Probiotic treatment of vancomycin-resistant enterococci: a randomised controlled trial

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ABSTRACT

Objective: To determine whether eating *Lactobacillus rhamnosus* GG (LGG) in the form of commercially available yoghurt improves clearance of vancomycin-resistant enterococci (VRE).

Design: Double-blind, randomised, placebo-controlled trial.

Setting: Renal ward of Austin Health, a tertiary hospital, Feb–Oct 2005.

Participants: 27 VRE-positive patients, 14 receiving active treatment and 13 controls.

Interventions: Subjects were randomly assigned to either a treatment group (receiving 100 g daily of yoghurt containing LGG for 4 weeks) or a control group (receiving standard pasteurised yoghurt). Faecal samples were obtained three times at about weekly intervals. Treated patients were tested for VRE again at 8 weeks. Patients in the control group who had failed to clear VRE after 4 weeks were then given LGG-containing yoghurt for 4 weeks, as an open continuation.

Main outcome measure: Number of faecal specimens clear of VRE.

Results: Of the 27 patients enrolled, 23 completed the study. Two patients were lost to follow-up, one died and one withdrew. All 11 patients in the treatment group who completed the study cleared VRE. Three subjects reverted to VRE positivity after using antibiotics to which LGG is sensitive, while all others remained negative for at least 4 weeks after trial completion. Twelve control subjects completed the study, of whom one cleared VRE and 11 remained VRE-positive. Eight of these 11 patients were subsequently crossed over to receive LGG yoghurt, and all cleared VRE within 4 weeks.

Conclusion: To our knowledge, this is the first description of a probiotic therapy to successfully treat gastrointestinal carriage of VRE in renal patients. Further investigation of the use of LGG in VRE-positive patients is warranted.

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Enterococci are normal flora of the gastrointestinal tract. Over the past 10–15 years, there has been a rapid increase in the prevalence of vancomycin-resistant enterococci (VRE).[1,2] The two most common species, *Enterococcus faecalis* and *E. faecium*, can harbour *vanA* and *vanB* genes, which encode resistance to vancomycin and have been implicated in the development of persistent, transmissible nosocomial infections that may be associated with poor outcomes.[3] The increasing prevalence of VRE has been associated with widespread use of broad-spectrum antibiotics and with cross-infection.[4] Because of the ability of VRE to transfer their antibiotic resistance factors to other microorganisms, and the threat of clinical infections with VRE in susceptible patient groups, prevention and control measures are critical.

Probiotics are living microbial food ingredients designed to have a beneficial effect on human health. As almost all strains of lactobacilli are resistant to vancomycin[5,6] and probiotics have been used with some success in preventing colonisation by enteric pathogens during treatment for *Clostridium difficile*,[7] we attempted to determine whether probiotics might reduce bowel colonisation by VRE.

METHODS

Subjects and testing protocol

Our hypothesis was that VRE-positive patients receiving *Lactobacillus rhamnosus* GG (LGG) would clear VRE from their stool within a 4-week period. We performed a randomised, placebo-controlled, blinded study in VRE-positive patients in the renal ward at Austin Health. Rectal swabs for VRE screening are routinely obtained once a week from patients in the renal ward. All patients with a positive VRE screening swab between February and October 2005 were invited to enrol in our study. Subjects were excluded if they were unwilling or unable to give informed consent, were unable to eat, or had received a renal transplant within the previous 3 months. They were then randomly allocated on a 1 : 1 basis to receive one of two commercially available yoghurts, one containing LGG (Vaalia yoghurt [Parmalat Australia]) and the other not (Bulla yoghurt [Bulla Dairy Foods]). Patient randomisation was based on randomised numbers prepared and kept by the School of Exercise and Nutrition Sciences, Deakin University, Melbourne, and obtained by phone call as each patient was allocated to a group.

A power calculation based on differences in proportions suggested that a total sample size of 30 was required to demonstrate a reduction in VRE carriage from 100% (only VRE-positive patients were enrolled) to 50%, with $\alpha = 0.05$ and power 0.80.

Over a period of 4 weeks, 100 g of either Vaalia or Bulla yoghurt was administered at breakfast each day. The yoghurt was placed in measured, unlabelled containers. Patients, nursing staff, medical staff and microbiology staff were blinded to the randomisation. Three faecal samples were obtained from all patients as close to weekly as possible (within 0–4 days) over 4 weeks, and another was obtained at about 8 weeks in patients receiving LGG-containing yoghurt. Patients in the control group who had failed to clear VRE after 4 weeks were then given LGG-containing yoghurt for 4 weeks, as an open continuation.

Yoghurt intake was recorded on fluid balance charts by nursing staff. All patients received standard VRE ward management, including isolation. Antibiotic use was monitored. Seven subjects were discharged from hospital, but all continued with the protocol. The primary endpoint of the study was VRE-colonisation status at the completion of the 4-week treatment period.

Microbiology cultures and clonality faecal cultures were directly inoculated onto Enterococcosel agar (BD, Sparks, Md, USA) containing 6 μg vancomycin. Any VRE present were then detected using previously published methods.[8]

Ethics approval

Our study was approved by the Human Research Ethics Committee of Austin Health.

RESULTS

Twenty-nine VRE-positive patients were invited to enter the study (Box 1), of whom one refused and one ceased dialysis. Patient characteristics at entry were similar. The majority were receiving one or more antibiotics immediately before entry to the study (Box 2). All but two patients had renal failure. These two belonged to other medical units, but were treated on the renal ward. Compliance with treatment was excellent, although compromised by "nil orally" orders.

VRE clearance

All 11 treatment-group subjects who completed the study cleared VRE — six within 1 week of consuming the LGG yoghurt, two within 2 weeks, and three within 3 weeks. At Week 8, 1 month after completion of the study, eight treatment subjects remained clear of VRE, while three patients had reverted to VRE positivity after receiving antibiotic treatment to which the probiotic was sensitive.

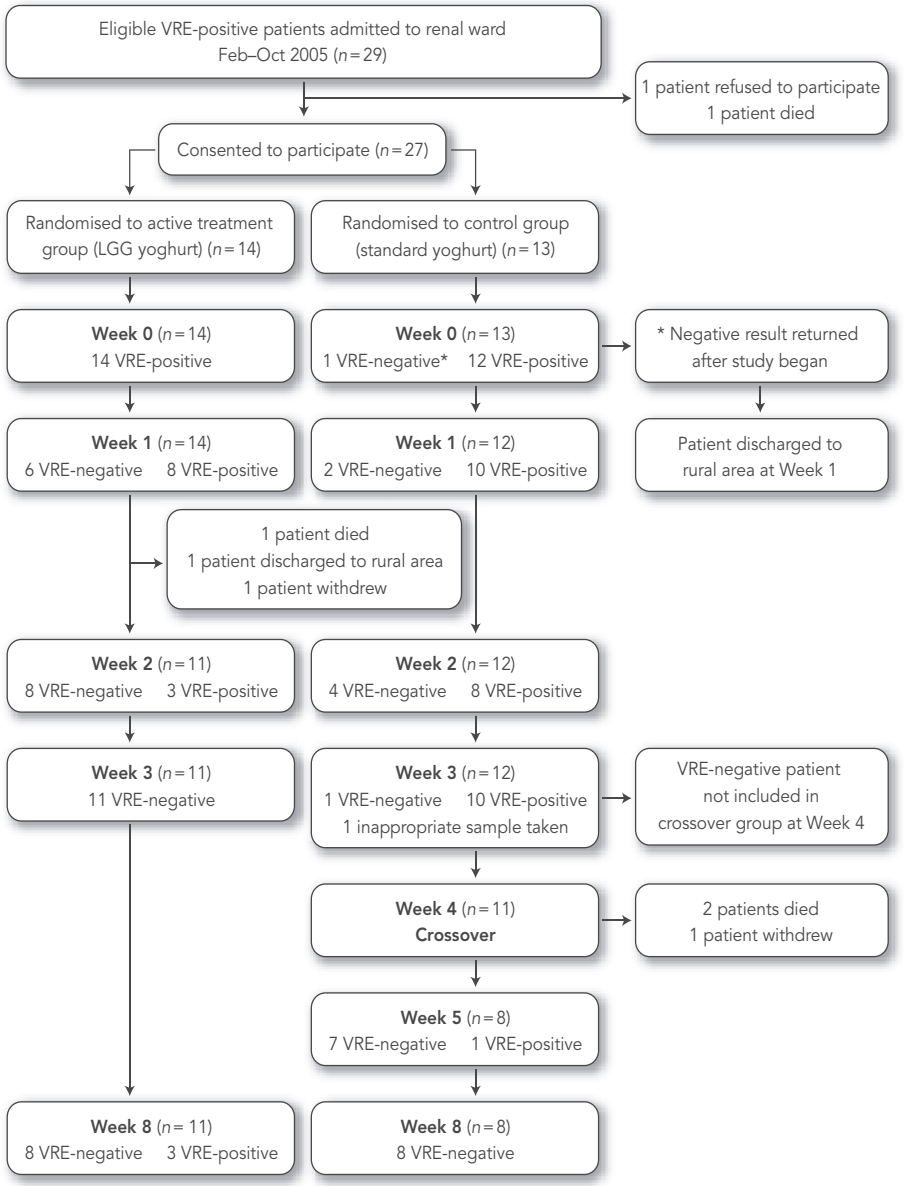
Of the 12 VRE-positive patients in the control group who completed the study, 10 remained positive at Week 4 and one had cleared VRE. (One patient was excluded from statistical analysis at Week 3 because a rectal swab instead of a faecal sample was inadvertently processed.) At the time of crossover to LGG treatment, two patients died before the first faecal sample was obtained and one declined to continue with the study. Of the eight remaining VRE-positive patients in the control group who were crossed over, seven cleared VRE within a week and one within 4 weeks.

The proportion of samples positive for faecal VRE at weekly intervals during the study is summarised in Box 3.

Antibiotic usage

There was greater antibiotic usage by patients in the LGG treatment group, with 10/14 receiving one or more antibiotics compared with 5/13 in the control group (Box 4). Two subjects were prescribed linezolid, which is effective against VRE, and both were in the LGG treatment group.

1 Participant flow diagram



LGG = *Lactobacillus rhamnosus* GG. VRE = vancomycin-resistant enterococci. ◆

DISCUSSION

Our study demonstrated a significant reduction in the detection of VRE in faecal specimens of patients receiving a probiotic yoghurt (containing LGG), whether given as initial treatment or following control treatment.

While all strains of lactobacilli are resistant to vancomycin, most do not survive stomach acid and duodenal bile acids in sufficient numbers to reach the bowel. However, live LGG bacteria can survive the stomach and bile acids to remain in sufficient numbers in the bowel. Thus, treatment with LGG has been proposed as a means of preventing overgrowth of bacteria that are resistant to the antibiotics used regularly in renal patients.[9,10] The LGG strains are resistant to vancomycin but susceptible to a broad range of other antibiotics.

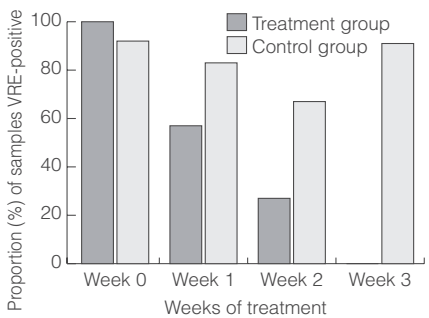
Potential mechanisms for the effectiveness of LGG in clearing intestinal infections include competitive colonisation, wherein LGG binds to the enteric epithelium and inhibits adhesion of pathogens such as *Escherichia coli* (a process that has been demonstrated in vitro).[11] Secondly, LGG may possess antimicrobial activity against VRE, similar to that of *L. ruminus* SPM0211,[12] although this has not been demonstrated. Thirdly, LGG may compete with VRE for consumption of monosaccharides, thereby slowing VRE growth. This mechanism has been shown to be partly responsible for the effectiveness of LGG against *C. difficile*.[13] Finally, lactobacilli produce short-chain fatty acids that lower the colonic pH and favour the growth of less pathogenic organisms.

2 Baseline characteristics of participants*

	Treatment group ($n = 14$)	Control group ($n = 13$)
Patients completing study	11	12
Mean age in years (range)	68 (46–84)	67 (28–88)
Male : female ratio	10 : 4	8 : 5
Renal status	5 CKD, 8 HD, 1 Tx	2 CKD, 8 HD, 1 Tx, 2 normal renal function
VRE type	12 VanB *Enterococcus faecium*, 2 VanB *E. faecalis*	13 VanB *E. faecium*
Antibiotic usage before study		
None	2	1
Glycopeptides	3	5
Penicillins	7	9
Carbapenems	4	0
Sulfonamides	1	1
Cephalosporins	3	3
Quinolones	2	1
Nitroimidazoles	0	2
Linezolid	1	0
Aminoglycosides	0	2

CKD = chronic kidney disease. HD = haemodyalysis. Tx = renal transplant. VRE = vancomycin-resistant enterococci. * Numbers represent number of patients, except where otherwise specified. ◆

3 Proportion of samples positive for faecal VRE at Weeks 0–3 ($n = 27$)*



VRE= vancomycin-resistant enterococci.
* There was a statistically significant difference between the two groups at Week 3 ($P < 0.001$, Fisher's exact test). ◆

4 Antibiotic usage during study

Antibiotic usage	Treatment group ($n = 14$)	Control group ($n = 13$)
None	4	8
Glycopeptides	1	3
Penicillins	4	3
Carbapenems	3	0
Sulfonamides	1	0
Cephalosporins	2	0
Quinolones	3	1
Rifamycins	0	1
Linezolid	2	0
Aminoglycosides	0	1
Nitroimidazoles	1	0

All patients taking no antibiotic or receiving antibiotics to which LGG was resistant cleared VRE. Three subjects reverted to VRE-positive status soon after the end of the study, after being prescribed an antibiotic to which LGG was sensitive, but none of these subjects had continued taking the probiotic yoghurt.

Studies have shown that no strains of *Lactobaccillus* possess the *vanA*, *vanB* or *vanC* gene, which provides some reassurance about the safety of LGG.[5] However, the safety of the probiotic in patients who are extremely unwell or immunocompromised is uncertain. There were no adverse effects of LGG detected in our study, but *Lactobacillus* bacteraemia has been described in severely ill patients with cancer, gastrointestinal disease or liver disease.[14,15]

While the sample size in our study was small, it was sufficient to demonstrate a significant difference between the treatment and control groups. We can not be sure whether absolute clearance of the organism occurred or whether the numbers of VRE were reduced to below the level of detection. This treatment approach to VRE clearance is only useful if the patient tolerates yoghurt. Concomitant use of antibiotics to which LGG is sensitive may negate the effect of the probiotic.

CONCLUSION

Our results suggest that the use of commercial yoghurt containing the probiotic LGG may be a worthwhile treatment for VRE colonisation, where there are few alternatives. Larger trials and further research are required to investigate the effectiveness of LGG in preventing primary infection, the kinetics of elimination, and factors associated with relapse in patients receiving antibiotics.

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COMPETING INTERESTS

None identified.

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