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A Pilot Randomized Trial to Determine the Tolerability of a Probiotic in Patients Colonized with Vancomycin-Resistant Enterococcus

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Abstract

Background: Vancomycin-resistant enterococcus (VRE) is an organism of major concern in hospital settings because of transmission in healthcare facilities.

Purpose: To examine the feasibility and tolerability of a probiotic, VSL#, to reduce colonization among subjects at risk of VRE infection.

Methods: Randomized double blind placebo-controlled trial.

Results: Fifty subjects were enrolled and randomized. Over half of the subjects were solid organ transplant recipients and/or immune compromised. The probiotic was well tolerated in the study population except for minor side effects such as nausea and bloating. A 30% withdrawal rate in this population was found.

Conclusion: Probiotics were well tolerated in our study population of largely immune compromised subjects with multiple comorbidities. Adherence to the intervention was low but not unexpected due to complexity of the of the study population. Future studies should examine ways to improve adherence to probiotics and subject retention in treatment trials in immune compromised patients.

Keywords: Vancomycin-resistant enterococcus (VRE); Probiotics; Immuno compromised patients; Healthcare-associated infection (HAI); Methicillin-resistant *Staphylococcus aureus* (MRSA); Standard deviation (SD)

Introduction

Vancomycin-resistant enterococcus (VRE) is a major healthcare associated pathogen [1]. Data from the National Healthcare Safety Network at the Centers for Disease Control and Prevention show that in 2009-2010, 13.9% of bacterial healthcare-associated infections (HAIs) were caused by Enterococcus species, including VRE. Ninety percent of VRE are *E. faecium* species [2]. Infection by VRE typically begins by colonization in the gastrointestinal tract; prospective studies have shown that some patients colonized with VRE are far more likely to develop VRE infection than patients not colonized with VRE [3]. Thus, while enterococci are gut commensals, the adverse consequences of VRE infection means that prevention or eradication of colonization by VRE should be explored for prevention of invasive infection with these multidrug resistant bacteria [3]. Currently, there are no available treatments for reducing VRE colonization in the GI tract. Colonization by VRE is facilitated when the microbiome is perturbed due to antibiotic use, surgery, transplantation or hospitalization. Thus restoring the normal gut microbiome may be important in reducing colonization by VRE.

Probiotics are a potential promising means of restoring microbiome balance, reducing VRE colonization and subsequent infection. In animal and human studies, probiotics have been shown to inhibit intestinal colonization by pathogenic bacteria [4,5], both in the intestine and at sites distant from the intestinal tract. This is due to the probiotic bacteria lowering the luminal pH [4] or production of biosurfactants [5]. In addition, probiotics have been shown to decrease time required to restore the normal flora after infection with *Campylobacter jejuni* [6]. However, little research is available on the acceptability and tolerance in transplant and other immunocompromised populations-which are disproportionately vulnerable to VRE colonization and infection [7]. We undertook a pilot randomized trial to examine the feasibility and tolerability of a probiotic, VSL#, containing a combination of 8 strains of bacteria: *Bifidobacterium breve*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, *Lactobacillus plantarum*, *Bifidobacterium infantis*, *Lactobacillus casei*, *Streptococcus thermophilus*, and *Lactobacillus bulgaricus*. This probiotic was chosen because it is well tolerated in non-transplant subjects with pouchitis and ulcerative colitis [8,9], is used extensively for prevention for pouchitis, includes well characterized strains and has the ability to increase the diversity of gut microbiota [10] which may offer resistance against colonization by antibiotic resistant bacteria, though this is not known.

Materials and Methods

Setting: The University of Wisconsin Hospital is a 566 bed tertiary care, academic medical center, including a large solid organ transplant program. No systematic screening for VRE is undertaken.

Trial Design: This was a randomized double blind placebo-controlled trial. The trial was registered on clinicaltrials.gov (#NCT00933556). Subjects were enrolled between October 2008 and April 2010. Potential subjects included women and men ≥ 18 years that tested positive for VRE according to hospital microbiology laboratory census list and were not currently receiving antimicrobial therapy at the time of enrollment. Subjects at high risk of colonization for VRE were also eligible and screened by research personnel to determine colonization status after informed consent. Patients at high risk were defined as any of the following: having current hospitalization or hospitalization within the last 2 years, solid organ transplant recipient, history of having been colonized by VRE or methicillin-resistant *Staphylococcus aureus* (MRSA) in the last 5 years, bone marrow transplant recipient or hematologic malignancy, hemodialysis patients, colonization by other drug resistant bacteria, or *Clostridium difficile*. Subjects with an active infection were excluded. Other exclusion criteria included pregnancy, the inability to take oral medications, inability to follow up in clinic, and those already taking probiotics.

Approval was received from the University of Wisconsin-Madison Institutional Review Board and written informed consent was obtained from all participants prior to enrollment. Subsequently, enrolled subjects were randomized to either VSL#3 (Sigma-Tau Pharmaceuticals, Inc., Gaithersburg, MD) or placebo taken orally provided by the manufacturer of VSL#3.

Baseline information was collected including demographic data, details of MRSA colonization and comorbid illnesses. VSL#3 and placebo were stored at the University of Wisconsin Hospital pharmacy which dispensed capsules according to the randomization schedule. Subjects and investigators were blinded to the treatment assignment.

Intervention: Participants were randomized to receive VSL#3 or placebo-one packet once daily for 28 days. Active sachets contained 450 billion live probiotic bacteria. Subjects were instructed to open the packets of probiotic/placebo and dissolve the contents in a cold or room temperature liquid once daily. Subjects were provided enough packets for 28 days.

Study Procedures: Study medication was started following randomization day and taken daily for 28 days. During the study, subjects were contacted twice per week encourage compliance, to remind subjects to submit specimens, and to collect information on potential adverse effects. Subjects were determined to be free from bacteremia unless they developed symptoms of an infection. Therefore, blood cultures were not routinely drawn. To determine factors that might affect tolerability of the probiotic, baseline data included comorbidities, recent antimicrobial use, and admission to a healthcare institution, surgical procedures, and invasive devices.

Subjects returned for follow-up after 4 weeks for perirectal swabs for detection of VRE and lactobacilli (perirectal only) (Becton Dickinson, Sparks, MD). If possible, a stool sample was obtained at that time. Alternatively, subjects could mail their stools to the laboratory. Though researchers were blinded to the intervention groups, compliance to the study was measured by counting capsules and laboratory identification of colonies of one or more species in VSL#3 from the subject's stool at the four-week time point.

Specimens were kept refrigerated or on ice packs until testing could be performed. We also undertook procedures to identify vancomycin-resistant enterococci from perirectal swabs or stool specimens using bile esculin azide broth (Remel, Lenexa, KS). After incubating aerobically overnight at 37°C broths indicating esculin hydrolysis were plated onto bile esculin azide agar with 6 mg/L vancomycin (Remel, Lenexa, KS). VRE was identified using Gram stain, catalase, PYR (Remel, Lenexa, KS) and E-Test susceptibility (Bio-Merieux, Marcy l'Etoile, France) [11].

Outcomes: The major outcomes were to determine the feasibility, and tolerability of a probiotic. Other outcomes included detection of VRE colonization at four weeks.

Statistical analysis: Means and standard deviations (SDs) or frequencies and percentages were used to summarize subject characteristics. Fisher's exact test or chi-squared tests were used to assess variable between study groups. To assess continuous variables, two-sample t-tests were used. All reported P values were two-sided, and a type I error level of 5% was used. Analyses were per protocol and evaluable patients were defined as those who completed at least half of the study medication. All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, NC, USA, 2007).

Results

Demographic Information: Between October 2008 and April 2010, 50 subjects were enrolled and randomized. Twenty-six of the 50 subjects were randomized to VSL#3 and 24 to placebo. Baseline characteristics of the study populations are shown in Table 1. A majority of subjects were ambulatory and 64% were transplant recipients. Over half of the subjects were female. Of the 50 subjects initially recruited, 14 withdrew, finished less than half of their study medication, or were unreachable (seven from each group) and two later died of causes unrelated to the study. Subjects took the study drug for a mean of 25.8 days (SD=2.3) in the probiotic group and 26.9 days (SD=1.6) in the placebo group. Yogurt consumption was also reported at the 4-week time point among 7/19 (37%) subjects in the probiotic group versus 3/17 (18%) subjects in the placebo group.

Variable	Placebo, all subjects (n=24) (%)	Placebo, minus withdrawals (n=17)	VSL#, all subjects (n=26)	VSL#, minus withdrawals (n=19)
Male	11 (46)	11 (65)	10 (38)	7 (37)
Average age (SD=Standard deviation)	51 (SD: 17.21)	51 (SD: 19.8)	58 (SD: 10)	57 (SD: 9.4)
Diabetes	17 (71)	11 (65)	15 (58)	13 (68)
Transplant	18 (75)	13 (76)	14 (16)	12 (63)
Cancer treatment (chemotherapy)	0 (0)	0 (0)	2 (8)	2 (11)
Coronary artery disease	3 (13)	1 (6)	8 (31)	8 (42)
Immunosuppression	18 (78)	14 (82)	16 (62)	14 (74)
PVD	3 (13)	1 (6)	5 (19)	4 (21)
Malnutrition	5 (22)	4 (24)	7 (29)	6 (32)
Renal failure	9 (40)	6 (35)	6 (23)	5 (26)
Vascular Catheter	5 (23)	4 (24)	3 (12)	3 (16)

Withdrawals or finished less than half of study meds in the first 4 weeks (based on total enrollment. 24 randomized to placebo, 26 to Vsl#3)	7 (29)	N/A	7 (27)	N/A
Withdrawals after 4 weeks	1 (6)	N/A	1 (5)	N/A
Selected reasons for withdrawal	Problems coordinating study medication, side effects, incarceration, too busy, changed mind	N/A	Occurrence of clinical illness, nausea, diarrhea, particating in another study	N/A

Table 1: Characteristics of Study Participants at Baseline (n=50) with changes due to withdrawals.

Outcomes and monitoring of compliance with study medication: Subjects who completed at least half of the study medication (the first 2 weeks) and those who did not withdraw until after the first four weeks of the study were evaluated. In the intervention group, 19/26 (73%) subjects completed at least the first half of probiotic treatment. Among these 19 subjects, the average number of pills missed was 2.3 (range 1-7). Data was unavailable for 4 subjects, 2 of whom withdrew. In the placebo group, 17/24 (71%) completed 4 weeks of the study, taking the placebo for an average of 27 days. Subjects missed an average of 1 pill (range 1-5). Pill count data was unavailable for 9 participants, 3 of whom withdrew from the study.

VRE colonization: Given that our inclusion criteria allowed subjects with a recent history of VRE, data on VRE colonization at the time of study enrollment (baseline) is available only for the subjects who were actively screened for VRE colonization by a perirectal swab, which was a minority of the study population. In subjects completing at least half of assigned medication, VRE was detected at baseline in 4/17 (24%) subjects in the placebo group and 5/19 (26%) in the intervention group. At the 4-week time point, 6/17 (38%) subjects in the placebo group remained positive for VRE and 4/19 (21%) in the intervention group were positive (P=0.37).

Adverse effects: Overall, adverse effects were mild and not serious (Table 2). Nausea or vomiting and an unpleasant taste were the most common. One subject taking probiotic developed fever but it was unrelated to the probiotic.

*Variable	Placebo (n=17)	%	VSL#3 (n=19)	%
Fever	0	0	1	5
Cough	1	6	2	11
Nausea	0	0	2	11
Vomiting	0	0	0	0
Unpleasant Taste	1	6	2	11
Abdominal Pain	0	0	1	5
Other	1	6	0	0
Problems taking medication	0	0	0	0
Other adverse effects	3 (low blood sugar, even with insulin)	18	1 (constipation-like symptoms)	5
# of subjects who missed at least 1 dose	6	46	14	78
Average number of doses (days) taken (SD)	26.6 (SD: 1.7)	N/A	25.6 (SD: 2.3)	N/A
Average missed doses/ subject	1	N/A	2	N/A
Range of missed doses (# packets missed)	1-5	N/A	1-7	N/A

Table 2: Adverse Effects at 4 Week Time Point.

Discussion

In our pilot study, we found that most subjects who participated tolerated VSL#3 and there were no major adverse effects in our population of individuals with multiple comorbidities. Moreover, we found that we were able to successfully recruit the required number of subjects at our single site. We observed a 30% drop out rate which is useful data to have for sample size calculations for future probiotic intervention studies in this patient population. The withdrawal rate is not unexpected given that are important our study population consisted largely of solid organ transplant patients who have multiple comorbid illnesses and complicated treatment and medication schedules.

Tolerability of lactobacilli based probiotics has been demonstrated [12-15] and compliance of 75-85% has been observed [1,15] in studies of bacterial pathogens Furthermore, a two-year trial on the effects of VSL#3 on *C. difficile* associated diarrhea showed the probiotic was well tolerated though issues with adherence occurred, for reasons similar to our findings [15]. In a recent trial examining the acceptability and tolerance of probiotics for patients with carriage by Methicillin-resistant *Staphylococcus aureus*, we found that the chosen probiotic (*L. rhamnosus* HN001) was well tolerated [16].

The safety of probiotics deserves mention. When used in generally healthy adults or children, probiotics appear to have a good safety profile for a variety of indications. Tapiovaara et al. examined (*Lactobacillus rhamnosus* GG (LGG) alone or LGG in combination with *L. rhamnosus* Lc70, Propionibacterium freudenreichii JS, *Bifidobacterium lactis* BB1, or *Bifidobacterium breve* 99) used in six clinical trials and found that probiotic ingestion did not result in statistically significant differences in adverse events (AE) in different groups when compared to placebo. There was no difference between the intervention groups or for different probiotic combinations [17]. However, when used in immune compromised or critically ill populations, the safety profile of probiotics needs to be carefully examined both in pre-clinical and clinical studies because of disparate results regarding the safety profile. In a trial of an oral probiotics used for prevention of ventilator-associated pneumonia in the intensive care

unit, Morrow et al. found no adverse effects in their critically ill population [18]. Similarly, multiple trials of probiotics in pregnant women have found no major safety issues. However, other studies [19] show an increased risk of infectious and non-infectious complications with probiotics [20-22]; therefore, this issue is unresolved and needs careful attention. In our study, we did not identify major AEs among the patients who remained in the study; it is possible that those who withdrew may have experienced AEs that remained undetected.

Our study had several limitations. Given that this was a pilot study, we were mainly interested in tolerability, feasibility of recruitment, and retention. Some subjects forgot their pills at home when re-hospitalized or subjects did not receive pills upon discharge, leading to occasional interruptions in treatment. In addition, recruitment took longer than expected for this study, largely because our facility is a referral center; patients travelled great distances and were reluctant to return only for research reasons. Transplant recipients proved to be a challenging study population in that 6/19 and 3/17 in the probiotic and placebo groups respectively, were already hospitalized when enrolled or required hospitalization during the study, for reasons unrelated to the study. This made tracking of medication adherence challenging, though weekly phone calls mitigated this to some extent. Though the probiotic population did not have a significantly higher rate of illness than the placebo group, they had a higher rate of missed pills. This could be explained by chance in a small study population.

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