Supplemental Online Content

Łukasik J, Dierikx T, Besseling-van der Vaart I, de Meij T, Szajewska H; the Multispecies Probiotic in AAD Study Group. Multispecies probiotic for the prevention of antibiotic-associated diarrhea in children: a randomized clinical trial. *JAMA Pediatr*. Published online June 21, 2022. doi:10.1001/jamapediatrics.2022.1973

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

eTable 1. Recruitment centres.

Location	**Amsterdam UMC, location VUmc** **De Boelelaan 1117 Amsterdam, NL**	**Amsterdam UMC, location AMC** **Meibergdreef 9, 1105 Amsterdam, NL**	**OLVG location East** **Oosterpark 9, 1092 Amsterdam, NL**	**OLVG location West** **Jan Tooropstraat 164, 1061 Amsterdam, NL**	**University Clinical Center of the Medical University of Warsaw,** **Żwirki i Wigury 63A, 02091 Warsaw, PL**	**St. Jadwiga Śląska Hospital** **Prusicka 53-55, 55100 Trzebnica, PL**
Number of the included participants	14	59	31	44	198	4

eTable 2: Patient characteristics depending on the country of recruitment

Clinical values	Poland	The Netherlands
Total	202	148
Lost to follow-up, n(%)	31 (15.1)	6 (4.1)
Compliant participants, n(%)	128 (63.4)	101 (68.2)
Median age in months (range)	27 (3-212)	32 (3-204)
Sex		
Female, n(%)	100 (49.5)	58 (39.2)
Male, n(%)	102 (50.5)	90 (60.8)
Setting		
Inpatient, n(%)	200 (99)	71 (48)
Outpatient, n(%)	2 (1)	77 (52)
Reason for antibiotic treatment		
Lower respiratory tract infection, n(%)	62 (30.7)	48 (32.4)
Upper respiratory tract infection, n(%)	83 (41.1)	18 (12.2)
Urinary tract infection, n(%)	27 (13.4)	32 (21.6)
Skin infection, n(%)	3 (1.5)	21 (14.2)
Lymphadenitis, n(%)	9 (4.5)	4 (2.7)
Nervous system infection, n(%)	2 (1)	5 (3.4)
Gastrointestinal infection, n(%)	3 (1.5)	7 (4.7)
Joint infection, n(%)	1 (0.5)	4 (2.7)
Other, n(%)	12 (5.9)	9 (6.1)
Antibiotic administration route		
Only oral, n(%)	31 (15.3)	113 (76.4)
Only intravenous, n(%)	43 (21.3)	10 (6.8)
Intravenous followed by oral , n(%)	128 (63.4)	25 (16.9)
Antibiotic type		
2nd generation cephalosporin, n(%)	48 (23.8)	3 (2)
3rd generation cephalosporin, n(%)	51 (25.2)	18 (12.2)
Aminopenicillin, n(%)	90 (44.6)	50 (33.8)
Amoxicillin+clavulanic acid, n(%)	36 (17.8)	86 (58.1)
Clindamycin, n(%)	29 (14.4)	2 (1.4)
Cloxacillin/flucloxacillin, n(%)	2 (1)	4 (2.7)
Gentamicin, n(%)	0	4 (2.7)
Other, n(%)	5 (2.5)	7 (4.7)
Two concomitant antibiotics, n(%)	31 (15.3)	8 (5.4)
Change of antibiotic class n(%)	28 (13.9)	18 (12.2)
Median treatment duration days (range)	10 (1-21)	7 (2-36)
Median hospital stay duration (range)	5 (2-21)	4 (1-45)

eTable 3: Characteristics of patients lost to follow-up

Clinical values	Placebo	Probiotic
Total	19	18
Median age in months (range)	26 (3-144)	25 (6-161)
Sex		
Female, n(%)	9 (47)	9 (50)
Male, n(%)	10 (53)	9 (50)
Setting		
Inpatient, n(%)	16 (84)	17 (94)
Outpatient, n(%)	3 (16)	1 (6)
Reason for antibiotic treatment		
Lower respiratory tract infection, n(%)	10 (53)	6 (33)
Upper respiratory tract infection, n(%)	5 (26)	7 (39)
Urinary tract infection, n(%)	1(5)	2 (11)
Nervous system infection, n(%)	1 (5)	-
Lymphadenitis	-	1 (6)
Other, n(%)	2 (10)	2 (11)
Antibiotic type		
2nd generation cephalosporin, n(%)	3 (16)	5 (28)
3rd generation cephalosporin, n(%)	2 (11)	2 (11)
Aminopenicillin, n(%)	10 (53)	9 (50)
Amoxicillin+clavulanic acid, n(%)	4 (21)	2 (11)
Clindamycin, n(%)	4(21)	4 (22)
Two concomitant antibiotics, n(%)	4 (21)	4 (22)
Median treatment duration days (range)	10 (5-21)	10 (3-14)
Median hospital stay duration (range)	4 (3-14)	4 (2-9)

eTable 4. Results of the per protocol analysis including 119 patients in probiotic group and 110 patients in placebo group.

Outcome	Probiotic group no. of events (%)	Placebo group no. of events (%)	Relative Risk (95% CI)
AAD	16 (13.4)	18 (16.4)	0.82 (0.45 to 1.52)
Severe AAD	13 (10.9)	12 (10. 9)	1 (0.49 to 2.07)
Mild AAD	29 (24.4)	25 (22.7)	1.07 (0.67 to 1.71)
Diarrhea	20 (16.8)	27 (24.5)	0.68 (0.41 to 1.14)
C. difficile diarrhea	1 (0.84)	2 (1.8)	0.46 (0.06 to 3.49)
Hospitalization due to diarrhoea	0 (0)	1 (0.9)	n/a
Antibiotic cessation due to diarrhea	0 (0)	0 (0)	n/a
Intravenous rehydration due to diarrhea	0 (0)	1 (0.9)	n/a
Adverse events			
Readmission to the hospital	3 (2.5)	1 (0.9)	2.77 (0.29. 26.27)
Abdominal pain	3 (2.5)	0 (0)	n/a
Vomiting	2 (1.7)	0 (0)	n/a
Rash	1 (0.84)	0 (0)	n/a
Trace of blood in the stool	1 (0.84)	0 (0)	n/a
	Probiotic group median (IQR)	**Placebo group median (IQR)**	**Median difference (95% CI)**
Diarrhea duration in days	3 (3-5.75)	4 (3-6)	1 (-1 to 2)

eTable 5. Available case analysis by the country of recruitment.

Available case analysis - Poland (probiotic n = 84, placebo n= 87)			
Outcome	**Probiotic group no. of events**	**Placebo group no. of events**	**Relative Risk (95% CI)**
AAD	13	16	0.84 (0.44 to 1.62)
Severe AAD	8	7	1.18 (0.46 to 3.02)
Mild AAD	21	25	0.87 (0.53 to 1.42)
Diarrhoea	18	28	0.67 (0.4 to 1.1)
C. difficile diarrhea	1	2	0.52 (0.07 to 3.89)
Hospitalization	0	2	n/a
Antibiotic cessation	0	0	n/a
Intravenous rehydration	0	5	n/a
Adverse events[a]	10	5	2.07 (0.77 to 5.61)
	Probiotic group median (IQR)	**Placebo group median (IQR)**	**Median difference (95% CI)**
Diarrhea duration	3 (2 to 5,5)	4 (3 to 6)	1 (-1 to 2)
[a]Including: rash (2), readmission to the hospital (2), vomiting (1) in the placebo group and vomiting (3), rash (2), readmission to the hospital (1), gag reflex (2), trace of blood in the stool (1), abdominal pain (1) in the probiotic group.			
Available case analysis - The Netherlands (probiotic n = 74, placebo n= 68)			
Outcome	**Probiotic group no. of events**	**Placebo group no. of events**	**Relative Risk (95% CI)**
AAD	10	12	0.77 (0.36 to 1.63)
Severe AAD	10	12	0.77 (0.36 to 1.63)
Mild AAD	19	13	1.34 (0.73 to 2.5)
Diarrhoea	15	22	0.63 (0.36 to 1.09)
C. difficile diarrhea	0	1	n/a
Hospitalisation	1	0	n/a
Antibiotic cessation	0	0	n/a
Intravenous rehydration	0	0	n/a
Adverse events[a]	6	5	1.03 (0.37 to 3.28)

	Probiotic group median (IQR)	Placebo group median (IQR)	Median difference (95% CI)
Diarrhea duration	5 (3-12)	6 (4-7)	0 (-2 to 3)

[a]Including: readmission to the hospital (4), abdominal pain (2) in probiotic group and readmission to the hospital (2), abdominal pain (2), rash (1) in placebo group.

eTable 6. Sensitivity analyses

Outcome	Probiotic group no. Of events (%)	Placebo group no. of events (%)	Relative Risk (95% CI)
AAD cases + diarrhea cases where the testing for pathogens was not performed	29 (18.4)	39 (25.2)	0.73 (0.48 to 1.11)
Infectious diarrhea excluding *C. difficile* diarrhoea	4 (2.5)	11 (7.1)	0.36 (0.02 to 0.65)
Rotaviral diarrhoea	1 (0.6)	9 (5.8)	0.11 (0.2 to 0.65)[a]
Norovirus diarrhea	3 (1.9)	0 (0)	n/a
Adenovirus diarrhea	0 (0)	1 (0.6)	n/a
Salmonella diarrhea	0 (0)	1 (0.6)	n/a
Diarrhea: plausible assumption[c] 5:1	51 (29)	56 (32.2)	0.9 (0.66 to 1.23)
Diarrhea: plausible assumption[c] 2:1	41 (23.3)	56 (32.2)	0.72 (0.51 to 1.02)
Diarrhea: plausible assumption[c] 1,5:1	39 (22.2)	56 (32.2)	0.69 (0.48 to 0.97)[b]
AAD: plausible assumption[c] 5:1	36 (20.5)	31 (17.8)	1.15 (0.75 to 1.77)
AAD: plausible assumption[c] 1:1	26 (14.8)	31 (17.8)	0.83 (0.52 to 1.33)

[a]p=0.01 [b]p=0.04

[c]Explanation of plausible assumption: we performed a sensitivity analysis assuming that the incidence of events among participants lost to follow-up is equal to, or higher by a specific ratio relative to the observed event incidence among participants followed up, For example, 'plausible assumption 5:1' means that we assumed the incidence of diarrhea among missing patients in the probiotic group to be 5 times higher than that in the probiotic group patients who were followed-up, and the incidence of diarrhea among missing patients in the placebo group to be equal to the incidence of diarrhea in the placebo group patients who were followed up.

eTable 7. Results of logistic regression analysis.

A. Logistic regression – AAD outcome

Predictor	**Model with covariates**		
	Odds Ratio	**95% CI**	**p**
Allocation to probiotic group	0.8	0.42 to 1.52	0.49
Age in months	0.99	0.98 to 1	0.006
Male sex	0.94	0.49 to 1.81	0.85
2nd gen. cephalosporin	0.83	0.24 to 2.91	0.78
3rd gen. cephalosporin	2.02	0.72 to 5.7	0.18
Aminopenicillin	0.76	0.24 to 2.45	0.65
Amoxicillin with clavulanic acid	2.07	0.68 to 6.31	0.2
Clindamycin	0.61	0.17 to 2.23	0.45
Other antibiotic	0.49	0.1 to 2.57	0.4
Intravenous antibiotic	1.36	0.40 to 4.62	0.62
Oral antibiotic	0.62	0.26 to 1.49	0.29
Hospital stay duration	1.04	0.97 to 1.12	0.26
Antibiotic treatment duration	1.05	0.96 to 1.14	0.28
	Model without covariates		
	Odds Ratio	**95% CI**	**p**
Allocation to probiotic group	0.77	0.42 to 1.41	0.4

B. Logistic regression – Diarrhea outcome

Predictor	Model with covariates		
	Odds Ratio	**95% CI**	**p**
Allocation to probiotic group	0.55	0.32 to 0.96	0.04
Age in months	0.99	0.98 to 0.99	<0.001
Male sex	1.05	0.60 to 1.82	0.86
2nd gen. cephalosporin	1.75	0.59 to 5.15	0.31
3rd gen. cephalosporin	2.44	0.98 to 6.05	0.05
Aminopenicillin	1.43	0.52 to 3.93	0.48
Amoxicillin with clavulanic acid	2.63	1 to 6.9	0.05
Clindamycin	0.72	0.23 to 2.24	0.57
Other antibiotic	1.65	0.45 to 6.02	0.45
Intravenous antibiotic	2.37	0.83 to 6.81	0.11
Oral antibiotic	0.78	0.38 to 1.61	0.5
Hospital stay duration in days	1.02	0.95 to 1.09	0.65
Antibiotic treatment duration in days	1	0.92 to 1.08	0.98
	Model without covariates		
	Odds Ratio	**95% CI**	**p**
Allocation to probiotic group	0.55	0.33 to 0.92	0.02