Tolerability of a probiotic in subjects with a history of methicillin-resistant Staphylococcus aureus colonisation

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Received: 17 October 2013 / Accepted: 3 April 2014 © 2014 Wageningen Academic Publishers

Abstract

Methicillin-resistant Staphylococcus aureus (MRSA) is a pathogen of major public health importance. Colonisation precedes infection; thus reducing MRSA carriage may be of benefit for reducing infection. Probiotics represent a novel approach to reducing MRSA carriage. We undertook a pilot feasibility randomised controlled trial of the tolerability and acceptability of probiotics for reducing nasal and intestinal carriage of MRSA. In addition, subjects were screened for vancomycin-resistant enterococci (VRE). Subjects with a history of MRSA were recruited from a large, academic medical center and randomised to take either a placebo or probiotic (Lactobacillus rhamnosus HN001). Subjects returned to the clinic after four weeks for further testing to determine adherence to the probiotic regimen and colonisation of MRSA. 48 subjects were enrolled and randomised. Nearly 25% were transplant recipients and 30% had diabetes. The probiotic was well tolerated in the study population though minor side effects, such as nausea and bloating, were observed. A majority of the subjects randomised to HN001 had good adherence to the regimen. At the four week time point among subjects randomised to the probiotic, MRSA was detected in 67 and 50% of subjects colonised in the nares and the gastrointestinal tract, respectively. Three subjects who initially tested positive for VRE were negative after four weeks of probiotic exposure. Probiotics were well tolerated in our study population of largely immunocompromised subjects with multiple comorbidities. Adherence to the intervention was good. Probiotics should be studied further for their potential to reduce colonisation by multidrug resistant bacteria.

Keywords: MRSA, probiotics, Lactobacillus rhamnosus HN001

1. Introduction

70% of healthcare-associated infections (HAIs) are caused by antimicrobial resistant bacteria (Fraimow and Abrutyn, 1995; Gold and Moeller, 1996; Maki, 1989; Virk and Steckelberg, 2000). Methicillin-resistant Staphylococcus aureus (MRSA) is one of the most important multidrug resistant organisms causing HAIs, which are associated with prolonged hospitalisation, readmissions, costs and mortality (Emerson et al., 2012; Hidron et al., 2008; Landrum et al., 2012; Maranan et al., 1997). Data from the National Healthcare-associated Infection Surveillance Study (now the National Healthcare Safety Network (NHSN)) of the Centers for Disease Control and Prevention show that in 2002, healthcare-associated infections in intensive care unit (ICU) patients caused by MRSA in the nearly 300 member US hospitals participating in this long-term, nation-wide surveillance network had increased to 57.1% of all S. aureus infections (Anonymous, 1999). Between 1998 and 2002, MRSA was the most common cause of skin and soft tissue infections in the community, resulting in increased antimicrobial usage, morbidity and mortality. MRSA remains a major challenge both in the healthcare setting but also, increasingly in community acquired skin and soft tissue infections (Hidron et al., 2008; Kallen et al., 2010; Landrum et al., 2012).
Several studies have found that colonisation is a prerequisite for infection with MRSA in the hospital environment (Garrouste-Orgeas et al., 2001; Von Eiff et al., 2001). Approximately 2-7% of patients admitted to acute care hospitals are colonised with MRSA at admission while 2-10% acquire it during their hospital stay in the nares, intestine, axilla or groin (Fishbain et al., 2003; Marshall et al., 2003). Between 30 to 50% of patients colonised with MRSA will go on to develop invasive infection with this organism; the risk of infection is higher with colonisation by MRSA compared with methicillin-sensitive S. aureus (Chang et al., 1998; Corbella et al., 1997; Davis et al., 2004; Huang and Platt, 2003; Pujol et al., 1996). Recent studies show that colonisation may be present at multiple sites with the intestinal tract an important reservoir in addition to the anterior nares (McKinnell et al., 2013).

Prevention of colonisation is the first step toward prevention of invasive infection with MRSA. Mupirocin may be of use in the short term, such as preoperatively, to eradicate nasal carriage but selection for mupirocin-resistant MRSA remains a concern (Dupeyon et al., 2002; Fujimura and Watanabe, 2003; Kampf and Kramer, 2004; Loeb et al., 2003). Chlorhexidine bathing may reduce the microbial burden of organisms, including MRSA, on the skin but does not address the multiple other sites, such as the gastrointestinal tract, which may be colonised by MRSA.

Probiotics containing strains of lactobacilli or bifidobacteria represent a novel approach to the prevention and control of antimicrobial resistance. In animal and human studies, probiotics have been shown to inhibit intestinal colonisation by pathogenic bacteria, both in the intestine and at sites distant from the intestinal tract (Alm, 1983; Asahara et al., 2004; Gan et al., 2002; Gluck and Gebbers, 2003; Rayes et al., 2002; Tojo et al., 1987). Therefore, we attempted to examine the tolerability and feasibility of an oral probiotic, Lactobacillus rhamnosus HN001, by recruiting inpatients and outpatients at our institution that were previously colonised by MRSA.

2. Materials and methods

Setting

The University of Wisconsin Hospital and Clinics (UWHC) is a 566 bed tertiary care academic medical centre, and has a large solid organ transplant program. No systematic screening for MRSA colonisation is undertaken and at the time of this study, preoperative decolonisation with mupirocin for MRSA colonised patients was not routinely performed.

Trial design

This was a randomised double blind placebo-controlled trial. The trial was registered on ClinicalTrials.gov and approval was received from the Institutional Review Board. Subjects were enrolled between December 2009 and September 2011. Potential subjects included women and men ≥18 years with a history of MRSA within the last five years and who had received no antimicrobial therapy in the seven days prior to study enrolment. Subjects with an active infection were not enrolled because they were likely to be receiving antibiotic therapy which could have negated the effect of the probiotic.

Subjects who were unable to take oral medication, pregnant, children, or unable to comply were excluded. In addition, those who currently received MRSA specific topical antimicrobial therapy, such as mupirocin, topical chlorhexidine, and tea-tree oil, or were on such therapy seven days prior were excluded as well.

Written informed consent was obtained from all participants prior to enrolment. Subsequently, enrolled subjects were randomised to either HN001 or placebo taken orally. Baseline information was collected including demographic data, details of MRSA colonisation and comorbid illnesses. HN001 and placebo were stored at the University of Wisconsin Hospital pharmacy which dispensed capsules according to the randomisation schedule. Subjects and investigators were blinded to the treatment assignment.

Intervention

Participants were randomised to receive HN001 or placebo – one capsule one time daily for 28 days. In an attempt to expose the nasopharynx to the probiotic without direct application, subjects were instructed to open the capsules, dissolve the contents in a cold or room temperature liquid, and drink the mixture. Active capsules contained L. rhamnosus strain HN001 (DuPont, Madison, WI, USA) in the amount of 1.0×10^{10} colony forming units (cfu)/capsule. Bacterial counts were verified monthly and ranged from 4.3×10^4 cfu/ml down to 3×10^3 cfu/ml with an average of 6.8×10^9 cfu/ml. There was a decline in colony counts over the course of the study.

Study procedures

Study medication was started following randomisation day. During the study, subjects were contacted twice per week to 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 drawn.
To determine factors that might affect tolerability of the probiotic, baseline data included comorbidities, recent antimicrobial use, admission to a healthcare institution, surgical procedures, and invasive devices.

Subjects returned to the clinic after four weeks for nasal and perirectal swabs for detection of MRSA and lactobacilli (perirectal only) (BD, Sparks, MD, USA). If possible, a stool sample was obtained at that time. Alternatively, subjects could mail their stools and nasal swabs to the laboratory. Though researchers were blinded to the intervention groups, compliance to the study was measured by counting capsules and laboratory identification of _L. rhamnosus_-like colonies from the subject’s stool at the four week time point. In addition, PCR specific for HN001 was performed on isolates obtained at this time from control and probiotic subjects.

Specimens were kept refrigerated or on ice packs until testing could be performed. MRSA was detected from the nasal swab and perirectal swab or stool using tryptic soy broth with 6.5% sodium chloride for enrichment (Remel, Lenexa, KS, USA, and Fisher Scientific, Fair Lawn, NJ, USA) (Safdar et al., 2003). After incubating aerobically overnight at 37°C, this was plated onto mannitol salt agar containing 4 mg/l cefoxitin (Sigma, St. Louis, MO, USA) (Smyth and Kalameter, 2005). _S. aureus_ was identified using Gram stain, catalase, and coagulase tests (BD) (Forbes et al., 1998). Using breakpoints from the Clinical and Laboratory Standards Institute (CLSI, 2006, 2007), MRSA was identified via Kirby-Bauer disc diffusion susceptibility testing using cefoxitin discs (BD). We also undertook procedures to identify vancomycin-resistant enterococci (VRE) from perirectal swabs or stool specimens using bile esculin azide broth (Remel). After incubating aerobically overnight at 37°C, brothss indicating esculin hydrolysis were plated onto bile esculin azide agar with 6 mg/l vancomycin (Remel). VRE was identified using Gram stain, catalase, PYR (Remel) and E-Test susceptibility (Bio-Merieux, Marcy l’Etoile, France) (AB Biodisk, 2005; CLSI, 2007; Smyth and Kalameter, 2005).

To detect _L. rhamnosus_, serial dilutions were made of 0.1 g stool and plated onto De Man, Rogosa and Sharpe (MRS) agar (Difco, Sparks, MD, USA) (Hedberg et al., 2008). Plates were incubated anaerobically at 37°C for at least 48 h. Colonies appearing morphologically similar to HN001 were further identified using Gram stain, catalase, and PCR. Isolates were frozen in trypticase soy broth + 20% glycerol (BD) at -80°C. For PCR, frozen isolates were grown anaerobically at 37°C for at least 48 hours in 2-5 ml of MRS broth and plated onto MRS agar once growth appeared. One to two colonies were transferred from MRS agar to 50 µl nuclease-free water (Promega, Madison, WI, USA) and boiled for 10 min to lyse the cells. PCR specific for _L. rhamnosus_ was performed using phosphoglucone isomerase (_pgi_) gene primers (forward: CTCAATCTCCGCTTTACTTAGCTGAT and reverse: CAGCAACTGCATTGGCAATAAG). Those isolates positive for _L. rhamnosus_ _pgi_ were further screened against primers for the clustered regularly interspaced short palindromic repeats (CRISPR) region specific for HN001 (forward: TGTATGTAAGCTTACGTGTCAGTC and reverse: CCTAGACGTTCGCTTTGAT). Primers were obtained from IDT DNA (Coralville, IA, USA). The reaction mixture consisted of 12 µl nuclease free water (Promega), 10 µl ExTaq Takara Premix (Takara, Bio Inc., Otsushiga, Japan), 1 µl of each primer (each at 20 µM) and 1µl DNA. The running conditions were: 5 min at 95°C, followed by 30 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s, followed by a 5 min 72°C final extension. Product DNA was amplified using an Applied Biosystems 2720 Thermal Cycler (Foster City, CA USA) and detected on a 1.2% FlashGel (Lonzza, Rockland, ME, USA). Reaction mix and running conditions for the CRISPR PCR were the same as those for _pgi_ with the following exceptions: 13 µl water and 0.25 µl each primer was added to the reaction mixture. The annealing temperature was 54°C.

### Outcomes

The major outcomes were to determine the feasibility and tolerability of a probiotic. Other outcomes included detection of MRSA and VRE colonisation at four weeks.

### Statistical analysis

Means and standard deviations (SDs) or frequencies and percentages were used to summarise 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 1 error level of 5% was used. Analyses were intention to treat. All statistical analyses were carried out using SAS (SAS Institute Inc., Cary, NC, USA).

### 3. Results

#### Demographic information

Between December 2009 and September 2011, several hundred subjects were screened and ultimately, 48 subjects were enrolled and randomised as shown in Figure 1. 317 subjects cited other reasons to decline participating in the study. Many of these subjects lived a great distance from the facility, and were unwilling to travel for follow up. 24 of the 48 subjects were randomised to HN001 and 24 to placebo. Only one subject (in the probiotic group) was an inpatient.

Baseline characteristics of the study populations are shown in Table 1. All subjects were ambulatory and 23% were transplant recipients. Half of the subjects were men. Of the 48 subjects, five withdrew or were unreachable (four from...
In the intervention group, 20/24 (83%) subjects took the drug for an average of 27 days after enrolment. The average number of pills missed was 1.0 (range 1–11). Data was unavailable for four subjects, two of whom withdrew. In the placebo group, 15/24 (63%) took the drug for an average of 2.9 days after enrolment. The average number of pills missed was 3.1 (range 1–14). Pill count data was unavailable for nine participants, three of whom withdrew from the study. Lactobacillus was detected via culture in 11/21 (52%) placebo subjects at four weeks, though none were positive for HN001 by PCR. Among the probiotic group, Lactobacillus colony morphology was identified in 14/22 (64%) subjects. Subsequent PCR indicated 5/22 (23%) subjects took the drug at the four week time point. For those subjects, the quantitative counts of HN001 ranged from 1.4×10⁶ to 4.0×10⁶ cfu/g stool.

### Outcomes

Baseline and follow up MRSA carriage (Table 2): in the control group, MRSA was detected in the gastrointestinal tract (GIT) in 4/24 (17%) and in the nares of 6/24 (25%) subjects at baseline. In the HN001 group, 5/24 (21%) and 8/24 (33%) were colonised in the GIT and nares, respectively. Using intention to treat analysis, MRSA nasal carriage was detected in 5/21 (24%) in subjects in the HN001 group and

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**Figure 1. Enrolment process and follow-up of subjects.**

**Table 1. Characteristics of study participants at baseline.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HN001, n (%)</th>
<th>Placebo, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>11/24 (46)</td>
<td>16/24 (67)</td>
</tr>
<tr>
<td>Average age (±SD)¹</td>
<td>53.9 (±12.4)</td>
<td>54.4 (±12.0)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>7 (29)</td>
<td>8 (33)</td>
</tr>
<tr>
<td>Transplant</td>
<td>6 (25)</td>
<td>5 (21)</td>
</tr>
<tr>
<td>Cancer treatment</td>
<td>3 (13)</td>
<td>3 (13)</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>5 (21)</td>
<td>7 (29)</td>
</tr>
<tr>
<td>Immunosuppression</td>
<td>7 (30)</td>
<td>7 (30)</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>1 (4)</td>
<td>2 (8)</td>
</tr>
<tr>
<td>Malnutrition</td>
<td>2 (8)</td>
<td>2/23 (9)²</td>
</tr>
<tr>
<td>Renal failure</td>
<td>6 (25)</td>
<td>7 (30)</td>
</tr>
<tr>
<td>Vascular catheter</td>
<td>5 (21)</td>
<td>1 (4)</td>
</tr>
</tbody>
</table>

1 SD = standard deviation.  
2 Malnutrition data not available for all subjects.
Assessing the tolerability of a regimen of probiotics

in 3/21 (14%) in the placebo group at the four week time point. MRSA in the GIT was detected in 4/22 (18%) in the intervention group and 1/22 (5%) in the placebo group. In those subjects who had MRSA nasal carriage at baseline, 4/6 (67%) were positive in the treatment group at four weeks and 3/6 (50%) were positive in the placebo group at four weeks in the nares. In the gastrointestinal MRSA carriage group, 2/4 (50%) were positive in the treatment group at four weeks and 1/4 (25%) positive in the placebo group.

Vancomycin-resistant enterococci carriage

At baseline, VRE was detected in 4/24 (17%) subjects in the placebo group and 3/24 (13%) in the intervention group. At the four week time point, 2/22 (10%) subjects in the placebo group remained positive for VRE and 0/23 (0%) in the intervention group were positive ($P=0.10$).

Table 2. Colonisation at baseline and four week time point.

<table>
<thead>
<tr>
<th>Variable</th>
<th>HN001 Baseline, n (%)</th>
<th>Four week, n (%)</th>
<th>Placebo Baseline, n (%)</th>
<th>Four week, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA (nasal)</td>
<td>8/24 (33)</td>
<td>5/21 (24)²</td>
<td>5/24 (21)</td>
<td>3/21 (14)⁴</td>
</tr>
<tr>
<td>MRSA (gastrointestinal)</td>
<td>5/24 (21)</td>
<td>4/22 (18)³</td>
<td>4/24 (17)</td>
<td>1/22 (5)⁵</td>
</tr>
<tr>
<td>VRE gastrointestinal</td>
<td>3/24 (13)</td>
<td>0/23 (0)</td>
<td>4/24 (17)</td>
<td>2/22 (10)⁶</td>
</tr>
</tbody>
</table>

¹ MRSA = methicillin-resistant Staphylococcus aureus; VRE = vancomycin-resistant enterococci.
² 1 subject negative at baseline, 4 subjects previously positive at baseline. Follow-up not available for 2 subjects who were previously positive.
³ 1 subject negative at baseline, 1 subject previously positive in nares, 2 subjects positive gastrointestinal at baseline.
⁴ All 3 subjects positive at baseline. Follow-up not available on 2 subjects who were positive at baseline.
⁵ 1 subject positive at baseline.
⁶ Both subjects were positive for VRE at baseline. Follow-up not available on additional subject who was positive at baseline.

Adverse effects

No subjects in either group developed bacteremia (assessed via phone call as blood cultures were not drawn in asymptomatic subjects). The probiotic was, in general, well tolerated. Mild nausea and abdominal bloating were the major side effects reported (Table 3).

4. Discussion

In our pilot study, we found that most subjects who participated tolerated *L. rhamnosus* HN001 well and there were no major adverse effects in our population of individuals with multiple comorbidities. Moreover, we found that we were able to recruit and retain a majority of the subjects and had a high rate of compliance, particularly among those randomised to the probiotic. These are important findings because our study population contained several transplant patients who have multiple comorbid illnesses and many competing demands on their time. Additional randomised placebo controlled trials on *L. rhamnosus* and other probiotics support our findings, though none focused specifically on using HN001 in subjects with a history of MRSA. Tolerability of these other lactobacilli based probiotics has been demonstrated (Barraud *et al.*, 2010; Drago *et al.*, 2011; Roos *et al.*, 2010; Wickens *et al.*, 2008) and compliance of 75-85% has been observed (Drago *et al.*, 2011; Wickens *et al.*, 2008).

Our study had several limitations. Given that this was a pilot study, we were mainly interested in tolerability, feasibility of recruitment, and retention. Subjects would forget their pills at home when re-hospitalised, leading to occasional interruptions in treatment. In addition, recruitment took longer than expected for this study, largely because of UWHC is a referral centre; patients travelled great distances.

Table 3. Side effects at four week time point.¹

<table>
<thead>
<tr>
<th>Variable</th>
<th>HN001 group (%)</th>
<th>Placebo group (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>1/21 (4.8)</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td>Cough</td>
<td>3/21 (14.3)</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td>Nausea</td>
<td>1/21 (4.8)</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0/21 (0)</td>
<td>0/17 (0)</td>
</tr>
<tr>
<td>Unpleasant taste</td>
<td>0/21 (0)</td>
<td>1/17 (5.9)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0/21 (0)</td>
<td>1/17 (5.9)</td>
</tr>
<tr>
<td>Other²,³</td>
<td>5/21 (24)</td>
<td>1/17 (5.9)</td>
</tr>
<tr>
<td>Problems taking medication</td>
<td>0/20 (0)</td>
<td>0/14 (0)</td>
</tr>
</tbody>
</table>

¹ Data not available for all subjects.
² Other side effects for probiotic: (1) dry mouth; (2) cracked feet; (3) acid reflux; (4) fatigue; (5) slight headache; (6) loose stool; (7) throat, chest, sinus, and ear congestion.
³ Other side effects for placebo group: dizzy, low blood pressure.
and were reluctant to return only for research reasons. Transplant recipients proved to be a challenging study population in that they required frequent hospitalisation, for reasons unrelated to the study, which made tracking of medication adherence challenging, though weekly phone calls mitigated this to some extent. Though the placebo population did not have a significantly higher rate of illness than the probiotic group, they had a higher rate of missed pills. This could be explained by chance in a small study population. Finally, though the probiotic was refrigerated in the UWHC pharmacy, this may not have been the case in subject homes. Furthermore, we saw a decrease in colony counts of the probiotic over time suggesting that viability during long-term storage might be an issue.

Though the study was not powered to assess differences in colonisation by MRSA and VRE in the treatment and placebo groups, we found that there was a trend toward reduced VRE colonisation. Fewer subjects in the treatment group tested positive for VRE at four weeks when compared with the placebo group. These findings are biologically plausible given that, unlike MRSA, the primary site of VRE colonisation is the intestinal tract. However, these findings need to be examined further in subsequent studies. These studies should examine probiotics for their impact on S. aureus colonisation. Adherence may be better tracked by using innovative methods, such as electronic monitoring caps, in addition to frequent communication by phone, email or text message. Finally, it is possible that for reducing nasal colonisation of S. aureus, a probiotic may need to be applied topically (Roos et al., 2010). This approach and effect of probiotic delivered in this manner deserves further study.

Acknowledgements

The authors wish to thank Buffy Stahl and the DuPont Nutritional Health team for sharing the PCR primers and conditions for identifying L. rhamnosus BN001. This research was funded by a New Investigator Award from the University of Wisconsin Partnership Program to Nasir Safdar.

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