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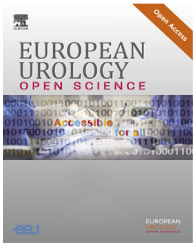

European Association of Urology

Infections

Multidrug-resistant Uro-associated *Escherichia coli* Populations and Recurrent Urinary Tract Infections in Patients Performing Clean Intermittent Self-catheterisation

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Abstract

Background: The AnTIC trial linked continuous low-dose antibiotic prophylaxis treatments to a lower incidence of symptomatic urinary tract infections (UTIs) among individuals performing clean intermittent self-catheterisation (CISC).
Objective: To explore potential mechanisms underlying the protective effects of low-dose antibiotic prophylaxis treatments, blood and urine samples and uro-associated *Escherichia coli* isolates from AnTIC participants were analysed.
Design, setting, and participants: Blood samples (n = 204) were analysed for *TLR* gene polymorphisms associated with UTI susceptibility and multiple urine samples (n = 558) were analysed for host urogenital responses. *E.coli* sequence data for 45 temporal isolates recovered from the urine samples of 16 trial participants in the prophylaxis (n = 9) and no-prophylaxis (n = 7) study arms, and characterised by multidrug resistance (MDR), were used to classify individual strains.
Outcome measurements and statistical analysis: *TLR* polymorphism data were analysed using Poisson regression. Concentrations of urine host defence markers were analysed using linear mixed-effects models, which accounted for repeated urine samples.
Results and limitations: Urine samples from CISC users, irrespective of antibiotic treatment regimens, were associated with robust urothelial innate responses. No links were identified between *TLR* genotype and CISC user susceptibility to recurrent UTIs. Microbiological study data were limited to the predominant MDR *E. coli* population; participants prescribed low-dose prophylactic antibiotics were

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predominantly colonised by a single uro-associated *E. coli* strain, while participants given acute antibiotic treatments were each colonised by more than one *E. coli* strain.

Conclusions: Antibiotic treatments did not impact urogenital responses to infection in CISC users. Host genetics in terms of *TLR* polymorphisms played no role in determining CISC user susceptibility to or protection from recurrent UTIs. Prophylactic antibiotic treatments associated with MDR *E. coli* were associated with colonisation by stable uro-associated *E. coli* genotypes.

Patient summary: Our findings show that the natural urogenital defences of clean intermittent self-catheterisation (CISC) users were not impacted by antibiotic treatments. For some CISC users, prophylaxis with low-dose antibiotics selected for a stable, predominantly, *Escherichia coli* rich uromicrobiota.

1. Introduction

Clean intermittent self-catheterisation (CISC) is an important management option for individuals, including those in a home setting, who are unable to empty their bladder naturally and involves inserting a sterile urethral catheter using an aseptic technique. Clinically, CISC is used for bladder emptying in patients with a range of pathologies, including spinal cord injury, multiple sclerosis, non-neurogenic bladder dysfunction, and incomplete bladder emptying due to infravesical obstruction [1]. Although CISC allows for regular complete bladder emptying, recurrent urinary tract infections (rUTIs) are common [2]. Despite strategies to reduce UTI risk, including single-use hydrophilic catheters and antiseptic washes, recurrent infections remain a key clinical and health economics problem. One approach to reduce UTI incidence involves prescribing low-dose prophylactic antibiotics [3], with data from the recent randomised open-label AnTIC trial reporting that prophylaxis reduced UTI frequency by 48% [4].

The anatomy of the urogenital tract means that the bladder is particularly vulnerable to microbial contamination, particularly in females, for whom colonisation of the periurethral mucosa with gut microbes is common, and catheter use further increases the risk of bacterial inoculation [5,6]. Constitutive and induced innate defence systems play a role in protecting the urinary tract and reducing the risk of infection [7,8]. Induced mechanisms include the urothelial synthesis and rapid release of host innate defence molecules, including cytokines and antimicrobial agents such as IL-8 (CXCL8), neutrophil gelatinase-associated lipocalin (NGAL), human β-defensin-2 (BD2) and secretory leukocyte peptidase inhibitor (SLPI), that work collectively to restrict and clear potential infections. Underpinning these defence mechanisms are collections of microbial sensors, including the Toll-like receptors (TLRs), located on host urothelial and antigen-presenting cells [9].

UTIs can be described as uncomplicated or complicated; the latter are usually associated with either structural or functional urinary tract abnormalities [10]. UTIs in CISC users are, by definition, complicated. Uropathogenic *Escherichia coli* is the most frequent pathogen [11] and current diagnostics involve documenting symptoms and obtaining a urine specimen for microbiological culture. CISC users often exhibit positive urine cultures, which in the absence of symptoms are classified as asymptomatic bacteriuria (ASB) and do not support antibiotic treatments [12]. When UTI symptoms are associated with significant positive urine cultures, acute antibiotic treatment is usually recommended. However, the frequent use of antibiotics to treat those with persistent or recurrent episodes can facilitate the emergence of antibiotic resistance among uropathogens, including uropathogenic *E. coli* [13].

Population study data suggest that polymorphisms in the genes encoding TLRs 1, 2, 4, and 5 are involved in the susceptibility of individuals to uncomplicated recurrent (r) UTIs. The genotypes TLR1_G1805T (S602I) and TLR4_A896G (D299G) are associated with greater protection from rUTIs, while TLR5_C1174T (R392STOP) is associated with greater vulnerability and TLR2_G2285A (R753Q) is linked to a higher risk of bladder colonisation with Gram-positive organisms [14]. In the case of TLR5_C1174T, UTI susceptibility is associated with lower urothelial innate responses, including IL-8 and BD2 expression [15]. To date, the roles of host genetics in the susceptibility of catheterised patients to complicated UTIs have largely been ignored, presumably because of assumptions that problems related to the use of indwelling catheters, including biofilms, over-ride any advantages or disadvantages associated with host genetics. CISC involves the use of sterile catheters that are present in the patient's bladder only transiently, but despite the lack of an indwelling catheter these patients still present as a high-risk patient cohort susceptible to recurrent symptomatic infections [2]. AnTIC trial data indicated that this figure can be reduced by using continuous low-dose antibiotic prophylaxis treatments [4], but the actual mechanisms that reduce UTI incidence remain unknown. Suggestions include the possibility that low-dose antibiotics preferentially target uropathogens and/or induce changes in the patient's urogenital defences that promote tolerance of non-pathogenic colonising bacteria.

The aim of this study was to explore potential factors underpinning the effectiveness of continuous low-dose antibiotic treatments in protecting CISC patients from rUTIs. To this end, blood, urine, and uro-associated *E. coli* samples banked from participants of the randomised open-label

AnTIC trial were analysed to obtain a retrospective picture of their *TLR* genetics, urothelial host responses, and microbial colonisation/infection profiles. In addition, to provide a further understanding of the lower number of UTIs associated with a prophylaxis strategy, the microbial colonisation profiles of participants, specifically characterised by multidrug-resistant (MDR) uro-associated *E. coli* isolates, were also investigated.

2. Patients and methods

2.1. Study design

The study protocol was approved by the North of Scotland Research Ethics Service (reference REC-19/NS/0024; protocol number 09020; IRAS project ID 243903) and used blood and urine samples and clinical data from CISC patients who participated in the AnTIC trial. In accordance with the original trial protocol, blood donation was optional. While all uro-associated MDR isolates were recorded clinically, only *E. coli* isolates recovered from participants' urine samples were banked [16].

2.2. TLR single-nucleotide polymorphism analyses

Genomic DNA was extracted from whole blood using the Reliaprep Blood gDNA Miniprep System (Promega, Madison, WI, USA). A polymerase chain reaction (PCR) fragment spanning the targeted single-nucleotide polymorphism (SNP) region was generated using PCR primers and cycling followed by melt curve analysis (Supplementary Table 1). SNPs were analysed using LightCycler 480 software and confirmed by sequencing a random selection of samples (Eurofins, Hamburg, Germany).

2.3. Urine analyses

Urine samples stored previously at −80°C were analysed via enzyme-linked immunosorbent assay for host defence agents (Supplementary material).

2.4. Microbiological analyses and genotyping of E. coli

All urine specimens associated with asymptomatic and symptomatic infections were analysed microbiologically by the central trial laboratory [4]. Bacterial isolates recovered from urine during UTIs and asymptomatic periods were assessed for antimicrobial resistance in accordance with the standards set by Public Health England and the European Committee on Antimicrobial Susceptibility Testing [4]. Only the single predominant *E. coli* isolate was banked and stored. Genomic DNA samples extracted from *E. coli* isolates were sequenced on an Illumina NextSeq500 platform at the Genomic Core Facility, Newcastle University. Assembled genomes and raw data can be accessed using the European Nucleotide Archive (https://www.ebi.ac.uk/ena/browser/home, accession number PRJEB39670).

Core genome multilocus sequence typing (MLST) of *E. coli* isolates was performed using chewBBACA [17] and consisted of 404 loci. The Achtman MLST scheme was used to type the sequenced AnTIC *E. coli* isolates [18]. PCR-based typing of strains was performed as previously described [19].

2.5. Statistical analyses

2.5.1. TLR SNPs

For each SNP, Poisson regression was performed with the number of UTIs as the outcome variable and genotype, coded according to the relevant model of genetic association, as a covariate. Models were adjusted for arm (prophylaxis/no prophylaxis) and coefficients from the regression model are reported along with 95% confidence intervals and *p* values.

2.5.2. Urine samples

Analyses of urine measurements were performed using the lme package in R v3.6.0. Linear mixed-effects models were fitted for each host agent with a random effect included to account for repeated samples among participants. Each model included the following fixed effects: infection status (level of bacterial growth, dichotomised as $<10^4$ or $\geq 10^4$ colony-forming units [CFU]/ml urine), sex, trial arm (prophylaxis/no prophylaxis), and genotype (for each of *TLR1*, *TLR2*, *TLR4*, and *TLR5*). Owing to the skewed distributions for the urine measurements, models were fitted to log-transformed data.

3. Results

3.1. Analyses of urinary bacteria

Semi-quantitative urine cultures were carried out for 2075 urine samples from AnTIC participants, of which 1098 were from the no-prophylaxis cohort and 977 were from the prophylaxis cohort. Laboratory records indicated that 51% of the no-prophylaxis urine samples were culture-negative or had no significant bacterial growth ($<10^4$ CFU/ml urine), compared to 65% of the prophylaxis samples. Positive urine samples ($\geq 10^4$CFU/ml urine) from both study arms contained a mix of species, with *E. coli* the most common bacterium identified (Fig. 1A). While the bacterial profiles of individual participants varied, *E. coli* was less prevalent among participants receiving prophylaxis treatment (48% of isolates) than among those treated with no prophylaxis (63% of isolates). Treatment data (Fig. 1A) also suggested that prophylaxis involving β-lactam antibiotics and nitrofurantoin selected against *E. coli* infections.

The AnTIC trial banked approximately 500 urine-associated *E. coli* single-colony isolates, 25% of which were randomly chosen and genotyped. The results revealed a mix of sequence types, with ST131 being the most frequently isolated (Supplementary Table 2). Most isolates aligned phylogenetically to clade B2, although clade A, B1, D, E, and F members were also identified.

3.2. Antibiotic therapy and emergence of MDR E. coli

Continuous low-dose antibiotic prophylaxis was associated with greater antibiotic resistance and the emergence of MDR uro-associated bacteria. TTo explore this further, a sub-group of 50 participants (13.8% of the patient cohort) and from whom isolates were defined as acquiring MDR [20] were selected for further study (Fig. 2A). These participants were from both the prophylaxis (n = 27) and no-prophylaxis (n = 23) study arms and were identified via retrospective analyses of urine microbiology reports (Fig. 2B). Sets of temporal *E. coli* isolates (45 samples in total) were available for 16 participants (Fig. 2B, green dots) and these were used to explore urogenital colonisation patterns of the predominant *E. coli* microflora. Nine of these 16 participants received prophylactic antibiotic treatments including nitrofurantoin (1 patient), trimethoprim (7 patients), and cefalexin (1 patient), while the seven participants in the no-prophylaxis cohort were treated intermit-

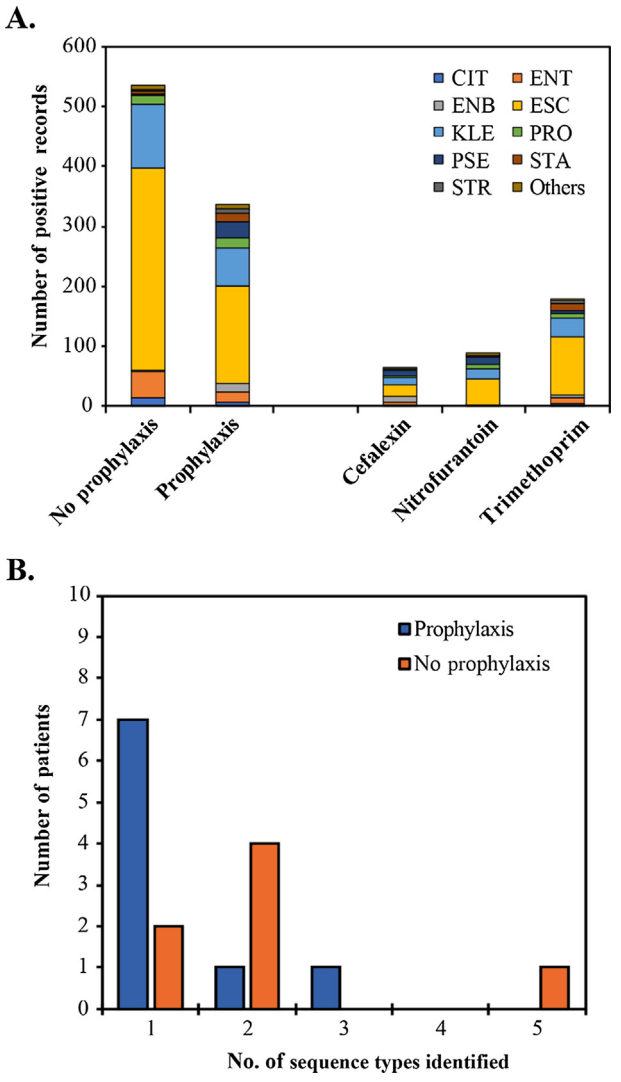

Fig. 1 – Bacterial species isolated from the urogenital tracts of AnTIC participants using clean intermittent self-catheterisation. (A) AnTIC participants submitted urine samples at baseline and 3, 6, 9, and 12 months, and if they sought antibiotic treatment for a suspected urinary tract infection. Microbiological data were extracted from the clinical records of 361 AnTIC participants [4] and stratified according to trial arm (no prophylaxis vs prophylaxis antibiotic treatment), with the prophylaxis group information further stratified to show the impact of cefalexin, nitrofurantoin, and trimethoprim treatments. CIT = *Citrobacter* sp.; ENB = *Enterobacter* sp.; KLE = *Klebsiella* sp.; PSE = *Pseudomonas* sp.; STR = *Streptococcus* sp.; ENT = *Enterococcus* sp.; ESC = *Escherichia coli*; PRO = *Proteus* sp.; STA = *Staphylococcus* sp. (B). Number of sequence types identified in the prophylaxis (blue) and no-prophylaxis (orange) patients carrying multidrug-resistant *E. coli*.

tently for acute UTIs with a range of antibiotics. Core genome MLST analyses showed that over the trial period, participants suffering *E. coli* infections in the no-prophylaxis study arm were generally colonised by more than one predominant *E. coli* strain; for example, patient 2433 (no prophylaxis) was infected chronologically by strains ST569, ST131, ST1629, ST131, and ST59 (Fig. 1B and Fig. 3). By contrast, participants receiving prophylaxis harboured a single predominant *E. coli* strain, although these strains differed between individual patients. For example, participants 2470 and 1260 were predominantly colonised by strains ST95 and ST90, respectively.

3.3. Host responses in CISC patients

In total, 558 urine samples from 144 trial participants were available for analyses, although variable volumes meant that not all samples could be analysed for multiple markers. The results (Fig. 4) suggest that urine concentrations of IL-8 (β =1.233; $p < 0.001$), NGAL (β = 1.717; $p < 0.001$), and BD2 (β = 0.258; p = 0.022) were significantly higher in samples characterised by bacterial infection ($\geq 10^4$ CFU/ml) than in samples with no infection (negative culture), but SLPI was significantly lower (β = −0.461; $p < 0.001$). Statistical analysis revealed no evidence that other variables in the model including sex, age, and antibiotic treatment regimen were significantly associated with alterations in the urinary innate defences except for age (NGAL: β = 0.023; p = 0.014) and sex (male; BD2: β = −0.341; p = 0.044; Supplementary Table 3).

3.4. TLR genotypes, host innate responses, and UTI incidence

Blood samples were collated from 204/361 (56.5%) of the AnTIC participants. Those genotyped for *TLR* polymorphisms comprised 104 of the 181 participants (57.5%) in the prophylaxis arm and 100 of the 180 participants (55.6%) in the no-prophylaxis arm. *TLR* allele frequencies (Table 1) were comparable to those reported for a European population [21]. The incidence rates of symptomatic antibiotic-treated UTIs (per person per year) were comparable across the different genotypes for *TLR1*, *TLR2*, *TLR4*, and *TLR5* SNPs. Statistical analysis revealed no significant links between *TLR* genotype and participants' host responses (Supplementary Fig. 1 and Supplementary Table 3).

4. Discussion

CISC provides patients with the independence to periodically fully empty their bladders to mimic normal bladder function, but users often suffer from rUTIs that are debilitating. To try and reduce the incidence of infections, prophylactic antibiotic treatments have been trialled [3] with success [4], although the concomitant increase in antimicrobial resistance remains a concern. The mechanism by which prophylaxis benefits patients has not been explored, although suggestions from the AnTIC trial were that prophylaxis is linked to either the selection and/or host tolerance of less pathogenic bacterial strains.

Despite the urogenital microbial diversity shown by AnTIC participants, the trial protocol meant that only *E. coli* isolates were curated. *E. coli* genotyping identified multiple lineages that clustered into six phylogenetic groups (A, B1, B2, D, E, and F) that support previous bacterial characterisation studies [10], and MDR uro-associated *E. coli* were identified from each lineage (Fig. 3 and Supplementary Table 2). Focussing specifically on participants from whom MDR isolates were obtained, results indicate that those receiving prophylactic antibiotics generally harboured the same *E. coli* MDR strain, while those receiving intermittent antibiotics in response to acute infections were, over time, colonised by different *E. coli* genotypes (Fig. 1B). Although counterintuitive, harbouring one MDR *E. coli* strain appeared to be beneficial, as it was associated

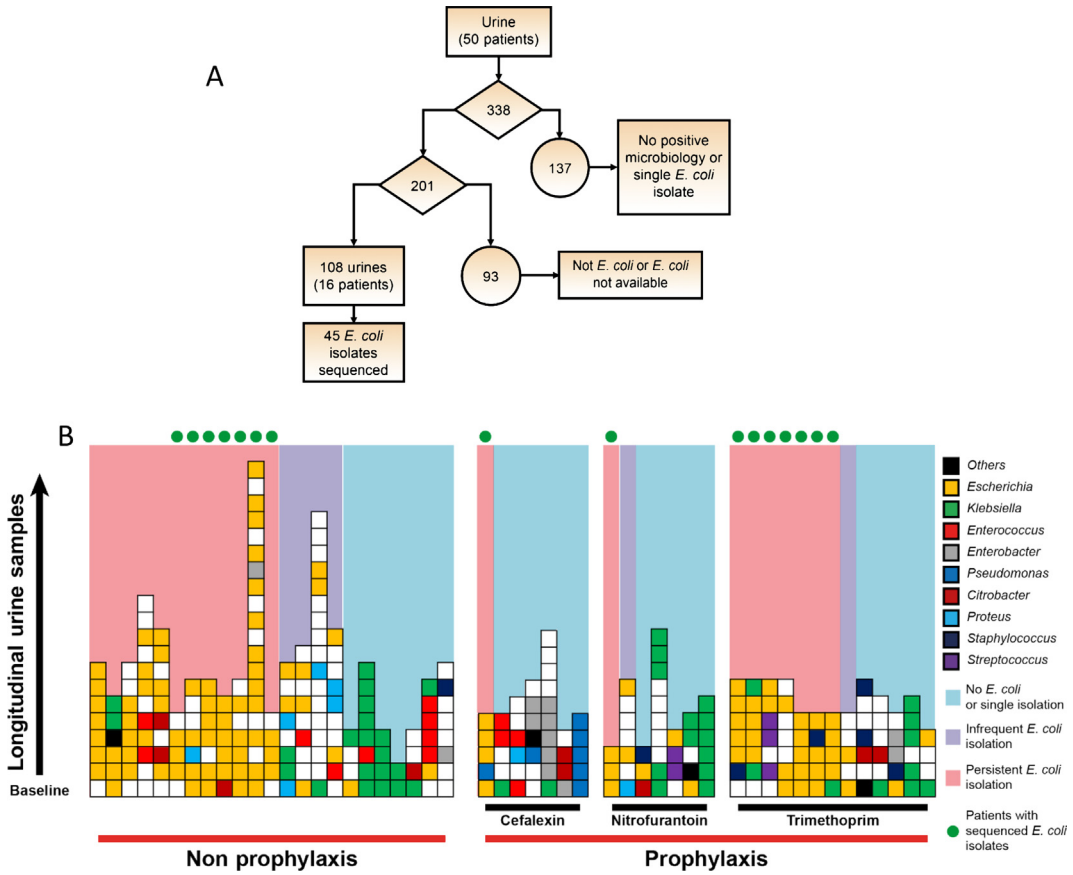

Fig. 2 – Selection of multidrug-resistant (MDR) *Escherichia coli* isolates for sequencing. (A) Flow chart showing selection of MDR *E. coli* isolates for DNA sequencing. Microbiological records for AnTIC trial participants were screened for *E. coli* antibiotic sensitivity profiles. Fifty participants who showed MDR^{+} *E. coli* after 0–3 months were selected for further study. There were data available for 338 urine samples from these participants, of which 230 were excluded as they were negative for microbiology, the isolates identified were not *E. coli,* the *E. coli* isolates identified had not been banked, and/or only one *E. coli* sample was available, preventing temporal analyses. This resulted in 108 urine samples from 16 patients and 45 *E. coli* isolates; these isolates were subjected to whole-genome sequencing. (B) Urine microbial profiles for the participants. Each column represents a single patient (50 patients in total) with trial participants grouped according to their respective treatment. Each square within a column represents a urine sample and microbes, if any, identified. White boxes indicate no bacteria detected. Urine samples are arranged in chronological order starting at baseline. The background colour of each column indicates whether patients carried no *E. coli* or a single isolate (blue), were infrequently colonised with *E. coli* (purple), or were persistently colonised with *E. coli* (red). Columns identified by green dots (16 in total) represent patients with sequenced *E. coli* isolates.

with a lower number of symptomatic infections (Fig. 3), in agreement with the AnTIC trial outcome [4]. These new observations, although limited to a small subset of participants carrying MDR *E. coli* (n = 9), suggest that continuous prophylaxis antibiotic treatment can stabilise a patient's *E. coli* uromicrobiota, creating a defensive barrier that protects against other uropathogens. While not directly comparable to the deliberate inoculation of UTI-prone individuals with *E. coli* 83972 to protect against symptomatic infections with more virulent strains [22], the outcome of stable microbial colonisation and a reduction in acute UTI episodes appears very similar. In contrast, uro-associated *E. coli* MDR isolates recovered from the no-prophylaxis cohort (n = 7) were genetically different, suggesting that these patients were being infected or colonised by different *E. coli* strains, possibly as a result of their intermittent antibiotic treatment regimens. Therefore, discrete acute antibiotic treatment courses in these CISC patients appeared to select for an unstable *E.coli* uromicrobiota resulting in the lack of a protective microbial barrier and greater susceptibility to UTIs, with the latter observation again reflective of the AnTIC trial outcome.. Although the study was limited to *E. coli* and small subsets of preselected CISC users (ie, those carrying MDR *E. coli*), the colonisation patterns observed help to explain the reduction in UTIs in a subset of participants receiving antibiotic prophylaxis. However, future studies monitoring the uromicrobiota diversity of such patients for periods longer than 12–18 months are needed to consolidate these observations.

AnTIC also provided a unique platform to examine the impact of antibiotic treatments on urogenital innate responses to potential infections among CISC patients. Urine analyses for host defence agents showed that the host urothelial responses were robust, regardless of treatment regimen, suggesting that continuous low-dose antibiotic prophylaxis did not have any dampening effects. It has been reported that greater susceptibility to and/or protection from uncomplicated rUTIs is linked to *TLR* SNPs [14,23] and data from 204 CISC participants allowed us to examine whether such relationships also exist in those suffering from complicated UTIs. Stratification for *TLR* genetics and infection status did not support any trends, and no signifi-

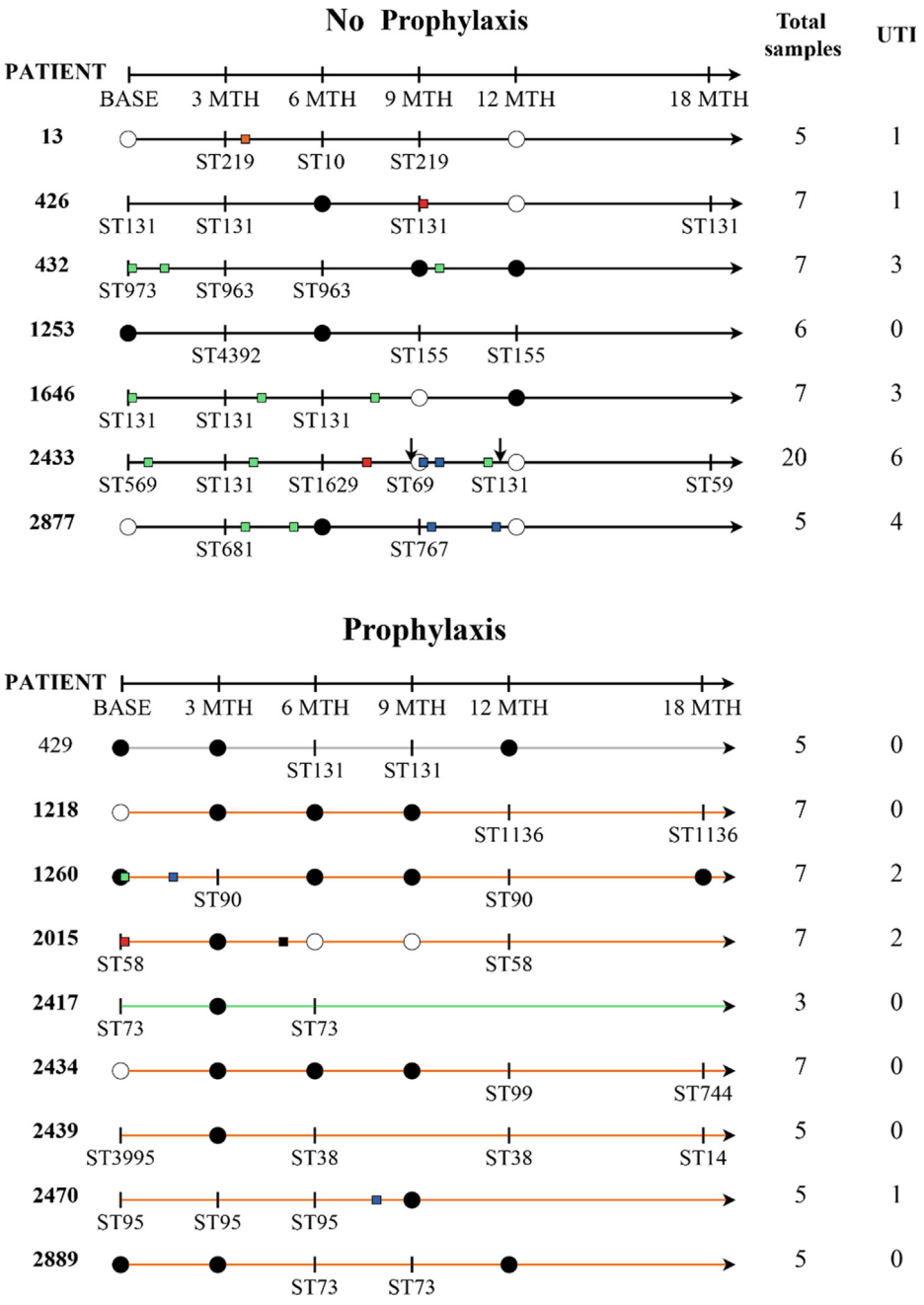

Fig. 3 – Timeline analysis of antibiotic therapy and emergence of multidrug-resistant *Escherichia coli*. Antibiotic therapies, colonisation and infection timelines of 17 patients in no-prophylaxis and prophylaxis treatment arms. Urine sampling is defined by solid vertical lines at baseline (BASE) and 3, 6, 9, 12, and 18 months (MTH). Open circles denote no microbiological record in clinical database. Closed circles denote positive microbiological results recorded as not *E. coli* or *E. coli* not available for analysis. Squares denote the time points for antibiotic treatment for symptomatic episodes, with colours representing the antibiotic prescribed (black = cephalosporin; red = co-amoxiclav or amoxicillin; light blue = ciprofloxacin; green = nitrofurantoin; orange = trimethoprim; white = no infection). Two acute infections (participant 2433) are indicated by arrows. For the horizontal timelines for the prophylaxis group, grey denotes cefalexin, orange denotes trimethoprim, and green denotes nitrofurantoin. All *E. coli* isolates sequenced are identified by their sequence type (ST) using the Achtman multilocus sequence typing scheme. Numbers denote the total number of samples available for each participant and the number of symptomatic infections registered clinically and requiring antibiotics.

cant associations between *TLR* polymorphisms and susceptibility to UTIs were detected. This observation suggests that any advantages or disadvantages associated with host *TLR* genetics were abolished by either structural and/or functional urinary tract abnormalities and/or the introduction of a catheter, albeit for short period of time, into the urinary tract. One suggestion is that the catheters function as conduits that allow direct access to the bladder for bacteria colonising the periurethral regions [24,25], which immunologically allows uropathogens such as *E. coli* to circumvent the urothelial TLR defences and facilitates bladder infection.

It has also been proposed that uropathogenic *E. coli* reside in specific bladder niches from which they can seed reinfections. Studies using animal models have reported the presence of such intracellular bacterial communities

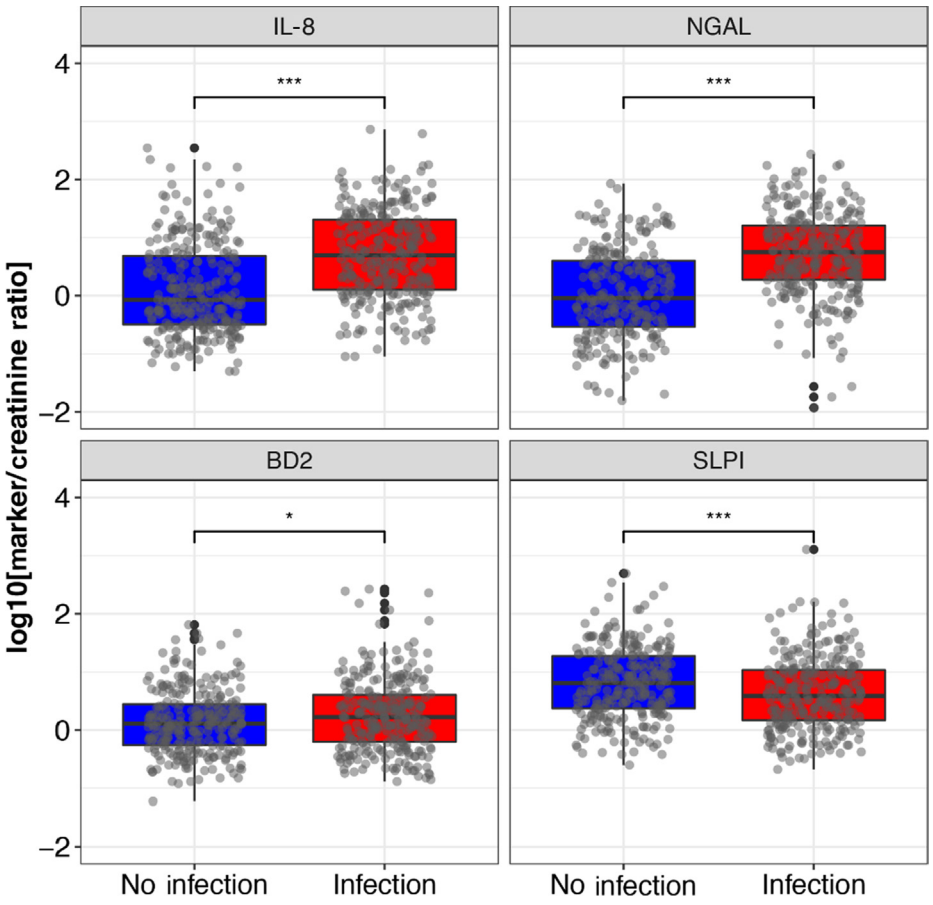

Fig. 4 – Urogenital responses in trial participants using clean intermittent self-catheterisation (CISC). IL-8, NGAL, HBD2 and SLPI concentrations in urine samples from CISC users (log scale; bars denote the median values). * $p < 0.05$; *** $p < 0.001$. Urine samples: no infection (<10^4 CFU/ml urine), $n = 274$; infection (≥10^4 CFU/ml urine), $n = 284$.

Table 1 – Incidence rates and incidence rate ratios of symptomatic antibiotic-treated UTIs compared between TLR genotypes (n = 204)[a]

SNP	Genotype	Cases, *n* (%)	satUTI incidence rate, per person-year (95% CI)	Incidence rate ratio [b] (95% CI)
TLR1 G1805T	GG	141 (69)	2.0 (1.8–2.3)	0.94 (0.69–1.3)
	GT/TT	63 (31)	1.9 (1.6–2.2)	
TLR2 G2258A	AG	193 (95)	2.0 (1.8–2.2)	1.1 (0.78–1.7)
	GG	11 (5)	2.2 (1.4–3.7)	
TLR4 A896G	AA	175 (86)	2.0 (1.8–2.2)	0.98 (0.66–1.5)
	AG/GG	29 (14)	2.1 (1.4–3.1)	
TLR5 C1174T	CC	179 (88)	2.0 (1.8–2.2)	1.0 (0.66–1.5)
	CT	25 (12)	2.1 (1.4–3.1)	

SNP = single-nucleotide polymorphism; UTI = urinary tract infection; satUTI = symptomatic antibiotic-treated UTI; CI = confidence interval.
[a] All AnTIC population data were in Hardy-Weinberg equilibrium, as determined via a χ^2 test (*TLR1*, $\chi^2 = 0.001$; *TLR2*, $\chi^2 = 0.160$; *TLR4*, $\chi^2 = 0.270$; *TLR5*, $\chi^2 = 0.864$).
[b] Adjusted for arm (prophylaxis vs no prophylaxis).

(IBCs) within urothelial cells [26]. There is some evidence to support these IBC structures in the human bladder of patients with uncomplicated UTIs [27], although this has not been corroborated in CISC patients. If IBCs do exist, then physical tissue damage linked to catheter use could promote the release of these bacteria to facilitate UTI development. However, the strain switching observed in the no-prophylaxis cohort does not lend support to this infection model. More recently, *E. coli* L-forms have been identified in urine samples from older patients suffering from uncomplicated rUTIs [28], suggesting a novel *E. coli* reinfection mechanism that potentially warrants further investigation.

5. Conclusions

In conclusion, these data showed that antibiotic treatments did not impact urogenital responses to infection in AnTIC participants. In addition, host genetics, linked to TLR polymorphisms, played no role in determining either CISC user susceptibility to or protection from recurrent UTIs. How-

ever, low-dose prophylactic antibiotic treatments associated with a predominant MDR *E. coli* population were associated with stable colonisation of the urogenital tract among study participants.

Author contributions: Judith Hall had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
Study concept and design: Hall, Pickard, Aldridge.
Acquisition of data: Chadwick, Mowbray, Tan, Vallée, Fisher, Walton, Brennand.
Analysis and interpretation of data: Aldridge and Tan, Chadwick, Brennand, Mowbray, Fisher, Harding, Hall, Walton.
Drafting of the manuscript: Hall, Aldridge, Harding, Walton, Mowbray.
Critical revision of the manuscript for important intellectual content: Walton, Harding, Chadwick.
Statistical analysis: Chadwick, Fisher, Mowbray, Aldridge, Tan.
Obtaining funding: Hall, Pickard.
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Supervision: Hall, Aldridge, Harding, Pickard, Walton.
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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.euros.2021.12.015.

References

[1] Prieto J, Murphy CL, Moore KN, Fader M. Intermittent catheterisation for long-term bladder management. Cochrane Database Syst Rev 2014;2014:CD006008.
[2] Wyndaele JJ. Complications of intermittent catheterization: their prevention and treatment. Spinal Cord 2002;40:536–41.
[3] Niel-Weise BS, van den Broek PJ, da Silva EM, Silva LA. Urinary catheter policies for long-term bladder drainage. Cochrane Database Syst Rev 2012;2012:CD004201.
[4] Fisher H, Oluboyede Y, Chadwick T, et al. Continuous low-dose antibiotic prophylaxis for adults with repeated urinary tract infections (AnTIC): a randomised, open-label trial. Lancet Infect Dis 2018;18:957–68.
[5] Hooton TM, Scholes D, Hughes JP, et al. A prospective study of risk factors for symptomatic urinary tract infection in young women. N Engl J Med 1996;335:468–74.
[6] Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med 2002;113(Suppl 1A):5S–13S.
[7] Abraham SN, Miao Y. The nature of immune responses to urinary tract infections. Nat Rev Immunol 2015;15:655–63.
[8] Ali AS, Townes CL, Hall J, Pickard RS. Maintaining a sterile urinary tract: the role of antimicrobial peptides. J Urol 2009;182:21–8.
[9] Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol 2005;17:1–14.
[10] Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol 2015;13:269–84.
[11] Lane MC, Alteri CJ, Smith SN, Mobley HL. Expression of flagella is coincident with uropathogenic Escherichia coli ascension to the upper urinary tract. Proc Natl Acad Sci U S A 2007;104:16669–74.
[12] Colgan R, Nicolle LE, McGlone A, Hooton TM. Asymptomatic bacteriuria in adults. Am Fam Phys 2006;74:985–90.
[13] Cai T, Verze P, Brugnolli A, et al. Adherence to European Association of Urology guidelines on prophylactic antibiotics: an important step in antimicrobial stewardship. Eur Urol 2016;69:276–83.
[14] Hawn TR, Scholes D, Li SS, et al. Toll-like receptor polymorphisms and susceptibility to urinary tract infections in adult women. PLoS One 2009;4:e5990.
[15] Ali ASM, Mowbray C, Lanz M, et al. Targeting deficiencies in the TLR5 mediated vaginal response to treat female recurrent urinary tract infection. Sci Rep 2017;7:11039.
[16] Brennand C, von Wilamowitz-Moellendorff A, Dunn S, et al. Antibiotic treatment for intermittent bladder catheterisation with once daily prophylaxis (the AnTIC study): Study protocol for a randomised controlled trial. Trials 2016;17:276.
[17] Silva M, Machado MP, Silva DN, et al. chewBBACA: a complete suite for gene-by-gene schema creation and strain identification. Microb Genom 2018;4:e000166.
[18] Wirth T, Falush D, Lan R, et al. Sex and virulence in Escherichia coli: an evolutionary perspective. Mol Microbiol 2006;60:1136–51.
[19] Drage LKL, Robson W, Mowbray C, et al. Elevated urine IL-10 concentrations associate with Escherichia coli persistence in older patients susceptible to recurrent urinary tract infections. Immun Ageing 2019;16:16.
[20] Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect 2012;18:268–81.
[21] Sherry ST, Ward MH, Kholodov M, et al. dbSNP: the NCBI database of genetic variation. Nucleic Acids Res 2001;29:308–11.
[22] Wullt B, Svanborg C. Deliberate establishment of asymptomatic bacteriuria—a novel strategy to prevent recurrent UTI. Pathogens 2016;5:52.
[23] Tabel Y, Berdeli A, Mir S. Association of TLR2 gene Arg753Gln polymorphism with urinary tract infection in children. Int J Immunogenet 2007;34:399–405.
[24] Cass AS, Ireland GW. Antibacterial perineal washing for prevention of recurrent urinary tract infections. Urology 1985;25:492–4.
[25] Chen SL, Wu M, Henderson JP, et al. Genomic diversity and fitness of *E. coli* strains recovered from the intestinal and urinary tracts of women with recurrent urinary tract infection. Sci Transl Med 2013;5:184ra60.
[26] Justice SS, Hunstad DA, Seed PC, Hultgren SJ. Filamentation by Escherichia coli subverts innate defenses during urinary tract infection. Proc Natl Acad Sci U S A 2006;103:19884–9.

[27] De Nisco NJ, Neugent M, Mull J, et al. Direct detection of tissue-resident bacteria and chronic inflammation in the bladder wall of postmenopausal women with recurrent urinary tract infection. J Mol Biol 2019;431:4368–79.

[28] Mickiewicz KM, Kawai Y, Drage L, et al. Possible role of L-form switching in recurrent urinary tract infection. Nat Commun 2019;10:4379.