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ARTICLE

Effect of probiotics on multi-resistant organism colonisation in persons with spinal cord injury: secondary outcome of ProSCIUTTU, a randomised placebo-controlled trial

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Abstract

Study design Randomised double-blind placebo-controlled trial.

Objectives Multi-resistant organism (MRO) colonisation is common in people with SCI. We aimed to determine whether *Lactobacillus reuteri* RC-14 + *Lactobacillus* GR-1 (RC14-GR1) and/or *Lactobacillus rhamnosus* GG + *Bifidobacterium* BB-12 (LGG-BB12) are effective in preventing or clearing MRO colonisation.

Setting New South Wales, Australia.

Methods The 207 SCI participants were randomised to one of four arms: (i) RC14-GR1 + LGG-BB12, (ii) RC14-GR1 + placebo, (iii) LGG-BB12 + placebo or (iv) double placebos for 6 months. Microbiological samples of nose, groin, urine and bowel were taken at baseline, 3 and 6 months. Analysis was conducted for the presence of methicillin-resistant *Staphylococcus aureus* (MRSA), multi-resistant gram-negative organisms (MRGNs) and vancomycin-resistant enterococcus (VRE). The outcomes were clearance of, or new colonisation with MRSA, MRGN, VRE or MROs and whether participants remained free of MRSA, MRGN, VRE or MROs throughout the study. Risk factors associated with an outcome were adjusted for using nominal or binary logistic regression.

Results There was a significant reduction in new MRGN colonisation compared with placebo for participants treated with RC14-GR1 (OR 0.10, 95% CI, 0.01–0.88, $P = 0.04$), after allowing that inpatients were more likely to be newly colonised (OR 21.41, 95% CI, 3.98–115.13, $P < 0.0001$). Participants who intermittent self-catheterised (IMC) were more likely to remain MRO-free than those utilising SPC or IDCs (OR 2.80, 95% CI, 1.41–5.54, $P = 0.009$).

Conclusions Probiotics are ineffective at clearing MROs in people with SCI. However, RC14-GR1 is effective at preventing new colonisation with MRGNs. The use of IMC significantly improves the chance of remaining MRO-free.

Introduction

Antimicrobial resistance has been recognised by the World Health Organisation as a public health threat of global concern. The discovery of newer classes of antibiotics has not kept pace with the emergence of multidrug-resistant bacteria, which could lead to increased mortality due to untreatable

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infections [1]. Spinal cord injured (SCI) patients have a high prevalence of multi-resistant organism (MRO) colonisation or infection. Colonisation refers to the presence of MROs cultured from microbiology specimens without evidence of tissue invasion or inflammation at that body site. Infection refers to the invasion of the body tissues by microorganisms resulting in disease [2]. As there is no universally accepted definition of multi-resistance [3], for the purpose of this manuscript, it is defined as bacteria that are resistant to two or more commonly used antibiotics from different classes to which they would normally be susceptible [4].

It has been demonstrated that SCI patients are at high risk of MRO colonisation or infection resulting from prolonged hospitalisation, need for foreign instrumentation e.g. urinary catheterisation, tracheostomy, central venous access and the risk of readmissions to hospitals due to complications like pressure ulcers, urinary or respiratory tract infections [5, 6]. A prospective study by Mylotte et al. showed that 43% of inpatients with SCI in an acute rehabilitation unit carried one or more resistant organisms [7]. Waites et al. found that 33% of bacterial isolates in urine specimens of community-dwelling SCI patients were resistant to two or more classes of antimicrobial agents [8]. Suda et al. reported that methicillin-resistant *Staphylococcus aureus* (MRSA) and multi-resistant gram negatives (MRGNs) were significantly more frequent in SCI patients admitted to a veterans hospital compared with patients without SCI [9]. Our own unpublished data from a retrospective analysis of microbiological samples from all inpatients admitted over 2001–2007 at the Prince of Wales spinal unit found that 43% of acute SCI patients were colonised or infected with an MRO. MRSA was the most common MRO, identified in 34% of patients.

In 2007, the New South Wales (NSW) state-wide health service initiated an infection control policy for the management of MROs [4, 10]. Patients who were colonised or infected with the following organisms were deemed at high risk of transmitting the organisms to other patients, so appropriate isolation precautions were to be implemented:

- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Extended spectrum beta-lactamase producing Enterobacteriaceae (ESBL)
- Multi-resistant gram negatives (MRGN)
- Vancomycin-resistant *Enterococci* species (VRE)

ESBL are bacteria that can produce a beta-lactamase enzyme which hydrolyses penicillin and first, second or third generation cephalosporins, rendering them ineffective [11].

Probiotics are defined as "live organisms that, when administered in adequate amounts, confer a health benefit on the host" [12]. Probiotics have been postulated to have beneficial effects in controlling pathogenic bacteria. They possess the ability to improve intestinal barrier function thereby reducing the adherence of pathogenic bacteria. The organic acids which probiotics produce allow them to inhibit the growth of pathogenic bacteria by enhancing their ability to compete for resources within the gastrointestinal tract (competitive exclusion) [13]. There is some evidence that probiotics may have a role in preventing or eradicating MROs. The largest amount of evidence for probiotics concerns their role in preventing antibiotic-associated diarrhoea, based on a meta-analysis of many randomised controlled trials (RCTs) [13]. Manley et al. reported the clearance of VRE in stools after treatment with *Lactobacillus rhamnosus* GG (LGG) [14]. This trial was also supported by Szachta et al. who showed that the administration of LGG for 3 weeks in colonised children led to the clearance of VRE in stools for up to 4 weeks [15]. Several RCTs have shown that probiotics can assist in eradicating multi-resistant *Helicobacter pylori* in conjunction with antibiotics [16–18]. Another RCT showed that *Lactobacillus casei* delayed respiratory tract colonisation or infection by *Pseudomonas aeruginosa* [19].

We therefore performed a placebo-controlled RCT to investigate the effectiveness of combination oral probiotic therapy [*Lactobacillus reuteri* RC-14 + *L. rhamnosus* GR-1 (RC14-GR1) and/or *Lactobacillus* GG + *Bifidobacterium* BB-12 (LGG-BB12) capsules] in clearing MRO colonisation or infection in people with SCI.

Methods

ProSCIUTTU was a prospective multi-site randomised, double-blind, double-dummy, placebo-controlled factorial design trial conducted in the state of NSW in Australia. Prior to trial commencement, approval was granted from the lead human ethics committee covering the eastern seaboard of Australia. Research governance approval was also granted in all hospitals involved in the trial. The trial was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12610000512022). The trial protocol, full methodology and results for the primary outcome have been published previously, so we will only present a synopsis here [20].

Participants

Participants were recruited from three NSW SCI units (Prince of Wales Hospital, Royal North Shore Hospital and Royal Rehabilitation Centre Sydney), including their rural affiliations. Participants were over 18 years of age, with SCI and stable neurogenic bladder management. Exclusion criteria were complex bladder disturbances requiring surgical intervention, known urinary tract calculi, having received bladder education within the last 4 weeks, pre-existing infection on intervention commencement, known long-standing osteomyelitis, long-

term antibiotic therapy, adverse reaction to yoghurt products, severe renal and hepatic failure, full mechanical ventilation and immunosuppression. Participants provided written consent before enrolment.

Interventions

Participants were enroled for a 6-month study period, which included 24 weeks of treatment. Each randomised participant was required to take two capsules orally each day consisting of either:

(1) Group 1: active RC14-GR1 (concentration per capsule is 5.4×10^9 colony-forming units) + LGG-BB12 (concentration per capsule is 7×10^9 colony-forming units);
(2) Group 2: active RC14-GR1 (concentration as above) + matched placebo (no LGG-BB12);
(3) Group 3: active LGG-BB12 (concentration as above) + matched placebo (no RC14-GR1); or
(4) Group 4: double-matched placebo capsules (no LGG-BB12 or RC14-GR1).

Primary outcome

The primary outcome was the time from randomisation to the first symptomatic urinary tract infection (UTI). The conclusion was that there was no effect of RC14-GR1 or LGG-BB12 in preventing UTI in people with SCI [21].

Secondary outcome

Microbiological cultures of the bowel (rectal swabs or stool cultures), nose and groin as well as urine cultures were obtained from all enrolled participants at baseline, 3 months and 6 months. These samples were analysed at a central laboratory. Specific instructions and an information sheet for sampling were given by the study co-ordinator to research assistants and community nurses performing the cultures to ensure consistency.

Cultures were directly plated using CHROMagar media for the detection of MRSA, ESBL and VRE. Incubation was at 35 °C for 24 h for MRSA, 48 h for VRE and all other plates for 18 h.

A urine culture was also performed if participants developed symptoms of UTI. The endpoint urine cultures were analysed at the participant's local microbiological laboratory due to logistics and clinical reasons. We requested all urine samples to be collected from a new single-use catheter, suprapubic catheter (SPC) or indwelling catheter (IDC).

The secondary outcome measure was defined in the original protocol as a change of MRO colonisation status based on cultures performed at baseline, 3 and 6 months. Clearance was originally defined as MRO status change within the first 3 months followed by two consecutive negative screens at least 2 weeks apart that was sustained until the end of study (Supplementary Table 2) [20]. Due to shortage of funding, we could not perform additional cultures to confirm sustained clearance, requiring a change in the definition of the outcome measure. The logistical changes to the protocol required the additional MRO categories of "unchanged" and "new colonisation" to be defined. In addition, as the intervention was for the whole 6-month duration, we altered the definition of clearance to reflect status at the 6 month (see Table 1).

The presence of the following organisms was recorded:

- MRSA in groin, nose and urine cultures. For the purpose of this trial, MRSA is defined as a *Staphylococcus* isolate that is resistant to methicillin and by inference also resistant to flucloxacillin, dicloxacillin, cephazolin and cephalothin [4, 10];
- MRGN organisms in urine and bowel cultures. MRGNs were defined as gram-negative bacteria identified by the laboratory that were multi-resistant, e.g. EBSL, multi-resistant *Acinetobacter* species (carbapenem resistant), multi-resistant *P. aeruginosa* (resistant to one or more aminoglycoside antibiotics and one or more anti-pseudomonal beta-lactams) [4, 10];
- VRE in bowel cultures. VRE was defined as any *Enterococcus faecalis* or *Enterococcus faecium* isolate that was resistant to vancomycin [4, 10].

For combined MRGN results, if MRGN was only tested for either the bowel or urine but not both, then it was

Table 1 Definitions for VRE or combined MRSA, MRGN and MRO status and screening time points.

VRE/combined MRSA, MRGN and MRO status	Baseline test	Month 3 test	Month 6 test
Clearance	Either or both tests positive for MRSA/MRGN/MRO/VRE		Negative
New colonisation	Negative	Either positive or negative	Positive
No change	Positive for all periods		
	Negative for all periods		
	Positive	Negative	Positive

assumed that if one sample tested positive, the other would also be positive. If the tested sample was negative for MRGN, it was assumed that the untested sample was negative only if there was at least one other sample from the same site that tested negative in a subsequent screen (e.g. if baseline urine was not tested for MRGN but baseline bowel was negative, baseline urine was only considered negative for MRGN if urine at third and/or sixth month was also negative for MRGN) (Supplementary Table 1).

For combined MRSA results, it was assumed that MRSA for that period was positive if one sample (groin, nose or urine) tested positive. It was only assumed that a sample was negative for MRSA if at least two out of three samples tested negative at each sampling time point and if there was at least another sample of the same site that tested negative at a subsequent screen (e.g. if baseline urine was not tested for MRSA but baseline nose and groin were negative, baseline urine was only assumed negative for MRSA if urine at third and/or sixth month were also negative for MRSA) (Supplementary Table 1).

MRO colonisation status was deemed positive if a participant had any of the above organisms. For combined MRO, if any sample tested positive for MRGN, MRSA or VRE at any period, the entire period was considered to be positive for MRO (e.g. if MRGN and MRSA negative but VRE positive at baseline, then status was MRO positive at baseline) (Supplementary Table 1).

Combined MRSA, combined MRGN, MRO and VRE status at the end of the trial was classified into three categories (Table 1):

(i) No change.
(ii) Clearance.
(iii) New colonisation.

Supplementary Table 2 lists in more detail how status was determined if either one sample was missing or not tested for baseline and 3 months. The assumptions that we have adopted are conservative as they only affect a participant's status at baseline and can only change them from inconclusive/missing to negative, and hence their status at the end of the trial from missing to no change or new colonisation. It is also pragmatic, because it reflects current clinical practice in NSW: patients are not isolated if they have no culture evidence of colonisation/infection.

Participants who remained free of MRSA, MRGN, VRE or MRO throughout the duration of the trial were also studied. MRSA, MRGN or VRE-free was defined by respective negative MRSA, MRGN or VRE cultures at baseline, 3 and 6 months. Their endpoint urine should have also been negative for MRSA, MRGN or VRE. If all of their cultures were negative for MRSA, MRGN and VRE throughout the entire study, then participants were classified as being MRO-free.

Sample size

An initial sample size of 372 was required, with 93 participants randomly allocated to each of the four study groups. The sample size calculation was powered for the primary outcome. We used simple computer-generated randomisation, stratified by bladder management type and inpatient/outpatient status [20].

Statistical methods

Analysis of all outcomes was by intention to treat. A blinded analysis was unable to be performed due to unblinding for analysis of the primary outcome [21].

Analysis was performed using SAS 9.4. The chi-square test was conducted to assess the effect of each probiotic on MRSA, MRGN, VRE or MRO status and MRSA-, MRGN-, VRE- or MRO-free status.

Pre-specified variables deemed as risk factors for MRSA, MRGN, VRE and MRO colonisation were similarly analysed using the chi-square test to assess their association with each outcome. The eight variables were: bladder management (SPC/IDC vs. intermittent self-catheterisation [IMC] vs. condom drainage/reflex voiding [ECD]), inpatient status, completeness of injury (American Spinal Injury Association Impairment Scale Grade A) [22], gender, level of injury, time since injury, UTI and hospitalisation for UTI 6 months prior to trial.

Multivariate nominal logistic regression modelling was performed to determine the effects of the two probiotics, while allowing for effects of any significant risk factors, on MRSA, MRGN, VRE and MRO clearance and new colonisation, compared with no change. Multivariate binary logistic regression modelling was similarly performed to determine the effects of the probiotics and any significant risk factors on remaining MRSA, MRGN, VRE and MRO-free. Due to the low frequencies in some categories, the most significant risk factors were progressively added to the model containing both probiotics. The results were summarised using odds ratios (OR) and their 95% confidence intervals (CI). *P* values were obtained from likelihood ratio tests comparing models.

Results

Baseline

Baseline nose swabs found 27/207 (13%) of participants were colonised with MRSA. Baseline groin swabs showed 17/206 (8%) of participants colonised with MRSA (Supplementary Table 3). Of the samples collected, 147/207 urine cultures were reported to be either mixed,

predominant or pure growth of organisms (greater or equal to $\geq 1 \times 10^6$ colony-forming units/L). Hence the rate of asymptomatic bacteriuria in our cohort of participants was 71%. Organisms were identified in 91 urine cultures. The predominant organisms were *Escherichia coli* (19/93) and *Klebsiella sp*. (18/93).

Only 196/207 urine cultures were tested for MRSA at baseline, and 10/196 samples (5%) were positive. For MRGN, 195/207 urine cultures were tested at baseline, with 8/195 samples (4%) positive. In total, 16 participants had MROs cultured from their urine of whom two were positive for both MRSA and MRGN. All 16 participants with MRO in their urine were using SPC/IDC for bladder management. Baseline bowel cultures revealed 2/207 (1%) of participants had VRE. In addition, 176/207 bowel cultures were tested for MRGN, of which 9/176 were positive (5%). The combined MRO cultures showed that 48/207 (23%) of participants had an MRO at baseline.

Sixth month

Nose swabs at the sixth month found 20/177 (11%) of participants were colonised with MRSA while groin swabs indicated that 12/180 (7%) of participants were colonised with MRSA (Supplementary Table 3). There were 28 missing urine cultures at the sixth month. One urine culture was not tested for MRSA or MRGN. Of the 178 urines tested, 10 (6%) were MRSA-positive and 9 (5%) were MRGN-positive. Overall, 17 participants had an MRO in their sixth month urine cultures, all of whom were using SPC/IDC for emptying their bladders. There were 30 missing bowel cultures at the sixth month. Only 2/177 (1%) bowel cultures tested positive for VRE. For MRGN, 165/177 bowel cultures were tested, of which 7/165 (4%) were MRGN-positive. Based on previous MRO definitions, 35/177 (20%) of participants remaining in the study had MRO colonisation.

Outcomes and estimation

MRSA clearance and colonisation

After 6 months on the RCT, results for 179/207 participants were available for analysis. MRSA status was unchanged from baseline for 153/179 participants (85%) (Table 2 and Supplementary Table 4). More participants who were inpatients at trial enrolment (5/36, 14%) had new MRSA colonisation than outpatients (5/143, 3%).

Multivariate nominal logistic regression analysis, adjusted for inpatient status at enrolment, showed no evidence that RC14-GR1 or LGG-BB12 was effective in clearing MRSA or preventing new colonisation with MRSA. After adjusting for both probiotics, those with inpatient status at trial enrolment were more likely to become colonised with MRSA than outpatients (OR 4.72, 95% CI, 1.25–17.78) (Table 3).

MRGN clearance and colonisation

For MRGN, following 6 months of RCT treatment, the results of 176/207 participants were available for analysis; 158/176 (90%) participants had unchanged MRGN status compared with baseline (Table 2 and Supplementary Table 4). Only 1/86 (1%) participants receiving RC14-GR1 (groups 1 and 2) developed new MRGN colonisation compared with 8/90 (9%) in the no RC14-GR1 group (groups 3 and 4).

Eight out of 105 (8%) participants in the SPC/IDC group developed new colonisation of MRGN compared with 1/62 (2%) in the IMC group and 0/9 (0%) in the ECD group. However, participants with SPC/IDC were also more likely to clear MRGN (9/105 participants) compared with none in the IMC or ECD group. Participants who enrolled as inpatients were more likely to clear MRGN (5/36, 14%) than outpatients (4/140, 3%), and were also more likely to become newly colonised (7/36, 19%) than outpatients (2/140, 1%).

Bladder management could not be included in the multivariate nominal logistic regression model as there were too many categories with no participants. After adjusting for inpatient status and RC14-GR1, there was no significant effect of LGG-BB12 in preventing new colonisation by MRGN. In contrast, RC14-GR1 was significantly effective in preventing new colonisation of MRGN (OR 0.10, 95% CI, 0.01–0.88; $P = 0.04$) after adjusting for LGG-BB12 and inpatient status at enrolment. After adjusting for probiotics, participants who enroled as inpatients were more likely to clear MRGN (OR 8.08, 95% CI, 1.94–33.70) or more likely to develop new colonisation of MRGN (OR 21.41, 95% CI, 3.98–115.13, $P = < 0.0001$) (Table 3).

VRE clearance and colonisation

Only 177/207 participants' results were available for analysis for VRE clearance, with the majority of participants (167/177, 94%) having no change in VRE status from baseline (Table 2 and Supplementary Table 4). Nominal logistic regression modelling showed no effect of either probiotics in preventing or clearing VRE colonisation. Multivariate modelling was not performed as none of the risk factors was statistically significant.

MRO clearance and colonisation

The results of 177/207 participants were available for analysis and 141/177 participants (80%) had an unchanged

Table 2 Frequency table for VRE, combined MRSA, MRGN and MRO status at trial completion.

Covariates	MRSA status					MRGN status					VRE status					MRO status				
	Clearance	No change	New colonisation	Total	χ^2 test P value	Clearance	No change	New colonisation	Total	χ^2 test P value	Clearance	No change	New colonisation	Total	χ^2 test P value	Clearance	No change	New colonisation	Total	χ^2 test P value
N	16	153	10	179		9	158	9	176		8	167	2	177		22	141	14	177	
RC14-GR1																				
Active	6	78	3	87	0.28	4	81	1	86	0.06	4	80	1	85	0.99	10	70	5	85	0.59
Placebo	10	75	7	92		5	77	8	90		4	87	1	92		12	71	9	92	
LGG-BB12																				
Active	8	78	4	90	0.80	2	80	6	88	0.15	3	85	1	89	0.76	9	74	6	89	0.51
Placebo	8	75	6	89		7	78	3	88		5	82	1	88		13	67	8	88	
Bladder management																				
Suprapubic/indwelling catheter	11	88	8	107	0.48	9	88	8	105	0.05	5	99	1	105	0.88	16	78	11	105	0.29
Intermittent catheter	5	55	2	62		0	61	1	62		2	59	1	62		5	54	3	62	
External condom draining	0	10	0	10		0	9	0	9		1	9	0	10		1	9	0	10	
Inpatient status at trial enrolment																				
Yes	3	28	5	36	0.053	5	24	7	36	<0.001	3	33	0	36	0.37	4	27	5	36	0.33
No	13	125	5	143		4	134	2	140		5	134	2	141		18	114	9	141	
Years post injury																				
0 to <2 years	6	41	5	52	0.47	6	39	7	52	0.001	3	49	0	52	0.52	8	38	6	52	0.46
2 years to 20 years	6	71	4	81		1	78	1	80		4	74	2	80		7	67	6	80	
>20 years	4	41	1	46		2	41	1	44		1	44	0	45		7	36	2	45	
UTI 6 months before trial																				
None	4	65	4	73	0.50	2	68	2	72	0.04	5	65	2	72	0.31	7	59	6	72	0.51
1	5	36	4	45		6	36	3	45		1	44	0	45		8	32	5	45	
2 or more	7	52	2	61		1	54	4	59		2	58	0	60		7	50	3	60	

Table 3 Multivariate nominal logistic regression modelling of combined MRSA and combined MRGN status with probiotics and other covariates.

Covariates	Adjusted Odds Ratio				
	Clearance	(95% CI)	New colonisation	(95% CI)	*P* value
Combined MRSA status					
LGG-BB12	0.95	(0.34–2.67)	0.56	(0.15–2.14)	0.69
RC14-GR1	0.58	(0.20–1.67)	0.40	(0.10–1.65)	0.27
Inpatient at trial enrolment	1.04	(0.29–3.91)	4.72	(1.25–17.78)	0.08
Combined MRGN status					
LGG-BB12	0.24	(0.05–1.24)	1.50	(0.31–7.30)	0.13
RC14-GR1	0.66	(0.16–2.73)	0.10	(0.01–0.88)	0.04
Inpatient at trial enrolment	8.08	(1.94–33.70)	21.41	(3.98–115.13)	<0.0001

MRO status compared with baseline (Table 2 and Supplementary Table 4). Nominal logistic regression modelling showed that both RC14-GR1 and LGG-BB12 were ineffective in preventing to clearing colonisation with MROs. Multivariate modelling was not performed as none of the risk factors was statistically significant.

In summary, we found that both probiotics were ineffective at clearing any MROs be it MRSA, MRGN or VRE. RC14-GR1 could prevent new MRGN colonisation. Being an inpatient at trial enrolment is a risk factor for new MRSA and MRGN colonisation. Conversely, inpatient status at trial enrolment increased the likelihood of clearing MRGN colonisation.

Maintaining MRSA-free status

A total of 137/189 participants (72%) remained MRSA-free throughout the trial period (Table 4 and Supplementary Table 5). There was a statistically significant difference by level of injury, with only 56/87 (64%) participants with cervical injury being MRSA-free throughout the trial compared with 81/102 (79%) with thoracolumbar injuries ($P = 0.02$).

After adjusting for level of injury, logistic regression showed no effect of LGG-BB12 or RC14-GR1 in maintaining MRSA-free status. However, after adjusting for both probiotics, participants with thoracolumbar injuries were significantly more likely to maintain MRSA-free status than those with cervical level injuries (OR 2.14, 95% CI, 1.12–4.10; $P = 0.02$) (Table 5).

Maintaining MRGN-free status

A total of 149/177 (84%) participants had no positive MRGN cultures throughout the entire trial (Table 4). Bladder management, inpatient status at enrolment, level of injury, years post injury, UTI and hospitalisation 6 months prior to trial commencement were associated with maintaining a MRGN-free status (Table 4 and Supplementary Table 5).

The logistic regression model included both probiotics, bladder management, inpatient status at enrolment and UTI 6 months prior to trial. Level of injury was not included as it was strongly associated with bladder management ($\chi^2 = 51.39$, df = 2; $P < 0.0001$). After adjusting for the above risk factors, there was no evidence that LGG-BB12 or RC14-GR1 is associated with MRGN-free status (Table 5).

There was a significantly increased likelihood of maintaining a MRGN-free status for participants who used IMC compared with SPC/IDC as a form of bladder management (OR 16.24, 95% CI, 3.17–83.29; $P < 0.0001$) after adjusting for probiotics and other risk factors. Participants who were less likely to maintain MRGN-free status throughout the trial include those who were inpatients at trial enrolment (OR 0.13, 95% CI, 0.04–0.38; $P = 0.0001$) and those with a UTI in the 6 months prior to trial commencement (OR 0.17, 95% CI, 0.05–0.65; $P = 0.01$).

Maintaining VRE-free status

Only 11/178 (6%) participants were positive for VRE in their bowel cultures throughout the entire duration of the trial. There was no effect of RC14-GR1 or LGG-BB12 or any other factor in maintaining a VRE-free status (Table 4 and Supplementary Table 5).

Maintaining MRO-free status

Using the definitions above, 119/190 participants (63%) had MRO-free status throughout the entire study. Neither probiotic was protective against MRO. With regard to type of bladder management, 62/114 (54%) participants who had SPC/IDC were MRO-free compared with 50/65 (77%) participants who performed IMC and 7/11 (64%) who had ECD ($P = 0.01$) (Table 4 and Supplementary Table 5).

Multivariate logistic regression modelling, adjusted for bladder management, showed no effect of LGG-BB12 or RC14-GR1 for protection against MRO. After adjusting for both probiotics, IMC was significantly more protective

Table 4 Frequency table for maintaining MRSA-free, MRGN-free, VRE-free and MRO-free status throughout the trial.

Covariates	MRSA-free throughout trial				MRGN-free throughout trial				VRE-free throughout trial				MRO-free throughout trial			
	No	Yes	Total	χ^2 test P value	No	Yes	Total	χ^2 test P value	No	Yes	Total	χ^2 test P value	No	Yes	Total	χ^2 test P value
N	52	137	189		28	149	177		11	167	178		71	119	190	
RC14-GR1																
Active	25	69	94	0.78	10	76	86	0.14	5	80	85	0.87	34	59	93	0.82
Placebo	27	68	95		18	73	91		6	87	93		37	60	97	
LGG-BB12																
Active	28	68	96	0.61	14	75	89	0.97	4	85	89	0.35	35	60	95	0.88
Placebo	24	69	93		14	74	88		7	82	89		36	59	95	
Bladder management																
Suprapubic/indwelling catheter	36	77	113	0.23	25	79	104	0.001	7	99	106	0.79	52	62	114	0.01
Intermittent catheter	13	52	65		2	61	63		3	59	62		15	50	65	
External condom draining	3	8	11		1	9	10		1	9	10		4	7	11	
Inpatient status at trial enrolment																
Yes	14	24	38	0.15	13	24	37	0.0003	3	33	36	0.55	18	20	38	0.15
No	38	113	151		15	125	140		8	134	142		53	99	152	
Level of injury																
Cervical	31	56	87	0.02	20	59	79	0.002	3	77	80	0.22	40	47	87	0.02
Thoracolumbar	21	81	102		8	90	98		8	90	98		31	72	103	
UTI 6 months before trial																
None	16	60	76	0.09	5	66	71	0.02	7	65	72	0.23	22	53	75	0.06
1	19	30	49		12	33	45		1	44	45		25	25	50	
2 or more	17	47	64		11	50	61		3	58	61		24	41	65	

Table 5 Multivariate binary logistic regression modelling for maintaining MRSA-free, MRGN-free and MRO-free status with probiotics and other covariates.

Covariates	Adjusted odds ratio (95% CI)	*P* value
MRSA-free		
LGG-BB12	0.84 (0.44–1.61)	0.59
RC14-GR1	1.09 (0.57–2.09)	0.79
Thoracolumbar injury	2.14 (1.12–4.10)	0.02
MRGN-free		
LGG-BB12	0.84 (0.32–2.22)	0.52
RC14-GR1	2.40 (0.90–6.44)	0.06
Bladder management		
Intermittent catheter	16.24 (3.17–83.29)	<0.0001
External condom drainage	1.64 (0.17–15.82)	
Inpatient at trial enrolment	0.13 (0.04–0.38)	0.0001
UTI 6 months before trial		
1	0.17 (0.05–0.65)	0.01
2 or more	0.24 (0.07–0.85)	
MRO-free		
LGG-BB12	1.02 (0.56–1.86)	0.92
RC14-GR1	1.07 (0.59–1.96)	0.81
Bladder management		
Intermittent catheter	2.80 (1.41–5.54)	0.009
External condom draining	1.47 (0.41–5.31)	

against MRO than SPC/IDC (OR 2.80, 95% CI, 1.41–5.54; $P = 0.009$) (Table 5).

In summary, we found that neither probiotic was effective in maintaining MRO-free status. There is more likelihood of maintaining MRSA-free status with thoracolumbar level injuries. Factors effective in maintaining MRGN-free status were IMC, being an outpatient at trial enrolment and not having a UTI in the 6 months leading to the trial. IMC was also found to be effective in maintaining MRO-free status.

Study endpoint urine culture results

Of the 207 participants, 53 met the study endpoint criteria for UTI, where 27/53 were participants with tetraplegia and 31/53 used SPC/IDCs. For urine endpoint cultures, 36/53 were positive for gram-negative organisms, the predominant species being *E. coli* (14/53).

MRSA was present in 3/53 endpoint urine cultures. All three participants with MRSA used SPC/IDC for bladder management. As the endpoint urine cultures were not analysed in the central laboratory, MRGNs were not specifically stated. However, examining the antibiotic susceptibility profile accompanying the culture reports, 2/53 had gram-negative organisms that could be classified as MRGN. Both these participants used either an SPC or IDC for bladder management. One participant's endpoint urine grew *P. aeruginosa*, which was resistant to at least three aminoglycosides and two beta-lactams. Another participant's endpoint urine grew *E. coli* that was only sensitive to a beta-lactam and was resistant to third generation cephalosporins.

Ancillary analysis

Post hoc analysis showed that there was no association between having a symptomatic endpoint UTI during the trial and changes in MRSA, MRGN, VRE or MRO status, or MRO-free status. Participants who were lost to follow-up were more likely to have a cervical level of injury (20/31, 65%) than the remaining participants (77/176, 44%) ($\chi^2 = 4.56$, df = 1; $P = 0.03$). However, there was no significant difference in bladder management between the missing and remaining participants.

Harms

Side effects from either intervention were infrequent and have been reported in the primary outcome paper [21]. The double placebo group appeared to have more adverse events than the other groups. The majority of adverse events were due to bowel complaints like accidents and increased frequency of bowel movements.

Discussion

Overall, the MRSA, MRGN and VRE positive rates were lower than for the general spinal population [7, 8], which was probably a result of our exclusion criteria. The majority of participants (80%) also had no change in their MRO status throughout the trial. More than 60% of participants remained MRO-free throughout the 6-month trial period, suggesting that change in MRO colonisation takes longer than 6 months to develop. Our rate of asymptomatic bacteriuria of 71% is similar to that reported by Kang et al. [23]; in their retrospective study, they found that 73% of patients had asymptomatic bacteriuria.

In summary, once a participant was colonised with MRSA, MRGN, VRE or MROs, neither LGG-BB12 nor RC14-GR1 was effective in clearing the respective bacterial colonisation. However, RC14-GR1 was shown to be effective in preventing new colonisation of MRGN over the 6-month duration of the trial. Longer term studies would be important to determine the extent of duration of the effect of RC14-GR1 in preventing MRO colonisation. The results should be treated with caution as the numbers of participants who had new colonisation was small. However, these results are consistent with our post hoc analysis for the

primary outcome (time to first symptomatic UTI) where there appeared to be a longer UTI-free survival when RC14-GR1 was used alone [21].

The finding that inpatient status at enrolment led to participants being more likely to have colonisation with MRGN and less likely to be MRGN-free may be explained by a higher risk of exposure to MRGN in a hospital setting. Participants may also be more likely to clear MRGN as they may be treated with antibiotics.

Having one or more UTI prior to trial commencement is associated with a lower likelihood of being MRGN-free throughout the trial which could be explained by more frequent antibiotic exposure leading to the emergence of more MRGNs. Recurrent UTIs and exposure to any antibiotics are risk factors for MROs [24–26].

Both LGG-BB12 and RC14-GR1 were ineffective in ensuring that participants remain free of MRSA, MRGN, VRE and MRO. The only clinical factor associated with participants remaining free of MRSA was having a thoracolumbar injury.

Results from our trial indicate that IMC and ECD as a form of neurogenic bladder management increase the likelihood of a participant being protected from MRGN and MRO colonisation. This conclusion is further supported by our findings that all participants who had endpoint MRO UTIs in the trial were using SPC/IDC for bladder management. Our results are consistent with the report from Kang et al. that SCI patients with SPC/IDC had more than twice the risk of MRO of IMC patients [23].

MRGNs have been viewed globally as an increasing health threat due to their association with treatment failures, morbidity and mortality [1, 26, 27]. Currently in NSW, for a patient with known colonisation with an MRO, some of the infection control measures include performing hand hygiene, using personal protective equipment (gowns and gloves), isolation in a single room, thorough cleaning of all equipment and surfaces that a patient uses after therapy sessions. Equipment such as wheelchairs, commodes or shower chairs, can be used for one patient only [10]. Considering all the above measures, a patient with MRO has a huge impact on health resources, equipment and cost. Cost-analysis studies found that the higher costs involved in the management of patients with MRGN infections are due to longer length of stay, antimicrobial drugs and higher readmission rates [27–29]. In fact, one study reported that just being colonised rather than infected with MROs in a hospital setting incurred a much higher cost due to the need for isolation [30]. Thus, treatment strategies, such as using IMCs or probiotic application could reduce MRO development in patients, which would potentially alleviate some of these costs as well as improve patient survival.

One main weakness in our study was the departure from protocol. Due to limited funding, underestimation of the cost of microbiological cultures, and logistical reasons mentioned in our primary outcome paper, we could not conduct further screening to confirm sustained clearance. As the trial went on for 6 months, it was difficult to interpret results based only on status at baseline and 3 months. However, the definitions of the categories "clearance", "new colonisation", "no change" and being MRSA-, MRGN-, VRE- or MRO-free were specified prior to data analysis. Another criticism might be that our screening cultures were too far apart, months rather than weeks, which again was due to the fact that the trial was funded for the outcome of UTI prevention. For logistical and clinical reasons, the endpoint urine cultures had to be performed at the participants' local microbiological laboratory which did not lead to a standardisation of MRGN reporting. We had to conclude whether a gram-negative should be classified as an MRGN based on the antibiogram reported.

Another weakness was that almost 15% of participants had results that were inconclusive for MRO clearance due to missing cultures at 6 months. Analysis of participants who were lost to follow-up revealed that they were more likely to have tetraplegia and therefore use SPC/IDC; we can only postulate that their rate of MRSA, MRGN and MROs may be higher.

The analysis for this paper was performed unblinded following analysis of the primary outcome. As the null hypothesis was not rejected for probiotics in maintaining MRO-free status or in clearing MROs, it is highly unlikely that these results would have been different with blinding. Although our trial co-ordinator and research assistants were contacting participants to check on their well-being every 2 weeks, we did not systematically monitor the participants' antibiotic and topical antiseptic exposure throughout the duration of the study. Microbiological analysis from other sites secondary to e.g. intercurrent pressure ulcers, diarrhoea or pneumonia that may have developed during the trial was also not recorded. Mylotte et al. suggested that presence of an ulcer predicted carriage of resistant organisms [7]. Manley et al. showed decolonisation of VRE within a group of patients with renal impairment [14], but the total number of patients with VRE within our clinical trial was low.

Our results with regard to the failure of probiotics to clear MRGNs is consistent with two other RCTs. Both trials had fewer patients overall and in each arm. Salomao et al. reported that *L. rhamnosus* and *L. bulgaricus* administered twice daily for 7 days in 116 participants did not decolonise patients harbouring MRGN bacilli in their gastrointestinal tract [31]. Tannock et al. found that a 5-week administration of twice daily *E. coli Nissle* 1917 (Mutaflor) in 69 participants did not change the carriage of norfloxacin-resistant *E. coli* [32].

There is a paucity of literature on trials investigating the role of probiotics in preventing new colonisation of MROs.

Dall et al. reported that *LGG* did not prevent the colonisation of ESBL in travellers to India. However, participant numbers were small ($n = 61$) and not blinded [33]. There was also no placebo arm. De Regt et al. conducted a prospective cohort cross-over trial in which ten species of probiotics were administered twice daily for 4.5 months to see if the probiotics could prevent colonisation with ampicillin-resistant *Enterococcus faecium* (ARE) in patients admitted for >48 h to the gastroenterology, renal or geriatric wards. They found that the multi-species probiotics did not prevent ARE acquisition in the 110 participants who were treated [34].

Other measures have been more effective at preventing MRSA colonisation and MRSA clearance. Kappel et al. adopted a pre-emptive approach of isolation with contact precautions to prevent intra-hospital transmission until MRSA swabs were negative for 14 days. They also undertook closure of pressure ulcers through plastic surgery to eradicate MRSA colonisation [35]. There is some evidence that daily bathing with chlorhexidine solution reduces the carriage of gram-positive pathogens [36]. Cassir et al. have also reported a reduction in healthcare related infections caused by gram-negative bacteria with daily bathing using disposable cloth saturated with 2% chlorhexidine [37].

Our trial results indicate that RC14-GR1 may have a role in preventing MRGN colonisation. Given the high risk of MRO colonisation after SCI [7, 8], the ideal time to commence such prophylaxis would be early after injury, prior to colonisation. Despite this, the solution to preventing colonisation of MROs in SCI patients remains complex with the need to employ a multi-faceted approach of isolation, contact precautions, antibiotic stewardship, closure of pressure ulcers, reduction in post-SCI pneumonia rates with efficient endotracheal-tracheostomy weaning and reduced catheter dwell-time within the urinary system through the use of intermittent catheterisation. It is hoped that future research with more robust microbiology methodology can be carried out to test our findings.

Conclusion

Our RCT showed that LGG-BB12 and RC14-GR1 are not effective in clearing MRSA, MRGN, VRE or MROs, although RC14-GR1 may be able to prevent new colonisation of MRGN, at least for 6 months. IMC is associated with maintaining an MRGN-free and MRO-free status. Based on these results, it is suggested that a paradigm shift to IMC for management of spinal neurogenic bladder regardless of level of spinal injury would yield significant benefits for patients in terms of UTI and MRO status.

Data availability

Raw data and datasets generated or extracted are archived in NEURA for 15 years. More detailed extracted data can be found in Supplementary Table 3–5.

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Author contributions Trial protocol was developed by S-LT, BBL, JMS, OM, GK, SR, KC, GW, JK, CBR, SG, JM and MT over a series of teleconferences and workshops in Sydney, Australia in the late 2009 and early 2010 from an original study design developed by BBL and JMS. OM was responsible for designing and maintaining trial database. Data analysis was conducted by S-LT and checked by JMS. S-LT was responsible for initial manuscript preparation. All authors reviewed and were involved in writing up the final version of the manuscript prior to submission.

Compliance with ethical standards

Conflict of interest BBL, JMS, SG and JM have received competitive research funding support from the NHMRC. BBL, CBR, JMS, KC and S-LT are also authors on the Cochrane review—probiotics for preventing UTI in people with neuropathic bladder. The Coloplast company has provided nursing support to several of BBL's community patients with recurrent UTIs from January 2018 to July 2019 and continues to provide occasional community support to some of these patients. However, Coloplast has had no financial or editorial input into the design, analysis or write up of this trial. The rest of the authors have no competing financial or non-financial interests.

Ethics We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were following during the course of this research.

References

1. Shallcross LJ, Davies SC. The World Health Assembly resolution on antimicrobial resistance. J Antimicrob Chemother. 2014;69:2883–5.
2. Casadevall A, Pirofski L-A. Host–pathogen interactions: basic concepts of microbial commensalism, colonization, infection, and disease. Infect Immun. 2000;68:6511–8.
3. Schwarz S, Silley P, Simjee S, Woodford N, van Duijkeren E, Johnson AP, et al. Assessing the antimicrobial susceptibility of bacteria obtained from animals. J Antimicrob Chemother. 2010;65:601–4.

4. NSW Department of Health. PD2007 084: Infection control policy: prevention and management of multi-resistant organisms. NSW Department of Health: New South Wales, Australia; 2007.
5. Girard R, Mazoyer M, Plauchu M, Rode G. High prevalence of nosocomial infections in rehabilitation units accounted for by urinary tract infections in patients with spinal cord injury. J Hospital Infect. 2006;62:473–9.
6. Lepainteur M, Ogna A, Clair B, Dinh A, Tarragon C, Prigent H, et al. Risk factors for respiratory tract bacterial colonization in adults with neuromuscular or neurological disorders and chronic tracheostomy. Respir Med. 2019;152:32–6.
7. Mylotte JM, Kahler L, Graham R, Young L, Goodnough S. Prospective surveillance for antibiotic-resistant organisms in patients with spinal cord injury admitted to an acute rehabilitation unit. Am J Infect Control. 2000;28:291–7.
8. Waites KB, Y-yC, DeVivo MJ, Canupp KC, Moser SA. Antimicrobial resistance in gram-negative bacteria isolated from the urinary tract in community-residing persons with spinal cord injury. Arch Phys Med Rehabil. 2000;81:764–9.
9. Suda K, Patel U, Sabzwari R, Cao L, Ramanathan S, Hill J, et al. Bacterial susceptibility patterns in patients with spinal cord injury and disorder (SCI/D): an opportunity for customized stewardship tools. Spinal Cord. 2016;54:1001.
10. Gallard J, Greig S. Management of Multi-resistant Organisms (MROs). New South Wales: South Eastern Sydney Illawarra Area Health Service Infection Control Manual Working Party; 2010.
11. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev. 2005;18:657–86.
12. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014; 11:506–14.
13. Ouwehand AC, Forssten S, Hibberd AA, Lyra A, Stahl B. Probiotic approach to prevent antibiotic resistance. Ann Med. 2016;48:246–55.
14. Manley KJFM, Maynall BC, Power DA. treatment of vancomycin-resistant enterococci: a randomised controlled trial. Med J Aust. 2007;186:454–7.
15. Szachta P, Ignys I, Cichy W. An evaluation of the ability of the probiotic strain Lactobacillus rhamnosus GG to eliminate the gastrointestinal carrier state of vancomycin-resistant enterococci in colonized children. J Clin Gastroenterol. 2011;45:872–7.
16. Plummer SF, Garaiova I, Sarvotham T, Cottrell SL, Le Scouiller S, Weaver MA, et al. Effects of probiotics on the composition of the intestinal microbiota following antibiotic therapy. Int J Antimicrob Agents. 2005;26:69–74.
17. Sheu B-S, Cheng H-C, Kao A-W, Wang S-T, Yang Y-J, Yang H-B, et al. Pretreatment with Lactobacillus-and Bifidobacterium-containing yogurt can improve the efficacy of quadruple therapy in eradicating residual Helicobacter pylori infection after failed triple therapy. Am J Clin Nutr. 2006;83:864–9.
18. Sýkora J, Valecková K, Amlerová J, Siala K, Dedek P, Watkins S, et al. Effects of a specially designed fermented milk product containing probiotic Lactobacillus casei DN-114 001 and the eradication of H. pylori in children: a prospective randomized double-blind study. J Clin Gastroenterol. 2005;39:692–8.
19. Forestier C, Guelon D, Cluytens V, Gillart T, Sirot J, De Champs C. Oral probiotic and prevention of *Pseudomonas aeruginosa* infections: a randomized, double-blind, placebo-controlled pilot study in intensive care unit patients. Crit Care. 2008;12:R69.
20. Lee BB, Toh S-L, Ryan S-L,Simpson JM, Clezy K, Bossa L. et al. Probiotics [LGG-BB12 or RC14-GR1] versus placebo as prophylaxis for urinary tract infection in persons with spinalcord injury [ProSCIUTTU]: a study protocol for a randomised controlled trial. BMC Urol. 2016;16:18
21. Toh S-L, Lee BB, Ryan S, Simpson JM, Clezy K, Bossa L, et al. Probiotics [LGG-BB12 or RC14-GR1] versus placebo as prophylaxis for urinary tract infection in persons with spinal cord injury [ProSCIUTTU]: a randomised controlled trial. Spinal Cord. 2019;57:550–61.
22. Kirshblum SC, Burns SP, Biering-Sorensen F, Donovan W, Graves DE, Jha A, et al. International standards for neurological classification of spinal cord injury (revised 2011). J Spinal Cord Med. 2011;34:535–46.
23. Kang MS, Lee BS, Lee HJ, Hwang SW, Han ZA. Prevalence of and risk factors for multidrug-resistant bacteria in urine cultures of spinal cord injury patients. Ann Rehabil Med. 2015;39:686–95.
24. Almomani BA, Hayajneh WA, Ayoub AM, Ababneh MA, Al Momani MA. Clinical patterns, epidemiology and risk factors of community-acquired urinary tract infection caused by extended-spectrum beta-lactamase producers: a prospective hospital case-control study. Infection. 2018;46:495–501.
25. García-Tello A, Gimbernat H, Redondo C, Meilán E, Arana DM, Cacho J, et al. Prediction of infection caused by extended-spectrum beta-lactamase-producing Enterobacteriaceae: development of a clinical decision-making nomogram. Scand J Urol. 2018;52:70–5.
26. Malcolm W, Fletcher E, Kavanagh K, Deshpande A, Wiuff C, Marwick C, et al. Risk factors for resistance and MDR in community urine isolates: population-level analysis using the NHS Scotland Infection Intelligence Platform. J Antimicrob Chemother. 2017;73:223–30.
27. Giske CG, Monnet DL, Cars O, Carmeli Y. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob Agents Chemother. 2008;52:813–21.
28. Tabak YP, Sung AH, Ye G, Vankeepuram L, Gupta V, McCann E. Attributable clinical and economic burden of carbapenem-non-susceptible Gram-negative infections in patients hospitalized with complicated urinary tract infections. J Hospital Infect. 2019;102:37–44.
29. Vallejo-Torres L, Pujol M, Shaw E, Wiegand I, Vigo JM, Stoddart M, et al. Cost of hospitalised patients due to complicated urinary tract infections: a retrospective observational study in countries with high prevalence of multidrug-resistant Gram-negative bacteria: the COMBACTE-MAGNET, RESCUING study. BMJ Open. 2018;8:e020251.
30. Engler-Hüsch S, Heister T, Mutters NT, Wolff J, Kaier K. In-hospital costs of community-acquired colonization with multidrug-resistant organisms at a German teaching hospital. BMC Health Serv Res. 2018;18:737.
31. Salomão MCC, Heluany-Filho MA, Menegueti MG, Kraker MEAD, Martinez R, Bellissimo-Rodrigues F. A randomized clinical trial on the effectiveness of a symbiotic product to decolonize patients harboring multidrug-resistant Gram-negative bacilli. Rev da Soc Brasileira de Med Tropical. 2016;49:559–66.
32. Tannock GW, Tiong S, Priest P, Munro K, Taylor C, Richardson A, et al. Testing probiotic strain Escherichia coli Nissle 1917 (Mutaflor) for its ability to reduce carriage of multidrug-resistant E. coli by elderly residents in long-term care facilities. J Med Microbiol. 2011;60:366–70.
33. Dall LB, Lausch KR, Gedebjerg A, Fuursted K, Storgaard M, Larsen CS. Do probiotics prevent colonization with multi-resistant Enterobacteriaceae during travel? A randomized controlled trial. Travel Med Infect Dis. 2019;27:81–6.
34. de Regt MJ, Willems RJ, Hené RJ, Siersema PD, Verhaar HJ, Hopmans TE, et al. Effects of probiotics on acquisition and spread of multiresistant enterococci. Antimicrob Agents Chemother. 2010;54:2801–5.
35. Kappel C, Widmer A, Geng V, Von Arx P, Frei R, Koch H, et al. Successful control of methicillin-resistant Staphylococcus aureus in a spinal cord injury center: a 10-year prospective study including molecular typing. Spinal Cord. 2008;46:438.

36. Donskey CJ, Deshpande A. Effect of chlorhexidine bathing in preventing infections and reducing skin burden and environmental contamination: a review of the literature. Am J Infect Control. 2016;44:e17–e21.

37. Cassir N, Thomas G, Hraiech S, Brunet J, Fournier P-E, La Scola B, et al. Chlorhexidine daily bathing: Impact on health care–associated infections caused by gram-negative bacteria. Am J Infect Control. 2015;43:640–3.