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Rifamycin SV-MMX® for treatment of travellers' diarrhea: equally effective as ciprofloxacin and not associated with the acquisition of multi-drug resistant bacteria

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Abstract: Background The novel oral antibiotic formulation Rifamycin SV-MMX®, with a targeted delivery to the distal small bowel and colon, was superior to placebo in treating travellers' diarrhea (TD) in a previous study. Thus, a study was designed to compare this poorly absorbed antibiotic with the systemic agent ciprofloxacin. Methods In a randomized double-blind phase 3 study (ERASE), the efficacy and safety of Rifamycin SV-MMX® 400 mg twice daily (RIF-MMX) was compared with ciprofloxacin 500 mg twice daily in the oral treatment of TD. Overall, 835 international visitors to India, Guatemala or Ecuador with acute TD were randomized to receive a 3-day treatment with RIF-MMX (n = 420) or ciprofloxacin (n = 415). Primary endpoint was time to last unformed stool (TLUS), after which clinical cure was declared. Stools samples for microbiological evaluation were collected at the baseline visit and the end of treatment visit. Results Median TLUS in the RIF-MMX group was 42.8 h versus 36.8 h in the ciprofloxacin group indicating non-inferiority of RIF-MMX to ciprofloxacin (P = 0.0035). Secondary efficacy endpoint results including clinical cure rate, treatment failure rate, requirement of rescue therapy as well as microbiological eradication rate confirmed those of the primary analysis indicating equal efficacy for both compounds. While patients receiving ciprofloxacin showed a significant increase of Extended Spectrum Beta Lactamase Producing-Escherichia coli (ESBL-E. Coli) colonization rates after 3-days treatment (6.9%), rates did not increase in patients receiving RIF-MMX (-0.3%). Both drugs were well-tolerated and safe. Conclusion The novel multi-matrix formulation of the broad-spectrum, poorly absorbed antibiotic Rifamycin SV was found non-inferior to the systemic antibiotic ciprofloxacin in the oral treatment of non-dysenteric TD with the advantage of a lower risk of ESBL-E. Coli acquisition.

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Robert Steffen[1,2]*, Zhi-Dong Jiang[2], Mónica L. Gracias Garcia[3], Prithi Araujo[4], Michael Stiess[5], Tanju Nacak[5], Roland Greinwald[5], and Herbert L. DuPont[2]

[1]Department of Public Health, Epidemiology, Biostatistics and Prevention Institute, WHO Collaborating Centre for Travellers' Health, University of Zurich, CH-8001 Zurich, Switzerland, [2]Division of Epidemiology, Human Genetics & Environmental Sciences and Center for Infectious Diseases, University of Texas School of Public Health, Houston, TX 77030, USA, [3]Private Clinica, Quetzaltenango 09001, Guatemala, [4]Medical Department, NUSI Wockhardt Hospital, Cuncolim, Margao, Goa 403701, India and [5]Research and Development, Dr Falk Pharma GmbH, Freiburg 79108, Germany

*To whom correspondence should be addressed. Tel: +41-79 2927832; Fax: +41-44-9108949. Email: robert.steffen@uzh.ch

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Abstract

Background: The novel oral antibiotic formulation Rifamycin SV-MMX®, with a targeted delivery to the distal small bowel and colon, was superior to placebo in treating travellers' diarrhea (TD) in a previous study. Thus, a study was designed to compare this poorly absorbed antibiotic with the systemic agent ciprofloxacin.

Methods: In a randomized double-blind phase 3 study (ERASE), the efficacy and safety of Rifamycin SV-MMX® 400 mg twice daily (RIF-MMX) was compared with ciprofloxacin 500 mg twice daily in the oral treatment of TD. Overall, 835 international visitors to India, Guatemala or Ecuador with acute TD were randomized to receive a 3-day treatment with RIF-MMX (n = 420) or ciprofloxacin (n = 415). Primary endpoint was time to last unformed stool (TLUS), after which clinical cure was declared. Stools samples for microbiological evaluation were collected at the baseline visit and the end of treatment visit.

Results: Median TLUS in the RIF-MMX group was 42.8 h versus 36.8 h in the ciprofloxacin group indicating non-inferiority of RIF-MMX to ciprofloxacin (P = 0.0035). Secondary efficacy endpoint results including clinical cure rate, treatment failure rate, requirement of rescue therapy as well as microbiological eradication rate confirmed those of the primary analysis indicating equal efficacy for both compounds. While patients receiving ciprofloxacin showed a significant increase of Extended Spectrum Beta Lactamase Producing—*Escherichia coli* (ESBL-*E. Coli*) colonization rates after 3-days treatment (6.9%), rates did not increase in patients receiving RIF-MMX (–0.3%). Both drugs were well-tolerated and safe.

Conclusion: The novel multi-matrix formulation of the broad-spectrum, poorly absorbed antibiotic Rifamycin SV was found non-inferior to the systemic antibiotic ciprofloxacin in the oral treatment of non-dysenteric TD with the advantage of a lower risk of ESBL-*E. Coli* acquisition.

Key words: Travel, diarrhea, anti-bacterial therapy, rifamycin, ciprofloxacin, ESBL, antibiotic resistance

Introduction

Travellers' diarrhea (TD) remains the most common health problem experienced by travellers in lower income regions of the world.[1] Depending on the destination, up to 30% of travellers develop TD within the first 2 weeks abroad.[2,3] TD is often accompanied by other symptoms, such as abdominal cramps, faecal urgency, nausea, vomiting, and, if the pathogen has been invasive, by fever and blood in the stools. The most

common agents isolated from patients with TD are enteric diarrhea-producing *E. coli* (DEC), especially enterotoxigenic *E. coli* (ETEC) and enteroaggregative *E. coli* (EAEC). Other causes are mucosa-invasive bacteria including *Campylobacter jejuni*, *Shigella* spp. and *Salmonella* spp. At least, 80% of TD cases are of bacterial origin and noroviruses and protozoan parasites such as *Giardia lamblia* and *Cryptosporidium* play a minor role. Presence of more than one pathogen is common.[1,4,5]

Antimotility (mainly loperamide) and antimicrobial drugs, so far mainly fluoroquinolones and azithromycin, are the main therapeutic options for TD.[6,7] Increased anti-bacterial resistance to fluoroquinolones,[8] the detection of extended spectrum β-lactamase-producing *E. coli* (ESBL-*E. coli*) strains with subsequent risk of transmission,[9,10] and concern for tendon, nervous system, ocular and vascular complications[11–14] have recently resulted in restricted recommendations for the use of fluoroquinolones in TD patients. In contrast, orally administered poorly absorbable antibiotics such as rifaximin have been suggested for therapy of TD,[15] as they act in the lumen of in the gastrointestinal tract and thus have a low-toxicity profile. Rifaximin is widely approved in the treatment of TD caused by non-invasive bacterial pathogen strains.[16]

Closely related to rifaximin, rifamycin SV is an oral poorly absorbable, broadspectrum antibiotic belonging to the class of ansamycins.[17] Rifamycin SV-MMX® (RIF-MMX) tablets contain the active ingredient rifamycin SV at a concentration of 200 mg/tablet. The new oral formulation[18] starts to release active compound only after reaching intestinal pH-levels of pH ≥ 7 with an additional 1-h delay upon reaching this pH, thereby targeting the distal small bowel and colon where pH-levels are ≥7. The anti-bacterial activity of rifamycin sodium against the most frequent microorganisms causing TD as well as *Clostridium difficile* has been demonstrated.[19] In humans, Rifamycin SV <1% of the administered dose is absorbed after oral administration of RIF-MMX.[20]

RIF-MMX proved to be superior over placebo by shortening the duration of TD in patients with a broad range of pathogens.[21] In addition, the unique pharmacokinetic properties of the drug offer evidence that TD pathogens worked at the level of the distal small bowel and colon.[21] Thus, it was decided to design a second pivotal study and to compare the efficacy and safety of RIF-MMX with ciprofloxacin for treatment of TD.

Methods

Study design

A randomized, double-blind, double-dummy, multi-center, comparative 4/5-day non-inferiority phase III clinical trial (Evaluation of Rifamycin as a topically acting Antibiotic for Safety and Efficacy in travellers' diarrhea (ERASE)) was conducted between 23 November 2010 and 15 February 2016, in 17 study centers in India and 2 centers in Latin America (Ecuador, Guatemala). The study design was based on two arms in the form of a parallel group comparison with the goal to assess efficacy and safety of a 3-day, twice daily, oral treatment with Rifamycin SV-MMX® versus ciprofloxacin in TD patients.

The study was conducted in accordance with good clinical practice, the Declaration of Helsinki, all applicable national laws and regulations, and it was approved by independent ethics committees at each of the centers prior to starting recruitment. All patients gave written informed consent prior to participating. This study is registered with ClinicalTrials.gov (NCT01208922).

Population

Men and women aged at least 18 years who arrived within the past 4 weeks from an industrialized country were eligible if they had acute moderate to severe TD, defined as at least three unformed, watery or soft stools accompanied by symptoms within 24 h preceding randomization with duration of illness ≤72 h. Presence of one or more signs or symptoms of enteric infection (gas/flatulence, nausea, vomiting, abdominal cramps or pain, rectal tenesmus, faecal urgency) of moderate to severe intensity was mandatory. Symptoms were considered moderate if they interfered with planned activities and as severe if they completely prevented planned activities.

Excluded were patients who were residents of any country with high incidence rates of diarrhea within the past 6 months, or at the time of presentation diarrhea of >72 h duration, fever (>38.0°C), passage of grossly bloody stools, known or suspected infection with a non-bacterial pathogen (e.g. HIV or viral hepatitis), moderate or severe dehydration, history of inflammatory bowel disease or celiac disease. In addition, patients were excluded if they had taken more than two doses of an antidiarrheal medication within 24 h or received an antibiotic within 7 days prior to randomization. The use of these medications during the study was also prohibited.

Randomization and procedures

Eligible patients were randomized in a 1:1 ratio to receive either two Rifamycin SV-MMX® 200-mg tablets twice daily or one ciprofloxacin 500-mg capsule twice daily for 3 days. The study drugs were administered orally at breakfast and dinnertime. For allocation of patients, a computer-generated list of random numbers was prepared using a block size of four. Randomization was concealed by packaging the study medication using the double-dummy technique to guarantee blinding for all patients, investigators and any other persons involved in the conduct of the study. The study medication was consecutively numbered for each patient according to the randomization schedule, and investigators dispensed the study medication as per the randomization schedule. Patients recorded for 5 days the precise time of each drug administration in their diaries, time and consistency of each stool (watery, soft, formed), detailed quantitative information of gastrointestinal symptoms (abdominal pain/cramps, intensity of gas/flatulence, tenesmus, faecal urgency, nausea, vomiting), any AE occurring in between visits or any intake of concomitant medication. Safety and efficacy were assessed at Visit 2 (Day 2), Visit 3 (Day 4 or 5) and the final visit (Day 6). Stool samples were collected before treatment (Visit 1), and on the day after the last dose of trial drug (Visit 3) and sent to a central laboratory for blinded pathogen identification and antibiotic susceptibility testing (University of Texas, USA). If a patient received rescue therapy within the 120-h after ingestion of the first dose of the study drug, the patient was considered a treatment failure.

Study endpoints

The study was designed to prove non-inferiority of Rifamycin SV-MMX® to ciprofloxacin in terms of time from first dose of

study drug to the last unformed stool. The primary endpoint time to last unformed stool (TLUS) was defined as the interval in hours between the first dose of study drug and the last unformed stool passed, after which clinical cure was declared, i.e. time between the first dose of study medication and the last unformed stool before the end of the clinical cure period. TLUS was also calculated using the last unformed stool before the start of the first clinical cure period (modified TLUS). Patients receiving rescue therapy, patients who terminated the study early due to lack of efficacy, or patients who terminated the study without clinical cure were considered to have a TLUS of 120 h.

Secondary efficacy endpoints included clinical cure rate (24-h period with no clinical symptoms except mild flatulence, no fever, no watery stools and no more than two soft stools OR 48-h period with no stools or only formed stools, and no fever, with or without symptoms of enteric infection), improvement (≥50% reduction in the number of unformed stools passing during a 24-h period compared with number of stools passed during 24 h before first intake of study medication), treatment failure rate (clinical deterioration after ≥24 h of study treatment or illness continuing 120 h after start of study treatment or use of antimicrobial prohibited concomitant medication), modified TLUS (the time (h) between the first dose of the study drug and the last unformed stool before the start of the clinical cure period), number of unformed stools passed, gastrointestinal symptoms, requirement of rescue therapy, minimum inhibitory concentration (MIC_{50} and MIC_{90}), microbiological eradication rate (pathogen eradication in post-treatment stool). The frequency of adverse events (AEs), clinically relevant changes in any laboratory parameters, vital signs and physical examination were assessed.

Microbiological analyses

For analysis purposes, the following groups of pathogens were defined:

– Diarrheagenic *E. coli* group: positive for at least one of the following tests: enterotoxigenic *E. coli* (heat stable toxin, heat labile toxin or heat stable/heat labile toxin) or enteroaggregative *E. coli* without any positive result for pathogens of the potentially invasive/non-bacterial group.
– Potentially invasive/non-bacterial group: positive for at least one of the following pathogens: *Giardia lamblia, Cryptosporidium parvum, Entamoeba histolytica, Shigella* spp., *Salmonella* spp., *Campylobacter jejuni, Aeromonas* spp., *Plesiomonas* spp., *Vibrio* spp. or *Norovirus*.
– Pathogen-negative illness group: no positive pathogen identification at baseline.

To analyse the primary endpoint TLUS in more detail, we further split the potentially invasive/non-bacterial group in the following subgroups:

– Potentially invasive bacteria group: positive for at least one of the following pathogens: *Shigella* spp., *Salmonella* spp., *Campylobacter jejuni, Aeromonas* spp., *Plesiomonas* spp. or *Vibrio* spp. and negative for DEC, protozoa and norovirus.
– Protozoa group: positive for at least one of the following pathogens: *Giardia lamblia, Cryptosporidium parvum or Entamoeba histolytica* and negative for DEC, potentially invasive bacteria and norovirus.
– Norovirus group: positive for norovirus and negative for DEC, potentially invasive bacteria, protozoa and norovirus.

The presence of enteropathogens in stool samples was evaluated using published methods.[22–24] In short, the stools blinded as to treatment group were shipped on dry ice to the central laboratory at Houston/USA. Colonies from each stool culture were screened for enterotoxigenic *E. coli* (ETEC) by showing that the organism produced heat-labile enterotoxin (LT) and/or heat-stable enterotoxin (ST) by DNA hybridization and for the presence of enteroaggregative *E. coli* (EAEC) by a HEp-2 assay. *Shigella* species, *Salmonella* species, *Vibrio* species, *Campylobacter jejuni*, *Aeromonas* species and *Plesiomonas* species were analyzed using six standard media: MacConkey, Tergitol, Hektoen enteric, *Yersinia*, TCBS and *Campylobacter* agar plates. Stools were examined for enteric protozoal parasites, including *Giardia lamblia*, *Cryptosporidium* spp. and *Entamoeba histolytica*, by use of ELISAs. Norovirus was detected by RT-PCR. The MICs of the following antibiotics were evaluated: ciprofloxacin (Sigma-Aldrich, St. Louis, MO, USA), rifamycin SV (MP biomedical, Solon, OH, USA) and rifaximin (Sigma-Aldrich). The MICs were determined by the agar dilution method as standardized by the Clinical and Laboratory Standards Institute.[25] For quality control of antimicrobial potency, the MICs of the recommended control strains (*E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853 and *Enterococcus faecalis* ATCC 29212) were determined with the test strains for each antimicrobial agent and the MICs were within published ranges. To look for extended spectrum beta lactamase (ESBL) resistance of *E. coli* strains, the stools blinded as to treatment group were stored at −80°C before analysis. The stools were thawed and plated directly onto three MacConkey agar plates with ceftazidime (1 μg/ml), ceftriaxone (1 μg/ml) or cefotaxime (1 μg/ml) and incubated at 37°C for 24 h. *E. coli*-like colonies growing were tested for synergistic resistance profile in Mueller–Hinton agar using ceftazidime (30 μg), clavulanate–amoxicillin (10 μg) and cefotaxime (10 μg) disks using published methods.[25,26] Quality control stains *Klebsiella pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 were included in the study. *E. coli* was recovered in 91% of the frozen stool samples and, thus the freezing step is unlikely to affect the analysis.

Sample size and statistical analysis

The sample size calculation was based on the primary efficacy variable TLUS. The median TLUS for ciprofloxacin patients was assumed as 27.5 h and 28.85 h for RIF-MMX patients. Assuming an exclusion rate of 10% from the per protocol population, 388 patients had to be included in each treatment group to attain a target power of 80%. The study protocol prespecified a three-stage group-sequential adaptive design with possible sample size adjustment or early stopping of the study for efficacy, futility or safety after the interim analysis. The interim analyses were planned to be performed at 50% and 75% of the initially planned sample size. Interim analyses were conducted by an independent data monitoring committee (IDMC) established by the sponsor prior to the interim analysis. Upon recommendation of the IDMC after second interim analysis, the total sample size was increased to up to 1032 patients. This is based on the fact that the observed response rates were different from the originally expected rates. Therefore, in this

adaptive group sequential design, the revised sample size was needed to show a significant non-inferiority with a power of 80% at the end of the study within the pre-specified, tight non-inferiority margin. The confirmatory non-inferiority test of the primary efficacy variable was performed for the per-protocol (PP) analysis set with a sensitivity test for the ITT population. The non-inferiority margin acceptable was defined by a maximally acceptable difference in the median TLUS of 8.5 h (and corresponding delta = 0.764 for the hazard ratio) between Rifamycin SV-MMX® and the reference drug ciprofloxacin. Patients with lack of compliance, intake of forbidden concomitant medication, violation of eligibility criteria or early discontinuation due to AEs without causal relationship with study drug, were excluded from the PP population. Safety analyses were performed for the safety population. Statistical testing of the primary endpoint was done via the ADDPLAN system. All other analyses were conducted using the SAS statistical package for Windows (SAS Institute, Cary, NC).

Results

Patients

In total, 835 patients were randomized and treated with study medication and thus, comprised both the safety and ITT population; 420 patients received RIF-MMX and 415 patients ciprofloxacin (Fig. 1). The population included 805 patients in India (96.4%; 405 RIF-MMX, 400 ciprofloxacin) and 30 patients in Latin America (3.5%; 15 in each group). The study was completed by 814 patients (97.5%); the most frequent reason for premature termination was lack of patient's co-operation, followed by lack of efficacy and intolerable adverse events (AEs) (Fig. 1 A). The respective proportions were similar in both treatment groups. As 68 (8.1%) patients had to be excluded from the PP population (36 RIF-MMX, 32 ciprofloxacin), the resulting PP population consisted of 767 patients (91.9%; 384 RIF-MMX, 383 ciprofloxacin). The most frequent reasons for exclusion included lack of compliance, use of prohibited medication and violation of inclusion/exclusion criteria (Fig. 1B).

Demographics including type of traveller, country of residence and disease-specific history did not differ between treatment groups (Table 1). Most patients suffered from moderate (78.7%), a smaller proportion from severe TD (21.2%). The presence of at least one pathogen at baseline was similar in both treatment groups with an overall rate of 62.3% in the ITT population; multiple pathogens were identified in 23.7% of the patients. The Diarrheagenic *E. coli* (DEC) group comprised 38.0% of the patients with ETEC/ST (33.5%) and EAEC (25.1%) as the most frequent microorganisms, whereas potentially invasive/non-bacterial pathogens were isolated in 24.3% of the patients (Table 2). In 37.1% of the patients, no pathogen could be isolated.

Efficacy

In the PP analysis, median TLUS was 42.8 h (IQR [21.3, 66.5]) in the RIF-MMX group as compared with 36.8 h (IQR [20.4, 65.5]) in the ciprofloxacin group. This indicated non-inferiority of RIF-MMX to ciprofloxacin ($P = 0.0035$) (Table 3A; Fig. 2). Results from the ITT analysis confirmed the PP analysis: median TLUS in the RIF-MMX group was 44.3 h (IQR [21.5, 68.2]) vs. 40.3 h (95% CI [20.5, 67.0]) in the ciprofloxacin group with $P = 0.0011$ for non-inferiority in the ITT population.

Subgroup analyses of the median TLUS by region and duration of disease symptoms prior to treatment did not reveal any significant differences between the two treatments (Table 3B). However, TD patients positive for potentially invasive bacteria had a statistically significant shorter TLUS in ciprofloxacin group compared with RIF-MMX group (Table 3B). All other comparisons in relation to pathogen status did not reveal any significant differences between the two treatment groups. In addition, subgroup analysis revealed that in both treatment groups, the median TLUS was the longer the later the treatment was started after the onset of symptoms (Table 3B). While this

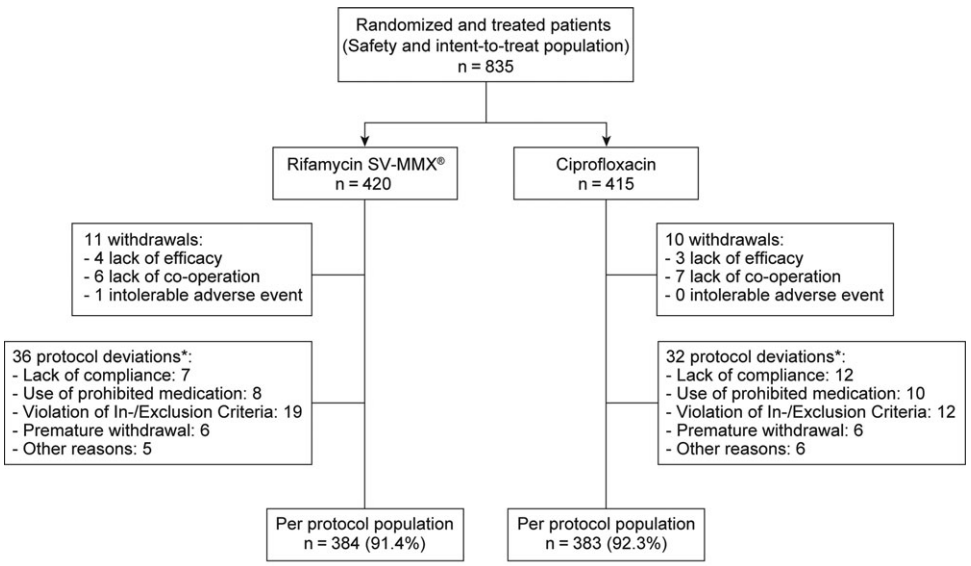

Figure 1. Disposition of patients.

Table 1. Demographic and TD characteristics (ITT population)

	Rifamycin SV-MMX® (*N* = 420)	Ciprofloxacin (*N* = 415)
Sex, *n* (%)		
Male	205 (48.8)	218 (52.5)
Female	215 (51.2)	197 (47.5)
Race, *n* (%)		
White	342 (81.4)	344 (82.9)
Asian	75 (17.9)	68 (16.4)
Black	0 (0.0)	1 (0.2)
Other	3 (0.7)	2 (0.5)
Age [years], mean (SD)	40.0 (16.1)	40.4 (16.6)
BMI [kg/m^2], mean (SD)	24.6 (3.8)	24.8 (4.0)
Type of traveller, *n* (%)		
Tourist	354 (84.3)	360 (86.7)
Business person	24 (5.7)	18 (4.3)
Student	26 (6.2)	21 (5.1)
Visiting friends/relatives	16 (3.8)	16 (3.9)
Country of residence[a], *n* (%)		
UK	136 (32.4)	141 (34.0)
Japan	35 (8.3)	37 (8.9)
Russia	38 (9.0)	30 (7.2)
Israel	22 (5.2)	33 (8.0)
Germany	23 (5.5)	31 (7.5)
France	21 (5.0)	14 (3.4)
South Korea	17 (4.0)	18 (4.3)
Spain	14 (3.3)	20 (4.8)
Duration (mean (SD)) between		
Arrival at country and first symptoms, days	12.4 (6.9) *n* = 420	12.0 (6.5) *n* = 415
First symptoms and randomization, h	28.8 (15.0) *n* = 419	28.4 (14.5) *n* = 414
Number unformed stools during 24 h prior to randomization, mean (SD)	5.5 (1.8) *n* = 420	5.4 (1.8) *n* = 415
Patients (*n* (%)) with	420 (100.0)	415 (100.0)
Maximum severity 'mild'[b]	0 (0.0)	1 (0.2)
Maximum severity 'moderate'[b]	328 (78.1)	329 (79.3)
Maximum severity 'severe'[b]	92 (21.9)	85 (20.5)
Macroscopic stool findings at baseline, *n* (%)		
Presence of blood and/or mucus	127 (30.2)	119 (28.7)

[a]Only countries listed if number of patients was at least 3% of the ITT population.

[b]Grading of severity: mild: not severe enough to change patient activity level; moderate: caused a change in the patient's daily activities; severe: rendered the patient disabled or he/she had to stay in bed due to the gastrointestinal symptom.

effect was independent of the treatment arm, using a cox regression model we found that patients with a treatment start 0–24 h after onset of symptoms had a shorter TLUS than patients with a disease duration prior of treatment of 24–<48 h (hazard ratio (HR): 0.475; 95% CI: 0.401, 0.563; $P < 0.0001$) or 48–<72 h (HR: 0.355; 95% CI: 0.278, 0.453; $P < 0.0001$). Subgroup analysis of the median TLUS by patients with and without presence of blood and/or mucus in the macroscopic stool analysis did also not reveal any statistical difference (Table 3B). No difference in the median modified TLUS between the RIF-MMX group (32.3 h; 95% CI [28.7, 39.4]) and the ciprofloxacin group (31.0 h; 95% CI: 28.5, 34.0) emerged (Log Rank test: $P = 0.7047$; PP analysis). The analysis of the secondary endpoints clinical cure rate, treatment failure rate and requirement of rescue therapy revealed similar results in both treatment groups with no statistically significant difference (Table 3C). Furthermore, both treatments not only rapidly reduced the number of unformed stools in a similar manner, but also resolved the gastrointestinal symptoms of the enteric infection (Fig. 3).

Microbiological eradication rate and MICs

Independent of the pathogen species, the pathogens identified at baseline could be eradicated in around half of the patients in both treatment groups (Table 4). Similarly, no difference in terms of the microbiological eradication rates by isolate was found. MICs were determined for all pathogens and both treatment groups as well as for pre- and post-treatment (Table 5). For the DEC group, increases of MIC_{50} and MIC_{90} between pre- and post-treatment became apparent in the RIF-MMX group for Rifamycin and rifaximin (data not shown), but not for ciprofloxacin. In the ciprofloxacin group, increases of MIC_{50} and MIC_{90} became apparent for ciprofloxacin, but not for Rifamycin SV. For the potentially invasive and non-bacterial pathogens, the numbers of available microbiological samples after treatment were too small to provide results of sufficient robustness.

ESBL-*E. coli* colonization

A post-hoc analysis of ESBL-*E. coli* colonization at baseline and after treatment was performed in both treatment groups. Of note, patients positive for ESBL-*E. coli* at baseline were only found at sites in India (103/662), whereas none of the tested patients from Latin America was positive (0/21). At baseline, both treatment groups had similar ESBL-*E. coli* colonization rates (Table 6). Interestingly, ESBL-*E. coli* rates did not rise after 3 days of RIF-MMX treatment. In contrast, patients randomized to ciprofloxacin showed a significant increase at the end of the 3-days treatment period (Table 6). Furthermore, among patients ESBL-*E. coli* negative at enrolment, a significantly higher number of patients receiving ciprofloxacin newly acquired ESBL-*E. coli* compared with those in the RIF-MMX group, resulting in an odds ratio of 1.84 (Table 6).

Safety

The incidence of AEs and adverse drug reactions (ADRs) was similar in both treatment groups. In total, 124/835 patients (14.9%) experienced AEs (RIF-MMX: 14.8%; ciprofloxacin: 14.9%). ADRs were reported by 34/420 patients (8.1%) in the RIF-MMX group and by 31/415 (7.5%) patients in the ciprofloxacin group. One patient in the RIF-MMX group withdrew the study drug after 1 day of treatment due to the intolerable AE 'worsening of diarrhea', that was considered non-serious and was of moderate intensity. The relationship to the study drug was considered unlikely and the patient recovered. No serious AE or death was reported. No further safety concerns arose from the results for vital signs, laboratory and physical examinations, and no meaningful differences between the treatment groups became obvious.

Discussion

Even if there is a decreasing trend in the incidence rate of TD,[1] TD at many destinations remains the most frequent health problem abroad in rapidly increasing numbers of travellers.[27,28] Only few among these profit of a decreased risk associated with previous exposure to enteric pathogens in low-income countries.[29] Particularly in this journal, there is an ongoing argumentation on the indications and limitations of antimicrobial therapy against TD[30,31] despite the fact that a graded expert panel report has been published just a few years ago.[6] This report does not only present a novel agent against TD, but also responding to a recent request on priorities towards travel-related research,[32] it offers a first piece of evidence that not all antimicrobials are equal with respect to increasing the risk for multiresistant enteropathogens. Not only in the domain of vaccine preventable diseases do we need regular updates,[33] in future such data should become available also with respect to pathogen resistance associated with TD and TD therapy.

This study demonstrated the efficacy of the oral multi-matrix formulation of the broad-spectrum, poorly absorbed antibiotic Rifamycin SV-MMX® (RIF-MMX) for the oral treatment of non-dysenteric TD to be non-inferior to the systemic antibiotic ciprofloxacin which until recently has been the antimicrobial of choice for the treatment of TD. Non-inferiority was shown in terms of the primary efficacy variable median TLUS. In this study, TLUS was defined as the interval in hours between the first dose of study drug and the last unformed stool (watery or soft) passed, after which clinical cure was declared. In other words, TLUS was defined as the time between the first dose of the study drug and the last unformed stool before the end of the clinical cure period. This is the most conservative way for determination of TLUS as it points to the end of the clinical cure period, i.e. a time the patient has overcome TD. This is in contrast to the TLUS definition of other trials in the field of TD, which defined TLUS as the time between the first dose of study drug and the last unformed stool before the start of the clinical cure period, which points to a time a patient starts to overcome TD.[16,21,34] We also analyzed the data using this TLUS definition (modified TLUS), but also found no difference. Nevertheless, the definition of TLUS affects the outcome quite strongly, with a TLUS difference of 10.5 h and 5.8 h for the PP analysis of RIF-MMX and ciprofloxacin groups, respectively, making the definition of TLUS an important factor when comparing trials. Interestingly, the obtained median in the modified TLUS results resembled those from earlier trials with rifaximin.[16]

Interestingly, subgroup analysis clearly showed that median TLUS was the shorter the earlier antibiotic treatment was started after the onset of symptoms, suggesting that early therapy of TD should be recommended at least in those patients who need a rapid cure to assure their travel plans.

TLUS varies depending on where the study has been conducted, as shown in this study by differing median TLUS in patients recruited in India and Latin America (Table 3B). Thus, comparison of TLUS between treatment groups within one study is considered more meaningful than a comparison across different studies.

In a previous clinical study, RIF-MMX showed a numerically, but due to the low number not statistically significant higher efficacy compared with placebo against potentially invasive pathogens.[21] Despite the trend of being more effective against invasive bacteria compared with placebo, treatment with RIF-MMX resulted in the current study into a statistically significant longer TLUS compared with the ciprofloxacin group. However, considering the low number of patients in this subgroup analysis (n = 22 vs. 23), one has to interpret this result carefully. In

Table 2. Baseline pathogen detection (ITT population)

	Rifamycin SV-MMX® (N = 420) n (%)	Ciprofloxacin (N = 415) n (%)
Patients with at least one pathogen at baseline	266 (63.3)	254 (61.2)
Diarrheagenic *E. coli* group[a]	159 (37.9)	158 (38.1)
ETEC heat stable toxin	142 (33.8)	138 (33.3)
ETEC heat labile toxin	16 (3.8)	11 (2.7)
ETEC heat stable/labile toxin	17 (4.0)	9 (2.2)
EAEC	112 (26.7)	98 (23.6)
Potentially invasive/non-bacterial group[b]	107 (25.5)	96 (23.1)
Shigella spp.	9 (2.1)	8 (1.9)
Salmonella spp.	7 (1.7)	12 (2.9)
Campylobacter jejuni	20 (4.8)	26 (6.3)
Aeromonas spp.	8 (1.9)	9 (2.2)
Plesiomonas spp.	4 (1.0)	3 (0.7)
Vibrio spp.	0 (0.0)	0 (0.0)
Norovirus	11 (2.6)	12 (2.9)
Giardia lamblia	48 (11.4)	35 (8.4)
Cryptosporidium parvum	14 (3.3)	6 (1.4)
Entamoeba histolytica	1 (0.2)	3 (0.7)
Pathogen-negative group[c]	152 (36.2)	158 (38.1)

[a]Positive for at least one of the following tests: enterotoxigenic *E. coli* (ETEC heat stable toxin, heat labile toxin or heat stable/heat labile toxin) or enteroaggregative *E. coli* without any positive result for pathogens of the invasive/non-bacterial group.

[b]Positive for at least one of the following pathogens: *Shigella* spp., *Salmonella* spp., *Campylobacter jejuni*, *Aeromonas* spp., *Plesiomonas* spp., *Vibrio*, Norovirus, *Giardia lamblia*, *Cryptosporidium parvum* or *Entamoeba histolytica*.

[c]Patients without positive detection of an enteric pathogen.

Table 3A. Primary endpoint (TLUS)

	TLUS [h]						One-sided P-value (for non-inferiority)
	Rifamycin SV-MMX®			Ciprofloxacin			
	n	Median	IQR	n	Median	IQR	
PP	384	42.8	21.3, 66.5	383	36.8	20.4, 65.5	0.0035
ITT	420	44.3	21.5, 68.2	415	40.3	20.5, 67.0	0.0011

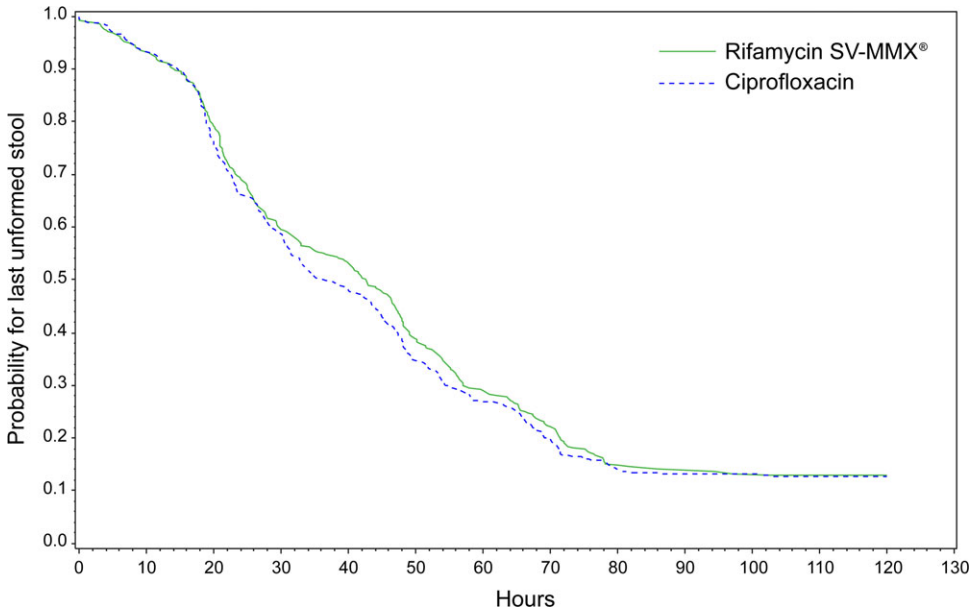

Figure 2. Kaplan–Meier plot of time to last unformed stool (TLUS) during the first 5 days after randomization (ITT population).

Table 3B. Subgroup analysis of primary endpoint (TLUS) (ITT population)

	TLUS [h]					
	Rifamycin SV-MMX® N = 420		Ciprofloxacin N = 415		Comparison	
	n	Median [IQR]	*n*	Median [IQR]	Hazard ratio [95% CI]	Log Rank test
Region						
India	405	45.0 [21.5, 69.0]	400	41.0 [21.1, 67.1]	0.965 [0.830, 1.121]	*P* = 0.6367
Latin America	15	28.8 [18.4, 50.2]	15	29.5 [16.6, 49.3]	1.017 [0.470, 2.197]	*P* = 0.9664
Pathogen status[a]						
Diarrheag. *E. coli* group	159	44.8 [23.0, 67.3]	158	42.3 [21.7, 65.0]	0.915 [0.722, 1.160]	*P* = 0.4644
Pot. invasive bacteria group	22	56.2 [32.0, 120.0]	23	35.3 [18.0, 47.0]	0.370 [0.187, 0.732]	*P* = 0.0031
Protozoa group	18	24.3 [15.3, 47.9]	9	35.0 [21.3, 44.8]	1.085 [0.458, 2.568]	*P* = 0.8528
Norovirus group	6	40.8 [32.5, 48.2]	4	45.2 [43.4, 82.8]	2.067 [0.505, 8.462]	*P* = 0.3030
Pathogen-negative group	152	43.0 [21.5, 68.4]	158	44.0 [22.8, 71.3]	1.164 [0.913, 1.484]	*P* = 0.2207
Onset of symptoms to baseline visit						
0 to <24 h	201	27.5 [19.2, 47.9]	192	27.7 [19.4, 48.5]	1.006 [0.819, 1.236]	*P* = 0.9526
24 to <48 h	149	50.2 [26.1, 75.5]	163	44.1 [21.5, 80.3]	0.977 [0.761, 1.255]	*P* = 0.8573
48–72 h	69	65.4 [48.3, 120.0]	58	64.6 [48.0, 79.6]	0.856 [0.573, 1.278]	*P* = 0.4465
>72 h	0	–	1	76.5 [76.5, 76.5]	–	
Macroscopic stool findings						
Presence of blood and/or mucus	127	48.2 [22.8, 69.2]	119	47.0 [26.0, 65.0]	0.902 [0.690, 1.180]	*P* = 0.4528
No findings	293	40.5 [21.3, 67.3]	296	33.5 [20.3, 68.5]	0.983 [0.825, 1.173]	*P* = 0.8523

[a]Diarrheag. *E. coli* group: includes patients with *E. coli* and no concurrent invasive pathogens. Pot. invasive bacteria group: positive for at least *Shigella* spp., *Campylobacter jejuni, Salmonella* spp., *Yersinia enterocolitica, Aeromonas* spp., *Plesiomonas* spp. or *Vibrio* spp. and no other analyzed enteric pathogens. Protozoa group: positive for at least *Giardia lamblia*, *Cryptosporidium parvum* or *Entamoeba histolytica* and no other analyzed enteric pathogens. Norovirus group: positive for norovirus and no other analyzed enteric pathogens.

contrast, subgroup analysis of the TLUS by patients with and without presence of blood and/or mucus in the macroscopic stool analysis, indicative of at least slight intestinal mucosa damage, revealed no difference in efficacy between the luminal acting Rifamycin SV-MMX and the systemic ciprofloxacin.

RIF-MMX showed a similar antimicrobial activity as ciprofloxacin against a broad range of pathogens involved in TD. The rates of pathogen eradication were not different between both treatment groups. This is in contrast to rifaximin, which had statistically significantly lower eradication rates than ciprofloxacin.[16] As RIF-MMX targets the distal small bowel and colon, the study further showed that these regions are not only important reservoirs for the common bacterial pathogens associated TD, but also represent valid targets for topical antibiotic therapy.

Table 3C. Secondary endpoints (Clinical Cure Rate, Treatment Failure Rate and Requirement of Rescue Therapy) (ITT population)

	Number (%) of patients		
	Rifamycin SV-MMX® ($N = 420$)	Ciprofloxacin ($N = 415$)	χ^2 test *P*-value
Clinical cure rate	357 (85.0)	352 (84.8)	0.942
Treatment failure rate	62 (14.8)	63 (15.2)	0.865
Requirement of rescue therapy	11 (2.6)	4 (1.0)	0.072

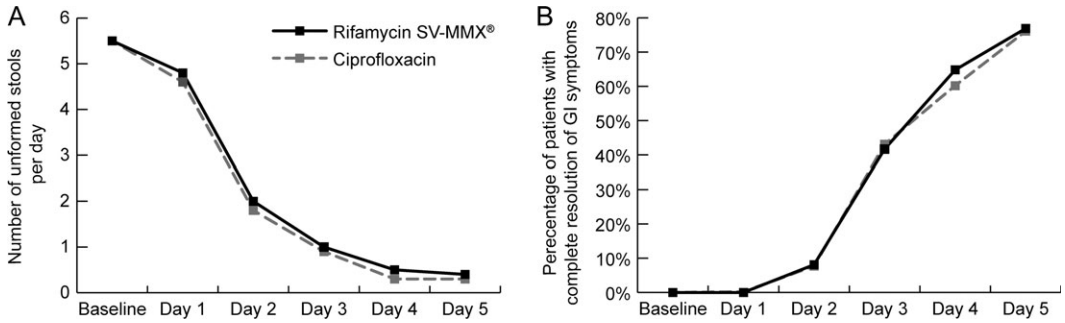

Figure 3. (A) Number of unformed stools per day during the first 5 days after randomization. (B) Percentage of patients with complete resolution of clinical symptoms of enteric infection per day.

Table 4. Microbiological eradication rate

	Rifamycin SV-MMX® ($N = 420$)		Ciprofloxacin ($N = 415$)		χ^2 test
	n/N'	%	n/N'	%	*P*-value
All patients	**131/266**	**49.2**	**126/254**	**49.6**	**0.935**
Diarrheagenic *E. coli* group	**77/159**	**48.4**	**80/158**	**50.6**	**0.695**
ETEC-ST	65/142	45.8	69/138	50.0	0.479
ETEC-LT	9/16	56.3	3/11	27.3	0.137
ETEC-ST/LT	11/17	64.7	6/9	66.7	0.920
EAEC	67/112	59.8	66/98	67.3	0.259
Potentially invasive/non-bacterial group	**54/107**	**50.5**	**46/96**	**47.9**	**0.717**
Shigella spp.	6/9	66.7	7/8	87.5	0.312
Salmonella spp.	7/7	100.0	10/12	83.3	0.253
Campylobacter jejuni	17/20	85.0	19/26	73.1	0.331
Aeromonas spp.	5/8	62.5	8/9	88.9	0.200
Plesiomonas spp.	3/4	75.0	3/3	100.0	0.350
Norovirus	8/11	72.7	6/12	50.0	0.265
Giardia lamblia	27/48	56.3	20/35	57.1	0.935
Cryptosporidium parvum	13/14	92.9	5/6	83.3	0.515
Entamoeba histolytica	1/1	100.0	3/3	100.0	

Note: n = number of patients with at least one isolate at baseline and no isolate at Visit 3, N' = number of patients with at least one isolate at baseline.

Although eradication rates were lower for both groups compared with previous trials,[16,21] it is difficult to draw clinical conclusions as there seems a lack of correlation between eradication and clinical outcome.[35] In addition, it has to be taken into account that rifamycin SV also has remarkable anti-inflammatory and immunomodulatory properties, particularly through the PXR receptor and NFkB signalling pathway (Caridad Rosette, personal communication), independent of its bactericidal activity.[36–38] These *in vitro* activities were superior when compared with rifaximin tested in parallel in the same assays. Additionally, rifamycin SV appeared to be less cytotoxic.[37]

Increases in MIC_{50} and MIC_{90} at Visit 3 became apparent for rifamycin in the RIF-MMX group and for ciprofloxacin in the Ciprofloxacin group. However, the increased MICs for Rifamycin are still largely below the high intraluminal and faecal concentrations of RIF-MMX.[20] Also, the high MICs for Campylobacter have to be seen in this context. In contrast, fluoroquinolone resistance has expanded from Campylobacter-

Table 5. Rifamycin SV and Ciprofloxacin $MIC_{50/90}$ for bacterial isolates before and after treatment

		Rifamycin SV-MMX® (*N* = 420)				Ciprofloxacin (*N* = 415)			
		Visit 1		Visit 3		Visit 1		Visit 3	
		n	$MIC_{50/90}$ [μg/ml]	*n*	$MIC_{50/90}$ [μg/ml]	*n*	$MIC_{50/90}$ [μg/ml]	*n*	$MIC_{50/90}$ [μg/ml]
EAEC	Ciprofloxacin	112	0.03/16	22	0.01/8	98	0.06/16	24	0.01/64
	Rifamycin SV	112	16/64	22	16/256	98	16/64	24	16/64
ETEC-LT	Ciprofloxacin	16	0.06/16	6	0.02/0.25	11	0.13/16	7	1024/1024
	Rifamycin SV	16	24/64	6	384/512	11	32/32	7	32/64
ETEC-ST	Ciprofloxacin	142	0.13/32	60	0.09/32	138	0.09/32	57	0.13/64
	Rifamycin SV	142	16/64	60	16/1024	138	16/64	57	16/32
ETEC-ST/LT	Ciprofloxacin	17	0.06/0.25	6	0.14/16	9	0.13/16	3	16/1024
	Rifamycin SV	17	16/64	6	264/1024	9	16/32	3	64/64
Aeromonas spp.	Ciprofloxacin	8	0.06/0.25	2	0.31/0.50	9	0.25/2		–/–
	Rifamycin SV	8	4/8	2	8/8	9	8/64		–/–
Campylobacter jejuni	Ciprofloxacin	20	128/1024	3	8/1024	26	128/512	7	128/1024
	Rifamycin SV	20	128/1024	3	1024/1024	26	64/1024	7	64/128
Plesiomonas spp.	Ciprofloxacin	4	0.13/0.13	1	0.01/0.01	3	0.13/0.25		–/–
	Rifamycin SV	4	3/4	1	256/256	3	4/4		–/–
Shigella spp.	Ciprofloxacin	9	2/4	1	4/4	8	1.50/4	1	4/4
	Rifamycin SV	9	16/16	1	64/64	8	16/64	1	32/32
Salmonella spp.	Ciprofloxacin	7	0.01/0.02		–/–	12	0.01/0.13		–/–
	Rifamycin SV	7	32/128		–/–	12	32/128		–/–

Table 6. *ESBL-E.coli* colonization before and after treatment

A) *ESBL-E. coli* by treatment group before and after treatment

	Visit 1				Visit 3				*P*-value[b]
	Positive for ESBL-*E. coli*			*P*-value[a]	Positive for ESBL-*E. coli*			*P*-value[a]	
	n/*N*′	%	95% CI		*n*/*N*′	%	95% CI		
Rifamycin SV-MMX®	55/345	15.9	12.2, 20.2	0.5931	54/347	15.6	11.9, 19.8	0.0758	1.000 (*N* = 313)
Ciprofloxacin	48/338	14.2	10.7, 18.4		72/342	21.1	16.9, 25.8		0.0319 (*N* = 306)

[a]Fisher's exact test (Rifamycin SV-MMX® vs. ciprofloxacin).
[b]McNemar's test (Visit 1 vs. Visit 3). Only patients with a positive or negative result for ESBL-E at Visit 1 and Visit 3 were considered.
Note: *N*′ = number of patients with a positive or negative result for ESBL-E at Visit 1, *N*′ = number of patients with a positive or negative result for ESBL-E at Visit 3.

B) *ESBL-E. coli* by treatment group for Visit 3, results at Visit 1 negative

	Visit 3						
	Positive for ESBL-*E.coli*		95% CI	*P*-value	Value	Odds ratio	*P*-value
	n/*N*′	%				95% CI	
Rifamycin SV-MMX®	27/263	10.3	6.9, 14.6	0.0221	1.84	1.10, 3.07	0.0197
Ciprofloxacin	45/259	17.4	13.0, 22.6				

Note: *N*′ = number of patients with a negative result for ESBL-E at Visit 1.

C) *ESBL-E. coli* by treatment group for Visit 3, result at Visit 1 positive

	Visit 3			
	Positive for ESBL-*E. coli*		95% CI	*P*-value*
	n/*N*′	%		
Rifamycin SV-MMX®	23/50	46.0	31.8, 60.7	1.000
Ciprofloxacin	21/47	44.7	30.2, 59.9	

*Fisher's exact test (Rifamycin SV-MMX® vs. ciprofloxacin).
Note: *N*′ = number of patients with a positive result for ESBL-E at Visit 1 and with a positive or negative result for ESBL-E at Visit 3.

associated cases in Southeast Asia to widespread occurrence, and increases among other common bacterial enteropathogens including ETEC, EAEC, *Shigella* and non-typhoidal *Salmonella*.[8,39,40] In this short-term study, however, no development of cross-resistance was observed for either antibiotic.

ESBL-*E. coli* carriage and acquisition in the context of travel and TD has been reported in recent years by studies from many European countries.[9,10,41–44] Travel to Asia, in particular South Asia, and TD have been consistently found to be associated with the highest risk for carriage and acquisition of ESBL-*E. coli* in all studies. Use of antibiotics, and particularly of ciprofloxacin is reported to be an independent risk factor for ESBL-*E. coli* acquisition[9,10,44,45] and this has been confirmed by this trial. The clinical relevance of these findings is enhanced by the fact that co-resistance of ESBL-*E. coli* to other non-beta-lactam systemic antibiotics including fluoroquinolones (ciprofloxacin), cefotaxime, TMP-SMX, gentamicin and cotrimoxazole is common and ESBL-*E. coli* co-resistance to ciprofloxacin was found in ~51–53% of isolates.[10,44,46] In contrast, RIF-MMX did not increase EBSL-*E. coli* carriage rate and did not promote new acquisition of ESBL-*E. coli*. Furthermore, resistance of rifamycin is usually encoded chromosomally and not on plasmids making horizontal gene transfer and co-selection far less likely to occur.[47–49]

Rifamycin SV-MMX® at a dose of 800 mg/day and ciprofloxacin at a dose of 1000 mg/day were safe and well-tolerated during this short-term study. However, fluoroquinolones were recently associated with disabling and potentially permanent side effects of the tendons, muscles, joints, nerves and central nervous system occasionally even after just a single dose and various of these AEs can occur together in the same patient.[13,14] These serious AEs were not detected with ciprofloxacin in this study as they are uncommon. But the FDA released a Boxed Warning, FDA's strongest warning, for fluoroquinolones and has advised restricted use of these antibiotics in certain uncomplicated infections considering that associated serious side effects generally outweigh the benefits.[11,12] Therefore, current guidelines for the treatment of moderate to severe TD recommend as systemic antibiotic now primarily azithromycin.[6,7] However, increasing resistance is reported also for this antimicrobial agent[40,50] and systemic side effects may occur, albeit rarely, such as sustained ventricular tachycardia in patients with prolonged QTc.[51] In contrast, poorly absorbed antibiotics not only do not cause systemic side effects, but they also contribute to reserve systemic antimicrobials to cure more severe infections than TD. Therefore, RIF-MMX offers an advantage, which may be particularly beneficial in subjects with co-morbidities and co-medications.

In conclusion, RIF-MMX is a safe and effective non-absorbable antibiotic to treat TD. Compared with systemic antibiotics, it has the advantage of not causing any systemic AEs. In addition, it does not lead to an increased acquisition of ESBL-*E. coli* while this is a relevant problem with ciprofloxacin. Thus, this novel multi-matrix formulation of the broad-spectrum, poorly absorbed antibiotic Rifamycin SV may be considered as a first-line treatment for afebrile, non-dysenteric TD.

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Authors' Contribution

Conception and design of study: R.S., H.L.D, T.N. and R.G.

Recruitment of patients and acquisition of data: R.S., H.L.D, Z.J., M.G. and P.A.

Assembly, analysis and interpretation of data: R.S., H.L.D, M.S., T.N. and R.G.

Drafting and revision of the manuscript: R.S., H.L.D, M.S., T.N. and R.G.

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