Association between use of different antibiotics and trimethoprim resistance

Pouwels, Koen B; Freeman, Rachel; Muller-Pebody, Berit; Rooney, Graeme; Henderson, Katherine L; Robotham, Julie V; Smieszek, Timo

Published in:
Journal of Antimicrobial Chemotherapy

DOI:
10.1093/jac/dky031

Publication date:
2018

J Antimicrob Chemother 2018; **73**: 1700–1707
doi:10.1093/jac/dky031 Advance Access publication 31 January 2018

Journal of Antimicrobial Chemotherapy

Association between use of different antibiotics and trimethoprim resistance: going beyond the obvious crude association

Koen B. Pouwels[1–3]*, Rachel Freeman[4], Berit Muller-Pebody[4], Graeme Rooney[4], Katherine L. Henderson[4], Julie V. Robotham[1]† and Timo Smieszek[1,3]†

[1]Modelling and Economics Unit, National Infection Service, Public Health England, London, UK; [2]PharmacoTherapy, -Epidemiology & -Economics, Department of Pharmacy, University of Groningen, Groningen, The Netherlands; [3]MRC Centre for Outbreak Analysis and Modelling, Department of Infectious Disease Epidemiology, Imperial College School of Public Health, London, UK; [4]Department of Healthcare-Associated Infection and Antimicrobial Resistance, National Infection Service, Public Health England, London, UK

*Corresponding author. Modelling and Economics Unit, National Infection Service, Public Health England, 61 Colindale Avenue, London NW9 5EQ, UK. Tel: +44-(0)20-8327-6377; E-mail: koen.pouwels@phe.gov.uk
†Contributed equally as last authors.

Received 27 September 2017; returned 13 November 2017; revised 2 January 2018; accepted 15 January 2018

Objectives: To evaluate the association between use of different antibiotics and trimethoprim resistance at the population level.

Methods: Monthly primary care prescribing data were obtained from NHS Digital. Positive Enterobacteriaceae records from urine samples from patients between April 2014 and January 2016 in England were extracted from PHE's Second Generation Surveillance System (SGSS). Elastic net regularization and generalized boosted regression models were used to evaluate associations between antibiotic prescribing and trimethoprim resistance, both measured at Clinical Commission Group level.

Results: In total, 2487635 (99%) of 2513285 urine Enterobacteriaceae samples from 1667839 patients were tested for trimethoprim resistance. Using both elastic net regularization and generalized boosted regression models, geographical variation in trimethoprim resistance among Enterobacteriaceae urinary samples could be partly explained by geographical variation in use of trimethoprim (relative risk = 1.14, 95% CI = 1.02–1.75; relative influence = 4.1) and penicillins with extended spectrum (mainly amoxicillin/ampicillin in England) (relative risk = 1.19, 95% CI = 1.11–1.30; relative influence = 7.4). Nitrofurantoin use was associated with lower trimethoprim resistance levels (relative risk = 0.83, 95% CI = 0.57–0.96; relative influence = 9.2).

Conclusions: Use of amoxicillin/ampicillin explained more of the variance in trimethoprim resistance than trimethoprim use, suggesting that co-selection by these antibiotics is an important driver of trimethoprim resistance levels at the population level. Nitrofurantoin use was consistently associated with lower trimethoprim resistance levels, indicating that trimethoprim resistance levels could be lowered if trimethoprim use is replaced by nitrofurantoin.

Introduction

A clear link exists between antimicrobial consumption and resistance rates of bacteria.[1–3] Studies linking antibiotic usage and resistance at the population level usually focus on crude associations between the resistance against a specific antibiotic and the use of that specific antibiotic or antibiotic group.[2,4,5] Confounding by use of other antibiotics and the fact that (multiple) resistance genes are often linked, thereby allowing co-selection, are typically ignored. Confounding by other antibiotics may occur if a reduction in one antibiotic is accompanied by an increase in another (related) antibiotic. For example, if one were to replace amoxicillin with ampicillin, one would not expect a change in amoxicillin resistance given the near complete cross-resistance between these two antibiotics. Additionally, treatment with one agent may increase the density of organisms resistant to another agent within a patient, by killing off competing bacterial flora.[6] Co-selection can occur when resistance genes are linked on the same mobile genetic element, which acts as a resistance 'vector'. For example, it is well-known that ampicillin and trimethoprim resistance genes are often linked on such elements.[7–9] Therefore, when prescribing ampicillin one not only selects for amoxicillin resistance genes, but also for the linked trimethoprim resistance genes.

Some studies, the majority using individual patient data, have incorporated at least some potential confounders/determinants in regression models, including patient demographics and/or a

selected group of other antibiotics.[10–14] Nevertheless, these studies adjusted for only a small, selected subset of other antibiotics or all other antibiotics grouped together, thereby missing the opportunity to adequately control for confounding by other antibiotics and potentially identify co-selection.

If the use of other antibiotics is simultaneously put in a model, one can not only adjust for such confounding, but also identify potential (unknown) co-selection. The latter is especially relevant since microbiological studies often cite co-selection as an important cause of increasing, or maintenance of existing, resistance prevalence levels. Moreover, microbiological studies often test only for cross-resistance between routinely tested antibiotics, thereby limiting the possibility to identify potential co-selection by antibiotics for which resistance is not routinely tested because these other antibiotics are rarely used to treat the infection of interest. Nevertheless, prescribing for other conditions can also result in an increase in resistance among the pathogens causing the infection of interest due to co-selection. Evaluating the associations between the use of different antibiotic groups and resistance prevalence could substantially improve our understanding of the role of co-selection in the development and maintenance of resistance at the population level, and potentially therefore insight into the potential impact on resistance of reductions in antibiotic use.

Given a recent change to guidelines in England, which recommend prescribing trimethoprim only if there is low risk of resistance, a decrease in trimethoprim use can be expected in the primary care setting. To be able to predict what impact this may have on trimethoprim resistance, the co-selection potential of other antibiotics needs to be identified.

We aimed to evaluate which antibiotic groups may select for trimethoprim resistance and to what extent in Enterobacteriaceae isolated from urinary samples in England. We hypothesized we would find associations between (i) trimethoprim use and trimethoprim resistance[1,4,13] and (ii) ampicillin use and trimethoprim resistance,[7–9] but potentially also (iii) identify unknown co-selection mechanisms.

Methods

Ethics

All data were collected as part of routine surveillance and were anonymized; Ethics Committee approval was therefore not required.

Data

Monthly prescribing data were obtained from NHS Digital, who collate for all general practices in England the total number of items that are prescribed and then dispensed (http://digital.nhs.uk/). Data on hospital use of antibiotics are not available. Prescribed units were measured using DDDs and items. To facilitate a more direct comparison with other countries, we created antibiotic groups based on the first five characters of the Anatomical Therapeutic Chemical (ATC) classification system. For the primary analysis, we analysed the data on a quarterly basis at the clinical commission group (CCG) level. CCGs are NHS organizations set up by the Health and Social Care Act 2012 to organize the delivery of NHS services in England. All general practices in England belong to one of 211 CCGs.

Reports of Enterobacteriaceae isolated from urine samples from general practice and hospital patients between April 2014 and January 2016 in England were extracted from PHE's Second Generation Surveillance System (SGSS). SGSS is a national voluntary laboratory surveillance system and captures antibiogram (i.e. antimicrobial susceptibility) data of all microorganisms tested. This national database contains laboratory data supplied electronically by approximately 98% of hospital microbiology laboratories in England. We used data from April 2014 onwards, because data coverage before this date was much more incomplete. During the study period, the vast majority of laboratories used BSAC disc diffusion methodology to test for trimethoprim resistance. A few laboratories used EUCAST or CLSI methodology. In our primary analysis, repeat specimen reports received from the same patient with matching causative agents were excluded if the specimen dates were within 14 days. Using different cut-offs (30 and 365 days) gave very similar results (data not shown).

Initial inspection of the data indicated that a standard Poisson generalized linear model would suffer from multi-collinearity and data sparsity issues. When two or more predictors in the regression model are highly correlated (multi-collinearity), the high obtained variances will make the results of a standard regression model essentially worthless. Moreover, given the relatively high number of potential predictors in the model, there is a risk of substantial bias due to a lack of sufficient numbers for some combinations of exposure and outcome levels (sparsity bias).[15]

To be able to address potential overfitting and multicollinearity we used two different methods: elastic net regularization and generalized boosted regression models.[16–18]

Elastic net regularization

Elastic net regularization combines the advantages of both least absolute shrinkage and selection operator (lasso)[19] and ridge regression[20] (see Section S1, available as Supplementary data at *JAC* Online, for further details). Such regularization or shrinkage techniques are especially useful when encountering situations with high collinearity and a relatively large number of variables compared to the amount of observations.[15,21]

For the current analyses, we fitted a Poisson model with elastic net regularization. The number of Enterobacteriaceae isolates from urine samples reported to be resistant to trimethoprim by quarter was included as a dependent variable. To take into account that some CCGs tested more samples than others, the natural logarithm of the number of Enterobacteriaceae urinary samples tested was included as an offset variable. As potential explanatory variables we considered all antibiotic groups prescribed in the year before each quarterly measured trimethoprim resistance prevalence (which are listed in Table 1) and quarter of calendar year (Jan–Mar, Apr–Jun, Jul–Sep and Oct–Dec), year and the test rate (i.e. the number of Enterobacteriaceae urinary samples tested for trimethoprim resistance divided by the population in that area). The test rate was included, because if only a few samples are submitted the proportion of resistant samples will be likely higher than when samples are routinely tested. Continuous variables were standardized by mean-centring and dividing by two standard deviations. To keep antibiotics on the same scale, all antibiotics were mean-centred and divided by two standard deviations of total antibiotic use instead of using the standard deviations of individual antibiotics. All elastic net analyses were performed using the 'glmnet' package in R version 3.2.2.[17,22] To reduce the false discovery rate often observed with standard application of regularization methods, we estimated the optimal shrinkage parameter λ using the Akaike information criterion (AIC).[23] In sensitivity analysis, alternative ways of obtaining a more conservative shrinkage parameter were evaluated.[23] CIs were obtained by taking 1000 clustered bootstrap samples.

Generalized boosted regression models

The second method we used to model associations between different antibiotic groups and trimethoprim resistance was the generalized boosted regression model, or boosted regression trees (BRT).[18] BRT models are more robust to multicollinearity than standard regression by giving each of the

correlated predictors a chance to be used in different trees.[24] Similarities with the lasso have been used to explain the success of boosting algorithms.[25]

For the current analyses, we fitted a Poisson BRT. The number of Enterobacteriaceae isolates from urine samples reported to be resistant to trimethoprim by quarter was included again as the dependent variable. The number of samples tested for trimethoprim resistance was included as an offset variable.

Results are presented in terms of the relative influence and using partial dependence plots. Predictors are ranked using their relative influence, which is the contribution of each predictor to the model relative to the other predictors. The relative influence is defined as the reductions in the sum of squared error of the outcome (SSE) attributable to splits on predictors over all K trees. The relative influence of each variable is expressed as the percentage of the total reductions (100%) in the SSE.[26]

All BRT analyses were performed using the 'gbm' and 'dismo' package in R version 3.2.2.[18,27]

Sensitivity/secondary analyses

Several sensitivity analyses were performed. First, in addition to expressing antibiotic use in DDDs/1000 inhabitants/day, antibiotic use at the CCG level was expressed as items dispensed. In addition, because resistance genes carried may differ between different species belonging to the Enterobacteriaceae family, we repeated the analyses separately for *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*.

To address potential concerns about lack of independence between quarters of measurements within CCGs, we performed an additional cross-sectional analysis using the average use for each antibiotic and the average trimethoprim resistance in each CCG over the entire study period. These data were also used to assess relationships between antibiotic groups and trimethoprim resistance using conventional Poisson regression with a separate model for each antibiotic (Table S1).

Results

For most antibiotic groups there was substantial variation in the amount of dispensed DDDs/1000 inhabitants/day (Table 1). These variations were mainly due to variation between different CCGs and less due to variation between different points in time. Between April 2014 and January 2016, 2487635 (99%) of 2513285 Enterobacteriaceae isolates from urine samples from 1667839 patients were tested for trimethoprim resistance. After removing repeated samples occurring within 14 days within the same patient, 2294826 Enterobacteriaceae samples were available for analysis. The most common pathogens identified were *E. coli* ($n = 1746013$), *K. pneumoniae* ($n = 70331$) and *P. mirabilis* ($n = 40905$). The data were subsequently grouped into quarters for each CCG, resulting in 1672 observations. There was substantial variation between the rate of samples tested for trimethoprim resistance per 100000 persons (median = 5.3, 25th–75th percentile = 2.5–7.7). There was also variation in the percentage of Enterobacteriaceae isolates from urine samples that were resistant to trimethoprim (median = 35%, 25th–75th percentile = 33%–37%) (Figure S1).

Poisson model with elastic net regularization

Antibiotic groups that were associated with increased trimethoprim resistance in at least 95% of the bootstrap samples were penicillins with extended spectrum (e.g. amoxicillin and ampicillin) and trimethoprim and derivatives (Table 2). Other antibiotic groups (combination of penicillins, including β-lactamase inhibitors; β-lactamase-sensitive penicillins; and second-generation cephalosporins) were associated with increased trimethoprim resistance, but not in 95% of the bootstrap samples. Nitrofurantoin and macrolides were the only antibiotic groups that were significantly associated with reduced trimethoprim resistance (Table 2). Similar results were obtained when alternative methods to estimate the optimal shrinkage parameter were used (Table S2) or antibiotics were expressed as items dispensed (Table S3). While results were similar when restricting the analyses to *E. coli* (Table S4), no antibiotic group had a significant association with trimethoprim resistance among *K. pneumoniae* or *P. mirabilis* samples (Section S2 and Table S5).

BRTs

While elastic net regularization can be successfully used in situations with highly correlated variables and sparse data, its interpretation can be difficult. Therefore, we also applied BRTs to the data, as results obtained using this method can intuitively be expressed as the relative importance of predictor variables.

The antibiotic groups that had the highest relative influence in the BRT are listed in Table 3 (the complete list can be found in Table S6). The antibiotic groups with high relative influence in the BRT were also selected by elastic net regularization, except lincosamides. However, this antibiotic group was selected by elastic net regularization if alternative methods to estimate the optimal shrinkage parameter were used (Table S2). The antibiotics that were significantly associated with trimethoprim resistance were among the variables with the highest relative influence in all sensitivity analyses (Tables S3 to S9).

The effect of the 10 antibiotic groups listed in Table 3 and the test rate were plotted using partial dependence plots, which show the effect of a variable on trimethoprim resistance after accounting for the average effects of all other variables in the full model. In line with the results from the elastic net regularization, nitrofuran derivatives and macrolides had a negative association with trimethoprim resistance (Figure 1). Especially trimethoprim derivatives and penicillins with extended spectrum were associated with a clear pattern of increasing trimethoprim resistance with increasing antibiotic use.

Discussion

Geographical variation in trimethoprim resistance among Enterobacteriaceae isolates from urine samples could be partly explained by geographical variation in trimethoprim use, even when taking into account confounding by use of other antibiotics using elastic net regularization and BRTs.

Using these techniques, we also observed the expected association between ampicillin/amoxicillin use and trimethoprim resistance.[7–9] In virtually all analyses, ampicillin/amoxicillin use had a similar or larger influence than trimethoprim use, suggesting that co-selection by these antibiotics is an important driver of trimethoprim resistance levels among Enterobacteriaceae at the population level.

Nitrofurantoin was clearly associated with lower resistance levels in virtually all analyses. This antibiotic is only used to treat urinary tract infections[28–32] and hence may be associated with less

Table 1. Variation in DDDs/1000 inhabitants/day between 2013 and 2015

Antibiotic group (ATC code)	Median DDDs/1000 inhabitants/day (25th–75th percentile)	Minimum DDDs/1000 inhabitants/day	Maximum DDDs/1000 inhabitants/day
Intestinal antibiotics (A07AA)	6.1×10^{-4} (2.7×10^{-4}–0.001)	0	0.0057
Tetracyclines (J01AA)	4.5 (4.0–5.1)	2.4	8.4
Amphenicols (J01BA)	0 (0–3.9×10^{-5})	0	0.0019
Penicillins with extended spectrum (J01CA)	4.2 (3.7–4.9)	2.3	7.0
β-Lactamase-sensitive penicillins (J01CE)	0.75 (0.68–0.84)	0.51	1.4
β-Lactamase-resistant penicillins (J01CF)	1.4 (1.2–1.5)	0.73	2.2
Combinations of penicillins, including β-lactamase inhibitors (J01CR)	0.91 (0.70–1.2)	0.13	2.6
First-generation cephalosporins (J01DB)	0.22 (0.17–0.32)	0.037	0.80
Second-generation cephalosporins (J01DC)	0.012 (0.0069–0.021)	1.6×10^{-4}	0.14
Third-generation cephalosporins (J01DD)	0.0012 (4.8×10^{-4}–0.0028)	0	0.022
Monobactams (J01DF)	0 (0–0)	0	2.6×10^{-4}
Carbapenems (J01DH)	3.0×10^{-5} (0–4.5×10^{-4})	0	0.022
Trimethoprim and derivatives (J01EA)	1.2 (0.98–1.3)	0.31	1.8
Short-acting sulphonamides (J01EB)	0 (0–0)	0	0.0095
Intermediate-acting sulphonamides (J01EC)	0.0012 (0–0.0038)	0	0.057
Long-acting sulphonamides (J01ED)	0 (0–0)	0	0.025
Combinations of sulphonamides and trimethoprim, including derivatives (J01EE)	0.050 (0.040–0.061)	0.012	0.16
Macrolides (J01FA)	2.8 (2.4–3.1)	1.2	5.5
Lincosamides (J01FF)	0.024 (0.016–0.036)	0.0032	0.22
Streptogramins (J01FG)	0 (0–0)	0	0.0033
Other aminoglycosides (J01GB)	0.012 (0–0.021)	0	0.084
Fluoroquinolones (J01MA)	0.32 (0.27–0.38)	0.14	0.55
Other quinolones (J01MB)	0 (0–0)	0	0.0043
Glycopeptide antibacterials (J01XA)	1.6×10^{-4} (0–9.2×10^{-4})	0	0.039
Polymyxins (J01XB)	0.050 (0.037–0.069)	0.0021	0.17
Steroid antibacterials (J01XC)	4.0×10^{-4} (8.4×10^{-5}–0.0012)	0	0.012
Imidazole derivatives (J01XD)	0 (0–0)	0	0.0017
Nitrofuran derivatives (J01XE)	0.81 (0.70–0.94)	0.24	1.4
Other antibacterials (J01XX)	0.015 (0.0076–0.035)	0	0.73

For each practice the average DDDs/1000 inhabitants/day was calculated on a quarterly basis during 2013–15.

use of trimethoprim and therefore less direct selection pressure on trimethoprim. In addition, nitrofurantoin resistance genes are, in contrast to trimethoprim resistance genes, not frequently found on mobile genetic elements with multiple resistances, presumably due to the relatively high fitness costs.[6,33] Hence, by treating with nitrofurantoin one may actually select for bacteria that are susceptible to trimethoprim.

Based on the low resistance profile for nitrofurantoin in most European countries, low potential for co-selection of other resistances with this antibiotic,[6] and clinical efficacy equivalent to that of other antibiotics indicated for uncomplicated lower urinary tract infections, [28] nitrofurantoin has been adopted as the first-line treatment for uncomplicated urinary tract infections in many countries.[28–32] Our data suggest that shifting towards more use of nitrofurantoin instead of trimethoprim for these types of infections may reverse trimethoprim resistance prevalence levels, if sustained for long enough and not accompanied by compensatory increases in antibiotics that co-select for trimethoprim resistance.[34]

The effect of lowering trimethoprim use on trimethoprim resistance has previously been studied in a 2 year intervention study from Sweden[34] An 85% decrease in trimethoprim-containing drugs during the intervention was accompanied by a disappointingly small effect on trimethoprim resistance[34] These results, together with other studies,[35] have increased scepticism against the effectiveness of interventions targeted at lowering antibiotic use, which are the cornerstone of many strategies aiming to tackle antibiotic resistance.

However, the overall antibiotic use was not affected during the intervention and especially pivmecillinam use increased. At the start of the intervention in 2004, the proportions of trimethoprim-susceptible and -resistant isolates also resistant to mecillinam were 4% and 26%, respectively,[34] suggesting that there was potential for co-selection.

Table 2. Associations between antibiotics and trimethoprim resistance among Enterobacteriaceae urinary samples using elastic net regularization

Antibiotic group (ATC code)[a]	Beta (2.5th–97.5th percentile of bootstrap)	Relative risk (2.5th–97.5th percentile of bootstrap)
Penicillins with extended spectrum (J01CA)	0.1701 (0.1084 to 0.2635)	1.185 (1.114 to 1.301)
Trimethoprim and derivatives (J01EA)	0.1298 (0.0206 to 0.5616)	1.139 (1.021 to 1.753)
Combinations of penicillins, including β-lactamase inhibitors (J01CR)	0.0682 (−0.3103 to 0.2653)	1.071 (0.773 to 1.304)
β-Lactamase-sensitive penicillins (J01CE)	0.0534 (−0.435 to 0.6284)	1.055 (0.957 to 1.875)
Second-generation cephalosporins (J01DC)	0.0002 (−0.0882 to 0.4678)	1.000 (0.916 to 1.597)
Polymyxins (J01XB)	−0.0086 (−0.7455 to 0.0659)	0.991 (0.475 to 1.068)
Other antibacterials (J01XX)	−0.0098 (−0.4359 to 0.2606)	0.990 (0.647 to 1.298)
Tetracyclines (J01AA)	−0.0203 (−0.0875 to 0.0367)	0.980 (0.916 to 1.037)
β-Lactamase-resistant penicillins (J01CF)	−0.0327 (−0.4593 to 0.1526)	0.968 (0.632 to 1.165)
Fluoroquinolones (J01MA)	−0.0700 (−0.9187 to 0.0463)	0.932 (0.399 to 1.047)
First-generation cephalosporins (J01DB)	−0.0728 (−0.8650 to 0.0318)	0.930 (0.421 to 1.032)
Macrolides (J01FA)	−0.1672 (−0.2879 to −0.0608)	0.846 (0.750 to 0.941)
Nitrofuran derivatives (J01XE)	−0.1847 (−0.5589 to −0.0397)	0.831 (0.572 to 0.961)

[a]Antibiotics were expressed as DDDs/1000 inhabitants/day and subsequently standardized by mean-centring and dividing by 2 standard deviations of total antibiotic use.

Table 3. Associations between antibiotics and trimethoprim resistance among Enterobacteriaceae urinary samples using generalized BRTs

Antibiotic group (ATC code)	Relative influence
Nitrofuran derivatives (J01XE)	9.2
First-generation cephalosporins (J01DB)	8.3
Penicillins with extended spectrum (J01CA)	7.4
Fluoroquinolones (J01MA)	6.2
Macrolides (J01FA)	5.7
Second-generation cephalosporins (J01DC)	4.2
Lincosamides (J01FF)	4.1
Trimethoprim derivatives (J01EA)	4.1
Tetracyclines (J01AA)	4.1
Combinations of penicillins, including β-lactamase inhibitors (J01CR)	4.1

The results in this table are obtained for antibiotics expressed as DDDs/1000 inhabitants/day using generalized BRTs. The top 10 antibiotics with the highest relative influence are listed.

In our study, antibiotics from the J01CA (extended-spectrum penicillins) group were particularly associated with higher trimethoprim resistance, even more so than trimethoprim. Although resistance mechanisms differ for antibiotics within the J01CA group and pivmecillinam is used much more frequently in Sweden than in England, co-selection by antibiotics from this J01CA group, including pivmecillinam and ampicillin, might partly explain the disappointing results from the Swedish study. Given the much lower potential for co-selection for nitrofurantoin—resistance of trimethoprim-susceptible and -resistant isolates to nitrofurantoin was 0.5% and 2%, respectively, in the Swedish study[34]—our findings indicate that replacing trimethoprim with nitrofurantoin for uncomplicated urinary tract infections may have a more beneficial impact from a resistance point of view. These findings may encourage clinicians to adhere to the recent guideline change in England recommending nitrofurantoin as first-line treatment for uncomplicated urinary tract infections and trimethoprim use only if there is a low risk of resistance.[36]

A major strength of this study is that, in contrast to previous studies, we took into account confounding by use of other antibiotic groups when evaluating associations between use of specific antibiotics and trimethoprim resistance. The methodology we used allowed us not only to control for confounding by other antibiotics, but also identified potential co-selection mechanisms. Our research is timely, given the recent change in English guidelines, recommending nitrofurantoin as first-line treatment for uncomplicated urinary tract infections and use of trimethoprim only if there is low risk of resistance.[36]

In addition we used advanced methodology, i.e. elastic net regularization and BRTs, which are better able to deal with sparse data and multicollinearity issues than the crude analyses or standard regression models applied in previous studies. Furthermore, the application of both elastic net regularization and BRTs and the high number of sensitivity analyses (e.g. antibiotic use based both on DDDs and items dispensed), strengthen the confidence in our results.

Finally, we focused on trimethoprim resistance among Enterobacteriaceae because for this combination there are several *in vivo*, *in vitro* and database studies that could be used to sense-check our results from a biological perspective. For future work it would be interesting to apply the same methodology for less well researched resistances. As such, the methodology we applied here could also identify potential areas of interest.

There are also some factors that complicate the interpretation of our results. The most important consideration is that our results represent associations and are not necessarily causal. We acknowledge that we could not take into account confounding by unmeasured factors, such as antibiotic use in hospitals. However, almost 80% of antibiotics are prescribed in primary care in

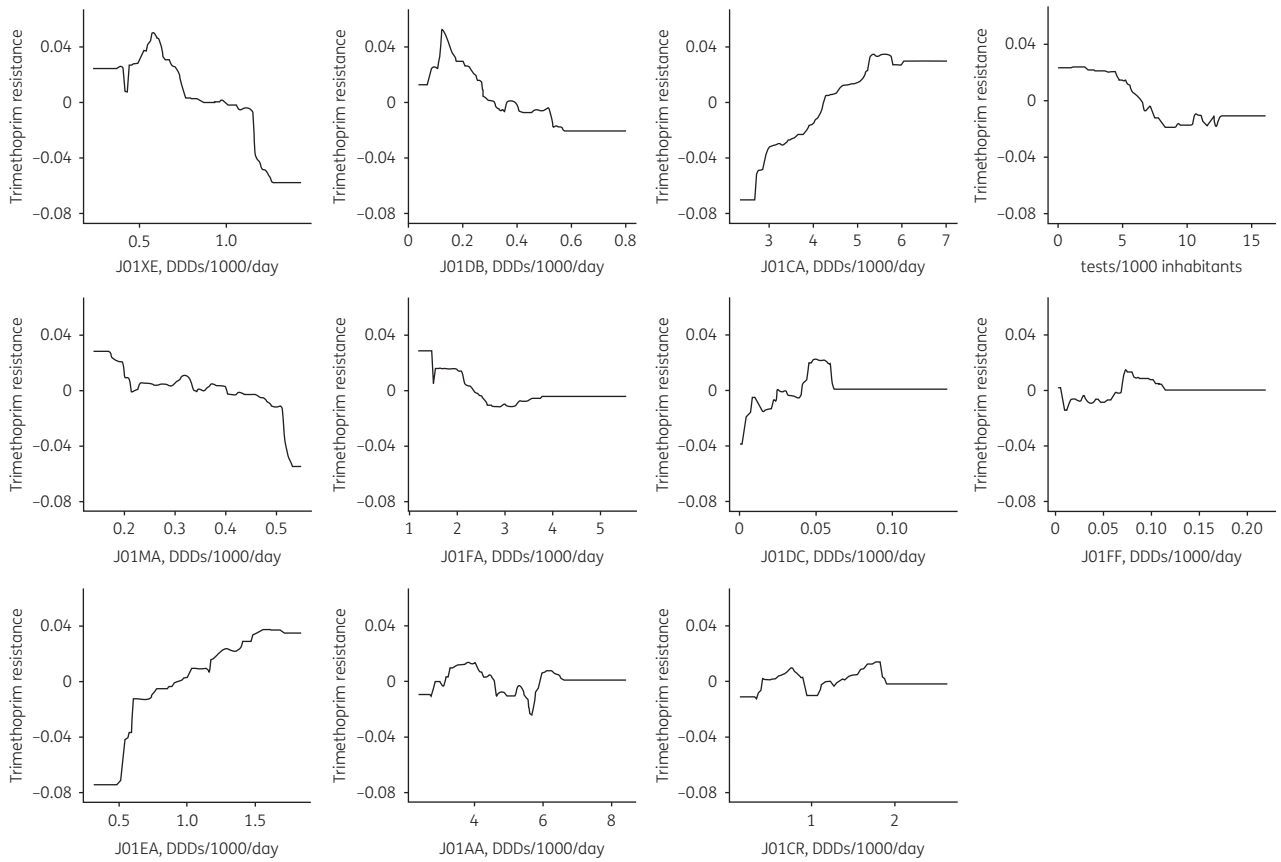

Figure 1. Partial dependence plots for the 11 most influential variables (10 antibiotics and the test rate) in the generalized boosted regression model assessing the association between antibiotics expressed as DDDs/1000 inhabitants/day (DDDs/1000/day) and trimethoprim resistance among Enterobacteriaceae urinary samples. *y*-Axes are centred to have zero mean over the data distribution. A line with a positive slope indicates that regions with higher use of that antibiotic also have a higher trimethoprim resistance prevalence among Enterobacteriaceae urinary samples.

England.[37] Although we do not have information about whether patients actually took the antibiotics, it is unlikely that there is strong geographical variation in the percentage of prescriptions that actually lead to antibiotic consumption. CLSI methodology has higher MIC breakpoints than EUCAST or BSAC methodology; this may have led to some bias if CCGs with more samples being tested using CLSI (associated with lower resistance rates) also had different patterns in antibiotic use. However, the majority of samples were tested using BSAC methodologies, thereby minimizing the influence of such bias.

Because urine samples are more likely to be sent for testing in situations where there is an increased likelihood of the pathogen being resistant—English guidelines recommend against routine sending of samples from adult women—the proportion of resistance is likely overestimated. However, by adjusting for the testing rate per population we have likely removed most potential confounding due to differences in tendency to send samples between different CCGs.

Prescribing may partly differ as a consequence of trimethoprim resistance instead of the other way around. Reverse causation would occur if higher trimethoprim resistance proportions would result in altered antibiotic prescribing patterns. This is mainly relevant for conditions where trimethoprim is being used as an important treatment option, i.e. urinary tract infections. If such reverse causation did play an important role, one would expect nitrofurantoin, the most common treatment for urinary tract infections besides trimethoprim, to be associated with higher trimethoprim resistance levels. However, in contrast we found that higher nitrofurantoin prescribing rates were associated with lower trimethoprim resistance levels, suggesting that the potential influence of reverse causation is limited.

Future work is needed to confirm/falsify some of the associations we observed. Moreover, results may not be generalizable to countries other than England, because of differences in (co-)resistance and antibiotic use patterns.

Conclusions

In conclusion, elastic net regularization and generalized boosted regression models both identified trimethoprim use as a predictor of geographical variation in the proportion of Enterobacteriaceae urinary samples resistant to trimethoprim. Importantly, ampicillin/amoxicillin use seemed to have a larger influence than trimethoprim use, suggesting that co-selection by these antibiotics is an important driver of trimethoprim resistance levels at the population level. The observation that nitrofurantoin was consistently

associated with lower trimethoprim resistance levels may indicate that trimethoprim resistance levels could be reversible if trimethoprim use is replaced by antibiotics that have low co-selection potential, such as nitrofurantoin.

Acknowledgements

An abstract of this manuscript (# P1274) has been presented as a poster at the Twenty-seventh European Congress of Clinical Microbiology and Infectious Diseases, Vienna, Austria, 2017.

Funding

This study was carried out as part of our routine work.

Transparency declarations

None to declare.

Author contributions

K. B. P., R. F., B. M.-P., J. V. R. and T. S. designed the study and analysis. G. R. and K. L. H. contributed data. All authors participated in interpreting the results and writing the manuscript. All authors read and approved the final manuscript.

Supplementary data

Sections S1 and S2, Tables S1 to S9 and Figure S1 are available as Supplementary data at *JAC* Online.

References

1 Costelloe C, Metcalfe C, Lovering A *et al*. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ* 2010; **340**: c2096.

2 Goossens H, Ferech M, Vander SR *et al*. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. *Lancet* 2005; **365**: 579–87.

3 Garcia-Rey C, Fenoll A, Aguilar L *et al*. Effect of social and climatological factors on antimicrobial use and *Streptococcus pneumoniae* resistance in different provinces in Spain. *J Antimicrob Chemother* 2004; **54**: 465–71.

4 Priest P, Yudkin P, McNulty C *et al*. Antibacterial prescribing and antibacterial resistance in English general practice: cross sectional study. *BMJ* 2001; **323**: 1037–41.

5 Magee JT, Pritchard EL, Fitzgerald KA *et al*. Antibiotic prescribing and antibiotic resistance in community practice: retrospective study, 1996-8. *BMJ* 1999; **319**: 1239–40.

6 Lipsitch M, Samore MH. Antimicrobial use and antimicrobial resistance: a population perspective. *Emerg Infect Dis* 2002; **8**: 347–54.

7 Kahlmeter G, Menday P. Cross-resistance and associated resistance in 2478 *Escherichia coli* isolates from the Pan-European ECO.SENS Project surveying the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections. *J Antimicrob Chemother* 2003; **52**: 128–31.

8 Amyes SG. The success of plasmid-encoded resistance genes in clinical bacteria. An examination of plasmid-mediated ampicillin and trimethoprim resistance genes and their resistance mechanisms. *J Med Microbiol* 1989; **28**: 73–83.

9 Kahlmeter G, Poulsen HO. Antimicrobial susceptibility of *Escherichia coli* from community-acquired urinary tract infections in Europe: the ECO·SENS study revisited. *Int J Antimicrob Agents* 2012; **39**: 45–51.

10 Van de Sande-Bruinsma N, Grundmann H, Verloo D *et al*. Antimicrobial drug use and resistance in Europe. *Emerg Infect Dis* 2008; **14**: 1722–30.

11 Blommaert A, Marais C, Hens N *et al*. Determinants of between-country differences in ambulatory antibiotic use and antibiotic resistance in Europe: a longitudinal observational study. *J Antimicrob Chemother* 2014; **69**: 535–47.

12 Donnan PT, Wei L, Steinke DT *et al*. Presence of bacteriuria caused by trimethoprim resistant bacteria in patients prescribed antibiotics: multilevel model with practice and individual data. *BMJ* 2004; **328**: 1297.

13 Vellinga A, Murphy AW, Hannahoe B *et al*. A multilevel analysis of trimethoprim and ciprofloxacin prescribing and resistance of uropathogenic *Escherichia coli* in general practice. *J Antimicrob Chemother* 2010; **65**: 1514–20.

14 Bergman M, Nyberg ST, Huovinen P *et al*. Association between antimicrobial consumption and resistance in *Escherichia coli*. *Antimicrob Agents Chemother* 2009; **53**: 912–7.

15 Greenland S, Mansournia MA, Altman DG. Sparse data bias: a problem hiding in plain sight. *BMJ* 2016; **352**: i1981.

16 Zou H, Hastie T. Regularization and variable selection via the elastic net. *J R Stat Soc Ser B Stat Methodol* 2005; **67**: 301–20.

17 Friedman J, Hastie T, Tibshirani R. Regularization paths for generalized linear models via coordinate descent. *J Stat Soft* 2010; **33**: 1–22.

18 Elith J, Leathwick JR, Hastie T. A working guide to boosted regression trees. *J Anim Ecol* 2008; **77**: 802–13.

19 Tibshirani R. Regression shrinkage and selection via the lasso. *J R Stat Soc Ser B Stat Methodol* 1996; **58**: 267–88.

20 Hoerl AE, Kennard RW. Ridge regression: biased estimation for nonorthogonal problems. *Technometrics* 1970; **12**: 55.

21 Greenland S. Invited commentary: variable selection versus shrinkage in the control of multiple confounders. *Am J Epidemiol* 2008; **167**: 523–9. discussion 530–1.

22 Hastie T, Qian J. *Glmnet Vignette*. https://web.stanford.edu/~hastie/glmnet/glmnet_alpha.html.

23 Ternes N, Rotolo F, Michiels S. Empirical extensions of the lasso penalty to reduce the false discovery rate in high-dimensional Cox regression models. *Statist Med* 2016; **35**: 2561–73.

24 Hayes T, Usami S, Jacobucci R *et al*. Using classification and regression trees (CART) and random forests to analyze attrition: results from two simulations. *Psychol Aging* 2015; **30**: 911–29.

25 Friedman J, Hastie T, Rosset S *et al*. Discussion of boosting papers. *Ann Stat* 2004; **32**: 102–7.

26 Miller PJ, McArtor DB, Lubke GH. *metboost: Exploratory Regression Analysis With Hierarchically Clustered Data*. arXiv 2017. https://arxiv.org/pdf/1702.03994.pdf.

27 Elith J, Leathwick J. *Boosted Regression Trees for Ecological Modeling*. https://cran.r-project.org/web/packages/dismo/vignettes/brt.pdf.

28 Huttner A, Verhaegh EM, Harbarth S *et al*. Nitrofurantoin revisited: a systematic review and meta-analysis of controlled trials. *J Antimicrob Chemother* 2015; **70**: 2456–64.

29 Pouwels KB, Visser ST, Bos HJ *et al*. Angiotensin-converting enzyme inhibitor treatment and the development of urinary tract infections: a prescription sequence symmetry analysis. *Drug Saf* 2013; **36**: 1079–86.

30 Pouwels KB, Visser ST, Hak E. Effect of pravastatin and fosinopril on recurrent urinary tract infections. *J Antimicrob Chemother* 2013; **68**: 708–14.

31 Dolk FCK, Pouwels KB, Smith DRM *et al*. Antibiotics in primary care in England: which antibiotics are prescribed and for which conditions? *J Antimicrob Chemother* 2018; **73** Suppl 2: ii2–ii10.

32 Rosello A, Pouwels KB, Domenech de Celles M *et al*. Seasonality of urinary tract infections in the United Kingdom in different age groups, analysis of The Health Improvement Network (THIN). *Epidemiol Infect* 2018; **146**: 37–45.

33 Sandegren L, Lindqvist A, Kahlmeter G *et al*. Nitrofurantoin resistance mechanism and fitness cost in *Escherichia coli*. *J Antimicrob Chemother* 2008; **62**: 495–503.

34 Sundqvist M, Geli P, Andersson DI *et al*. Little evidence for reversibility of trimethoprim resistance after a drastic reduction in trimethoprim use. *J Antimicrob Chemother* 2010; **65**: 350–60.

35 Pouwels KB, Batra R, Patel A *et al*. Will co-trimoxazole resistance rates ever go down? Resistance rates remain high despite decades of reduced co-trimoxazole consumption. *J Glob Antimicrob Resist* 2017; **11**: 71–4.

36 PHE. *Managing Common Infections: Guidance for Primary Care*. 2017. https://www.gov.uk/government/publications/managing-common-infections-guidance-for-primary-care.

37 PHE. *English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR) Report* 2016. https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/575626/ESPAUR_Report_2016.pdf.