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Original Article

Saccharomyces boulardii and bismuth subsalicylate as low-cost interventions to reduce the duration and severity of cholera

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We conducted a randomised single-blinded clinical trial of 100 cholera patients in Port-au-Prince, Haiti to determine if the probiotic *Saccharomyces cerevisiae* var. *boulardii* and the anti-diarrhoeal drug bismuth subsalicylate (BS) were able to reduce the duration and severity of cholera. Subjects received either: *S. boulardii* 250 mg, *S. boulardii* 250 mg capsule plus BS 524 mg tablet, BS 524 mg, or two placebo capsules every 6 hours alongside standard treatment for cholera. The length of hospitalisation plus the number and volume of emesis, stool and urine were recorded every 6 hours until the study subject was discharged (n=83), left against medical advice (n=11), or requested removal from the study (n=6). There were no reported deaths or adverse study-related events. There were no statistically significant differences between the study arms and the outcomes of interest.

Keywords: Cholera, Bismuth subsalicylate, *Saccharomyces boulardii,* Diarrhoea, Cholera toxin, Treatment, Probiotic

Introduction

Vibrio cholerae is a gram-negative bacterium that causes clinical cholera by a producing cholera toxin (CT). Cholera toxin increases adenylate cyclase activity resulting in increased intracellular cyclic adenosine monophosphate (cAMP) leading to an efflux of chloride ions and secretion of water, sodium, potassium and bicarbonate into the intestinal lumen. Cholera is characterised by a sudden onset of watery diarrhoea that may be accompanied by vomiting.[1] Cholera is highly contagious, and in severe disease stool volumes of 500–1000 ml/hour can result in dehydration.[1]

In January 2010, Haiti suffered a 7.0 magnitude earthquake. In October 2010, an outbreak of cholera ensued infecting about 700 000 Haitians and killing 8500.[2] Early in the cholera outbreak Operation Blessing International, a non-governmental organisation working with St. Luc hospital outside Port-au-Prince Haiti, established a cholera rehydration centre.

Cholera affects several million people each year in developing countries without access to clean water or sanitation.[3] Untreated cholera mortality can approach 50%, but treatment with oral rehydration solution (ORS) to prevent dehydration drops the mortality rate to below 4%.[3] Oral rehydration solution contains salts and glucose to augment fluid absorption from the intestine by the sodium glucose cotransporter-1. The addition of zinc supplements and antibiotics also have been shown to be beneficial in the treatment of cholera.[4,5]

For decades, probiotics – defined as a live microorganism that confers benefit to the host – have been utilised in humans to treat and prevent gastrointestinal diseases. *Saccharomyces cerevisiae* var. *boulardii* hence referred to as *S. boulardii* is one of the best-studied probiotics that has been used to treat diarrhoea by many millions of people worldwide as an over-

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the-counter supplement. Lyophilised *S. boulardii* is resistant to antibiotics, survives optimally at 37°C, is resistant to gastric acidity and proteolysis, reaches steady state concentrations within the stool in 3 days, and is cleared from the stools within 2–5 days.[6] While *S. boulardii* has never been evaluated clinically to determine efficacy for treatment against cholera in humans, it has been the subject of multiple clinical trials, meta-analysis, and expert consensus guidelines for managing gastrointestinal illnesses in children and adults.[7–13]

S. boulardii augments gastrointestinal immunity by reducing inflammation and improving humoural immunity, inhibits some bacterial toxins, and enhances the activity of the sodium glucose cotransporter-1.[14] When pretreated with *S. boulardii*, Fischer rats are largely protected from the clinical effects of CT.[15,16] The effects of CT on isolated loop jejunum secretion was reduced by the addition of *S. boulardii*.[17,18] *S. boulardii* also protects cultured intestinal cells against CT-induced cAMP elevation.[17,19,20]

Bismuth subsalicylate (BS) is sold over the counter in the United States under several brand names including Pepto-Bismol®. Bismuth subsalicylate is used to treat diarrhoea, acid reflux, gastritis and *Helicobacter pylori* infection in individuals over the age of 12 years. Bismuth subsalicylate is postulated to provide benefit by a number of mechanisms including coating the intestines to prevent intestinal secretion and promote fluid absorption, reducing inflammation, binding toxins and killing some bacteria. Fluid accumulation in ligated and unligated rabbit and pig intestinal segments was substantially reduced when BS was added before, but not after, the addition of CT.[21] Similarly, BS significantly reduces the effects of CT when BS administered before CT receptor binding in Y-1 adrenal cell tissue and rabbit ligated intestinal loops.[22]

Since cholera affects individuals living in economically disadvantaged areas of the world, interventions to reduce the duration and severity of disease must be inexpensive, safe, non-technical and readily available. Two medications that fit these requirements are BS and *S. boulardii*, both of which already have been shown to be safe and efficacious in treating other diarrhoeal diseases. The purpose of our study was to evaluate BS and *S. boulardii* to determine if they would be efficacious against cholera.

Methods

Ethical considerations

We received institutional review board (IRB) approval from Eastern Virginia Medical School (11-04-FB-0097). We were unable to identify a local ethical review board affiliated with St. Luc hospital in Port-au-Prince, Haiti, so administrators at both St. Luc and Operation Blessing International reviewed and approved the study. The study took place from September to October 2011 at St. Luc hospitals.

Experimental

The primary objectives of this study were to determine if the study interventions were able to reduce the duration of cholera-symptoms as defined by the total number of hours in the cholera rehydration centre, reduce the mean number of stools, and reduce the volume of stools when compared to placebos. The study inclusion criteria were: (1) non-pregnant and non-breastfeeding persons aged 12–89 years old; (2) onset of cholera symptoms for <3 days; and (3) subjects had not taken any medications to treat cholera. Study exclusion criteria included: (1) clinical diagnostic uncertainty about having cholera; (2) unable to obtain informed consent from study subject (including the subject's guardian if under age 18 years old); (3) allergy to BS, aspirin, doxycycline, promethazine, non-steroidal anti-inflammatory drugs or yeast; or (4) currently taking methotrexate due to a potential drug interaction with BS.

A Haitian research assistant fluent in Creole, English, and French approached potential study subjects within 4 hours of arrival to the rehydration centre. Study participation did not interfere with clinical management and subjects too ill to consent were excluded. Subjects were consented using otherwise identical consent forms in Creole, English and French. All study subjects had a positive stool-based rapid enzyme-linked immunoassay (ELISA) test for *V. cholerae* (Crystal VC, Span Diagnostics, Ltd., Surat, Gujarat, India) before study enrolment. All patients screened for study enrolment with a diagnosis of clinical cholera tested positive by the cholera ELISA test. In addition, all female study subjects of reproductive age had a negative urine pregnancy test. Routine laboratory tests are not performed as part of the standard management for cholera patients at St. Luc hospital, and aside from urine pregnancy tests and stool ELISA tests for cholera, we did not perform additional laboratory investigations on our subjects.

Study subjects were randomised to one of four treatment arms using a random number generator. Study drugs were given by mouth every 6 hours by a nurse from an envelope labelled with a number corresponding to the treatment arm that the subject was randomised. The study subject, nurse and physician, but not the research assistant recording the data, were blinded to the treatment arm of the study subject. The treatment arms were: (1) *S. boulardii*

250 mg capsule (Advanced Orthomolecular Research, Calgary, AB, Canada) plus a placebo capsule (empty gel capsule); (2) *S. boulardii* 250 mg capsule plus two BS (Pepto-Bismol®, Proctor and Gamble, Cincinnati, OH, USA chewable 262 mg tablets); (3) two BS 262 mg tablets plus a placebo capsule; or (4) two placebo capsules. All study subjects received zinc 5 mg by mouth every 6 hours along with the study drugs.

A cholera treatment protocol developed and approved by St. Luc hospital prior to initiation of this research study was followed for the management of all patients with cholera, including our study subjects. All study interventions were provided in additional to the standard of care treatment provided at the hospital. The cholera treatment protocol consisted of the administration of ORS *ad lib* to all patients. If the treating physician determines that the person is not severely dehydrated based on clinical criteria, but the patient vomits, then that patient will receive 2 L of intravenous ringer's lactate over 60 minutes with additional intravenous fluids given at the discretion of the treating physician. Patients with severe dehydration receive 4 L of intravenous ringer's lactate over 2 hours and then 4 L over 4 hours with additional intravenous fluids administered as clinically indicated by the treating physician. All patients, including our study subjects, received a single 300 mg dose of oral doxycycline when admitted to the rehydration centre.

In summary, study subjects received the same resuscitation and medical treatment for cholera as non-study subjects with cholera with the exception that study subjects received: (1) possible urine pregnancy test; (2) stool ELISA to confirm cholera; (3) optional single-dose oral promethazine 6.25 mg at the time of study enrolment; (4) study drugs every 6 hours; (5) zinc 5 mg orally every 6 hours; (6) measurement of the number and volume of stool, urine and emesis every 6 hours; (7) record of self-reported duration of cholera-symptoms prior to arrival at the cholera rehydration centre and (8) the time and reason for leaving the study.

Results

One hundred fifteen persons were approached for study participation, of which 100 study subjects were enrolled for greater than 6 hours. The baseline characteristics for our study subjects were similar (Table 1). Based on the average heart rates, initial reported urine frequency and urine volumes suggest that our study subjects were not severely dehydrated. The mean outcome measurements for each treatment arm, recorded every 6 hours, is displayed in Table 2.

Study subjects left the study for one of the following reasons: death (n=0); discharge from the hospital

Table 1 Characteristics of the study subjects

	SB and BS (n = 25)	SB (n = 26)	BS (n = 25)	Placebo (n = 24)
Age	39 (SD = 15)	35 (SD = 15)	28 (SD = 10)	32 (SD = 16)
Percentage of study subjects that were male	48%	62%	68%	54%
Percentage of study subjects receiving promethazine at enrolment	48%	58%	44%	58%
Number of hours of diarrhoea prior to study enrolment	23 (SD = 18)	33 (SD = 18)	26 (SD = 17)	29 (SD = 17)
Heart rate at time of enrolment	80 (SD = 12)	83 (SD = 13)	84 (SD = 13)	84 (SD = 18)
Number of hours study subject participated in the study	41 (SD = 22)	42 (SD = 28)	42 (SD = 31)	32 (SD = 18)
Number of study subjects ultimately discharged from study	22	19	23	19
Number of study subjects asking to be taken out of the study	0	3	1	2
Number of study subjects leaving the before hospital discharge (i.e. left against medical advice or eloped)	3	4	1	3

SB = *S. boulardii*, BS = bismuth subsalicylate

Table 2 Mean value for each treatment arm, (standard deviation), and *n* = number of study subjects with available data

Outcome	Treatment	6 Hours	12 Hours	18 Hours	24 Hours	30–36 Hours	42-48 Hours	54–60 Hours	66–72 Hours	78–84 Hours
Mean number of stools	BS	4 (2.8), $n = 25$	3.3 (2.2), $n = 20$	2.6 (1.8), $n = 17$	3.2 (2.6), $n = 10$	3.1 (1.7), $n = 8$	3.3 (2.3), $n = 2$	6 (2.1), $n = 1$	11 (3.5), $n = 1$	NA
	P	4.5 (1.8), $n = 23$	5.6 (8.4), $n = 23$	3.7 (2.8), $n = 19$	3.2 (1.3), $n = 13$	2.7 (2.4), $n = 10$	3.5 (2.4), $n = 6$	4.25 (3.6), $n = 5$	4.5 (2.1), $n = 2$	2 (1.7), $n = 2$
	SB	4.3 (2.4), $n = 26$	4.0 (2.4), $n = 25$	4.8 (3.1), $n = 21$	4.1 (3.4), $n = 17$	4.2 (3.5), $n = 15$	2.7 (2.8), $n = 9$	2.8 (3.6), $n = 5$	5.4 (1.3), $n = 2$	1.5 (1.5), $n = 2$
	SB + BS	3.8 (2.2), $n = 25$	3.7 (2.5), $n = 24$	3.8 (2.5), $n = 20$	3.2 (2.8), $n = 19$	3.2 (2.3), $n = 14$	2.9 (2.5), $n = 8$	3.1 (2.3), $n = 3$	5 (4.5), $n = 2$	NA
Mean stool volume (ml)	BS	1646 (1147), $n = 24$	1485 (1439), $n = 20$	1600 (2537), $n = 16$	1758 (2665), $n = 10$	1790 (1632), $n = 8$	3667 (2566), $n = 2$	2900 (2404), $n = 1$	4425 (2227), $n = 1$	NA
	P	1907 (1199), $n = 23$	1758 (1384), $n = 20$	1534 (1052), $n = 14$	1455 (1270), $n = 13$	1081 (891), $n = 10$	1447 (1171), $n = 6$	633 (631), $n = 5$	1300 (578), $n = 3$	1000 (1075), $n = 2$
	SB	2132 (1769), $n = 25$	2892 (3379), $n = 25$	1587 (1235), $n = 18$	1363 (1070), $n = 15$	2748 (2158), $n = 14$	718 (768), $n = 9$	2038 (1255), $n = 4$	1325 (339), $n = 2$	578 (492), $n = 2$
	SB + BS	1384 (1080), $n = 25$	1550 (1056), $n = 25$	1685 (1254), $n = 20$	1502 (1245), $n = 18$	1644 (1263), $n = 14$	903 (822), $n = 7$	2958 (2940), $n = 3$	1667 (1155), $n = 2$	NA
Mean number of emesis	BS	0.6 (1.6), $n = 25$	0.2 (0.7), $n = 20$	0 (0), $n = 17$	0 (0), $n = 10$	0.1 (0.3), $n = 8$	1.5 (1.7), $n = 2$	1 (0.7), $n = 1$	0 (0), $n = 1$	NA
	P	0.6 (1.3), $n = 25$	0.2 (0.7), $n = 20$	0 (0), $n = 7$	0 (0), $n = 10$	0.1 (0.3), $n = 8$	1.5 (1.7), $n = 2$	1 (0.7), $n = 1$	0 (0), $n = 1$	NA
	SB	0.5 (1.3), $n = 26$	0.4 (1.0), $n = 25$	0.4 (1.7), $n = 21$	0.4 (1.2), $n = 17$	0.5 (1.2), $n = 15$	0.7 (1.0), $n = 9$	0.1 (1), $n = 5$	0 (0), $n = 3$	100 (115), $n = 2$
	SB + BS	0.3 (1), $n = 25$	0.1 (0.4), $n = 24$	0.1 (0.3), $n = 20$	0 (0), $n = 19$	0.1 (0.3), $n = 14$	0 (0), $n = 8$	0 (0), $n = 3$	3 (3.5), $n = 2$	NA
Mean emesis volume (ml)	BS	138 (512), $n = 24$	71 (296), $n = 20$	0 (0), $n = 17$	0 (0), $n = 10$	100 (200), $n = 8$	750 (866), $n = 2$	900 (636), $n = 1$	0 (0), $n = 1$	NA
	P	65 (184), $n = 23$	241 (803), $n = 22$	88 (223), $n = 17$	15 (55), $n = 13$	0 (46), $n = 10$	0 (288), $n = 6$	0 (83), $n = 5$	0 (0), $n = 3$	0 (125), $n = 2$
	SB	72 (192), $n = 25$	76 (176), $n = 22$	53 (182), $n = 20$	23 (90), $n = 15$	93 (168), $n = 13$	357 (541), $n = 9$	9.5 (142), $n = 5$	0 (0), $n = 3$	100 (115), $n = 2$
	SB + BS	58 (182), $n = 25$	29 (143), $n = 24$	0.1 (0.3), $n = 19$	0 (0), $n = 19$	0 (0), $n = 12$	0 (0), $n = 8$	0 (0), $n = 3$	125 (144), $n = 2$	NA
Mean number of urine	BS	3.1 (1.9), $n = 25$	2.6 (2.1), $n = 20$	3.4 (2.2), $n = 17$	2.5 (1.4), $n = 10$	2.9 (1.6), $n = 8$	2.2 (0.6), $n = 2$	4.5 (0), $n = 1$	11 (3.5), $n = 1$	NA
	P	3.3 (2.1), $n = 25$	2.9 (1.8), $n = 23$	2.8 (2.1), $n = 19$	2.9 (1.4), $n = 13$	2.3 (1.7), $n = 10$	3.7 (2.2), $n = 6$	4.5 (2.4), $n = 5$	4.5 (1.8), $n = 3$	3.5 (1.0), $n = 2$
	SB	3.3 (2.9), $n = 26$	2.9 (2.5), $n = 25$	3.8 (2.2), $n = 21$	2.8 (2.2), $n = 14$	3.3 (2.7), $n = 15$	1.6 (1.9), $n = 9$	2.8 (1.6), $n = 5$	4.4 (1.2), $n = 3$	1.5 (1.5), $n = 2$
	SB + BS	2.4 (1.3), $n = 25$	3 (2.5), $n = 24$	3.3 (2.5), $n = 20$	2.8 (2.3), $n = 19$	2.2 (2.2), $n = 14$	4.4 (3.3), $n = 8$	2.8 (1.4), $n = 3$	2.5 (2.1), $n = 2$	NA
Mean urine volume (ml)	BS	389 (276), $n = 24$	496 (625), $n = 20$	1884 (5713), $n = 17$	444 (379), $n = 10$	605 (608), $n = 8$	933 (839), $n = 2$	1400 (283), $n = 1$	2210 (1117), $n = 1$	NA
	P	338 (203), $n = 23$	410 (255), $n = 20$	340 (256), $n = 13$	458 (480), $n = 13$	309 (228), $n = 5$	966 (672), $n = 3$	533 (366), $n = 3$	1035 (541), $n = 2$	200 (150), $n = 1$
	SB	452 (466), $n = 24$	360 (356), $n = 22$	496 (355), $n = 19$	352 (201), $n = 15$	1109 (1512), n = 14	187 (225), $n = 9$	365 (159), $n = 4$	410 (80), $n = 3$	179 (128), $n = 2$
	SB + BS	337 (236), $n = 25$	456 (489), $n = 24$	552 (703), $n = 20$	560 (579), $n = 18$	402 (367), $n = 13$	758 (1088), $n = 7$	1097 (1110), $n = 3$	150 (100), $n = 2$	NA

BS = bismuth subsalicylate, *P* = placebo, SB = *S. boulardii*

(n=83); left against medical advice (n=11); transferred out of the cholera rehydration ward to another part of the hospital due to ongoing other medical concerns (n=0); suffered a clinical adverse event from study participation as determined by the treating physician (n=0); a subject study's request to be removed from the study (n=6); anuric for 24 hours (n=0); no stool for 24 hours (n=0) or in the study for a total of 14 days (n=0). There were no reported adverse events and study investigators did not contact study subjects after leaving the study.

We calculated correlations between stool number and volume, urine number and volume and emesis number and volume, and those correlations with a P-value of <0.05 were the number of stools recorded every 6 hours was correlated with the volume of stool, the number and volume of emesis and number of times the study subject urinated. The volume of stool was correlated with the number and volume of emesis and the number and volume of urine. The number of times there was emesis was correlated with the volume of emesis. The number of times a person urinated was correlated with the volume of urine. There were no other correlations between our measured variables that were statistically significant.

The average time that subjects were in the study for each treatment arm was 19.8 hours for BS, 26.4 hours for placebo, 34.2 for *S. boulardii*. and 33 hours for *S. boulardii* + BS. A Kruskal-Wallis test statistic for the maximum reported hours between the different treatment groups was 10.87 with a P-value of 0.012 indicating that patients in the different treatment arms stayed in the hospital for different lengths of time. Figure 1 shows the length of time study subjects participated in the study separated by their treatment arm.

We did repeated measures analysis with the 6, 12, 18, and an average of the values for 24 hours and beyond for the number and volume of stool, urine and emesis of the study subjects with the covariates as treatment, time, and the intersection between treatment and time. The analysis was done with the help of Generalised Estimating Equations (GEE). Three of the six outcomes were frequencies, so we utilised a Poisson regression model. For the volume measurements we used a normal (Gaussian) regression. The P-values indicate no statistically significant effect of the treatment interventions on the length of stay in the study (Table 3). Figures 2 and 3 show the mean number and volume of stool respectively for each treatment arm reported every 6 hours until there were less than three study subjects remaining in the arm for that time point.

We utilised a regression model for each of the six response variables (number and volume of stool, number and volume of emesis, and number and volume of urination) up until the study subject either left against medical advice, requested removal from the study, or was discharged. We determined that the different treatment arms did not have any statistically significant effects on the average length of stay in the study. The number of stools, emesis and urine every 6 hours was modelled as Poisson variables and the volume measurements as normal variables. The analysis showed insignificant P-values indicating that the interventions did not have statistically different effects on he length of stay in the study (Table 4).

Discussion

We report the first human clinical trial using BS and *S. boulardii* in cholera patients. In the current study, neither BS, *S. boulardii* nor BS plus *S. boulardii* was shown to be effective at reducing the duration and severity of cholera. Our initial power calculations overestimated the level of sickness of our study subjects. It is possible that a larger sample size would show that one of our treatment arms had beneficial effect on the reduction and duration of symptoms. There may have been a trend for BS to reduce the duration and number of stools in cholera patients compared with placebo however it did not reach statistical significance. There were no reported adverse events from study participation, and no deaths occurred in our study subjects while they were participating in our study.

Our data do not support the small number of animal and *in vitro* studies that suggest that *S. boulardii* and BS are effective at reducing the effects of CT. In the animal studies, the benefits of *S. boulardii* and BS came from pretreatment or concurrent exposure to CT. It is possible that either *S. boulardii* or BS could have benefit in prophylaxis against cholera, although that hypothesis was not tested in this study.

Limitations

The initial physician impression on the level of dehydration at presentation to the cholera rehydration centre was not available to study investigators. One person on the placebo arm was recorded as having 42 stools between 6 and 12 hours of being in the study, and while this was likely recorded in error it was reported in our final analysis.

Patients were asked to remember the number of stools, emesis and urine in the previous 6 hours introducing recall bias. The time of day that the data was recorded may have affected the number of stools recorded (e.g. study subjects may have produced less stools at night). On occasion a research assistants had to estimate volumes when

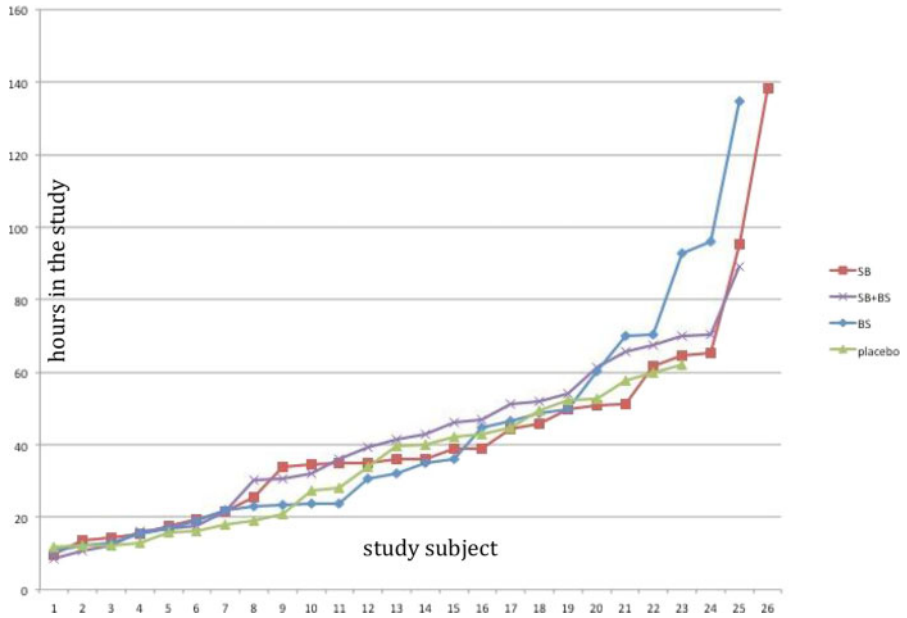

Figure 1 The number of hours each study subject was enrolled in the study separated by treatment arm. SB = *S. boulardii*, BS = bismuth subsalicylate

Table 3 Analysis of variables using the generalised estimating equations (GEE).

	Chi-square (*P*-value)		
Outcome	Treatment (df=3)	Time (df = 1)	Treatment*time (df = 3)
Stool frequency	1.48 (0.68)	13.33 (0.0003)	3.63 (0.30)
Stool volume	2.73 (0.44)	9.74 (0.0018)	1.58 (0.66)
Emesis frequency	Model did not converge		
Emesis volume	2.12 (0.55)	5.36 (0.02)	1.66 (0.65)
Urine frequency	0.6 (0.90)	0.1 (0.75)	0.96 (0.80)
Urine volume	2.1 (0.55)	1.45 (0.22)	3.71 (0.29)

df = degrees of freedom. (*P*-values)

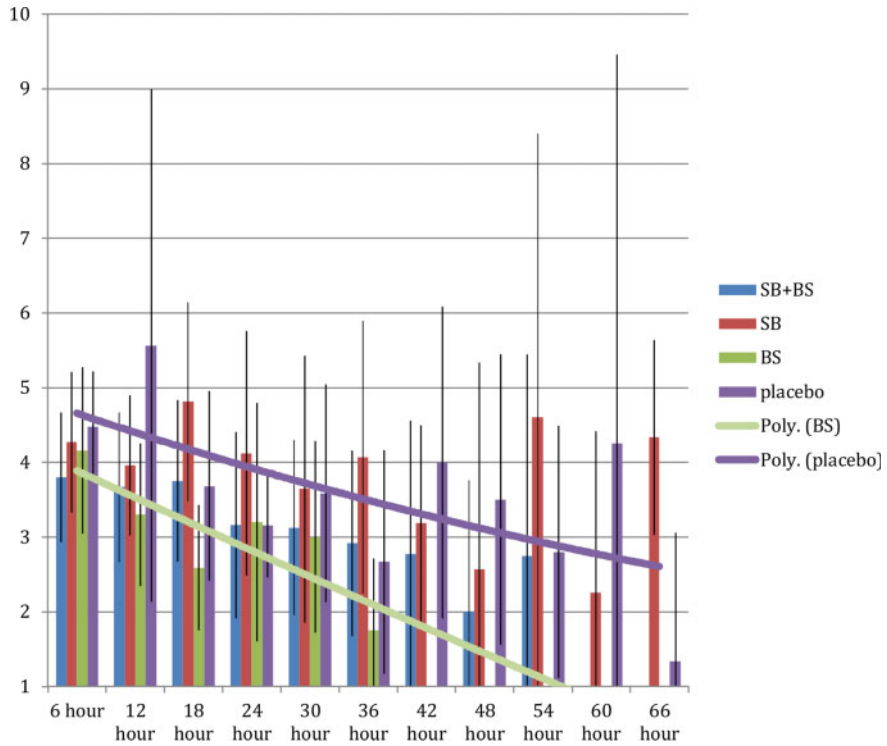

Figure 2 The mean number of stools for each treatment arm reported every 6 hours until there were less than three study subjects remaining in the arm for that time point. A two order polynomial trendline is recorded for the placebo arm and the bismuth subsalicylate (BS) arm. Error bars show the 95% confidence interval with an alpha of 0.05. SB = *S. boulardii*

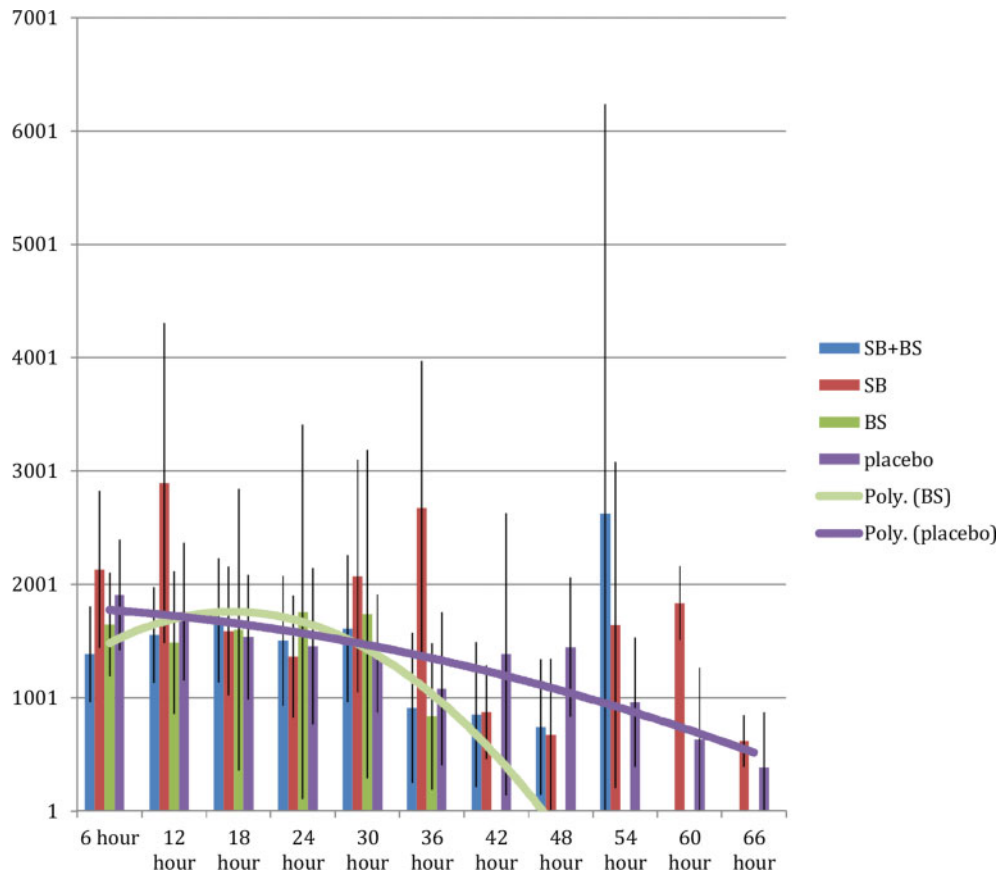

Figure 3 The mean volume of stool in millilitres for each treatment arm reported every 6 hours until there were less than three study subjects remaining in each arm for that time point. A two order polynomial trendline is recorded for the placebo arm and the bismuth subsalicylate (BS) arm. Error bars show the 95% confidence interval with an alpha of 0.05. SB = *S. boulardii*

Table 4 Results of a regression model for each response variable to determine if there is an affect on length of study participation

Outcome	Chi-Square (*p*-value) Treatment (df = 3)
Stool frequency	0.41 (0.9382)
Stool volume	4.66 (0.1981)
Emesis frequency	6.37 (0.0949)
Emesis volume	2.01 (0.5707)
Urine frequency	1.76 (0.6238)
Urine volume	0.99 (0.8035)

stool was mixed with urine. Our study population was not as sick as we had initially anticipated and our subjects did not remain in the study for as long as we originally expected. The study was underpowered to determine a smaller effect of our treatment interventions on the duration and severity of cholera

Conclusion

While BS and *S. boulardii* appear safe in cholera patients, our study does not support their use as adjunct treatments. Our study was underpowered to identify smaller effects of these treatments on the duration and severity of cholera.

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Disclaimer Statement

Contributors

Johnathan Sheele: study PI, developed protocol, reviewed data, assisted with data analysis, wrote manuscript. Jessica Cartowski: assisted with protocol development, assisted with data analysis, reviewed manuscript. Angela Dart: assisted with protocol development, oversaw involvement of Operation Blessing in this project, reviewed manuscript. Arjun Poddar: assisted with data analysis. Shikha Gupta: assisted with obtaining data and reviewed manuscript. Eric Stashko: assisted with protocol

development assisted with data analysis. Bhaskara S. Ravi: assisted with data analysis. Crawford Nelson: assisted with data collection. Ajay Gupta: assisted with biostats and mechanism to obtain informed consent. also reviewed of manuscript.

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Conflicts of interest

The authors report no conflict of interests.

Ethics approval

We received institutional review board (IRB) approval from Eastern Virginia Medical School (11-04-FB-0097). We were unable to identify a local ethical review board affiliated with St. Luc hospital in Port-au-Prince, Haiti, so administrators at both St. Luc and Operation Blessing International reviewed and approved the study.

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