by bacteria making them resistant to beta-lactam antibiotics. ESBL producing organisms are often also resistant to quinolones and aminoglycosides.

7.4 Discussion

The main findings of this study were that increased previous prescribing increases the individual odds of being diagnosed with a resistant *E.coli* UTI and the importance of the practice in this propensity. The increasing odds ratios with an increasing number of antimicrobial prescriptions supports the causality principle of dose-response relation. The large odds ratio for trimethoprim after two previous prescriptions and even more so for ciprofloxacin with significant and high odds ratios after only one previous prescription, warrants prudent use of these antimicrobials. It should also be noted that the data analysis did not differentiate between the types of infection for which the antimicrobial was prescribed, which in particular was important for ciprofloxacin as this agent is also prescribed for respiratory infections.

Another variable significant in the ciprofloxacin model was nursing home residence. The increased odds for nursing home residence was associated with the higher prevalence of ESBL infections in nursing homes. The problem of nursing homes as a reservoir for these highly resistant *E.coli* has been described previously, and independent risk factors associated with increased ESBL carriage were found to be increased ciprofloxacin use and UTI infections (Rooney, O'Leary *et al.* 2009). The finding that all other factors (except age) were not significant showed that the chance of being diagnosed with a resistant *E.coli* UTI is mainly driven by previous individual prescribing and practice related factors.

This analysis allowed for the clustering of data at practice level which was shown to be of importance in a previous published paper (Vellinga, Murphy *et al.* 2010). Even though the clustering was quantified as substantial in the mOR, this practice level variation in the outcome could not be explained by either practice prescribing or practice resistance levels. A multilevel analysis of retrospective data, including practice prescribing instead of individual prescribing, showed similar results for practice level variation. This suggests

that with only the individual prescribing significant in the prospective model, it is not practice level prescribing (or resistance) conveying the practice propensity of a resistant *E.coli* UTI. This could be a result of the relatively small numbers in the database. The data used for the retrospective study were much larger (72 practices and more than 14,000 individuals) which increased the chance of finding significant associations. Secondly, other practice-related factors, not included in this study, might be associated with practice variation in the outcome. An example of such a factor could be the geographical spread and overlap with other practices of the practice population.

As mentioned, the main limitation of the study lies in the limited number of practices and the relatively low cluster size, even though the power of the study was sufficient. It has been shown that small group size may lead to type II error while the fixed effect parameters were unbiased (Moineddin, Matheson *et al.* 2007; Theall, Scribner *et al.* 2009). Publication of our data might urge other research groups to share their data in an aim to increase the overall sample size. Additionally, as the mOR is time- and place-specific (Merlo, Ohlsson *et al.* 2009), the importance of practice level variation can be further analysed by the inclusion of data from other centres.

Selection bias threatens the validity of many epidemiological studies. In our study, however, a selection bias is not expected to be of importance as an opt-out methodology was used for the inclusion of patients. We showed that the use of this methodology resulted in an opt-out of 14% and that there was no difference in opt-out between patients with and without an organism isolated from their urine sample (Vellinga, Cormican *et al.* 2011).

Practice variation is a common phenomenon that may reflect different therapeutic approaches to a similar health problem (Dalemo, Hjerpe *et al.* 2010; Hjerpe, Ohlsson *et al.* 2010) or may reflect an underlying, possibly geographical, trait (Chaix, Merlo *et al.* 2005). Even though our multilevel approach controlled for confounding by including practice and patient level factors, none of the included factors at the practice level could explain the

variation between practices. Identification of these unidentified factors is necessary to understand the occurrence and spread of resistance as well as to design appropriate interventions. It can be suggested that an administrative boundary like the practice is not appropriate to study antimicrobial resistance but more data to aggregate at different levels are necessary to investigate this suggestion. However, it is clear that prudent prescribing of trimethoprim and ciprofloxacin to individual patients is important as it is highly associated with the probability of a resistant *E.coli* UTI.

7.5 How this compares

Most papers include the time since the last prescription of the antimicrobial in their analysis and generally show higher odds ratios for prescriptions given closer to the episode (Donnan, Wei *et al.* 2004; Hillier, Roberts *et al.* 2007). None of these studies looked at previous ciprofloxacin use. For trimethoprim, increased previous prescribing of trimethoprim and subsequent resistance was identified by Hillier *et al.* (Hillier, Roberts *et al.* 2007) who also found an association between number of courses of trimethoprim and resistance, even though they did not find a significant trend (OR 2.08 (95% CI 1.34-3.22) for one dose, 2.05 (95% CI 0.85-4.94) for two doses and 7.53 (95% CI 2.71-20.88 for three or more doses). The sample size in their study was comparable to ours but the prevalence of trimethoprim resistance in our study was higher (17% vs 36%) which might explain the lack of a significant trend in their study. Metlay *et al.* also found a strong relationship between the number of any antimicrobial courses received in the preceding six months and the likelihood of a trimethoprim-sulfamethoxazole resistant UTI in male veterans, but not for other antimicrobials (Metlay, Strom *et al.* 2003). Hay *et al.* (Hay, Thomas *et al.* 2005) did not find an increase in resistance with increasing previous courses, but found an increase of 1% in the odds of resistance for every trimethoprim tablet prescribed. Additionally, this study was a population cross sectional study, while only patients with suspected UTI were included in the other studies. Even though some of these studies included practice variables, all data were analysed at the individual level only and none of the analyses allowed for the cluster effect by separating the practice and individual levels. Similarly, to our knowledge, no other study has simultaneously included practice and individual level prescribing in the analysis of individual level resistance data. Studies analysing individual resistance data include either individual prescribing of an antimicrobial agent (Metlay, Strom *et al.* 2003; Hay, Thomas *et al.* 2005; Hillier, Roberts *et al.* 2007; Colgan, Johnson *et al.* 2008; Colodner, Kometiani *et al.* 2008) or practice (area) prescribing (Steinke,

Seaton *et al.* 2001; Donnan, Wei *et al.* 2004). Our study showed that when previous practice prescribing as well as previous individual prescribing is included, only the individual prescribing is a significant predictor of a UTI with a resistant *E.coli*. More studies are necessary to confirm our findings.

However, if it can be replicated in other studies and other areas, such results may also help to identify other practice or area factors that are of importance in explaining practice or area level variance.

7.6 Conclusion

The more trimethoprim or ciprofloxacin prescribed in the previous year, the higher the odds of an *E.coli* UTI resistant to this agent. Visiting a different practice can also influence this chance of a resistant *E.coli* UTI. None of this variation could be explained by practice resistance or prescribing levels. The chance of being diagnosed with a resistant *E.coli* UTI is mainly driven by previous prescribing and practice related factors.

Chapter 8: Bringing it all together

8.1 Summary of key findings

- The retrospective multilevel analysis of trimethoprim and ciprofloxacin showed that practice prescribing of trimethoprim and ciprofloxacin was associated with the individual probability of an *E.coli* UTI resistant to the respective agent; the higher practice prescribing, the higher the probability. (Primary research objective 1).
- Practice level variation was higher for ciprofloxacin than it was for trimethoprim, suggesting that before resistance to an antimicrobial agent becomes widely disseminated in the community, variations in prescribing behaviour might have a greater impact on selection for resistance.
- The analysis of recurrent UTI confirmed the impression that susceptibility test results from a previous UTI can guide subsequent UTI treatment. A test result from a previous UTI showing *E.coli* resistant to trimethoprim or ciprofloxacin warrants against the use of this antimicrobial in a subsequent episode for a period up to a year. Similarly, a susceptible previous infection indicates the likelihood that treatment of the current UTI with these antimicrobials will be successful. (Secondary research objective 2).
- Nitrofurantoin resistance was very uncommon and if a resistant infection was detected, the chance of a subsequent infection with a resistant *E.coli* within three months was negligible which promotes nitrofurantoin as a beneficial first line agent for initial and repeat presentations.
- The opt-out methodology was generally well accepted. Participation reached 86%. The 14% of patients opting out suggests that patients understood the process and effectively felt assured to decline participation
- Antimicrobial prescribing for UTI in general practice is variable. Whereas 56% of patients receive empirical antimicrobials, only 37% of these are according to

the recommended first line guidelines for prescriptions (trimethoprim or nitrofurantoin). Only 21% of the submitted urine samples showed significant growth. (Secondary research objective 1).

- Empiric treatment of UTI as prescribed by the GP would give similar results to standard prescribing of nitrofurantoin only or ciprofloxacin only. However, standard prescribing of trimethoprim only would not reach the same level of appropriately treated patients due to higher resistance levels in the community. Treatment with nitrofurantoin only was cheapest while treatment with ciprofloxacin only was the most expensive empiric treatment.
- An increased number of prescriptions of trimethoprim or ciprofloxacin to an individual was associated with an increased probability of that individual being diagnosed with a UTI with an *E.coli* resistant to the respective agent. (Primary research objective 2).
- Between practice variation was important and a patient's probability of a resistant *E.coli* infection depended on the practice he or she visits. This practice variation could not be explained by resistance or prescribing levels of the practice.

8.2 Study set-up

The study was set up in two distinct parts, a retrospective and a prospective part. This set-up has shown a number of advantages. Firstly, the retrospective analysis study informed the set-up of the prospective study. Data collection was guided by the significance of variables in the retrospective analysis. Secondly, the quantification of the variation between practices showed similar mOR in the retrospective and prospective studies. The consistency of the mOR between the results from the retrospective study and the prospective study was particularly interesting as the mOR is time- and place-dependent. Third, the correlation between the aggregated practice prescribing according to the HSE-PCRS and the detailed prospective study information showed that the HSE-PCRS is a good proxy, but also that this practice prescribing is explained by the individual level prescribing; a model including both practice prescribing and individual prescribing showed that individual prescribing explains all the variation between antimicrobial prescribing and resistance.

8.3 Prescribing and resistance

The retrospective study showed that for every prescription per 1000 practice population the odds for a trimethoprim resistant *E.coli* increased by 1.02 (95% CI 1.01-1.04) and for ciprofloxacin the odds increased by 1.08 (95% CI 1.04-1.11) for every prescription of ciprofloxacin per 1000 practice population.

The retrospective study calculated a prescription rate per month per 1000 practice population from the overall number of prescriptions prescribed by GPs associated with the practices. The number of prescriptions was obtained from the HSE-PCRS, a database of all medication prescribed to medical card patients. The practice population was calculated from the sum of the number of medical card patients registered with each practice. Similar to other ecological studies, our retrospective analysis showed an association between prescribing and resistance and in addition quantified this association for the individual patient with the use of multilevel statistical methods. However, a main concern with the use of the medical card database was highlighted in the prospective study on management of UTI in general practice. Not all practices have an equal distribution of medical card patients and private patients. In addition, the data indicated that prescribing to medical card and private patients might be different. This detail could not emerge from the retrospective analysis as no data on the ratio of private/medical card patients were available. The retrospective study showed clearly though, that the number of prescriptions per patient per month, increases the patient's chance of having a resistant *E.coli*.

To understand the detail of this association, the prospective study was set up to quantify the direct association between prescribing and resistance. The prospective study found an association between previous individual prescribing and odds for the individual of a subsequent resistant *E.coli*. The odds ratio was 1.4 (95% CI 0.8-2.2) for patients with one previous prescription of trimethoprim, increasing to 4.7 (95% CI 1.9-12.4) and 6.4 (95% CI 2.0-25.4) for two and three prescriptions of trimethoprim in the

previous year compared to patients with no previous prescriptions of trimethoprim. For ciprofloxacin, the odds were 2.7 (95% CI 1.2-5.6) and 6.5 (95% CI 2.9-14.8) for one and two previous prescriptions of ciprofloxacin respectively compared to patients with no exposure to ciprofloxacin in the previous year. The combination of the results from the prospective and retrospective studies clearly indicate that prescribing in general, as well as individual prescribing of antimicrobials, influences the individual risk of a resistant *E.coli* to this agent.

8.4 Increased prescribing and resistance

An indirect as well as a direct association between antimicrobial prescribing and resistance exists for the individual, and this association was shown to be dose dependent. According to the retrospective study, increasing practice prescribing from 10 to 20 trimethoprim prescriptions per 1000 patients increased the patients' chance of a trimethoprim resistant *E.coli* from 27% to 32%. Increasing ciprofloxacin practice prescribing from 10 to 20 prescriptions per 1000 patients increased the patients' chance of a ciprofloxacin resistant *E.coli* from 6% to 11%. According to the prospective study, at the individual level, the probability of a trimethoprim resistant *E.coli* increased from 33% for an individual without any trimethoprim prescriptions to 40% with one prescription of trimethoprim, to 68% for two and to 74% with three or more trimethoprim prescriptions in the previous year. For ciprofloxacin, the probability of a ciprofloxacin resistant *E.coli* increased from 7% for an individual without any ciprofloxacin prescriptions to 18% for one and to 34% for two or more ciprofloxacin prescriptions in the previous year. The dose dependent association adds evidence to the direct and indirect causal relation between antimicrobial prescribing and resistance.

8.5 Persistence of resistance

The retrospective study showed in the analysis of the variance between practices that this was higher for ciprofloxacin compared to trimethoprim, and a similar conclusion could be made in the prospective study. Additionally, the prescribing of ciprofloxacin has a relatively higher substantial impact on subsequent ciprofloxacin resistance than was seen for trimethoprim. The analysis of repeated UTI infections adds to this knowledge as it shows that the individual ‘wash-out’ of resistance is slow; once an individual has a trimethoprim resistant *E.coli*, a subsequent infections within three months is very likely to be still resistant (80%) as is one at 9-12 months (60%). For ciprofloxacin, 86% of the subsequent infections within 3 months are still resistant and 46% up to a year. The higher three month positive predictive value and lower 9-12 month PPV of a resistant ciprofloxacin *E.coli* compared to trimethoprim could also be due to this higher substantial impact of ciprofloxacin prescribing compared to trimethoprim. Additionally, the higher overall resistance levels for trimethoprim (the wider dissemination of trimethoprim) also resulted in smaller differences between practices.

Antimicrobial cycling is sometimes suggested to contain the spread of antimicrobial resistance. However, the combination of factors as described above suggests that antimicrobial cycling would not be an effective method as the rise of resistance and the subsequent ‘wash out’ would need to be discrete as well as non overlapping time periods. The time period for ‘wash-out’ has been shown to be much longer than the time period in which antimicrobial resistance emerges.

8.6 Cross-over effects

Practice prescribing according to the HSE-PCRS was associated with individual risk of a resistant *E.coli* as well as with practice risk of a resistant *E.coli*. Previous individual prescribing was not limited to UTI and showed increased risk of a resistant *E.coli* infection with an increasing number of previous prescriptions. This shows that it is antimicrobial prescribing in general, for any type of infection, which affects the risk of a resistant *E.coli* UTI.

The strength of the association would potentially be higher if only patients without any previous prescriptions were taken into account (as the measurement of exposure would be more extreme). Similarly, potential cross-resistance would potentially be eliminated if controls (for the prospective study) were only patients with an *E.coli* infection susceptible to all tested antimicrobials. However, the sample size of the prospective study would not allow such a comparison. Also, the comparison as made in our analyses better reflects the actual clinical situation.

8.7 Practice variation

Practice variation was demonstrated in a number of ways. Firstly, the variation in resistance between practices was different for ciprofloxacin and trimethoprim, as shown in the caterpillar plot in both the prospective and retrospective studies (Figure 3.8, page 64 and Figure 7.3, page 142). These caterpillar plots showed the average resistance at practice level and rank the practices in their deviance from this average. The variation was markedly higher for ciprofloxacin compared to trimethoprim for both models. Practice variation was also calculated by the mOR (median odds ratio) which can be interpreted as a higher level (practice) odds ratio for the average patient; patients could theoretically increase or decrease their chance of a resistant *E.coli* by moving practice. The outcome of both studies showed similar results; there was important variation between practices, the mOR was higher for ciprofloxacin compared to trimethoprim and the size of the mOR was also comparable. The consistency of the findings in the prospective and retrospective studies confirms that higher level variation is of importance in the risk of a resistant *E.coli* UTI. The difference in practice variation was however not significant for the prospective study, which was most likely due to the small cluster size for some of the practices.

Finally, differences between practices were clear when comparing practices in their prescribing behaviour. Guidelines for antimicrobial prescribing in general practice were not always followed; variation in prescribing between practices was high and showed a high level of autonomy in the choice of antimicrobial prescribing behaviour of the GP.

The correlation between prescribing according to the HSE-PCRS and practice prescribing as recorded in the prospective study was significant for all antimicrobials except ampicillin. This means that the percentage use of each antimicrobial according to the HSE-PCRS did broadly overlap with the prescribing recorded in the practice. Differences between these databases were due to the unknown ratio of private and

medical card patients as well as the type of infection the antimicrobial was prescribed for. The difference in prescribing was not expected to be due to differences in practice population as the mOR was stable and decreased with adding individual and practice level factors (an increasing mOR could hide a different individual composition of the practice).

8.8 Methodological considerations

8.8.1 Issues concerning sampling

The selection of the practices in the prospective study was based on the data from the year previous to the set up of the study. Some of the practice composition changed dramatically due to the loss or inclusion of a GP. Both had an impact. Due to the loss of a GP the number of patients seen and therefore the number of samples collected decreased, resulting in a lower number of urine samples and isolates than originally anticipated. The (temporary) replacement of a GP was also highlighted as an issue as prescribing patterns of a replacement GP could be very different from the patterns found in the retrospective study. Even though worth mentioning, such changes could not be incorporated into the analysis of the results.

Sample size for the retrospective study was sufficient. However, the numbers in the prospective study were limited. Power calculations showed that, including the design effect, sample size was sufficient to find statistically significant differences in the occurrence of trimethoprim/ciprofloxacin resistant *E.coli* UTI between patients who did and patients who did not have prescriptions of trimethoprim/ciprofloxacin in the previous year. However, power and sample size calculation simulations for multilevel studies have shown that minimum cluster sizes and minimum numbers of clusters are advisable to be able to detect contextual effects (effects on the individual outcome resulting from group level variables) (Moineddin, Matheson *et al.* 2007; Theall, Scribner *et al.* 2009). These papers suggested that small group size may lead to type II error (failing to find a true significant result). The borderline significance of resistance and/or prescribing levels of the practice might be an example. Conversely, in the retrospective study, with more than 14,000 individual patient results, a borderline significant result would be given relatively less weight due to the higher chance of finding a significant result with increased sample size.

8.8.2 Opt-out methodology

The application and acceptability of the opt-out methodology has been one of the major successes of this thesis. The process of obtaining approval for this method of patient enrolment through the research ethics committee was cumbersome. However, as a result of the implementation of this opt-out methodology participation was particularly high and the results representative. Additionally, this method resulted in a minimal increase in workload for the general practitioner as well as the laboratory, and data collection was completed within a relatively short time frame. The combination of easy applicability and general acceptability are reflected in the completeness of the data as well as the population sample which was considered representative. The positive responses, in particular phone calls and letters from patients, were a welcome reinforcement of the relevance of the study.

The use of an opt-out methodology has resulted in a participation of 86% in the prospective study.

8.8.3 External generalisability

The findings from the study on re-infection are generalisable to the general population. For the multilevel analyses, both retrospective and prospective, the fixed effects can also be generalised. However, the analysis of variance and its interpretations are constricted by time and space. From the multilevel analyses it can be concluded that there is an association between the practice and the risk of a resistant *E.coli* UTI (in general), but for quantifying the relevance of the practices, an analysis of variance (mOR) is needed and its interpretation is specific to the West of Ireland. Inclusion of data from similar studies would be very interesting in this respect.

An additional remark regarding generalisability is on the inclusion of males and females in our study population. Many studies report on females and males separately

(for instance (Lipsky 2000; Metlay, Strom *et al.* 2003; Hummers-Pradier, Koch *et al.* 2005; Hummers-Pradier, Ohse *et al.* 2005)); however, in our study both males and females were included and differences in outcome were controlled for by the inclusion of gender in the statistical analyses.

8.8.4 Internal validity

Issues concerning selection bias

A potential bias, raised by the editor in response to the retrospective study paper, was that the use of the laboratory could vary between practices and over time. According to the editor ‘It is known that GPs do not send samples from all patients presenting with UTIs. Some may only send samples, for example, when there is a recurrence, which would of course show a higher rate of resistance than unselected pre-treatment samples. If there were a change in practice over the period of the study then the apparent increase in resistance might therefore be entirely or partially an artefact’.

With the prospective study this concern was proven to be unfounded. During the prospective study, GPs were asked to send in urine samples from all patients with suspected UTI. The number of samples during the prospective study was similar to the number during the same period in previous years.

Issues related to an information bias

The microbiological analyses were performed at the Department of Medical Microbiology, GUH which is an accredited diagnostic laboratory (ISO 15189). No information bias was therefore anticipated in the microbiological measurements. Prescription data from the HSE-PCRS are also unlikely to be influenced by information bias as the collection of these data happens in a standard fashion, irrespective of any research study or study outcome.

General issues concerning bias in the prospective study

Prescription data and other patient's characteristics for the prospective study were collected from medical records in general practice. The accuracy of data recorded in general practice has been shown to be high (Hassey, Gerrett *et al.* 2001).

Additionally, using the recorded data reflects the reality of decision making in prescribing (based on the recording of previous prescriptions and results of previous urine samples) by the GP.

The fact that only one researcher recorded data, guaranteed the consistency in data collection. A potential observer bias due to knowledge of the outcome (resistance or not) was avoided by blinding the researcher at the time of data collection. A common bias in case-control studies is the Berkson's bias, a systematic difference due to the selection of cases and controls. However, as the cases and controls in our prospective study originated from the same source population and were selected simultaneously within the same surveillance system, a Berkson's bias was unlikely.

The Hawthorne effect, the effect of being under study, has been described in research on antimicrobial prescribing by community paediatricians. The antimicrobial prescriptions for viral infections fell by 29 percentage points during the observational study when compared to the retrospective file analysis (Mangione-Smith, Elliott *et al.* 2002). If such an effect existed in our prospective study, this would be limited as contact with the GP was kept to an absolute minimum (maximum three visits) and the duration of the study was nine months. In the invitation letter (Appendix 6) emphasis was on the study of the interaction between prescribing and resistance and did not mention interventions or restrictions.

Confounding

Confounding is caused by variables that cause or prevent the outcome without being an intermediate. In our study, with the use of multilevel analysis, adjustments for confounding variables could be made, without necessarily knowing the precise

variables; the effects of processes at different levels were separated and distortion was accounted for. Residual confounding is however always a possibility as there may be other unmeasured and unknown factors that have affected the outcome of the study. For instance, the indirect measure of socio-economic status was the SAHRU index (Kelly 2009) in the retrospective study but this was measured directly through medical card status of the patient in the prospective study. Both measures showed to be of little significance but might distort the effect of another exposure on the outcome through an association with this other unknown factor (differential prescribing). This could not be assessed within the analyses of this thesis.

Reverse causation

Reverse causation is a methodological weakness of retrospective studies. This complex phenomenon questions the time relation between an exposure and outcome by assessing whether the cause (exposure to antimicrobials) precedes the effect (resistance to antimicrobials) (Kummeling and Thijs 2008). Antimicrobial prescribing in the retrospective study was obtained from the HSE-PCRS at the practice level and no individual interference could be made regarding the timing of the antimicrobial intake. In the prospective study however, the timing of the antimicrobial intake was recorded and the sequence of events was clear. Additionally, reverse causality was eliminated as a possible explanation by the increasing effect of increasing dose, suggesting a dose-response effect.

8.8.5 Boundary issues

A critical point in multilevel analysis is the level at which data were aggregated, in this study the practice level. For an individual the limits that define the human body are obvious, the boundaries of geographic environments are, however, less obviously delineated (Subramanian, Glymour *et al.* 2007; Merlo, Ohlsson *et al.* 2009). The validity of administrative boundaries in the study of antimicrobial resistance could be

criticised and even though the practice level was a convenient measure to aggregate data, this did not seem to be enforced by the results of the analysis resulting in relatively small measures of association (ICC).

Unpublished, preliminary results from 104 *E.coli* isolates from patients from two of the participating practices were further analysed using Pulse Field Gel Electrophoresis (PFGE) to determine the degree of similarity (Tansey 2010). Twenty clusters of two or more isolates with >85% homology were identified including four (comprising two isolates each) that were indistinguishable. The two practices were in roughly the same geographical area but patients would generally not be shared.

These results add to the suggestion posed in the discussion of the prospective study that the practice level might not be important as such and that the multilevel analysis may need a higher level transcending the practice level. It would be interesting to do a similar retrospective study to see the extent to which it is the practice or the area that can explain the practice/higher level variation. An expansion of the analyses would be to introduce a (third) higher level variable aggregating the practices within geographical areas.

8.9 Discussion

The tragedy of the commons (Hardin 1968; Foster and Grundmann 2006) depicts the urgency of the need to understand how antimicrobial resistance spreads in a population, which factors influence this trend, and maybe most importantly, how to communicate this urgency to patients and health-care practitioners. The idea that antimicrobials are a limited source, like oil, depleted by its own use has given rise to papers like ‘How you can reduce your “resistance footprint”’ (Patrick and Hutchinson 2009). Even though it was clear that factors related to the individual as well as to the population at large were important, existing statistical methods and appropriate data were not available. The first published ‘resistance’ studies used population level studies (ecological studies) in which exposure (prescribing) and outcome (resistance) were measured at an aggregated level. An ecological analysis is, however, prone to errors in inference because associations may be artefactually created or masked by the aggregation process (Last 2003; Porta and Last 2008). Subsequent individual level studies produce direct associations between prescribing and resistance for the individual but do not take the importance of its impact on a wider population into account. Resistance may have emerged from the prescription of antimicrobials, but the spread can be through various routes, from food to personal contacts (Steinke and Davey 2001; Gupta, Hooton *et al.* 2011). This thesis has made a significant contribution to the use of statistical methods which model the possible influence of different levels on the outcome. The application of multilevel logistic regression allowed for the separation of group and individual levels in the analysis of the odds of a resistant *E.coli* UTI and showed that both previous individual prescribing

The use of multilevel regression techniques that separate the group and individual levels showed to be an important methodological improvement in the analysis of (the spread of) antimicrobial resistance.

and practice variation were important factors in the propensity of a resistant *E.coli* UTI.

For the community, the probability of a resistant *E.coli* UTI does not only depend on factors related to the individual. Practice or area level factors are involved in the chance of being diagnosed with a UTI caused by a resistant organism. In both the retrospective and prospective studies the difference between practices in their odds of resistant *E.coli* infections could be quantified and were similar in both studies; however none of the practice factors related to this difference could be identified. The higher variability between practices for ciprofloxacin compared to trimethoprim was previously suggested to be related partly to the mechanisms of resistance (Enne 2010; Vellinga, Murphy *et al.* 2010). Trimethoprim resistance in *E.coli* is associated with horizontal transmission of plasmids (Huovinen 2001; Skold 2001) and these may encode for resistance to one or more agents other than trimethoprim (Blahna, Zalewski *et al.* 2006). Resistance may be selected for and maintained by trimethoprim as well as other antimicrobial agents. Resistance to ciprofloxacin in *E.coli* is classically associated with point mutations in the chromosome (Strahilevitz, Jacoby *et al.* 2009). Additionally, the time since the introduction of trimethoprim (1962) is much longer than that of ciprofloxacin (1980s). The ‘opportunities’ for genetic determinants of trimethoprim resistance to spread and maintain themselves may be greater compared to those of ciprofloxacin. This in turn might be a factor in the success of interventions; antimicrobials with less established and disseminated resistance levels, i.e. more variation in resistance levels between practices, might be more likely to show an impact of changing prescribing and vice versa (Gottesman, Carmeli *et al.* 2009; Sundqvist, Geli *et al.* 2010). The success of an intervention in

This thesis poses the hypothesis that interventions could be more successful for antimicrobials with less established and disseminated resistance levels.

limiting or reversing resistance depends on previous extent of use of the agent (time and volume) as well as on specific proportions of the agent related to the mechanism of action/resistance. Although counter-intuitive, this is not in disagreement with our finding from the prospective study that practice resistance or prescribing levels were not found to be of importance in the probability of a resistant *E.coli* UTI. The practice level might not be important as such, and maybe factors influencing the probability that a UTI is caused by a resistant *E.coli* are not related to the practice level but to a higher (geographical) level.

Findings from the prospective study also revealed that antimicrobial treatment guidelines are not widely implemented in general practice. At the same time, it is not clear at which point guidelines should be altered to adjust for increasing resistance levels in the community. A cut-off of 10-20% has been suggested in relation to trimethoprim, above which empiric treatment of UTIs with this agent should be switched to another antimicrobial (Warren 2001; Gupta, Hooton *et al.* 2011). The rationale for this cut-off is mainly related to clinical and economic considerations but it does not take into account other factors that may influence these decisions, such as the effect that antimicrobial use has on antimicrobial susceptibility in the community or other societal costs (Miller and Tang 2004; Foster and Grundmann 2006; Gupta, Hooton *et al.* 2011). Depending on the antimicrobial and its mechanisms of resistance, it seems that a critical group/population level of resistance exists above which the resistance is widespread and persistent and interventions limiting antimicrobial use are likely to be less effective. As a result, efforts to decrease resistance might not show any change in resistance levels in the community (Enne 2010; Sundqvist, Geli *et al.* 2010). Guidelines should reflect the balance between individual treatment efficiency and the spread of antimicrobial resistance in the community and include more specific community information or additional information on empiric treatment.

Guidelines on antimicrobial treatment are not widely implemented in general practice.

The practical implication of this thesis can be translated into prescribing guidelines. For an individual with a suspected UTI the results from this study show that the first empiric treatment to consider should be nitrofurantoin, irrespective of the resistance of a previous *E.coli* infection. Nitrofurantoin is also the cheapest option to treat the patient. Additional efficiency can be obtained by dipstick analysis of the urine sample to exclude prescribing of nitrofurantoin for patients with alkaline urine (often resulting from an infection with *Proteus spp.* and for which nitrofurantoin would not be advised) (Simerville, Maxted *et al.* 2005). If trimethoprim is preferred, consideration should be given to the level of trimethoprim resistance in the community, previous trimethoprim resistant *E.coli* UTIs and previous trimethoprim prescriptions. If one or more of these factors are met, the likelihood that treatment with trimethoprim will not be successful is high. With two good antimicrobials for the treatment of infections as well as further guidelines on their application, ciprofloxacin should be prescribed prudently in the treatment of uncomplicated UTI (Foster and Grundmann 2006). Not only is treatment with ciprofloxacin more expensive, the odds of resistance increases dramatically after just one prescription. Even though the chance of successful treatment of the individual is high, this will lead to increasing population resistance levels, thereby decreasing the applicability of the antimicrobial in the long run. The IDSA recommendations on treatment of UTI with fluoroquinolones once a community resistance cut-off level for trimethoprim of 20% is reached need more consideration beyond the economic and clinical features (Naber 2000; Miller and Tang 2004; Gupta, Hooton *et al.* 2011).

Nitrofurantoin should be considered as first line empiric antimicrobial treatment of UTI, as it has very low resistance levels, resistance is not widely disseminated and treatment is cheap.

8.10 Recommendation for intervention

Interventions aimed at curbing the spread of resistance in the community are, for practical reasons, mainly at the level of prescription of antimicrobials. The link between exposure to antimicrobials and the development of resistance has been reiterated at individual and at population level, separately and in combination (Arnold and Straus 2005). According to this Cochrane review on interventions up to 2005, one approach to reducing the incidence of infections caused by antimicrobial resistant organisms is to reduce the inappropriate use of antimicrobials in hospital and community settings. The review concludes that there is no single intervention that can be recommended to improve antimicrobial prescribing; multifaceted interventions with educational material for the prescriber and the patient as well as the use of delayed prescription can potentially decrease the use of antimicrobials. These conclusions were reiterated in a similar review on interventions to reduce unnecessary antimicrobial prescribing (Ranji, Steinman *et al.* 2008). Most studies addressed prescribing for acute respiratory infections and only these multifaceted interventions showed effect sizes big enough to potentially reduce the incidence of antimicrobial resistant bacteria; only four studies assessed the effect of the intervention on antimicrobial resistance in the community. An educational intervention resulted in a significant reduction of macrolide resistance in group A streptococcus isolates over two years (Seppala, Haanpera *et al.* 2003) but the other three community, educational interventions showed no significant correlation between reduced penicillin use and carriage or rate of penicillin non-susceptible streptococcus pneumonia (Belongia, Sullivan *et al.* 2001; Hennessy, Petersen *et al.* 2002; Perz, Craig *et al.* 2002).

Policy interventions seem to be more successful and two recent interventions are of particular interest in this context. Firstly, a 24 month voluntary restriction of trimethoprim in a Swedish county showed a disappointing, though very small, increase in resistance of *E.coli* from UTI (Sundqvist, Geli *et al.* 2010). In contrast, in a

retrospective, ‘natural’ experiment the overall proportion of quinolone susceptible *E.coli* from UTI was compared between the periods before, during, and after a nationwide restriction on ciprofloxacin. It was found that reducing quinolone consumption lead to an immediate increase in the proportion of quinolone susceptible *E.coli* isolates (Gottesman, Carmeli *et al.* 2009). A possible implication of this could be that the choice of antimicrobial on which an intervention focuses, is more likely to be an antimicrobial which is less disseminated and with resistance levels still relatively low.

The communication of the importance of population consequences of individual prescribing is a major challenge in the combat against the spread of resistance. A paper describing tensions in antimicrobial prescribing investigated preferred patterns of antimicrobial prescribing by physicians for patients with community-acquired pneumonia. Not only did the physicians prefer newer, broader spectrum agents, they also rated the issue of contributing to antimicrobial resistance lowest among seven determinants influencing their choice (Metlay, Shea *et al.* 2002). At the same time all participating physicians endorsed statements reflecting the physician’s concern over the societal influence of antimicrobial resistance.

Before setting up an intervention, consideration should be given to the methodology for measuring its effectiveness. A successful intervention in infectious diseases, which can be applied to the problem of antimicrobial resistance, will result in direct and indirect effects. The direct effect of an intervention received by an individual is the difference between the outcome in the individual with the intervention and what the outcome would have been without the intervention, all other factors being equal. The indirect effect of an intervention in an individual is the difference between what the outcome is in an individual not receiving the intervention in a population with an intervention programme and what the outcome would have been in the individual, again not receiving the intervention, but in a comparable population without an intervention programme (Halloran and Struchiner 1991; Diez Roux and Aiello 2005). To assess a potential impact of an intervention, burden of disease studies would be most

appropriate as they measure the rate of resistance which is independent of the susceptible population (Schwaber, De-Medina *et al.* 2004) (see also explanation in Text box 2, page 37). Consideration should also be given to the period over which the intervention is implemented as well as the area covered by the intervention.

Literature shows that nitrofurantoin is a safe and appropriate antimicrobial for first line treatment of UTI and obtains a high clinical cure rate of 88% - 93% and a bacterial cure rate of 81% - 92%. (Gupta, Hooton *et al.* 2011). Additionally, the emergence and persistence of resistance in *E.coli* UTI to nitrofurantoin is low and collateral damage, a term describing ecological adverse effects of antimicrobial therapy, is small. Studies comparing nitrofurantoin with trimethoprim or β -lactam empiric treatment in clinical trials showed no differences in short-term or long-term symptomatic or bacteriological cure. No studies have compared nitrofurantoin with ciprofloxacin empiric treatment. However, collateral damage has been described for fluoroquinolones. Moreover, studies of placebo for treatment of uncomplicated cystitis demonstrate that clinical cure can be achieved in 25%-42% of women who are not treated (Christiaens, De Meyere *et al.* 2002) or similar outcomes for ibuprofen compared to ciprofloxacin (Bleidorn, Gagyor *et al.* 2010). Our results added that nitrofurantoin resistance is not persistent and often disappears in subsequent UTIs whereas resistance against other antimicrobial agents, like trimethoprim and ciprofloxacin, is likely to be present in a subsequent infection.

A possible intervention should include decision trees and clear recommendations on the prescription of empiric antimicrobials in a user-friendly format (De Souza, MacFarlane *et al.* 2006). It was shown that there was a lot of variation in prescribing between different practices, suggesting that recommendations of the SARI committee (Strategy for the control of Antimicrobial Resistance in Ireland (SARI) 2008) were not followed. The co-operation of GPs in such an intervention study, by adhering to guidelines, would be vital for its success.

An intervention can be designed in which the first option for empiric prescribing for suspected UTI in general practice is nitrofurantoin. This intervention can be monitored and outcomes measured by GMS prescribing rates as well as a similar prospective study over a similar period of time which will allow for the comparison of rates (Schwaber, De-Medina *et al.* 2004).

8.11 Further research

A number of suggestions have been made throughout the thesis to improve and add to this research.

- Combining our prospective database with other databases with similar information on prescribing and resistance. This will increase the power of the study, in particular for the quantification of practice variation in resistance and/or prescribing. It will also add to the interpretation (and generalisability) of the variation (mOR) between practices as the mOR is time- and place-specific. An increased sample size might also allow for the aggregation of the data at a higher area level; by combining geographically close practices.
- No data on children were included due to the restriction of ethical approval to adults only. The inclusion of data on children will give information on the increase in antimicrobial resistance in individuals and a practice/area effect will be easier to study as antimicrobial history and interactions will be limited, particular in younger children. Setting up a cohort of children to follow up will allow the study of the increase and spread of antimicrobial resistance as well as colonisation of individuals with resistant organisms. The use of multilevel analysis techniques in this context has great potential for disentangling individual and area level influences in the risk of resistant infections.
- Further detailed analysis of the relative impact of patients' socio-economic status (medical card or private patient) on the prescription of antimicrobial treatment for UTI. Data are available from the prospective study.
- Analysis of re-infections in the prospective database. One of the limitations of the paper on 'Predictive value of antimicrobial susceptibility from previous urinary tract infection in the treatment of re-infection' (Vellinga, Cormican *et al.* 2010) was that previous prescribing would have an impact on the predictive value. The positive predictive value was defined as the proportion of patients with an *E.coli* resistant to an antimicrobial at first isolate that remain resistant to this

antimicrobial at the subsequent isolate. This calculation can be corrected/stratified for prescribing in between these episodes with the available data from the prospective study.

- During data collection a sense grew that some patients whose urine samples had mixed growth (no predominance of any particular organism) and to whom antimicrobials were empirically prescribed seemed more prone to showing an organism resistant to this empirically prescribed agent at a subsequent episode of UTI. Around 150 patients who had a urine sample with mixed growth were identified in the database. A comparison can be made between patients 'with mixed growth' who were empirically prescribed a specific antimicrobial, and those who did not receive antimicrobials for this episode of suspected UTI. Even though the sample size might be too small (due to stratification according to antimicrobial), exploratory analysis might reveal some patterns.

8.12 Dissemination and other achievements during the project

Since the start of the project on antimicrobial prescribing and resistance in uropathogenic *E.coli* infections in general practice in September 2008, a number of outputs were achieved. To date (April 2011), three papers have been published resulting directly from the study and abstracts have been presented at various conferences. Additionally, help and advice was given in a number of other projects and these also had outcomes. A chronological list of papers, abstracts, courses and conferences is presented below. The presenter of an abstract is indicated by (P).

- Abstract: Trimethoprim and ciprofloxacin resistance and prescribing in *E.coli* associated urinary tract infection: a multilevel analysis. Akke Vellinga (P), Andrew W Murphy, Belinda Hanahoe, Kathleen Bennett, Martin Cormican. Society of Social Medicine, Warwick, September 2011.
- Abstract: Antimicrobial management of UTI in general practice. Akke Vellinga, Martin Cormican, Belinda Hanahoe, Kathleen Bennett, Andrew W Murphy (P). Society Academic Primary Care, Bristol. July 2011. Poster presentation.
- Paper: Rational Testing: Interpreting Asymptomatic Bacteriuria (ASB). Martin Cormican, Andrew W Murphy, Akke Vellinga. Accepted for publication in British Medical Journal. May 2011.
- Paper submitted: Antimicrobial management of urinary tract infection in general practice in Ireland. Akke Vellinga, Martin Cormican, Belinda Hanahoe, Kathleen Bennett, Andrew W Murphy. Submitted to the BMC Family Medicine. April 2011.
- Paper: Opt-out as an acceptable method of obtaining consent in medical research: a short report. Akke Vellinga, , Martin Cormican, Belinda Hanahoe, Kathleen Bennett, Andrew W Murphy. BMC Medical Research Methodology, April 2011 (Vellinga, Cormican *et al.* 2011).
- Paper submitted: Progressive increase in prevalence of ESBL production among Enterobacteriaceae from clinical specimens from 2004 to 2008. Jérôme

Fennell, Akke Vellinga, Belinda Hanahoe, Dearbhaile Morris, Fiona Boyle, Francis Higgins, Maura Lyons, Karina O'Connell, Deirbhile Keady, Martin Cormican. Submitted to Eurosurveillance. April 2011.

- Abstract: Antimicrobial management of UTI in general practice. Akke Vellinga (P), Martin Cormican, Belinda Hanahoe, Kathleen Bennett, Andrew W Murphy. Association of University Departments of General Practice in Ireland, Annual Conference, Dublin. January 2011. Oral presentation.
- Abstract: Opt out as an acceptable method of obtaining consent in a prospective study on antimicrobial resistance and prescribing. Akke Vellinga (P), Martin Cormican, Belinda Hanahoe, Kathleen Bennett, Andrew W Murphy. Federation of Infection Society, Edinburgh. Nov 2010. Poster presentation.
- Paper: WestREN: A description of an Irish Academic General Practice Research Network. Kim Kavanagh, Niamh O' Brien, Liam Glynn, Akke Vellinga, Andrew Murphy. BMC Family Practice. October 2010 (Kavanagh, O'Brien *et al.* 2010).
- Abstract: The STOP questionnaire is the best screener for Obstructive Sleep Apnoea Syndrome at the Sleep Clinic. Keshaf Sharma (P), Imran Sulaiman, Melissa McDonnell, Akke Vellinga, JJ Gilmartin. European Respiratory Society, September Congress 2010. Oral presentation.
- Paper: Predictive value of antimicrobial susceptibility from previous urinary tract infection in the treatment of re-infection. Akke Vellinga, Martin Cormican, Belinda Hanahoe, Andrew W Murphy. British Journal of General Practice, July 2010 (Vellinga, Cormican *et al.* 2010).
- Paper: Enumeration and characterization of antimicrobial resistant *E.coli* in effluent from municipal, hospital and secondary treated sources. Sandra Galvin, Fiona Boyle, Patrick Hickey, Akke Vellinga, Dearbhaile Morris, Martin Cormican. Applied and Environmental Microbiology, June 2010 (Galvin, Boyle *et al.* 2010).
- Paper: Frequency and risk factors associated with emergency medical readmissions in Galway University Hospitals. Josephine Gorman, Akke Vellinga,

JJ Gilmartin, Sean O’Keeffe. Irish Journal of Medical Science, June 2010 (Gorman, Vellinga *et al.* 2010).

- Paper: A multilevel analysis of trimethoprim and ciprofloxacin prescribing and resistance of uropathogenic *Escherichia coli* in general practice. Akke Vellinga, Andrew W Murphy, Belinda Hanahoe, Kathleen Bennett, Martin Cormican. Journal of Antimicrobial Chemotherapy. April 2010 (Vellinga, Murphy *et al.* 2010).
- Abstract: Sleep Apnoea AT Your Fingertip. Ali Khan Kashif (P), Akke Vellinga, Amoia Maurizio, Katherine Finan, JJ Gilmartin. European Respiratory Society, Congress September 2009. Oral presentation.
- Abstract: Predictive value of antimicrobial susceptibility from previous urinary tract infection in the treatment of re-infection. Akke Vellinga, Martin Cormican, Belinda Hanahoe, Andrew W Murphy (P). North America Primary Care Research Group, Seattle, USA. Nov 2010. Oral presentation.
- Abstract: Opt out as an acceptable method of obtaining consent in medical research. Akke Vellinga, Martin Cormican, Belinda Hanahoe, Kathleen Bennett, Andrew W Murphy. Society of Social Medicine, Belfast. Sept 2010. Poster presentation.
- Course: Introduction to Cochrane reviews. HRB. October 2010.
- Postgraduate Certificate in Teaching & Learning in Higher Education (10 ECTS). Centre for Excellence in Learning and Teaching (CELT). Conferred November 2010.
- Abstract: Trimethoprim and Ciprofloxacin prescribing and resistance in Irish general practice: A cross-sectional comparison. Akke Vellinga (P), Andrew W Murphy, Belinda Hanahoe, Kathleen Bennett, Martin Cormican. Association of University Departments of General Practice in Ireland, Annual Conference, Dublin. March 2010. Oral presentation.
- Abstract: Predictive value of antimicrobial susceptibility from previous urinary tract infection in the treatment of re-infection. Akke Vellinga (P), Martin

Cormican, Belinda Hanahoe, Andrew W Murphy. Association of University Departments of General Practice in Ireland, Annual Conference, Dublin. March 2010. Oral presentation.

- Abstract: Increased prescribing of Trimethoprim and Ciprofloxacin within a practice increases the risk of resistance: A multilevel analysis of uropathogenic *E.coli* in Irish general practice. Akke Vellinga (P), Andrew W Murphy, Belinda Hanahoe, Kathleen Bennett, Martin Cormican. Federation of Infection Society, Birmingham. Nov 2009. Oral presentation.
- Abstract: Antimicrobial prescribing and resistance in urinary tract pathogens in Irish General Practice: A cross sectional comparison. Akke Vellinga, Andrew W Murphy (P), Belinda Hanahoe, Kathleen Bennett, Martin Cormican. Society for Academic Primary Care, St Andrews, Scotland. July 2009. Poster presentation.
- Abstract: Trimethoprim and Ciprofloxacin prescribing and resistance in Irish general practice: A cross sectional comparison. Akke Vellinga, Andrew W Murphy, Belinda Hanahoe, Kathleen Bennett, Martin Cormican. Infectious Disease Society Ireland, Dublin. June 2009. Poster presentation.
- Course: Multilevel modelling in MLWiN. Bristol, March 2009.
- CMS website design. December 2008. Subsequent design and maintenance of websites of Discipline of General Practice, the project and WestREN (www.nuigalway.ie/general_practice, www.antibiotics.nuigalway.ie, www.westren.nuigalway.ie)

'Perplexity is the beginning of knowledge'

Kahlil Gibran

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Appendix 1

Initial response of the ethical committee of the ICGP

3rd October 2007

Prof. Andrew Murphy
Clinical Sciences Institute
National University of Ireland
Galway



Re: Antibiotic prescribing and bacteriuria caused by trimethoprim and quinolone resistant bacteria in the community: a multilevel study with prospective patient and retrospective practice data.

**The Irish College of
General Practitioners**

Coláiste Dhochtúirí
Teaghlaigh Éireann

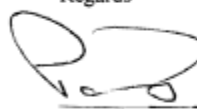
Dear Prof. Murphy,

I wish to advise that the above study was reviewed by the Research Ethics Committee on 12th September 2007. The committee approved the study subject to an opt in consent arrangement. The committee read with care the argument for the opt-out method and this was defended by co-applicant Prof. Bradley. The committee accepts the scientific argument that it is possible that an opt-in requirement could create a bias in response. However, the supportive evidence refers to a different type of project and the committee felt strongly that it could only approve an opt-out arrangement, which runs counter to precedent, should it be demonstrated that recruitment to the study was being adversely affected.

Alternatively the committee highlighted that were there a pilot study to prove that response was affected by the opt-in method, the decision of the Committee could be reconsidered.

If you have any queries please do not hesitate to contact me.

Regards


Pauline Tierney
REC Administrators



4-5 Lincoln Place, Dublin 2

Tel: (01) 676 3705/06

Fax: (01) 676 5850

e-mail: info@icgp.ie

www.icgp.ie

Regd. in Ireland at The Irish College
of General Practitioners Limited

Registration Number 100456

F Ó Cuinneagáin
Chief Executive

Appendix 2

Resubmission of the application for ethical approval

Galway, January 15th, 2009

Re: Re-submission of application:

‘Antibiotic prescribing and bacteriuria caused by trimethoprim and quinolone resistant bacteria in the community: a multilevel study with prospective patient and retrospective practice data’.

Dear Members of the ICGP Research Ethics Committee,

Thank you for your consideration of the above application and your letter of the 3rd of October 2007.

This resubmission refers to the prospective patient based component only; the retrospective practice based study received approval as outlined in your letter of 14th November 2007. The original application is also attached for your information.

We understand and appreciate the concerns of the REC regarding the original prospective opt-out recruitment method. We now propose a thoroughly

revised opt out recruitment method which, we believe, is in keeping with current best ethical practice and research conduct. The specifics of this revised approach are outlined on page 3 of this letter.

The comments of the REC were most helpful in developing this revised approach.

In your letter of the 3rd October 2007, you referred to:

- The supportive evidence for the opt-out method, regarding selection bias and recruitment difficulties, as being unconvincing.
 - A review of the literature regarding opt-out methodology with specific reference to selection bias and recruitment difficulties is now supplied in Appendix 1.
- The need for additional information such as pilot data.
 - A summary of a focus group study specifically reviewing our proposed recruitment strategy, nested in a HRB funded project, is included in Appendix 2.

A review of the literature regarding opt-out methodology (Appendix 1)

Various studies have shown that people are generally favourable and committed to research. Of particular interest, is a landmark study of Irish women who had contracted hepatitis C through contaminated blood products. The response rate was 61% when interviewees received a letter and a follow-up phone call compared to a 25% response by an opt-in strategy (McGee H 2000). This opt-out method was approved by the REC of five hospitals. Additionally, when contacted, patients were asked if the contact and the method of contact was acceptable to them. All 124 patients indicated the contact acceptable, even though 39% declined to participate in the study. If this method of contact was deemed acceptable to participants in the Hepatitis C study, a highly sensitive and vulnerable patient population with high risk of

stigmatisation, this method should also be acceptable for participants in the presented study. Similarly, in a Chlamydia screening trial where additional sample testing was requested by letter, an opt-out approach resulted in 2 out of 2500 women declining participation (Nathan, Thacker *et al.* 2008).

The review of the literature suggests that changing to an opt-in strategy, as suggested by the REC, is likely to result in a significantly lower response rate and a selection bias. As outlined in the original application, a clear strength of the proposed study was the potential generalisability of study *E.coli* resistance patterns to the general Irish population. If this generalisability is imperilled through selection bias, the extrapolation of the findings may be limited.

Pilot data

As suggested by the REC we have performed two pilot studies.

1) Four focus groups with 27 participants in total were set up to investigate the Irish public's attitudes to the use of personal information, from their general practice medical records, in research and service development (Appendix 2). This study is a three year project grant funded by the Health Research Board and led by Brian Buckley, a departmental researcher.

One of the scenarios presented to the participants depicted our proposed revised recruitment strategy. In summary, participants would hypothetically not have any difficulty with that type of information being passed to researchers due to the “greater good” effect. Differentiation was made between this scenario and one concerning more sensitive information. Assurance around confidentiality and security of data was deemed important.

These concerns about strict guidelines on the storage of the personal information and confidentiality agreements, are addressed by the commitment of the department to best practice guidelines outlined by the UK MRC Report 'Personal information in medical research' (<http://www.mrc.ac.uk/index.htm>).

2) The recruitment method in which patients are identified from the central microbiology records of University Hospital Galway (UHG) has been successfully piloted over a two month period in the practice of one of the applicants (Appendix 3). Telephone follow up showed that all twenty patients with *E.coli* had no difficulty with the confidential transfer of identification details from the microbiology laboratory to the researcher.

Proposed revised opt-out recruitment method

Reflecting the advice of the REC, the literature review and pilot data, we now propose a revised opt-out recruitment method as outlined in the flowchart (page 5). The key sequential steps are:

- A **website** (www.antibiotics.nuigalway.ie) devoted to the proposed research will be developed. This will describe the proposed study with particular emphasis on the ethical aspects of the opt-out recruitment strategy. Contact details for the lead researcher, will be provided.
- **Notices** will be placed in the waiting rooms of all participating practices. These notices will describe the proposed study with particular emphasis on the ethical aspects of the opt-out recruitment strategy. Contact details for the lead researcher, will be provided.
- Patients, identified in the lab of UHG from participating practices, whose MSU's grow *E.coli* will receive a **written letter** outlining the study and how to opt-out (Appendix 4).

- Patients can opt-out through **the website, a Freephone number, email or prepaid written response.**
- There will be a ‘cooling-off’ period from receipt of letter to accessing patient medical records.
- As previously approved by the Data Protection Commission and the ICGP REC, the lead researcher will sign **data confidentiality agreements** and **data agent nomination** forms with each participating practice.
- Each participating practice will receive **audit data** of their prescribing and E Coli resistance patterns. This will potentially contribute to direct gain for the individual patient and practice.

This approach closely resembles that of McGee’s study on Hepatitis C which can be considered a much more sensitive issue.

Conclusion

We believe that the revised opt-out recruitment strategy, informed by a literature review and relevant pilot data, balances the needs to both protect the confidentiality of individual patients and generate a generalisable study sample.

We look forward to your response and thank you for your consideration.

Yours sincerely,

Ms Akke Vellinga

Lead Researcher

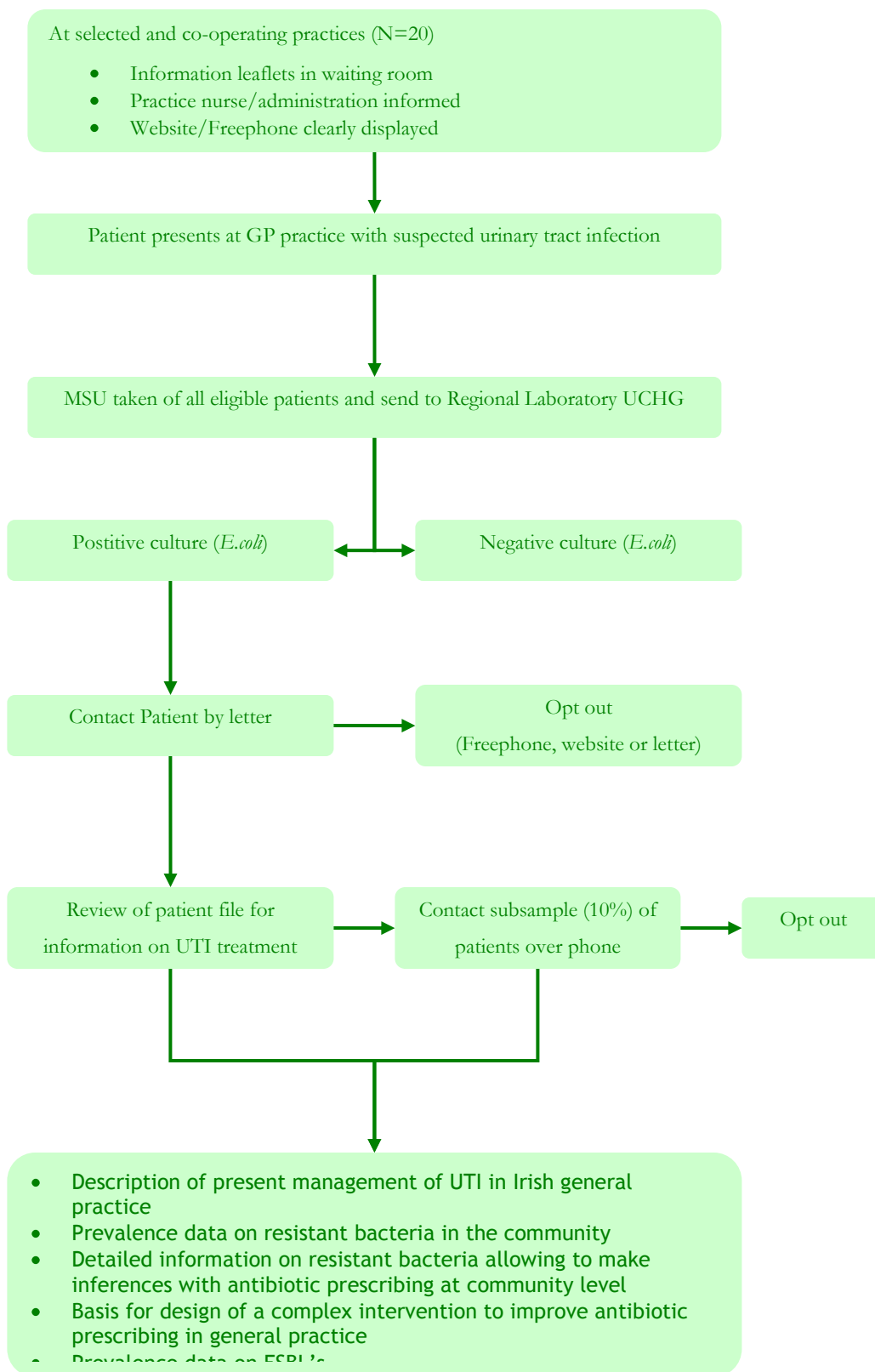
Prof Andrew W Murphy

Prof of General Practice

Prof Martin Cormican

Prof of Medical Bacteriology

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Appendix 3

*Literature review accompanying the ethical re-application***Literature Review on Opt-out methodology***Appendix of re-submission of ethical approval*

Due to changing privacy laws, the ethical approach to clinical trials and medical treatment is also applied to ethical approval for further analysis of databases. The golden standard in ethical approval is still considered to be explicit written consent. This type of explicit consent only applies for the specific trial/treatment and includes the retention of medical records. Within this framework, subsequent re-use of data is not possible even though it is considered preferable to re-use data rather than re-collect it ¹.

Research in health and health services is often based on the use of datasets available. Data are collected at various levels, prescription data by GMS, hospital admission data in HIPE, census data by the CSO. These data usually have a different primary function but can, especially when combined with other datasets, open a wealth of information in subsequent secondary analyses ¹. Due to the application of privacy laws originating from ethical approval for clinical trials, the demand for written explicit consent is requested for this type of research. In this overview of literature, we aim to show that for low risk non invasive studies, privacy laws are not breached when permission to study anonymised data is obtained through passive consent after providing public information.

The evaluation of health service and practice requires the information on patients using these services as they will also be the ones most affected by changes resulting from research. If ethical approval requires active consent by reading and signing information sheets, this might discriminate against more vulnerable groups and work against the principle of equal opportunities for health. To aim for equality of access to quality health and social services, this equality should be reflected in its research. Without equal representation of all population groups, the outcome of research cannot reflect the real life situation and inevitably will result in population groups missing and their problems not being appropriately addressed ².

*Re-submission of application: Appendix 1: Literature review
Antibiotic prescribing and bacteriuria caused by trimethoprim and quinolone resistant bacteria in the community
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In low risk studies consent can be considered as an indication of willingness rather than refusal. Various studies have shown that most patients do not have a preference for active consent ³. If it can be assumed that people will have no objections to taking part in studies and if risks for people are very low, an opt-out arrangement or passive consent is the most efficient procedure without violating the option of providing choice ⁴ which will serve the central ethical dilemma of balancing respect for the individual freedom and liberty with public health ⁵.

Active consent or opt-in has been shown to limit participation ⁶ and introduce bias into studies ⁷. The resulting selection bias is reported to influence results due to higher participation of certain age groups, gender, socio-economic background and ethnicity⁸ ⁹.

Furthermore, people who actively consent and people who do not consent differ from each other in various demographic characteristics, but both also differ from people who do not answer ¹⁰. This shows that active consent is prone to bias, resulting in conclusions not necessary representative for the population intended. The requirement of individual informed consent for organisational research is misleading and inappropriate as it will lead to unrepresentative outcomes ².

A number of studies explicitly researched objection to the opt-out system. In the Southampton women's study 75% of the women contacted agreed to join the study after being directly approached and the public concern about this approach was minimal ¹¹. The use of opt-out in a Chlamydia screening trial where additional sample testing was required, resulted in 2 out of 2500 women to opt-out of the study ¹². Interestingly, even though not required, 133 women formally responded of whom some included positive comments explicitly expressing their motivation to co-operate. A national survey on British public views on the use of identifiable medical data by the cancer registry concluded that the use of personal, identifiable patient information for the purpose of public health research and surveillance was not considered to be an invasion of privacy. An Irish study on public perceptions of biomedical research reports on telephone interviews with over 2,000 members of the public in 2004/2005

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(response rate 65%) for their views on various ethical issues including preferences for informed consent procedures¹³. The report found that the public is generally aware of and committed to making a contribution to research and related activities in the healthcare system for their benefit and for the benefit of future patients.

The public's knowledge about research is low even though there is a general support for research¹⁴ but education about research has shown to increase patients' willingness to participate¹³. Information should be available to participants to allow for informed consent. Informed consent is then seen in the sense of providing option and helping to make the optimum choice in light of their circumstances, needs and values, including perceptions of the benefits from medical research¹⁵.

Good methodology should include safeguards to protect privacy and necessary information should be made available to make an informed decision^{16 17}. This methodology should respect personal autonomy by allowing the right to inform and agree without unnecessary administrative burden on patient or researcher¹. Such a strategy is served by an opt-out policy with additional educational brochures, description of the observational research project and contact names and telephone numbers for further information or to request exclusion.

Based on advice given by the United Kingdom Data Protection Act and General Medical Council an opt-out strategy was designed for a project to improve delivery of cardiac health care between primary and secondary providers⁴. The principle of the strategy is the need to take all reasonable measures to inform those whose personal data could be stored regarding the registry's purpose, data set, storage method, and users. This comprehensive public-awareness campaign fulfils the ethical imperative of informed consent and the implied right of individual people to refuse inclusion while maintaining a registry that is complete. A similar strategy was used for the registry of the Canadian Stroke network. Information leaflets and posters describing the purposes of the registry, including how to opt-out, are available on the stroke and general medical wards at each participating hospital¹⁸. The opt-out rate has been very low.

In conclusion, research by analysis of databases for the benefit of the community has been severely obstructed by the implementation of individual privacy laws. The implementation of research ethics and confidentiality agreements should allow public health researchers to access personal records without violating privacy laws. The requirement for informed consent could be better served by providing information through the distribution of leaflets, provision of websites providing references and further information and contact names and phone numbers for additional requirements.

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Appendix 4

Summary of focus group study

Summary of Focus Group:

UTI Antibiotic Resistant Research Scenario

Patients' attitudes from Inform Study focus groups.

Brian Buckley, Pauline Clerkin

Background and methods

As part of the Inform study, which is ongoing and which is investigating the Irish public's attitudes to the use of personal information from their general practice medical records in research and service developments, 27 men and women aged over 18 have been involved in four focus groups in Galway, Spiddal, Corofin and Ballyvaughan.

As part of these focus groups, various scenarios were discussed which described different types of data access and use. One scenario depicted the proposed use of identifiable data to contact patients with anti-biotic resistant Urinary Tract Infections.

The scenario was described in the following way:

- Some urinary tract infections (UTIs) are resistant to antibiotics, so that extra treatment is needed.
- Laboratory researchers want to study whether being prescribed with antibiotics previously by GPs can lead to antibiotic-resistant UTIs.
- But of the very many samples sent by GPs to hospital laboratories for testing, only a few will have an infection and even fewer will have a resistant infection.
- The researchers want the hospital laboratory to provide the name and address of patients whose laboratory tests show they have an antibiotic resistant UTI, so that they can contact them and ask for their permission to use medical and prescribing information from their GP records in the study.
- If a patient does not want to allow their records to be used in the study, they can opt out.
- But even to ask whether the patients' information might be used, the researchers would have to be able to link personal medical information (the urine analysis results) and the

*Re-submission of application: Appendix 2: Summary of focus group study
Antibiotic prescribing and bacteriuria caused by trimethoprim and quinolone resistant bacteria in the community
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patients' identities: i.e. the researcher would know that everyone they write to has had a UTI.

- Would patients be upset that someone unknown to them has already linked their name to personal medical information in order to invite them to participate in the study?
- If NOT allowed to see these data the research will not take place because the only other option is to write to invite everyone who has had a urine sample tested and then identify those with UTI's of interest - and this would be too expensive and impractical.

Results

Across all the groups, most participants reported that in general they would have “no problem” with their contact information being passed to researchers in the scenario given. Opinions varied from general acceptance of personal information being available for research in general, with or without permission, to acceptance of this particular scenario given that the amount of information being made available to the researchers is limited.

“Because you might help somebody.. And if it was going to be of benefit to people then ...other people, then it’s great...”

“so the only data that any of these researchers know about me is the fact that I’ve had UTIs that did not react to antibiotics, right? They DON’T know that I’m also a schizophrenic and that I’m on half a dozen drugs for that, they don’t know that I’ve got a heart condition or anything else about me ... so they know nothing ...”

Some pointed out that if they thought that if it were pointed out that they personally were to benefit from the research, or that the purpose of the research was made more pertinent to them they were less likely to be concerned about their details being passed on to researchers.

“If I got the letter and like they were doing research on it and I was personally to gain by the outcome of the research...that there might be some, you know, benefit for me as such...I’d be all for it...”

“that’s where the benefit is...they can go straight to the...straight to the carrier, right, and go look it, you have a problem, these hundred people in the West of Ireland have a problem...you ARE resistant...what we’re doing is attacking this problem...”

The fact that the scenario concerned a topic deemed to be less sensitive was a factor in whether or not participants would be concerned. It was suggested that they would be less likely to approve of this scenario if it involved a more sensitive condition e.g. S.T.D.

“what if you ... you’d got an STD, for example...now if you got a letter in the post from researchers saying we know that you’ve had this at such and such a time...I mean it’s going to be a bit of a shock to the system isn’t it?”

“But a UTI is a UTI ...it’s not like you have ...you know..”

While in general most people were happy with the scenario and wouldn't have any objections some pointed out that it would need to be undertaken under strict guidelines and criteria to ensure that personal information was secure.

“I think the research in itself is extremely important and it’s very valid to have to do it....but I think the conditions under which it is done are also very important... and the controls are very important... they have to have clearly defined criteria though to make sure that that doesn’t happen...so that people know exactly what the rules are...exactly what they’re allowed to do and not allowed to do...because once that information leaks from a doctors surgery and is in someone else’s hands, there has to be very careful control”.

Security arrangements around where the identifying details are stored were seen as important as was the signing of confidentiality agreements by research staff.

“Access by other people whoever they are. And also the kind of place in which it’s stored. And the working environment for people to actually use it in so it’s not moved to other places.

“Because they’d sign the confidentiality...and I’m hoping that like everyone else in the practice they’d abide by that...”

Conclusion

In summary participants overall were happy to consider the UTI antibiotic resistant research scenario and would hypothetically not have any difficulty with that type of information being passed to researchers due to the “greater good” effect. Differentiation was made between this scenario and one concerning more sensitive information and assurance around confidentiality and security of data was deemed important.

Appendix 5

Irish College of General Practitioners, ethical approval



19th February 2009

**The Irish College of
General Practitioners**

Coláiste Gnóchtair
Teaghlach Éireann

Prof. Andrew Murphy
Department of General Practice
Clinical Sciences Institute
NUIG
Galway

**Re: Antibiotic prescribing and bacteriuria caused by trimethoprim and quinolone
resistant bacteria in the community: a multilevel study with prospective patient
and retrospective practice data**

Dear Prof. Murphy,

I wish to confirm that the resubmission relating to the patient based component of the above
study was reviewed by the Research Ethics Committee on 11th February 2009.

The committee approved the change in the recruitment method as requested.

If you have any queries please contact Ms. Pauline Tierney – pauline.tierney@icgp.ie

Yours sincerely,



✱ Prof. Colin Bradley
Chairman REC

4-5 Lincoln Place, Dublin 2
Tel: (01) 676 3705/06
Fax: (01) 676 5850
e-mail: info@icgp.ie
www.icgp.ie

Regd. in Ireland at The Irish College
of General Practitioners Limited

Registration Number 1004

F.O. Cunniffe
Chief Executive

Appendix 6

*Invitation letter to practice****Antibiotic prescribing and urinary tract infections in the community***

Galway, September 2009

Dear Dr. NAME,

Antibiotic resistance is a growing clinical problem in general practice. Although there is general acceptance that the problem of resistance is linked to antibiotic prescribing, details of this link are not clear. We wish to invite you to take part in a study on antibiotic prescribing and urinary tract infections in general practice. This is a HRB funded project led by the NUI Galway Departments of General Practice and Bacteriology with full ethical approval from the ICGP. Further details are available at www.antibiotics.nuigalway.ie.

Background

In the first part of our study we looked, for the first time in Ireland, at the association between antibiotic prescribing and resistance at the practice level. This multilevel analysis showed a clear association between prescribing of Trimethoprim and Ciprofloxacin and resistance of *E.coli* from urine to these antibiotics. The second part of the study is to look for evidence of this link between antibiotic prescribing and antibiotic resistance of urinary *E. coli* at the individual level.

Why our practice and what is being asked of us?

We are asking practices that have, in the past, submitted the most urine samples to UHG to participate in the study. It is likely that these practices already submit urine samples on most or all patients with clinical features of UTI and we anticipate that participation in the project will not have an impact on workload.

For the duration of the project we would like to ask you to send a urine sample from every patient with suspected UTI. Upon receipt of this sample in the microbiology laboratory of UHG, a letter will be sent out by us to inform every patient about the study. We will also wish to access additional information on antibiotic prescribing from the patient record which will be done by our researcher.

What is being asked of patients?

Only a selection of these patients will be asked to answer some specific questions on their UTI. Patients can opt-out at any stage of the study. A website has been set up to give additional information to patients as well as an easy opt-out form to facilitate patients who do not wish to participate. Leaflets to inform patients of this study will be distributed to the practice waiting room.

www.antibiotics.nuigalway.ie

091/495192

akke.vellinga@nuigalway.ie

What happens next?

We will call you during the next ten days to answer any queries you may have about the study. Alternatively, you can contact Martin Cormican (087 833 9881), Andrew Murphy (087 2731188) or Akke Vellinga (091/495192) to discuss the study. If you prefer, a visit by one of us can be set up. This visit will be brief and can be arranged at a time chosen by you. All staff are welcome to attend. Alternatively if you do not wish to participate, just contact Akke by phone or email (see below).

At the conclusion of the study you will receive a newsletter with an overview of the results, together with a profile of specific *E. coli* resistance patterns for your practice.

Yours sincerely,

Prof. Andrew Murphy
General Practice

Prof. Martin Cormican
Bacteriology

Akke Vellinga
Researcher

Appendix 7

Invitation letter to patient, including opt-out return letter

Antibiotic prescribing and urinary tract infections in the community

A study conducted by the Departments of General Practice and Bacteriology, NUI Galway

Dear NAME,

You are invited to take part in the above research study which is funded by the Health Research Board.

Why am I being asked to participate?

- At your last visit with your general practitioner, you were asked to provide a urine sample which was then forwarded to the laboratory in University Hospital Galway.
- All such patients are being asked to participate in the study. The lead researcher is a member of the microbiology team and is now contacting you.

What is the study about?

- The study has been set up to understand the relationship between the prescribing of antibiotics in general practice and community urinary tract infections.
- The study will contribute to a better understanding of how best to manage urinary infections in the Irish community.

What are you being asked to do?

- You are being asked to give your permission to allow us to look at your charts in your general practice for information specific to this research study. We will only record information relating to urinary tract infections. No personal information that would allow you to be identified will be taken. Any information will be kept strictly confidential and will be seen only by the researcher.
- If you are happy to give your permission, you do not need to do anything. However, if you would prefer that we do not use your information then please let us know by returning the enclosed form in the prepaid envelope, calling the phone number or logging onto our website www.antibiotics.nuigalway.ie where a special opt-out button can be clicked. Should you decide not to take part, your care will not be affected in any way.

More information on this study can be obtained by visiting our website www.antibiotics.nuigalway.ie, contacting the researcher at 091/495192 or emailing akke.vellinga@nuigalway.ie

Thank you for considering this request.

Prof. Andrew Murphy
General Practice

Prof. Martin Cormican
Bacteriology

Akke Vellinga
Researcher

Antibiotic prescribing and urinary tract infections in the community
www.antibiotics.nuigalway.ie 091/495192 akke.vellinga@nuigalway.ie

OPT-OUT

Name: _____

Date: ____ / ____ / ____

GP: _____

I would like to opt-out of this study:

- ☐ I do NOT give permission to search my charts to get information for this research study

Additional comments:

Please return this form to:
 Akke Vellinga
 Department of General Practice
 NUI Galway
 1 Distillery Road
 Galway

Appendix 8

Practice information sheet (A3 for waiting room, A5 for reminder cards in the consultation room)



This general practice has agreed to co-operate with the study:

Antibiotic prescribing and urinary tract infections in the community.

This is a study conducted by the Departments of *General Practice* and *Bacteriology* of the **National University Ireland, Galway** and funded by the *Health Research Board*.

The study will contribute to a better understanding of how best to manage urinary infections in the Irish community. For more information about the study and how this research can have an impact, please log on to our website www.antibiotics.nuigalway.ie

The study obtained approval from the ethical committee of the *Irish College of General Practitioners*. All data will be handled with respect to privacy and confidentiality.

If you would prefer that we do **not** use your information then please let us know by calling the phone number below or logging onto our website where a special opt-out button can be clicked. Should you decide not to take part, your care will not be affected in any way.

Thank you for considering this request.

Akke Vellinga
Researcher


Prof. Andrew Murphy
Department of General Practice


Prof. Martin Cormican
Department of Bacteriology

www.antibiotics.nuigalway.ie
Telephone number: 091/495192

Appendix 9

Website





NUI Galway
OÉ Gaillimh

You are here → **HOME > FACULTIES & DEPARTMENTS > GENERAL PRACTICE**

Colleges & Schools

ANTIBIOTIC PRESCRIBING AND URINARY TRACT INFECTIONS IN THE COMMUNITY

This is a [HRB](#) funded project from the Departments of [General Practice](#) and [Bacteriology](#) from the NUI Galway.

The project aims to understand the relation between prescribing antibiotics in primary care and antibiotic resistance. The study focuses on urinary tract infections.

There are two parts to the study:


- Linking the database on prescribing behaviour of general practices in the West of Ireland and results from urinary samples sent to the microbiology laboratory of the University Hospital Galway. This part of the study is now finished and the results are published and can be viewed by clicking on the "Findings" button.
- Collection of samples from patients in a selection of general practices and linking this with individual characteristics related to antibiotic prescriptions

Antibiotics and Resistance

The Project **Findings**

Contact us **Links**

Opt Out



Appendix 10

Practice agent nomination form

AGENT NOMINATION FORM

Name of Practice:

As a General Practitioner representing the practice named above, I hereby nominate Akke Vellinga as an agent of this practice for the duration of the practice's involvement in '*Antibiotic prescribing and urinary tract infections in the community*' study.

As an agent of the practice, Akke Vellinga will be bound by the normal procedures governing patient confidentiality in this practice. Information about individual patients will be treated confidentially and will be used solely for the purpose of the research study. Akke Vellinga will remove personal identifiers from the data to ensure that only anonymised data is disclosed from the practice to the study.

GP Signature: _____ Date: _____

GP Name (block capitals): _____

Appendix 11

*Confidentiality agreement***CONFIDENTIALITY AGREEMENT**

As a researcher working with the '*Antibiotic prescribing and urinary tract infections in the community*', I agree to be bound by the normal procedures governing patient confidentiality. Information about individual patients will be treated confidentially and will be used solely for the purpose of the research study.

Researcher Signature: _____

Researcher Name: Akke Vellinga

Date: / /2009

Witnessed by (member of staff):

Appendix 12

A multilevel analysis of trimethoprim and ciprofloxacin prescribing and resistance of uropathogenic Escherichia coli in general practice. Vellinga A, Murphy AW, Hanahoe B, Bennett K, Cormican M. J Antimicrobial Chemotherapy 2010; 65(7): 1514-20.

A multilevel analysis of trimethoprim and ciprofloxacin prescribing and resistance of uropathogenic *Escherichia coli* in general practice

Akke Vellinga^{1*}, Andrew W. Murphy¹, Belinda Hanahoe², Kathleen Bennett³ and Martin Cormican^{2,4}

¹Discipline of General Practice, School of Medicine, NUI Galway, Ireland; ²Department of Medical Microbiology, University Hospital, Galway, Ireland; ³Department of Pharmacology & Therapeutics, Trinity Centre for Health Sciences, Dublin, Ireland; ⁴Discipline of Bacteriology, School of Medicine, NUI Galway, Ireland

*Corresponding author. Tel: +353-91-495192; E-mail: akke.vellinga@nuiagalway.ie

Received 22 March 2010; accepted 28 March 2010

Objectives: A retrospective analysis of databases was performed to describe trimethoprim and ciprofloxacin prescribing and resistance in *Escherichia coli* within general practices in the West of Ireland from 2004 to 2008.

Methods: Antimicrobial susceptibility testing was performed by disc diffusion methods according to the CLSI methods and criteria on significant *E. coli* isolates (colony count $>10^5$ cfu/mL) from urine samples submitted from general practice. Data were collected over a 4.5 year period and aggregated at practice level. Data on antimicrobial prescribing of practices were obtained from the national Irish prescribing database, which accounts for ~70% of all medicines prescribed in primary care. A multilevel model (MLwiN) was fitted with trimethoprim/ciprofloxacin resistance rates as outcome and practice prescribing as predictor. Practice and individual routinely collected variables were controlled for in the model.

Results: Seventy-two general practices sent between 13 and 720 (median 155) samples that turned out to be *E. coli* positive. Prescribing at practice level was significantly correlated with the probability of antimicrobial-resistant *E. coli* with an odds ratio of 1.02 [95% confidence interval (CI) 1.01–1.04] for every additional prescription of trimethoprim per 1000 patients per month in the practice and 1.08 (1.04–1.11) for ciprofloxacin. Age was a significant risk factor in both models. Higher variation between practices was found for ciprofloxacin as well as a yearly increase in resistance. Comparing a 'mean' practice with 1 prescription per month with one with 10 prescriptions per month showed an increase in predicted probability of a resistant *E. coli* for the 'mean' patient from 23.9% to 27.5% for trimethoprim and from 3.0% to 5.5% for ciprofloxacin.

Conclusions: A higher level of antimicrobial prescribing in a practice is associated with a higher probability of a resistant *E. coli* for the patient. The variation in antimicrobial resistance levels between practices was relatively higher for ciprofloxacin than for trimethoprim.

Keywords: quinolones, UTIs, community

Introduction

Ireland is one of only three countries in Europe where outpatient antimicrobial prescribing is increasing.^{1,2} For every 1000 members of the Irish population, 21 daily defined doses (DDDs) of antibiotics were prescribed in 2006 compared with 11 for the Netherlands and 14 for Austria, the lowest prescribers of antimicrobial agents.³ Use of antimicrobials is recognized as the main selective pressure driving the emergence and spread of antimicrobial resistance in human pathogens. Higher rates of antimicrobial resistance were shown in high consuming countries in a cross-national ecological study.⁴ Associations have also been shown between prescribing and resistance for

individual prescribing of trimethoprim⁵ as well as at practice level for ampicillin and trimethoprim prescribing.⁶

Prescribing of antimicrobials in general practices is often targeted to control and limit the spread of resistance of pathogens, but general practitioners (GPs) are not always convinced of their contribution to overall antimicrobial resistance levels.⁷ A small-scale study of antimicrobial prescribing in the Irish general practice showed that >80% of the GPs agreed that they overprescribe antimicrobial agents and almost 70% felt under pressure by patients to prescribe antimicrobial agents.⁸ Efforts to reduce the level of prescribing of antimicrobial agents at national and patient level can be expected to have an effect on resistance levels, but evidence to support this hypothesis is limited.⁹

Analysis of trimethoprim and ciprofloxacin

Urinary tract infection (UTI) is one of the most common bacterial infections presenting in general practice and most episodes are treated with antimicrobial agents.¹⁰ Some guidelines for empirical treatment of uncomplicated cystitis suggest trimethoprim as first-line agent and ciprofloxacin as an alternative as the level of resistance to ciprofloxacin is generally still low.¹¹ A single pathogen (*Escherichia coli*) accounts for a high proportion of UTI in the community.¹² Most clinical laboratories perform susceptibility testing to a standard panel of antimicrobial agents, usually including trimethoprim and ciprofloxacin, on routine isolates.

A multilevel modelling approach separates the effects of individual- and group-level variables on the outcome. This approach is applied to assess the association between antimicrobial prescribing of a practice and patient laboratory data on resistance to trimethoprim and ciprofloxacin of *E. coli* from urinary samples.

Methods

Isolates

Urine samples submitted from general practice to the Microbiology Laboratory at Galway University Hospitals were cultured by semi-quantitative methods. Isolates from specimens yielding $>10^5$ cfu/ml were identified by standard methods and susceptibility testing was performed by the disc diffusion method according to CLSI methods.¹³ Susceptibility testing to trimethoprim, ciprofloxacin, ampicillin, co-amoxiclav, cefpodoxime and nitrofurantoin was performed on all isolates. Data from all cultures over a 4.5 year period (April 2004–September 2008) were collected. For each sample, gender, age and general practice address were available. Duplicate isolates were defined as repeat isolates from the same patient within 91 days of the first isolate and were excluded from analysis.⁶ The number of urine samples and the number of isolates for each practice were calculated. Data were only included when available for the whole period and at least 10 positive samples were submitted by a practice each year. Only one practice did not submit the minimum 10 samples per year.

Prescribing data

Data on practice antimicrobial prescribing were obtained from Health Service Executive–Primary Care Reimbursement Services (HSE-PCRS). In Ireland 70% of the population is covered by private insurance and 30% (who are means tested) have free healthcare and free medication (termed ‘GMS eligible’, where GMS stands for General Medical Services).¹⁴ For the duration of this study all patients >70 years of age were GMS eligible. While the HSE-PCRS population cannot be considered representative of the entire population, as the elderly, the young and the socially disadvantaged are over-represented, it is estimated to account for ~70% of all medicines dispensed in primary care.

The HSE-PCRS provided the number of prescriptions per practice for the study period. Data were obtained for all antimicrobial prescriptions and, to allow corrections for prescribing behaviour of the practice, the overall numbers of prescriptions, oral contraceptives, hormone replacement therapy, selective serotonin re-uptake inhibitors (SSRIs) and benzodiazepines were obtained.

Practice data

Practice-level data were obtained from the HSE: GMS panel size; location; nurse availability; number of partners; dispensing status; distance to the hospital laboratory; percentage of female GPs; mean age of GPs; and relative affluence of practice area. To assess the relative affluence, the Small Area Health Research Unit (SAHRU) index was used.¹⁵ This index

is based geographically on district electoral divisions, and rates indices of relative poverty on a scale of 1 to 10, where 1 is the most affluent.

Data analysis

Average practice prescription rates were calculated per 1000 patients (panel size) per month (see Table 1). Socio-economic status (SES) was categorized into high and low SES, with scores of ≥ 8 being deprived. Age was categorized into five age groups.

The resistance of *E. coli* to trimethoprim and ciprofloxacin was compared with the pooled prescriptions of these antimicrobials within the practice. The association was studied with a multilevel logistic model in which variables are controlled for at practice level or at individual level. The odds ratio (OR) [and its 95% confidence interval (CI)] gives the increase in the probability of having a resistant *E. coli* for every additional prescription of the antimicrobial (per 1000 patients per month) or, for the other variables, for one category compared with the reference category. The relative importance of practice and individual variables was interpreted by comparing the amount of variation explained at each step. The median OR (mOR) shows the relevance of practice compared with individual variables; the larger the mOR the more important the practice is in predicting the probability of having a resistant *E. coli* (see Table 2).

A caterpillar plot showing the estimated residuals for all the practices in rank order was made based on the final model. The horizontal line at zero is the average resistance at practice level. Similarly, based on the final model, a prediction of the overall level of resistance was made with the varying prescribing per month. For each practice the same prediction was made varying the prescribing within the practice limits.

Predictions [and 95% CI] were calculated based on the final models, with all variables set at their mean value and practice prescribing ranging from 0 to 20 prescriptions per 1000 patients per month.

Additional statistical explanation

Data were analysed using a multilevel logistic regression model with trimethoprim and ciprofloxacin resistance as binary outcomes (yes/no). This approach allows the separation of practice- and patient-level factors. A model was built starting with an empty model and introducing the main factor of interest, antimicrobial prescribing, patient variables (age and gender) and practice-level variables at each step.

Prescription rates were specified as random effects (varying intercept for each practice), which allows rates to be specific for each practice. Prescription rates were also tested for random slopes to see whether the effect of prescribing differs between practices, but this did not improve the models.

An alternative model in which ‘cohort’ (year) was included as a level (individuals within cohorts within practices) was not significantly better than the two-level model. Models were tested for interactions, but these did not show any statistical significance.

The adjusted ORs with 95% CI for resistance to the antimicrobial agents were calculated for the explanatory variables. In order to quantify the variability between practices in antimicrobial resistance level, an mOR was calculated using Larsen’s mOR.^{16,17} The mOR can be interpreted as the increase in risk in the imaginary event of a patient moving from a practice with low to a practice with high resistance. An mOR equal to one means no differences between practices in the probability of resistance. For the mOR a Bayesian credible interval was calculated based on the distribution of the mOR to distinguish it from a fixed effects OR CI.

The multilevel logistic regression models were estimated with a Markov Chain Monte Carlo (MCMC) method using MLWin software (version 1.2) developed by the Goldstein research group.^{18,19} The deviance information criterion (DIC), which combines the deviance with information about the number of parameters in the model, was used to compare different models.

Results

Our study sample consisted of 14 181 *E. coli* isolates from 72 practices. The number of samples per practice ranged from 44 to 567 (median 175) over the 4.5 year study period. The panel size (number of GMS patients) ranged from 71 to 3195 with a median of 1066 per practice. Table 1 shows the basic characteristics of the individual- and practice-level variables. An overview of the final models for trimethoprim- and ciprofloxacin-resistant *E. coli* for individual- and practice-level variables is shown in Table 2. The specific correlation for each practice and the overall correlation (thick red line) between prescribing and predicted resistance are shown in Figure 1 for trimethoprim and Figure 2 for ciprofloxacin.

Trimethoprim prescribing is significantly correlated with trimethoprim resistance of *E. coli* in urinary isolates with an increase of 1.02 (95% CI 1.01–1.04) for every additional

prescription of trimethoprim per 1000 patients per month (Table 2). Increasing age increased the odds of resistance for trimethoprim, but this is only significant for ages over 60. No differences were observed between males and females within practices. No yearly changes were observed in trimethoprim resistance at practice level, except for a small increase for 2008 compared with the reference year 2004 [OR 1.19 (95% CI 1.04–1.36)]. No practice-level variables were found to be significantly associated with trimethoprim resistance. Overall prescribing of medication at the practice level was kept in the model, but did not show a significant effect.

Ciprofloxacin prescribing is significantly associated with ciprofloxacin resistance of *E. coli* in urinary isolates with an increase of 1.08 (95% CI 1.04–1.11) for every additional prescription of ciprofloxacin per 1000 patients in the practice. The OR for year (cohort) showed an increase in ciprofloxacin resistance with increasing year. The OR of resistance for an individual in 2008

Table 1. Basic characteristics of individual- and practice-level variables

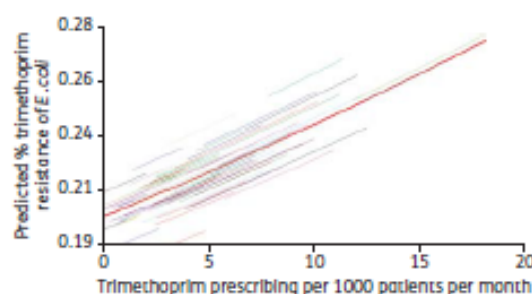
	Percentage or mean	Median	SD	Min–max
Individual level variables n = 14 181				
Gender				
male	7.9%			
female	88.9%			
unknown	3.5%			
Age (years)	46.3	45	25.0	0–102
<18	12.3%			
18 to <40	31.4%			
40 to <60	21.5%			
60 to <80	24.1%			
≥80	10.7%			
Practice level variables n = 72				
number of samples	197	175		44–567
panel size (patients)	1081	1066	660	71–3195
age of GMS GPs (years)	52.3	51.7	7.0	38.9–68.7
number of GMS GPs	1.6	1	0.9	1–5
SES of area	6.8	8	3.3	1–10
distance to hospital (km)	25.0	21.6	25.2	0–85
practice nurse	55.6%			
dispensing	13.9%			
rural allowance	20.8%			
single handed	63.3%			
Predictor variables—prescription				
trimethoprim prescribing per month (mean no. of prescriptions/1000 practice population)	4.7	4.3	3.2	0–18.1
ciprofloxacin prescribing per month (mean no. of prescriptions/1000 practice population)	4.6	4.1	3.4	0–17.9
antibiotics per year (no. of prescriptions)	1037	1058	461	4–2412
overall medication per year (no. of prescriptions)	20604	21869	8976	4–44 576
Dependent				
antibiotic resistance rate at practice level, trimethoprim	25.9%		5.0	15.0–40.0
antibiotic resistance rate at practice level, ciprofloxacin	5.5%		3.5	0.8–18.6

Analysis of trimethoprim and ciprofloxacin

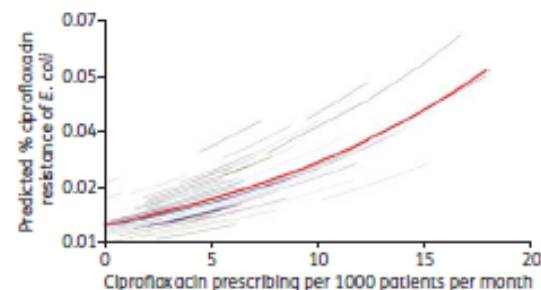
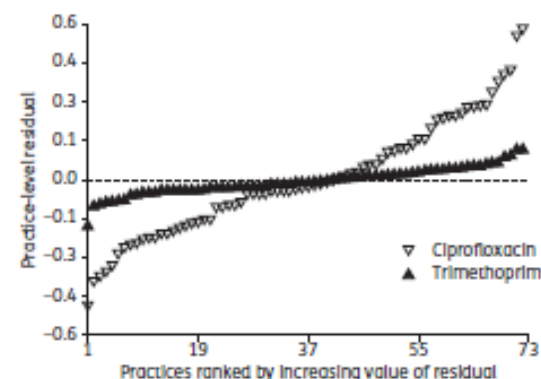
Table 2. Overview of the final multilevel models for trimethoprim- and ciprofloxacin-resistant *E. coli* in urinary isolates

	Trimethoprim		Ciprofloxacin	
	OR	95% CI	OR	95% CI
Prescribing per 1000 patients per month	1.02	1.01–1.04	1.08	1.04–1.11
Gender				
male	reference		reference	
female	0.96	0.96–1.10	0.50	0.40–0.63
unknown	1.17	0.92–1.50	0.80	0.48–1.27
Age, years				
<18	reference		reference	
18–<40	1.09	0.81–1.06	1.38	0.94–2.05
40–<60	1.04	0.91–1.20	2.14	1.47–3.18
60–<80	1.43	1.25–1.64	4.69	3.31–6.80
≥80	2.25	1.94–2.62	11.52	3.00–16.61
Year				
2004	reference		reference	
2005	0.99	0.87–1.12	1.05	0.78–1.41
2006	1.09	0.96–1.23	1.70	1.29–2.28
2007	1.09	0.96–1.23	1.89	1.42–2.52
2008	1.19	1.04–1.36	2.18	1.62–2.98
SES area	1.05	0.94–1.17	1.04	1.01–1.08
Overall prescribing				
low	reference		reference	
medium	1.01	0.90–1.14	0.78	0.59–1.02
high	0.82	0.71–0.96	0.77	0.51–1.14
Practice mOR ^a	1.10	1.03–1.16	1.37	1.22–2.59

^aThe median value of the OR between the practice at highest risk and the practice at lowest risk when randomly picking out two practices.

**Figure 1.** Predicted practice and overall (thick line) correlation between trimethoprim prescribing and resistance. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.

is double the odds of 2004 [OR 2.18 (95% CI 1.62–2.98)]. The linear trend for year was statistically significant ($P < 0.01$, results not shown). Age was the most important individual-level factor; compared with a person <18 years old, the odds of a

**Figure 2.** Predicted practice and overall (thick line) correlation between ciprofloxacin prescribing and resistance. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.**Figure 3.** Practice-level residuals showing the difference in variance between the trimethoprim and ciprofloxacin model. The ciprofloxacin model shows more unexplained variation at practice level.

ciprofloxacin-resistant isolate for a person >40 years old doubles and increases to 11.5 (3.0–16.6) for ≥80 years of age. Females were significantly less likely to have a ciprofloxacin-resistant *E. coli* infection compared with males. Socio-economic area was found to be borderline significant, with lower socio-economic class (i.e. higher Sahru index) increasing the odds of resistant isolates.

Differences in resistance between practices were smaller for trimethoprim than for ciprofloxacin (mOR 1.10 compared with 1.37) and the credible interval for the trimethoprim model is narrower than is the case for ciprofloxacin. Figure 3 shows the variance of the practice-level residuals for each practice compared with the overall resistance (horizontal line) in increasing order of this difference from left to right. The practice-level residuals are greater for ciprofloxacin, showing a higher variability in antimicrobial resistance between practices for ciprofloxacin compared with trimethoprim.

Figure 4 shows the predicted probability of having a resistant *E. coli* for the 'mean' patient in the 'mean' practice for every increase in prescription of the antimicrobial agent per 1000 patients per month. The probability of a 'mean' patient having

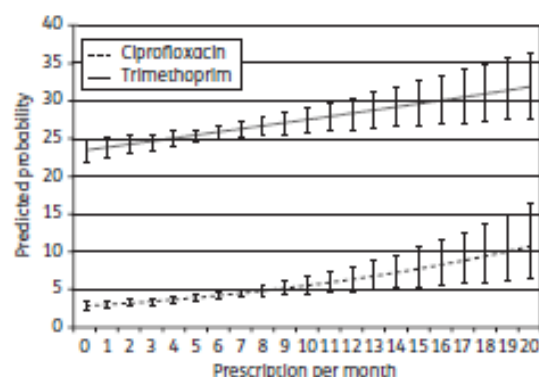


Figure 4. Predicted probability of resistance for every increase in prescription per month (based on the final model for 'mean' patient and 'mean' practice).

a resistant *E. coli* in a 'mean' practice with 1 trimethoprim prescription per 1000 patients per month was 23.9% (95% CI 22.6%–25.2%); with 10 prescriptions per 1000 patients per month, 27.5% (95% CI 26.0–29.2); and with 20 prescriptions per 1000 patients per month, 32.0% (95% CI 27.7%–36.5%). Similarly for ciprofloxacin the probability was, respectively, 3% (95% CI 2.5%–3.5%) for 1 prescription, 5.5% (95% CI 4.5%–6.7%) for 10 prescriptions and 10.7% (95% CI 6.4%–16.4%) for 20 prescriptions.

Discussion

Our results demonstrate that increased prescribing of both trimethoprim and ciprofloxacin within a practice is associated with an increased probability of a resistant *E. coli* for the patient, independent of other risk factors. This means that a patient consulting a practice with a high antimicrobial prescribing pattern will have a higher probability of infection with antimicrobial-resistant *E. coli* than a patient visiting a low prescribing practice.

The additional information obtained from the use of a multilevel logistic regression showed that the variation between practices was higher for ciprofloxacin than for trimethoprim. None of the practice-level variation was explained by any of the practice variables included in the model, which were mainly related to structure of care, such as the presence of a practice nurse or the number of GPs associated with the practice.

The association between antimicrobial use and antimicrobial resistance in the community is well described internationally, in correlation as well as individual-level studies. Goossens et al.⁴ reported in an international ecological study a high correlation between resistance levels and prescribing pattern in 26 European countries. Whereas the strength of this evidence was relatively weak due to the study design, the consistent patterns were striking. Various other studies also identified a correlation with resistance at the ecological level^{20–22} and with the use of multilevel studies for β -lactam antibiotics and trimethoprim^{5,6} in bacteriuria. A multilevel study by Donnan et al.⁵ showed an increase in OR of 1.22 (95% CI 1.16–1.28) for every prescription of trimethoprim at the patient level after adjusting for practice-level

variables. Butler et al.⁶ used a multilevel linear model in which changes in antimicrobial resistance at practice level were found to be associated with prescribing. Our study combined the approaches in these studies as it showed an association of practice-level prescribing and individual-level resistance for trimethoprim with the addition that this also applied to fluoroquinolone (ciprofloxacin) resistance. The opportunity to compare the predictions from the two models demonstrated that the scale of the impact was relatively more influential for ciprofloxacin compared with trimethoprim. A practice with 1 prescription per 1000 patients per month compared with one with 10 prescriptions per 1000 patients per month showed a difference in predicted resistance of 3.6% for trimethoprim and 2.5% for ciprofloxacin. For ciprofloxacin this represented an almost 2-fold increase in the predicted probability of resistance.

Geographical variation in resistance levels was shown for β -lactam and macrolide resistance of *Streptococcus pneumoniae* reflected in the differences between the proportions of resistance within individual serotypes.²³ Goossens et al.⁴ supported the hypothesis that selection pressure was the main factor in the geographical variation in resistance patterns and not clonal dissemination. A paper from Garcia-Rey et al.²⁴ however, showed a reverse association between fluoroquinolone consumption and resistance in both *S. pneumoniae* and *E. coli* between provinces. They performed a linear regression analysis in which an inverse relationship was found between consumption and resistance after removal of outliers. The lower geographical level (practice) used in our multilevel analysis might be the reason why our results show evidence supporting the overall hypothesis that increasing antimicrobial use in practices is associated with increased resistance in isolates of patients attending that practice.

It is biologically plausible that differences in practice prescribing of trimethoprim and ciprofloxacin have a different impact on the frequency of resistance in *E. coli* to these antimicrobials. Trimethoprim was introduced in clinical practice in 1975 and resistance levels in *E. coli* have remained stable at ~30% for some years.²⁵ Trimethoprim resistance in *E. coli* is associated with plasmid-encoded resistance determinants capable of horizontal transmission. As the plasmids may encode resistance to one or more agents other than trimethoprim, resistance may be selected for and maintained by antimicrobial agents other than trimethoprim. Ciprofloxacin was introduced into clinical practice more recently and resistance levels in *E. coli* were low at 2.5% in 2003, but are increasing.²⁶ Although plasmid-encoded low-level ciprofloxacin resistance is well described, high-level resistance to ciprofloxacin in *E. coli* is generally associated with point mutations in the chromosomal housekeeping gene *gyrA*.²⁷ Therefore, the 'opportunities' for trimethoprim resistance genes to spread and maintain themselves may be greater compared with those of ciprofloxacin.

Irrespective of how resistance emerges, the time scale of emergence of resistance under constant selective pressure was found to be much shorter than the decay time after cessation or decline in the level of prescribing.²⁸ A major decrease in sulphonamide prescribing in the UK did not have an effect on the prevalence of resistance to this drug in *E. coli* within a useful time frame.²⁹ The prevalence of sulphonamide-resistant *E. coli* in this study was very high (46%) and prescribing was established. Nasrin et al.³⁰ looked at the effect of β -lactam antibiotic

use in children on pneumococcal resistance (14%) to penicillin in a cohort study and showed that reduction in antimicrobial use could result in a reduction in resistance rates within 6 months. A recent study from Sundqvist *et al.*²¹ did not show a decrease in trimethoprim resistance in *E. coli* after a 24 month intervention that decreased trimethoprim prescribing by 85%. In contrast, Gottesman *et al.*²² showed in a retrospective ecological study an immediate effect of quinolone restriction on the susceptibility of *E. coli* in community urine cultures. The quinolone resistance level of *E. coli* decreased from 12% before to 9% during the intervention restricting ciprofloxacin. Our study may explain the differences in outcome between these interventions as it suggests that antimicrobials with less established and disseminated resistance levels, i.e. more variation in resistance levels between practices, might be more likely to show an impact of changing prescribing, and vice versa.

Our data support the following message to GPs with respect to antimicrobial prescribing; not only is prudent use of antimicrobial agents of general value to the community as a whole in limiting the emergence and dissemination of infection, but conservative antimicrobial prescribing is of specific benefit to patients within a practice by reducing the likelihood of infection with antimicrobial-resistant bacteria. Also, interventions aimed at reducing resistance against antimicrobials should take its potential impact into account as this could be different for more or less established antimicrobials.

Limitation of the study

The main limitation of the study is the use of the HSE-PCRS database for the prescribing data. Ireland does not have a comprehensive national prescribing database; the HSR-PCRS database covers 70% of all primary care prescriptions. The missing 30% is largely prescribing to middle-aged and relatively affluent patients. Possible biases resulting from this exclusion are difficult to identify, and for this reason our conclusions need to be interpreted with some caution.

A second limitation of the study refers to the possible impact of routine laboratory sampling on the results. Overall, urine sample submission did not show any discernible changes over time in a stable population and it appears reasonable to suggest that no change in sampling behaviour has occurred.

A prospective individual-based study is currently being conducted in 22 practices in the West of Ireland (the laboratory results from these practices were also part of this analysis) (www.antibiotics.nuigalway.ie). The 22 practices were requested to send in urine samples of all patients with suspected UTI. Preliminary results from the first 6 months of this study showed no increase in the number of urine samples submitted to the laboratory, suggesting that generally all patients with suspected UTIs have a sample submitted for culture.

Furthermore, the statistical modelling used in this paper takes 'time' and 'number of samples' into account as independent variables and only the variable time remained a significant factor in the model.

From these results it is expected that urine samples are routinely submitted from all suspected UTI patients and no differences in resistance levels are likely to be due to changes in submissions.

Conclusions

This multilevel analysis with patient- and practice-level data showed that an increase in trimethoprim and ciprofloxacin prescribing within a practice is associated with an increase in the probability of diagnosis of a uropathogenic *E. coli* resistant to these antimicrobials for the patients. Other practice variables appeared to have little impact on resistance rates. The variation between practices was higher for ciprofloxacin than for trimethoprim, which suggests that before resistance to an antimicrobial agent becomes widely disseminated in the community, variations in prescribing behaviour may have a greater impact on selection for resistance.

Acknowledgements

Sincere thanks to Deirdre Goggin, Department of Public Health, Health Service Executive West, Galway and to the laboratory staff of the Department of Medical Microbiology, University Hospital, Galway for providing data for this analysis.

Funding

This work was supported by a grant from the Health Research Board, Ireland.

Transparency declarations

None to declare.

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Appendix 13

Predictive value of antimicrobial susceptibility from previous urinary tract infection in the treatment of re-infection. Vellinga A, Cormican M, Hanahoe B, Murphy AW. British Journal of General Practice 2010; 60: 511-513.

Predictive value of antimicrobial susceptibility from previous urinary tract infection in the treatment of re-infection

Akke Vellinga, Martin Cormican, Belinda Hanahoe and Andrew W Murphy

ABSTRACT

Recurrent urinary tract infections are often re-infections; therefore, antimicrobial susceptibility test results from a previous episode may guide the empiric therapy in subsequent episodes. This analysis provides predictive values of the antimicrobial susceptibility of previous *Escherichia coli* isolates for the treatment of re-infections in routine clinical practice. If resistance to ampicillin, trimethoprim, or ciprofloxacin is detected, re-prescription within 3 months is imprudent. Susceptibility to nitrofurantoin, ciprofloxacin, or trimethoprim in a previous sample supports their prescription for a re-infection within 3 months and up to a year. Resistance to nitrofurantoin is low and, once detected, decays relatively quickly. Nitrofurantoin should be considered as a first-line agent for initial and repeat treatment.

Keywords

antibiotic resistance; *Escherichia coli*; general practice; predictive value; urinary tract infection.

INTRODUCTION

Urinary tract infection (UTI) is considered to be one of the most common bacterial infections.¹ Approximately 5% of young adults have bacteriuria at any one time,² with up to 50% of adult women reporting a UTI at some time in their life.¹ The incidence of UTI increases with age, at the rate of 1–2% per decade.² Recurrence happens frequently, in 27–48% of healthy women,³ after spontaneous clearance as well as after antimicrobial treatment.^{2,4} Prospective studies have shown that the vast majority of recurrent UTIs are re-infections by a previously identified strain.⁵

Guidelines on empirical treatment of acute uncomplicated UTI suggest that agents may not be suitable for empirical use when the community prevalence of resistance to the antimicrobial in *Escherichia coli* exceeds 10–20%.⁶ Empirical antimicrobial therapy requires a balance between the need to achieve effective therapy as well as to limit the use of broad-spectrum antimicrobial agents. Antimicrobial susceptibility test results from previous episodes of UTI may guide the decision-making process in the selection of empiric therapy in a subsequent episode of UTI, and indicate the use of antimicrobials with high community resistance levels.

There are limited data to confirm or quantify the predictive value of the antimicrobial resistance pattern of previous isolates. This analysis assesses the value of the antimicrobial susceptibility of a previously isolated *E. coli* on predicting the susceptibility of a subsequent isolation of *E. coli* from a UTI in routine clinical practice.

METHOD

The laboratory at Galway University Hospital is the main regional laboratory for over 200 000 patients (of a total Irish population of around 4 million) and provides a microbiology service to GPs in the West of Ireland as well as to the hospital.

All records from general practices of patients with more than one sample of significant bacteriuria

A Vellinga, MSc, MEd, researcher; AW Murphy, MD, FRCP, MRCP, professor, Discipline of General Practice, School of Medicine, NUI Galway, Ireland. M Cormican, MD, MRCP, FRCP, professor, Discipline of Bacteriology, School of Medicine, NUI Galway and consultant microbiologist, Galway University Hospitals, Galway, Ireland. B Hanahoe, MSc, surveillance scientist, Department Medical Microbiology, University Hospital, Galway.

Address for correspondence

Mrs Akke Vellinga, Discipline of General Practice, School of Medicine, 1 Distillery Road, NUI Galway, Ireland. E-mail: akke.vellinga@nuiigalway.ie

Submitted: 14 January 2010; Editor's response: 9 March 2010; final acceptance: 25 March 2010.

©British Journal of General Practice 2010; 60: 511–513.

DOI: 10.3399/bjgp10X514765

How this fits in

Although commonly practised by GPs there is no evidence that previous episodes of UTI caused by *E. coli* can guide selection of empiric antimicrobial therapy for subsequent episodes of UTI. This database analysis of *E. coli* re-infections shows that previous episodes can provide guidance in the treatment of subsequent infections.

(>10⁶ pure culture/ml), that is, recurrent infections, were extracted from the database over a 4.5-year period (April 2004 to September 2008). The first isolate during this period was identified for each patient, and the time to each subsequent isolate was calculated. A recurrence was defined as a re-infection if it was caused by the same species and if it occurred more than 2 weeks after the original UTI.² Relapse isolates, defined as recurrence with the same species within 2 weeks from the previous sample, were excluded from this analysis.³ If more than one sample was given during a 3-month period, only the first of these samples was considered.

The positive predictive value (PPV) was calculated as the proportion of patients with an *E. coli* resistant to an antimicrobial at first isolate that remain resistant to this antimicrobial at the subsequent isolate. Similarly, the negative predictive value (NPV) gives the proportion of patients with an *E. coli* infection susceptible to an antimicrobial at the first isolate, who show the same susceptibility in a subsequent isolate. As the PPV and NPV are directly proportional to the prevalence of resistance in the population, a correction (Bayes theorem) is applied,⁴ with a correction for the variability introduced by the prevalence according to Zou.⁷ The PPV is calculated as $P(\text{disease}|\text{+test}) = \frac{(\text{sensitivity} \times \text{prevalence})}{[(\text{sensitivity} \times \text{prevalence}) + \{(1 - \text{specificity}) \times (1 - \text{prevalence})\}]}$. PPV and NPV and their 95% confidence intervals (CIs) were calculated using WINPEPI.⁸ The prevalence was obtained from the full database (all general practice samples over the period of 4.5 years). For simplicity, PPV and NPV

are only presented for re-infection within 3 months and between 9 and 12 months.

RESULTS

Over the 4.5-year period, 147 306 urine samples were analysed; in 21.3% an organism and in 14.4% *E. coli* was identified. A total of 3413 patients provided at least two *E. coli*-positive samples over the study period. The mean age of the prospective cohort was 51.7 years (standard deviation [SD] 25.7 years) and median 56.0 years. The study population consisted of 90.9% females and 11.0% were under 18 years of age. No changes in age or sex were observed over the time period.

A total of 1092 of patients had a re-infection within 3 months, 693 patients had a re-infection between 3 and 6 months after the first sample, 543 between 6 and 9 months, and 450 between 9 and 12 months. Little difference was found between age and sex when comparing the full database from the 4.5-year period to the re-infection database (Table 1). Table 2 gives an overview of the PPV and NPV of the first sample when the subsequent sample is within 3 months and between 9 and 12 months; the PPV and NPV for the periods in between (3–6 months and 6–9 months) declines gradually between these periods.

There is an 84.6% probability that a patient with an ampicillin-resistant *E. coli* in a previous sample will still have an ampicillin-resistant *E. coli* in a subsequent episode of bacteriuria within 3 months.

Table 1. Comparison of full and re-infection database.

	Full database	Re-infection database
<i>n</i>	14 405	3413
Mean age, years	46.8	51.7
Sex, %		
Female	86.4	90.2
Male	9.1	8.2
Unknown	4.5	1.6

Table 2. Overview of prevalence of resistance, positive predictive value and negative predictive value and 95% confidence interval for each antimicrobial for a re-infection within 3 months and after 9–12 months.

	Prevalence of resistance, %	Within 3 months				9–12 months			
		PPV	95% CI	NPV	95% CI	PPV	95% CI	NPV	95% CI
Co-amoxiclav	23.0	54.5	49.8 to 59.1	87.3	85.7 to 88.7	43.1	35.9 to 50.4	82.4	80.0 to 84.7
Ampicillin	60.7	84.9	82.2 to 87.2	77.6	74.2 to 80.7	75.9	71.4 to 80.0	60.1	54.7 to 65.2
Ciprofloxacin	5.7	83.8	71.7 to 90.7	98.3	97.8 to 98.7	43.4	30.1 to 56.9	96.8	96.0 to 97.5
Nitrofurantoin	2.6	20.2	12.3 to 31.3	98.0	97.6 to 98.3	5.7	1.5 to 26.3	97.5	97.1 to 97.9
Trimethoprim	26.4	78.3	73.1 to 82.5	91.3	89.9 to 92.5	59.2	51.9 to 66.0	86.3	83.6 to 88.0

NPV = negative predictive value. PPV = positive predictive value.

Brief Reports

PPVs are obtained for ciprofloxacin (83.8%) and trimethoprim (78.3%). The probability of a nitrofurantoin resistant re-infection within 3 months if the previous isolate is resistant is particularly low (20.2%). The probability that a re-infection between 9 months and a year remains resistant is high, at 75.9% for ampicillin and 59.2% for trimethoprim. In contrast, the probability that a re-infection within 3 months and up to a year is susceptible if the initial *E. coli* was susceptible is nearly 100% for ciprofloxacin and nitrofurantoin, and 86.3% for trimethoprim.

DISCUSSION

Summary of main findings

If a patient presents with a recurrent UTI within 3 months and their previous sample showed an *E. coli* resistant to ampicillin, trimethoprim, or ciprofloxacin, this recurrent UTI is most likely associated with an organism that is still resistant. If a patient with a recurrent UTI was diagnosed in the previous year with an *E. coli* that was susceptible to nitrofurantoin, ciprofloxacin, or trimethoprim, the organism associated with this recurrent episode is likely to be still susceptible.

Limitations of the study

The use of routine laboratory urine samples as the basis for this analysis may influence the results due to varying request behaviour or changes in laboratory procedures. However, the number of urine samples submitted did not change over time (data available from authors). Also, the GPs' follow-up of patients with a laboratory-confirmed UTI as well as its potential effect on the data is unknown.

Comparison with existing literature

Occurrences of re-infection are common and clinicians often look at the results of previous urine testing to help guide their antimicrobial choice when patients represent. To the authors' knowledge there has been no research, to date, which either supports or opposes this practice.

Implications for clinical practice and future research

These results may help GPs to conserve broad-spectrum agents by using antimicrobial test results

from previous episodes of UTI to prescribe more narrow-spectrum agents such as trimethoprim, even when community resistance levels are high. The high PPV of previous ampicillin, trimethoprim, and ciprofloxacin resistance warrants against the re-prescription of these agents within 3 months, while the high NPV indicates prescription of these antimicrobials if susceptibility for these antimicrobials was shown in a previous sample of the patient. The low prevalence of resistance and high NPV of nitrofurantoin at both 3 and 12 months promotes nitrofurantoin as a beneficial first-line agent for initial and repeat presentations.

More in-depth research into patients presenting with another positive *E. coli* UTI, in particular within 3 months, would be of interest to further improve prescribing practice. An ongoing prospective study will address these concerns (www.antibiotics.nuigalway.ie).

Funding body

This work was conducted as part of a project funded by the Health Research Board of Ireland.

Ethical approval

Ethical approval was granted by the Irish College of General Practitioners.

Competing Interests

The authors have stated that there are none.

Acknowledgements

Sincere thanks to the laboratory staff of the Department of Medical Microbiology, University Hospital, Galway for providing the data for this analysis.

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Appendix 14

Opt-out as an acceptable method of obtaining consent in medical research: a short report.
Vellinga A, Cormican M, Hanahoe B, Bennett K, Murphy AW. *BMC Health Research Methodology* 2011, 11: 40.

RESEARCH ARTICLE

Open Access

Opt-out as an acceptable method of obtaining consent in medical research: a short report

Akke Vellinga^{1,2*}, Martin Cormican^{2,3}, Belinda Hanahoe², Kathleen Bennett⁴ and Andrew W Murphy¹

Abstract

Background: A prospective cohort study was set up to investigate a possible association between antibiotic prescribing and antibiotic resistance of *E. coli* urinary tract infection in the community. Participation of patients with urinary tract infection was obtained through an opt-out methodology. This short paper reports on the acceptability of the opt-out recruitment approach.

Methods: Participating practices (22) were requested to send a urine sample from all patients presenting with symptoms of urinary tract infection. Upon receipt of the sample in the laboratory, a letter explaining the study, an opt-out form and a freepost envelope were sent to all adult patients. A website with additional information and including an 'opt-out' button was set up for the study.

Results: A total of 1362 urine samples were submitted by the 22 participating practices representing 1178 adult patients of whom 193 actively responded to the letter: 142 opted out by letter, 15 through the website, 2 by phone and 12 sent the letter back without indication, making a total of 171 patients or 14.5% opt-out; the remaining 22 patients (1.9%) explicitly opted in. The total group consisted of 80% women and the mean age was 50.9 years (sd 20.8). No significant differences were found between patients who participated and those who opted out in terms of age, gender or whether the urine sample was positive or not.

Conclusions: Overall the opt-out method was well received and participation in the study reached 85.5%. The low number of complaints (2) indicates that this is a generally acceptable method of patient recruitment. The 14.5% opt-out shows that it effectively empowers patients to decline participation. The similarity between patients opting out and the rest of the patients is reassuring for extrapolation of the results of the study.

Background

The gold standard with respect to ethical recruitment of participants in research is explicit written consent, although various studies have shown that most patients do not have a preference for active consent [1]. An Irish study on public perceptions of biomedical research found that the public is generally aware of and committed to making a contribution to research and related activities in the healthcare system for their benefit and for the benefit of future patients [2]. In our study on management of urinary tract infection (UTI) in general practice, it was important to get a representative sample of patients. Concern was raised that an opt-in method for recruitment could cause bias as this approach is time consuming for the general practitioner (GP) which

could impact on the participation of the GP and the patient. This short paper gives an overview of the application of an opt-out recruitment approach and its acceptability for consideration in other health-related studies.

Overview of the justification to support opt-out consent

Active consent or opt-in has been shown to limit participation [3] and introduce bias into studies [4]. If consent is considered an indication of willingness rather than refusal and if risks for the participants are very low, an opt-out arrangement or passive consent is generally the most efficient procedure without violating the option of providing choice [5]. This methodology will most likely result in a more representative population reflecting the real life situation [6].

A limited number of studies explicitly researched possible objections to the opt-out system and not only

* Correspondence: akke.vellinga@nuigalway.ie

¹Discipline of General Practice, School of Medicine, NUI Galway, Ireland
Full list of author information is available at the end of the article



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found this method to be generally accepted but additionally identified patients' appreciation of participation in research [7,8].

Good methodology should respect personal autonomy by providing the necessary information to make an informed decision and include safeguards to protect privacy [9-11]. The challenge in applying an opt-out methodology is to provide easily accessible information to all patients to facilitate informed consent without interfering with the medical consultation. This principle was applied to our study on antimicrobial resistance and prescribing in adults with urinary tract infections.

Methods

A prospective cohort study was set up to describe the management of UTI in Irish general practice as well as to investigate a possible association between antimicrobial prescribing and resistance of *E. coli* isolated from patients with urinary tract infection.

Following extensive dialogue with the Research Ethics Committee of the Irish College of General Practitioners, approval for an opt-out consent method was given. The study received ethical approval for the use of an opt-out methodology based on the low risk to the patient and the potential benefit for the patient of adequate management of UTI based on unbiased information [5,12]. In this interpretation consent is an indication of willingness rather than refusal and informed consent is obtained by generally accessible information as well as easy modes to opt out [11].

Only one laboratory provides microbiological services in this region. After a retrospective analysis of laboratory data from all practices submitting to this laboratory [13], a list of practices was generated based on the number of positive urine samples in 2007. In an effort to limit the workload of a potential increase in the number of samples due to the study and in consultation with the laboratory, the highest ranking practices were selected. The top 25 practices were invited and 22 agreed to participate in the study, two practices did not have computerised records and one practice declined. Practices were located in rural as well as urban locations with a variety of patient populations. Practices had different patient (age and gender) profiles as well as different proportions of private and medical card patients. At the time of the study about 30% of the population was eligible for a medical card. Medical card eligibility is determined by income as well as age (all pensioners over the age of 70 years are eligible). Medical card patients have free medical care and medication [14]. The participating general practices were considered representative of all Irish general practices.

All practices received posters informing patients of the study to display in the waiting room, as well as little

reminder cards for the consultation rooms. Participating practices were requested to send a urine sample from all patients presenting with symptoms suggestive of urinary tract infection. Upon receipt of the sample in the laboratory, a letter explaining the study (supplementary file), an opt-out form and a freepost envelope were sent to all adult patients who supplied a urine sample.

In the letter patients were informed of the objectives of the study and permission for the researcher to look at their GP records was requested. Additionally, it was explained that they were free to opt out of participation in the study by filling out the included opt-out form, by phone or through the website.

A dedicated website was set up with detailed information on the study as well as on the problem of antimicrobial resistance in general <http://www.antibiotics.nuigalway.ie>. The index page included an 'opt-out' button which linked to a form that could be filled out by the patient. The website was clearly laid out to avoid confusion and ensure easy opt-out. Regular updates on the study, as well as study results were added to the website when available.

Results

A total of 1362 urine samples were submitted by the 22 participating practices during the study period. The samples were from 1178 adult patients. The 22 practices sent in between 15 and 115 samples.

In total 193 patients actively responded to the letter: 142 opted out by letter, 15 through the website, 2 by phone and 12 sent the letter back without indication, making a total of 171 patients (14.5%) who opted out; the remaining 22 patients (1.9%) explicitly opted in (Figure 1). The letters of 24 patients had a wrong address and were returned.

Two patients expressed concerns regarding the use of the opt-out method. Both questioned the way their address was obtained and whether this interfered with the confidentiality of their patient data. An individual response to these concerns was sent to their GP with a request to forward this to the patient. No further concerns were expressed.

Patients consisted of 941 women (79.9%) and 237 men (20.1%). Their mean age was 50.9 years (sd 20.8) and the median age was 47 years. Patients who opted out were slightly older (52.8 vs 50.4 years) and the percentage of females was slightly higher (83% vs 79.5%) but these differences were not statistically significant. Patients who opted out through the website were significantly younger than those who used the letter (non-parametric, 53.5 vs 38.7 years, $p < 0.05$).

A significant isolate (pure culture at greater than 10^5 colony forming units/ml) was identified from the urine sample of 402 (34.1%) patients. Patients with a positive

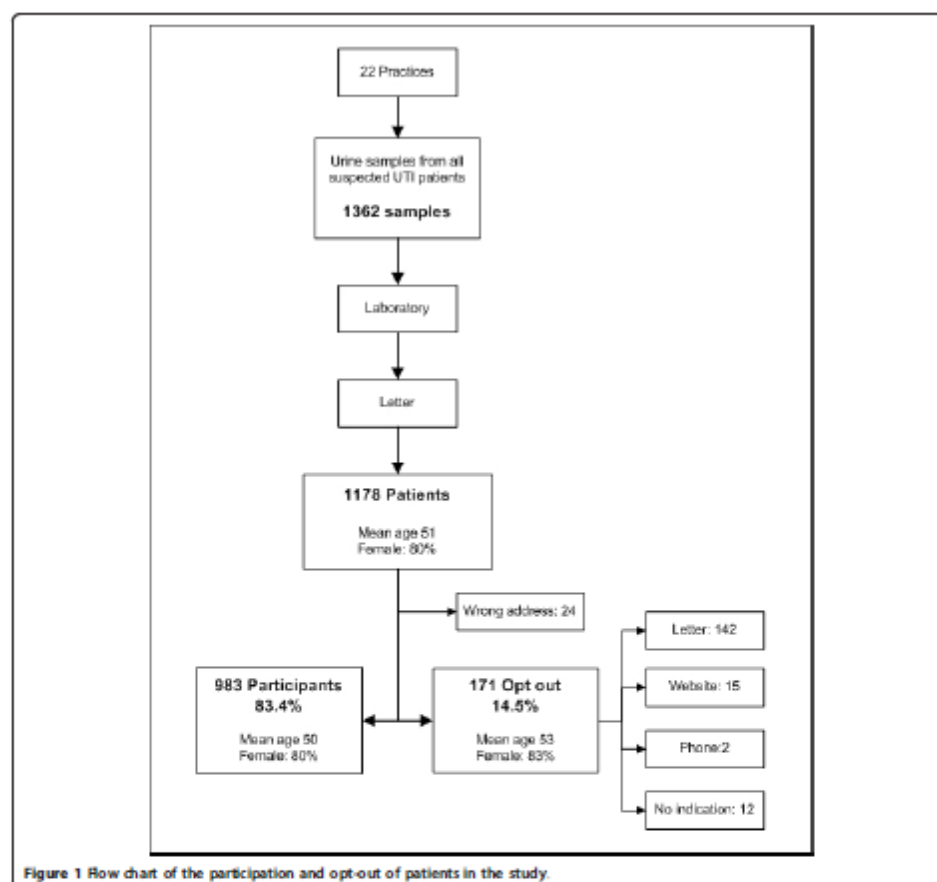


Figure 1 Flow chart of the participation and opt-out of patients in the study.

culture were no more likely to opt out compared to those with a negative culture.

Discussion and Conclusions

Overall the opt-out method was well received by both general practitioner and patients and achieved a high level of participation in the study at 83.4%. The low number of complaints indicates that this is a generally acceptable method of patient recruitment. The 14.5% opt-out of patients shows that the process effectively empowered patients to decline participation. The high similarity between patients opting out and the participating patients with respect to age, gender and isolation of a positive culture is reassuring for extrapolation of

the results of the study. However, as no other potentially important variables were available about the patients who opted out, it cannot be ruled out that other factors were of importance for participation in the study. Similarly, even though every effort was made to inform patients of the study, it cannot be guaranteed that all patients received this information through the different media offered by us.

Our findings are in line with other studies which have shown that an opt-out methodology is generally well accepted and will result in high participation rates [7,8,15]. A recent Cochrane review looked at ways to increase recruitment into clinical studies and also identified opt-out as a possible method [16]. The lack of

further involvement in the study by participants and general practitioners, acknowledged in the ethical approval given to the study, favours this type of recruitment which might be less applicable for studies with more involvement or risk. For non-interventional, low-risk studies in which rigorous measure to inform patients and protect patient confidentiality are in place, recruitment by opt-out is an easy and acceptable methodology for patients, GPs and researchers. As earlier stated by Junghans et al. [4], the opt-out approach should be the default recruitment strategy for studies with low risk to participants.

Funding

This research project was funded by a grant from the Health Research Board, Ireland

Acknowledgements

We thank Mary Byrne for proof reading the final manuscript.

Author details

- ¹Discipline of General Practice, School of Medicine, NUI Galway, Ireland.
²Discipline of Bacteriology, School of Medicine, NUI Galway, Ireland.
³Department of Medical Microbiology, University Hospital, Galway, Ireland.
⁴Department of Pharmacology & Therapeutics, Trinity Centre for Health Sciences, Dublin, Ireland.

Authors' contributions

AV set up and coordinated the study, analysed the results and drafted the manuscript. MC and AWM conceived of the study and critically revised the manuscript. BH acquired the data for the study and approved the final manuscript. KB has been involved in the conception of the study, acquisition of data and has approved the final manuscript. All authors read and approved the final draft.

Competing interests

The authors declare that they have no competing interests.

Received: 10 November 2010 Accepted: 6 April 2011

Published: 6 April 2011

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Pre-publication history

The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2288/11/40/prepub

doi:10.1186/1471-2288-11-40

Cite this article as: Vellings et al.: Opt-out as an acceptable method of obtaining consent in medical research: a short report. *BMC Medical Research Methodology* 2011 **11**:40.

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Appendix 15

Newsletter for participating practices

NUI Galway
OÉ Gaillimh

July 2010



NUI GALWAY - GENERAL PRACTICE AND BACTERIOLOGY

Antimicrobial resistance and urinary tract infections in the community

The study on antimicrobial prescribing and resistance of UTI in General Practice

First we would like to take the opportunity to thank you all very sincerely for your participation in our study and for all that you have done to make the data collection as easy as possible. This newsletter includes an overview of antibiotic resistance levels in *E.coli* of your practice and some of the main findings to date. A final newsletter with results will be sent at the end of the study, next summer.

The aim of this project is to understand the relationship between prescribing antibiotics in primary care and antibiotic resistance of *E.coli* isolated from a urine sample. There are two parts to the study, a retrospective and a prospective part.

The first part of the study linked the database of the medical card prescriptions with the results from urine samples sent to the microbiology laboratory at University Hospital Galway (UHG). This retrospective part of the study is now complete.

The results from the first part of the

study have fed into the second part that is currently underway. In this study we are looking at a possible link between antibiotic prescribing and antibiotic resistant *E. coli* at the level of the individual patient rather than at the practice level.

A total of 22 practices participated in the prospective second part of the study. Using the laboratory system at the UHG eligible patients were enrolled in the study. A letter explaining the study and an opt-out form were sent to each patient. The charts from the participating patients were checked for information on the prescription of antibiotics in the year before the urine sample was submitted.

Initially the chart review included all patients from whom a urine sample was sent. After three months only the charts of patients with a positive urine culture were included. Data collection for this prospective part of the study is now nearly completed.

Find out how your practice compares on page 3

Special points of interest:

- The more antimicrobials are prescribed in a practice, the higher the chance that a patient with a UTI will have an antibiotic resistant *E. coli**read more on page 2 and page 4*
- Results from previous UTI can guide empiric prescribing.....*read more on page 2*
- Nitrofurantoin should be considered for first line empirical prescribing in uncomplicated UTI as resistance is very uncommon in *E. coli**read more on page 2 and page 4*
- Patients were generally very happy to participate in our study.....*read more on page 2*
- More than 2000 patient's charts will have been checked by the end of this study

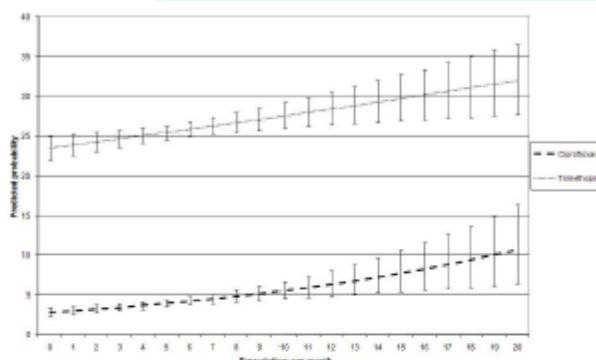
Does practice prescribing influence patients resistance?

The easy answer is yes! But it is a little more complicated than that. In the retrospective study the overall practice prescribing to medical card patients was linked with the antibiotic resistance patterns of the UTI *E.coli* from each practice. Even though the database only holds data on medical card patients, this is representative of overall frequency and type of prescribing of the practice. We focused our attention on 2 antibiotics, trimethoprim and ciprofloxacin.

An interesting finding was that, when controlling for the individual impact of prescribing on resistance, an underlying practice resistance remained. This means that a patient moving from a practice with low to one with a high resistance will increase his or her chances of being diagnosed with a resistant *E.coli*.

Journal of Antimicrobial Chemotherapy 2010 65(7):1514-1520;
doi:10.1093/jac/dkq149

The results showed that with every increase in trimethoprim prescription in the practice, the odds of a patient from the practice having a trimethoprim resistant *E.coli* increased slightly (to 1.02) and a similar but more marked trend was apparent for ciprofloxacin (1.08). This increase does not look very big but when comparing practices with 1 prescription per 1000 medical card patients per month with a practice with 10 prescriptions per 1000 patients, the predicted probability of having a resistant *E.coli* increased from 24% to 28% for trimethoprim and from 3% to nearly 6% for ciprofloxacin.



Recurrent urinary tract infections

In a database analysis of *E.coli* re-infections we showed that antibiotic sensitivity test results from previous episodes of UTI can provide guidance in the treatment of subsequent infections.

If a patient presents with a recurrent UTI within 3 months and their previous sample showed an *E.coli* resistant to ampicillin, trimethoprim or ciprofloxacin, this recurrent UTI is most likely still resistant. If a patient with a recurrent UTI was diagnosed in the previous year

with an *E.coli* that was susceptible to nitrofurantoin, ciprofloxacin or trimethoprim, this recurrent episode is likely to be still susceptible.

This study is published in the British Journal of General Practice, July 2010, volume 60, Nr 576, 511-513(3).

*Nitrofurantoin resistance is very uncommon in the community.
Nitrofurantoin is a generally safe drug and it should be considered
for first line treatment of uncomplicated UTI (cystitis)*

Opt-out ethical approval

Ethical approval for the prospective study in which your practice participated was granted by the ICGP based on an "opt-out" approach. It was important to have approval to use this opt-out method because it avoided adding an additional burden to the doctor-patient contact. With this method the GP did not have to allocate time to explain the study to patients, which could have resulted in lower participation in the study.

The opt-out method has proven to be a generally accept-

able method of patient recruitment. Overall 13% of the patients opted-out, by letter, phone or through the website. This shows that it effectively empowers patients to decline participation. No differences were found for age or gender between patients opting out compared to the rest of the patients which is reassuring for the extrapolation of the results of the study.

87% participation !

How does your practice compare with the others?

In 2009 a total of 29,341 urine samples came into the laboratory of the University College Hospital. No organisms were identified in 65% of these samples, mixed organisms in 13%, *Escherichia coli* in 17%, *Proteus* species in 1% and a variety of other organisms in the remaining 4%.

The overall percentage resistance of *E. coli* in the full database (only including one sample per patient) was 63% for ampicillin, 21% for co-amoxycylav, 11% for ciprofloxacin, 5% for nitrofurantoin and 31% for trimethoprim.

In 2009, the 22 practices in the study participating practices sent 11,189 samples to the lab. Of these, 65% yielded no organism, 14% mixed organisms and 17% *E. coli*. This is similar to the breakdown for all specimens submitted. The 1880 *E. coli* from the participating practices were from 1321 patients. In the picture below an overview of antimicrobial resistance of these urinary *E. coli* is shown. For comparison, the overall resistance of the 22 participating practices is shown with its standard deviation.

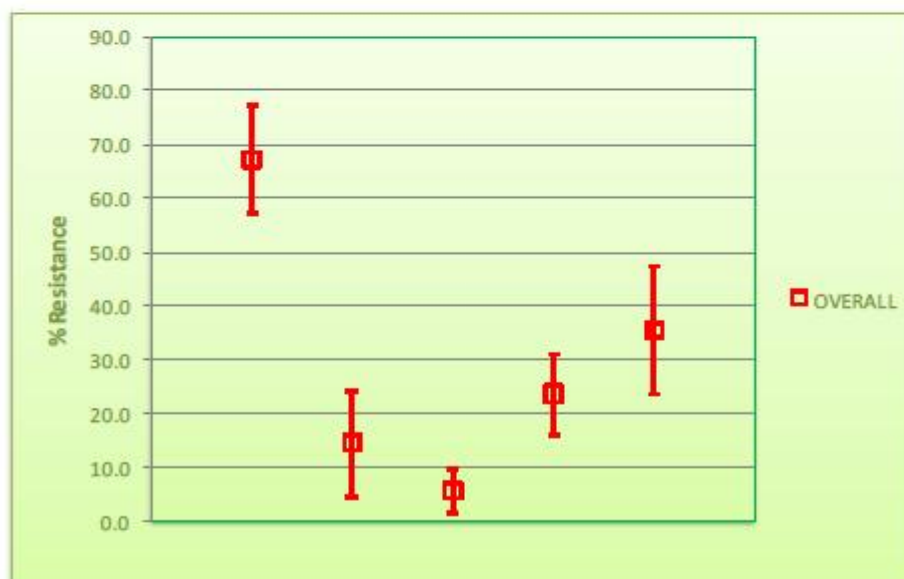
If you have more questions about this or if you like to get more advice on prescribing, please let us know and we can set

up a practice visit by a member of our research team or a telephone call can be arranged to discuss your question whichever is more convenient for you.



A picture of the flooding last year, due to which some appointments were cancelled; one of the river interruptions. (Courtesy of C. Kinnane)

Overall practice resistance and 95% confidence interval



Effect of antibiotic prescribing in primary care on antimicrobial resistant in individual patients: a systematic review. BMJ May 2010

A recent systematic review found that antibiotic resistance is greatest in the month after treatment but may last for up to a year, possibly driving high levels of resistance in the community. British researchers analysed 24 published studies of antibiotic resistance in primary care patients, mainly for respiratory or urinary infections. Of the included studies, 22 involved patients with symptomatic infection and two involved healthy volunteers. Nineteen were observational studies (two prospective) and five were randomized. Five studies of urinary tract bacteria (14,348 participants) found the pooled odds ratio (OR) for resistance was 2.5 (95% CI, 2.1 to 2.9) within two months of antibiotic treatment and 1.33 (95% CI, 1.2 to 1.5) within 12 months. Seven studies of respiratory tract bacteria (2,605 participants) found

pooled ORs of 2.4 (95% CI, 1.4 to 3.9) within two months and 2.4 (95% CI, 1.3 to 4.5) within 12 months.

Researchers found greater rates of resistance associated with higher doses of amoxicillin and longer courses of trimethoprim. Also, longer duration and multiple courses of these antibiotics were associated with higher resistance rates.

Authors concluded that the only way to avoid resistance is to avoid using antibiotics whenever possible.



http://www.bmj.com/cgi/content/abstract/340/may18_2/c2096

Prescribing of Nitrofurantoin

The dose for adults is 50mg every 6 hours (best taken with food or milk)

~For simple cystitis in non-pregnant women 3 days treatment is enough.

~Treatment for 7 days may be required in some patients.

CAUTION:

- Nitrofurantoin should not be used in late pregnancy (from 38 weeks) or in patients with renal failure
- It should not be used in children less than 3 months of age
- Long term (> 6 months) continuous use of nitrofurantoin (e.g. for prophylaxis of UTI) may be associated with lung and liver toxicity and requires careful monitoring.

Who is involved?

Prof Andrew Murphy, Discipline of General Practice, School of Medicine, NUI Galway and GP at Turloughmore Health Centre

Prof Martin Cormican, Discipline of Bacteriology, School of Medicine, NUI Galway and Consultant Microbiology, Galway University Hospitals

Belinda Hanahoe, Surveillance Scientist, Department of Microbiology, Galway University Hospitals

Dr Kathleen Bennett, Department of Pharmacology & Therapeutics, Trinity Centre for Health Sciences, Dublin

Breda Kelleher, Administrative support, Discipline of General Practice, School of Medicine, NUI Galway

Akke Vellinga, Epidemiologist, Discipline of General Practice, School of Medicine, NUI Galway

My personal journey

You have all seen me in your practice, where I usually sat quietly at a computer, trawling through charts and avoiding even a chat. It was important for the study to describe current management of urinary tract infection in general practice without any interference. This provides an important baseline. At this stage we have finished the data collection and I have learned a lot; locating practices, interpreting charts, how practices are run and

most importantly, prescribing.

I am going into the next stage now where the data will be analysed, interpreted and written up. I would like to thank you all for participating and allowing me to get to this stage. It has been a great experience and I hope to get back to you all with the results next year.

I will keep in touch.

Akke

akke.vellinga@nuigalway.ie

www.antibiotics.nuigalway.ie



NUI Galway
OE Gaillimh



A 'wordle' of this PhD thesis



A wordle is a word cloud in which more prominence is given to words that appear more frequently in the text (Courtesy of www.wordle.net).

