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Fluoroquinolone resistance in *Escherichia coli* isolates after exposure to non-fluoroquinolone antibiotics: a retrospective case-control study

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Running title: Fluoroquinolone resistance in *E. coli* after exposure to other antibiotics

Synopsis

Objectives: To investigate whether prior exposure to non-fluoroquinolone antibiotics increases the risk of fluoroquinolone resistance in *Escherichia coli* (*E. coli*).

Methods: This was a secondary analysis of data collected retrospectively in a case-control study linking microbiological test results (isolated bacteria and their susceptibility) of urine samples routinely collected in primary, secondary and tertiary care patients in Belgium with information on prior antibiotic use at the patient level up to one year prior.

Results: In urine samples from 6125 patients, 7204 *E. coli* isolates were retrieved; 1949 fluoroquinolone resistant isolates (cases) and 5255 fluoroquinolone susceptible isolates (controls). After adjusting for potential confounders (including fluoroquinolone use) and correcting for multiple testing, there was lower odds of fluoroquinolone resistance in *E. coli* isolates after exposure to cefazolin (OR = 0.65; 95% CI: 0.52 – 0.81; $p = 0.00034$) and higher odds after exposure to trimethoprim/sulfamethoxazole (OR = 1.79; 95% CI, 1.38 - 2.31; $p < 0.001$) and after exposure to nitrofurantoin (OR: 1.41, 95% CI: 1.14 - 1.76; $p < 0.002$). A sensitivity analysis excluding samples with antibiotic use in the 6 months prior to the sampling date, confirmed the higher odds of fluoroquinolone resistance after exposure to trimethoprim/sulfamethoxazole and nitrofurantoin.

Conclusions: Assuming no residual confounding or other biases, this study suggests that exposure to non-fluoroquinolone antibiotics, i.e. trimethoprim/sulfamethoxazole and nitrofurantoin, might be causally related to fluoroquinolone resistance in *E. coli* isolates from urinary samples. Future prospective research is needed to confirm non-fluoroquinolone antibiotics as potential drivers of fluoroquinolone resistance.

Introduction

Fluoroquinolones, a major class of antibiotics, belong to the WHO list of highest priority antimicrobials for resistance surveillance in human medicine.[1] In various European countries, considerable increases in ciprofloxacin resistance in *Escherichia coli* (*E. coli*) causing uncomplicated urinary tract infections were found between 2000 and 2014.[2] Although a slight decrease in fluoroquinolone resistance has been reported in Belgium (from 26.7% in 2014 to 23.8% in 2017) it was still high compared to other northern European countries, such as the Netherlands (14.2% in 2017) and Denmark (12.8% in 2017).[3]

While fluoroquinolone use is likely the main driver of resistance to fluoroquinolones,[4-11] we need to consider other risk factors. Previous studies in *Enterococcus* species*, K. pneumoniae, P. aeruginosa* and *E. coli* found older age,[4] long term care facility residency,[5] recent hospitalization,[9] the use of urinary catheter,[7] and previous exposure to non-fluoroquinolone antibiotics,[5, 10, 12-14] as independent risk factors for fluoroquinolone resistance. The link between non-fluoroquinolones and fluoroquinolone resistance can be explained by the phenomenon of co-selection.[15, 16]

Even though investigators have identified several non-fluoroquinolone antibiotics as independent risk factors to fluoroquinolone resistance,[4, 5, 12-14, 17] most of these studies had no information on prior antibiotic use, i.e. before hospitalization, only included hospitalized patients and had small sample sizes, and in some case-control studies, controls were taken from the same hospital, with a high probability of selection bias and resulting in a persistent effect of a previous selection pressure of an antimicrobial.

Identification of a causal relationship between non-fluoroquinolone antibiotic use and fluoroquinolone resistance could guide treatment options to reduce fluoroquinolone resistance and enhance our understanding of co-selection. Therefore, we investigated the increased risk of fluoroquinolone resistance in urinary *E. coli* samples collected from primary, secondary and tertiary health care settings in Belgium by the use of non-fluoroquinolone antibiotics in the year prior to the sampling date. We hypothesized that we would find associations between non-fluoroquinolone antibiotic use and

fluoroquinolone resistance. Additionally, we assessed effect modification of this relation by exposure to fluoroquinolones.

Methods

Study design and setting

We performed a secondary analysis of data collected retrospectively from primary, secondary and tertiary care patients in Belgium during a case-control study. Microbiological test results (1st January 2011 to 31st December 2012) were linked with data on prior antibiotic use at the patient level (1st January 2010 until 31st December 2012) up to one year before sample collection and susceptibility testing for fluoroquinolones.[18] More information on the data sources, linkage, data access and cleaning methods, is available as Supplementary material S1.

Study population

In the linked dataset, including 197,393 urine samples, in 21,569 (10.9%) samples *E. coli* was isolated. For 16,593 (76.9%) of these *E. coli* strains, ciprofloxacin susceptibility was tested, and in 1,743 (10.5%), 106 (0.6%), 3,415 (20.6%), and 11,329 (68.3%) the test result was unknown, intermediate, resistant, and susceptible, respectively. After selecting only antibiotic use one year prior to sampling testing, we obtained a total of 8,400 samples. From this data, we excluded samples from the same patient within the next 30 days as this cut-off point is often used to differentiate between the same and new urinary tract infection episode[16]; resulting in a total of 7,204 samples. For the purpose of this study, *E. coli* strains with unknown susceptibility test results were excluded from the analysis, cases were defined as *E. coli* isolates with intermediate and resistant test results and controls were defined as *E. coli* isolates with susceptible test results.

Exposure and outcome assessment

The exposures of interest were the consumption of antibacterials for systematic use, i.e. substances with Anatomical Therapeutic Chemical (ATC) code J01.[19] These antibiotics were studied at the chemical substance level, e.g. doxycycline (ATC J01AA02), and at the chemical subgroup level, e.g. tetracyclines (ATC J01AA), separately. We use the terms *antibiotics alone* for the chemical substance level and *group of antibiotics* for the chemical sub-group level. Should a causal relation between exposure to an antibiotic and fluoroquinolone resistance exist, one would expect a similar relation for the group of antibiotics to which it belongs. We considered a patient's sample exposed to an antibiotic if that antibiotic was reimbursed to the patient up to one year prior to their fluoroquinolone susceptibility testing. The inputs for all exposures were categorical (Yes/No).

The primary outcome was fluoroquinolone resistance indicated as resistant or intermediate in the ciprofloxacin (fluoroquinolone) susceptibility test of *E. coli* isolates from urine samples. The susceptibility testing was mainly done by the Kirby Bauer disk diffusion technique according to CLSI guidelines. Modifications were present according to the manufacturer for deviations in disk charge or diameter. The majority of Belgian hospitals worked with Neosensitabs for producing these antibiograms.[20]

Covariates

We considered potential confounders to be any variable suspected to be linked to both fluoroquinolone resistance and non-fluoroquinolone antibiotic use. Confounding variables were extracted from the antibiotic use and socio-demographic data, and included prior or current use of fluoroquinolones (Yes/No), whether the urine sample was taken in a hospital (Yes/No), the total number of days that a patient stayed in the hospital the last 6 months before the urine sample was taken (zero if the patient was not hospitalized in the last 6 months), the most recent time between any antibiotic use and the susceptibility test for fluoroquinolones resistance (in days), age (in years), gender (female/male) and any other antibiotic use. We also assessed modification of the relationship between

non-fluoroquinolones and fluoroquinolone resistance by fluoroquinolone use for the secondary aim of the study.

Statistical methods

Categorical variables were described using proportions, while continuous variables were summarized using medians and IQRs. Significance of differences in median values (for continuous variables) or proportions (for categorical variables) among cases and controls were assessed using Wilcoxon rank sum or Fisher's exact tests, respectively. The (2x2) contingency table analyses included only antibiotics with a least 5 counts per cell.

To quantify the relation between fluoroquinolone resistance and the use of each of the non-fluoroquinolone antibiotics (both alone and as group), a generalized estimating equation (GEE) approach for a binary outcome was used to accommodate dependence in observations related to the same individual,[21] thereby producing crude ORs with their 95% CIs.

In model 1, a multivariable GEE approach was used to adjust for confounding variables. Since multicollinearity was not an issue when adding all covariates in an initial full model (to check for multicollinearity we used (generalized) variance inflation factors, calculated using the car package in R, which had values smaller than 4) and our sample size was large enough, we conducted model building in a backward fashion by removing all non-significant ($p<0.15$) covariates. In model 2, we further examined effect modification of fluoroquinolone use only for non-fluoroquinolone antimicrobials that were found to be significant in model 1.

The Quasi-likelihood under the Independence model Criterion (QIC) was used to choose between an independence or exchangeable working correlation structure. A Bonferroni correction for multiple testing was applied to control for the overall Type I error. As a sensitivity analysis, we re-analyzed the available data excluding samples with antibiotic use in the 6 months prior to the sampling date to minimize the 'memory-like' correlations of resistance.[22]

All statistical analyses were performed using the statistical R software, version 3.5.1.[23] Unless stated otherwise (cf. in case of a Bonferroni correction), a 5% significance level was used for inference. The final data for this study consisted of complete cases for which no missing information regarding the determinants of the outcome under study was present.

Ethical statement

The study protocol were approved by the Sectorial committee Social Security and Health of the former Privacy Commission (now Data Protection Authority; SCSZG/13/274) as well as by the ethics committee of Antwerp University Hospital (reference: 14/4/27).[24] This manuscript adheres to the STROBE and the RECORD-PE reporting guidelines.[25 26]

Results

The final data used for this study provides information on the fluoroquinolone resistance status for 7,204 *E. coli* isolates identified in urine samples of 6,125 patients. Most patients were female (77%). The number of isolates per patient widely varied, where the minimum number was one and the maximum was 10. Our analysis included 1,949 case samples from 1,601 patients and 5,255 control samples from 4,710 patients.

Significant differences in characteristics between cases and controls were observed (Table 1). Cases were older than controls, with a difference of 10 years in median values. The median length of stay at the hospital was longer in cases than controls, with a difference of 12 days in medians. The median time between any antibiotic use and a susceptibility test for fluoroquinolones was longer in controls (77 days) than cases (38 days), with a difference of 39 days in median values (Figure 1). The proportion of samples from females was lower in cases than in controls (66.70% and 80.36%, respectively). The proportion of urine samples taken in the hospital was slightly lower in cases than in controls (12.98% and 13.17%, respectively). Moreover, the proportion of fluoroquinolone use was higher in cases than in controls (73.01% and 32.33%, respectively). Among the different types of fluoroquinolones used, ciprofloxacin was the most frequently used and its use was higher in cases than in controls (52.03% and 19.12%, respectively).

Univariable analysis

Exposures: Table 2 shows the crude ORs with 95% CIs. For *antibiotics alone*, only the following antibiotics showed a significant association with fluoroquinolone resistance in *E. coli* isolates: tetracycline, ampicillin, amoxicillin, temocillin, benzylpenicillin, cefadroxil, cefuroxime, ceftazidime, ceftriaxone, meropenem, trimethoprim/sulfamethoxazole, amikacin, nitrofurantoin, nifurtoinol, and fosfomycin. For *group of antibiotics*, only penicillin with extended spectrum, penicillin & β-lactamase

inhibitors, second and third generation cephalosporins, lincosamides, other aminoglycosides, glycopetides, nitrofuran derivatives and other antibacterials did.

Multivariable analysis

Model 1: For *antibiotics alone*, after adjusting for the predefined confounders and correcting for multiple testing (the number of tests performed was 14 based on the covariates finally included in the multivariable model; Bonferroni correction: 0.05/14 = *0.004*), only cefazolin, trimethoprim/sulfamethoxazole and nitrofurantoin showed a significant association with fluoroquinolone resistance. Next to these three antibiotics, the final model also adjusted for fluoroquinolone use, age, time of length of stay at the hospital, most recent time between any antibiotic use and the susceptibility test, gender, the use of vancomycin, temocillin, metronidazole, roxithromycin, cefadroxil and cefuroxime. After exposure to cefazolin, there was lower odds of fluoroquinolone resistance in *E. coli* isolates (OR = 0.65; 95% CI: 0.52 – 0.81; *p = 0.00034*) as compared to no exposure to this antibiotic. There was a higher odds of fluoroquinolone resistance in *E. coli* isolates after exposure to trimethoprim/sulfamethoxazole (OR = 1.79; 95% CI: 1.38 - 2.31; *p = 0.00023*) and after exposure to nitrofurantoin (OR: 1.41; 95% CI: 1.14 - 1.76; *p = 0.00013*) compared to no exposure to these antibiotics, respectively (Table 2).

As for *group of antibiotics*, after adjusting for confounders and correcting for multiple testing (Nine covariates were finally included in the multivariable model; Bonferroni correction: 0.05/9 = *0.006*), only first generation cephalosporins and nitrofurantoin derivates were significantly associated with fluoroquinolone resistance in *E. coli* isolates in urine samples. Next to these two groups of antibiotics, the final model also adjusted for fluoroquinolones use, age, time of length of stay at the hospital, most recent time between any antibiotic use and the susceptibility test, gender, whether the sample was taken at hospital, the use of trimethoprim/sulfamethoxazole and imidazole. The odds of fluoroquinolone resistance in *E. coli* isolates was lower after exposure to first generation

cephalosporins (OR = 0.65; 95% CI: 0.53 – 0.81; $p < 0.0001$) and higher after exposure to nitrofuran derivates (OR = 1.38; 95% CI: 1.17 - 1.64; $p = 0.00015$) (Table 2).

Table 3 shows the results of fitting model 2 to the data and stratifying by fluoroquinolone use. Only the interaction term between the use of fluoroquinolones and trimethoprim/sulfamethoxazole exposure was significant ($p = 0.004$). In those using fluoroquinolones, the association between exposure to trimethoprim/sulfamethoxazole and fluoroquinolone resistance was found to be non-significant (OR = 1.13; 95% CI: 0.87 - 1.46), whereas the association was significant in those that did not use fluoroquinolones (OR = 2.75; 95% CI: 1.87 - 4.05). The estimated exposure effects of non-fluoroquinolone antibiotics on fluoroquinolone resistance were always smaller in those who used fluoroquinolones than in those who did not use fluoroquinolones. However, fluoroquinolone resistance was always higher in those exposed to fluoroquinolones as compared to those not exposed to fluoroquinolones regardless of the use of non-fluoroquinolone antibiotics.

Sensitivity analysis

In 1445 cases and 3821 controls there were no samples with antibiotic use in the 6 months prior to the sampling date. Only trimethoprim/sulfamethoxazole and nitrofurantoin were consistently associated with fluoroquinolone resistance and no substantial changes were found in the association between fluoroquinolone resistance and non-fluoroquinolone antibiotic use, after adjusting for confounders and correcting for multiple testing (the number of tests performed in the final model was 14; corrected *p-value* = 0.004): trimethoprim/sulfamethoxazole: OR = 1.63; 95% CI; 1.19 - 2.23; $p = 0.0023$; nitrofurantoin: OR = 1.51; 95% CI, 1.18 - 1.93; $p = 0.0011$.

Discussion

Our study suggests a potentially causal relation between non-fluoroquinolone antibiotics use and fluoroquinolone resistance in *E. coli* isolates identified in urine samples which were routinely collected in primary, secondary and tertiary care patients in Belgium. Particularly, exposure to trimethoprim-sulfamethoxazole and nitrofurantoin in the year prior to the sample collection and susceptibility testing was significantly associated with fluoroquinolone resistance. These relations were confirmed when excluding samples with antibiotic use in the 6 months prior to sampling and susceptibility testing. Regarding the effect of fluoroquinolone use, the effect of exposure to non-fluoroquinolone antibiotics to fluoroquinolone resistance was smaller in those who also used fluoroquinolones than in those who did not use fluoroquinolones. Fluoroquinolone resistance was always higher in those who used fluoroquinolones as in those who did not.

Comparison with other studies

Previous studies found associations between use of several non-fluoroquinolone antibiotics and fluoroquinolone resistance. The use of ceftriaxone was correlated with a decreased susceptibility of *E. coli* isolates to ciprofloxacin in in-patients;[12] the use of aminoglycosides in the last 30 days increased the risk of ciprofloxacin more than fivefold,[5] whereas treatment with aminoglycosides in a year prior to sampling increased the risk of infection with levofloxacin resistance by ten-fold[13] in nosocomial bacteremia due to extended-spectrum β-lactamase-producing *E.coli* and *Klebsiella pneumoniae (K. pneumoniae).* In another study, aminoglycoside use in the previous 30 days was found to increase levofloxacin resistance by over eight-fold in nosocomial *E. coli* and *K. pneumoniae* infections.[4] Aminoglycosides and ceftriaxone were less frequently used compared to other antibiotics in our study population, which might explain why we were not able to show an association with fluoroquinolone resistance.

A three-fold risk of levofloxacin resistance with the use of “other types of antibiotics” was found in *Enterococcus faecalis* isolates from in-patients with urinary tract infection (UTI).[10] Another study

showed that the use of β-lactamase inhibitors and extended spectrum cephalosporins and clindamycin, in the previous 30 days, increased levofloxacin resistance by over nine-fold in hospitalized patients with UTI caused by Enterococcus species.[14] Meanwhile, we did not observe associations between fluoroquinolone resistance and any of these antibiotics, despite having a large sample size.

In line with our findings, a recent large study that analyzed data at a higher geographical level and explored whether higher levels of non-fluoroquinolone antibiotics were associated with higher fluoroquinolone resistance in *E. coli* isolates, found that the use of trimethoprim and derivates was associated with increased ciprofloxacin resistance, i.e. use over one year, three and one month before an antibiotic susceptibility test (AST), but not for the use of nitrofurantoin.[16] Another recent large study at the patient level observed that the purchase of trimethoprim was associated with ciprofloxacin resistance which is also consistent with our findings.[22] The association between the use of sulfamethoxazole/trimethoprim and fluoroquinolone resistance might be explained by the selection of resistance genes that code for resistance against both fluoroquinolones and sulfamethoxazole/trimethoprim by the use sulfamethoxazole/trimethoprim, as for example found in *E. coli* sequence type ST131 isolates.[27] On the other hand, and given that the strains were not available for further investigations, we are not able to provide an biological mechanisms for the higher odds of fluoroquinolone resistance after exposure to nitrofurantoin found in our study since, to our knowledge, no previous studies found such associations and neither found co-selection resistant genes in urinary pathogens coding for both nitrofurantoin and fluoroquinolone resistance.

Strengths and limitations

We used data at patient level for each antibiotic use and for each corresponding susceptibility test in a large linked dataset from primary, secondary and tertiary healthcare settings. Our study findings fit with previous studies,[4, 5, 10, 12-14] although not exactly the same non-fluoroquinolones antibiotics were identified. In turn, we attempted to minimized reverse causality by excluding samples with antibiotic use in the 6 months prior to a susceptibility test.

Furthermore, we used a longer patient history of antibiotic use in a larger sample of patients. This enabled a more granular disaggregation of the effects of specific antibiotic classes in different periods before a formal susceptibility test was performed and using hospitalized and non-hospitalized patients from which a urinary sample was taken and where *E. coli* was identified, and hence, helps to narrow the possible effect of exposure to antibiotics on resistance to fluoroquinolones. Another strength is that we used a GEE approach which considers clustering of samples from the same patient, while other approaches such as logistic regression would have required selection of one sample per patient, by doing this, we would have lost very valuable and from an evolutionary microbiological perspective relevant information.

Even though one can argue that the potential for confounding by indication, contraindication or disease severity could mislead our results, statistical control for age and gender to some extent covers these possible confounders, for instance, co-morbidities require most often antibiotic treatment which is linked to age; regarding morbidity such as UTIs, older and female patients in the database are more likely to have more than one urinary sample tested, and women are also more vulnerable to UTIs. Finally, because there is a concern when using multiple samples from patients as prior resistance is one of the main predictors of future resistant samples,[22] we have conducted an additional sensitivity analysis using only the first sample per patient, which provided similar results as well (data not shown). Moreover, as a main sensitivity analysis we excluded samples with antibiotic use in the 6 months prior to a sampling date to reduce potential memory-like correlations of resistance,[22] considering that the choice of antibiotic for treatment may be influenced by prior AST results. Yet, given the nature of our data reverse causality cannot be completely excluded, despite using individual-level data

Regarding potential limitations, our study is a retrospective observational study. Hence, we missed other potential confounders, such as the presence of a urinary catheter.[7] Even though we adjusted for the setting in which the urinary sample was taken, though more likely, not all hospital samples are catheter specimens of urine, and not all outpatient samples are midstream specimens. Additionally,

we treated patients as independent individuals without considering a broader range of important factors such as household membership,[28] food intake, socioeconomic factors,[29] and variation in prescribing among practices and areas,[30, 31] thus limiting the chances of meeting the Stable Unit Treatment Value Assumption (SUTVA) that underlies standard regression models.[16] Nevertheless, it is a strength that the previous study on data grouped at higher levels[16] and ours on data at the individual level, each having their strengths and weaknesses, agreed that trimethoprim and derivates (trimethoprim/sulfamethoxazole) use may select for fluoroquinolone resistance.

We were not able to assess the effect of all non-fluoroquinolone antibiotics present in the dataset due to limited use of these antibiotics in our study sample and not being able to cope with small cell counts by using statistical techniques, e.g. Firth correction.[16] Misclassification bias of the measurement of the exposures is possible as we only have information on the reimbursement of purchased antibiotics and not on actual consumption and antibiotics can be purchased and consumed without being reimbursed. However, we believe overall compliance to antibiotic treatment in Belgium is high, particularly starting a course of purchased antibiotics, and patients do not need to end the course for antibiotics to select for resistant bacteria. Hence, misclassification bias of the exposures is less likely to affect our findings. Since our study population consisted of samples that are more likely to be taken and sent for a resistance test, e.g. in case of treatment failure,[16, 32] this might have confounded our estimates. Another relative issue is that we did not measure the exposure using DDD, which would rather have included the volume of use in the model enabling the demonstration of a dose-response effect, which consequently could have led to more convincing evidence in favour of a causal relation as described in one of the Bradford-Hill criteria.[33] Finally, the generalizability of our findings might not be applicable to other European countries since co-resistance and antibiotic use trends vary across countries.

Implications for future studies

Future studies should be conducted prospectively, collecting information on all potential confounders, not only at the hospital level but also at the community level or geographical level, and ideally measure actual exposure. The fact that microorganisms can share resistance genes even without the trigger of exposure to antibiotics, requires assessment of a broader range of factors that could lead to increase resistance, (i.e. household membership,[28] food intake, socioeconomic factors,[29] and variation in prescribing among practices and areas.[30, 31]). Another consideration is that after exposure the time to selection of resistant bacteria will be more similar across antibiotics than the persistence of resistance,[34, 35] while the latter might also differ by pathogen. Regarding the assessment of a dose-response relationship, both the number of DDD per treatment and the number of treatments should be assessed. Finally, further studies should be performed in patients that were not tested for antibiotic resistance for at least two years prior to a first AST result to assess fluoroquinolone resistance in UTIs due to *E. coli,*[22] to prevent confounding of the relation between non-fluoroquinolone use and fluoroquinolone resistance due to 'reverse causality'.

In conclusion, our study suggests a (causal) association between exposure to non-fluoroquinolone antibiotics, specifically to trimethoprim/sulfamethoxazole and nitrofurantoin, and fluoroquinolone resistance in *E. coli* isolates from urinary samples. Assuming no residual confounding or other biases previously discussed, this study implies that co-selection could drive fluoroquinolone resistance after exposure to non-fluoroquinolone antibiotics especially in those not exposed to fluoroquinolones. Further prospective evidence however is quintessential to confirm which non-fluoroquinolone antibiotics increase the risk of fluoroquinolone resistance.

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Transparency to declare

The funders had an advisory role in the design and data collection of the overall study but were not involved in any aspect of the current study.

Author contributions

L.C-P., S.C, R.B., S.A., B.C., designed the study and analysis. B.C., K.L. contributed data. L.C-P., R.B., B.C., K.L., S.A, H.G., and S.C, participated in the interpretation of the results and writing the manuscript. All authors read and approved the final manuscript.

Data accessibility

Original databases at patient level are no longer available in accordance with restriction from the Sector Committee. However, final data used for this study can be requested for further analyses upon formal request and under strict supervision of Sciensano.

Code availability

Code used for data analysis is available upon request.

Supplementary material

More information on the data sources, linkage, data access and cleaning methods is available in Supplementary material S1.

The RECORD statement for pharmacoepidemiology (RECORD-PE) checklist, extended from the STROBE and RECORD statements is reported as Supplementary material S2.

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Table 1.Characteristics of 7204 *E. coli* isolates identified in urine samples of 6125 patients in primary, secondary, and tertiary healthcare settings in Belgium, 2011-2012. Controls are susceptible to fluoroquinolones according to applied disk diffusion test, cases are not (intermediate + resistant).

Exposure one year prior to susceptibility test	**Cases (*N*= 1949), n (%)**	**Controls (*N= 5255*), n (%)**	***P***
Median age (IQR), years	76 (63 - 83)	66 (37 - 81)	< 0.0001[a]
Gender, female	1300 (66.70)	4223 (80.36)	< 0.0001
Urine sample taken in the hospital	253 (12.98)	692 (13.17)	0.88
Median length of stay at the hospital (IQR), days	21 (5 - 54)	9 (2 -34)	< 0.0001[a]
Median of time between any antibiotic use and the susceptibility test (IQR), days	38 (14 - 104)	77 (25 – 175)	< 0.0001[a]
Fluoroquinolones (J01MA) use	1423 (73.01)	1699 (32.33)	< 0.0001
Ofloxacin	85 (4.36)	92 (1.75)	< 0.0001
Ciprofloxacin	1014 (52.03)	1005 (19.12)	< 0.0001
Norfloxacin	152 (7.80)	219 (4.17)	< 0.0001
Levofloxacin	205 (10.52)	189 (3.60)	< 0.0001
Moxifloxacin	400 (20.52)	426 (8.11)	< 0.0001

[a] Wilcoxon sum rank test; all other comparisons were made by Fisher's exact test.

Table 2. Exposure to non-fluoroquinolone antibiotics, controls are susceptible to fluoroquinolones, cases are not. Univariable and multivariable analysis of non-fluoroquinolone antibiotic use one year prior to a susceptibility test for fluoroquinolones in 7204 *E. coli* isolates identified in urine samples of 6125 patients in primary, secondary and tertiary healthcare setting in Belgium (2011-2012).

Exposure one year prior to susceptibility test	Cases (N= 1949), n (%)	Controls (N= 5255), n (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
Antibiotics alone				
Doxycycline	45 (2.31)	114 (2.17)	1.07 (0.73 - 1.55)	-
Tetracycline	8 (0.41)	40 (0.76)	0.47 (0.24 - 0.95)	-
Minocycline	19 (0.97)	57 (1.08)	1.15 (0.67 - 1.96)	-
Ampicillin	19 (0.97)	91 (1.73)	0.96 (0.57 - 1.60)	-
Amoxicillin	434 (22.27)	1552 (29.53)	0.68 (0.60 - 0.78)	-
Temocillin	45 (2.31)	42 (0.80)	2.93 (1.85 - 4.64)	1.83 (1.07 - 3.14)
Benzylpenicillin	7 (0.36)	10 (0.19)	2.91 (1.08 - 7.81)	-
Oxacillin	6 (0.31)	17 (0.32)	0.95 (0.40 - 2.29)	-
Flucloxacillin	102 (5.23)	219 (4.17)	1.27 (0.97 - 1.66)	-
Amoxicillin - clavulanic acid	766 (39.30)	1940 (36.92)	1.11 (0.98 - 1.25)	-
Piperacillin - clavulanic acid	60 (3.07)	90 (1.71)	1.38 (0.96 - 1.99)	-
Cefazolin	200 (10.26)	534 (10.16)	1.01 (0.83 - 1.23)	0.65 (0.52 - 0.81)[a]
Cefadroxil	9 (0.46)	78 (1.48)	0.31 (0.15 - 0.62)	0.43 (0.18 - 1.01)
Cefuroxime	296 (15.19)	601 (11.44)	1.39 (1.16 - 1.65)	1.24 (1.01 - 1.53)
Ceftazidime	48 (2.46)	57 (1.08)	1.65 (1.04 - 2.60)	-
Ceftriaxone	45 (2.31)	68 (1.29)	1.80 (1.20 - 2.71)	-
Meropenem	28 (1.44)	39 (0.74)	2.31 (1.40 - 3.82)	-
Trimethoprim/sulfamethoxazole	266 (13.65)	368 (7.00)	2.10 (1.73 - 2.54)	1.79 (1.38 - 2.31)[b]
Erythromycin	16 (0.82)	42 (0.80)	1.18 (0.68 - 2.04)	-
Roxithromycin	10 (0.51)	18 (0.34)	1.50 (0.62 - 3.63)	2.07 (0.84 - 5.12)
Clarithromycin	113 (5.80)	288 (5.48)	1.06 (0.83 - 1.35)	-
Azithromycin	106 (5.44)	333 (6.34)	0.85 (0.67 - 1.09)	-
Clindamycin	117 (6.00)	248 (4.72)	1.27 (0.97 - 1.66)	-
Lincomycin	12 (0.62)	20 (0.38)	1.62 (0.77 - 3.42)	-
Gentamicin	11 (0.56)	21 (0.40)	1.41 (0.65 - 3.09)	-
Amikacin	30 (1.54)	44 (0.84)	1.70 (1.03 - 2.80)	-
Vancomycin	45 (2.31)	76 (1.45)	1.61 (1.04 - 2.49)	0.61 (0.37 - 1.01)
Metronidazole	40 (2.05)	94 (1.79)	1.30 (0.90 - 1.90)	0.62 (0.38 - 1.01)
Nitrofurantoin	296 (15.19)	480 (9.13)	1.78 (1.49 - 2.13)	1.41 (1.14 - 1.76)[c]
Nifurtoinol	180 (9.24)	285 (5.42)	1.77 (1.44 - 2.19)	-
Fosfomycin	348 (17.86)	825 (15.70)	1.17 (1.00 - 1.36)	-
Group of antibiotics				
Tetracyclines	73 (3.75)	213 (4.05)	0.92 (0.69 - 1.23)	-
Penicillin with extended spectrum	475 (24.37)	1629 (31.00)	0.72 (0.63 - 0.82)	-
β-lactamase sensitive penicillin	10 (0.51)	30 (0.57)	1.54 (0.51 - 2.27)	-
β-lactamase resistant penicillin	106 (5.44)	232 (4.41)	1.25 (0.96 - 1.61)	-
Penicillin & β-lactamase inhibitors	790 (40.53)	1969 (37.47)	1.14 (1.01 - 1.28)	-
First generation cephalosporins	208 (10.67)	603 (11.47)	0.92 (0.76 - 1.11)	0.65 (0.53 - 0.81)[d]

Second generation cephalosporins	296 (15.19)	604 (11.49)	1.38 (1.16 - 1.64)	-
Third generation cephalosporins	83 (4.26)	122 (2.32)	1.87 (1.36 - 2.67)	-
Macrolides	243 (12.47)	694 (13.21)	0.94 (0.79 - 1.11)	-
Lincosamides	125 (6.41)	266 (5.06)	1.29 (1.00 - 1.65)	-
Other aminoglycosides	42 (2.15)	61 (1.16)	1.68 (1.11 - 2.54)	-
Glycopeptides	48 (2.46)	76 (1.45)	1.72 (1.12 - 2.64)	-
Imidazoles derivatives	43 (2.21)	97 (1.85)	1.43 (0.98 - 2.10)	-
Nitrofuran derivatives	447 (22.93)	738 (14.04)	1.82 (1.57 - 2.11)	1.38 (1.17 - 1.64)[e]
Other antibacterials	352 (18.06)	828 (15.76)	1.18 (1.01 - 1.37)	-

[a] p-value: 0.00016; [b] p-value: 0.0000098; [c] p-value: 0.00166; [d] p-value: 0.000083, [e] p-value: 0.00015

Table 3. Effect modification by fluoroquinolone antibiotic use of the relation between fluoroquinolone resistance and non-fluoroquinolone antibiotic use in 7204 *E. coli* isolates identified in urine samples of 6125 patients in primary, secondary, and tertiary health care settings in Belgium (2011-2012).

Exposure one year prior to susceptibility test	Not use of fluoroquinolones = 0	Use of fluoroquinolone = 1	*P*[a]
	Adjusted OR (95%CIs)	Adjusted OR (95%CIs)	
Antibiotic alone			
Cefazolin	0.65 (0.46 – 0.92)	0.74 (0.56 – 0.99)	0.15
Trimethoprim/sulfamethoxazole	2.75 (1.87 – 4.05)	1.13 (0.87 – 1.46)	0.004
Nitrofurantoin	1.49 (1.05 – 2.10)	1.44 (1.13 – 1.84)	0.96
Group of antibiotics			
First generation cephalosporins	0.61 (0.44 – 0.86)	0.71 (0.54 – 0.94)	0.15
Nitrofuran derivatives	1.28 (0.94 – 1.73)	1.40 (1.15 – 1.72)	0.44

[a] *p* value of the interaction term

Figure 1. Histogram for the most recent time between any antimicrobial use and a susceptibility test for fluoroquinolones (one year prior) (days), with a median line stratified by cases and controls in *E. coli* isolates from urine samples retrieved from 6125 patients in primary, secondary, and tertiary healthcare centers in Belgium (2011-2012).