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Original article

Effect of outpatient antibiotics for urinary tract infections on antimicrobial resistance among commensal *Enterobacteriaceae*: a multinational prospective cohort study

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ABSTRACT

Objectives: We quantified the impact of antibiotics prescribed in primary care for urinary tract infections (UTIs) on intestinal colonization by ciprofloxacin-resistant (CIP-RE) and extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-PE), while accounting for household clustering.
Methods: Prospective cohort study from January 2011 to August 2013 at primary care sites in Belgium, Poland and Switzerland. We recruited outpatients requiring antibiotics for suspected UTIs or asymptomatic bacteriuria (exposed patients), outpatients not requiring antibiotics (non-exposed patients), and one to three household contacts for each patient. Faecal samples were tested for CIP-RE, ESBL-PE, nitrofurantoin-resistant *Enterobacteriaceae* (NIT-RE) and any *Enterobacteriaceae* at baseline (S1), end of antibiotics (S2) and 28 days after S2 (S3).
Results: We included 300 households (205 exposed, 95 non-exposed) with 716 participants. Most exposed patients received nitrofurans (86; 42%) or fluoroquinolones (76; 37%). CIP-RE were identified in 16% (328/2033) of samples from 202 (28%) participants. Fluoroquinolone treatment caused transient suppression of *Enterobacteriaceae* (S2) and subsequent two-fold increase in CIP-RE prevalence at S3 (adjusted prevalence ratio (aPR) 2.0, 95% CI 1.2–3.4), with corresponding number-needed-to-harm of 12. Nitrofurans had no impact on CIP-RE (aPR 1.0, 95% CI 0.5–1.8) or NIT-RE. ESBL-PE were identified in 5% (107/2058) of samples from 71 (10%) participants, with colonization not associated with antibiotic exposure. Household exposure to CIP-RE or ESBL-PE was associated with increased individual risk of colonization: aPR 1.8 (95% CI 1.3–2.5) and 3.4 (95% CI 1.3–9.0), respectively.
Conclusions: These findings support avoidance of fluoroquinolones for first-line UTI therapy in primary care, and suggest potential for interventions that interrupt household circulation of resistant *Enterobacteriaceae*. **A.J. Stewardson, Clin Microbiol Infect 2018;24:972**

Introduction

Antimicrobial resistance (AMR) imposes an important health and economic burden and the threat of a post-antibiotic future

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requiring major changes to contemporary healthcare provision [1,2]. Antibiotic exposure is a key factor in the selection and dissemination of AMR and most human antibiotic use occurs in the community [3]. In addition to infection control measures and the development of new antibiotic agents, antibiotic stewardship should optimize the use of existing antibiotics to minimize AMR [4]. Yet stewardship interventions are faced with a relative scarcity of evidence to quantify the relative merits of agent selection and duration of therapy. Moreover, recent studies have demonstrated the importance of accounting for the colonization status of household contacts when assessing the impact of antibiotics on ambulatory patients treated with antibiotics [5].

Our primary aim was to determine the impact of antibiotic class and treatment duration on the carriage of antibiotic-resistant *Enterobacteriaceae* among individuals consuming antibiotics for urinary tract infections (UTIs), while accounting for household transmission of commensal microbiota. As secondary aims, we sought to assess epidemiological factors associated with carriage of antibiotic-resistant *Enterobacteriaceae*; and to determine the impact of antimicrobial use on the carriage of any *Enterobacteriaceae*.

We adapted a conceptual model to develop *a priori* hypotheses regarding the impact of different antibiotic classes on the emergence of antimicrobial resistance [6,7] (Table 1), and also hypothesized that any effects would increase with increasing treatment duration.

Materials and methods

This trial is registered with the ISRCTN registry, number ISRCTN26797709.

Design, setting and population

We performed a multinational prospective cohort study. From January 2011 to August 2013, ambulatory patients were recruited from established general practice networks in Antwerp (Belgium) and Łódź (Poland) [8], and from ambulatory care clinics at the Geneva University Hospitals (Geneva, Switzerland). We recruited—as the 'exposed' index patient group—a convenience sample of patients prescribed antibiotics for suspected upper or lower UTIs or asymptomatic bacteriuria (see Supplementary material, Table S1, for definitions). Antibiotic agent and duration were determined by the treating physician. We recruited an unmatched group of 'non-exposed' index patients presenting to the same clinics for an indication that did not require antibiotic therapy. Inclusion criteria applied to all index patients were: age ≥18 years and current residence in a household with at least one other person. Exclusion criteria were: treatment with systemic antibiotics or hospitalization within the previous 30 days; residence in a long-term care facility; presence of an indwelling urinary catheter; renal transplant or renal replacement therapy; or if follow up was unlikely to be possible. Non-exposed index patients were also excluded if they, or any member of their household, were currently being treated with antibiotics. We recruited one to three household contacts for each index patient. There were no age restrictions or exclusion criteria for household contacts.

Data collection

Investigators at each site completed a case report form at the time of index participant recruitment. Each participant also completed a self-administered baseline paper questionnaire. Participants provided three faecal samples: baseline (Sample 1 (S1)); completion of antibiotic therapy (S2); and 28 days after the second sample (S3). For all participants from non-exposed households, S2 was 7–10 days after S1. Participants collected their own samples using a disposable Protocult™ kit (Ability Building Center, Rochester, MN, USA), and these were kept on ice for a maximum of 24 h before being collected in person and frozen at −80°C until analysis.

Variables

The exposure of interest was antibiotic therapy, stratified by class and duration. We used chemical subgroups from the Anatomical Therapeutic Chemical classification system to define antibiotic class [9], including J01MA (fluoroquinolones), J01XE (nitrofuran derivatives), J01XX01 (fosfomycin) and J01EE (trimethoprim-sulfamethoxazole). Clinically relevant thresholds were used to dichotomize duration into 'short' and 'long' where relevant. The main outcomes were detectable intestinal colonization by extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-PE) and ciprofloxacin-resistant *Enterobacteriaceae* (CIP-RE), defined as detection of such organisms in faecal samples taken at the end of antibiotic therapy (S2) and 28 days after the end of therapy (S3). As a summary measure for the primary outcome,

Table 1
Summary of *a priori* hypotheses regarding the impact of fluoroquinolones and nitrofurans on the emergence of antimicrobial resistance

Antibiotic exposure	Resistance type	Predicted impact	Rationale (potential mechanisms)
Fluoroquinolone	Ciprofloxacin	Strong	• Individuals are usually colonized by ciprofloxacin-susceptible *Enterobacteriaceae* AND resistance is conferred by single mutation(s). • Ciprofloxacin suppresses the endogenous flora that otherwise tends to block acquisition of the resistant organism AND individuals are exposed to infectious sources of the resistant organism during or shortly after the period of treatment. • Individuals may be colonized by both ciprofloxacin-resistant and susceptible *Enterobacteriaceae* AND treatment increases the load of resistant organisms by killing the competitive susceptible strains.
Fluoroquinolone	Extended-spectrum β-lactamase	Moderate	• Ciprofloxacin suppresses the endogenous flora AND individuals are exposed to extended-spectrum β-lactamase-producing *Enterobacteriaceae* during or shortly after the treatment period. • Extended-spectrum β-lactamase-producing *Enterobacteriaceae* can be resistant to ciprofloxacin, and treatment shifts the balance of colonizing organisms from mostly susceptible to mostly resistant.
Nitrofurans	Nitrofurantoin	Weak	• Resistance can be conferred by single mutations, however high fitness cost and low gastrointestinal antibiotic levels reduce impact
Nitrofurans	Extended-spectrum β-lactamase	Negligible	• Nitrofurantoin resistance uncommonly conveyed by extended-spectrum β-lactamase plasmids
Nitrofurans	Ciprofloxacin	Negligible	• No potential mechanisms likely to have significant role

we computed colonization prevalence by dividing the number of colonized participants by number of participants (according to participant type (index/contact) and antibiotic exposure) at each time-point.

Secondary outcomes were detectable intestinal colonization by nitrofurantoin-resistant *Enterobacteriaceae* (NIT-RE) for those participants receiving nitrofurantoin, and by any *Enterobacteriaceae* for all participants. Baseline covariates included age and sex, birth in or recent travel (within 12 months) to a high-risk country, animal contact, meat preparation and education level. High-risk countries were defined by location in the following geographic areas: Indian subcontinent, Southeast Asia, and Africa [10]. Colonization of one or more household member with *Enterobacteriaceae* with the resistance phenotype of interest (dichotomous) was recorded as a time-varying covariate at each time point.

Microbiological methods

Microbiological analyses of samples from all sites were performed at a central laboratory (Laboratory of Medical Microbiology, University of Antwerp, Antwerp, Belgium). Faecal samples from all three time points were quantitatively screened for presence of resistant organisms. Stool suspensions (10%) were prepared in sterile physiological water with a stomacher (BagMixer 100 MiniMix, Interscience, Saint Nom la Bretèche, France), serially diluted (up to 10^{-5}), with two- to three-fold dilutions inoculated on the following media by spiral plating 100 µl in a logarithmic mode (Eddy Jet, IUL Instruments, Barcelona, Spain): blood agar, CHROMagar Orientation (CHROMagar, Paris, France), CHROMagar ESBL, CHROMagar KPC and CHROMagar Orientation supplemented with 0.12 mg/L and 2 mg/L ciprofloxacin (CHROMagar CIP). Samples from households of patients receiving nitrofurantoin and control households were additionally cultured on CHROMagar Orientation supplemented with 64 mg/L nitrofurantoin. Cultures were read and quantified after being incubated at 37°C for 18–24 h and 24 h, respectively. In case of no growth, these were re-incubated for 24 h. Bacterial loads (CFU/mL of stool) were calculated separately for each colony colour.

The relative abundance of resistant *Escherichia coli* in the gastrointestinal tract was determined by dividing the counts of resistant *E. coli* (sum of bacterial loads of pink colonies on supplemented CHROMagar) by the counts of all *E. coli* (sum of bacterial loads of pink colonies on CHROMagar Orientation) in each stool sample. Ten colonies of each morphology type were sampled from selective plates. Strains not identified as *E. coli* by coloration on the chromogenic agar underwent species identification by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Antibiotic susceptibility and phenotypic ESBL confirmation for all strains was determined by the disk diffusion method according to CLSI guidelines.

Sample size

The null hypothesis was that there is no difference between the control and fluoroquinolone-treated index participants with regard to the increase in prevalence of detectable intestinal colonization with CIP-RE from S1 to S3. With a power of 0.8 and two-sided α of 0.05, we would need approximately 40 patients in each group to reject this null hypothesis with an absolute colonization prevalence increase of 25% in the treated group and negligible increase (1%) in the control group. To facilitate multivariable analysis, we aimed for 70 households in the control, fluoroquinolone and nitrofuran groups.

Statistical methods

The impact of antimicrobial class and duration on the colonization status was evaluated using mixed-effects generalized linear regression models. We used Poisson models for the binary colonization outcome to compute prevalence ratios [5]. Antibiotic class (categorized as 'nitrofuran', 'fluoroquinolone' or 'other'), household exposure to the organism of interest, and potential confounders were included as fixed effects. Household exposure was a dichotomous variable for each participant at each time point to indicate whether one or more participants in the same household (excluding that participant) was colonized by *Enterobacteriaceae* with the resistance phenotype of interest. Potential confounders were chosen on the basis of existing evidence [11], with final model selection performed using Akaike's information criterion [12]. To evaluate the impact of fluoroquinolone treatment duration, we selected 7 days as a clinically relevant threshold for 'short' duration [13]. We accounted for repeated measurements and the clustered study design by including random intercepts for participant, household and study site [14]. Households were included in the analysis if at least one faecal sample was collected at each time point and the case report form and questionnaire were available. We used multiple imputation for missing outcome values. We estimated the number-needed-to-harm for antibiotic classes and resistant phenotypes. See Appendix S1 for further details regarding statistical analyses.

All analyses were performed using R, version 3.4.0 (R Foundation for Statistical Computing, Vienna, Austria), including the 'lme4', 'MASS', 'mitml' and 'tidyverse' packages.

Ethics

The study was approved by each centre's institutional review board: Geneva University Hospitals (protocol 10-123), Antwerp University Hospital (B30020109056), and Medical University of Łódź (RNN/127/10/KE z 13 lipca 2010 r). Written informed consent was obtained from all participants.

Results

Participants

Recruitment is outlined in Fig. 1. A total of 300 households (205 antibiotic-exposed and 95 non-exposed) consisting of 716 participants were included in the analysis: 69, 105 and 126 households in Antwerp, Geneva and Łódź, respectively. Baseline characteristics and sample collection details are presented in Table 2.

Among the exposed index patients, 73% (149/205), 20% (42/205) and 7% (14/205) had presumptive diagnoses of lower UTI, upper UTI and asymptomatic bacteriuria, respectively. Two antibiotic classes accounted for 79% (162/205) of prescriptions to these patients: nitrofuran derivatives (ATC code J01XE; 47 (23%) nitrofurantoin and 39 (19%) furazidin) and fluoroquinolones (ATC code J01MA; 68 (33%) ciprofloxacin and 8 (4%) norfloxacin). Fosfomycin (J01XX01) and trimethoprim-sulfamethoxazole (J01EE) accounted for 15 (7%) and 9 (4%) of the remaining prescriptions. Fluoroquinolones were more common among patients with upper UTI (86%; 36/42) than lower UTI (22%; 33/149) or asymptomatic bacteriuria (50%; 7/14). As they account for the bulk of prescriptions, we focused our analysis on fluoroquinolone and nitrofuran treatment.

The proportion of participants—stratified by participant-type, exposure and time point—with any *Enterobacteriaceae* (regardless of antibiotic susceptibility), CIP-RE and ESBL-RE isolated from stool samples is presented in Fig. 2. Detailed results from faecal samples are presented in the Supplementary material (Table S2).

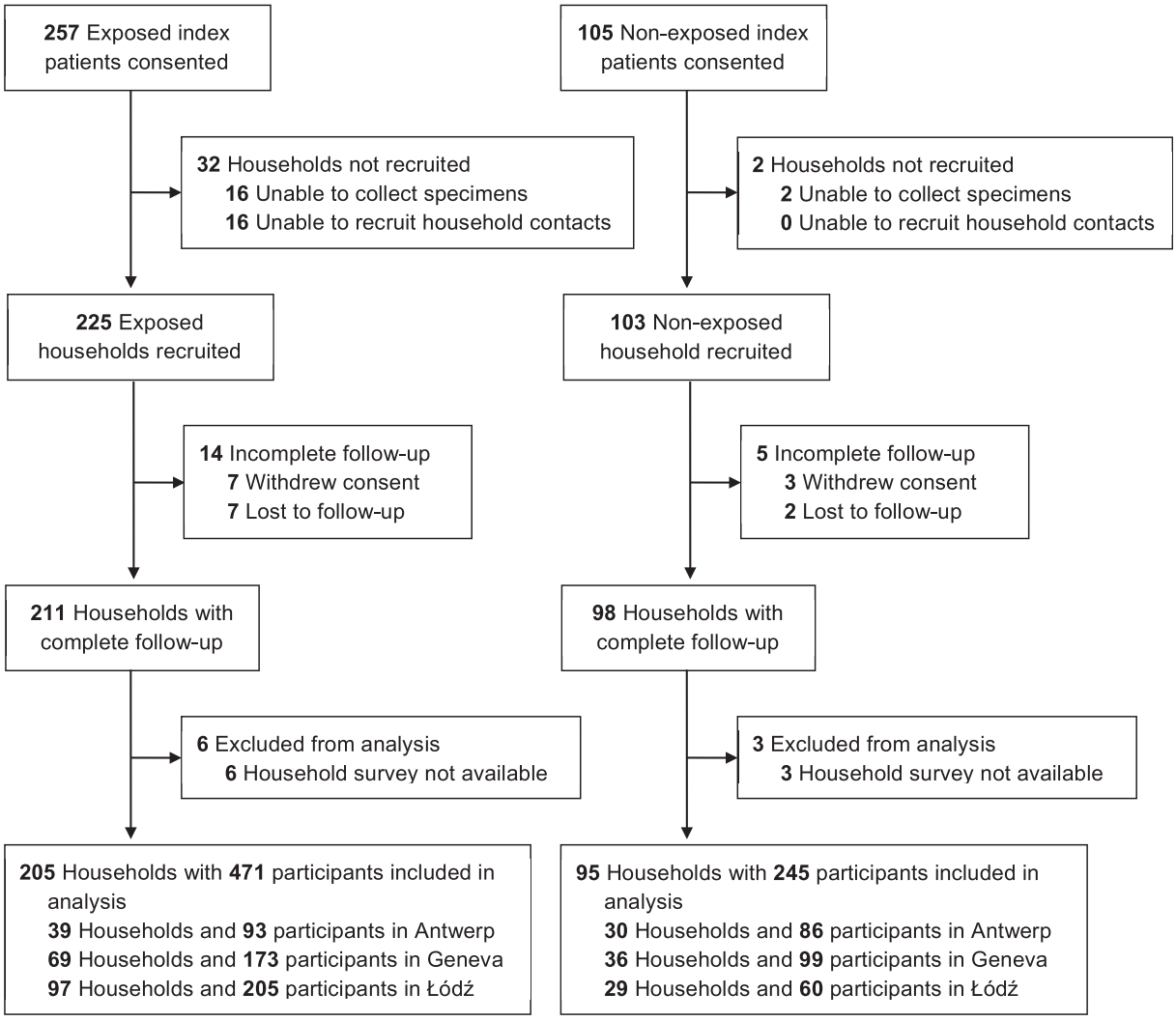

Fig. 1. Study flow diagram.

Ciprofloxacin-resistant Enterobacteriaceae

The final model is presented in Table 3. The prevalence of CIP-RE was doubled 28 days after treatment with fluoroquinolones (adjusted prevalence ratio (aPr) 2.00; 95% CI 1.18–3.36), but not increased by nitrofurans (aPR 0.98; 95% CI 0.53–1.81) or other antibiotics (aPR 1.48; 95% CI, 0.66–3.31). Given the CIP-RE prevalence of 8% among non-exposed patients at S3, 12 patients would need to be treated with a fluoroquinolone in order to have one additional patient colonized by CIP-RE. Other factors associated with risk for colonization by CIP-RE included household exposure to CIP-RE, age and recent travel to highly endemic region (Table 3, and see Supplementary material, Fig. S1). When travel to high-risk region was further categorized into specific geographic regions, none was individually associated with increased prevalence of colonization. There was an increased relative abundance of ciprofloxacin-resistant *E. coli* 1 month following the end of fluoroquinolone treatment (see Supplementary material, Fig. S2).

Fluoroquinolones were the only class of antibiotics with sufficient variation in treatment duration to explore the impact of duration on emergence of resistance. Of 76 patients receiving fluoroquinolones, 33 (43%) and 43 (57%) received short and long treatments, respectively. The impact of treatment duration on emergence of ciprofloxacin resistance—as well as the presence of any *Enterobacteriaceae*—is presented graphically in the Supplementary material (Fig. S3). There was no statistically significant difference between 'long' and 'short' duration with regard to the prevalence of CIP-RE at the final follow-up sample.

ESBL-producing Enterobacteriaceae

In contrast with CIP-RE, no epidemiological risk factors were identified for colonization by ESBL-PE, nor was fluoroquinolone treatment significantly associated with an increase in the prevalence of ESBL-PE colonization within 28 days: aPR at 1.36 (0.35–5.20) (see Supplementary material, Table S3). However, as with CIP-RE, we found evidence of household clustering of ESBL-PE, with exposure to one or more household contacts colonized with ESBL-PE being associated with a 3.38-fold (95% CI 1.27–9.01) increase in risk of ESBL-PE colonization.

Nitrofurantoin-resistant Enterobacteriaceae

There were insufficient samples with NIT-RE to support a regression model. Of the 12 participants with positive samples, 11 belonged to control households. Three control households had two participants with NIT-RE samples.

Colonization with any Enterobacteriaceae

Compared with S1, the proportion of samples from which any *Enterobacteriaceae* were detected decreased significantly at S2 among

Table 2
Characteristics of households and participants included in the analysis

Household-level characteristics	Household type	
	Non-exposed households	Exposed households
	(n = 95)	(n = 205)
Study site		
Antwerp	30 (32)	39 (19)
Geneva	36 (38)	69 (34)
Łódź	29 (31)	97 (47)
Residents		
2	25 (26)	81 (40)
3–4	56 (59)	98 (48)
>4	14 (15)	26 (13)
Children in household		
Any age <18 years	62 (65)	101 (49)
<5 years and attends day-care	16 (17)	22 (11)
Highest education level		
Primary	0 (0)	7 (3)
Secondary	21 (22)	95 (46)
Undergraduate tertiary	17 (18)	41 (20)
Postgraduate tertiary	57 (60)	62 (30)
Farm location	2 (2)	5 (2)

Participant-level characteristics	Participant type			
	Non-exposed households		Exposed households	
	Index patients	Household contacts	Index patients	Household contacts
	(n = 95)	(n = 150)	(n = 205)	(n = 266)
Demographics				
Age, median (interquartile range)	40 (33–49.5)	16.5 (7–39)	39 (30–53)	29 (13–49)
Female sex	71 (75)	67 (45)	190 (93)	84 (32)
Healthcare exposures in previous 12 months				
Hospitalization	9 (9)	26 (17)	24 (12)	30 (11)
Antibiotic exposure	28 (29)	39 (26)	91 (44)	58 (22)
Urinary tract infection	14 (15)	NA	71 (35)	NA
Urinary catheter	2 (2)	NA	2 (1)	NA
Social exposures				
High-risk travel[a]	11 (12)	16 (11)	19 (9)	25 (9)
Companion animal contact	43 (45)	71 (47)	91 (44)	122 (46)
Farm animal contact	4 (4)	7 (5)	9 (4)	12 (5)
Vegetarian	2 (2)	2 (1)	2 (1)	8 (3)
Raw meat preparation	77 (81)	63 (42)	163 (80)	116 (44)
Health and co-morbidities				
Current pregnancy	2 (2)	NA	10 (5)	NA
Chronic kidney disease	0 (0)	NA	1 (0)	NA
Cardiovascular disease	8 (8)	NA	29 (14)	NA
Diabetes	3 (3)	NA	17 (8)	NA
Hemiplegia	0 (0)	NA	2 (1)	NA
Chronic skin condition	1 (1)	NA	2 (1)	NA
Chronic airways disease	1 (1.1)	NA	2 (1)	NA
Autoimmune disease	3 (3)	NA	2 (1)	NA
Liver cirrhosis	0 (0)	NA	1 (0)	NA
Neoplasia	1 (1)	NA	3 (1)	NA
Faecal sample collection				
Sample 1 collected	95 (100)	149 (99)	184 (90)	262 (98)
Sample 2 collected	95 (100)	148 (99)	204 (100)	264 (99)
Sample 3 collected	94 (99)	147 (98)	203 (99)	263 (99)

Abbreviation: NA, not applicable.
Result reported as n (%).
[a] Within 12 months prior to recruitment.

individuals with UTIs treated with fluoroquinolones (aPR 0.55; 95% CI 0.40–0.77) (Fig. 2). One month later (S3), the prevalence of *Enterobacteriaceae* returned to baseline (aPR 1.00; 95% CI 0.78–1.27). The prevalence of *Enterobacteriaceae* remained stable throughout for all other groups, including household contacts of patients treated with fluoroquinolones, patients treated with nitrofurantoin and their household contacts, and participants from control households.

Multiple-resistance

The antimicrobial susceptibility profile of *E. coli* strains from the ESBL, ciprofloxacin and nitrofurantoin screening plates that were confirmed as having the resistance phenotype of interest (ESBL-positivity, ciprofloxacin resistance or nitrofurantoin resistance, respectively) are presented in the Supplementary material (Table S4). Among the 1842 ciprofloxacin non-susceptible *E. coli*, 216 (11.7%) were ESBL-positive. None of the 19 nitrofurantoin-resistant *E. coli* were ESBL-positive.

Discussion

This study confirmed that exposure to fluoroquinolone results in a significant reduction in the presence of *Enterobacteriaceae* in the gut immediately at the end of therapy. Though the numbers of

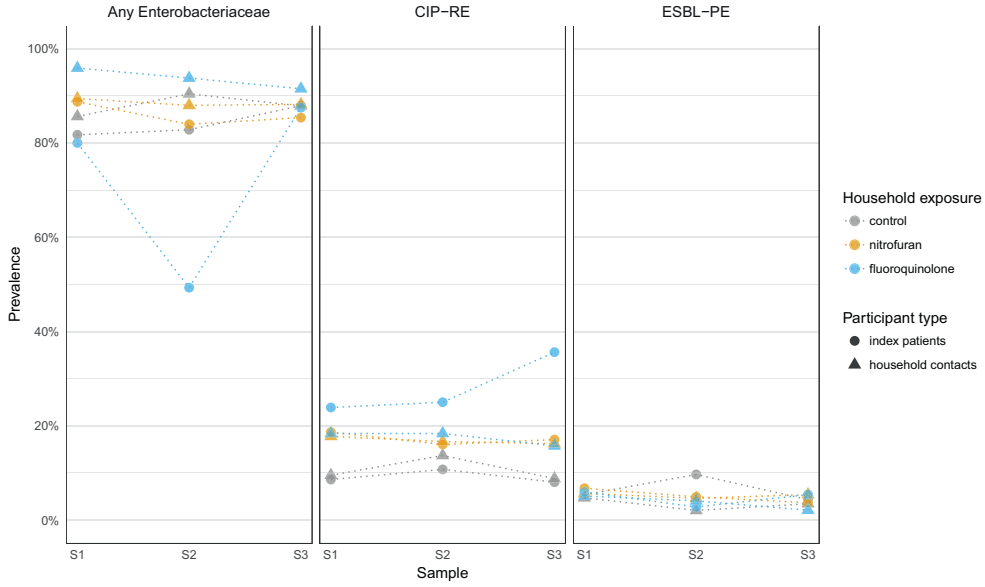

Fig. 2. Prevalence of any, ciprofloxacin-resistant, and ESBL-producing *Enterobacteriaceae*. Abbreviations: CIP-RE, ciprofloxacin-resistant *Enterobacteriaceae*; ESBL-PE, extended-spectrum β-lactamase-producing *Enterobacteriaceae*.

patients with cultivable *Enterobacteriaceae* recovered 28 days later, this recovery was accompanied by an increased prevalence of CIP-RE. By contrast, nitrofurantoin had minimal impact on total *Enterobacteriaceae* and was neither associated with emergence of ciprofloxacin nor nitrofurantoin resistance. These findings are consistent with our *a priori* hypothesis based on a mechanistic conceptual model of the link between exposure to specific antibiotics and emergence of resistance (Table 1) [6,7].

Table 3
Multivariable mixed-effects Poisson regression model for colonization by ciprofloxacin-resistant *Enterobacteriaceae*

Exposure	No. (%) of participants (n = 716)	Prevalence ratio (95% CI)
Antibiotic exposure		
Immediately post-treatment		
nitrofuran	86 (12)	0.91 (0.47–1.76)
fluoroquinolone	76 (11)	1.46 (0.83–2.59)
other antibiotic	43 (6)	1.54 (0.69–3.44)
28 days post-treatment		
nitrofuran	86 (12)	0.98 (0.53–1.81)
fluoroquinolone	76 (11)	2.00 (1.18–3.36)
other antibiotic	43 (6)	1.48 (0.66–3.31)
Household type		
Control	245 (34)	reference
Antibiotic: nitrofurantoin	198 (28)	1.59 (1.05–2.43)
Antibiotic: fluoroquinolone	176 (25)	1.66 (1.07–2.57)
Antibiotic: other	97 (14)	1.48 (0.86–2.56)
Age group (years)		
≥60	85 (12)	reference
40–59	204 (28)	0.76 (0.48–1.20)
19–39	254 (35)	0.56 (0.36–0.89)
5–18	128 (18)	0.59 (0.35–1.01)
<5	45 (6)	0.35 (0.15–0.80)
Travel to high-risk country within 12 months	71 (10)	1.92 (1.24–2.96)
Household exposure to ciprofloxacin-resistant *Enterobacteriaceae*	Time-varying	1.80 (1.28–2.54)

Note: Multiple imputation used to account for 115 of 2148 (5%) observations with missing ciprofloxacin-resistant *Enterobacteriaceae* colonization status. All other variables in the model were complete for all cases.

We were unable to detect a significant benefit in reducing the duration of fluoroquinolone treatment. Although it is contrary to the notion that duration of exposure is positively associated with selection resistance [15], this finding is consistent with a previous study in the hospital setting demonstrating that emergence of quinolone resistance was not associated with fluoroquinolone type or treatment duration [16]. As previously discussed by de Lastours et al. [16], this finding may be attributable to the relatively long half-life of ciprofloxacin in the intestinal tract and impact on the intestinal microbiota following even a single dose [17]. Indeed, with regards to the suppression of *Enterobacteriaceae*, we were equally likely to recover any *Enterobacteriaceae* at the end of treatment whether that treatment lasted for more or less than 1 week. Furthermore, if selection for resistant strains indeed occurs when fluoroquinolone levels fall below the MIC of least susceptible strains, and into the mutant selection window [18], then the crucial period would be following the cessation of treatment, regardless of its duration. Consistent with this concept is the greater increase in proportion of participants colonized with CIP-RE after the cessation of fluoroquinolones than during treatment. This pattern has previously been reported among healthy volunteers [19]. Together, these findings suggest that the 'damage is done' early during the fluoroquinolone treatment course, and that antibiotic stewardship should therefore focus on the avoidance of fluoroquinolones rather than shortened duration as has been recently advocated [20].

In addition to the emergence of ciprofloxacin resistance, fluoroquinolones resulted in an increase in the relative abundance of resistant strains. This finding is significant given that an increase in the relative abundance of resistant *Enterobacteriaceae* means that in the event of subsequent UTI there is a greater risk of infection by the resistant strain [21]. In addition, an increase in the relative abundance of antibiotic-resistant *Enterobacteriaceae* has been associated with a greater risk of environmental contamination by such strains in hospitalized patients [22]—and it is plausible that this may result in an increased risk of transmission in the community setting also [6].

In contrast to country-level ecological studies, we did not demonstrate an association between exposure to antibiotics and colonization by ESBL-producing *Enterobacteriaceae*. We propose

three explanations. First, two-thirds of ESBL-producing *E. coli* from faecal samples remained susceptible to ciprofloxacin, so co-selection by ciprofloxacin may not be sufficiently frequent. Second, the prevalence of ESBL-PE colonization in the community is lower than CIP-RE, so transmission events may be less likely to occur. Third, in contrast to ciprofloxacin resistance, ESBL are not the result of *de novo* mutation, so the 'acquisition' of ESBL-PE requires either pre-existing colonization below the level of detection or acquisition from an external source.

We noted household clustering for colonization by both CIP-RE and ESBL-PE. This finding has previously been reported for resistance to trimethoprim [23,24], ampicillin, trimethoprim-sulfamethoxazole, and doxycycline [25], and ESBL-PE [26–29]. Indeed, the transmission rate for ESBL-PE has previously been estimated as greater in the household than in the hospital setting [28], with neonates, infants and companion animals potentially favouring dissemination [24–26]. Although transmission of AMR strains is likely to represent the 'tip of the iceberg' with regard to the shared household microbiome [30], it is notable for at least two reasons. First, household transmission of pathogenic, antibiotic-resistant strains may result directly in negative health outcomes, such as has been suggested for *E. coli* ST131, which is associated with both multidrug resistance and robust pathogenicity [31,32]. Second, with the reservoir of Gram-negative resistance and the focus of its transmission shifting from the hospital to the community [33], interruption of household transmission represents a hitherto largely neglected opportunity for interventions to tackle this problem.

These findings should be interpreted within the context of the study design. In the absence of randomization, we cannot exclude residual confounding. In particular, patients receiving fluoroquinolones were more likely to be receiving treatment for upper UTI rather than cystitis. Second, our follow-up period of 28 days after end of treatment was relatively brief. However, quinolone-resistant *E. coli* selected from the intestinal microbiota of individuals exposed to ciprofloxacin are 'highly adapted to a commensal lifestyle' and may persist for long periods following emergence [34]. Third, the number of index patients receiving antibiotics other than nitrofurans and fluoroquinolones was too low to assess their impact. Finally, we have not performed molecular characterization of the ciprofloxacin resistance mechanisms or strain clonality. Important strengths of this study include the multinational participant recruitment, which supports the generalizability of our findings to countries with varying prevalence of resistance, and our hypothesis-driven approach.

Exposure to fluoroquinolones transiently suppresses intestinal *Enterobacteriaceae* with a subsequent increase in the probability of colonization by CIP-RE and the relative abundance of these resistant strains. This effect may not be attenuated by short treatment duration. These findings highlight the 'collateral damage' inflicted by fluoroquinolones and support recommendations to avoid their use in favour of agents with milder impact on commensal microbiota where possible [35]. Finally, we noted household clustering of CIP-RE and ESBL-PE, suggesting household transmission as a potential target for strategies to contain spread of AMR in the community.

Transparency declaration

All authors report no conflicts of interest relevant to this article.

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Contribution to authorship

All authors contributed to study design; NA, SC, AK, MG-C and AS contributed to recruitment and enrolment of participants; NA, SC, AK, MG-C, AS, JV, CL and SM-K contributed to acquisition of data; AS performed statistical analyses; HG, SM-K, MG-C and SH supervised the study; AS and SH drafted the manuscript and all authors reviewed and approved the final draft.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.cmi.2017.12.026.

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