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Clinical Microbiology and Infection (Article Type: 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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Keywords: fluoroquinolone, nitrofuran, urinary tract infection, Enterobacteriaceae, antimicrobial resistance, extended-spectrum beta-lactamase, microbiota, collateral damage

Running title: Outpatient antibiotics and commensal Enterobacteriaceae

Abstract word count: 250

Text word count: 3,049

Figures/tables: 5

ABSTRACT

Objectives: We quantified the impact of antibiotics prescribed in primary care for urinary tract infections (UTIs) on intestinal colonisation by ciprofloxacin-resistant (CIP-RE) and extended-spectrum beta-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 1–3 household contacts for each patient. Faecal samples were tested for CIP-RE, ESBLE-PE, nitrofurantoin-resistant Enterobacteriaceae (NIT-RE) and any Enterobactericeae 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 Enterobactericeae (S2) and subsequent 2-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 colonisation not associated with antibiotic exposure. Household exposure to CIP-RE or ESBL-PE was associated with increased individual risk of colonisation: 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 interrupting household circulation of resistant Enterobacteriaceae.

INTRODUCTION

Antimicrobial resistance (AMR) imposes an important health and economic burden and the threat of a post-antibiotic future 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 optimise use of existing antibiotics to minimise 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 colonisation 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 epidemiologic 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 hypothesised that any effects would increase with increasing treatment duration.

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 (**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 hospitalisation 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 1–3 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 (CRF) 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, USA), and these were kept on ice for a maximum of 24 hours before being collected in person and frozen at -80° Celsius 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 dichotomise

duration into ‘short’ and ‘long’ where relevant. The main outcomes were detectable intestinal colonisation by ESBL-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, we computed colonisation prevalence by dividing the number of colonised participants by number of participants (according to participant type [index/contact] and antibiotic exposure) at each time point.

Secondary outcomes were detectable intestinal colonisation 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, education level. High-risk countries were defined by location in the following geographic areas: Indian subcontinent, Southeast Asia, and Africa [10]. Colonisation of one or more household member with Enterobactericeae with the resistance phenotype of interest (dichotomous) was recorded as a time-varying covariate at each time point.

Microbiological methods

Microbiologic analyses 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 odd dilutions inoculated on the following media by spiral plating 100µl 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 0.12µg/ml and 2µg/ml ciprofloxacin (CHROMagar CIP). Samples from households of patients receiving nitrofurantoin and control households were additionally cultured on CHROMagar Orientation supplemented with

64µg/ml nitrofurantoin. Cultures were read and quantified after being incubated at 37°C for 18-24 hours and 24 hours, respectively. In case of no growth, these were re-incubated for 24 hours. Bacterial loads (CFU/ml of stool) were calculated separately for each colony colour.

The relative abundance of resistant *E. coli* in the gastrointestinal tract was determined by dividing the counts of resistant *E. coli* (sum of bacterial loads of pink colonies colour 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 colouration on the chromogenic agar underwent species identification by matrix-assisted laser desorption ionisation time-of-flight mass spectrometry. Antibiotic susceptibility and phenotypic ESBL confirmation for all strains was determined by the disc 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 alpha of 0.05, we would need approximately 40 patients in each group to reject this null hypothesis with an absolute colonisation 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 colonisation status was evaluated using mixed-effects generalised linear regression models. We used Poisson models for the binary colonisation outcome to compute prevalence ratios [5]. Antibiotic class (categorised 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 colonised by Enterobactericeae 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 clinical 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 CRF and questionnaire were available. We used multiple imputation for missing outcome values. We estimated the number-needed-to-harm (NNH) for antibiotic classes and resistant phenotypes. See supplementary material 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 **Figure 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 **Figure 2**. Detailed results from faecal samples are presented in **Table S2**.

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 treat with a fluoroquinolone in order to have one additional patient colonised by CIP-RE. Other factors associated with risk for colonisation by CIP-RE included household exposure to CIP-RE, age, and recent travel to highly endemic region (**Table 3** and **Figure S1**). When travel to high-risk region was further categorised into specific geographic regions, none were individually associated with increased prevalence of colonisation. There was an increased relative abundance of ciprofloxacin-resistant *E. coli* one month following the end of fluoroquinolone treatment (**Figure 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 treatment, respectively. The impact of treatment duration on emergence of ciprofloxacin resistance – as well as the presence of any Enterobacteriaceae – is presented graphically in **Figure 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 epidemiologic risk factors were identified for colonisation by ESBL-PE, nor was fluoroquinolone treatment significantly associated with an increase in the prevalence of ESBL-PE colonisation within 28 days: aPR at 1.36 (0.35−5.20) (**Table S3**). However, as with CIP-RE, we found evidence of household clustering of ESBL-PE, with exposure to one or more household contacts colonised with ESBL-PE being associated with a 3.38-fold (95% CI, 1.27-9.01) increase in risk of ESBL-PE colonisation.

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.

Colonisation with any Enterobacteriaceae

Compared with S1, the proportion of samples from which any Enterobacteriaceae were detected decreased significantly at S2 among UTI patients treated with fluoroquinolones (aPR, 0.55 [95% CI, 0.40−0.77]) (**Figure 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 **Table S4**. Amongst the 1,842 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 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].

We were unable to detect a significant benefit in reducing the duration of fluoroquinolone treatment. While 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 one 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 colonised 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 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 hospitalised 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 ecologic studies, we did not demonstrate an association between exposure to antibiotics and colonisation 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 colonisation 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 colonisation below the level of detection or acquisition from an external source.

We noted household clustering for colonisation 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]. While 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 randomisation, 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 characterisation of the ciprofloxacin resistance mechanisms or strain clonality. Important strengths of this study include the multinational participant recruitment which supports the generalisability 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 colonisation 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.

Acknowledgements

Local Investigators: Caroline Brossier, Cécile Delémont, Begoña Martinez de Tejada, Gesuele Renzi, Jacques Schrenzel, Sylvie Van Bylen, Niels Adriaenssens, Jonathan Vanbergen, Philip Koeck, Peter Leysen, Kim Vandercam, Jacques Kluijtmans, Agnieszka Borkiewicz, Frank Heyvaert, Nele Michels, Gerd Deswaef, Tine Beckx, Hadewijch Declerck, Katy Embrechts, Nina Verheyen, Tine Bauwens, Josefien Béghin, Liesbeth Verpooten, Katrien Bombeke, Tine Vandenabeele, Magdalena Muras, Joanna Świstak, Anna Wesołowska, Ewa Sterniczuk, Lidia Brzozowska, Krystyna Cichowska, Jolanta Szewczyk, Grażyna Krupińska, Honorata Błaszczyk, Urszula Stawińska, Maria Szyler, Marek Kasielski, Rafał Rydz, Agata Myszkowska, Maciek Godycki-Ćwirko, Lilianna Żebrowska

SATURN Bacterial Genetics and Functional Studies (WP1) group: Jascha Vervoort, MS, PhD, Christine Lammens, MS, Surbhi Malhotra-Kumar, MS, PhD, Herman Goossens, MD, PhD

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This work was supported by the European Commission under the 7th Framework Program [FP7-HEALTH-2009-SINGLE STAGE-SATURN, contract 241796]; and by the Methusalem grant of the Flemish government to Herman Goossens.

This study was presented in part at the 1st ASM Microbe conference, Boston, 20 June, 2016 (abstract MO-132).

Contribution to authorship

Study design: all authors; recruitment and enrolment of participants: NA, SC, AK, MG-C, AS; acquisition of data: NA, SC, AK, MG-C, AS, JV, CL, SM-K; statistical analyses: AS; study supervision: HG, SM-K, MG-C, SH; drafting of the manuscript: AS, SH; review and approval of final draft: all authors.

Transparency declaration

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

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468 **FIGURE LEGENDS**

469 **Figure 1.** Study flow diagram

470 **Figure 2.** Prevalence of any, ciprofloxacin-resistant, and ESBL-producing Enterobacteriaceae.

471 Abbreviations: CIP-RE, ciprofloxacin-resistant Enterobacteriaceae; ESBL-PE, extended-spectrum

472 beta-lactamase-producing Enterobacteriaceae

473 **TABLES**

474 **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 colonised 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 colonised by both ciprofloxacin resistant and susceptible Enterobacteriaceae AND treatment increases the load of resistant organisms by killing the competitive susceptible strains.
Fluoroquinolone	ESBL	Moderate	• Ciprofloxacin suppresses the endogenous flora AND individuals are exposed to ESBL-PE during or shortly after the treatment period. • ESBL-PE can be resistant to ciprofloxacin, and treatment shifts the balance of colonising organisms from mostly susceptible to mostly resistant.
Nitrofurans	Nitrofurantoin	Weak	• Resistance can be conferred by single mutations, however high fitness cost & low GI antibiotic levels reduce impact
Nitrofurans	ESBL	Negligible	• Nitrofurantoin resistance uncommonly conveyed by ESBL plasmids
Nitrofurans	Ciprofloxacin	Negligible	• No potential mechanisms likely to have significant role

475 **Table 2.** Characteristics of households and participants included in the analysis

Household-level characteristics	Household type	
	Non-exposed households (n=95)	Exposed households (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	62 (65)	101 (49)
<5 years & 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 (n=95)	Household contacts (n=150)	Index patients (n=205)	Household contacts (n=266)
Demographics				
Age, median (IQR)	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				
Hospitalisation	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 comorbidities				
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)

476 Result reported as *N* (%).

477 [a]Within 12-months prior to recruitment. NA, not applicable.

478 **Table 3.** Multivariable mixed-effects Poisson regression model for colonisation by ciprofloxacin-

479 resistant Enterobacteriaceae (CIP-RE)

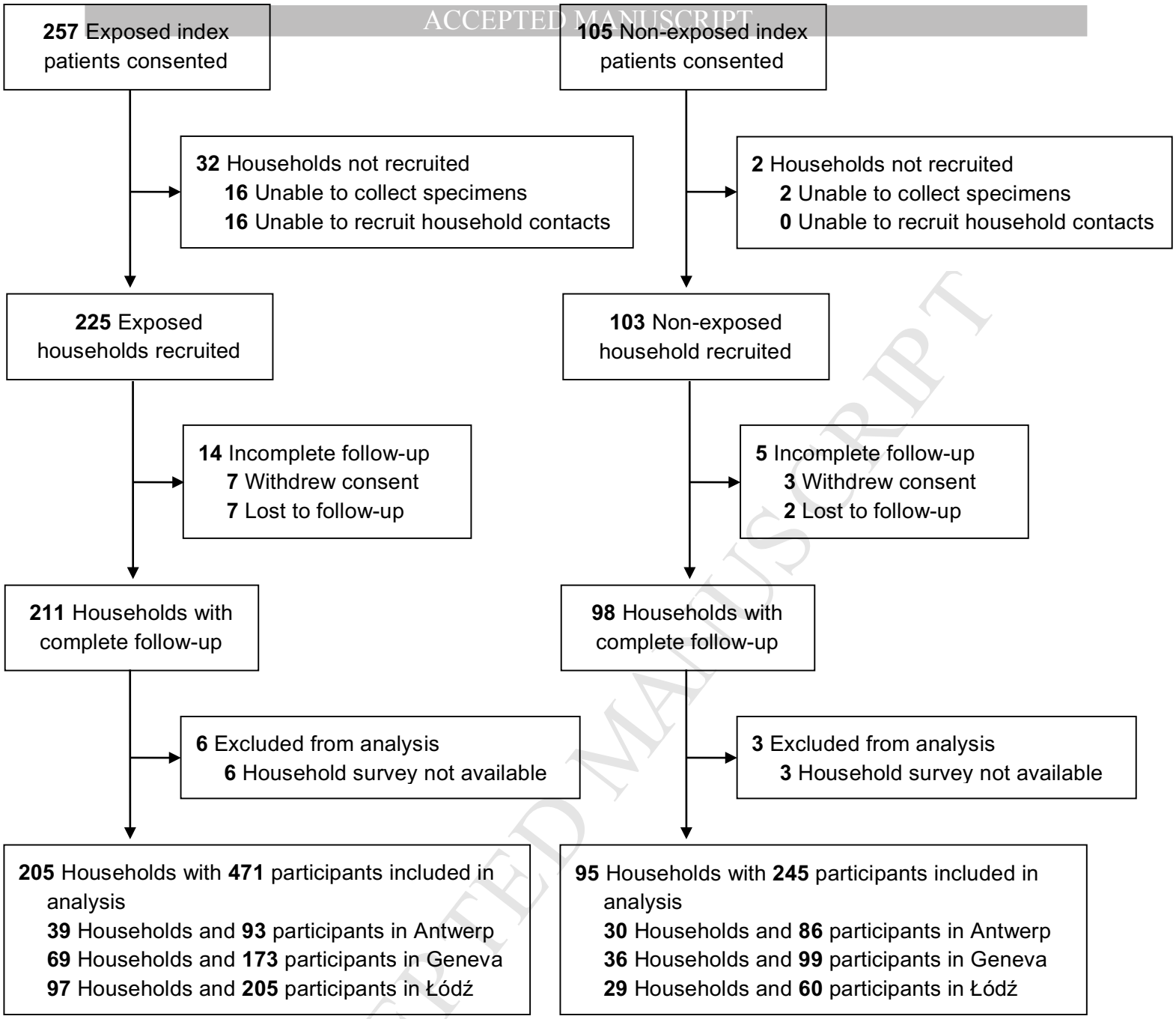
480

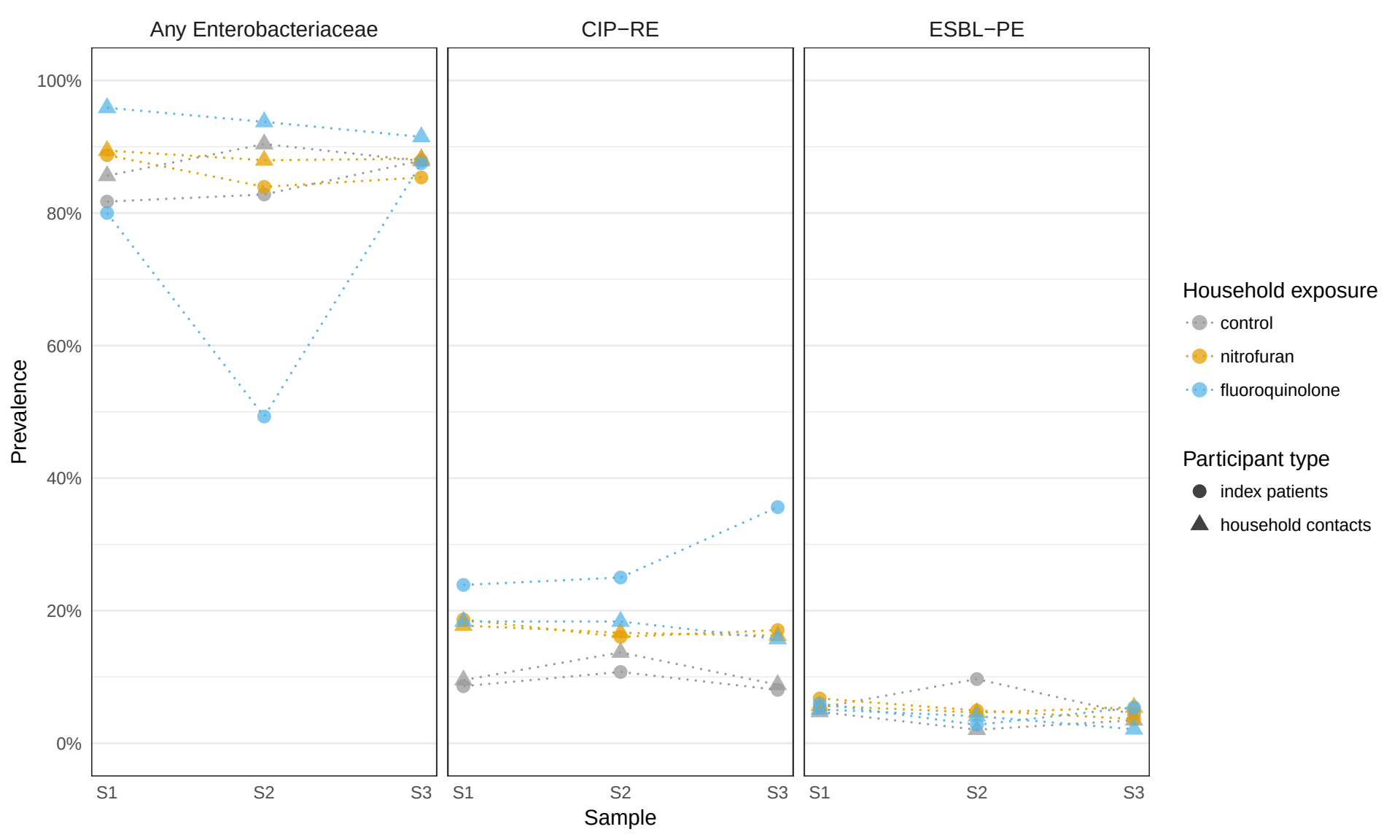
Exposure	No. (%) of participants (n = 716)	Prevalence ratio (95% CI)
Antibiotic exposure		
Immediately post-treatment		
nitrofuran	86 (12)	0.91 (0.47−1.76)
fluoroquinolone	76 (11)	1.46 (0.83−2.59)
other antibiotic	43 (6)	1.54 (0.69−3.44)
28-days post-treatment		
nitrofuran	86 (12)	0.98 (0.53−1.81)
fluoroquinolone	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		
≥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 CIP-RE	Time-varying	1.80 (1.28−2.54)

481

482 Note: Multiple imputation used to account for 115 of 2148 (5%) observations with missing CIP-RE

483 colonisation status. All other variables in the model were complete for all cases.




Any Enterobacteriaceae
CIP−RE
ESBL−PE
100%
80%
60%
40%
20%
0%
Prevalence
S1
S2
S3
Sample
Household exposure
control
nitrofuran
fluoroquinolone
Participant type
index patients
household contacts