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Trends, seasonality and the association between outpatient antibiotic use and antimicrobial resistance among urinary bacteria in the Netherlands

Evelyn Pamela Martínez [ID] [1,2]*, Joost van Rosmalen [ID] [3], Roberto Bustillos[1], Stephanie Natsch[4],
Johan W. Mouton[2]† and Annelies Verbon[2] on behalf of the ISIS-AR study group‡

[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Central del Ecuador, Quito, Ecuador; [2]Department of Medical Microbiology and Infectious Diseases, Erasmus University Medical Center, Rotterdam, The Netherlands; [3]Department of Biostatistics, Erasmus University Medical Center, Rotterdam, The Netherlands; [4]Department of Pharmacy, Radboud University Medical Center, Nijmegen, The Netherlands

*Corresponding author. E-mail: pmartinezl@uce.edu.ec
†Deceased.
‡Members of the ISIS-AR study group are listed in the Acknowledgements section.

Objectives: To determine trends, seasonality and the association between community antibiotic use and antimicrobial resistance (AMR) in *Escherichia coli* and *Klebsiella pneumoniae* in urinary tract infections.

Methods: We analysed Dutch national databases from January 2008 to December 2016 regarding antibiotic use and AMR for nitrofurantoin, trimethoprim, fosfomycin and ciprofloxacin. Antibiotic use was expressed as DDD/1000 inhabitant-days (DID) and AMR was expressed as the percentage of resistance from total tested isolates. Temporal trends and seasonality were analysed with autoregressive integrated moving average (ARIMA) models. Each antibiotic use–resistance combination was cross-correlated with a linear regression of the ARIMA residuals.

Results: The trends of DID increased for ciprofloxacin, fosfomycin and nitrofurantoin, but decreased for trimethoprim. Similar trends were found in *E. coli* and *K. pneumoniae* resistance to the same antibiotics, except for *K. pneumoniae* resistance to ciprofloxacin, which decreased. Resistance levels peaked in winter/spring, whereas antibiotic use peaked in summer/autumn. In univariate analysis, the strongest and most significant cross-correlations were approximately 0.20, and had a time delay of 3–6 months between changes in antibiotic use and changes in resistance. In multivariate analysis, significant effects of nitrofurantoin use and ciprofloxacin use on resistance to these antibiotics were found in *E. coli* and *K. pneumoniae*, respectively. There was a significant association of nitrofurantoin use with trimethoprim resistance in *K. pneumoniae* after adjusting for trimethoprim use.

Conclusions: We found a relatively low use of antibiotics and resistance levels over a 9 year period. Although the correlations were weak, variations in antibiotic use for these four antibiotics were associated with subsequent variations in AMR in urinary pathogens.

Introduction

Urinary tract infections (UTIs) are the most frequent bacterial infection in primary care, affecting 150 million people per year worldwide.[1,2] Women have higher risk of developing UTIs, and 60% of this group have at least one episode during their lifetime.[1,2] Approximately 60%–80% of these infections are caused by *Escherichia coli* and 3%–10% by *Klebsiella pneumoniae*.[3–5]

Currently, increasing antimicrobial resistance (AMR) in these urinary bacteria has led to treatment failures and has increased the societal cost to USD 3.5 billion per year in the USA alone.[1]

Antibiotic use is the key driver of AMR, and this association implies a dynamic process in which a time delay between antibiotic use and AMR may be involved.[6–8] Some studies have considered the influence of time on this association. For instance, a time delay of 1–3 months between ceftazidime use and imipenem use and

resistance to these antibiotics by Gram-negative bacteria was reported in Spain.[6] A similar time delay was found in the USA between high prescriptions of macrolides, β-lactams, fluoroquinolones and resistance in *E. coli*,[8] while a delay of 1–2 months between use of amoxicillin and resistance in urinary *E. coli* was observed in Australia.[7]

Seasonality of antibiotic use in outpatients has been observed in Europe and in the USA, where the highest consumption of antibiotics occurs in winter and spring compared with summer and autumn.[9–11] In high-consuming countries such as Greece, stronger seasonal fluctuations were observed than in low-consuming countries such as the Netherlands.[11] Strong seasonal fluctuations can induce rapid selective pressure and further selection of antibiotic resistance with short time delays.[8] However, it has not been elucidated what the time delay is between antibiotic use and resistance in low-consuming countries with weak seasonal variations. Therefore, we aimed to determine trends, seasonality and the time delay between antibiotic use and AMR in two clinically important urinary bacteria, *E. coli* and *K. pneumoniae*. We studied this using time-series analysis in Dutch national databases for a period of 9 years, which, unlike statistical methods that are commonly applied in medical research, such as cross-sectional methods and methods for repeated measurements, allowed us to take into account trends and seasonality in the time series as well as possible associations between observations taken at regular time intervals.[6]

Methods

Data collection

Outpatient antibiotic use

Outpatient antibiotic prescriptions from January 2008 to December 2016 were obtained from the Dutch Foundation for Pharmaceutical Statistics (SFK) database, which includes dispensing data of antibiotics from GPs, outpatient clinics and dentists.[12] Since 1990, SFK routinely collects data from more than 95% of community pharmacies serving around 15.8 million people, corresponding to 93% of the total Dutch population, and extrapolates the data to 100% of antibiotic prescriptions in the Netherlands.[12,13]

Antibiotic use data were expressed in DDD for each Anatomical Therapeutic Chemical (ATC) code at the fifth level. For this study, we analysed antibiotics mostly used in primary care to treat UTIs. According to the current guidelines of the Dutch College of General Practitioners (NHG), nitrofurantoin (ATC code J01XE01), fosfomycin (J01XX01) and trimethoprim (J01EA01) should be used as first, second and third-choice therapy options, respectively, for uncomplicated UTIs, and ciprofloxacin (J01MA02) as first-choice option for UTIs with tissue invasion.[14] We calculated the monthly DDD per 1000 inhabitant-days (DID) of these antibiotics according to the WHO ATC/DDD toolkit.[15]

Antimicrobial resistance

The Dutch national AMR surveillance system, named the Infectious Diseases Surveillance Information System for Antimicrobial Resistance (ISIS-AR), provided AMR data on *E. coli* and *K. pneumoniae* isolated from urine samples. The ISIS-AR surveillance system is a combined initiative of the Ministry of Health, Welfare and Sport and the Dutch Society of Medical Microbiology (NVMM), and is coordinated by the Centre for Infectious Disease Control (CIb) at the National Institute for Public Health and the Environment (RIVM) in Bilthoven.[16] Currently, ISIS-AR contains data from routine antimicrobial susceptibility testing in 46 laboratories distributed across the country serving hospitals, GPs, obstetrician practices, long-term care facilities and public health facilities.[16] The geographical distribution of laboratories is representative of the Netherlands.[12,16] Previously, the majority of laboratories based their antimicrobial susceptibility testing on the CLSI criteria, but between 2011 and 2013 most laboratories adapted their methods to EUCAST criteria.[16] For reporting, such as the yearly NethMap report on surveillance and use of antibiotics in the Netherlands by the Dutch Working Group on Antibiotic Policy (SWAB) and the CIb, all MIC data are reinterpreted using 2017 EUCAST criteria whenever possible.[12]

To exclude bias in determining trends, we included data from the 24 out of 40 laboratories that continuously provided information to ISIS-AR from 2008 to 2016. Data included isolates from all 12 provinces of the Netherlands. Resistance data included isolates selected under the following conditions: (i) only urinary samples from GPs; (ii) the first isolate per patient per year; and (iii) only data from laboratories that tested at least 50% of isolates for that specific antibiotic, for each pathogen–agent combination.[12] We calculated monthly resistance levels, and their binomial proportion 95% CIs, per year per antibiotic for *E. coli* and *K. pneumoniae* based on ISIS-AR and NethMap methodology.[12,16] Nitrofurantoin resistance levels were not calculated for *K. pneumoniae* due to the lack of a susceptibility breakpoint. For *E. coli* and *K. pneumoniae*, fosfomycin resistance data in 2008 were not available, and therefore resistance levels in that year were not calculated.

Data analysis

Univariate time-series analysis

R software 3.5.0 and RStudio version 1.0.153 were used for all analyses, notably the packages 'tseries', 'forecast', 'asta' and 'ggplot2'. In total we created 11 time series on a monthly basis, four for each antibiotic use expressed in DID, four for the percentage of resistance in *E. coli* and three for the percentage of resistance in *K. pneumoniae*. We analysed each time series with the methodology proposed by Box and Jenkins in 1976,[17] based on a three-stage modelling approach: (i) model identification; (ii) parameter estimation; and (iii) model diagnostics.

This methodology is explained in more detail in the Supplementary data (available at *JAC* Online). Briefly, we applied a decomposition procedure using an additive model with the moving average method (LOESS) to estimate trends, seasonal variation and irregularity from each time series. Stationarity of time series was reached by first-order differencing, followed by seasonal differencing, if a strong seasonality was observed. The autocorrelation structure within each time series was analysed to select the terms for an initial autoregressive integrated moving average (ARIMA) model. ARIMA models were fitted for each time series and residuals were checked for deviations from white noise.[17,18]

Cross-correlation analysis

To determine whether there was an association between resistance and antibiotic use, analyses were done in pairs comparing each resistance level with its corresponding antibiotic use. Cross-correlation analysis included the following steps:[8,19,20] (i) the coefficients of the cross-correlation function (CCF), with lags ranging from −12 to 12 months, were calculated with the residuals of the fitted ARIMA models with antibiotic use as independent variable and the corresponding resistance level as dependent variable; (ii) inspection of the autocorrelogram (ACF) plot to identify at what lags the correlation between the two series is the strongest [i.e. the cross-correlation coefficient (C_{XY}) peaking outside the 95% significance boundaries]; and (iii) construction of multiple linear regression models with lagged effects, seven in total, four for *E. coli* and three for *K. pneumoniae*.

Cross-correlation modelling allows the identification of associations between time series in both directions, i.e. positive and negative lags. The interpretation of the C_{XY} was as follows: a peak at a zero lag represents an immediate response of resistance to antibiotic use. A significant peak at a positive lag indicates that a change in antibiotic use would likely lag behind

(i.e. occur after) a change in resistance, so that antibiotic use would be considered a lagging variable. A significant peak at a negative lag indicates that a change in antibiotic use is followed by a change in resistance, so that antibiotic use would be considered a leading variable and a predictor of antibiotic resistance.

In the multiple linear regression analyses, residual values of the fitted ARIMA models for the resistance time series were considered as the dependent variables and the independent variables were the lagged residual values of antibiotic use. The negative lag lengths of 1, 3, 6 and 12 months were pre-selected to cover the short-term and long-term relationship between variables. The joint significance of these four lag lengths was tested using *F* tests. In addition, we performed an extra analysis to determine the influence of use of the analysed antibiotics on resistance to other antibiotics, specifically between trimethoprim and nitrofurantoin, for which an association has been found.[21,22] These extra analyses consisted of multiple linear regression analyses of trimethoprim and nitrofurantoin resistance with simultaneous adjustment for trimethoprim and nitrofurantoin use, using the same lag lengths as in the other regression analyses. The autocorrelation structure of residuals of the linear regression models was examined to check for deviations from white noise.[19]

Results

During the 9 year study period, *E. coli* was the most commonly found bacterium in urine samples, with a total of 487088 isolates (Table 1). Resistance to trimethoprim was common in both *E. coli* and *K. pneumoniae* (>20% prevalence of resistance). Nitrofurantoin was the most prescribed antibiotic (1.32 DID) followed by ciprofloxacin (0.54 DID) (Table 2).

Trends of outpatient antibiotic use and resistance levels

Temporal trends and changes in antibiotic use and resistance levels in urinary bacteria over the study period are presented in Figure 1 and Table 3. The use of fosfomycin increased from 0.01 DID in 2009 to 0.05 DID in 2016 (an increase of 400%), and the use of nitrofurantoin increased from 1.12 in 2008 to 1.41 in 2016 (a total increase of 25.9%). The use of trimethoprim showed a steady decreasing trend from 0.21 DID in 2008 to 0.15 DID in 2016 (a total decrease of 33.3%). The use of ciprofloxacin was variable, showing an increasing trend from 0.47 DID in 2008 to 0.61 DID in 2015, followed by a decrease until the end of 2016 (a total increase of 29.8%).

Fosfomycin resistance showed a steady increase from 0.6% in 2009 to 1.4% in 2016 in *E. coli* (a total increase of 133.3%), and from 16.2% to 32.6% for *K. pneumoniae* (a total increase of 101.2%). Also, nitrofurantoin resistance in *E. coli* showed an increasing trend between 2008 (1.8%) and 2014 (2.5%), followed by a decrease from 2015 onwards (a total increase of 5.6%). Furthermore, the prevalence of resistance to trimethoprim showed a slowly decreasing trend from 27.4% in 2008 to 24.8% in 2016 in *E. coli* (a decrease of 9.5%) and from 29.0% to 22.3% in *K. pneumoniae* (a total decrease of 23.1%). Ciprofloxacin resistance in *K. pneumoniae* showed a decreasing trend from 12.0% in 2008 to 9.9% in 2016 (a total decrease of 17.5%). In *E. coli*, ciprofloxacin resistance initially increased from 9.6% in 2008 to 10.5% in 2012, followed by a decrease from 10.4% in 2011 to 9.9% in 2016 (a total increase of 3.1%).

Table 1. Summary data on antibiotic resistance in the Netherlands from January 2008 to December 2016

Bacteria/antibiotic	Number of isolates tested		Proportion of isolates resistant (%) and 95% CI
	total	resistant	
E. coli			
Ciprofloxacin	484993	49207	10.2 (10.1–10.2)
Nitrofurantoin	487088	10242	2.1 (2.1–2.1)
Trimethoprim	482030	129032	26.8 (26.6–26.9)
Fosfomycin	328625	3393	1.0 (1.0–1.07)
K. pneumoniae			
Ciprofloxacin	50944	5360	10.5 (10.3–10.8)
Trimethoprim	50639	12568	24.8 (24.4–25.1)
Fosfomycin	34424	9602	27.9 (27.4–28.4)

Table 2. Summary data on antibiotic use in the Netherlands from January 2008 to December 2016

Antibiotic	DID ± SD
Ciprofloxacin	0.54±0.030
Nitrofurantoin	1.32±0.074
Trimethoprim	0.18±0.014
Fosfomycin	0.02±0.001

Seasonality of outpatient antibiotic use and resistance levels

We identified weak seasonal variation in both antibiotic use, peaking in summer/autumn, and resistance levels, peaking in winter/spring (Figures 2 and 3). Seasonal variation in *E. coli* was more pronounced for nitrofurantoin use than for other antibiotics, with use approximately 0.10 DID higher in summer/autumn. Seasonal variation of resistance levels in *E. coli* was more pronounced for trimethoprim, which was 1 percentage point higher in winter/spring, and ciprofloxacin, which was 0.6 percentage points higher in the same seasons. In *K. pneumoniae*, seasonal variation of resistance levels was more pronounced for fosfomycin: 2 percentage points higher in winter. For more detail see Tables S1–S3.

Association between outpatient antibiotic use and resistance levels

The final specification of the fitted ARIMA models is presented in Table S4. In general, we found weak associations between antibiotic use and resistance (Tables 3 and 4).

Cross-correlation analysis

Cross-correlation coefficients (based on univariate analysis) are shown in Figure 4 and significant coefficients are presented in Table 4. The highest cross-correlations were approximately 0.20 with a time delay (lag length) of 3–6 months. In *E. coli* combinations, significant negative and positive correlations at zero lag were observed for ciprofloxacin ($C_{XY} = -0.23$, $P = 0.02$), fosfomycin ($C_{XY} = 0.22$, $P = 0.03$) and trimethoprim ($C_{XY} = -0.22$, $P = 0.02$)
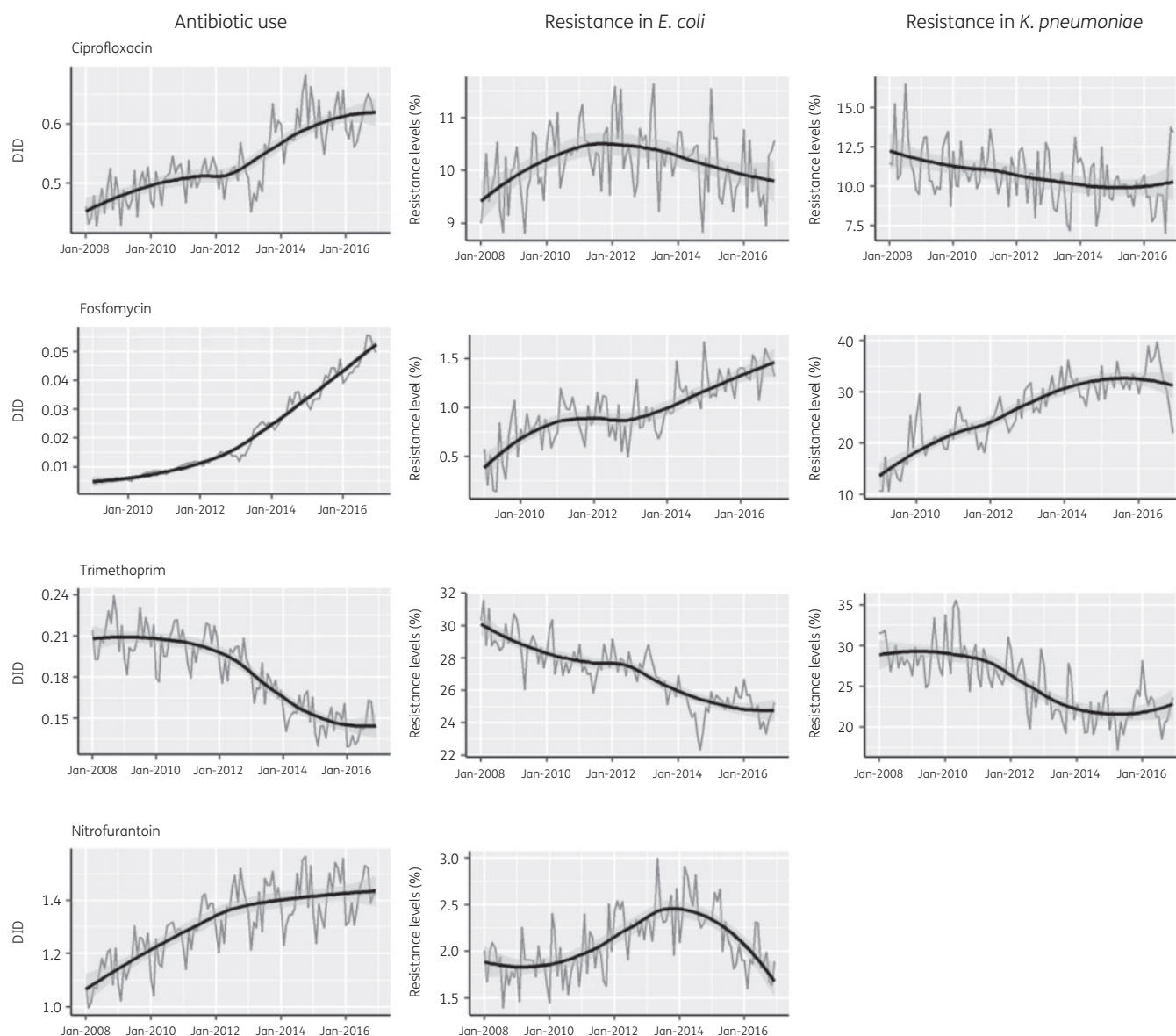
Figure 1. Trends of outpatient antibiotic use and antimicrobial resistance levels in *E. coli* and *K. pneumoniae* in the Netherlands from January 2008 to December 2016. Antibiotic use was expressed as DID and resistance levels as percentages. Smoothed trends (black solid line) and their 95% CIs (grey shading around the line) were estimated by seasonal and trend decomposition using local regressions (LOESS). Grey lines show the original time series. Fosfomycin resistance is presented from 2009 to 2018. No susceptibility breakpoint was available for nitrofurantoin in *K. pneumoniae* isolates. Antibiotic use data source: SFK. Resistance data source: ISIS-AR in the Netherlands.

(Table 4). Peaks at negative lags were found for nitrofurantoin and trimethoprim, suggesting that an increase in nitrofurantoin use was followed by an increase in resistance with a time delay of 6 months ($C_{XY}=0.22$, $P=0.02$). A decrease in trimethoprim use was followed by a decrease in resistance with a time delay of 3 months ($C_{XY}=0.22$, $P=0.03$). Furthermore, peaks at positive lags were found for fosfomycin, ciprofloxacin and trimethoprim, which represents the counterintuitive result that if resistance to fosfomycin, ciprofloxacin or trimethoprim rises, the use of these antibiotics will increase 6, 7 or 8 months later, respectively (Figure 4 and Table 4).

In *K. pneumoniae* combinations, significant associations at negative lags were found for ciprofloxacin and trimethoprim,

whereas for fosfomycin an association was identified at a positive lag of 10 months. A decrease in trimethoprim use was followed by a decrease in resistance with a time delay of 2 months ($C_{XY}=0.24$, $P=0.01$) and 5 months ($C_{XY}=0.21$, $P=0.03$). An increase in ciprofloxacin use was followed by a decrease of resistance with a time delay of 6 months ($C_{XY}=0.28$, $P=0.004$) (Figure 4 and Table 4).

Multiple linear regression analyses of antibiotic use on antibiotic resistance

In multivariate analysis using linear regression, which was performed to account for the effects of multiple testing of lag lengths,

Table 3. Relative change in outpatient antibiotic use and resistance levels in *E. coli* and *K. pneumoniae* during the period between January 2008 and December 2016 in the Netherlands

Antibiotic	Study period									Relative change (%)[a]
	2008	2009	2010	2011	2012	2013	2014	2015	2016	
Mean outpatient antibiotic use (DID)										
ciprofloxacin	0.47	0.48	0.51	0.51	0.52	0.54	0.6	0.61	0.61	29.8
nitrofurantoin	1.12	1.18	1.23	1.31	1.38	1.39	1.41	1.42	1.41	25.9
trimethoprim	0.21	0.21	0.20	0.20	0.19	0.17	0.16	0.15	0.14	−33.3
fosfomycin	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.04	0.05	400.0
Mean resistance level in *E. coli* (%)										
ciprofloxacin	9.6	9.9	10.4	10.4	10.5	10.4	10.1	10	9.9	3.1
nitrofurantoin	1.8	1.9	1.8	2.0	2.3	2.4	2.5	2.2	1.9	5.6
trimethoprim	27.4	28.7	28	27.5	27.6	26.8	24.6	25.5	24.8	−9.5
fosfomycin	NA	0.6	0.8	0.9	0.9	0.9	1.1	1.3	1.4	133.3
Mean resistance level in *K. pneumoniae* (%)										
ciprofloxacin	12	11.3	11	11.1	10.5	9.9	10.3	9.8	9.9	−17.5
trimethoprim	29	28.2	30.2	26.9	25.3	23.0	21.7	21.5	22.3	−23.1
fosfomycin	NA	16.2	21	22.3	25.6	29.9	30.9	32.3	32.6	101.2

NA, not analysed.
[a]Relative change was calculated by using 2008 as reference year for ciprofloxacin, nitrofurantoin and trimethoprim, and 2009 for fosfomycin.

the lagged effect of antibiotic use on resistance was only statistically significant for nitrofurantoin ($F = 2.52$, $P = 0.05$) in *E. coli* combinations and for ciprofloxacin ($F = 3.86$, $P = 0.01$) in *K. pneumoniae* combinations. In a separate analysis, significant co-resistance was found for nitrofurantoin use, showing that nitrofurantoin use predicts changes in resistance to trimethoprim in *K. pneumoniae* after adjustment for lagged trimethoprim use ($F = 4.56$, $P = 0.002$) (Table 5). We did not find significant co-resistance for the other analysed antibiotics.

Discussion

In Dutch databases covering a 9 year period, we found a relatively low use of antibiotics and relatively low resistance levels compared with southern European countries. Antibiotic use and resistance levels showed weak seasonal variations peaking in summer/autumn and winter/spring, respectively. We show that the use of nitrofurantoin, fosfomycin and ciprofloxacin increased over time, and so did resistance levels to these antibiotics. Conversely, trimethoprim use decreased and so did trimethoprim resistance. Use of nitrofurantoin was associated with a decrease in trimethoprim resistance in *K. pneumoniae*, at several lag lengths.

In contrast with previous studies, the evidence for an association between antibiotic use and resistance was the strongest, with a time delay of 3–6 months, probably due to low levels of antibiotic use in the Netherlands. Studies done in the USA, Australia and England have shown strong associations between high resistance levels in *E. coli* and high antibiotic use during winter, with a time delay of 1–2 months.[7,8,23] These associations were mainly found among antibiotics often prescribed for respiratory infections, such as β-lactams, macrolides and fluoroquinolones.[7,8,23] These antibiotics account for more than 40% of total antibiotic consumption in the USA and Europe, and their usage increases by a range of 24%–30% in winter.[8,10,11,24] In the Netherlands, a weak winter

seasonal variation of overall outpatient antibiotic use was described.[11,24] This was considered to be a result of a consistent low antibiotic consumption (mean of 10.2 DID) compared with high-consuming countries such as France (mean of 33.0 DID) and Belgium (mean of 25.4 DID). Similar to the findings of our study, weak seasonal differences among antibiotics used to treat UTIs were shown in England,[25] suggesting that the observed weak seasonal summer and autumn variation in antibiotic use could be influenced by the summer seasonality of the incidence of UTIs.[26–29]

We found that the association between nitrofurantoin use and resistance in *E. coli* was with a delay of 3 and 6 months. Similar results were found in a 4 year study in England, in which nitrofurantoin resistance lagged behind nitrofurantoin use at 6 months.[23] These results could be explained by studies showing that once the selective pressure is removed, the wild type of *E. coli* replaced nitrofurantoin-resistant strains due to the high fitness cost of resistance.[30] Nitrofurantoin has a multifactorial mechanism of action with activity against enzymes that damage vital processes in the bacterium,[31] and nitrofurantoin resistance genes are not often located on mobile genetic elements in bacteria.[30] In the Netherlands, nitrofurantoin is the first-choice therapy for uncomplicated UTIs and thus highly prescribed,[14] but AMR was not shown to increase at high rates.[12,16]

The cross-correlation coefficients found in this study were relatively low (all approximately 0.20 or smaller), similar to those found in a previous study.[8] This suggests that the contribution of antibiotic use to the dynamic of resistance in a low-consuming country like the Netherlands is small, meaning that other factors play an important role. Previous studies have found that patient-related factors such age, sex and nutritional habits were associated with resistance to ciprofloxacin in urinary *E. coli* isolates.[32,33] In addition, other factors, such as international travel, the spread
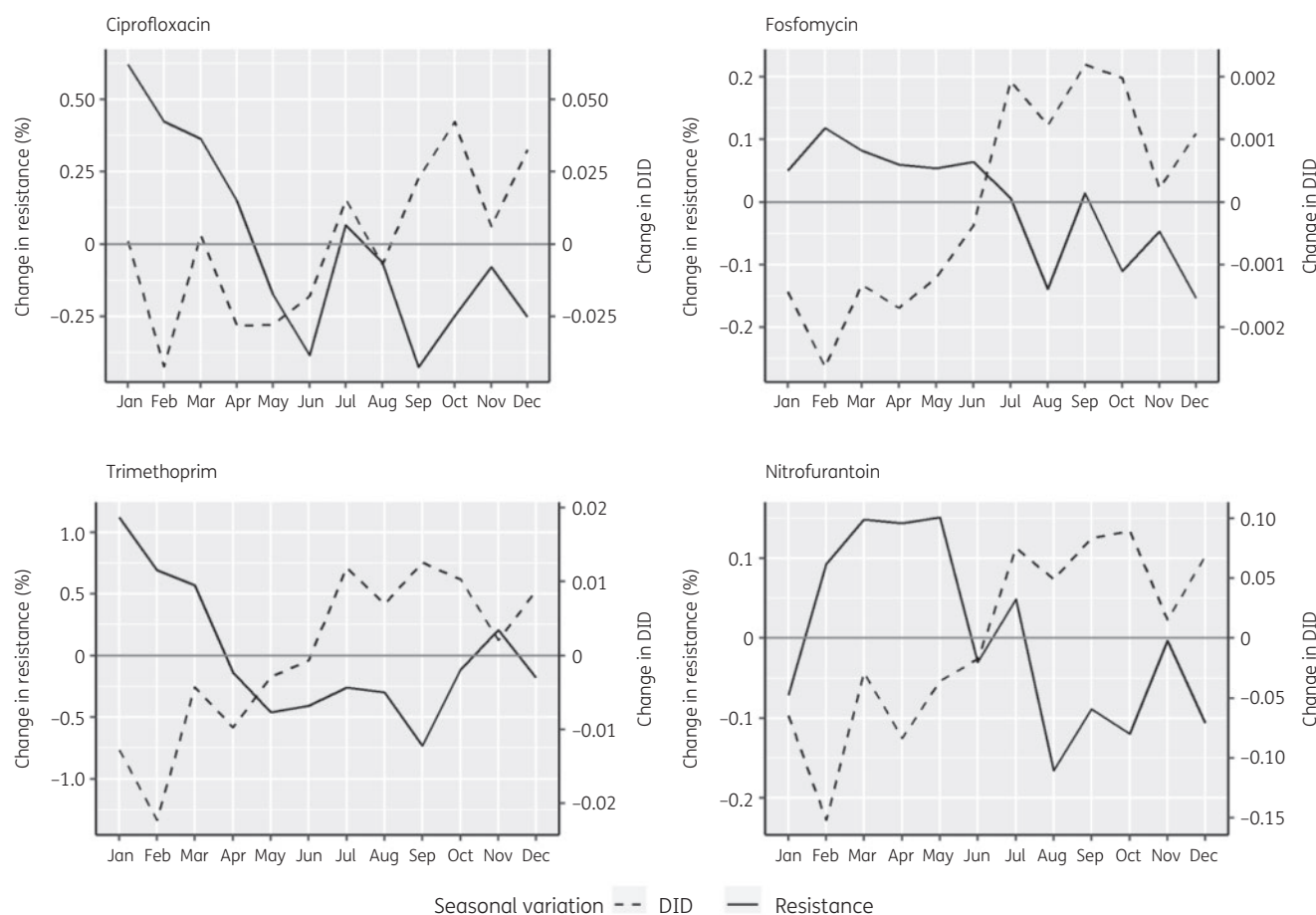
Figure 2. Seasonal variations of outpatient antibiotic use and resistance levels in *Escherichia coli* from 2008 to 2016 in the Netherlands. Seasonal variations were calculated by a decomposition procedure using an additive model with the moving average method. The *y*-axis refers to the monthly change around the mean (horizontal solid line) in the percentage of resistance levels and in antibiotic use expressed as DID. Antibiotic use data source: SFK; Resistance data source: ISIS-AR in the Netherlands.

of resistance genes in the community[34,35] and the interaction with the use of other antibiotics,[6] were found to be actively participating in the dynamic of resistance selection.

Moreover, it has been shown that having received two previous prescriptions of trimethoprim and ciprofloxacin increased the risk of resistance to these antibiotics in urinary *E. coli*.[33] In our study, the use of ciprofloxacin alone would explain about 11% of the resistance variation in urinary bacteria. It may be that use of other fluoroquinolones or use of β-lactams to treat respiratory infections contributes to the temporal changes in ciprofloxacin resistance. One study found an association between use of other fluoroquinolones and ciprofloxacin resistance in *E. coli* with a delay of 1 month,[8] while another study showed that levofloxacin use in the community was associated with ciprofloxacin-resistant *E. coli* in hospitals with a time delay of 12 months.[36] Resistance mechanisms to antibiotics differ between bacteria; unlike nitrofurantoin resistance, the levels of ciprofloxacin resistance can easily increase due to its multifactorial selection mechanism (e.g. target-site mutation, efflux pumps, and transmissible resistance on mobile genetic elements) and the demonstrated fitness advantage of resistant strains over susceptible strains.[37]

The latter could explain the significant immediate effect of ciprofloxacin use on resistance in *E. coli* found in this study. Interestingly, a study in England found an association between amoxicillin use and the increase of amoxicillin and ciprofloxacin resistance in urinary *E. coli*.[38] Therefore, the possible co-resistance needs further investigation to understand its influence on changes in trends and the lagged AMR development.

Furthermore, the strongest association between the decrease in trimethoprim use and resistance in urinary bacteria was with a delay of 2–5 months. The decrease in trimethoprim resistance could partly be explained by the decreased use of trimethoprim/sulfamethoxazole and increased nitrofurantoin use. In the USA and Spain, a significant association of approximately 20% between trimethoprim/sulfamethoxazole use and trimethoprim resistance in *E. coli* was found, with a time delay of 3–7 months.[8,20] Interestingly, in England and the Netherlands, an association with reduced trimethoprim resistance in urinary *E. coli* was shown for nitrofurantoin use.[21,22,38] Similarly, we found that an increase in nitrofurantoin use was associated with a decrease in trimethoprim resistance in *K. pneumoniae*. Possibly, nitrofurantoin use selects for strains that are susceptible to trimethoprim due to collateral susceptibility.[38]
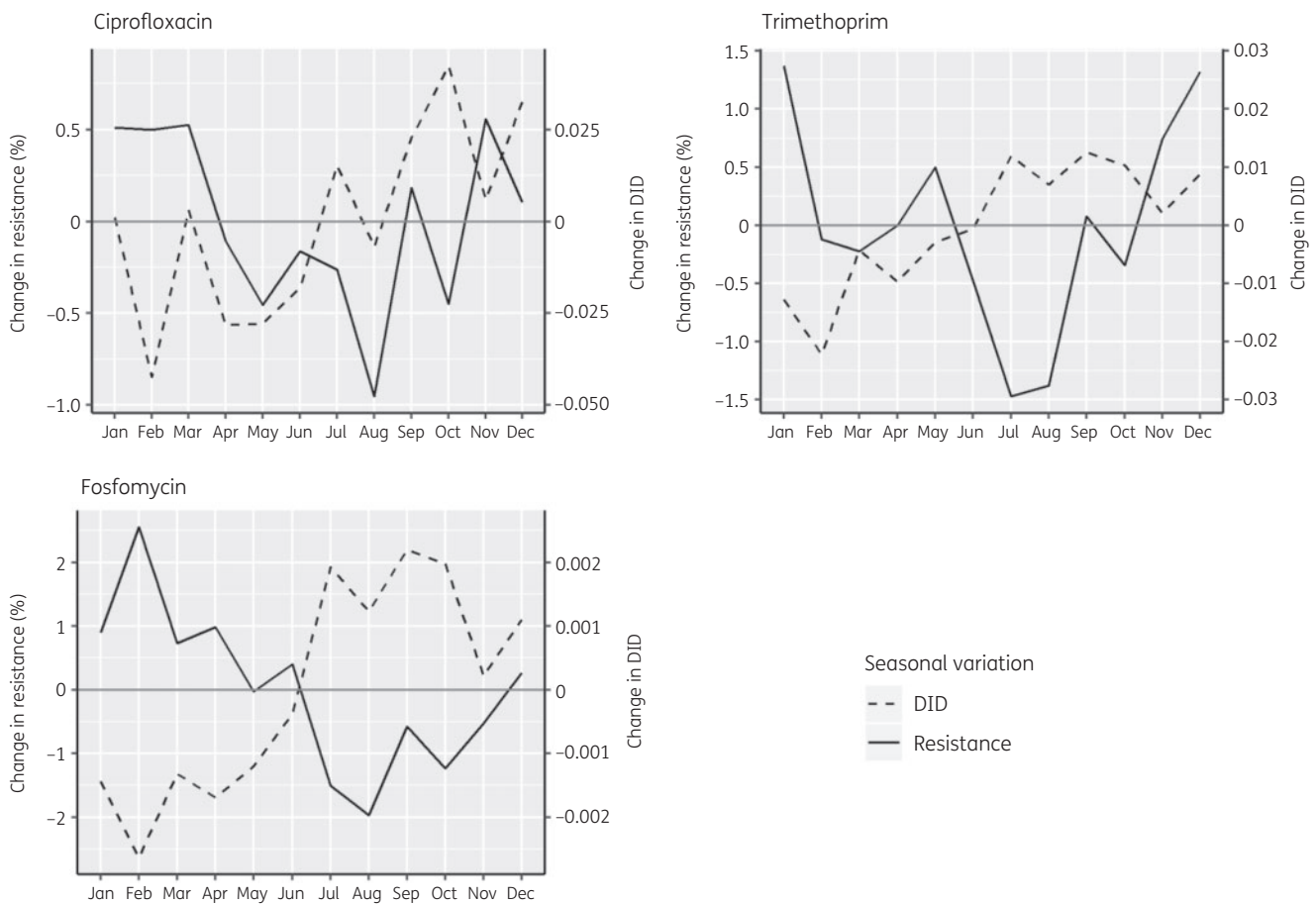
Figure 3. Seasonal variations of outpatient antibiotic use and resistance levels in *Klebsiella pneumoniae* from 2008 to 2016 in the Netherlands. Seasonal variations were calculated by a decomposition procedure using an additive model with the moving average method. The *y*-axis refers to the monthly change around the mean (horizontal solid line) in the percentage of resistance levels and in antibiotic use expressed in DID. Antibiotic use data source: SFK; Resistance data source: ISIS-AR in the Netherlands.

The temporal changes in fosfomycin resistance could not be explained by the lagged fosfomycin use, despite its dramatic increase of 400% from 2009 to 2016. The increase in fosfomycin use is possibly due to the update to the NHG guidelines in 2013, in which fosfomycin instead of trimethoprim was recommended as second-choice therapy for uncomplicated UTIs.[14] *In vitro* studies suggest that fosfomycin use is associated with rapid selection of resistance in Enterobacteriaceae,[39,40] which could explain the immediate selection response in *E. coli* found in this study. In Spain, the rapid increase in fosfomycin use in the community was found to be a risk factor for fosfomycin resistance in ESBL *E. coli*,[41] and it was associated with a 24.5% increase in fosfomycin resistance in *E. coli* with a time delay of 10 months.[20] Future studies are necessary to confirm this association in different multinational settings, in which patterns of antibiotic use may differ from those in the Netherlands.

Our study has substantial strengths, but also some limitations. This is the first outpatient-based study assessing trends, seasonality and the association between resistance in urinary bacteria and antibiotic use in a low-consuming country. The type of analysis applied, the construction of time series with short time intervals (i.e. months) over 9 years, and the unbiased collection of the information regarding antibiotic use and resistance are also substantial

strengths of this study. One possible limitation is the high level of aggregation of our study (i.e. national level), which can lead to a lower power for the analysis than a lower level of aggregation (e.g. provinces, municipalities) would provide. However, similar studies in other countries at the same level of aggregation found strong and significant correlations.[8,20] Moreover, selection bias is possible since Dutch GPs usually do not send urine samples for identification and susceptibility testing except in complicated UTI cases or if antibiotic treatment failure is suspected.[12] Therefore, resistance levels may be overestimated; however, resistance levels were found to be similar amongst unselected urinary cultures from uncomplicated UTI in general practice in the Netherlands.[5] A final limitation may be the ecological nature of this study; we could not rule out a fallacy in our results since the analysis did not control for patient-related factors. Patient demographic data were only available for antibiotic resistance, limiting the value of trying to include variables such as age, sex and location in our analysis. However, given the large sample size, effects of sampling variation in patient-level characteristics should theoretically be negligible. Moreover, the trends of antibiotic use were found to be similar among age and sex categories.[42] Similar figures have been found regarding resistance levels in *E. coli* and *K. pneumoniae*.[43]

Table 4. Cross-correlation and multiple linear regression results with lagged effects between antimicrobial resistance in *E. coli* and *K. pneumoniae* and antibiotics used to treat urinary tract infections in the Netherlands

Bacteria	ABU[a]	Cross-correlation			Multiple linear regression for lagged ABU						
		lag (months)[b]	coef.	P value	R^2 (%)	F	P value (F-test)[c]	ABU lag (months)[d]	coef.	95% CI	P value
E. coli	CIP	0	−0.23	0.02	6.6	1.39	0.25	CIP1	0.08	−0.27 to 0.42	0.65
		+7	0.21	0.03				CIP3	0.08	−0.26 to 0.43	0.64
								CIP6	0.16	−0.18 to 0.51	0.34
								CIP12	−0.32	−0.67 to 0.03	0.07
E. coli	FOS	0	0.22	0.03	2.5	0.42	0.79	FOS1	0.21	−1.36 to 1.79	0.79
		+6	0.20	0.04				FOS3	0.36	−1.26 to 1.97	0.66
								FOS6	−0.98	−2.59 to 0.62	0.23
								FOS12	−0.20	−1.85 to 1.44	0.80
E. coli	NIT	−6	0.22	0.02	11.3	2.52	0.05	NIT1	−0.48	−1.47 to 0.50	0.33
								NIT3	1.23	0.19 to 2.28	0.02
								NIT6	0.98	−0.02 to 1.98	0.05
								NIT12	−0.47	−1.56 to 0.62	0.39
E. coli	TMP	0	−0.22	0.02	5.7	1.19	0.32	TMP1	−0.02	−0.18 to −0.14	0.80
		−3	0.22	0.02				TMP3	0.15	−0.01 to 0.32	0.07
		+8	0.22	0.02				TMP6	0.10	−0.06 to 0.26	0.23
								TMP12	0.00	−0.17 to 0.17	0.99
K. pneumoniae	CIP	−6	0.28	0.004	16.3	3.86	0.01	CIP1	0.04	−0.75 to 0.82	0.93
								CIP3	0.56	−0.23 to 1.34	0.16
								CIP6	1.45	0.67 to 2.22	<0.001
								CIP12	0.36	−0.43 to 1.16	0.37
K. pneumoniae	FOS	+10	0.28	0.01	3.7	0.64	0.64	FOS1	0.04	−0.50 to 0.59	0.87
								FOS3	0.32	−0.24 to 0.88	0.26
								FOS6	−0.25	−0.81 to 0.30	0.37
								FOS12	−0.29	−0.85 to 0.28	0.32
K. pneumoniae	TMP	−2	0.24	0.01	5.6	1.17	0.33	TMP1	−0.01	−0.50 to 0.49	0.98
		−5	0.21	0.03				TMP3	−0.20	−0.71 to 0.31	0.45
								TMP6	0.48	−0.01 to 0.97	0.06
								TMP12	0.22	−0.29 to 0.74	0.39

ABU, antibiotic use; coef., coefficient; F, F test statistic; CIP, ciprofloxacin; NIT, nitrofurantoin; TMP, trimethoprim; FOS, fosfomycin.
[a]Combination of antimicrobial resistance and antibiotic use for *E. coli* and *K. pneumoniae*.
[b]Months of lag in which significant peaks were observed in the cross-correlation function plot. For the cross-correlation function, + and − signs show significant positive and negative lag lengths.
[c]The F-test assesses whether there is any association between lagged antibiotic use and the antimicrobial resistance level, for lags of 1, 3, 6 and 12 months combined.
[d]Lag length in months of antibiotic use in the linear regression models with lagged effects. The length was chosen to cover the short-term and long-term relationship between variables. The (negative) lag lengths of 1, 3, 6 and 12 months were pre-selected, irrespective of the P values. Lagged regression models used as dependent variables the residuals of ARIMA models for resistance time series, and as independent variables the lagged residuals from ARIMA models for antibiotic use time series.

In conclusion, in a low-consuming country like the Netherlands there was a weak association between antibiotic use and resistance, and the strongest evidence for this association was observed with a time delay of 3–6 months between changes in antibiotic use and changes in resistance.

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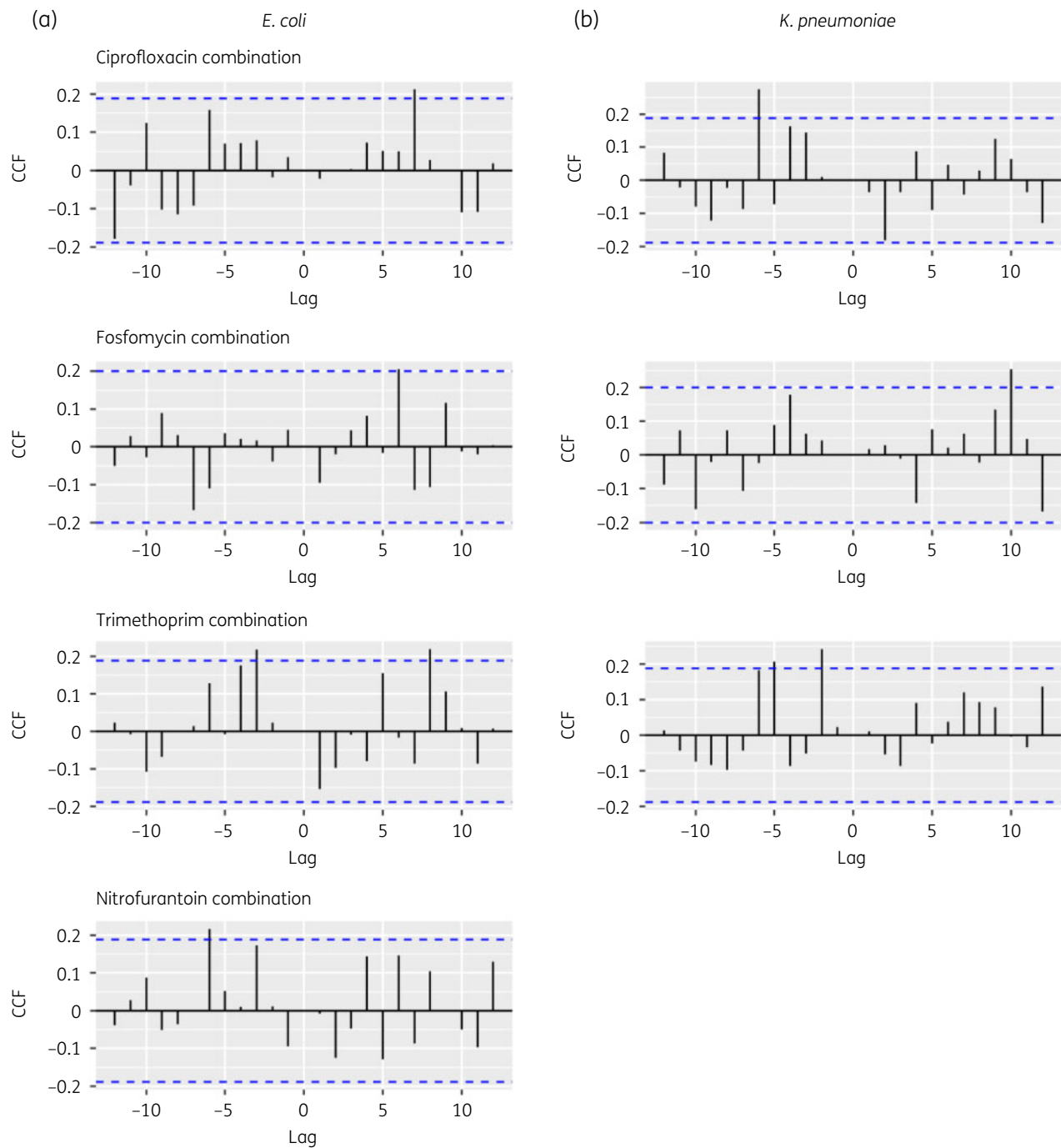
(a) *E. coli*

(b) *K. pneumoniae*

Figure 4. CCF of combinations between antibiotic use and resistance levels in *E. coli* and *K. pneumoniae*. The CCF represents lag lengths from −12 months (leading effect of antibiotic use) to 12 months (lagging effect of antibiotic use). The horizontal dotted lines show the cross-correlation significance limit at 95%. Vertical solid lines shown cross-correlation coefficients between the two time series. Cross-correlation peaks outside the limits are considered significant, thus a peak at negative or positive lag is statistically significant and shows that antibiotic use is associated with resistance. A negative lag implies that a change in antibiotic use is followed by a change in resistance (predictor variable), and a positive lag implies that a change in antibiotic use would likely occur after a change in resistance (lagging variable). The 95% CIs are not adjusted for multiple testing. This figure appears in colour in the online version of *JAC* and in black and white in the print version of *JAC*.

Laboratory, Amsterdam; B.C. van Hees, Gelre Hospitals, Department of Medical Microbiology and Infection prevention, Apeldoorn; B.M.W. Diederen, Bravis Hospital, Department of Medical Microbiology, Bergen op Zoom; J. Aguilar Diaz, J. Alblas, W. Altorf-van der Kuil, L. Blijboom, S.C. de Greeff, S. Groenendijk, R. Hertroys, J.C. Monen, W. van den Reek, A. Reuland, A.F. Schoffelen, C.C.H. Wielders, S.H.S. Woudt, National Institute for Public Health and the Environment (RIVM), Centre for Infectious Diseases, Epidemiology and Surveillance, Bilthoven; W. van den

Table 5. Results of multiple linear regression with lagged effects for co-resistance between AMR in *E. coli* and *K. pneumoniae* and two antibiotics used to treat urinary tract infections in the Netherlands

Bacteria	AMR	R^2 (%)	F	P value (F-test)[a]	ABU lag (months)[b]	coef.	95% CI	P value
E. coli	NIT	16.3	2.52	0.08	NIT1	0.02	−1.42 to 1.45	0.98
					NIT3	1.51	0.12–2.89	0.03
					NIT6	0.92	−0.49 to 2.33	0.20
					NIT12	−1.33	−2.82 to 0.16	0.08
			1.11	0.36	TMP1	−0.5	−1.35 to 0.35	0.24
					TMP3	−0.25	−1.09 to 0.58	0.55
					TIMP6	−0.03	−0.86 to 0.80	0.94
					TMP12	0.74	−0.11 to 1.59	0.09
E. coli	TMP	8.0	0.81	0.59	TMP1	0.03	−0.21 to 0.26	0.81
					TMP3	0.12	−0.11 to 0.35	0.29
					TIMP6	0.06	−0.17 to 0.29	0.59
					TMP12	−0.11	−0.34 to 0.37	0.37
			0.47	0.76	NIT1	−0.08	−0.47 to 0.32	0.71
					NIT3	0.02	−0.37 to 0.40	0.94
					NIT6	0.08	−0.31 to 0.47	0.69
					NIT12	0.27	−0.15 to 0.68	0.20
K. pneumoniae	TMP	24.1	2.97	0.01	TMP1	−0.43	−1.09 to 0.23	0.19
					TMP3	0.23	−0.43 to 0.88	0.49
					TMP6	0.68	0.04 to 1.33	0.04
					TMP12	−0.15	−0.81 to 0.51	0.65
			4.56	0.002	NIT1	1.21	0.09–2.32	0.03
					NIT3	−1.26	−2.34 to −0.18	0.02
					NIT6	−0.76	−1.85 to 0.34	0.17
					NIT12	1.34	0.18–2.49	0.02

ABU, antibiotic use; coef., coefficient; F, F test statistic; CIP, ciprofloxacin; NIT, nitrofurantoin; TMP, trimethoprim; FOS, fosfomycin.
[a]The F-test assesses whether there is any association between a lagged antibiotic use and the antimicrobial resistance level, for lags of 1, 3, 6 and 12 months, to each antibiotic combined.
[b]Lag length in months of antibiotic use used in the linear regression models with lagged effects. The length was chosen to cover the short-term and long-term relationship between variables. The (negative) lag lengths of 1, 3, 6 and 12 months were pre-selected, irrespective of the P values. Lagged regression models used as dependent variables the residuals of ARIMA models for resistance time-series, and as independent variables the lagged residuals from ARIMA models for antibiotic use time series.

Bijllaardt, Microvida Amphia, Laboratory for Microbiology and Infection Control, Breda; E.M. Kraan, IJsselland hospital, Department of Medical Microbiology, Capelle a/d Ijssel; E.E. Mattsson, Reinier de Graaf Groep, Department of Medical Microbiology, Delft; E. de Jong, Slingeland Hospital, Department of Medical Microbiology, Doetinchem; H.M.E. Frénay, B. Maraha, Albert Schweitzer Hospital, Department of Medical Microbiology, Dordrecht; A.J. van Griethuysen, Gelderse Vallei Hospital, Department of Medical Microbiology, Ede; G.J. van Asselt, A. Demeulemeester, SHL-Groep, Etten-Leur; B.B. Wintermans, Admiraal De Ruyter Hospital, Department of Medical Microbiology, Goes; M. van Trijp, Groene Hart Hospital, Department of Medical Microbiology and Infection Prevention, Gouda; A. Ott, Certe, Department of Medical Microbiology, Groningen; E. Bathoorn, M. Lokate, University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Groningen; J. Sinnige, Regional Laboratory of Public Health, Haarlem; D.C. Melles, St Jansdal Hospital, Department of Medical Microbiology, Harderwijk; E.I.G.B. de Brauwer, F.S. Stals, Zuyderland Medical Centre, Department of Medical Microbiology and Infection Control, Heerlen; W. Silvis, Laboratory of Medical Microbiology and Public Health, Hengelo; L.J. Bakker, J.W. Dorigo-Zetsma, CBSL, Tergooi Hospital, Department of Medical Microbiology, Hilversum; B. Ridwan, Westfriesgasthuis,

Department of Medical Microbiology, Hoorn; K. Waar, Izore Centre for Infectious Diseases Friesland, Leeuwarden; A.T. Bernards, Leiden University Medical Center, Department of Medical Microbiology, Leiden; S.P. van Mens, Maastricht University Medical Centre, Department of Medical Microbiology, Maastricht; N. Roescher, St Antonius Hospital, Department of Medical Microbiology and Immunology, Nieuwegein; M.H. Nabuurs-Franssen, Canisius Wilhelmina Hospital, Department of Medical Microbiology and Infectious Diseases, Nijmegen; E. Kolwijck, Radboud University Medical Center, Department of Medical Microbiology, Nijmegen; B.M.W. Diederen, Bravis Hospital, Department of Medical Microbiology, Roosendaal; L.G.M. Bode, Erasmus University Medical Center, Department of Medical Microbiology, Rotterdam; M. van Rijn, Ikazia Hospital, Department of Medical Microbiology, Rotterdam; S. Dinant, O. Pontesilli, Maasstad Hospital, Department of Medical Microbiology, Rotterdam; P. de Man, Sint Franciscus Gasthuis, Department of Medical Microbiology, Rotterdam; G.J. van Asselt, STAR Medical Diagnostic Center, Rotterdam; M.A. Leversteijn-van Hall, Haaglanden Medical Centre/Alrijne Hospital, Department of Medical Microbiology and Infection Control, 's-Gravenhage; E.P.M. van Elzakker, Haga Hospital, Department of Medical Microbiology, 's-Gravenhage; A.E. Muller, MCH Westeinde Hospital, Department of Medical Microbiology,

's-Gravenhage; N.H. Renders, Jeroen Bosch Hospital, Department of Medical Microbiology and Infection Control, 's-Hertogenbosch; D.W. van Dam, Zuyderland Medical Centre, Department of Medical Microbiology and Infection Control, Sittard-Geleen; B.M.W. Diederen, ZorgSaam Hospital Zeeuws-Vlaanderen, Department of Medical Microbiology, Terneuzen; A.G.M. Buiting, St. Elisabeth Hospital, Department of Medical Microbiology, Tilburg; A.L.M. Vlek, Diakonessenhuis, Department of Medical Microbiology and Immunology, Utrecht; E.A. Reuland, Saltro Diagnostic Centre, Department of Medical Microbiology, Utrecht; A. Troelstra, University Medical Center Utrecht, Department of Medical Microbiology, Utrecht; I.T.M.A. Overdevest, PAMM, Department of Medical Microbiology, Veldhoven; R.W. Bosboom, Rijnstate Hospital, Laboratory for Medical Microbiology and Immunology, Velp; T.A.M. Trienekens, VieCuri Medical Center, Department of Medical Microbiology, Venlo; G.J.H.M. Ruijs, M.J.H.M. Wolfhagen, Isala Hospital, Laboratory of Medical Microbiology and Infectious Diseases, ZwolleFunding.

Transparency declarations

None to declare.

Supplementary data

Tables S1 to S4 and additional Methods are available as Supplementary data at *JAC* Online.

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