Supplementary file 1: Eligibility criteria

Inclusion criteria:

1. Men / Women aged 18 and over (including adults who lacked mental capacity who had a legal representative)
2. Successful treatment of clinically diagnosed CDI using standard therapy (metronidazole or vancomycin given according to standard local hospital guidelines)

Exclusion criteria:

1. Women of child bearing potential and not willing to use at least one highly effective contraceptive method throughout the study
2. Male with spouse/partner of child bearing potential and not willing to use condoms
3. Pregnant or breast feeding
4. Unable to swallow tablets
5. Life expectancy of <4 weeks
6. Hypersensitivity to the active substance, to any rifamycin (e.g. rifampicin or rifabutin) or to any of its excipients (Tablet core: Sodium starch glycolate type A, glycerol distearate, colloidal anhydrous, silica, talc and microcrystalline cellulose. Tablet coating: hypromellose, titanium dioxide (E171), disodium edentate, propylene glycol and red iron oxide E172)
7. >5 days post standard therapy (metronidazole or vancomycin) for clinically diagnosed CDI
8. Taking ciclosporin

Amendments:

- Taking ciclosporin was also added as an exclusion in July 2015 due to a change in the Summary of Product Characteristics (SPC).
- Concomitant antibiotic use initially required exclusion but in December 2014 an amendment removed this criterion, other than in the case of rifamycins, to facilitate recruitment.
- Life expectancy of less than 4 weeks was added as a reason for exclusion following a clarifying amendment in June 2013.

Supplementary file 2: Adherence with trial medication

	Placebo (n =74)	Rifaximin (n = 77)
Percentage of prescribed dose taken		
Mean [sd]	80.0 [32.1]	88.9 [25.3]
Median [25th, 75th centile]	97.2 [68.3, 100]	100 [94.4, 100]
Min, max	0, 100	0, 100
n[1]	70	77
Study drug taken		
No	6 (8%)	4 (5%)
Yes	66 (89%)	73 (95%)
Not known	2 (3%)	0
Information on number of tablets remaining known (where taken)		
No	2 (3%)	0
Yes	64 (86%)	73 (95%)
Number of tablets remaining[2]		
Mean [sd]	40.8 [45.5]	17.6 [35.6]
Median [25th, 75th centile]	12.5 [1, 82]	0 [0, 9]
Min, max	0, 126	0, 126
n	70	77
Trial treatment completed (from CRF)		
Yes	34 (46%)	59 (77%)
No	39 (53%)	18 (23%)
Not known[3]	1 (1%)	0
Primary reason for discontinuation if trial treatment not completed		
Adverse event	5 (7%)	2 (3%)
recurrence of C difficile	15 (20%)	3 (4%)
Lost to follow-up	1 (1%)	1 (1%)
Non-adherence with trial treatment	8 (11%)	2 (3%)
Physician decision	0	5 (6%)

Protocol deviation	2 (3%)	2 (3%)
Withdrawal of consent	5 (7%)	3 (4%)
Death	3 (4%)	0
Adherence with trial treatment (derived)		
63 or less tablets remaining	48 (65%)	68 (88%)
More than 63 tablets remaining or no doses taken	22 (30%)	9 (12%)
Number of tablets remaining not known[4]	4 (5%)	0

All values are n (%) unless specified. 126 tablets are dispensed.
CRF = case report form
1 – Percentage of tablets taken not calculated for the four participants where the number of tablets remaining was unknown.
2 – Participants not starting the study drug derived as having 126 tablets remaining for this summary
3 – Not known if participant took any doses, participant only completed study visit up to week 2.
4 – Includes participants where it is not known if study drug was taken or it is not known how many tablets were remaining at the end of the treatment phase. These participants are considered as non-adherent with trial treatment.

Supplementary file 3: Sensitivity analyses for primary outcome: CDI recurrence within 12 weeks

Sensitivity analyses for the primary outcome were performed as follows: making further adjustment for variables with an imbalance at baseline, imputing missing outcome data (using multiple imputation and making simple assumptions), estimating the complier average causal effect (CACE) for participants considered adherent with trial medication (defined as return of 63 or fewer tablets remaining in the rifaximin group) and a per protocol analysis excluding participants who did not take any doses of trial medication and/or for whom standard therapy for initial *C difficile* was not successful.

	Placebo (n =74)	**Rifaximin (n = 77)**	**Risk difference (95% CI, p-value)**
Available case analysis with adjustment for gender[1] and where the participant was recruited from (n = 130)			-11.5% (-25.1% to 2.1%)
Complier averaged causal effect (n = 130)[2]			-14.6% (-30.1% to 0.9%)
Per protocol analysis (n = 125)[3]	16/58 (27.6%)	11/67 (16.4%)	-11.1% (-25.6% to 3.3%)
Using imputation for missing data (n = 151)			
Using multiple imputation assuming missing data is missing at random[4]	29.1% (n = 74)	16.3% (n = 77)	-12.8% (-27.0% to 1.4%)
Using simple imputation assuming all participants with missing data did recur	31 (41.9%)	19 (24.7%)	-17.1% (-31.7% to -2.6%)
Using simple imputation assuming all participants with missing data did not recur	18 (24.3%)	11 (14.3%)	-10.2% (-23.1% to 2.6%)

1- No adjustment was made for age as this did not differ between groups

2- Complier for this analysis was defined as less than 63 tablets remaining for those allocated to the Rifaximin group. Instrumental variable regression used to estimate complier averaged causal effect.

3 - Participants were excluded from the *per protocol* analysis if no doses of trial medication were taken and/or standard therapy for initial *C difficile* was not successful.

4 - Multiple imputation assumes that missing outcomes are missing at random i.e the unobserved outcomes depend on observed characteristics and not the unobserved outcomes. Multiple imputation using chained equations with 20 imputed datasets. Imputation model included treatment allocation, age, gender, previous CDI, where the participant was recruited from, antibiotic use (at randomisation or started during the study), proton pump inhibitor use in 30 days prior to randomisation and proton pump inhibitor started during the study

Supplementary file 4: Subgroup analysis for primary outcome: CDI recurrence within 12 weeks

	Placebo (n = 74)	Rifaximin (n = 77)	Risk difference (95% CI)	Interaction effect (95% CI, p value for interaction)
ANTIBIOTIC USED TO TREAT INITIAL CDI (planned)				
Metronidazole used to treat initial CDI	27	31		
Primary outcome data available	21	29		
No recurrence	16 (76.2%)	25 (86.2%)		
Recurrence	5 (23.8%)	4 (13.8%)	-10.2% (-32.4% to 12.1%)	
Vancomycin used to treat initial CDI	47	46		
Primary outcome data available	40	40		
No recurrence	27 (67.5%)	33 (82.5%)		
Recurrence	13 (32.5%)	7 (17.5%)	-15.1% (-33.9% to 3.7%)	-4.9% (-34.0% to 24.2%, p-value 0.74)
HISTORY OF CDI (post hoc)				
No previous diagnosis of CDI	61	57		
Primary outcome data available	50	52		
No recurrence	36 (72%)	45 (87%)		
Recurrence	14 (28%)	7 (13%)	-14.7 (-30.4% to 1.1%)	
Previously diagnosed with CDI	9	11		
Primary outcome data available	8	10		
No recurrence	5 (63%)	8 (80%)		
Recurrence	3 (38%)	2 (20%)	-18.2% (-60.2% to 23.7%)	-3.6% (-48.4 to 41.2%, p-value 0.88)
Unknown history of CDI	4	9		
Primary outcome data available	3	7		
No recurrence	2 (67%)	5 (71%)		
Recurrence	1 (33%)	2 (29%)		

Number of participants with primary outcome data available is used as the denominator for CDI recurrence in each subgroup

Risk difference, interaction effect (difference in risk differences) and 95% confidence intervals calculated using Generalised Estimating Equations (to account for centre) using the Binomial family. 120 participants where information known about history of CDI included in model for subgroup analysis according to history of CDI

Supplementary file 5: Serious adverse events frequency

	Placebo (n = 74)		Rifaximin (n = 77)	
	At least one dose taken (n = 68)	No doses taken (n = 6)	At least one dose taken (n = 73)	No doses taken (n = 4)
Number of participants with a serious adverse event starting up to 28 days post randomisation – n(%)	15 (22%)	2 (33%)	12 (16%)	0
Number of SAEs per participant				
1 SAE	13	2	10	
2 SAEs	2	-	2	
Total number of SAEs starting up to 28 days post randomisation - not related to an IMP	16	2	14	0
Clostridium difficile infection	3	-	3	
Clostridium difficile colitis	2	-	1	
Chronic obstructive pulmonary disease	2	-	-	
Pneumonia	-	1	1	
Ascites	1	-	-	
Cellulitis	-	-	1	
Chest pain	-	-	1	
Chronic hepatic failure	1	-	-	
Dehydration	1	-	-	
Diverticulitis	-	-	1	
Duodenal ulcer haemorrhage	-	1	-	
Dyspnoea	-	-	1	
Fall	1	-	-	
Gastroenteritis viral	-	-	1	
Gastrointestinal haemorrhage	-	-	1	
Hip fracture	-	-	1	
Megacolon	1	-	-	
Pancreatitis	-	-	1	
Pelvic fracture	-	-	1	
Renal failure acute	1	-	-	
Sepsis	1	-	-	
Urinary tract infection	1	-	-	
Urinary tract infection fungal	1	-	-	
Total number of SAEs starting up to 28 days post randomisation - related to an IMP not unexpected	1	0	0	0
Adverse drug reaction	1	-	-	-
Number of participants with a serious adverse event starting from 29 days post randomisation to end of study – n(%)	20 (29%)	0	26 (36%)	0
Number of SAEs per participant				
1 SAE	10		15	
2 SAEs	6		6	
3 SAEs	4		3	
4 SAEs	0		1	
5 SAEs	0		1	

	Placebo (n = 74)		Rifaximin (n = 77)	
	At least one dose taken (n = 68)	No doses taken (n = 6)	At least one dose taken (n = 73)	No doses taken (n = 4)
Total number of SAEs starting from 29 days post randomisation to end of study - not related to an IMP	34	0	45	0
Clostridium difficile colitis	4		2	
Clostridium difficile infection	1		5	
Pyrexia	3		2	
Cellulitis	-		4	
Renal failure acute	2		2	
Dyspnoea	1		2	
Gastroenteritis	2		1	
Lower respiratory tract infection	1		2	
Sepsis	1		2	
Abdominal pain	1		1	
Chest pain	-		2	
Chronic obstructive pulmonary disease	-		2	
Fall	2		-	
Urinary tract infection	-		2	
Urinary tract stoma complication	2		-	
Alcoholic liver disease	1		-	
Arthritis bacterial	1		-	
Asthenia	1		-	
Back pain	-		1	
Cardiac failure	-		1	
Cardiac failure congestive	-		1	
Confusional state	-		1	
Death	-		1	
Deep vein thrombosis	1		-	
Dementia Alzheimer's type	-		1	
Haematuria	-		1	
Hypoglycaemia	1		-	
Hypotension	-		1	
Infection	1		-	
Intestinal perforation	1		-	
Klebsiella infection	-		1	
Musculoskeletal chest pain	-		1	
Overdose	-		1	
Pneumonia	1		-	
Pneumonia aspiration	-		1	
Prepuce dorsal slit	1		-	
Renal injury	1		-	
Retroperitoneal fibrosis	-		1	
Septic shock	-		1	
Stent placement	1		-	
Subdural haematoma	-		1	
Suprapubic pain	1		-	
Toxicity to various agents	-		1	
Upper gastrointestinal haemorrhage	1		-	
Ureteric calculus removal	1		-	
Total number of SAEs starting from 29 days post randomisation to end of study - related to an IMP not unexpected	0	0	0	0

Preferred term for SAEs from medDRA coding presented at event level. Preferred terms sorted by total number (descending order) and preferred term name (alphabetical).
All serious adverse events were collected up to the 6 month follow-up visit for participants randomised on or prior to the 10th December 2015 and up to the 12 week visit for participants randomised after the 10th December 2015.

Supplementary file 6: Non serious adverse events frequency

	Placebo (n = 74)		Rifaximin (n = 77)	
	At least one dose taken (n = 68)	No doses taken (n = 6)	At least one dose taken (n = 73)	No doses taken (n = 4)
Number of participants with a non serious adverse event starting up to 28 days post randomisation – n (%)	18 (26%)	0	21 (29%)	1 (25%)
Number of AEs per participant				
1 AE	14	-	16	1
2 AEs	4	-	3	0
4 AEs	0	-	2	0
Total number of non serious AEs starting up to 28 days post randomisation	22	0	30	1
AE Relationship with IMP				
Definitely	0	-	0	0
Probably	1	-	1	0
Possibly	2	-	3	0
Not Related	19	-	25	1
Not known	0	-	1	0
AE severity				
Mild	11	-	18	1
Moderate	8	-	11	0
Severe	3	-	0	0
Not known	0	-	1	0
MedDRA preferred term of AE				
Diarrhoea	6	-	4	1
Urinary tract infection	2	-	4	-
Constipation	1	-	2	-
Abdominal pain	-	-	2	-
Cellulitis	1	-	1	-
Abdominal pain lower	1	-	-	-
Abdominal pain upper	-	-	1	-
Acute myocardial infarction	-	-	1	-
Back pain	1	-	-	-
Cholecystitis	1	-	-	-
Cystoscopy	1	-	-	-
Fall	1	-	-	-
Gastroenteritis viral	1	-	-	-
Gastrointestinal sounds abnormal	-	-	1	-
Gout	-	-	1	-
Haematuria	1	-	-	-
Herpes zoster	1	-	-	-
Hydronephrosis	-	-	1	-
Hydroureter	-	-	1	-
Hypoglycaemia	-	-	1	-
Irritability	-	-	1	-
Lower respiratory tract infection	-	-	1	-
Muscle spasms	1	-	-	-
Musculoskeletal chest pain	-	-	1	-
Neuralgia	-	-	1	-
Norovirus test positive	-	-	1	-

	Placebo (n = 74)		Rifaximin (n = 77)	
	At least one dose taken (n = 68)	**No doses taken (n = 6)**	**At least one dose taken (n = 73)**	**No doses taken (n = 4)**
Pleural effusion	1	-	-	-
Pollakiuria	-	-	1	-
Renal failure acute	-	-	1	-
Syncope	-	-	1	-
Tooth abscess	-	-	1	-
Uterine prolapse	1	-	-	-
Vomiting	-	-	1	-
Wound secretion	1	-	-	-
Number of participants with a non serious adverse event starting up to 28 days to 56 days post randomisation – n (%)	8 (12%)	0	6 (8%)	0
Number of AEs per participant				
1 AE	3	-	6	-
2 AEs	4	-	0	-
4 AEs	1	-	0	-
Total number of non serious AEs starting from 29 days to 56 days post randomisation	15	0	6	0
AE Relationship with IMP				
Definitely	0	-	0	-
Probably	0	-	0	-
Possibly	1	-	0	-
Not Related	14	-	6	-
AE severity				
Mild	10	-	6	-
Moderate	3	-	0	-
Severe	2	-	0	-
MedDRA preferred term of AE				
Urinary tract infection	2	-	1	-
Diarrhoea	1	-	1	-
Oral candidiasis	2	-	-	-
Wound secretion	2	-	-	-
Asthma	-	-	1	-
Blister	1	-	-	-
Excoriation	1	-	-	-
Fall	1	-	-	-
Haemorrhoids	-	-	1	-
Hyperkalaemia	-	-	1	-
Musculoskeletal chest pain	-	-	1	-
Neutropenic sepsis	1	-	-	-
Renal failure acute	1	-	-	-
Thrombocytopenia	1	-	-	-
Vomiting	1	-	-	-
Wound	1	-	-	-

Preferred term for AEs from medDRA coding presented at event level. Preferred terms sorted by total number (descending order) and preferred term name (alphabetical).

Supplementary file 7: Exploratory outcome summary for stool frequency and consistency

Outcome	Placebo (n = 74)	Rifaximin (n = 77)
Average daily stool frequency during weeks 1 to 4		
Mean [sd]	2.0 [1.1]	1.8 [0.6]
Median [25th, 75th centile]	1.6 [1.2, 2.4]	1.9 [1.3, 2.2]
Min, max	0.5, 4.8	0.6, 3.1
n	36	39
Average daily stool frequency during weeks 11 to 12		
Mean [sd]	1.4 [0.6]	1.5 [0.8]
Median [25th, 75th centile]	1.1 [1, 1.9]	1.3 [1, 1.9]
Min, max	0.5, 2.4	0.7, 4.4
n	23	25
Average stool consistency during weeks 1 to 4		
Mean [sd]	4.3 [0.8]	4.3 [0.8]
Median [25th, 75th centile]	4.3 [3.9, 4.7]	4.2 [3.8, 4.8]
Min, max	2.4, 6.1	1.5, 5.8
n	36	39
Average stool consistency during weeks 11 to 12		
Mean [sd]	3.9 [0.8]	3.6 [0.9]
Median [25th, 75th centile]	4.0 [3.4, 4.2]	4.0 [3.2, 4.1]
Min, max	2.7, 6	1.4, 4.9
n	23	25
Percentage of days during weeks 1 to 4 with at least one loose stool		
Mean [sd]	20.0 [26.0]	19.6 [20.2]
Median [25th, 75th centile]	8.9 [0, 25]	14.3 [3.6, 37]
Min, max	0, 100	0, 64.3
n	36	39
Percentage of days during weeks 11 to 12 with at least one loose stool		
Mean [sd]	5.8 [12.4]	8 [18.5]
Median [25th, 75th centile]	0 [0, 0]	0 [0, 14.3]
Min, max	0, 42.9	0, 85.7
n	23	25

Stool consistency was assessed using the Bristol Stool Chart, for a maximum of 8 stools in one day, on a scale of 1 to 7 (scores of 6 and 7 indicate loose stools).