PRINCESS Trial Protocol

Long title: A double-blind placebo controlled trial to evaluate the efficacy of probiotics (*Lactobacillus rhamnosus,* LGG and *Bifidobacterium animalis* subsp. *lactis,* BB-12) in reducing antibiotic administration for infection in care home residents.

Short title: **P**robiotics to **R**educe **I**nfections i**N** **C**ar**E** home re**S**ident**S**, which is abbreviated to **PRINCESS**

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This protocol has been authorised by:

Professor M Robling	Director of Population Health Trials		
Name	**Role**	**Signature**	**Date**
Professor C Butler	Chief Investigator		
Name	**Role**	**Signature**	**Date**

General Information This protocol describes the PRINCESS trial, and provides information about the procedures for entering participants into the trial. The protocol should not be used as a guide, or as an aide-memoire for the treatment/care of other patients/participants. Every care has been taken in drafting this protocol; however, corrections or amendments may be necessary. These will be circulated to the known Investigators in the trial, but centres entering participants for the first time are advised to contact the South East Wales Trials Unit (SEWTU) in Cardiff to confirm that they have the most up-to-date version of the protocol in their possession. Problems relating to the trial should be referred, in the first instance, to SEWTU.

Compliance This study will adhere to the conditions and principles outlined in the EU Directive 2001/20/EC, EU Directive 2005/28/EC and the ICH Harmonised Tripartite Guideline for Good Clinical Practice (CPMP/ICH/135/95). It will be conducted in compliance with the protocol, the Research Governance Framework for Health and Social Care (Welsh Assembly Government November 2001 and Department of Health 2nd July 2005), the Data Protection Act 1998, and other regulatory requirements as appropriate.

For those trial participants lacking capacity, the Mental Capacity Act (2005) will be adhered to.

Funding The PRINCESS trial is being funded by the Medical Research Council's Efficacy and Mechanism Evaluation Programme administered by the National Institute for Health Research.

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Please contact the Trial Manager for general queries and supply of trial documentation

Randomisations:

Randomisation

To randomise a patient log onto the following website:
https://ctu1.phc.ox.ac.uk/randomise
(See section 15 for more details)

Clinical queries:

Clinical queries

All clinical queries should be directed to the Trial Manager who will direct the query to the most appropriate clinical person.

Serious Adverse Events:

SAR reporting

SAEs will be reported by the RN on the PRINCESS Weekly Record and Weekly Record: Further Information CRF.

Where an adverse event meets the definition of a SAR a SAR form should be completed by the responsible person and faxed to the PRINCESS Trial Manager (See section 13 for more details).

Fax Number: 02031070875

Table of Contents

Glossary of abbreviations

AAD	Antibiotic Associated Diarrhoea
AE	Adverse Event
AMR	Antimicrobial Resistant/ce
CAAD	Cumulative Antibiotic Administration Days
CAPD	Cumulative Antibiotic Prescription Days
CI	Chief Investigator
CID	Common Infectious Diseases
CIS	Consultee Information Sheet
CHR	Care Home Resident(s)
CRF	Case Report Form
CTIMP	Clinical Trial of an Investigational Medicinal Product
CU	Cardiff University
EME	Efficacy and Mechanisms Evaluation Programme
EQ5D	EuroQol 5D
FBC	Full Blood Count
GCP	Good Clinical Practice
GI	Gastrointestinal Infections
GMP	Good Manufacturing Practice
GP	General Practitioner
HCAI	Health care associated infections
HI	Haemagglutinin Inhibition
IC	Informed Consent
ICECAP-O	ICEpop CAPability measure for Older people
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IDMC	Independent Data Monitoring Committee
MAR	Medication Administration Record
NHS	National Health Service
PAAD	Probiotics for Antibiotic Associated Diarrhoea study
PI	Principal Investigator
PIS	Participant/ Patient Information Sheet
PPI	Patient Public Involvement

QL (QoL)	Quality of Life
R&D	NHS Trust Research & Development Department
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
RN	Research Nurse
RTI	Respiratory Tract Infection
SAE	Serious Adverse Event
SEWTU	South East Wales Trials Unit
TLR	Toll Like Receptors
TMG	Trial Management Group
TSC	Trial Steering Committee
UTI	Urinary Tract Infection
VDR	Vitamin-D Receptors
VRE	Vancomycin resistant enterococci

1 Amendment History

Amendment No.	Protocol version no.	Date issued	Author(s) of changes	Details of changes made
1	2.0	12/07/2016	EOJ	Trial team details updated (pg.3). Details of randomisation website added (pg.4 & pg.27). Secondary outcome of GP visits, A&E visits, and prophylactic antibiotic use removed (pg. 10, pg. 19, pg. 39). Word "serum" has been added to clarify that this refers to vitamin D levels in blood rather than in faecal specimens (pg. 10 & pg. 21). Section 3.1 Trial Schema (pg.12) and 3.2 Participant Flow Diagram (pg. 13) updated Section 4.1 Background has been modified to further clarify the objectives of the study (pg. 13). Reference to "hospital records" changed to "discharge summaries" (pg. 20 & 39). Reference to "microbiome analysis" removed (pg. 21 & pg. 39). Reference to "record increased level of care required" via analysis of MAR sheets removed (pg. 20). Section 8.3 Exclusion criteria amended to include residents currently involved in another CTIMP or who have participated in a CTIMP during the last 30 days (pg. 23). Section 10.4 updated to change Unblinding SOP to unblinding procedure, and individual carrying out unblinding from QA Manager to suitable delegated individual (pg.27), and addition of 2 trial packs at initial allocation (pg.27). Clinical Rating Scale removed from the table on pg. 39 and text (pg.29).

				Section 13 Adverse Events (Safety) updated to include General Definitions (pg.29), Expectedness (pg.31), updated flowchart (pg.36) and removal of 'not assessable' outcome (pg.33), Reporting Procedures (pg.32). Section 14 Adverse Events (Human Tissue) added (pg.34).
N/A	2.1	01/09/2016	EOJ	ISRCTN added to cover page. In version 2.0 of the protocol the "Protocol version no." column under the Amendment History should be 2.0 and not 1.1 – this has been amended in version 2.1 protocol.
3	2.2	02/02/2017	EOJ	Richard Fuller removed as co-applicant; Victoria Shepherd replaced by Alison Edwards; additional secondary outcome added.
5	2.3	20/06/2017	EOJ	On P1 Prof Robling's details updated; text added to the following sentence on P26: All consultees who have not responded will be contacted by phone **or sent a follow-up letter** at least once by the individual delegated to undertake the declaration process; on P47 the three month follow-up window changed to -2/+4 weeks.
6	3.0	25/04/2017	EOJ	P3 Change of data manager, change of name of Senior Trial Manger and Research Nurse; P13 and 14 Changes to Trial Schema and Flow Diagram; P10 Changes to terminology and items in Synopsis table; P11, 20 and 21 Clarification and update to secondary endpoints/outcomes; P19-21 Table 5.1 Changes to terminology and items in table; P11, 14, 22 and 24 Change in wording to sample size to "Between 258 and 270 participants, with an upper limit of 330"; P39 "Description of outcome" amended under Secondary – mechanistic: Immune parameters heading; P14, 24, 28, 29 and 34 Change to timing and name of 12-month interview; P28 Timing of study product; P34-

				35 and P48 Detail of follow-ups amended; P40 Change to trial closure.
N/A	3.1	16/10/2018	EOJ	Figure 3.1 Updated – removal of Use of antimicrobials; Table 5.1 Removal of "Use of antimicrobials"; P20-P22 Clarification of outcome measures; Updates to sections 16, 17 and Section 17.2 Description of outcomes and method of statistical analysis.
7	4.0	15/04/2019	EOJ	P12, P15, P37 Section 16 and Appendix 1 (P53 onwards) Addition of qualitative sub-study

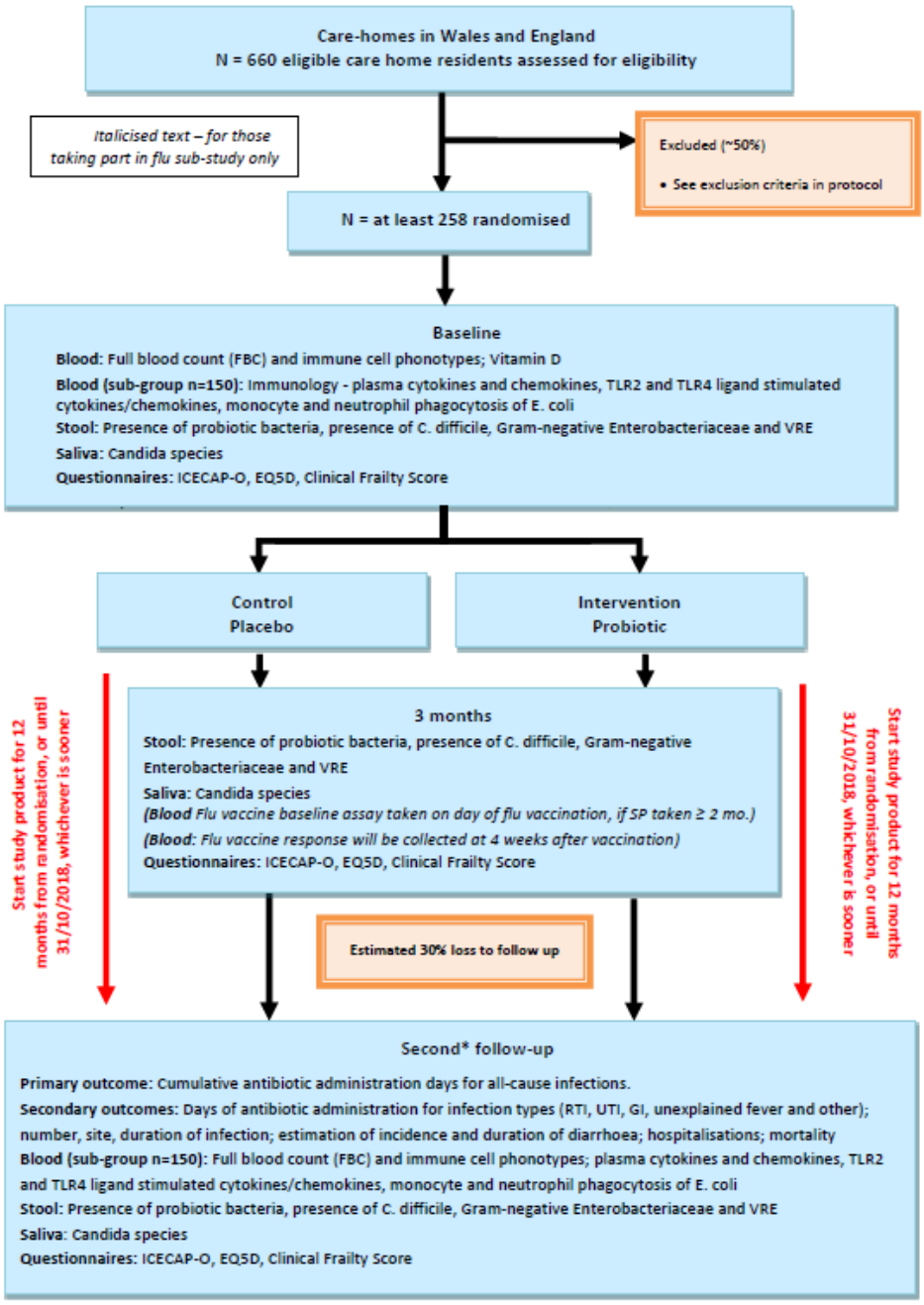
2 Synopsis

Short title	**P**robiotics to **R**educe **I**nfections i**N** **C**ar**E** home re**S**ident**S**
Acronym	PRINCESS
Internal ref. no.	
Trial/study design	Two arm, individually randomised, double blind placebo controlled efficacy trial.
Trial/study participants	Care home residents in Wales and England
Planned sample size	Between 258 and 270 participants, with an upper limit of 330
Follow-up duration	Follow-up schedule will depend on the length of time that a participant has been in the study. Where possible, participants will have a baseline assessment, and three- and 12-month follow-ups. Due to time limitations, some participants may have a truncated follow-up and will receive either a baseline assessment and 3 month follow-up, or a baseline assessment, 3 month follow-up and a second follow-up between 6-10 months post-randomisation.) Infection, antibiotic use, adverse events and study product use is also recorded at regular intervals by the RN from care home notes for 12 months post randomisation or until 31/10/18, whichever is sooner.
Planned trial/study period	Once daily oral probiotics or placebo for 12 months or until 31/10/18, whichever is sooner

Primary objective	To evaluate the effect of a dose of daily oral probiotics on cumulative systemic antibiotic administration days (CAAD) for all-cause, acute infections
Secondary objectives	There are secondary objectives in infection, health utility, wellbeing, service use, mortality, antimicrobial resistance, oral and gut microbiology, immunology, blood and mechanisms (see section 5)
Primary endpoint	CAAD for all-cause infections over 12 months
Secondary endpoints	CAAD for five sub-categories of CID (RTI, UTI, skin, GI unexplained fever); number, site, duration (mean and cumulative) of infection; incidence and duration of diarrhoea; EQ-5D (health utility), ICECAP-O (wellbeing); hospitalisations; death; Antimicrobial resistance (AMR) (Culture and antibiotic sensitivity of Gram-negative Enterobacteriaceae and VRE from stool sample and presence of probiotic species in stool to explore adherence and contamination); Antibiotic consumption **Mechanistic measures:** **Blood** – FBC and immune cell phenotypes; influenza vaccine response - Haemagglutination inhibition assay; immunology - plasma cytokines and chemokines, TLR2 and TLR4 ligand stimulated cytokines/chemokines, monocyte and neutrophil phagocytosis of E. coli; Vitamin D **Saliva** – presence of oral Candida species **Stool** – presence of probiotic bacteria L*actobacillus rhamnosus,* LGG and *Bifidobacterium animalis* subsp. *lactis,* BB-12, plus presence of AMR bacteria and *C. difficile* Tertiary outcome: To determine if level of serum vitamin D correlate with colonisation of AMR bacteria in faecal isolates (see section 5). Qualitative sub-study to understand how the trial was conducted within the care home context and identify the mechanisms which affect implementation of trial activities.
Interventions	Daily dose for 12 months of study product - probiotic or matching placebo, or until 31/10/18, whichever is sooner.

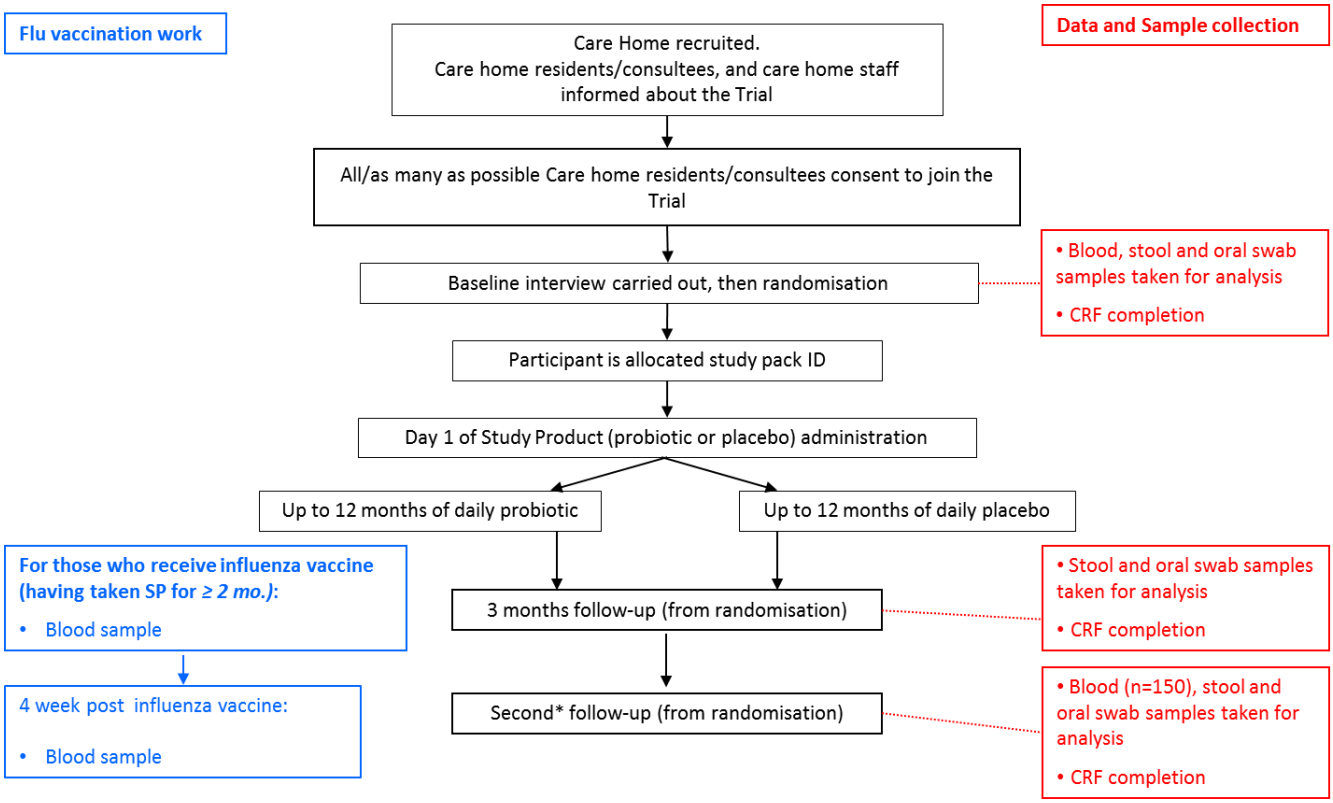
3 Trial summary and schema

3.1 Trial schema



* The follow-up schedule will depend on the length of time that a participant has been in the study. Where possible, participants will have a baseline assessment, and three- and 12-month follow-ups. Due to time limitations, some participants may have a truncated follow-up and will receive either a baseline assessment and 3-month follow-up, or a baseline assessment, 3-month follow-up and a second follow-up between 6-10 months post-randomisation. Infection, antibiotic use, adverse events and study product use is also recorded at regular intervals by the RN from care home notes for 12 months post-randomisation or until 31/10/2018, whichever is sooner

3.2 Participant flow diagram



* The follow-up schedule will depend on the length of time that a participant has been in the study. Where possible, participants will have a baseline assessment, and three- and 12-month follow-ups. Due to time limitations, some participants may have a truncated follow-up and will receive either a baseline assessment and 3-month follow-up, or a baseline assessment, 3-month follow-up and a second follow-up between 6-10 months post-randomisation. Infection, antibiotic use, adverse events and study product use is also recorded at regular intervals by the RN from care home notes for 12 months post-randomisation or until 31/10/2018, whichever is sooner

3.3 Trial summary

Care home residents (CHR) are prescribed far more antibiotics than the general population because of the high burden of infections they bear, caused by weakened immunity, close-proximity living and multi morbidity (1). In our previous research in care homes, we found CHR took antibiotics for an average of 17.4 days per year (2). High antibiotic use increases the risk of reservoirs of antimicrobial resistance (AMR) in care homes that can spread within care homes and to hospitals and the community (1). This will intensify as the population ages. Infections in CHR cost the NHS >£54 M/year in hospitalisation alone (3) and infections are the commonest reason for CHR to be hospitalised. AMR infections are generally more serious and costly, particularly in older people. Reduction in antibiotic use and AMR could improve quality of life, save money, and help preserve the usefulness of existing antibiotics.

Other than vaccination and hygiene methods, there are few interventions proven to prevent infection in CHR. Probiotics are live bacteria that may confer health benefit by improving immune function and reducing carriage of potentially harmful bacteria. Probiotics are safe and cheap and are available as supplements. Probiotics may mediate positive changes in gut bacteria and therefore decrease immunity decline and infection. Reviews of studies found that probiotics reduced antibiotic use and risk of respiratory tract infections in adults (4) and reduced antibiotic associated diarrhoea (AAD) (5). Probiotics also reduced carriage of AMR bacteria in a review of critically ill patients, and have reduced duration of CID (6) and enhanced immune response, including to flu vaccination (7). However, research in CHR is currently lacking.

The PRINCESS trial will trial a probiotic preparation containing *Lactobacillus rhamnosus*, LGG and *Bifidobacterium animalis subsp. lactis*, BB-12 to determine whether this product prevents infections over a 12 month period. Other probiotic species have not shown benefit in respiratory tract infection (RTI) or immune function in older people (8). We will assess total days on antibiotics for infections (including RTI, urinary tract infection (UTI), skin and gastro-intestinal infection (GI)), immune parameters (including influenza vaccine response), and changes in AMR in bowel flora. We will explore mechanisms, and test if probiotics reduce cumulative antibiotic prescribing days by 10% or more. To detect this we will recruit at least 258 CHR from care homes in Wales and England into an individually randomised trial of probiotic vs placebo, and follow them up for up to 12 months. We will seek informed consent from CHR with capacity, and consult with next of kin/representative for those lacking capacity.

A qualitative sub-study will be conducted at the end of data collection of the main study to understand how the trial was carried out within the care home context and identify the mechanisms which affect implementation of trial activities.

4 Introduction

4.1 Background

Introduction

The PRINCESS trial will evaluate the efficacy and mechanisms of a nutritional intervention (daily probiotics) on (i) cumulative antibiotic administration days (CAAD) for all-cause infections and (ii) incidence and duration of infections in care home residents. Antibiotic prescribing has been found to be associated with isolation of resistant organisms and subsequent infections that are resistant to antibiotics. Fewer and less severe infections may reduce the need for antibiotics in CHR. This may therefore reduce the driving influence on AMR and may reduce the likelihood of subsequent resistant infection.

Care home residents (CHR) are particularly vulnerable to common infectious diseases (CID) (1) which are the commonest cause of hospitalisation in this group, costing the NHS >£54 million/year, and often results in lasting health decline. Frailty, atypical presentation and bacterial colonisation patterns lead to high antibiotic use. Reducing antibiotic prescribing in the expanding care home sector is central to the challenge of containing antimicrobial resistance (AMR) in UK.

Probiotics

Probiotics are “live” microorganisms that, when administered in adequate amounts, confer a health benefit on the host. They are present in certain products available in supermarkets as foodstuffs and in formulations used for specific therapeutic purposes. They may prevent infection by blocking pathogenic colonisation and enhancing gut-immune interaction, with influence on mucosal and systemic immunity, leading to enhanced natural killer (NK) cell activity and vaccine response in the elderly (7). Probiotics are safe and well tolerated (9, 10), and systematic reviews found no serious adverse effects in participants of trials of probiotics for antibiotic associated diarrhoea. Some older people already use them regularly, despite an inadequate evidence base supporting their effect on CIDs. Probiotics are feasible to administer to CHR in the course of routine care: Carlsson and colleagues confirmed feasibility of serving a probiotic intervention to people in care homes with dementia for six months (11). They found it was easy to serve alongside usual diet, there were few side effects and that staff were able to complete the processes and measures. In contrast to antibiotic use, long term probiotic use does not result in resistance in commensal gut organisms (12).

The gastrointestinal tract may be a major reservoir for AMR bacteria. This is important because most urinary tract infections arise from auto-inoculation with organisms for the gut. We will directly assess gut colonisation with AMR bacteria, which may be reduced directly via probiotic-induced colonisation or indirectly via reduced cumulative antibiotic prescription days (CAPD). We will explore the underlying immunological mechanisms, including influenza vaccine response (diminished generally in CHR), probiotic modulation of toll-like receptor (TLR) expression and responses to *ex-vivo* pathogenic challenge.

Existing trials and the research gap

Small trials found benefits from probiotics on AMR colonisation, e.g. a systematic review of multidrug resistant gram negative organisms in critically ill patients (13) and a randomised controlled trial (RCT)

of vancomycin resistant enterococci (VRE) in renal dialysis patients (14). A Cochrane review of probiotics to prevent acute upper respiratory tract infections (URTIs) included 14 RCTs, ten of which were meta analysed (including a total of 3451 participants) (4). Probiotics reduced episodes of acute URTI (OR 0.58 95%CI0.36-0.92) and antibiotic prescribing (OR 0.67, 95% CI 0.45-0.98). Side effects of probiotics were minor. The review noted poor allocation concealment of several studies, and heterogeneity, and recommended that future RCTs should "focus on older people." This is because only four studies have compared probiotics to placebo in older people to prevent infections. Turchet and colleagues performed a single centre pilot study of otherwise well 'free living' elderly, randomised to receive a probiotic containing *L. casei* or placebo for three weeks (15). They found no differences in frequency of URTI (in this short study), but reduction in the severity and duration of URTIs. Guillemard randomised otherwise well people living in care homes in France to a probiotic containing *L. casei* or placebo for three months (6). Probiotics decreased the duration of CIDs, especially for URTIs. Makino and colleagues in Japan compared whether the intake of yogurt fermented with *Lactobacillus delbrueckii* over 12 weeks had an effect on the common cold, and found the risk was 2.6 times lower (OR 0.39) and there was increased in natural killer cell activity in the yoghurt group (16). However, randomisation and concealment may not have been adequate in this study. Van Puyenbroeck and colleagues found no difference in the duration of respiratory symptoms or the probability of respiratory symptoms in 'healthy older people' in nursing homes in Antwerp from a probiotic containing *L. casei* shirota for 176 days (8). However, relevant medical conditions and cognitive deficits were excluded. Antibiotic prescription rates were not reported. There was a high level of missing self-complete diary data that generated analytic challenges. The probiotic we plan to use will be different to the strain used in the Guillemard study. Effects of probiotics are thought to vary by strain due to differing resistance to gastric acid and bile, ability to colonise mucosa and susceptibility to antibiotics. Thus, overall, the evidence base supporting a recommendation for frailer elderly either to consume or not consume probiotics is insufficient to robustly guide care. Therefore, a new trial is warranted that is properly designed in terms of allocation and concealment, that focuses on antibiotic use of all cause infections in all CHR including those without capacity and who are most frail, with reliable, frequent external ascertainment.

PRINCESS will be the first rigorous efficacy RCT of daily probiotic vs. placebo probiotic over 12 months on cumulative antibiotic administration days (CAAD) for infections, and will provide data on cheap, safe and widely accessible interventions for the prevention of infection, antibiotic use and AMR in CHR. Whether or not the trial finds a positive effect, the results will help CHR make evidence based decisions either to take or not take this probiotic product in order to maintain their optimal health and wellbeing. Mechanisms will be explored, in terms of vaccine efficacy, cytokine/ chemokine response to TLR ligand stimulation and probiotic effect on cellular TLR expression. This will extend current mechanisms knowledge regarding the interplay between probiotics, gut microbiota and immune function.

Risks and benefits

Infections are a common cause of suffering and increased resource use in CRHs. Even so-called 'minor infections' such as urinary tract infections and upper respiratory tract infections can have an important negative impact on the health, wellbeing and dignity of older frail people. As people get older, the microbiological diversity in their gut reduces as they are less mobile, eat differently, and their immune systems become less active. Most urinary tract infections arise from infection by organisms in our own

gut. By increasing the diversity of microbiological organisms in the gut, taking probiotics regularly as a food supplement may reduce the prevalence of pathogenic bacteria and stimulate the immune system, adding to overall resilience and general wellbeing as well as reducing infections and antibiotic use. Health care associated infections (HCAI) include CIDs and cause significant debility, hospital admissions and death in CHR, burdening both the health service and care home staff. The risk of deterioration in general physical condition was twice as high in CHR in Norway who develop HCAI and were nine times more likely to be admitted to hospital (17). Ageing is independently associated with reduced immune response to infections (18). Probiotics reduced the incidence of RTIs in the general population, possibly by boosting immune mechanisms (4). Although probiotics carry theoretical risks of causing infection beyond the gut and transferring of antibiotic resistant genes, there have been no reports of bacteraemia or fungaemia attributable to the probiotics in trials (9, 10, 19). Gastrointestinal side effects and rash are generally no more common than in patients on placebo probiotic (5).

4.2 Rationale for current trial

The 2010 Adult Social Care Statistics reported that there were 229,900 people in residential care in England, with numbers predicted to steadily increase. The 2011 census reported that there were 291,000 people over the age of 65 resident in the care home population in England and Wales (20). CHR are particularly prone to infections. The year-long observational study (n=274) identified 609 infections that led to an antibiotic prescription (incidence of 2.16 antibiotic prescriptions per resident year, 95% CI 1.90-2.46). The most common indications were RTI (47% of prescriptions), urinary tract infections UTI (29%) and skin infections (18%) (2). CID in CHR led to suffering, loss of dignity, hospitalisation, GP visits and death. Health needs assessment of 240 CHR in South Tyneside PCT identified 167 hospital admissions accounting for 1595 bed days over a year (21). On average, at least 4 beds in the Acute Trust are used for CHR all year round costing around £400K. About 25% of admissions were due to infection costing £100K in one PCT, or an estimated £54M/year for the UK. CIDs in CHR increase GP and care home burden and costs, impacting on opportunities for other aspects of care. Antibiotic prescribing leads to HCAIs such as *C. difficile* and drives AMR. The greatest risk of being infected with a AMR compared to a sensitive bacteria is recent consumption of antibiotics, even after controlling for age, comorbidity and other risk factors (22). AMR infections are more serious, last longer and are more costly to manage (23). Probiotics are effective in preventing AAD (5, 19), but we have no adequate data on prevention of all-cause common infections and antibiotic prescribing in care home residents.

There is an urgent need to reduce AMR through infection prevention in care homes. Urinary tract infections (UTIs) are usually caused by auto-inoculation; that is infection from our own bowel organisms. Carriage of AMR bowel organisms increases the chances of an AMR UTI. AMR Gram-negative septicaemia and AMR UTI are on the increase in the community, especially among older people (24). Care homes are a reservoir for AMR that cycle between the community and hospitals (3, 25). There is a steady increase in the care home population, and evidence-based interventions are needed to improve their quality of life through reducing the incidence of CIDs and antibiotic use (26).

In PRINCESS we will study the long-term effect of administering a combination of two probiotics L*actobacillus rhamnosus* GG and *Bifidobacterium animalis* subsp. *lactis,* BB-12.

Lactobacillus rhamnosus GG has the most extensive number of human studies in a wide variety of populations including elderly individuals (27). These strains have been found to be safe. Although the effect of these probiotic strains on infections in care home residents or older people has not yet been assessed, relevant evidence proving efficacy in other populations supports its use in this trial. A meta-analysis of RCTs involving 1805 children in total found that *Lactobacillus* GG was associated with reduced risk of otitis media, upper respiratory tract infections and antibiotic treatments (28). This meta-analysis included a double blind, placebo controlled RCT of 742 hospitalised children found *Lactobacillus rhamnosus* GG reduced the risk of gastrointestinal (RR 0.40; 0.25-0.70) and respiratory tract infections (RR 0.38; 0.18-0.85) and duration of these infections (29). An RCT of 281 children in day care found that Lactobacillus GG reduced the risk of respiratory tract infections over three months (RR 0.66; 0.52-8.82, NNT=5), and reduced the time with a RTI (30). A double blind RCT found that *Lactobacillus* GG acts as an immune adjuvant to influenza vaccination as measured by levels of protective antibodies to the H3N2 flu strain. This study stresses the need for future studies of probiotics as immune adjuvants focusing on groups known to have poor response to influenza vaccination (31). When administered orally as lozenges the combination of probiotics has been found to have beneficial effect on oral and dental health: reducing plaque and gingival inflammation without affecting oral microbiota (32).

Many studies include more than one probiotic strain in the intervention, and in several studies, *Lactobacillus* GG and *Bifidobacterium animalis* subsp. *lactis,* BB-12 have been included in the same probiotic formulation (33, 34). A randomised placebo controlled trial of 231 college students taking a combination probiotic including *Bifidobacterium animalis* subsp. *lactis,* BB-12 for 12 weeks found that duration and severity of upper respiratory tract infections were improved by the active probiotic (35). A randomised, placebo-controlled, double blind, parallel dose-response study investigated the impact of 4-week commercial yoghurt consumption supplemented with *Bifidobacterium animalis subsp. lactis* (BB-12). The probiotic strain remained active during gut transit and was associated with an increase in beneficial bacteria and a reduction in potentially pathogenic bacteria (36). In a double-blind, placebo- controlled study, 109 new-born 1-month-old infants receiving *Bifidobacterium animalis subsp. lactis* BB-12 were reported to have experienced fewer respiratory infections (65 v. 94 %; risk ratio 0.69; 95 % CI 0.53, 0.89; P = 0.014) than the control infants (37). A randomised, double-blind, placebo-controlled study Infant formula supplemented with the probiotics *Lactobacillus rhamnosus GG* and *Bifidobacterium lactis Bb-12* or placebo was administered daily until the age of 12 months. Those receiving the active probiotic had fewer episodes of otitis media, received fewer courses of antibiotics in the course of routine care, and had fewer recurrent respiratory infections (38). A multispecies probiotic supplementation that included *Lactobacillus rhamnosus GG* and *Bifidobacterium animalis ssp. lactis Bb12* reduced symptoms of irritable bowel syndrome and stabilised the bowel microbiota (39). A randomised, double-blind, placebo-controlled trial in 12 wards in two nursing homes in Finland involving 209 residents received either 109 CFU/day *Bifidobacterium longum* strains or 2) 109 CFU/day *Bifidobacterium lactis Bb12* or 3) placebo for 7 months. Compliance was 85%, and the groups receiving active products had more frequent bowel movements than the placebo group (40). In a 7-week double-blind crossover study, 36 subjects were randomised to receiving yoghurt with *Bifidobacterium animalis subsp. lactis* BB-12 or placebo. BB-12 was safe. The defecation frequency during the BB-12 intake period was increased compared to the no-intake period for group, and comfort after defecation improved significantly (41).

Thus, while both of the proposed probiotic strains have never been evaluated for the purposes intended in the PRINCESS trial; there is evidence from other population groups that these strains have the effect of reducing frequency, severity and duration of infections and stabilising bowel flora. Many infections in the elderly result in autoinoculation from the bowel. It could be likely that the two strains may supplement each other in terms of efficacy due to different mode of actions.

5 Trial objectives and outcomes

HYPOTHESIS (Primary): Daily oral probiotic reduces CAAD for infection vs placebo in CHR.

HYPOTHESIS (Mechanisms): Daily oral probiotic reduces gastrointestinal colonisation with AMR bacteria, enhances influenza vaccine response and modulates *ex-vivo* cytokine and chemokine response to Toll Like Receptor (TLR) agonists vs placebo in CHR.

5.1 Primary and secondary objectives and outcome measures

	Objectives	**Outcome Measures**	**Timepoint(s)* of evaluation of this outcome measure**
Primary	To evaluate the effect of a daily dose of oral probiotics on cumulative systemic antibiotic administration days (CAAD) for all-cause, acute infections	CAAD for all cause infections; Total number of days of systemic antibiotic administration as recorded in care home medical records and discharge summaries if the participant is admitted to hospital	Over a 12* month period (data recorded at regular intervals by RN from care home records)
Secondary	**To determine the effect of daily probiotic intake;**		
Infection	On CAAD for the following sub-categories of infection; respiratory tract infections (RTI), urinary tract infections (UTI), skin infections, gastrointestinal infections (GI), unexplained fever and other	Total number of days of antibiotic administration for each infection type as recorded in care home medical records (collect generic name and mode of delivery) (to be recorded as defined daily dose multiples) Number, site, duration (mean and cumulative) of infection.	Over a 12* month period (data recorded at regular intervals by RN from care home records)
	On incidence and duration of diarrhoea in CHR who are being treated with oral antibiotics	Estimation of incidence and duration of all cause diarrhoea in CHR when taking (and also not taking) oral antibiotic treatment Estimation of incidence and duration of antibiotic-associated diarrhoea in CHR when taking oral antibiotic treatment	Question asked by RN and recorded on weekly record sheet Question asked by RN and recorded on weekly record sheet

	The site, incidence and duration of infection (RTI, UTI, skin infection, GI and unexplained fever) On prevalence of *C. difficile* infection (clinical and bacteriological evidence of *C. difficile* colonisation)	 Stool sample laboratory analysis	Over a 12* month period (data recorded at regular intervals by RN from care home records) At baseline, 3* and up to 12* months
Health Utility	On participants health utility	Self and/or proxy reported health related quality of life measurement EQ5D (5L)	At baseline, 3* and up to 12* months
Wellbeing	On participants wellbeing	Self and/or proxy reported ICEpop CAPability measure for Older people (ICECAP-O)	At baseline, 3* and up to 12* months
Hospitalisation	NHS service use	In relation to infections; number of hospital stays for all-cause hospitalisation (as recorded in care home records and discharge summaries)	In a 12 month* period (data recorded at regular intervals by RN from care home records)
Mortality	Mortality rates	Total number of deaths of trial participants (collected from care home records)	In a 12* month period (data recorded at regular intervals by RN from care home records)
Antimicrobial resistance	Gastrointestinal colonisation with antimicrobial resistant (AMR) bacteria	Culture and antibiotic sensitivity of Gram-negative Enterobacteriaceae and vancomycin resistant enterococci (VRE) from stool sample	Samples taken at baseline, 3* and 12* months
Oral microbiology	Levels of oral Candida	Semi quantitative analysis of oral rinse or saliva samples	Samples taken at baseline, 3* and up to 12* months
Influenza vaccine response (for those who have received influenza vaccine only)	Influenza vaccine efficacy	Blood sample - Haemagglutinatin inhibition assay and antibody titers	Sample taken on the day of (or up to 10 days prior to), and 4 weeks (28 days) post influenza vaccination (trial participants need to have been on Study Product for at least 2 months prior to influenza vaccination to take part in this sub-study)
Immunology (ex-vivo responses to	Participant's cytokine and chemokine response in whole	Response measured by laboratory analysis in whole blood (stimulated ex-vivo by TLR2 and TLR4 agonists)	Baseline and up to 12* months

pathogenic challenge) (n≈100)	blood samples stimulated *ex-vivo* by TLR2 and TLR4 agonists Participant's plasma cytokines and chemokines Participant's monocyte and neutrophil phagocytosis of *E.coli*	 Measurement of plasma cytokines and chemokines in plasma and whole blood Measurement of monocyte and neutrophil phagocytosis of *E.coli*	 Baseline and up to 12* months Baseline and up to 12 months*
Gut microbiology	Quantify the amount of probiotic in stool samples	Investigative work to analyse level of L*actobacillus rhamnosus,* LGG and *Bifidobacterium animalis* subsp. *lactis,* BB-12 from stool sample	Samples taken at baseline, 3* and up to 12* months
Haematology and Biochemistry	Haematology; biochemistry	Full blood count (FBC) including immune phenotyping; vitamin D	FBC and Vitamin D at Baseline (all participants); FBC on n≈100 (immunology participants only) and up to 12 months
Tertiary	To determine if the level of serum vitamin D at baseline correlate with colonisation of AMR bacteria in faecal isolates	Level of serum vitamin D and AMR colonisation within faecal sample	Baseline
Additional baseline measurements		Demographic information (to include age); clinical frailty score; use of proton pump inhibitors, laxatives and dose of Vitamin D	Baseline
Other		Record whether trial participant refused to give blood/saliva/stool sample	Over 12* months from care home resident record
		Ask care home staff what arm they think the trial participant is in	12 month CRF
		Record most common method of delivery of study product e.g. swallowed with water or sprinkled on food/drink	Over 12* months from MARS sheet
Qualitative sub-study	To understand how the trial was carried out within the care home context and to identify the mechanisms with affect implementation of trial activities	With trial participants; consultees; relatives or friends; care home managers; care home staff; and research nurses	At the end of data collection

* The follow-up schedule will depend on the length of time that a participant has been in the study. Where possible, participants will have a baseline assessment, and three- and 12-month follow-ups. Due to time limitations, some participants may have a truncated follow-up and will receive either a baseline assessment and 3-month follow-up, or a baseline assessment, 3-month follow-up and a second follow-up between 6-10 months post-randomisation. Infection, antibiotic use, adverse events and study product use is also recorded at regular intervals by the RN from care home notes for 12 months post-randomisation or until 31/10/2018, whichever is sooner

All tissue samples donated by trial participants will be taken on the understanding that they may be used for future research projects in the UK. The samples will be stored and subsequently distributed to approved projects in accordance with the Human Tissue Act and ethical legislation. This statement will form part of the consent procedure.

6 Trial design

A double blind, individually randomised two arm trial design to assess the effect of a daily oral probiotic versus placebo on CAAD for infection in at least 258 Care Home Residents.

6.1 Internal Pilot

A three month internal pilot phase will assess:

- CHR recruitment rate assumptions
- The suitability of the data collection tools and processes
- Study product administration rate
- The robustness of the sample collection and shipment processes

Information gathered during this period will be used to inform any changes to the main trial design or procedures. It is not anticipated that any substantial changes will be required. All trial procedures, participant visit and sample collection detailed below will be carried out during the internal pilot. All participants will be followed up for as long as possible to a maximum of 12 months.

We anticipate recruiting four care homes for the internal pilot – approximately two in the South Wales area and approximately two in the Oxford area. These four care homes should have at least 30 residents in each home.

7 Care Home and Investigator selection

We anticipate that a total of approximately 20 care homes located in Wales and England will be recruited to the trial. We will aim to recruit larger care homes with a minimum of 50 CHR in order to organise and plan recruitment. Care homes with less than 50 CHR may be selected if they have adequate recruitment potential. The manager of the care homes will be approached to obtain permission for the care home to take part in the trial and for all CHR to be approached about the trial.

The senior care home staff / nursing staff will ensure that the following documents have been received by the coordinating centre (see contact details on page 2):

- Site Specific Assessment approval for the care home
- A signed trial agreement (care home lead and sponsor signature)
- Completed signature list and roles and responsibilities document
- Completed contacts list of all site personnel working on the trial
- Participant/consultee information sheets and consent/declaration forms

Upon receipt of all the above documents, the coordinating centre will send a confirmation letter to the care home lead detailing that the care home is now ready to recruit participants into the trial. The coordinating centre will also provide the care home with a site file, in which the confirmation letter should be filed. The care home will also be provided with all documents required to recruit a participant into the PRINCESS trial and trial supplies.

8 Participant selection

8.1 Trial population

Care home residents in Wales and England.

Participants are eligible for the trial if they meet all of the following inclusion criteria and none of the exclusion criteria. All queries about patient eligibility should be directed to the coordinating centre before randomisation.

8.2 Inclusion criteria

- Currently living in a care home setting (residential, nursing or mixed)
- Participant is willing and able to give informed consent for participation in the trial OR if the participant lacks capacity, a consultee is willing to complete a consultee declaration form
- Aged 65 years or older

8.3 Exclusion criteria

The CHR may not enter the trial if **ANY** of the following apply:

- Is known to be immunocompromised (requiring immunosuppressants, long term high dose oral, intramuscular or intravenous steroids)
- Is currently taking regular probiotics and is not willing to adapt to trial protocol
- Is currently participating in a CTIMP, or has been a participant in a CTIMP in the last thirty days
- Is a temporary care home resident (i.e. less than 1 month of planned transitional/respite residential care)
- Death is thought to be imminent
- Lactose intolerant

9 Outcome measures

9.1 Primary outcome measure

Please see section 5 Trial objectives and outcomes.

9.2 Secondary outcome measure

Please see section 5 Trial objectives and outcomes.

10 Recruitment and randomisation

10.1 Number of participants

A total of between 258 and 270 participants, with an upper limit of 330 participants will be recruited.

10.2 Recruitment process

The PRINCESS trial will randomise at least 258 care home residents (CHR) to either receive probiotics or placebo treatment for 12 months or until 31/10/2018, whichever is sooner. In order to achieve this target we estimate that 660 care home residents from approximately 20 care homes will need to be invited to participate in the trial. Care home staff, nursing staff and/or PRINCESS Trial Research Nurses (RNs) will identify those CHR who are potentially eligible to join the trial.

A screening log of all ineligible and eligible but not consented/not approached CHR will be kept at each care home so that any biases from differential recruitment can be explored. The screening log should be sent to the coordinating centre on request (see section 21 for further detail on data monitoring/quality assurance).

The participant's GP will be informed of the participant's entry into the trial. Each GP will be provided with a summary of the trial and the participant information sheet (PIS) plus any other information they may require as appropriate. It will be made clear that the research team will collect data on infections but that all clinical assessments and management of any infections will remain the responsibility of the GP.

Care homes will be provided with information on prevalence of *C. difficile* infection arising at their care home.

Each Care Home will receive £750 to cover their involvement – this will consist of £400 to help with set-up costs, and £350 six months later if participants have been recruited.

10.3 Informed consent

There are two categories of CHR who are eligible to join the trial; those who have capacity and are able to consent for themselves and those unable to consent for themselves (lack capacity). Consent procedures will differ according to the mental capacity of the CHR. Informed consent or a consultee declaration (see below) must be obtained prior to any trial procedures being undertaken.

The following information sheets apply:

Participant

- Pictorial Participant Information Booklet
- Participant Information Booklet
- Participant Consent Form
- Participant Information Booklet Update^

Consultee

- Consultee Information Booklet
- Consultee Declaration Form
- Consultee Information Booklet Update^

^ These updates will be used in addition to the Booklets for those trial participants who will not be getting the full 12-month follow-up

Mental Capacity Assessment: Mental capacity will be assessed according to the Mental Capacity Act 2005, which provides a legal framework within which health and care professionals must act. As such all care homes have senior staff members who are fully trained to assess the mental capacity of CHR at any time. This Code of Practice (42) provides comprehensive advice on good practice for the assessment of capacity, which is time and decision-specific and depends on clinical judgement within a valid contestable process.

According to the Mental Capacity Act, the CHR will be assumed to have capacity unless it is established that they lack capacity, all practicable steps having been made to help them do so. Where there is a concern that a CHR lacks capacity to provide informed consent for themselves to participate in the trial, the CHR will be assessed for mental capacity by delegated individuals (e.g. senior care home staff/nursing staff/RNs). A standard template for recording of the mental capacity assessment will be provided to care homes, together with specific training on the use of this template. This document will be reviewed by a delegated individual (e.g. senior care home staff/RN) to enable a decision to be made regarding the mental capacity of the CHR, prior to taking consent from the CHR or a consultee declaration from their representative if the CHR is assessed as lacking capacity.

For both CHR with capacity and those lacking capacity: All potentially eligible CHR or their consultee will be fully informed about the trial through the PIS or CIS respectively supplemented with verbal explanations. The detailed PIS and CIS will include: the exact nature of the trial; what it will involve for the participant (and consultee, if applicable); the implications and constraints of the protocol; the known side effects and any risks involved in taking part. It will be clearly stated that the participant is free to withdraw/be withdrawn from the trial at any time for any reason without prejudice to future care, and with no obligation to give the reason for withdrawal.

The participant or consultee will be allowed as much time as they need to consider the information, and the opportunity to ask questions. Written Informed Consent (or exceptions as listed below) or Consultee Declaration will then be obtained by means of participant or consultee dated signature, respectively, and dated signature of the person who presented and obtained the Informed Consent/Consultee Declaration. The consent/declaration process will be undertaken by a suitably qualified and experienced delegated individual who has been authorised to do so by the Chief/Principal Investigator (e.g. care home staff/nursing staff/RN). A copy of the signed Informed Consent or Declaration Form will be given to the participant or consultee, respectively. The original signed form will be retained at the trial site.

Eligible CHR may participate in the trial even if they (or their consultee) prefer to opt out of the participant providing blood and/or stool and/or saliva samples.

Specific consent will be sought at trial entry from CHR with capacity to remain in the trial should capacity be lost during the trial.

CHR lacking capacity: Where a CHR lacks capacity to provide consent for themselves, a legal representative/guardian will be consulted who acts as their 'consultee'. The consultee may be a friend or unpaid carer or relative, an attorney acting under a lasting power of attorney, or a court appointed deputy who has a relationship or personal knowledge of the person who lacks capacity. The consultee will be provided with information about the trial and they will be asked their advice regarding the inclusion of the person and what the person's views would have been regarding inclusion in the research if they had capacity to make the decision for themselves. In the event that a consultee who has an unpaid or non-professional role in caring for the person cannot be identified, or is not willing to act, a nominated consultee will be appointed and consulted prior to including the resident in the trial.

In the event that a consultee themselves loses capacity or dies during the trial period, an alternative representative will be contacted to act as consultee and a new consultee Declaration Form will be completed. If there is no one to represent the CHR in the trial, the CHR will be excluded or withdrawn depending on whether a CHR has already been recruited to the trial.

If a participant regains capacity during the trial period, the participant will be fully informed about the trial and informed consent to remain in the trial will be obtained from the participant.

Consultee Declaration by post: If a consultee is required and they cannot attend a face-to-face interview for the declaration process, the above documents may be sent by post from the care homes. The delegated individual (e.g. care home senior staff/RN) will countersign the signed and dated consultee declaration form returned by the consultee and a copy of the completed consultee Declaration Form will then be sent to the consultee. The original consultee Declaration Form will be retained at the trial site. All consultees who have not responded will be contacted by phone or sent a follow-up letter at least once by the individual delegated to undertake the declaration process.

Verbal consent/declaration: In the event that a CHR with capacity or a consultee for a CHR lacking capacity cannot provide handwritten signatures on the Consent/Declaration Form, verbal consent will be taken. In such cases, a delegated individual (e.g. senior care home staff) will read and discuss the trial with the CHR or their consultee to ensure understanding of the trial protocol. A member of the research team will witness, sign and date the Consent/Declaration Form to approve that consent or a consultee declaration has been given.

10.4 Randomisation/registration and unblinding

Participants will be remotely randomised using an online computerised randomisation system created by the University of Oxford Primary Care Clinical trials Unit (PCCTU). The system will be operational 24 hours a day.

Randomisation will be performed by the RN only after the participant has signed the Consent Form (or their consultee has signed a consultee Declaration Form) and completed the baseline assessments.

The RNs will be provided with an individual login ID to the online system. Participants will be randomised to either probiotic or placebo.

As PRINCESS is a double-blind trial, the participants, care home staff, treating clinicians, and trial team (including the trial statistician and RNs conducting all assessments) will be unaware of the group to which the participant has been allocated for the duration of the trial.

Each Study Product pack (probiotic or placebo) will be labelled with a unique identification number (pack ID). The online system will allocate a pack ID for each participant. The pack will contain one month's supply of study product (see section 12). The first allocation of study product will be a two month supply (two trial packs), but will be for one month (one trial pack) thereafter.

The participant ID should be entered on the pack label and both the participant ID and pack ID should be entered onto the CRF by the randomiser. The coordinating centres will also be notified that a participant has been randomised via an automated e-mail alert mechanism.

When a new study product pack is required (i.e. on a monthly basis): the delegated individual should enter the participant ID into the online system and will be allocated another pack ID. The participant ID should then be entered onto the pack label and the pack ID entered onto the paper CRF.

The unique participant IDs and pack IDs will be linked in the randomisation file, which will only be accessible by a statistician who is independent of the trial.

For more details, please consult the PRINCESS randomisation protocol.

Randomisation

To randomise a patient log on to https://ctu1.phc.ox.ac.uk/randomise

Unblinding: There will not be an emergency unblinding procedure – care homes will be advised to stop study product administration if this is necessary. Unblinding will be available during normal office working hours.

In the event that the participant needs to be unblinded, the care home staff are directed to contact the PRINCESS team on 02920 687601. The PRINCESS unblinding procedure will be followed to unblind the participant and in this case the unblinding of a randomised trial participant can only be carried out by a suitable delegated individual at SEWTU.

10.5 Screening logs

A screening log of all ineligible and eligible but not consented/not approached will be kept at each centre so that any biases from differential recruitment will be detected. The screening log should be sent to the PRINCESS Trial Manager (see section 21 for further detail on data monitoring/quality assurance).

11 Withdrawal & loss to follow-up

Each participant/consultee has the right to withdraw from any aspect of the trial at any time. The participant's care will not be affected at any time by declining to participate or withdrawing from the trial. Clear distinction must be made as to what aspect of the trial the participant is withdrawing or is withdrawn from. These aspects could be:

- Withdrawal from entire trial and does not want any data or samples already collected relating to them to be used
- Withdrawal from trial, study product and all subsequent trial follow-up (sample collection and data collection), but data and samples already obtained up to this point can be used
- Withdrawal from study product but happy to continue with follow-up processes (to include data collection and/or sample collection) – this may be due to a request to withdraw, or as a result of a change in the participant's condition or circumstances which justifies the discontinuation of the study product
- Withdrawal from study product and all subsequent sample and questionnaire data collection, but happy for routine care record data to be collected.

If a participant wishes to withdraw from the trial or is withdrawn all trial data collected to that date will be included in the final analysis subject to the appropriate consent being in place. The reason for participant withdrawal will be detailed in a Case Report Form (CRF) and reviewed by the CI and independent committee. Any queries relating to potential withdrawal of a participant should be forwarded to the coordinating centre immediately.

12 Intervention

Participants in the PRINCESS trial will be asked to take an oral dose of probiotic (*Lactobacillus rhamnosus,* LGG and *Bifidobacterium animalis* subsp. *lactis,* BB-12) or a matched placebo once daily for 12 months or until 31/10/18, whichever is sooner. The probiotic or placebo (referred to as Study Product) will be administered by the CHR's normal care giver and provided in capsule form. The preferred route of administration will be in the following order:

1) The capsule swallowed whole with water
2) The capsule emptied into a small amount of cold or lukewarm liquid and then swallowed
3) The capsule opened and its contents sprinkled onto cold or lukewarm food (not hot food) and then eaten

Suitability for religious groups and dietary preferences

The capsules are suitable for vegans, are Halal and Kosher (Kosher dairy excluding Passover), do not contain any genetically modified organisms or genetically modified raw materials, and allergen labelling is not required. The capsules do contain lactose. Care homes will be provided with documentation detailing the composition of the capsules.

The capsules are stable at room temperature for two years and temperature monitoring is not required for short term (e.g. one month) storage.

Labelling and recording

The capsules will be provided to the care home labelled with a unique pack ID. Packs will be allocated to participants on a monthly basis. The delegated individual (e.g. RN) should enter the participant ID into the online pack allocation system (see section 10.4) and will be allocated a pack ID. The participant ID should then be entered onto the pack label and the CRF completed.

Participants admitted to hospital would not be expected to continue taking the study product during their hospital stay.

12.1 Adherence

Data regarding participant's adherence to the study product will be collected from a number of sources:

- MAR sheets - administration of the study product will be recorded by care home staff using Medication Administration Record sheets (MAR sheets) and monitored by RNs during their visits to each care home. MAR sheet are used in most care homes and will allow incorporation of trial procedures into routine practice easily. If MAR sheets or an equivalent recording system is not used routinely by any care home participating in the PRINCESS trial the research team will provide MAR sheets and training as appropriate.
- Presence of probiotic organisms in bowel - the presence of the probiotic organisms will be assessed in stool samples of participants at baseline, three and up to 12 months. This will give an indication of adherence, survival of the probiotic in the large bowl and potential contamination in the placebo arm.
- Capsule count - regular counts of unused study product will be undertaken by the PRINCESS trial research nurses.

13 Adverse Events (Safety)

13.1 General Definitions

Adverse Event (AE): Any untoward medical occurrence in a trial participant which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including abnormal laboratory finding), symptom, or disease.

Serious Adverse Event (SAE): Any adverse event that:

- Results in death
- Is life-threatening*
- Required hospitalisation or prolongation of existing hospitalisation**
- Results in persistent or significant disability or incapacity
- Consists of a congenital anomaly or birth defect
- Other medically important condition ***

* Note: The term "life-threatening" in the definition of serious refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

** Note: Hospitalisation is defined as an inpatient admission (at least an overnight stay), regardless of the length of stay, even if the hospitalisation is a precautionary measure, for continued observation. Pre-planned hospitalisation e.g. for pre-existing conditions which have not worsened or elective procedures does not constitute an adverse event.

*** Note: other events that may not result in death are not life-threatening, or do not require hospitalisation may be considered as a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

An SAE occurring to a research participant should be reported to the main REC where in the opinion of the CI the event was:

- Related – that is, it resulted from administration of any of the research procedures, and
- Unexpected – that is, the type of event is not listed in the protocol as an expected occurrence

Serious Adverse Reaction (SAR)

For ease of recording and reporting, in this study an SAE thought to be probably or definitely related to the trial/study or intervention (see causality section below) will be referred to as a Serious Adverse Reaction (SAR).

13.2 Causality

Causality should be assigned using the definitions in the table below.

For AEs, this assignment should be made by the delegated RN. For SAEs this assignment should be made by the delegated RN and delegated second assessor (e.g another RN or a clinician involved in the study) and the assessment confirmed by the Chief Investigator or a delegated Clinical Reviewer.

Relationship	Description
Unrelated	There is no evidence of any causal relationship with the trial/study or intervention
Unlikely	There is little evidence to suggest there is a casual relationship (e.g. the event did not occur within a reasonable time after intervention) with the study/trial or intervention. There is another reasonable explanation for the event (e.g. the participant's clinical condition, other treatment).
Possible	There is some evidence to suggest a causal relationship with the trial/study or intervention (e.g. because the event occurs within a reasonable time after intervention). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definite	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.

13.3 Expectedness

In this study only SARs will be assessed for expectedness by the CI (or delegated Clinical Reviewer).

Based on the manufacturer's information and review of the literature, at the time of writing, any SAR occurring in this study should be classed as unexpected. However, as the manufacturer's safety information is updated every two years and new literature maybe published at any time, when assessing expectedness the CI (or delegated Clinical Reviewer) should refer to the PRINCESS safety reporting management plan as a separate document to the protocol.

Summary of current manufacturer's information

CHR Hansen's safety and origin information sheet published in March 2015 states that the probiotic strains BB-12 and LGG have been used worldwide since 1985 and 1990 as an ingredient in food and dietary supplements with no reported consumer illness or injury, and that the BB-12 and LGG strains have been tested in more than 140 and 250 clinical studies respectively from new-born pre-term infants to elderly in doses up to at least 100 billion CFU/day with no reported adverse events. Therefore, we do not anticipate any adverse events based on the current manufacturers information.

Summary of current literature

Based on an extensive review of the literature the CI has concluded that probiotics, including the strains used in PRINCESS, are generally well tolerated by the elderly and the risks to CHR associated with the probiotics used in PRINCESS are very low. A recent paper summarises the current risks (43): Infections (e.g. septicaemia) caused by the probiotic bacteria have not been reported in trials; theoretical risks of gene transfer of antibiotic resistance and over stimulation of the immune response have not been reported; although deleterious metabolic activities e.g. bowel ischemia and D-lactic acidosis in patients with pancreatitis have been reported, the mode of probiotic delivery was different to PRINCESS. Therefore, if any of the above events were to occur in PRINCESS they would be unexpected.

Minor gastrointestinal symptoms such as abdominal cramping, nausea, soft stools, flatulence and taste disturbance have been reported and it is also possible that a minor allergic reaction could occur. If these events were to occur, they would be classed as expected AEs, but would be unexpected if they fulfilled the definition of a SAR.

Summary of current expected events related to the study procedures

There is a risk of haematoma at the site of venepuncture for a study blood sample; however this would only be expected to be an AE. If the event fulfilled a definition of a SAR, it would be classed as unexpected.

13.4 Reporting procedures

This study is not a CTIMP and the probiotic being used is a well-established food supplement. The study population will have a vast number of health events in the normal course of their care at this stage in their lives. Given the potential frailty of the trial population and the high incidence of hospitalisation and death in the course of routine care, we would aim to not cloud any true emerging safety profile by collection of unrelated data. The following reporting procedure should be used for

PRINCESS. Any queries concerning adverse event reporting should be directed to the PRINCESS Trial Manager in the first instance.

Adverse events will be initially identified either through the PI/Care home staff identifying that an adverse event has occurred and informing the RN/TM, or during routine weekly data collection by the RN.

- **Non-serious Adverse Events** with the causality classification of probably or definitely related to:
    - the study product (such as gastrointestinal symptoms, or ingredient-related allergic reaction)
    - or study procedures (such as a haematoma at the site of venepuncture for a study sample)

  will be collected as part of routine follow-up (recorded by the RN on the PRINCESS Weekly Record and Weekly Record: Further Information CRF) from the time of consent until the 6 to 12 month follow-up period.

- **Other non-serious AEs** will not be collected. The PI/CHS should manage AEs according to routine care home procedures.

- **All Serious Adverse Events (SAEs)** will be collected as part of routine follow-up (recorded by the RN on the PRINCESS Weekly Record and Weekly Record: Further Information CRF) from the time of consent until the 12 month follow-up period. SAEs will be discussed (in person, by phone or by email) by the RN with a second delegated assessor (e.g. another RN or a clinician involved in the study) to confirm the causality classification (definitely, probably, possibly, unlikely, not related). The details of the second assessment will be recorded on the PRINCESS Weekly Record: Further Information CRF. Where there is a difference in classification between the two assessors, the highest category of causality (most likely to be related) will be selected.

- **Serious Adverse Reaction (SAR):** If the Serious Adverse Event (SAE) is classified as being probably or definitely related to study procedures or the study product (such as septicaemia and the suspected pathogen is identified as either of the strains used in the PRINCESS study product) it is a classed as a **Serious Adverse Reaction (SAR).** A SAR reporting form should also be completed by the RN and returned directly to the TM (or delegate), or faxed to SEWTU on 02031 070875 with a follow up email (PRINCESS@Cardiff.ac.uk) within 4 days of the RN becoming aware of the event.

Further information may be required from reviewing the participant's care home records, or reported by care home staff liaising with clinical teams caring for the resident (GP, secondary care).

All SAR reporting forms received by SEWTU will be subject to clinical review by the CI or delegate to confirm causality and assess expectedness.

SEWTU will notify the Sponsor and main REC of all related and unexpected SAEs (i.e. all unexpected SARs) occurring during the trial within **15** calendar days of the CI becoming aware of the event. All

SARs will be reported to the monitoring committees (TMG, TSC and IDMC), sponsor and CHR Hansen (study product manufacturer) as required by the relevant committee/party. All unrelated SAEs will be reported to the TMG and included in the annual report to the IDMC, and any arising safety concerns will also be reported to the main REC as part of the annual progress report.

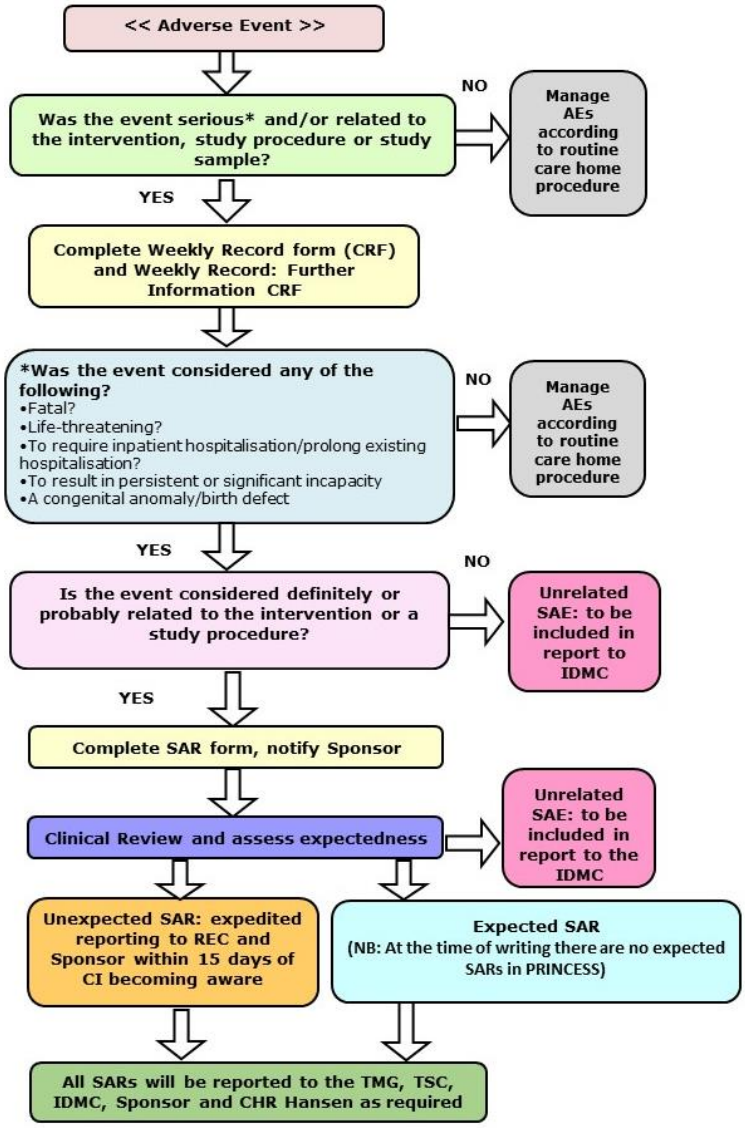
Contact details for reporting SARs

Please Fax to 020 3107 0875, attention Princess Trial Manager.

Queries

Tel: 029 2068 7601 (Mon to Fri 09.00 – 17.00)

SAE Flowchart



14 Adverse Events (Human Tissue)

14.1 General Definitions:

(Human Tissue) Adverse Event: Any untoward occurrence associated with the procurement, testing, processing, storage and distribution of relevant material and cells that might lead to loss or damage of relevant material or breach procedures protecting the donor and/or relevant material.

Where **Relevant Material** means any material, other than gametes, removed from the body which consists of or includes human cells. In the Human Tissue Act references to relevant material from a human body do not include:

- embryos outside the human body,
- hair and nail from the body of a living person,
- cell lines or any other human material created outside the human body,
- serum, plasma, DNA and RNA,

14.2 PRINCESS Reporting Procedures:

Any Human Tissue Adverse Event thought to be related to the stool, blood or saliva samples (such as sample being taken without consent, an incorrectly labelled or lost sample, or spillage during transport) will be collected as part of routine follow-up (recorded by the RN on the PRINCESS Weekly Record and Weekly Record: Further Information CRF and monitored by SEWTU) from the time of consent until the 12 month follow-up period.

SEWTU will notify the Sponsor of all human tissue adverse events occurring during the trial, and all other requirements as specified by the Human Tissue Act.

15 Trial procedures

15.1 Location

All trial appointments will take place at the care home where the participant is resident. All trial procedures will be carried out by either the participants' normal care providers or by RNs.

15.2 Data collection/assessment

Eligible participants or their consultee will have an appointment arranged at which informed consent or a consultee declaration (see section 10.3) will be obtained. A second baseline appointment will be made, at which baseline samples will be taken, and baseline data recorded.

The follow-up schedule will depend on the length of time that a participant has been in the study.

Where possible, participants will have a baseline assessment, and three- and 12-month follow-ups (participants randomised pre-December 2017).

Due to time limitations, some participants may have a truncated follow-up and will receive either a baseline assessment and 3 month follow-up (those randomised in May 2018), or a baseline

assessment, 3 month follow-up and a second follow-up between 6-10 months post-randomisation (those randomised between December 2017 and April 2018).

The target window for the 3-month follow-up is -2/+4 weeks.

The target window for the 12-month follow-up is 11 months +/- 1 month.

Infection, antibiotic use, adverse events and study product use will also be recorded by the RN from care home notes at regular intervals using the weekly record forms for 12 months post randomisation or until 31/10/18, whichever is sooner.

A sub-set of patients who have been taking the study product for a minimum of two months and who have had an influenza vaccination after the two month period will have an appointment arranged to attend a third appointment about four weeks (28 days) after the influenza vaccine.

Influenza vaccinations will be administered via routine care. The date of administration will be recorded.

15.3 Trial samples

All trial participants: All trial participants will be asked to provide blood samples at baseline - a full blood count and vitamin D levels will be measured for each participant - the volume of blood taken will be no more than 10ml. At 12 months a full blood count will be measured for around 150 participants - the volume of blood taken will be no more than 4ml.

Immunology: For a sample of around 100 trial participants some additional immunology work (detailed in section 5.1) will be done and an additional blood sample will be taken at baseline and up to 12 months – the volume of blood taken will be no more than 6ml at each timepoint. The sample will be taken at the same time as the other blood samples.

Flu vaccine response sub-study: A sub-set of patients will be asked to provide a blood sample on the day of (or up to 10 days prior to this), and four weeks (28 days) after they receive their routine seasonal influenza vaccination. For those agreeing to take part in this flu vaccine response sub-study, 5ml of blood will be taken on the day of, and four weeks after receipt of the influenza vaccination, giving a total of no more than 10ml of blood collected.

Blood samples will be taken by the RN who, if needed, will have undergone geriatric phlebotomy training, or by a care home staff member if for example, they are already taking a blood sample for routine care, and/or the care home resident prefers this. The total volume of blood collected during the 6 to 12 month duration of the trial on any one participant will be no more than 36ml.

Each participant will be asked to provide a stool sample at baseline, three months and up to 12 months. The participants normal care provider will be asked to collect the stool samples.

Each participant will be asked to provide a saliva samples (saliva sample or oral rinse) at baseline, three months and up to 12 months.

If participants prefer not to provide any or all of the trial samples listed above this will not be a barrier to their entry to the PRINCESS trial.

All samples will be labelled with the participant unique trial identification number before being sent for analysis. Only fully trained and authorised members of the research team and laboratory staff will have access to the samples. The CI will ensure that the storage, analysis and disposal of all clinical samples will meet the requirements of the Human Tissue Act, 2004.

All tissue samples donated by trial participants will be taken on the understanding that they may be used for future research projects in the UK. The samples will be stored and subsequently distributed to approved projects in accordance with the Human Tissue Act and ethical legislation.

Any blood/saliva/stool test results will not be reported back to the CHR GP as they are being collected only for research purposes and will not be analysed in real-time.

15.4 Follow-up

The three and 6 to 12 month follow-up visit is detailed above in section 15.3.

16 Qualitative sub-study

In addition to the main PRINCESS trial, a qualitative sub-study will be conducted after the end of data collection. The sub-study will consent PRINCESS trial participants, consultees, research nurses, care home staff and care home managers. The aim of the sub-study is to interview these groups of people about their involvement in the PRINCESS trial, and to examine how the PRINCESS research activities were carried out within the care home environment. The protocol for this sub-study can be found in Appendix 1.

16.1 Justification

The set-up and the implementation of the PRINCESS trial was informed through past experience of other studies undertaken in care homes, as well as research around alternative models of consent (44-46). This qualitative study aims to examine how PRINCESS researchers, care home staff, and study participants worked to enact PRINCESS research activities.

The findings will inform the interpretation of the main study findings and also provide important information for those conducting research (including randomised controlled trials) in the care home context with practical recommendations and potential models of working with and within care homes. More specifically, the qualitative data will: provide a better understanding of the quantitative results, including reasons for missing data, and information about adherence to the study product; provide rich detail regarding how the PRINCESS consultee model of recruitment worked in practice; and what were the means (or barriers) by which PRINCESS researchers were able to recruit a representative cross section of those living in the care home.

16.2 Aims

The overall aim of the study is to understand how the PRINCESS study was carried out within the care home context and identify the mechanisms which affected implementation of study activities.

16.3 Objectives

The following objectives aim to be achieved:

1. Obtain research professionals' perspectives on the PRINCESS study, and gain an understanding of how they conducted the PRINCESS research activities within the care home.
2. Obtain care home staff perspectives on PRINCESS and the research activities associated with the study.
3. Obtain resident and consultee perspectives on PRINCESS and the research activities associated with the study.
4. Gain an understanding of the mechanisms which facilitated (or limited) conducting research activities in the care home.
5. Understand and gather contextual information that can assist the implementation of future research studies (including randomised controlled trials) with older people in care homes, a vastly under-researched setting.

16.4 Methods

Design

Face-to-face or telephone semi-structured, qualitative interviews will be conducted with eligible participants - these are expected to take around 30 minutes. A topic guide will be developed which will be informed by relevant literature, and it will be piloted to ensure the interviews collect relevant data. Aspects of the research process of the RCT will be explored, with the aim to elucidate relevant contextual issues, barriers, and facilitators to the implementation of the RCT.

Participant identification

Participation will be limited to those who have already been involved in some capacity in the PRINCESS RCT. Ideally seven participants from each of the following groups will be interviewed:

1. PRINCESS RCT participants (care home residents) and family members/friends who a) acted as consultees for PRINCESS RCT participants who lacked capacity to provide informed consent or b) experienced/observed their relative/friend's participation in the PRINCESS RCT;
2. Managers of participating PRINCESS RCT care homes;
3. Care home staff that contributed to the research activities in the PRINCESS RCT;
4. Research professionals who conducted the research activities in the PRINCESS RCT.

Recruitment

The recruitment strategy will vary according to the potential participant group. Those within the care home environment (managers, staff, consultees and residents) will be 'snowball sampled'; i.e. managers will be approached initially. Managers can then approach staff, who in turn will then approach residents and consultees (47).

Consent

All participants will be provided with an information sheet for the sub-study, and have the opportunity to ask questions and receive satisfactory responses, prior to the lead researcher seeking informed consent. Written informed consent will be obtained using a study-specific consent form prior to any data collection. A copy of the consent form will be provided to the participant, and the original retained by the lead researcher.

Participants will be informed that they are able to withdraw from the sub-study, should they wish to do so, by contacting the lead researcher. Data from participants who have withdrawn will not be used in the analysis. Participants are able to request to withdraw from the sub-study at any point, up until analysis is concluded.

Analysis

The interviews will be digitally audio-recorded with consent and transcribed verbatim. All transcripts will be deidentified, and along with the source data will be stored confidentially on password protected servers maintained on the Cardiff University Network.

Data will be entered into Qualitative Data Analysis software (NVivo 11, QRS International) and iteratively coded using codes emerging from the data, and those identified *a priori*. Data will be analysed using framework analysis.

17 Statistical considerations

17.1 Randomisation

Participants will be individually randomised in a 1:1 ratio using minimisation. In order to achieve a balance of probiotic and placebo allocations within care homes, the care home from which a participant is recruited and gender of participant will be used as a minimisation variable. A random element, set at 80%, will be used in order to maintain the integrity of the minimisation process. The participants, treating clinicians, trial statistician and research nurses will remain unaware of the group to which participants are allocated during the trial. Pack IDs will be assigned probiotic / placebo allocations using randomly permuted blocks, and this list will be uploaded into the online system. This process will be carried out by a statistician independent of the trial team.

For more details, please consult the PRINCESS randomisation protocol.

17.2 Sample size

Primary Outcome

The original target for the PRINCESS trial was to randomise 330 participants from around 20 care homes in Wales and England. This would provide 90% power at the 5% level to demonstrate a 10% relative reduction in cumulative antibiotic administration days (CAAD) (assuming an average CAAD of 17.4 days and an absolute reduction in the probiotic arm to 15.6 days per resident-year). We consider that a 10% reduction is feasible and would be clinically important.

This sample size accounted for 30% of participants being lost to follow-up due to withdrawal or death during the study.

An interim assessment of primary outcome ascertainment revealed that the mean percentage of days for which there are valid antibiotic administration data (i.e. either no antibiotics administered, or the number of days antibiotics have been administered in a given week) is 77.4%, or 283 days out of a possible 365 on average. This percentage varies depending on the length of time participants have been in the study. However, it remains high for participants who have been in the study for over six-months, and does not take into account other data sources that might be used (e.g. hospital discharge summaries, medication administration records, etc.). This is likely to therefore reflect an underestimate of the availability of primary outcome data, but nevertheless is a more accurate reflection of the likely level of follow-up when compared to our original assumption.

Given slower than anticipated recruitment, and this new information regarding the trade-off between the number of participants required and average length of follow-up, we will aim to randomise between 258 and 270 participants. Assuming a mean number of days for which primary outcome data will be available (i.e. accounting for follow-up time and missing data) of approximately 250 days, this will provide at least 82% power to detect a 10% relative reduction in CAAD.

Secondary Mechanistic Outcomes

Previous research has found a 40% prevalence of multi-drug resistant *E. coli* in faecal samples of UK nursing home residents (3) and a 37% prevalence of oral candida in hospitalised elderly patients (48). A meta-analysis of 6 trials of probiotics in critically ill patients reported probiotics reduced colonisation with multi-drug resistant gram negatives (OR 0.39 (95% CI: 0.16 to 0.95) (13). Despite high prevalence of AMR colonisation in CHR few studies have measured the effect of probiotics on this outcome. Hatakka et al (2006) found probiotics reduced the risk of oral candida in 276 older people by 75% (OR = 0.25, 95%CI 0.10- 0.65) (49). Stool and saliva samples at 6 to 12 months will provide 90% power at the 5% level to detect a 19% absolute reduction antimicrobial resistant bacteria and oral candida, assuming a 30% drop-out rate.

18 Analysis

18.1 Main analysis

The primary analysis will be by intention-to-treat, and will consist of a between-group comparison of the mean cumulative antibiotic administration days (CAAD) using Poisson regression. As the randomisation will be stratified by care home, the regression model will control for the care home a participant was recruited from. Negative binomial regression will be used in the presence of over-dispersed count data.

For secondary outcomes, depending on the type of data, a mixture of Poisson, linear, logistic and Cox models will be used to appropriately compare trial arms with respect to rates, means, proportions and time to events (see table below for more details).

Adherence-adjusted analysis

While our primary analyses will be conducted using the intention-to-treat principle (i.e. a comparison of groups as randomised regardless of what happens after randomisation), we will monitor adherence to study product throughout the trial. Using this data, we will perform adherence-adjusted analyses, deriving estimates of treatment efficacy that maintain a comparison of groups as randomised, using structural mean models (50). A definition of adherence to study product will be defined prior to the commencement of any statistical analysis.

Missing data assumptions and adjustments

Given our proposed intensive monitoring schedule, missing data is likely to be minimal for participants who remain in the trial for the full duration. Where missing data is likely to occur, it will most likely be due to participant drop-out, with reasons for dropout falling into two broad categories, withdrawal from the trial and death.

Where responses are missing due to drop-out, these will be assumed to be missing at random given observed data, and appropriate modelling techniques will be used (e.g. likelihood-based methods or multiple imputation). Sensitivity analyses will be conducted using joint modelling approaches (e.g. selection and/or pattern mixture models) to explore departures from the missing at random assumption (51).

We will also explore stratifying deaths into two groups: death during an infection and death from any other cause. We will then investigate applying a missing at random-valid modelling approach to the deaths from any other cause and take a more extreme approach with those that died during an infection (e.g. assume that they would have remained on antibiotics for the remainder of the follow-up period).

Similarly, we will explore stratifying drop-out due to residents moving to another care facility into two groups: those who move for health-related reasons (e.g. from a residential home to a care home providing nursing care) and those who move for other reasons. A missing at random-valid modelling approach will be applied to the latter strata, with a more extreme approach taken for those dropping out due to moving from the care home for health-related reasons as this represents a decline in health, which may also be subject to unmeasured selection bias.

Analysis for mechanistic process

Further statistical modelling will explore the causal mechanisms by which the probiotic may have an effect. Mediation analyses will explore the effect of exposure to probiotics on CAAD and cumulative number of infection days, and this is mediated through an effect on antimicrobial resistance. These analyses will be performed using G-computation (52, 53).

18.2 Description of outcomes and method of statistical analysis

Outcome	Main analysis	Sensitivity analyses*
Primary		
Cumulative antibiotic administration days (CAAD)	Poisson regression.	Negative binomial regression (if Poisson over-dispersed).
Secondary		
CAAD by infection (UTI, RTI, skin, GI, unexplained fever)	Poisson regression, with trial arm interacted with infection.	Negative binomial regression (if Poisson over-dispersed).
Incidence of infection	Poisson regression.	Negative binomial regression (if Poisson over-dispersed).
Site of infection	Poisson regression, with trial arm interacted with infection.	Negative binomial regression (if Poisson over-dispersed).
Mean duration of infection	A two-level Cox PH (frailty) model with infections nested within participants.	
Cumulative number of infection days	Poisson regression.	Negative binomial regression (if Poisson over-dispersed).
Antibiotic-associated diarrhoea Incidence and duration	Poisson regression.	Negative binomial regression (if Poisson over-dispersed).
All- cause diarrhoea (incidence and cumulative days)	Poisson regression.	Negative binomial regression (if Poisson over-dispersed)
Mean duration of diarrhoea episodes	A two-level Cox PH (frailty) model with infections nested within participants.	
Secondary – Health Utility		
Health utility (EQ5D)	Two linear regressions (EQ5D at 3/12 months separately) controlling for baseline EQ5D.	Transformations for normality.
Well-being (ICECAP-O)	Two linear regressions (ICECAP-O at 3/12 months separately) controlling for baseline ICECAP-O.	Transformations for normality.
Hospital stays	Logistic regression.	Poisson/negative binomial regression of counts (mean rate of hospital stays).
Death	Logistic regression.	Cox PH model for time from randomisation to death.
Secondary - Antimicrobial		

Resistance		
Presence of Gram-negative isolates in stools	Two logistic regression (stool sample finding at 3/12 months separately) controlling for baseline stool sample finding.	
Vancomycin resistant enterococci in stools	Two logistic regressions (stool sample finding at 3/12 months separately) controlling for baseline stool sample finding.	
Secondary - Mechanistic		
Immune parameters -Immune cell phenotypes -TLR ligand stimulated cytokines/chem -Plasma cytokines/chemokines -Monocyte and neutrophil phagocytosis -Flu vaccine response	Linear regression.	Transformation for normality.
Clostridium difficile in stools	Two logistic regressions (stool sample finding at 3/12 months separately) controlling for baseline stool sample finding.	
Presence of oral candidiasis	Two logistic regressions (response at 3/12 months separately) controlling for baseline response.	
Amount of oral candidiasis present in saliva	Two ordinal regressions (response at 3/12 months separately) controlling for baseline response.	
L. rhamnosus GG in stools	Two linear regressions (stool sample finding at 3/12 months separately) controlling for baseline stool sample finding.	Transformations for normality.
Bifidobacterium animalis subsp. lactis (BB-12)	Two linear regressions (stool sample finding at 3/12 months separately) controlling for baseline stool sample finding.	Transformations for normality.
Full blood count	Linear regression	Transformations for normality.
Flu vaccine response - titer	Linear regression	Transformations for normality.
Flu vaccine response - seroconversion and seroprotection	Logistic regression	

* All sensitivity analyses will include a consideration of adherence to study product and the impact of missing data / deaths.

18.3 Data storage & retention

Access to Data

Direct access to trial data will be granted to authorised representatives from the sponsor or host institution for monitoring and/or audit of the trial to ensure compliance with regulations. This access, the reason for it and who has authorised it will be recorded by the trial team and reported to the DMC. Only authorised members of the PRINCESS research team will have access to the PRINCESS trial data in order to carry out their assigned trial role. Who has access, what level or access and for what purpose will be recorded in the trial Data Management Plan. All data will be kept for 15 years in line with Cardiff University's Regulations for clinical research. This data will be stored confidentially on password protected servers maintained on the Cardiff University Network.

Data Recording and Record Keeping

A trial specific Data Management Plan (DMP) will be developed for the PRINCESS trial outlining in detail the trial specific procedures that will be put in place to ensure that high quality data are produced for statistical analysis. All data recorded onto paper Case Report Forms (CRFs) or other paper data sources will be returned to the trial team and a copy will be held at the research site. Upon receipt all CRFs or other paper documentation containing clinical data will be date stamped and tracked until archiving. A full pre-entry review and electronic data validation for all data entered into the clinical database will be provided by trial specific programmed checks. Prior to database lock, a dataset review will be undertaken by the Data Manager and the Trial Statistician. An independent review of the quality of the data being produced will also be provided by the IDMC throughout the trial.

19 Trial closure

The end of trial is the date of the last data capture and once all pre-specified efficacy laboratory analyses have been completed.

20 Regulatory issues

20.1 Ethical and research governance approval

The study will be conducted in accordance with the recommendations for physicians involved in research on human participants adopted by the 18th World Medical Assembly, Helsinki 1964 as amended.

The protocol, informed consent form, participant information sheets, consultee declaration form, consultee information sheet and any proposed advertising material will be submitted for written approval to an appropriate Research Ethics Committee (REC); host institution(s); and for Research governance approval, prior to any trial procedures taking place. The Health Research Authority guidance on consent and participant information sheets will be followed as appropriate. The Chief Investigator (CI) or delegate will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

20.2 Consent

The PRINCESS trial will recruit participants both with capacity and those lacking capacity to provide informed consent. We anticipate that some care home residents may lose capacity during the course of the trial either temporarily or permanently and will make appropriate provision. Specific consent will be sought at trial entry for CHR to remain in the trial should capacity be lost during the trial and where CHR regain capacity, consent will be subsequently obtained. The CI will ensure that the consent process adheres to the Mental Capacity Act, 2005 as appropriate.

Full study specific training will be available to all care home staff. Only care home staff who have untaken the appropriate training for their trial role will take responsibility for taking informed consent or other trial procedures. The staff along with their trial role will be recorded in the trial delegation log and authorised by the CI and care home representative. The type of training needed and how it will be recorded will be noted as a trial specific risk and reviewed as part of the risk assessment process.

20.3 Risks and benefits

Due to this being a randomised placebo controlled trial, half of the participants have no chance of benefit from the trial intervention. All participants (or their consultee), however, may value contributing to the advancement of medical knowledge and appreciate the increased assessments and monitoring. Probiotics are classed as a food supplement (and this has been confirmed by the MHRA for this trial) and there are very few side effects (mainly bloating and flatulence if these occur). Probiotics carry theoretical risks including infection beyond the gut, and transfer of antibiotic resistant genes. However, so far, there have been no reports of bacteraemia or fungaemia attributable to the probiotics in trials included in the systematic reviews and mild to moderate gastrointestinal side effects and rash are generally no more common than in patients on placebo probiotic.

For participants receiving the probiotic, reducing the possibility of developing an infection that may require treatment with an antibiotic, we believe, outweighs the risk of the potential side effects. If our hypothesis is correct and probiotics reduce antibiotic prescribing for CHR, this may also reduce the number of antibiotics prescribed for other CHR and subsequent AMR. For all participants, the high level of monitoring for common infectious diseases may result in better-targeted treatment and added vigilance in general.

Residents lacking capacity will be included in the trial as our previous PAAD observational study data demonstrated that those who lack capacity are frailer and receive antibiotics more often and for different indications than those with capacity and, therefore, may be more likely to benefit from interventions aimed at stimulating and supporting the immune system. Interventions should be evaluated in populations for whom the intervention is intended, especially when there are relevant material differences (e.g. in frailty and in immune function).

20.4 Confidentiality

The CI will ensure all trial staff are fully trained and adhere to the principles of Good Clinical Practice (GCP) and the Data Protection Act, 1998. Patients will only be identified on trial documents by use of a unique trial ID which cannot be used to identify individual participants. The research team will store all CRFs and other trial data documents securely at the coordinating centre prior to data entry. CRFs

and all other documents holding patient identifiable information will be anonymised as soon as possible with the process of management being outlined in detail within the ethics application and in the Data Management Plan.

20.5 Indemnity

Cardiff University will provide indemnity and compensation in the event of a claim by, or on behalf of participants, for negligent harm as a result of the study design and/or in respect of the protocol authors/research team. Cardiff University does not provide compensation for non-negligent harm.

All participants will be recruited at Care Homes. Care Homes will arrange insurance cover for any harm to participants caused by the negligence of the Care Home.

20.6 Trial sponsorship

Cardiff University will act as sponsor for trial. Delegated responsibilities will be assigned to SEWTU, PCCTU and to the care homes taking part in this study.

20.7 Funding

The trial will be funded by Medical Research Council's Efficacy and Mechanism Evaluation Programme administered by the National Institute for Health Research by means of a research grant to the Nuffield Department of Primary Care Health Sciences. The research funding will be administered by the University of Oxford. The study product (probiotic/placebo) will be provided by CHR Hansen free of charge.

20.8 Audits & inspections

The trial is participant to inspection by National Institute for Health Research Efficacy and Mechanism Evaluation as the funding organisation. The trial may also be participant to inspection and audit by Cardiff University under their remit as sponsor.

21 Trial management

The Trial Management Group will be led by the Chief Investigator and will take responsibility for the on-going management of the PRINCESS trial. Members will be required to sign up to the remit and conditions as set out in the TMG Charter.

22 Data monitoring & quality assurance

The UKCRC accredited South East Wales Trials Unit (SEWTU) will have fully responsibility for all aspects of the PRINCESS trial. SEWTU will work closely with the Primary Care Clinical Trials Unit (PC-CTU) on all matters relating to the trial design and delivery. A formal division of responsibilities will be drafted between SEWTU and PC-CTU detailing which standard operating procedures will be followed when completing each controlled process. SEWTUs Quality Assurance Team will review these arrangements as appropriate to ensure compliance with the relevant regulations and any Sponsor requirements.

22.1 TSC (Trial Steering Committee)

An independent Trial Steering Committee (TSC) will be established to provide oversight of the PRINCESS trial. The TSC will include at least an independent chairperson, two independent members and a patient representative. The TSC will review the progress of the trial on a regular basis and provide advice to the TMG. Representatives of the Sponsor and the Funder will be invited to attend all meetings. Members will be required to sign up to the remit and conditions as set out in the TSC Charter.

22.2 IDMC (Independent Data Monitoring Committee)

An independent Data Monitoring Committee (DMC) will be established to provide oversight of all matters relating to patient safety and data quality. The DMC will be asked to convene at least annually and provide advice to the TSC. Members will be required to sign up to the remit and conditions as set out in the DMC Charter.

23 Publication policy

All publications and presentations relating to the trial will be authorised by the Trial Management Group and will follow the PRINCESS publication policy. The trial protocol will be published and the trial registered with the ISRCTN. The trial results will be published and all who meet the criteria for authorship will be listed as authors. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged. The results will be presented to the DMC prior to publication. Papers will be shared with the funders prior to submission. Funders will have 14 days in which to respond and to bring any matters of factual accuracy relating to the intervention to the attention of the trial team. The funders will have no role in decisions on publication. The funding source and other support will be acknowledged.

23.1 Feedback to participants and other stakeholders

Participants, care home staff and associated GPs will receive summaries of the trial findings and they will be made available to the general public via the trial website. The PPI representatives will be asked for their assistance in ensuring the material prepared for the care home residents and general public is comprehensive and appropriate.

24 References

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25 Schedule of Study Procedures

Assessment	**Baseline**	**Once Daily (for 12 months)**	**Regular intervals (for 12 months)**	**Monthly (for 12 months)**	**If vaccinated against flu, on day of vaccination or as close as possible**	**If vaccinated against flu, 4 weeks (28 days) afterwards**	**3 month***	**Second follow-up***
Assessment of eligibility	X							
Informed consent or consultee declaration"	X							
Demographic data	X							
Brief clinical history	X							
Clinical Frailty Score	X							
CAAD for infection			X				X	X (from MAR and hospital record, if applicable)
Infection details			X				X	X
Oral rinse or saliva culture"^ for oral candida	X						X	X
Blood sample"^ (FBC, Vitamin D)	X							
Blood sample"^ for serum cytokines (ex-vivo response) (n≈100)	X							X plus FBC blood sample
Blood sample"^ (influenza vaccine efficacy)					X	X		
Stool sample"+ (*C.diff*, antibiotic sensitivity of gram –ve enterobacteria and VRE, gut microbiota diversity or phylotype)	X						X	X
EQ-5D" (participant and/or proxy completion)	X						X	X
ICECAP-O" (participant and/or proxy completion)	X						X	X
NHS service use (Care Home note review)			X					X
NHS service use (Hospital discharge summaries)								(X)
Mortality								X
Randomisation	X							
Study product pack allocation				X				
Study Product administration		X						
Adherence to Study Product (MAR sheet)		X (MAR sheet completion as often as practicable)	X (transcribe to CRF)					
Adherence to study product (capsule count)				X				

Key

" requires participant or consultee/proxy involvement

(X) if applicable

+ Sample to be collected by Care Home staff

^ Sample to be taken by Research Nurse

Follow-up time windows:
3 month: 3 months -2/+4 weeks
12 month: 11 months +/- 1 month

* The follow-up schedule will depend on the length of time that a participant has been in the study. Where possible, participants will have a baseline assessment, and three- and 12-month follow-ups. Due to time limitations, some participants may have a truncated follow-up and will receive either a baseline assessment and 3-month follow-up, or a baseline assessment, 3-month

follow-up and a second follow-up between 6-10 months post-randomisation. Infection, antibiotic use, adverse events and study product use is also recorded at regular intervals by the RN from care home notes for 12 months post-randomisation or until 31/10/2018, whichever is sooner

26 Appendix 1

The PRINCESS qualitative interview study: understanding how a randomised controlled trial of probiotics was conducted in care homes

Contents **Page no.**

TABLE OF ABBREVIATIONS

CTR	Medicines for Human Use (Clinical Trials) Regulations 2004
ENRICH	National Institute Health Research 'ENabling Research In Care Homes'
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation, 2016
ICH GCP	Internationals Conference on Harmonisation Guideline for Good Clinical Practice
MCA	Mental Capacity Act 2005
PIS	Participant information sheet
PRINCESS	Probiotics to Reduce Infections iN CarE home reSidentS
RA	Risk Assessment
RCT	Randomised controlled trial
REC	Research Ethics Committee
SOP	Standard Operating Procedures

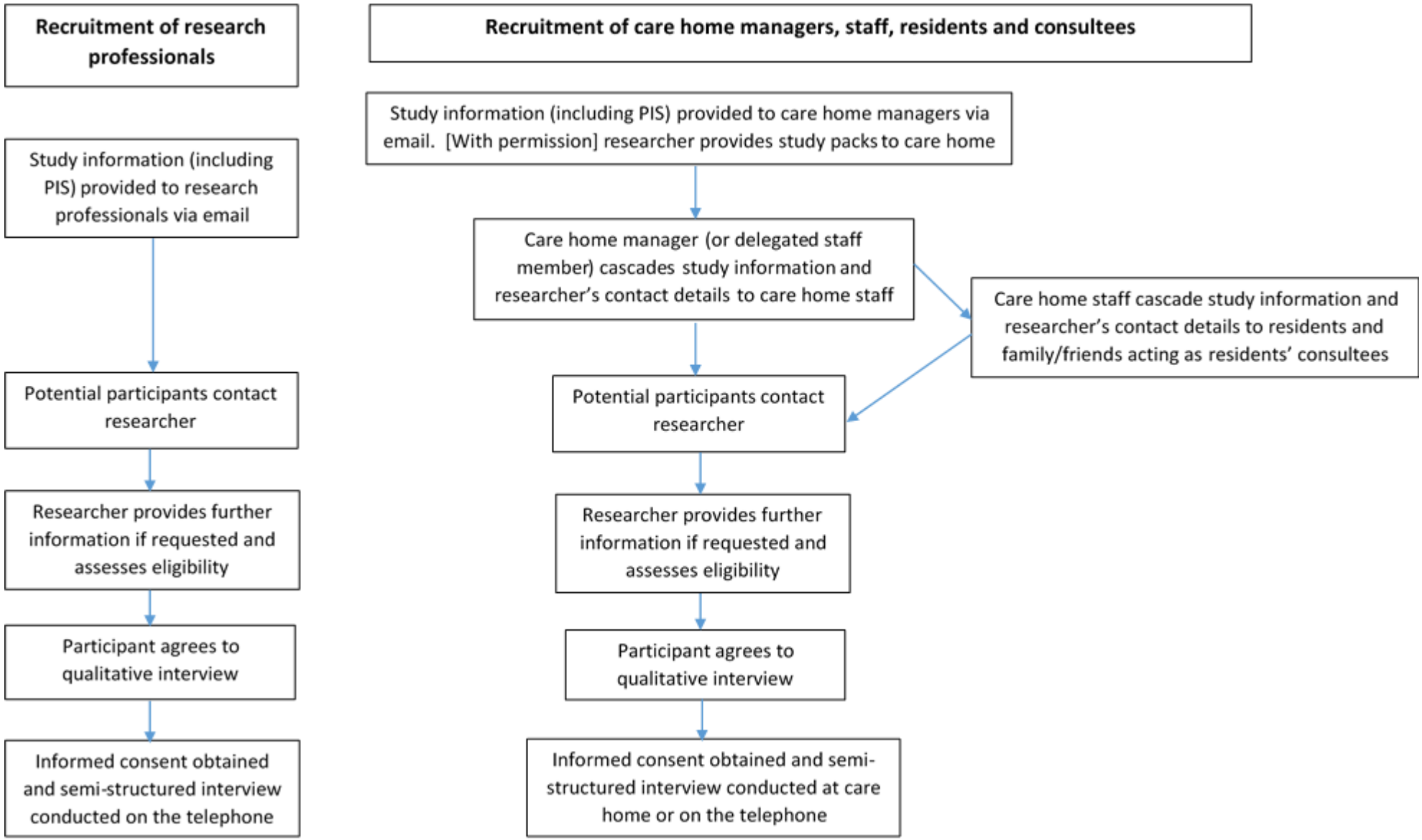
KEY STUDY CONTACTS

Lead researcher	Helen Stanton Centre for Trials Research College of Biomedical & Life Sciences, Cardiff University, 7th Floor, Neuadd Meirionnydd, Heath Park, Cardiff, CF14 4YS 029 20687142
Funder(s)	This interview study is funded via the PRINCESS RCT
Key Protocol Contributors	Helen Stanton, Victoria Shepherd, Dr Eleri Owen-Jones, Dr Jane Davies, Dr Rachel Lowe, Alison Edwards

STUDY SUMMARY

Study Title	The PRINCESS qualitative interview study: understanding how a randomised controlled trial (RCT) of probiotics was conducted in care homes
Internal ref. no. (or short title)	PRINCESS interview study
Study Design	Face-to-face or telephone semi-structured, qualitative interviews
Study Participants	1. PRINCESS RCT participants (care home residents) and family members/friends who a) acted as consultees for PRINCESS RCT participants who lacked capacity to provide informed consent or b) experienced/observed their relative/friend's participation in the PRINCESS RCT 2. Managers of participating PRINCESS RCT care homes 3. Care home staff that contributed to the research activities in the PRINCESS RCT 4. Research professionals who conducted the research activities in the PRINCESS RCT
Planned Size of Sample (if applicable)	25-30 (evenly spread between the above cohorts)
Follow up duration (if applicable)	N/A
Planned Study Period	6 months
Research Question/Aim(s)	To understand how the PRINCESS RCT was carried out within the care home context, and identify the mechanisms which affected implementation of study activities

STUDY FLOW CHART



The PRINCESS qualitative interview study: understanding how a randomised controlled trial of probiotics was conducted in care homes

1. BACKGROUND

The UK population is ageing, and the number of people living in long-term care homes is forecast to increase over the coming decades (54, 55). As such, there is a growing need to ensure that individuals living in care homes are recipients of high quality evidence-based care, where the unique needs of the population are considered (56, 57). However, the regulatory and operational complexities in involving elderly and frail care home residents in clinical research has served as a barrier to the development of research in the care home environment (58, 59).

Research programmes such as the National Institute Health Research Enabling Research in Care Homes (NIHR ENRICH) programme have begun to tackle the inverse relationship between the need for evidence-based approaches to healthcare provision, and the facility in which this evidence can grow. The ENRICH programme provides a 'toolkit' of resources for navigating research with residents, within the care home environment (60). While programmes such as ENRICH provide a route in to care home-based research, navigating the necessary regulatory and governance pathways in this largely research naïve environment can be complicated (59). The challenges of setting up a clinical trial in care homes includes complexities and severe time delays through navigating variations in research governance, difficulty recruiting care homes, and protracted discussions with ethics committees surrounding the inclusion of residents who lack the mental capacity to provide informed consent for themselves (59, 61). The majority of care home residents have some degree of cognitive impairment, and many will lack the decision-making capacity to provide informed consent to take part in research (62-64). As such, all aspects of the research process needs specific consideration in order for the best outcomes to be achieved with regards to involving the care home population in research.

The set-up and the implementation of the PRINCESS trial (Probiotics to Reduce Infections iN CarE home reSidentS) was informed through past experience of other studies undertaken in care homes, as well as research around alternative models of consent (58, 59, 65). The double-blind individually randomised controlled trial of a probiotic used a consent model based on obtaining advice from a consultee for residents who lacked capacity to provide informed consent, in accordance with the Mental Capacity Act 2005 (66). Residents and consultees provided separate consent for stool, saliva, and blood samples (67). The trial sample size allowed for expected attrition rates, and missing data due to hospitalisations (61, 67).

Research professionals (including research nurses) conducted the majority of research activities required to determine the primary and secondary outcomes, which included cumulative antibiotic use, and rates of infections. Despite the complexity and high demands of this randomised controlled trial, 310 residents were recruited and are being followed-up weekly (67) for up to 12 months. The successful implementation of this study deserves further in-depth exploration. This qualitative interview study aims to examine how PRINCESS researchers, care home staff, and study participants worked to enact PRINCESS research activities.

The findings will be used to provide those conducting research (including randomised controlled trials) in the care home context with practical recommendations and potential models of working with and within care homes. Enabling and empowering researchers to undertake research with older people in the care home setting is essential if evidence-based healthcare is to keep up with the changing health and social care demands of an ageing population.

2. RESEARCH QUESTION/AIM(S) AND OBJECTIVES

The overall aim of the study is to understand how the PRINCESS study was carried out within the care home context, and identify the mechanisms which affected implementation of study activities. The following objectives will serve as a framework for the interview topic guides, allowing the study aim to be achieved:

1. Obtain research professionals' perspectives on the PRINCESS study, and gain an understanding of how they conducted the PRINCESS research activities within the care home
2. Obtain care home staff perspectives on PRINCESS and the research activities associated with the study
3. Obtain residents'/consultees'/relatives'/friends' perspectives on PRINCESS and the research activities associated with the study
4. Gain an understanding of the mechanisms which facilitated (or limited) conducting research activities in the care home
5. Understand and gather contextual information that can assist the implementation of future research studies (including randomised controlled trials) in care homes

3. STUDY DESIGN and METHODS of DATA COLLECTION AND DATA ANALYSIS

Semi-structured interviews will be conducted with eligible participants. The interviews may vary in length, but are expected to take around 30 minutes. A topic guide will be developed which will be informed by relevant literature and discussions with the research team. The topic guide will be piloted to ensure interviews achieve relevant data. The topics will explore various aspects of the research process of the PRINCESS RCT. Interview topics will also aim to elucidate relevant contextual issues, and barriers and facilitators to the implementation of the PRINCESS RCT (68).

The interviews will be digitally audio-recorded with consent, and transcribed verbatim. The transcripts will be checked for accuracy and completeness against the source data. The transcripts will be de-identified using a Participant Identification Number. Transcripts and the original source data (audio file) will be stored confidentially on password protected servers maintained on the Cardiff University Network. All data will be archived securely and kept for no less than end of project + 5 years or at least 2 years post publication in line with Cardiff University's Guidance for Managing Research Records and Data.

Data will be entered into Qualitative Data Analysis software (NVivo 11, QRS International) and iteratively coded using codes emerging from the data, and those identified *a priori*. Data will be analysed using framework analysis. This is a systematic approach to a thematic qualitative analysis that allows for easy comparisons between and within cases, facilitates sharing and discussion of data, and allows for clear linking / access from developed themes to original data (69). Framework analysis involves five stages: 1.) familiarisation with the data; 2.) development of a thematic framework; 3.) applying thematic codes to all of the data (indexing); 4.) retrieving and summarising coded data in a chart; and 5.) interpreting the data by drawing inferences and pulling together relevant themes (70). Framework analysis is particularly useful when there are a number of clear research aims that have guided the questions, while allowing new themes to emerge from the data that are relevant to the research question. Dual coding will be carried out for 10% of the interviews to allow for an assessment of coding validity. NVivo qualitative analysis software will be used to assist coding. A sub-group of the research team with expertise in qualitative analysis will assess themes emerging from the data against the coding framework generated to determine at which point during analysis saturation has been achieved.

4. STUDY SETTING

Participation will be limited to those who have already been involved in some capacity in the PRINCESS RCT, i.e. participation in the PRINCESS RCT itself, acting as a consultee for a resident in the PRINCESS RCT, family members/friends who have experience of the resident's participation in the PRINCESS RCT, involvement in the research activities required to recruit residents, or collecting research data for the PRINCESS RCT.

5. PARTICIPANT SAMPLING

5.1 Participant selection

Sampling will take place at a care home level in order to get an in-depth understanding of how activities were enacted. We plan to interview up to 30 interviewees from across selected care homes. We plan to include research professionals (at least seven), care home managers (at least seven), care home staff who were involved in the PRINCESS RCT (at least seven), and residents and consultees who participated in the study (at least seven). We anticipate that this will be a sufficient number of participants for data saturation to be reached (i.e. where no new themes are emerging from the data) (71).

5.2 Eligibility Criteria

Eligibility will be determined by the lead researcher prior to a potential participant's inclusion in the study. A potential participant will be eligible if they meet all the inclusion criteria. A potential participant will not be eligible if any of the exclusion criteria apply.

5.2.1 Inclusion criteria

- a) A resident who participated in the PRINCESS RCT and retains the mental capacity (under the MCA 2005) to provide informed consent to participate in an interview about the RCT (66)
  OR
  b) A family member or friend who has acted as a consultee on behalf of an adult who lacks capacity for a decision regarding their participation in the PRINCESS RCT
  OR
  c) A family member or friend who did not act as a consultee on behalf of a resident who participated in the PRINCESS RCT but have first-hand experience of the resident's participation in the PRINCESS RCT.*
  OR
  d) Care home staff members (including management) who implemented any part of the PRINCESS RCT (e.g. initial set-up of trial, acted as Principal Investigator, recruitment of residents, data collection, sample collection etc.)
  OR
  e) Research professional who implemented any part of the PRINCESS RCT (e.g. initial set-up of trial, recruitment of residents, data collection, sample collection etc.)
- Agree to participate in an interview

5.2.2 Exclusion criteria

- Did not have any involvement in the PRINCESS RCT
- A participating resident of the PRINCESS RCT who has subsequently lost capacity under the MCA 2005 to provide informed consent to take part in an interview

- Are unable to understand English sufficiently to comprehend the study information and conduct an interview in English
- Are unable to communicate verbally to the extent that an interview cannot reasonably be conducted**

*Owing to care home residents' older age, some will have lost mental capacity since taking part in the PRINCESS RCT, or cannot recall participating in the PRINCESS RCT. Some residents may have passed away. Close family members/friends who **volunteer** to take part in an interview because they have experience of the resident's participation in the PRINCESS RCT should be included as their views can generate a more holistic understanding of how the PRINCESS RCT was carried out in care homes, thus improving the generalisability of the interview study results.

**The interviewer will take in to account any needs of the individual that may arise due to age-related issues, such as hearing loss, and age related changes in verbal communication. The interviewer will make efforts to provide a communication friendly environment in order to facilitate communication with individuals who want to be interviewed. The interviewer will make notes after the interview to document any communicative artefacts that may influence understanding, or future interpretation of the discussion.

6. PARTICIPANT RECRUITMENT

The recruitment strategy will vary according to the potential participant group. Those within the care home environment (managers, staff, consultees and residents) will be 'snowball sampled'; i.e. managers will be approached initially. Managers can then approach staff, who in turn can then approach residents and consultees (72).

6.1 Research professional recruitment:

Research professionals (e.g. research nurses) will be approached by email with a brief summary of the study. The participant information sheet (PIS) will be attached to the email. The email will advise that if they are interested in being interviewed to contact the researcher by telephone or email and the consent form can be sent in the post. If requested, hard copies of the participant information sheet (PIS) and the consent form can be sent with a stamp-addressed envelope. Once the signed consent form has been received a time will be arranged for a telephone interview.

6.2 Recruitment within care home environment:

The care home managers will be approached by email or telephone stating that we are hoping to gather the views and experiences of care home managers, staff, relatives/friends (who may have acted as consultees) and residents around their input on the PRINCESS RCT. A request will be made to the manager (or a delegated colleague) by email or telephone to put up a study poster and add some A6 study poster cards by the visitors book on the front desk and ask them to highlight the interview study to eligible staff/residents/consultees and to contact the researcher (using Helen Stanton's (lead researcher) details on the A6 poster card) if they are interested in taking part.

With the care home manager's (or delegated colleague) permission information packs will be sent to the care home and given to eligible staff/consultees/residents. These will contain a participant information sheet (PIS), contact details form, and consent form*. If the researcher is contacted via email or telephone and the potential participant is happy to be interviewed they will be asked to return the consent form and contact details form (in a stamp addressed envelope) and an interview will be arranged once the researcher has received the documents. Potential participants will be given

a choice of being interviewed face-to-face or on the telephone (depending on the proximity of the care home to the location of the researcher conducting the interviews).

*To aid information accessibility and self-completion of materials, A3 copies of forms with larger print will also be provided. Information on the PIS can also be verbally communicated if requested. In the event that a resident or consultee cannot provide handwritten signatures on the consent, verbal consent will be taken, and documented in the presence of a witness (member of care home staff, family, or friend).

6.3 Consent

All participants will be provided with an information sheet, and have the opportunity to ask questions and receive satisfactory responses, prior to the lead researcher seeking informed consent. Written informed consent will be obtained using a study-specific consent form prior to any data collection. A copy of the consent form will be provided to the participant, and the original retained by the lead researcher.

Participants will be informed that they are able to withdraw from the study, should they wish to do so, by contacting the lead researcher. Data from participants who have withdrawn will not be used in the analysis. Participants are able to request to withdraw from the study at any point, up until analysis is concluded.

7. ETHICAL AND REGULATORY CONSIDERATIONS

The study will be conducted in accordance with ICH GCP requirements, and in accordance with the recommendations for research on human participants adopted by the 18th World Medical Assembly, Helsinki 1964 as amended. The Health Research Authority guidance on consent and participant information sheets will be followed as appropriate. Participants will be provided with sufficient information prior to any decision whether to participate and informed that it is voluntary. Explicit consent for the interview to be audio-recorded will be sought.

Ethical issues raised by the project are anticipated to be limited due to the nature of the project. However, issues that are of concern to the participant may be raised during the interview, and there is the potential for the participant to become upset or distressed during the interview. The researcher has a background in research with older people, and those with chronic conditions, and therefore has experience of conducting interviews with care and sensitivity. In the event of a participant becoming upset or distressed, the interview will be suspended or discontinued as appropriate.

At the end of each interview, the researcher will take time to ensure that participants did not feel distressed by their participation.

7.1 Research Ethics Committee (REC)

The protocol, informed consent form, participant information sheets, and any proposed advertising material will be submitted for written approval to an appropriate Research Ethics Committee (REC) prior to the start of the study taking place. As NHS patients or users of the NHS, or their relatives, will not be involved in the study, ethical approval will be sought from the School of Medicine Research Ethics Committee, Cardiff University. Care home residents who lack capacity to provide informed consent will not be included in the study. Approval will also be sought regarding any amendments to the study.

7.2 Peer review

This interview study is an evaluation of the PRINCESS RCT and has been reviewed by the study team named in this protocol.

7.3 Patient & Public Involvement

The Patient and Public representative who formed part of the PRINCESS RCT trial management group will review and contribute to the interview topic guide and themes.

7.4 Data protection and patient confidentiality

The lead researcher will be fully trained and adhere to the principles of Good Clinical Practice (GCP) and the General Data Protection Regulation, 2016 (GDPR). Potential participants who express interest in being contacted and receiving further information about the study will send their contact details to the lead researcher by returning a contact details form, or contacting the lead researcher by phone or by email. Contact details will be stored electronically in an Excel spreadsheet which will be password protected, kept on the secure Cardiff University network and only accessed by named individuals within the research team as outlined in the delegation log. The research team will store all study data documents securely at Cardiff University. Interview transcripts and all other documents holding participant identifiable information will be anonymised as soon as possible. Participants will only be identified by use of a unique study ID which cannot be used to identify individual participants. Data encryption will be used for all portable media containing participant data.

All data will be kept for no less than end of project + 5 years or at least 2 years post publication in line with Cardiff University's Guidance for Managing Research Records and Data. This data will be stored confidentially on password protected servers maintained on the Cardiff University Network.

7.5 Indemnity

Cardiff University will provide indemnity and compensation in the event of a claim by, or on behalf of participants, for negligent harm as a result of the study design and/or in respect of the protocol authors/research team. Cardiff University does not provide compensation for non-negligent harm.

7.6 Access to the final study dataset

The researchers and administrators involved in the study will have access to the full dataset.

8. DISSEMINIATION POLICY

The study results will be published in peer-reviewed academic journals, and the results presented at conferences, and through other dissemination events or outputs. Participants will receive summaries of the study findings and information about how to access a copy of the full results (e.g. online journal article).

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