Supplementary Online Content

Butler CC, Lau M, Gillespie D, et al. Effect of probiotic use on antibiotic administration among care home residents: a randomized clinical trial. *JAMA*. doi:10.1001/jama.2020.8556

This supplementary material has been provided by the authors to give readers additional information about their work.

eAppendix 1. Eligibility Criteria
Residents were eligible for the trial if they met all of the following inclusion criteria and none of the exclusion criteria.

Inclusion criteria:

- Resident lived in a care home setting (residential, nursing or mixed)
- Resident was willing and able to give informed consent for participation in the trial OR if the resident lacked capacity, a consultee was willing to complete a consultee declaration form
- Aged 65 years or older

Exclusion criteria:

- Resident was known to be immunocompromised (requiring immunosuppressants, long term high dose oral, intramuscular or intravenous steroids)
- Resident was currently taking regular probiotics and not willing to adapt to trial protocol
- Resident was currently participating in a CTIMP, or participated in a CTIMP in the last thirty days
- Resident was 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

eAppendix 2. Further Details of Trial Intervention
The probiotic or placebo was administered by the resident's normal caregiver. The capsule was swallowed whole with water (preferred route), emptied into a small amount of cold or lukewarm liquid and swallowed, or contents sprinkled onto cold or lukewarm (not hot) food and eaten. Adherence was recorded on medication administration record sheets, specifically, how the study product was given, and whether it was fully, partially, or not consumed.

eAppendix 3. Details on Derivation of Outcome Measures

Primary outcome:
Cumulative systemic antibiotic administration days for all-cause infection was a rate variable, with the number of exposure days as the denominator and antibiotic administration days as the numerator. This was ascertained from the total number of days of systemic antibiotic administration as recorded in care home medical records and discharge summaries if the participant was admitted to hospital, collected retrospectively by the registered nurses (blind to participant group allocation) during weekly visits to care home residents.

Secondary outcomes:
Antibiotic associated diarrhea defined as diarrhea occurring following the start of a course of antibiotic treatment and up to eight weeks after stopping the antibiotic.

Cumulative day of antibiotic associated diarrhea was a rate variable, with the number of at-risk days as the denominator and number of days with antibiotic associated diarrhea as the numerator. Number of at-risk days defined as the number of days taking antibiotic and up to 8 weeks after the course ended.

Cumulative number of infection days was a rate variable, with the number of exposure days as the denominator and number of suspected infection days as the numerator.

eAppendix 4. Further Details of Statistical Analyses

Descriptive data:
Care home residents' characteristics and clinical measures were summarized using frequencies and percentages, means and standard deviations, or medians and interquartile ranges as appropriate. All analyses have been presented as estimates of treatment effects (adjusted incidence rate ratio, mean differences or odds ratios, as appropriate), with associated 95% confidence intervals and p-values.

Analysis populations:
All primary and secondary comparative analyses were based primarily on Intention to Treat (ITT) population, which included all randomized participants who provided outcome data, without imputation of missing values and regardless of protocol deviations or intervention received.

We conducted several sensitivity analyses for our primary outcome analysis, including all residents who initiated treatment, with missing diary data imputed under four different scenarios:

- Hypothetical scenario incorporating reason for death: in this scenario, we have assumed that any resident who died due to infection will have been taking antibiotics on all missing diary days, with the remainder having their rate imputed for the remaining missing days (i.e. their rate on missing days will be assumed to reflect their rate on observed days).

We also considered the extent to which residents not fully or partially taking study product on a given day may impact the conclusions drawn on the primary outcome. To investigate this, we modelled the CAAD rate as a continuous outcome and fitted a two-stage least squares instrumental variable regression model to CAAD, with randomization used as the instrument and the exposure being the percentage of study product fully or partially taken (with this set to zero in the placebo arm). We fitted the model adjusting for CHR sex and using cluster robust standard errors to account for residents within care homes. The interpretation from the model coefficients is the adjusted mean difference in cumulative systemic antibiotic administration days per percentage point increase in the percentage of study product fully/partially taken. For presentation purposes, we multiplied this coefficient (and associated 95% confidence interval) by 100, to estimate the effect of probiotic combination under the scenario whereby participants took their study product fully/partially 100% of the time.

Primary outcome analysis:

The mean cumulative systemic antibiotic administration days per resident-year was compared between arms by fitting a two-level negative binomial regression model, accounting for participants nested within care homes, the length of time observed, and the sex of the care home resident.

Secondary outcome analyses:
Similar to the analysis of the primary outcome, the majority of secondary outcomes analyses (cumulative systemic antibiotic administration days by infection type, rates of infections, rates of diarrhea) involved the between-arm comparison of rate variables using two-level Poisson or negative binomial regression (depending on the presence of over-dispersion). Where rates were low, single-level zero-inflated negative binomial regression models were fitted. Robust standard errors were used to account for clustering of care home residents within care homes. All models were adjusted for care home resident sex.
The mean duration of infection and mean duration of diarrhea episodes were compared between arms by fitting a two-stage hurdle model, whereby the presence/absence of at least one episode was compared between arms by fitting a two-level logistic regression model (care home residents nested within care homes, gender included in the model) and, in those with at least one episode, the mean episode was compared between arms by fitting a two-level linear regression model.

Mean differences for the EQ-5D and ICECAP-O measures were compared between arms by fitting two-level linear regression models, adjusting for care home resident sex. Any transformations required to fulfil modelling assumptions are described in table footnotes.

Differences between arms in the proportion of residents with Enterobacterales in stools, Enterobacterales in stools resistant to at least one of the tested antibiotics, vancomycin resistant enterococci in stools, oral candidiasis in saliva, and amount of oral candidiasis in saliva, at three-months and the second follow-up time point, were investigated by fitting two-level logistic regression models (two-level ordinal regression models for amount of oral candidiasis), adjusting for care home resident sex.

Subgroup analyses:
We explored the extent to which there were any differential treatment effects on cumulative systemic antibiotic administration days by several pre-specified subgroups (care home resident sex, capacity to provide informed consent for the trial at baseline, and level of clinical frailty at baseline) by extending the primary analysis and fitting a sub-group by trial arm interaction.

Sensitivity analyses:
We investigated the consistency of the conclusions drawn on our primary outcome by

- Including prophylactic antibiotic use in our definition of cumulative systemic antibiotic administration days
- Ignoring periods of hospitalization from consideration from both numerator and denominator of our outcome

Sensitivity Analyses for the Primary Outcome Measure:

eTable 1. Analysis of Primary Outcome Measure With Modified Definitions

Analysis set	Probiotic	Placebo	Absolute difference, 95% CI	Adjusted incidence rate ratio, 95% CI	p-value
Including prophylactic antibiotic use in cumulative systemic antibiotic administration days definition, mean (SD) N	14.6 (20.0) 152	12.7 (18.7) 153	1.95 (-2.40 to 6.30)	1.2 (0.83 to 1.67)	.36
Removing periods of hospitalization from the cumulative systemic antibiotic administration days definition, mean (SD) N	11.9 (16.7) 152	12.1 (20.8) 152	0.21 (-4.03 to 4.45)	1.1 (0.74 to 1.54)	.73

eTable 2. Demonstrates the results of complier average causal effect analysis. Given the high levels of adherence to study product (median percentage of taken study product either in full dose or partial dose was 97.8% (IQR 93.56 to 99.45)), there was minimal impact on our study findings when accounting for study product non-adherence.

eTable 2. Analysis of Primary Outcome Measure Accounting for Study Product Adherence

Analysis set	N	Adjusted coefficient, 95% CI	p-value
Complier average causal effect analysis	305	0.01 (-0.20 to 0.41)	.52

eTable 3. Demonstrates the most extreme approach for participants who died during an infection (i.e. assume that participants with missing cumulative systemic antibiotic administration days due to death during an infection would have remained on antibiotics for the remaining follow-up period).

eTable 3. Analysis of Primary Outcome Measure Accounting for Missing Data

Analysis set	Probiotic	Placebo	Absolute difference, 95% CI	Adjusted incidence rate ratio, 95 % CI	p-value
Death during infection*, mean (SD) N	**30 (66.2) 152**	**18 (40.7) 153**	**11.8 (-0.49 to 24.18)**	**1.62 (1.03 to 2.57)**	**.04**

*Death during infection will be assumed to have infection and taken antibiotic. Care home residents with missing data but not due to death will be imputed at the same rate as the primary cumulative systemic antibiotic administration days.

Analysis of Amount of Candidiasis Outcome Measures:

eTable 4. Between-Arm Differences for Amount of Oral Candidiasis Outcome Measures*

	Placebo	Probiotic	Adjusted odds ratio, 95% CI	p-value
3 months, n/N (%) N				
(-/+)	20/119 (16.8) 80	21/116 (18.1) 88		
(+)	20/119 (16.0) 80	26/116 (22.4) 88		
(++)	38/119 (31.9) 80	20/116 (17.2) 88		
(+++)	42/119 (35.3) 80	49/116 (42.2) 88	0.7 (0.20 to 2.17)	.49
Second follow-up, n/N (%) N				
(-/+)	14/81 (17.3) 57	15/98 (15.3) 70		
(+)	11/81 (13.6) 57	11/98 (11.2) 70		
(++)	23/81 (28.4) 57	31/98 (31.6) 70		
(+++)	33/81 (40.7) 57	41/98 (41.8) 70	0.5 (0.12 to 2.16)	.36

*Level of oral candidiasis was determined by semi-quantitative count as (-/+), (+), (++), and (+++).Ratio is probiotic arm / placebo arm. Adjusted for gender. Clustering of organisms within participants within care homes accounted for by fitting a three- level ordinal regression model (analysis at three-months based on 174 organisms within 138 participants within 22 care homes; analysis at second follow-up time point based on 131 organisms within 103 participants within 20 care homes).

Analysis of Microbiology Outcome Measures:

eTable 5. Between-Arm Differences for Microbiology Outcome Measures*

	Probiotic	Placebo	Absolute difference, 95% CI	Adjusted odds ratio, 95% CI	p-value
Presence of Clostridium difficile					
3 months, n/N (%)	**6/55 (10.9)**	**1/52 (1.9)**	**0.1 (-0.01 to 0.18)**	**6.5 (0.75 to 56.57)**	**.09**
Second follow-up, n/N (%)	**2/36 (5.6)**	**0/28 (0.0)**		**Not analyzable**	
Presence of Lactobacillus rhamnosus					
3 months, n/N (%)	**47/56 (83.9)**	**19/52 (36.5)**	**0.5 (0.29 to 0.67)**	**9.2 (3.51 to 24.07)**	**<.001**
Second follow-up, n/N (%)	**27/37 (73.0)**	**9/29 (31.0)**	**0.4 (0.18 to 0.66)**	**6.4 (2.14 to 19.20)**	**.001**
Presence of Bifidobacterium animalis ssp. Lactis					
3 months, n/N (%)	**29/56 (51.8)**	**2/52 (3.8)**	**0.5 (0.31 to 0.65)**	**26.9 (5.95 to 121.66)**	**<.001**
Second follow-up, n/N (%)	**21/37 (56.8)**	**2/29 (6.9)**	**0.5 (0.27 to 0.73)**	**22.0 (2.97 to 162.43)**	**.002**
Enterobacterales in stool					
3 months, n/N (%)	**55/56 (98.2)**	**52/52 (100.0)**		**Not analyzable**	
Second follow-up, n/N (%)	**36/37 (97.3)**	**29/29 (100.0)**		**Not analyzable**	
Enterobacterales in stool resistant to at least one of the tested antibiotics					
3 months, n/N (%)	**37/55 (67.3)**	**39/52 (75.0)**	**-0.1 (-0.25 to 0.10)**	**0.6 (0.24 to 1.56)**	**.30**
Second follow-up, n/N (%)	**23/33 (69.7)**	**19/27 (70.0)**	**-0.01 (-0.24 to 0.23)**	**0.8 (0.20 to 2.89)**	**.68**
Vancomycin-resistant enterococci in stools					
3 months, n/N (%)	**3/3 (100.0)**	**0/0 (0.0)**		**Not analyzable**	
Second follow-up, n/N (%)	**3/3 (100.0)**	**0/0 (0.0)**		**Not analyzable**	
Presence of oral Candidiasis					
3 months, n/N (%)	**88/113 (77.9)**	**80/105 (76.2)**	**0.02 (-0.10 to 0.13)**	**1.2 (0.54 to 2.83)**	**.62**
Second follow-up, n/N (%)	**70/85 (82.4)**	**57/76 (75.0)**	**0.1 (-0.05 to 0.20)**	**1.3 (0.50 to 3.21)**	**.62**
Weight of oral candida*					
3 months, n/N (%)	**88/113 (77.9)**	**80/105 (76.2)**	**0.02 (-0.10 to 0.13)**	**0.7 (0.20 to 2.17)**	**.49**

	Probiotic	Placebo	Absolute difference, 95% CI	Adjusted odds ratio, 95% CI	p-value
Second follow-up, n/N (%)	**70/85 (82.4)**	**57/76 (75.0)**	**0.1 (-0.05 to 0.20)**	**0.5 (0.12 to 2.16)**	**.36**

Subgroup Analyses:

We investigated differential intervention effects on primary outcome for three pre-specified subgroups by extending out regression models to include a sub-group by trial arm interaction. The sub-groups of interest were Sex of residents (female, male); resident capacity to consent to trial (lack of capacity or with capacity to consent); clinical frailty scale at baseline (very fit to managing well, vulnerable to moderately frail and severely frail to terminally ill). No formal adjustments for multiplicity were made.

eTable 6. Subgroup Effects for Cumulative Antibiotic Administration Days (Primary Outcome)

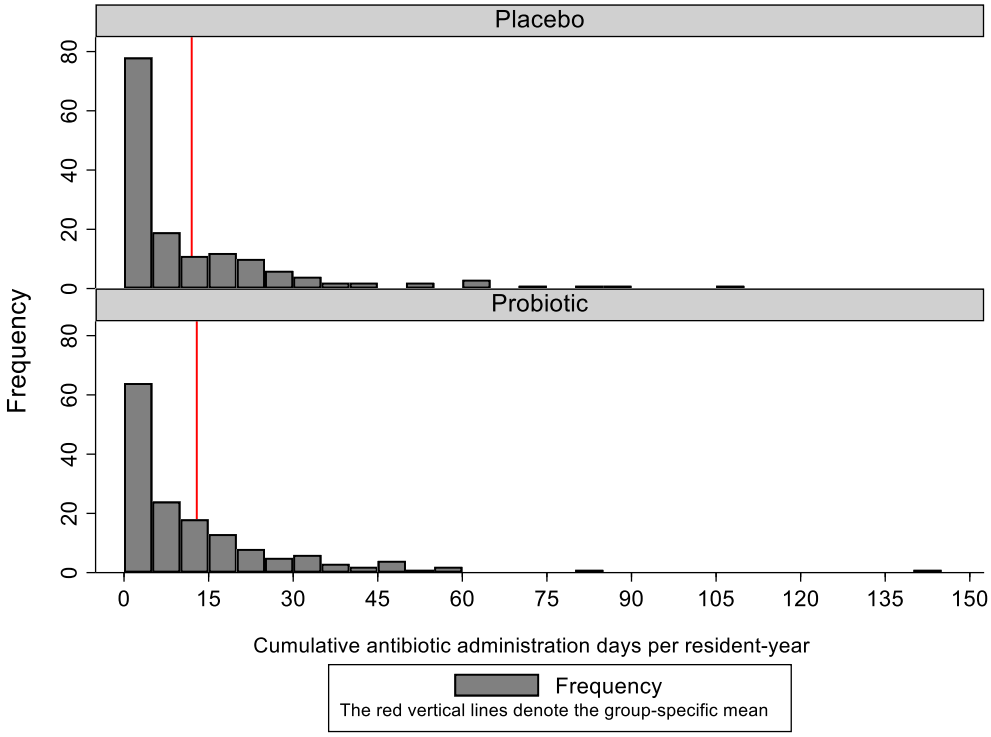
Subgroup analysis	Variable	Adjusted incidence rate ratio*, 95% CI	P-value
Sex of care home residents (n=305)	Placebo	Reference category for trial arm main effect (i.e. effect of trial arm for Female subgroup)	.95
	Probiotic	1.0 (0.65, 1.59)	
	Female	Reference category for gender main effect (i.e. effect of female residents allocated to the placebo arm)	.76
	Male	0.9 (0.52 to 1.62)	
	Probiotic x Female	Reference category for trial arm x gender	.41
	Probiotic x Male	1.4 (0.63 to 3.08)	
Subgroup analysis	**Variable**	**Adjusted incidence rate ratio*, 95% CI**	**P-value**
Baseline capacity to consent to the trial (n=305)	Placebo	Reference category for trial arm main effect (i.e. effect of trial arm for lack of capacity subgroup)	.41
	Probiotic	1.2 (0.77 to 1.91)	
	Lack of capacity	Reference category for capacity to consent to the trial (i.e. the effect of with capacity to consent for residents allocated to placebo arm)	.38
	With capacity	1.3 (0.73 to 2.28)	
	Probiotic x Lack of capacity	Reference category for trial arm x capacity at consent	.64
	Probiotic x with capacity	0.8 (0.38 to 1.80)	
Subgroup analysis	**Variable**	**Adjusted incidence rate ratio*, 95% Ci**	**P-value**
Baseline clinical frailty scale (n=305)	Placebo	Reference category for trial arm main effect (i.e. effect of trial arm for Severely frail to terminally ill subgroup)	.22
	Probiotic	1.4 (0.83 to 2.85)	
	Severely frail to terminally ill	Reference category for clinical frailty scale main effect (i.e. effect of Severely frail to terminally ill residents allocated to the placebo arm)	.20

Subgroup analysis	Variable	Adjusted incidence rate ratio*, 95% CI	P-value
	Very fit to managing well	0.5 (0.20 to 1.23)	
	Vulnerable to moderately frail	1.2 (0.66 to 2.07)	
	Probiotic x Severely frail to terminally ill	Reference category for trial arm x clinical frailty scale	.31
	Probiotic x Very fit to managing well	1.2 (0.34 to 4.55)	
	Probiotic x Vulnerable to moderately frail	0.6 (0.26 to 1.25)	

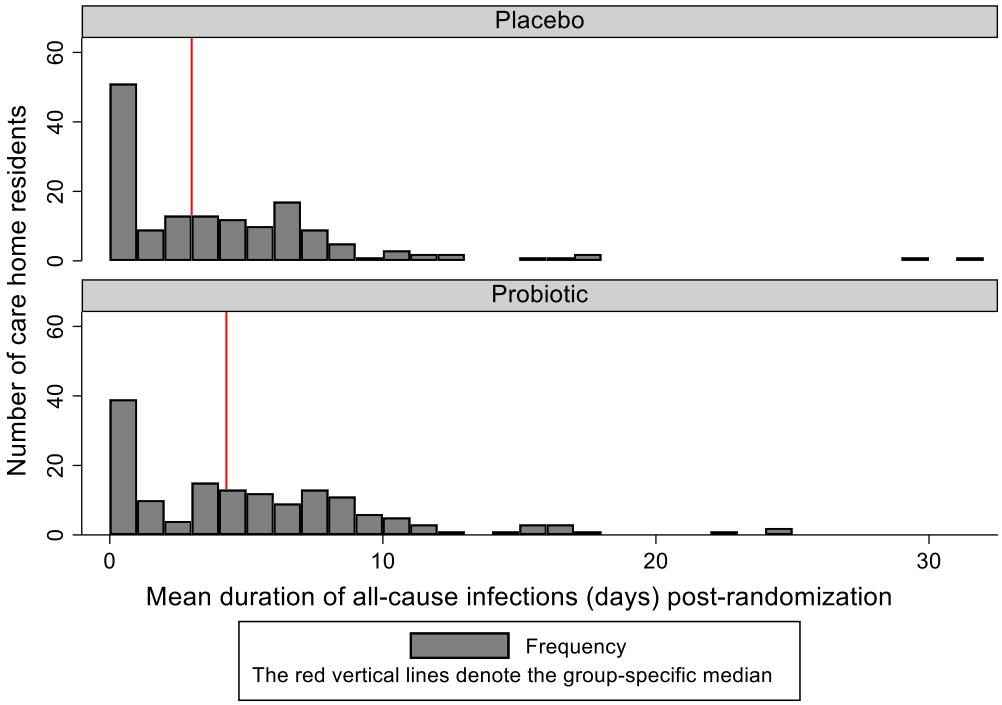
*Models adjust for gender (where it is not the subgroup of interest)

Additional Figures Illustrating Distribution of Outcomes Between Groups:

eFigure 1. Cumulative Antibiotic Administration Days by Group (N=305)



eFigure 2. Mean Duration of All-Cause Infections (Days) by Group (N=305)*



* Mean duration of infection was calculated by dividing the number of infection days by the total number of infections.