PROBIOTICS IN THE NICU: EVIDENCE AND CONTROVERSIES

Miami Neonatology 2021—45th Annual International Conference

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Archive of this course is made possible through an educational grant from
Mead Johnson Nutrition

Outline

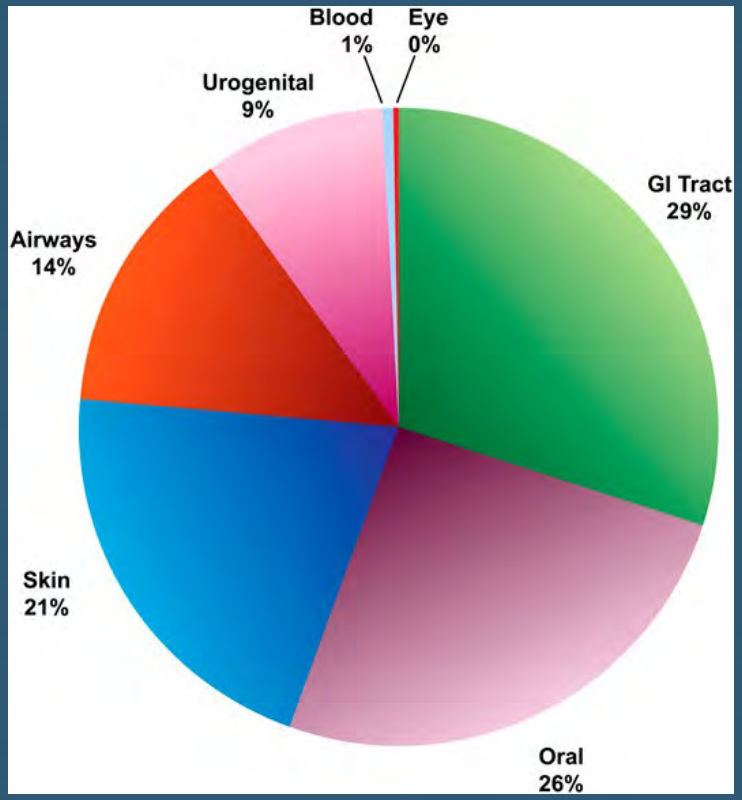
- Premature infant dysbiosis
- Manipulation of the microbiota:

  Probiotics

  - Evidence
  - Controversies

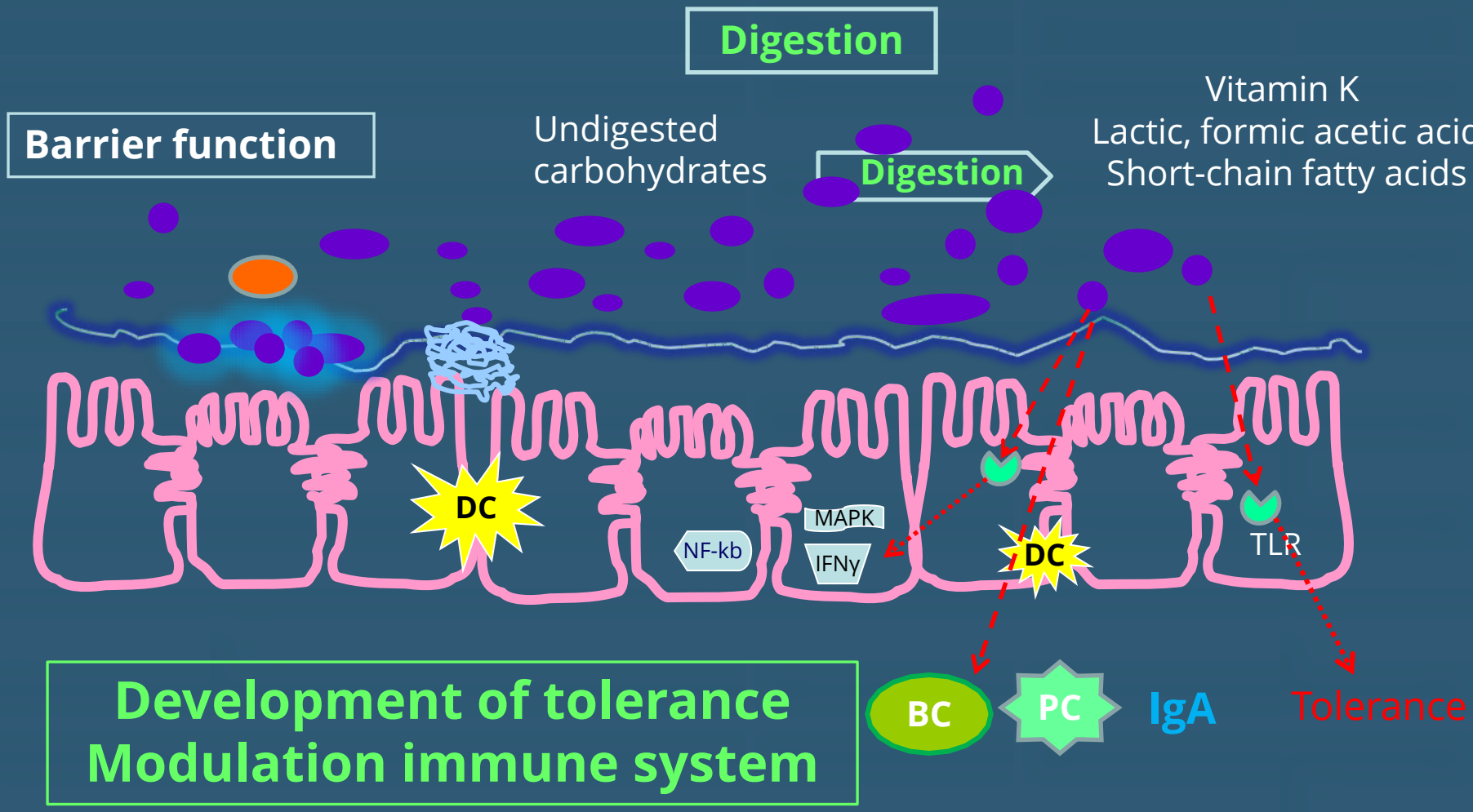
Probiotics, 100 years after Élie Metchnikoff's observation

Human Microbiome Project (HMP)



Bacterial distribution by body site. This figure shows the distribution by body site of bacteria that have been sequenced under the HMP or are in the sequencing pipelines.

Role of the Microbiota in the Immature Intestine



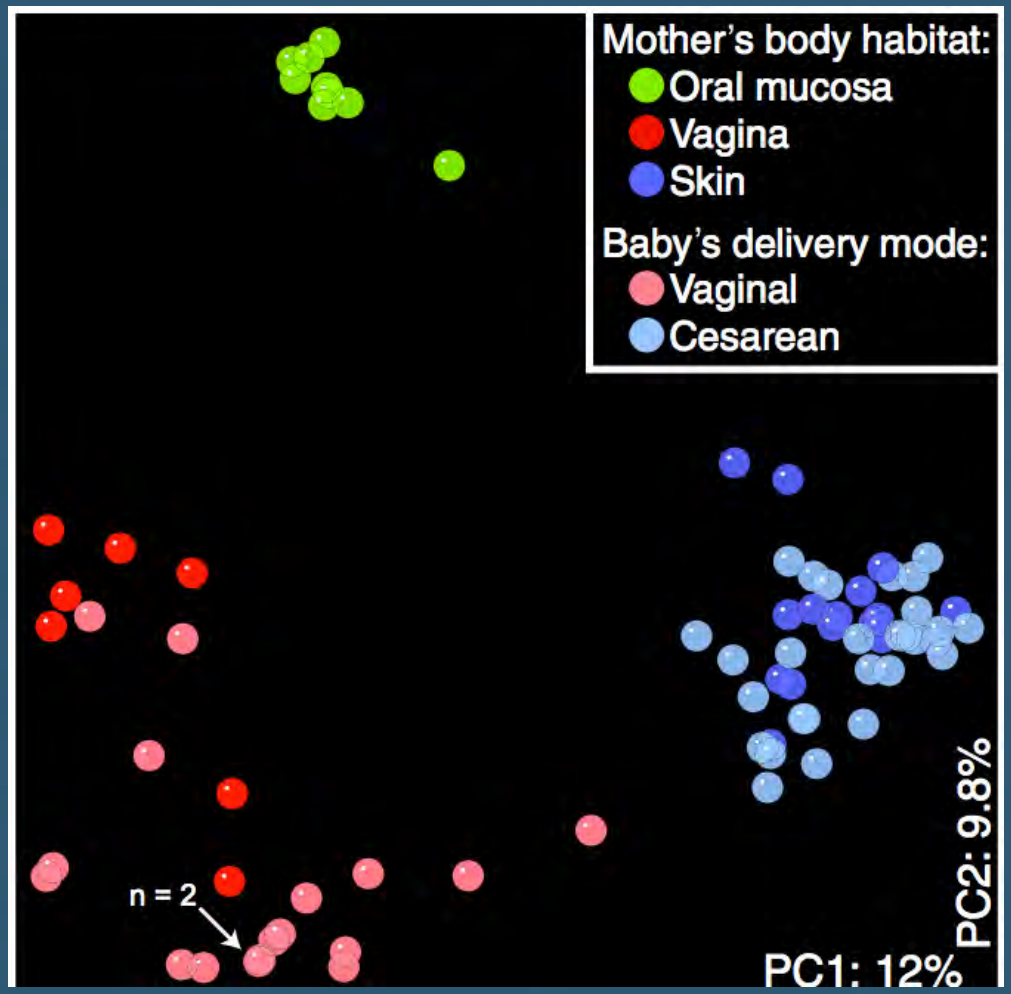
How does the newborn get colonized?

- Intrauterine: partial colonization?
- Delivery: vaginal
- Postnatal: breast feeding

- The placenta harbors a unique microbiome [1]
- Human gut colonization may be initiated in utero by distinct microbial communities in the placenta and amniotic fluid [2]

1. Aagaard K, et al. *Sci Transl Med.* 2014(6);237.
2. Reprinted from Collado MC, et al. *Sci Rep.* 2016;6:23129. Used under terms of the Creative Commons Attribution 4.0 International License without modification

Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns



Bacterial 16S rRNA gene surveys reveal that the first microbiotas of human newborns are primarily structured by delivery mode

Dominguez-Belloa MG, et al. *Proc Natl Acad Sci USA.*2010;107(26):11971-5. Figure used under the National Academy of Sciences terms for nonprofit educational use.

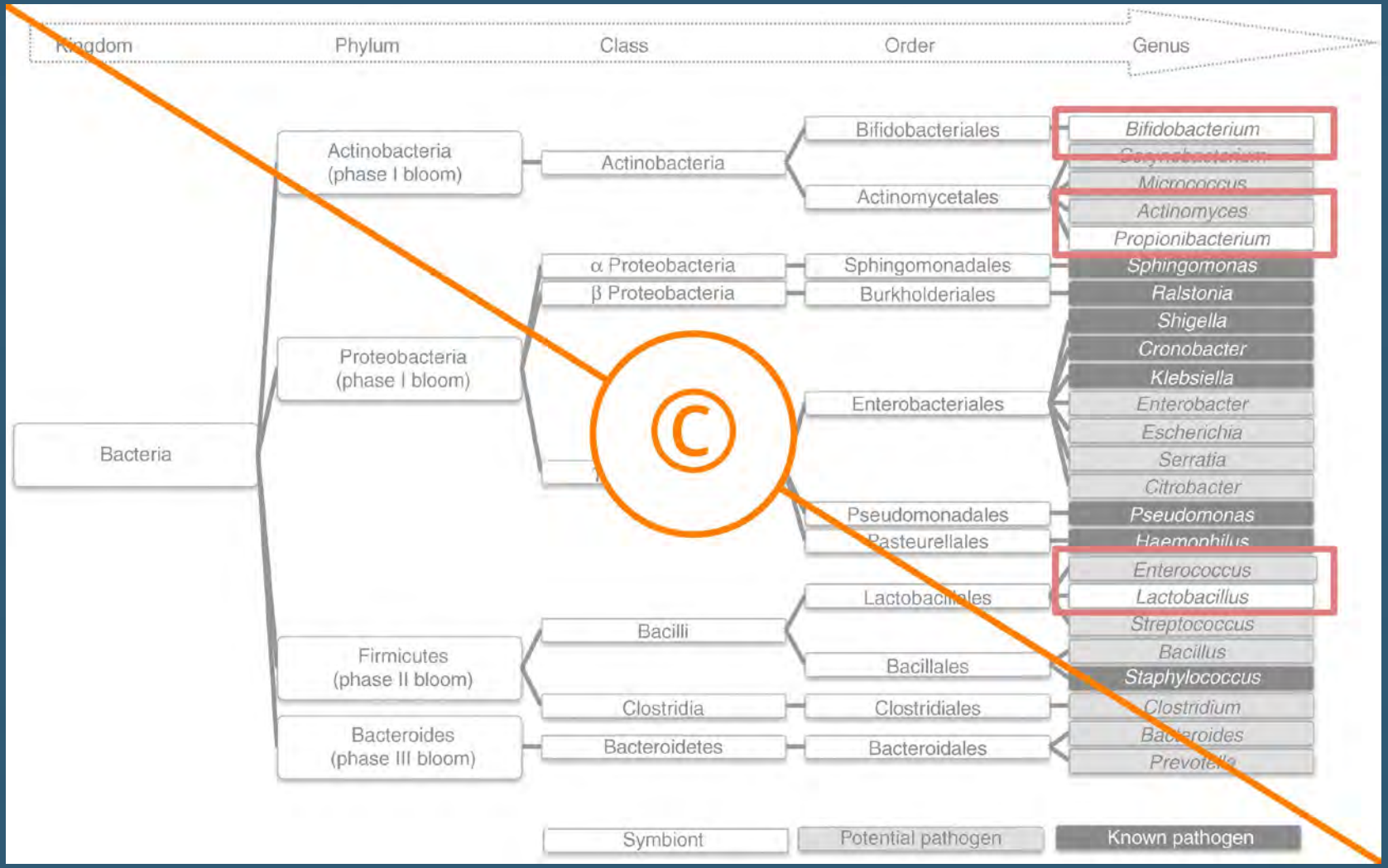
Studies: Translocation Mechanism

	Vaginal Swab				Breast milk				Infant feces			
Species	**V3**	**V4**	**V9**	**V10**	**BM3**	**BM4**	**BM9**	**BM10**	**F3**	**F4**	**F9**	**F10**
Lactobacillus jensenii	+	+	-	+	-	-	-	-	-	-	-	-
Lactobacillus iners	+	-	+	+	-	-	-	-	-	-	-	-
Lactobacillus crispatus	-	+	+	+	-	-	-	-	-	-	-	+
Lactobacillus casei	-	-	-	-	-	-	-	-	+	-	-	-
Lb paracasei	-	-	-	-	-	-	-	-	+	-	-	-
Lactobacillus rhamnosus	-	-	-	-	-	+	-	-	-	+	-	-
Lactobacillus gasseri	-	-	-	-	+	+	-	+	+	+	-	+
Lactobacillus fermentum	-	-	-	-	+	+	+	-	+	+	+	-
Lactobacillus plantarum	-	-	-	-	-	+	-	-	-	+	-	-
Weisella confusa	+	-	-	-	+	+	+	+	+	+	+	+
Leuconostoc fallax	-	-	-	-	-	-	-	+	-	-	-	-
Leuconostoc citreum	-	-	-	-	+	+	+	+	+	+	+	+
Aerococcus sp.	-	-	-	+	-	-	-	-	-	-	-	-

The bacterial flora present in human breast milk, including *lactobacillus* and *bifidobacteria*, are transferred and colonize the gut of the newborn infant.

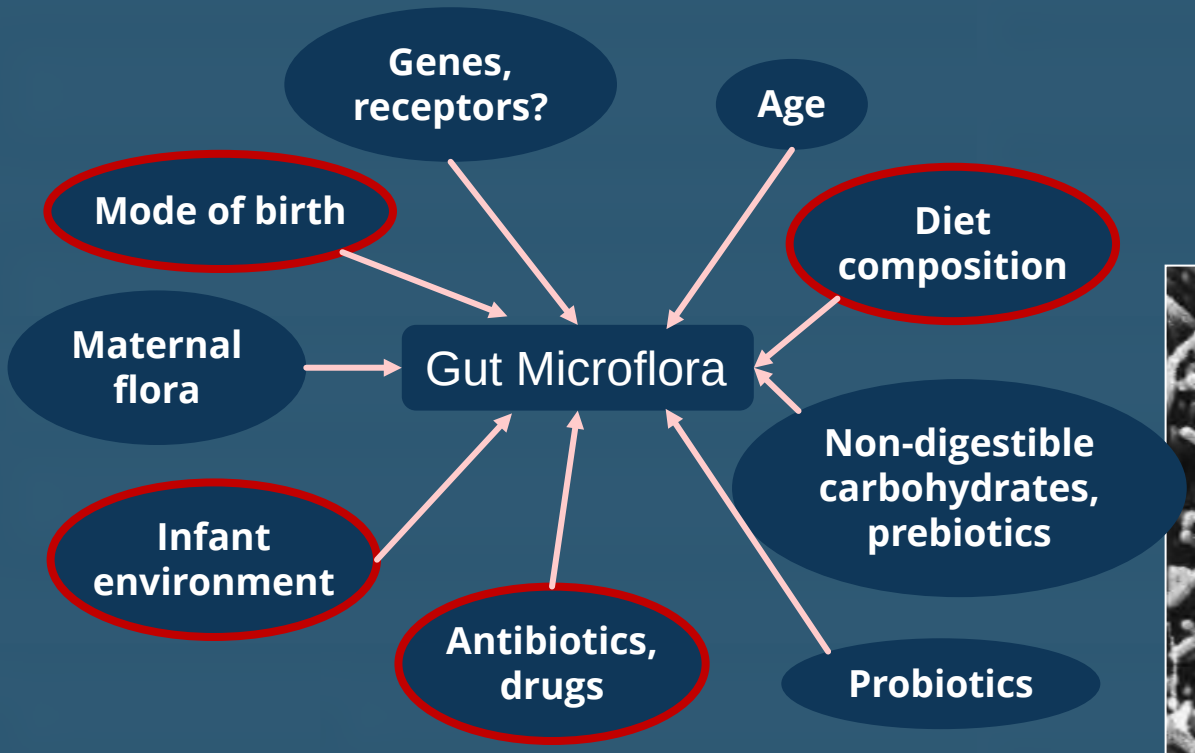
Martin R, et al. *J Appl Microbiol*. 2007;103:2638-2644. (figure replicated)

Gut Microbiota of the Very-Low-Birth-Weight Infant



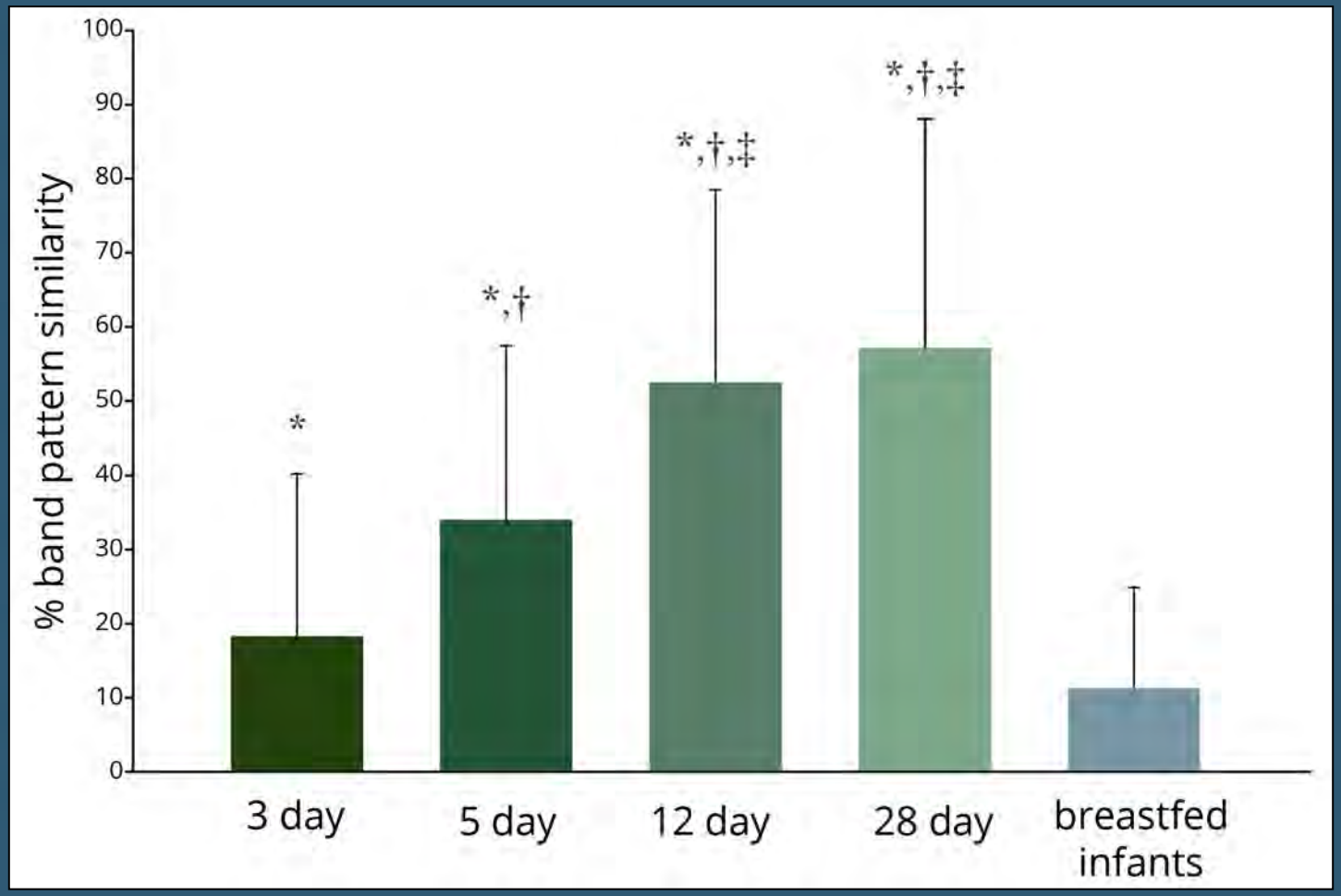
Unger S, et al. *Pediatr Res.* 2015;77:205-213.

Causes of Dysbiosis in Premature Infants



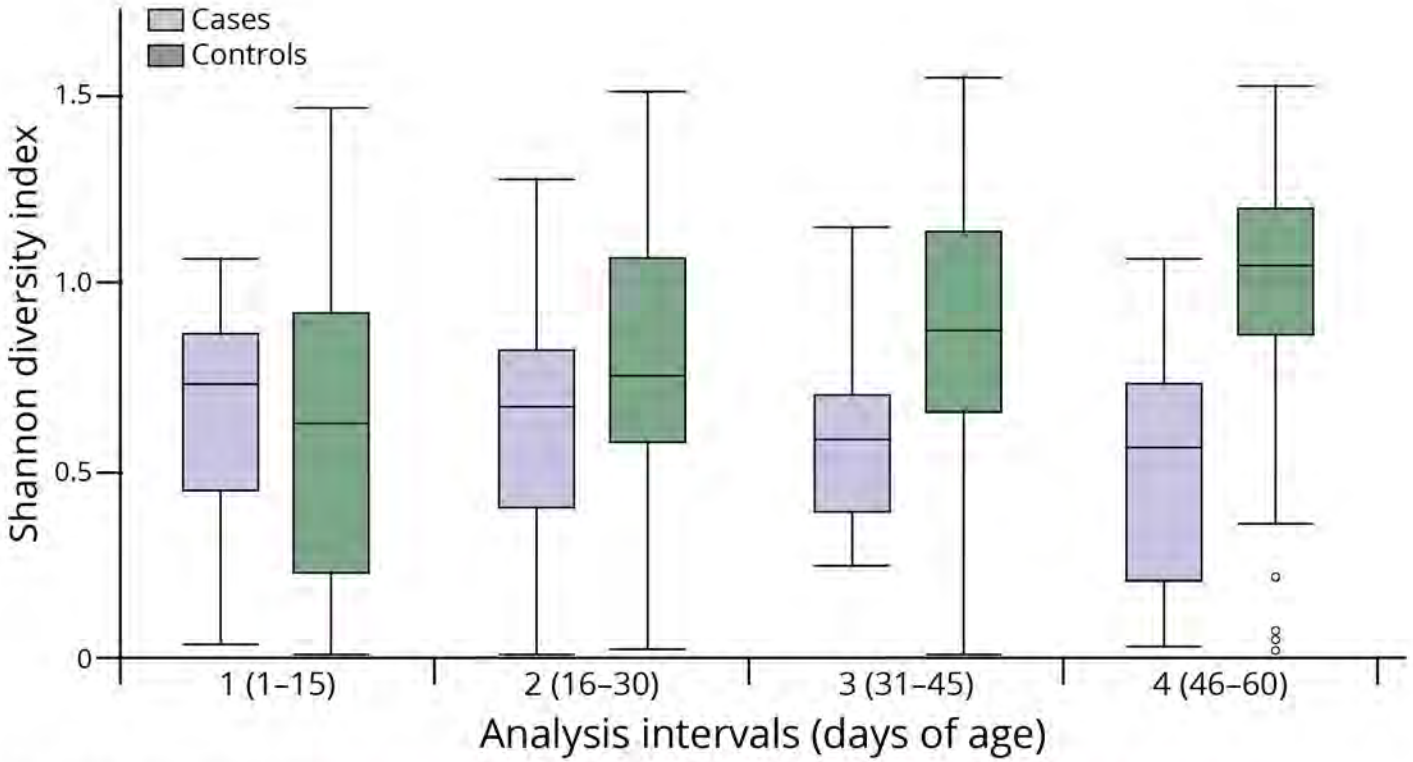
GUT MICROBIOTA DYSBIOSIS

Development of the Intestinal Bacterial Composition in Hospitalized Preterm Infants in Comparison With Breast-Fed, Full-Term Infants



Schwierts A, et al. *Pediatr Res.* 2003;54:393-399. (figure replicated)

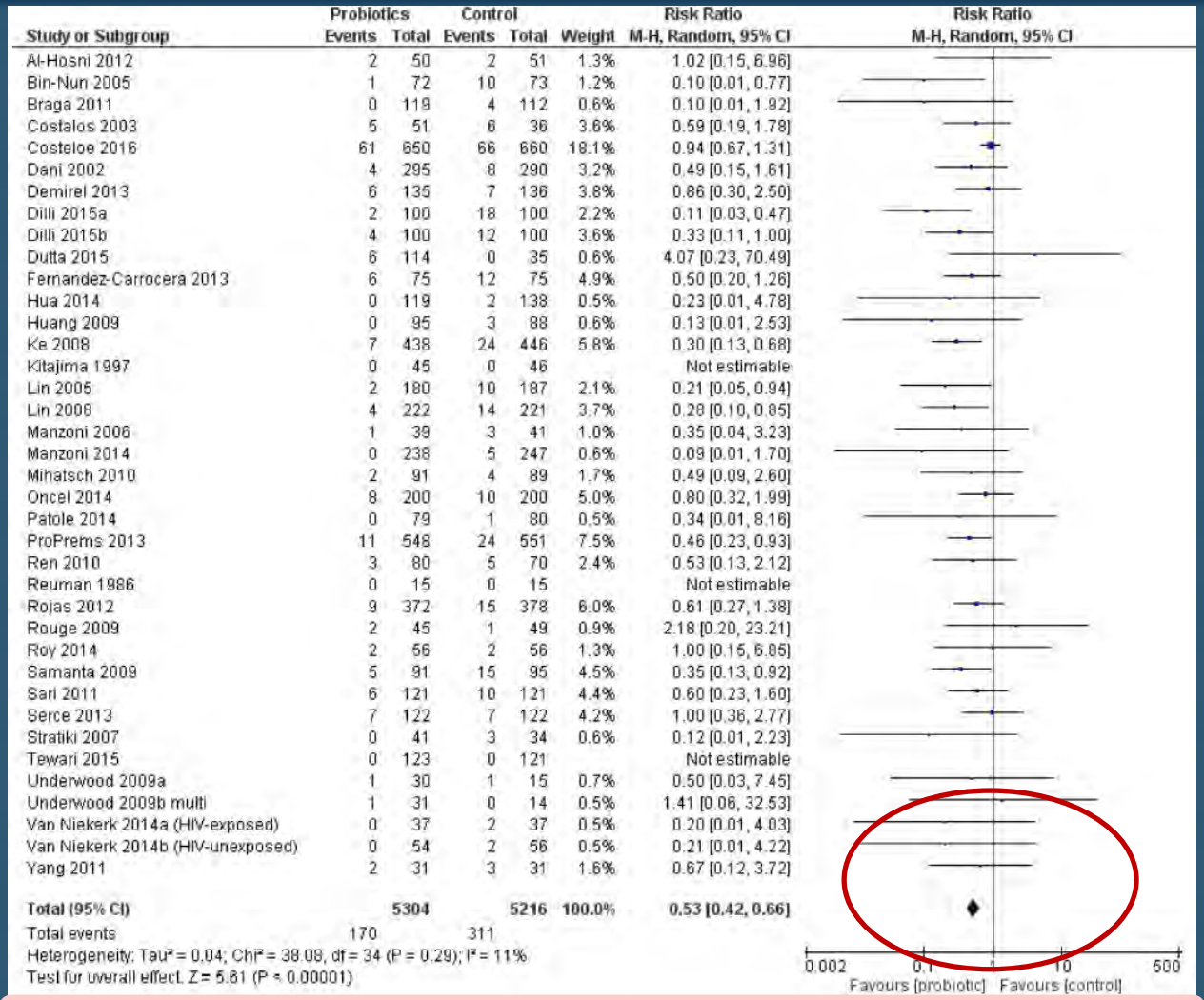
Gut Bacteria Dysbiosis and Necrotizing Enterocolitis in Very-Low-Birth-Weight Infants: A Prospective Case-Control Study



Shannon diversity indices in each 15 day analysis interval from the St. Louis cohort
Shows microbial diversity in stools from cases and controls. Horizontal line shows median, box boundaries show 25th and 75th percentiles, and whiskers show the differences between the 25th and 75th percentiles multiplied by 1.5. Values that exceed these boundaries are depicted as open circles. p=0.0004 for time-by-necrotizing-enterocolitis interaction indicating significantly discordant trends in bacterial diversity in stools from cases versus controls.

- Dysbiosis is prevalent in premature infants
- Dysbiosis is associated with morbidity
- Premature infants are one of the populations that can benefit the most from restoration of intestinal microbiota

Prevention of NEC with Probiotics: A Systematic Review and Meta-Analysis



Study or Subgroup	Probiotics Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
Al-Hosni 2012	2	50	2	51	1.3%	1.02 [0.15, 6.96]
Bin-Nun 2005	1	72	10	73	1.2%	0.10 [0.01, 0.77]
Braga 2011	0	119	4	112	0.6%	0.10 [0.01, 1.92]
Costalos 2003	5	51	6	36	3.6%	0.59 [0.19, 1.78]
Costeloe 2016	61	650	66	660	18.1%	0.94 [0.67, 1.31]
Dani 2002	4	295	8	290	3.2%	0.49 [0.15, 1.61]
Demirel 2013	6	135	7	136	3.8%	0.86 [0.30, 2.50]
Dilli 2015a	2	100	18	100	2.2%	0.11 [0.03, 0.47]
Dilli 2015b	4	100	12	100	3.6%	0.33 [0.11, 1.00]
Dutta 2015	6	114	0	35	0.6%	4.07 [0.23, 70.49]
Fernandez-Carrocera 2013	6	75	12	75	4.9%	0.50 [0.20, 1.26]
Hua 2014	0	119	2	138	0.5%	0.23 [0.01, 4.78]
Huang 2009	0	95	3	88	0.6%	0.13 [0.01, 2.53]
Ke 2008	7	438	24	446	5.8%	0.30 [0.13, 0.68]
Kitajima 1997	0	45	0	46		Not estimable
Lin 2005	2	180	10	187	2.1%	0.21 [0.05, 0.94]
Lin 2008	4	222	14	221	3.7%	0.28 [0.10, 0.85]
Manzoni 2006	1	39	3	41	1.0%	0.35 [0.04, 3.23]
Manzoni 2014	0	238	5	247	0.6%	0.09 [0.01, 1.70]
Mihatsch 2010	2	91	4	89	1.7%	0.49 [0.09, 2.60]
Oncel 2014	8	200	10	200	5.0%	0.80 [0.32, 1.99]
Patole 2014	0	79	1	80	0.5%	0.34 [0.01, 8.16]
ProPrems 2013	11	548	24	551	7.5%	0.46 [0.23, 0.93]
Ren 2010	3	80	5	70	2.4%	0.53 [0.13, 2.12]
Reuman 1986	0	15	0	15		Not estimable
Rojas 2012	9	372	15	378	6.0%	0.61 [0.27, 1.38]
Rouge 2009	2	45	1	49	0.9%	2.18 [0.20, 23.21]
Roy 2014	2	56	2	56	1.3%	1.00 [0.15, 6.85]
Samanta 2009	5	91	15	95	4.5%	0.35 [0.13, 0.92]
Sari 2011	6	121	10	121	4.4%	0.60 [0.23, 1.60]
Serce 2013	7	122	7	122	4.2%	1.00 [0.36, 2.77]
Stratiki 2007	0	41	3	34	0.6%	0.12 [0.01, 2.23]
Tewari 2015	0	123	0	121		Not estimable
Underwood 2009a	1	30	1	15	0.7%	0.50 [0.03, 7.45]
Underwood 2009b multi	1	31	0	14	0.5%	1.41 [0.06, 32.53]
Van Niekerk 2014a (HIV-exposed)	0	37	2	37	0.5%	0.20 [0.01, 4.03]
Van Niekerk 2014b (HIV-unexposed)	0	54	2	56	0.5%	0.21 [0.01, 4.22]
Yang 2011	2	31	3	31	1.6%	0.67 [0.12, 3.72]
Total (95% CI)		**5304**		**5216**	**100.0%**	**0.53 [0.42, 0.66]**
Total events	170		311			

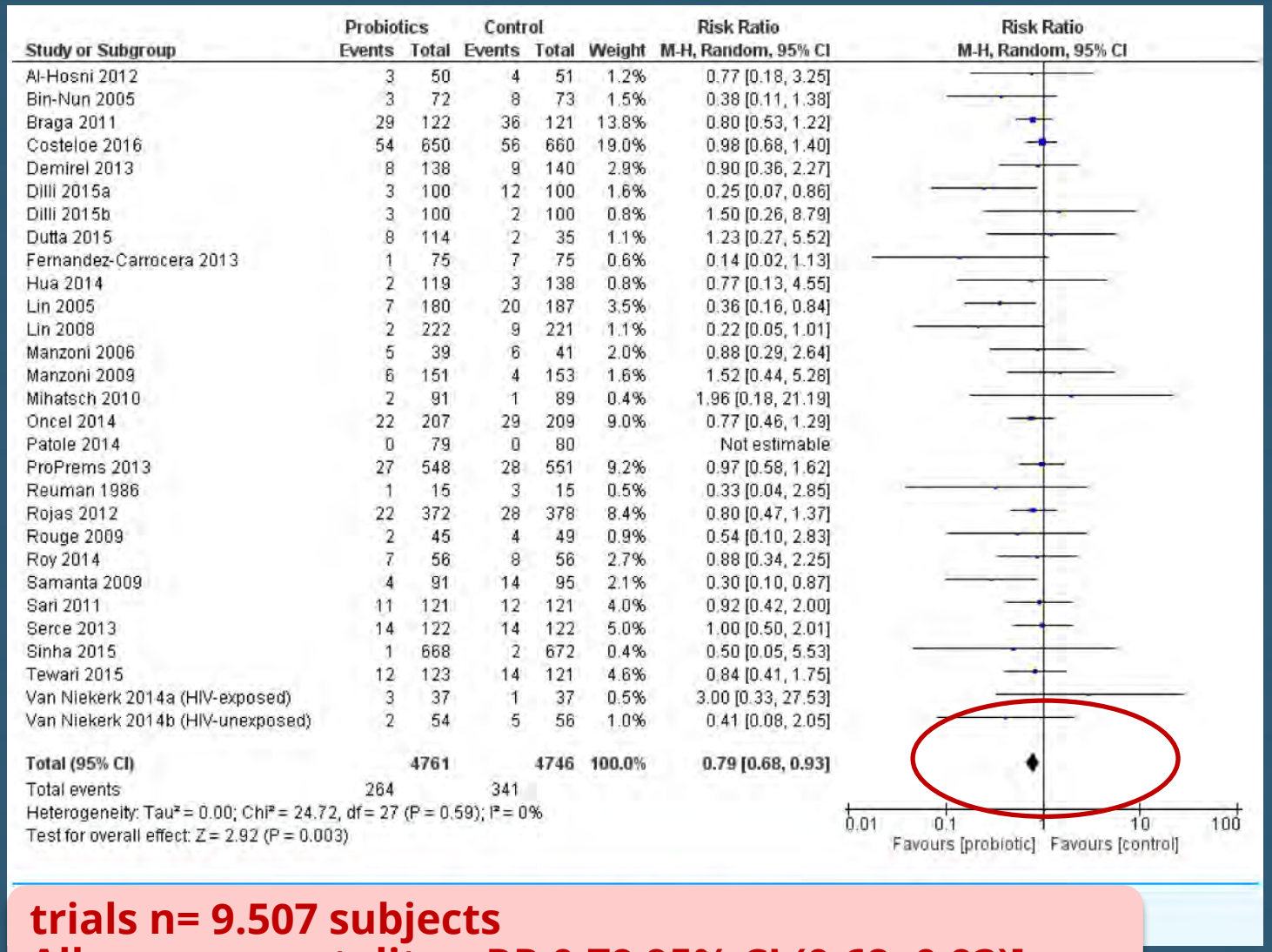
Heterogeneity: Tau² = 0.04; Chi² = 38.08, df = 34 (P = 0.29); I² = 11%
Test for overall effect: Z = 5.81 (P < 0.00001)

Risk Ratio M-H, Random, 95% CI

0.002 0.1 1 10 500
Favours [probiotic] Favours [control]

38 trials n = 10,520 subjects
Severe NEC in all infants. RR 0.53 95% CI (0.42-0.66)

Prevention of NEC with Probiotics: A Systematic Review and Meta-Analysis



Study or Subgroup	Probiotics Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI
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Bin-Nun 2005	3	72	8	73	1.5%	0.38 [0.11, 1.38]
Braga 2011	29	122	36	121	13.8%	0.80 [0.53, 1.22]
Costeloe 2016	54	650	56	660	19.0%	0.98 [0.68, 1.40]
Demirel 2013	8	138	9	140	2.9%	0.90 [0.36, 2.27]
Dilli 2015a	3	100	12	100	1.6%	0.25 [0.07, 0.86]
Dilli 2015b	3	100	2	100	0.8%	1.50 [0.26, 8.79]
Dutta 2015	8	114	2	35	1.1%	1.23 [0.27, 5.52]
Fernandez-Carrocera 2013	1	75	7	75	0.6%	0.14 [0.02, 1.13]
Hua 2014	2	119	3	138	0.8%	0.77 [0.13, 4.55]
Lin 2005	7	180	20	187	3.5%	0.36 [0.16, 0.84]
Lin 2008	2	222	9	221	1.1%	0.22 [0.05, 1.01]
Manzoni 2006	5	39	6	41	2.0%	0.88 [0.29, 2.64]
Manzoni 2009	6	151	4	153	1.6%	1.52 [0.44, 5.28]
Mihatsch 2010	2	91	1	89	0.4%	1.96 [0.18, 21.19]
Oncel 2014	22	207	29	209	9.0%	0.77 [0.46, 1.29]
Patole 2014	0	79	0	80		Not estimable
ProPrems 2013	27	548	28	551	9.2%	0.97 [0.58, 1.62]
Reuman 1986	1	15	3	15	0.5%	0.33 [0.04, 2.85]
Rojas 2012	22	372	28	378	8.4%	0.80 [0.47, 1.37]
Rouge 2009	2	45	4	49	0.9%	0.54 [0.10, 2.83]
Roy 2014	7	56	8	56	2.7%	0.88 [0.34, 2.25]
Samanta 2009	4	91	14	95	2.1%	0.30 [0.10, 0.87]
Sari 2011	11	121	12	121	4.0%	0.92 [0.42, 2.00]
Serce 2013	14	122	14	122	5.0%	1.00 [0.50, 2.01]
Sinha 2015	1	668	2	672	0.4%	0.50 [0.05, 5.53]
Tewari 2015	12	123	14	121	4.6%	0.84 [0.41, 1.75]
Van Niekerk 2014a (HIV-exposed)	3	37	1	37	0.5%	3.00 [0.33, 27.53]
Van Niekerk 2014b (HIV-unexposed)	2	54	5	56	1.0%	0.41 [0.08, 2.05]
Total (95% CI)		**4761**		**4746**	**100.0%**	**0.79 [0.68, 0.93]**
Total events	264		341			

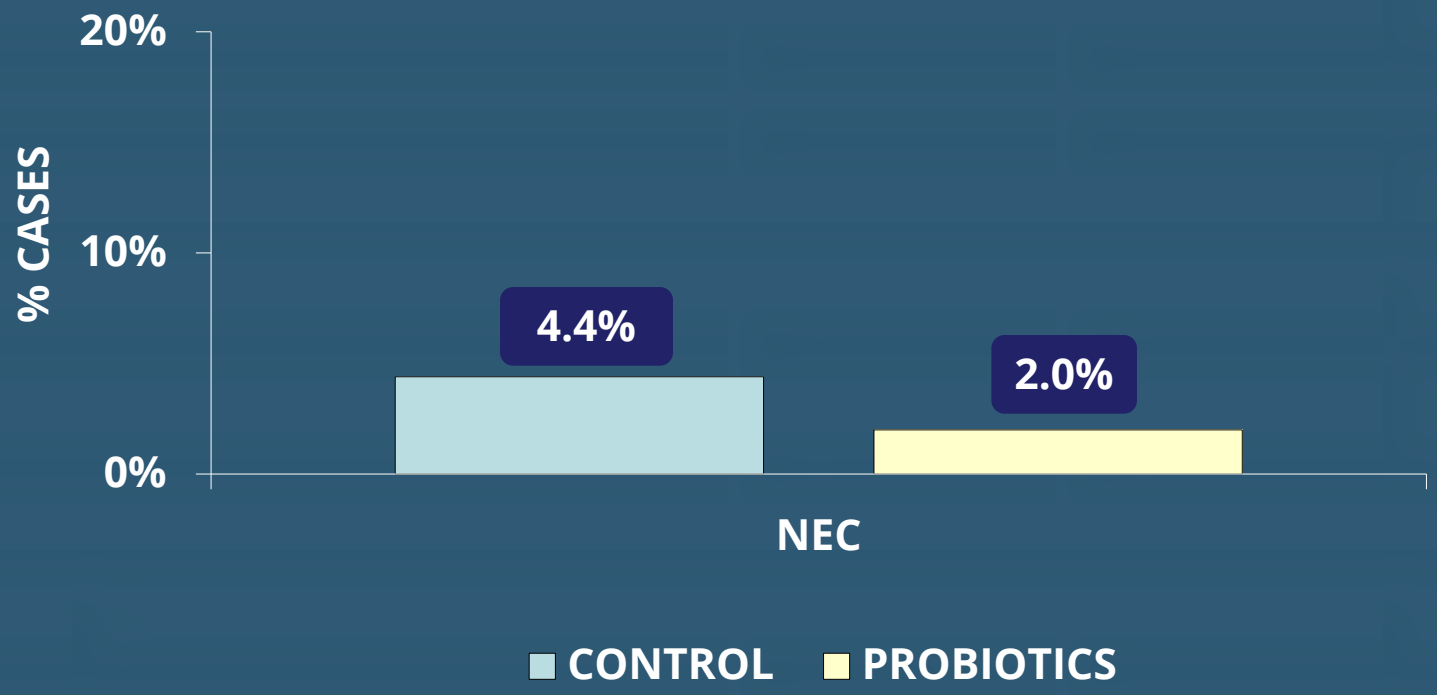
Heterogeneity: Tau² = 0.00; Chi² = 24.72, df = 27 (P = 0.59); I² = 0%
Test for overall effect: Z = 2.92 (P = 0.003)

Risk Ratio M-H, Random, 95% CI

0.01 0.1 1 10 100
Favours [probiotic] Favours [control]

trials n= 9.507 subjects
All causes mortality RR 0.79 95% CI (0.68- 0.93)]

The ProPrems Randomized Trial Investigating the Effects of Probiotics on Late Onset Sepsis in Very Preterm Infants



Bifidobacterium infantis, Streptococcus thermophilus, and *Bifidobacterium lactis*

Jacobs SE, et al. *Pediatrics.* 2013;132(6):1055–1062.

Bifidobacterium breve BBG-001 in Very Preterm Infants: A Randomized Controlled Phase 3 Trial

	Bifidobacterium breve **BBG-001 probiotic (n=650)**	**Placebo (n=660)**	**Adjusted [a] risk ratio (95% CI)**
Necrotizing enterocolitis [b]	61 (9%)	66 (10%)	0.93 (0.68–1.27)
Sepsis [c]	73 (11%)	77 (12%)	0.97 (0.73–1.29)
Death before discharge home [d]	54 (8%)	56 (9%)	0.93 (0.67–1.30)

Data are n (%), unless otherwise indicated.

a. Adjusted for sex, gestational age at birth, and randomization within 24 h of birth. Adjustment by center was excluded because the model did not converge. Allowances for correlations between multiple births are accounted for.

b. Necrotizing enterocolitis (Bell stage 2 or 3).

c. Sepsis is defined as bloodstream infection with non-skin commensals after 72 h postnatal age and before 46 weeks' postmenstrual age.

d. Includes three infants who remained on pediatric wards at the time of analysis and are included as survivors; all were later discharged home.

Bifidobacterium breve BBG-001 in Very Preterm Infants: A Randomized Controlled Phase 3 Trial

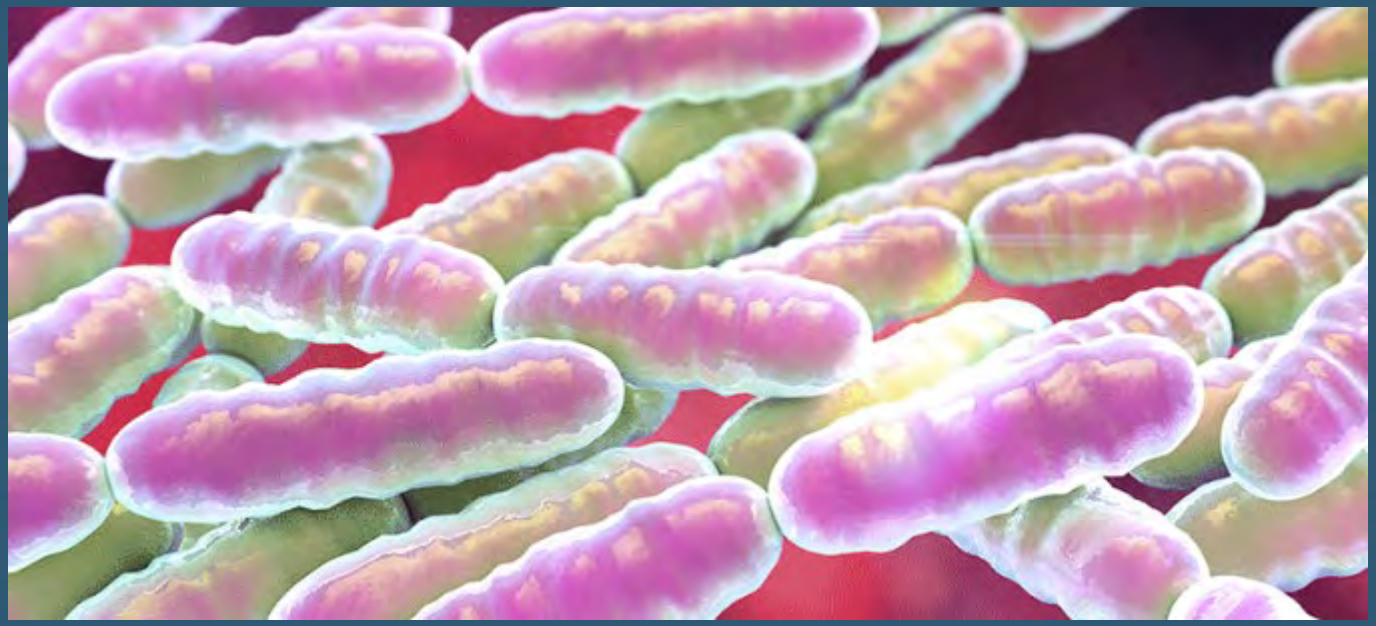
Stool PCR at 2 weeks' postnatal age			
PCR positive	416 (84%)	177 (35%)	2.42 (2.06–2.85)
B breve positive by culture or PCR	505 (85%)	219 (37%)	2.30 (1.99–2.66)
Stool culture at 36 weeks' postmenstrual age			
B breve	438 (84%)	253 (49%)	1.69 (1.50–1.91)
MRSA	1 (<1%)	0	Too few data
VRE	3 (1%)	1 (<1%)	2.97 (0.15–57.67)
ESßL	19 (4%)	18 (4%)	0.98 (0.44–2.18)

Probiotics in Very Preterm Infants: PiPS Trial

Unadjusted analysis of colonized infants versus non-colonized infants					
	Colonized infants (n=724)	Non-colonized infants (n=462)	Risk ratio (unadjusted, 95% CI)	Risk ratio (unadjusted, 99% CI)	Adjusted risk ratio (99% CI)
Necrotizing enterocolitis	47 (7%)	58 (13%)	0.52 (0.36–0.75) p =0.0005	0.52 (0.32–0.84) p =0.0005	0.68 (0.43–1.09)
Sepsis	67 (9%)	66 (14%)	0.65 (0.47–0.89) p =0.0082	0.65 (0.42–0.98) p =0.0082	0.88 (0.59–1.31)
Death before discharge	24 (3%)	33 (7%)	0.46 (0.28–0.77) p =0.0033	0.46 (0.24–0.91) p =0.0033	0.68 (0.35–1.29)

Deshpande G, et al. *Lancet.* 2016;388(10045):655. (table replicated)

Should the use of probiotics in the preterm infant be routine?



Millar M, et al. *Arch Dis Child Fetal Neonatal Ed.* 2003;88:F354-F358.

Survey of Clinical Use of Probiotics in USA

PROBIOTIC BRAND NAME	SPECIES INCLUDED	AMERICAN NICUs USING PROBIOTICS
Culturelle	*Lactobacillus rhamnosus GG*	27%
Biogaia	*Lactobacillus reuteri*	14%
Gerber Soothe	*L reuteri*	14%
Florababy	*Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium bifidum, Bifidobacterium longum* *L rhamnosus*	9%
Align	*B infantis*	7%
Floro-Q2	*Lactobacillus acidophilus* *Lactobacillus paracasei*	
Risaquad	*S thermophilus*	6%
VSL#3	*B breve, B longum, B infantis* *L acidophilus, Lactobacillus plantarum, L paracasei, Lactobacillus bulgaricus* *S thermophilus*	6%
Lactinex	*L acidophilus* *L bulgaricus*	4%
ABC Dophilus	*B infantis* *S thermophilus* *B bifidum*	3%
Udo's Choice	*Lactobacillus casei, L rhamnosus, L acidophilus* *B infantis, B bifidum, B breve*	3%

14% (70/500) NICUs using probiotics
16 different products
Only 2 validated by clinical trials

Viswanathan S, et al. *J Perinatol.* 2016;36:1106-1111. (table replicated)

Bifidobacterium infantis
Bifidobacterium bifidum
Bifidobacterium fecalis
Bifidobacteria longum
Bifidobacterium breve
Bifidobacterium lactis

Lactobacillus casei
Lactobacillus rhammosus
Lactobacillus acidophilus
Lactobacillus plantarum
Lactobacillus reuteri
Lactobacillus lactis

Streptococcus thermofilus
Shacaromices boulardii
Bacillus cereus

Bifidobacterium breve—0.005 to 5 billion(Yakult LB® - Sao Paulo, Brazil)

Saccharomyces boulardii

B. breve BBG-001 (Yakult Honsha Co Ltd –Tokyo, Japan)

L. rhamnosus GG (Dicoflor®)

S. boulardii (Reflor®)

Lactobacillus acidophilus—1 billion

L. rhamnosus—0.44 billion

L. casei—1 billion

Lactobacillus plantarum—0.176 billion

B. infantis—0.0276 billion

S. thermophillus—0.0066 billion

(Lactipan®)

Bifidobacterium longum

Lactobacillus bulgaricus

S. thermophiles (Golden Bifid®)

idobacterium adolescentis

infantis—0.35 billion

bifidus—0.35 billion

thermophilus—0.35 billion BC Dophilus®)

terococcus faecalis—1 billion

longum—1 billion

acidophilus—1 billion ifico®)

breve YIT4010 akult® Honsya Co. Ltd., Tok

B. b

L. a mg

B. i cap Farı

L. a mg

B. bifidum—1 billion/250 mg cap (Infloran®- Laboratorio Farmaceutico, Italy)

L. rhamnosus GG (Dicoflor®)

L. rhamnosus GG 6 billion + *Bovine Lactoferrin* 100 mg (Dicoflor®)

Bovine Lactoferrin 100 mg (Dicofarm®)

Bifidobacterium lactis—20 billion/g (Nestle®)

L. rhamnosus GG (Valio Finnish Co-operative Dairies Association®)

Bacillus cereus—0.0005 billion (*Bifidobacterium tetravaccine*)

L. ac Labo WI)

L. re (Bio

L. reuteri DSM 17938 in oil (Biogaia®)

L. rhamnosus GG (Dicoflor®)

L. rhamnosus GG—0.1 billion (Valio, Ltd®)

B. longum BB536—0.1 billion (Morinaga Milk Industry Co, Ltd®, Tokyo, Japan)

L. acidophilus—1.25 billion/g

B. longum—0.125 billion/g

B. bifidum—0.125 billion/g

—1 billion/g

l®)

ntis—2.5 billion

lum—2.5 billion

um—2.5 billion

ophilus—2.5 billion

s coagulans acillus sporogenes)

ITALIA SRL®, Rome,

S. boulardii (Reflor®)

Probiotic suitable for premature infant

- Strain-specific effects

Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low-birth-weight infants: an updated meta-analysis of 20 randomized, controlled trials

Summary of pooled RR with 95% CI in the subgroup analyses

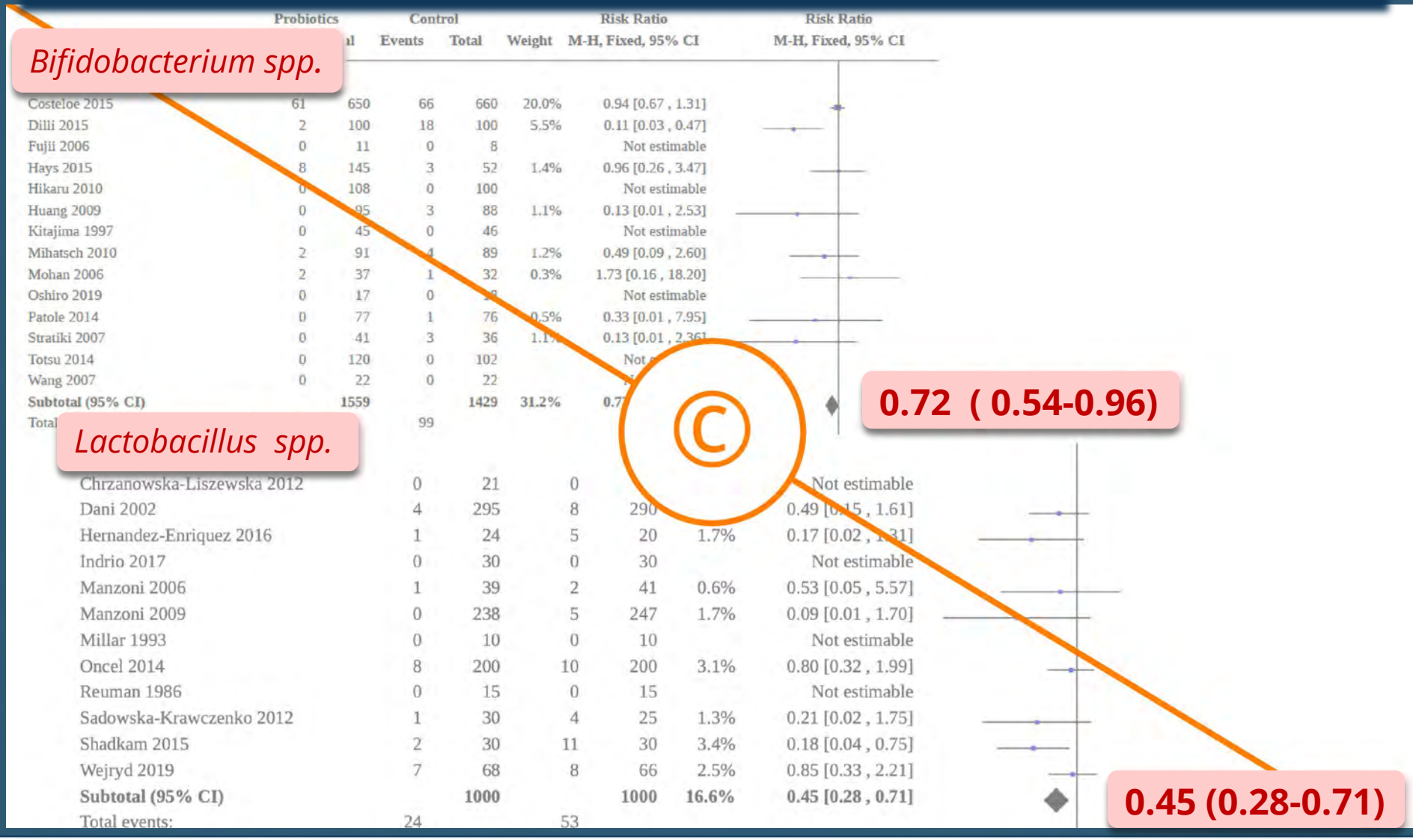
$I^2_{Heterogeneity}$

Subgroup analyses	Studies (no. in probiotics group/no. in placebo group)	RR				
		RR (95%)	P_{RR}	$I^2_{Heterogeneity}$	$P_{Heterogeneity}$	Model
Bifidobacteria						
NEC	8 (509/467)	0.30 (0.16–0.58)	0.0003	0	0.64	Fixed
Mortality	3 (174/166)	0.74 (0.18–2.97)	0.67	0	0.51	Fixed
Sepsis	3 (174/166)	0.84 (0.29–2.41)	0.74	0.21	0.28	Fixed
Lactobacillus and *Bifidobacteria*						
NEC	6 (714/689)	0.33 (0.19–0.58)	0.0001	0	0.51	Fixed
Mortality	5 (653/660)	0.47 (0.26–0.87)	0.02	49	0.09	Random
Sepsis	5 (653/660)	0.90 (0.60–1.36)	0.62	71	0.007	Random
Lactobacillus						
NEC	4 (595/610)	0.37 (0.19–0.73)	0.004	0	0.40	Fixed
Mortality	4 (595/610)	0.61 (0.38–0.97)	0.04	0	0.88	Fixed
Sepsis	4 (595/610)	0.79 (0.46–1.36)	0.40	71	0.01	Random

$I^2_{Heterogeneity}$ indicates the I^2 value for heterogeneity analysis; $P_{Heterogeneity}$, the P value for heterogeneity analysis.

Wang Q, et al. *J Pediatr.* 2012;47:241-248. (table replicated)

Probiotics to prevent necrotizing enterocolitis in very preterm or very low birth weight infants (Review)



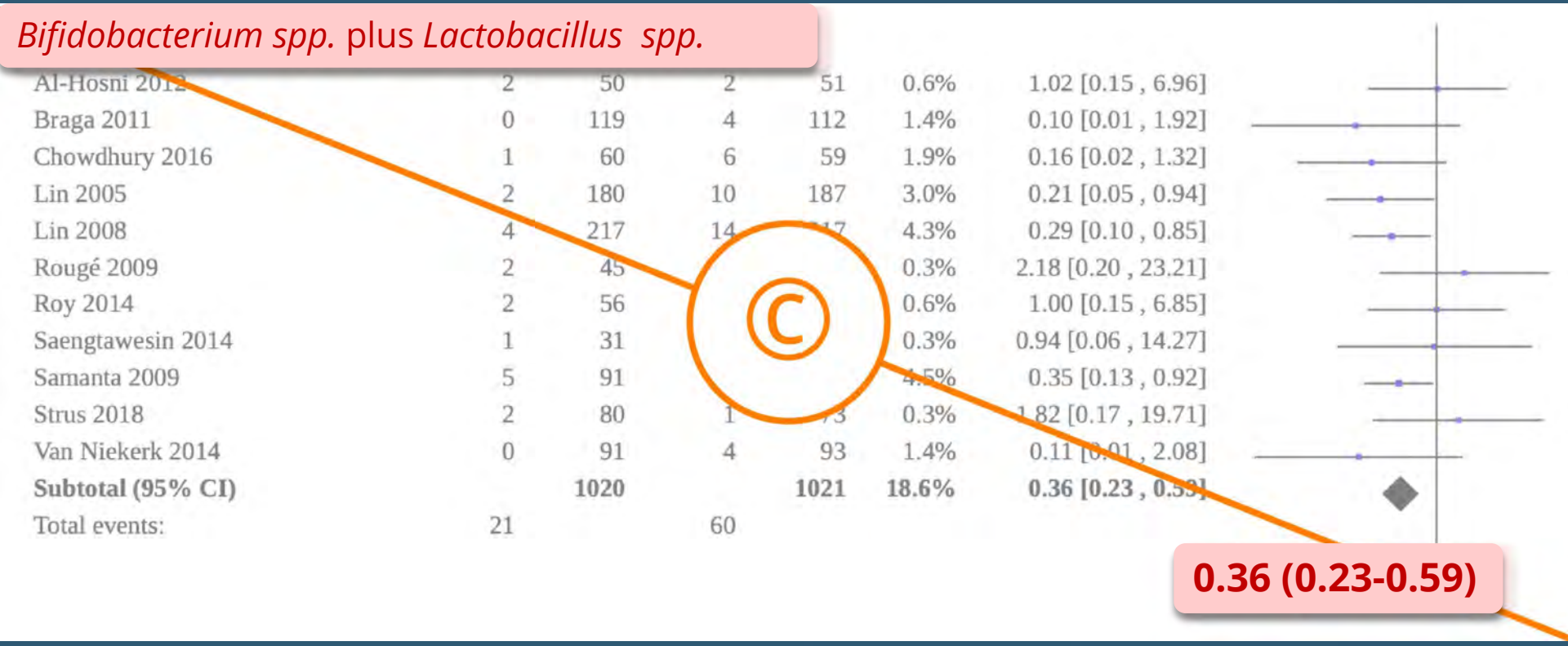
	Probiotics		Control			Risk Ratio	Risk Ratio
		Events	Total	Weight	M-H, Fixed, 95% CI	M-H, Fixed, 95% CI
Costeloe 2015	61	650	66	660	20.0%	0.94 [0.67 , 1.31]	
Dilli 2015	2	100	18	100	5.5%	0.11 [0.03 , 0.47]	
Fujii 2006	0	11	0	8		Not estimable	
Hays 2015	8	145	3	52	1.4%	0.96 [0.26 , 3.47]	
Hikaru 2010	0	108	0	100		Not estimable	
Huang 2009	0	95	3	88	1.1%	0.13 [0.01 , 2.53]	
Kitajima 1997	0	45	0	46		Not estimable	
Mihatsch 2010	2	91	4	89	1.2%	0.49 [0.09 , 2.60]	
Mohan 2006	2	37	1	32	0.3%	1.73 [0.16 , 18.20]	
Oshiro 2019	0	17	0			Not estimable	
Patole 2014	0	77	1	76	0.5%	0.33 [0.01 , 7.95]	
Stratiki 2007	0	41	3	36	1.1%	0.13 [0.01 , 2.36]	
Totsu 2014	0	120	0	102		Not	
Wang 2007	0	22	0	22			
Subtotal (95% CI)		**1559**		**1429**	**31.2%**	**0.7**	
Total			99				
Chrzanowska-Liszewska 2012		0	21	0			Not estimable
Dani 2002		4	295	8	290		0.49 [0.15 , 1.61]
Hernandez-Enriquez 2016		1	24	5	20	1.7%	0.17 [0.02 , 1.31]
Indrio 2017		0	30	0	30		Not estimable
Manzoni 2006		1	39	2	41	0.6%	0.53 [0.05 , 5.57]
Manzoni 2009		0	238	5	247	1.7%	0.09 [0.01 , 1.70]
Millar 1993		0	10	0	10		Not estimable
Oncel 2014		8	200	10	200	3.1%	0.80 [0.32 , 1.99]
Reuman 1986		0	15	0	15		Not estimable
Sadowska-Krawczenko 2012		1	30	4	25	1.3%	0.21 [0.02 , 1.75]
Shadkam 2015		2	30	11	30	3.4%	0.18 [0.04 , 0.75]
Wejryd 2019		7	68	8	66	2.5%	0.85 [0.33 , 2.21]
Subtotal (95% CI)			**1000**		**1000**	**16.6%**	**0.45 [0.28 , 0.71]**
Total events:		24		53			

Sharif S, et al. *Cochrane Database Syst Rev.* 2020;10(10):CD005496.

Probiotics to prevent necrotizing enterocolitis in very preterm or very low birth weight infants (Review)

Risk of NEC

Bifidobacterium spp. plus *Lactobacillus spp.*



Al-Hosni 2012	2	50	2	51	0.6%	1.02 [0.15 , 6.96]
Braga 2011	0	119	4	112	1.4%	0.10 [0.01 , 1.92]
Chowdhury 2016	1	60	6	59	1.9%	0.16 [0.02 , 1.32]
Lin 2005	2	180	10	187	3.0%	0.21 [0.05 , 0.94]
Lin 2008	4	217	14	217	4.3%	0.29 [0.10 , 0.85]
Rougé 2009	2	45			0.3%	2.18 [0.20 , 23.21]
Roy 2014	2	56			0.6%	1.00 [0.15 , 6.85]
Saengtawesin 2014	1	31			0.3%	0.94 [0.06 , 14.27]
Samanta 2009	5	91			4.5%	0.35 [0.13 , 0.92]
Strus 2018	2	80	1	73	0.3%	1.82 [0.17 , 19.71]
Van Niekerk 2014	0	91	4	93	1.4%	0.11 [0.01 , 2.08]
Subtotal (95% CI)		**1020**		**1021**	**18.6%**	**0.36 [0.23 , 0.59]**
Total events:	21		60			

0.36 (0.23-0.59)

Probiotics to prevent necrotizing enterocolitis in very preterm or very low birth weight infants (Review)

Risk of NEC

Saccharomyces spp.

Costalos 2003	5	51	6	36	2.2%	0.59 [0.19 , 1.78]
Demirel 2013	6	135	7	136	2.1%	0.86 [0.30 , 2.50]
Serce 2013	7	104	7	104	2.1%	1.00 [0.36 , 2.75]
Zeber-Lubecka 2016	0	27	0	28		Not estimable
Subtotal (95% CI)		**317**		**304**	**6.4%**	**0.82 [0.44 , 1.50]**
Total events:	18		20			

Heterogeneity: Chi² = 0.50, df = 2 (P = 0.78); I² = 0%

Test for overall effect: Z = 0.65 (P = 0.51)

0.82 (0.44-1.50)

Bacillus spp.

Sari 2011	6	110	10	111		3 , 1.61]
Tewari 2015	0	123	0	121		timable
Subtotal (95% CI)		**233**		**232**		**, 1.61]**
Total events:	6		10			

0.61 (0.23-1.61)

Bifidobacterium ssp. plus *Lactobacillus spp.* plus *Saccharomyces ssp.*

Chandrashekar 2018	0	70	3	70	1.1%	0.14 [0.01 , 2.72]
Dutta 2015	6	114	0	35	0.2%	4.07 [0.23 , 70.49]
Hariharan 2016	3	93	3	103	0.9%	1.11 [0.23 , 5.35]
Shashidhar 2017	2	49	6	49	1.8%	0.33 [0.07 , 1.57]
Subtotal (95% CI)		**326**		**257**	**4.0%**	**0.67 [0.28 , 1.58]**
Total events:	11		12			

0.67 (0.28-1.58)

Probiotic Suitable for Premature Infant

- Strain-specific effects
- Other possible effects

Probiotics for Preterm Infants: A Strain-Specific Systematic Review and Network Meta-analysis

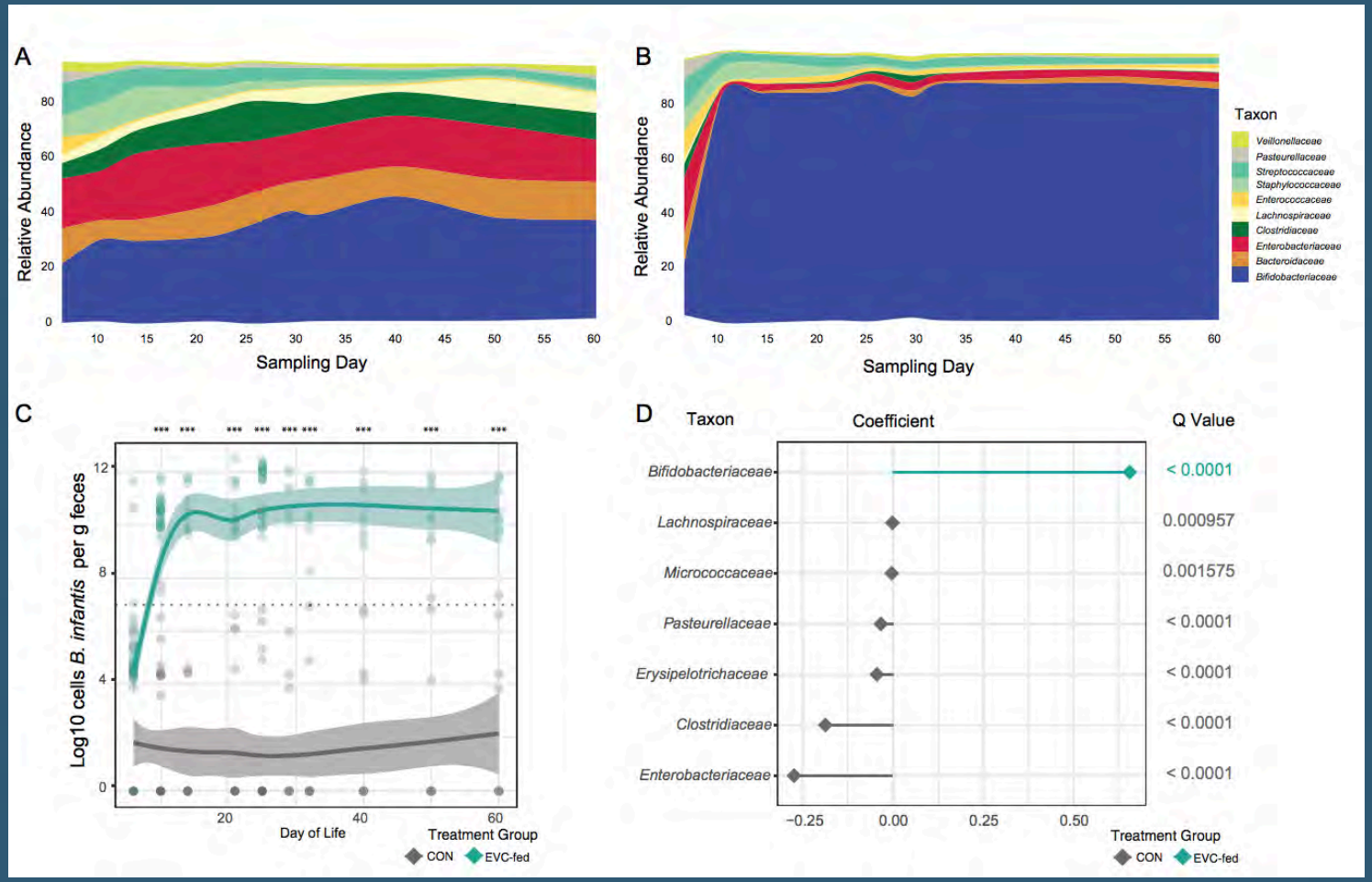
Time to full enteral feeding			
L Reuteri ATCC or DSM 17938	3	626	-3.3 (-6.4 to-0.62)
B bifidum B infantis B longun and L acidophilus	2	247	-4.7 (-8.6 to – 0.70)
B longum BB 536 *and L rhamosus GG*	1	94	-10 (-16 to – 3.6)

van den Akker CHP, et al. *J Pediatr Gastroenterol Nutr.* 2018;67(1):103-122. (table replicated)

Probiotic Suitable for Premature Infant

- Strain-specific effects
- Other possible effects
- Mechanisms of action

Persistence of Supplemented *Bifidobacterium longum* subsp. *infantis* EVC001 in Breastfed Infants



Colonization by *B. infantis* EVC001 modulates enteric inflammation in exclusively breastfed infants

Bethany M. Henrick[1,2], Stephanie Chew[1], Giorgio Casaburi[1], Heather K. Brown[1], Steven A. Frese[1,2], You Zhou[3], Mark A. Underwood[4,5] and Jennifer T. Smilowitz[4,6]

BACKGROUND: Infant gut dysbiosis, often associated with low abundance of bifidobacteria, is linked to impaired immune development and inflammation—a risk factor for increased incidence of several childhood diseases. We investigated the impact of *B. infantis* EVC001 colonization on enteric inflammation in a subset of exclusively breastfed term infants from a larger clinical study.
METHODS: Stool samples ($n = 120$) were collected from infants randomly selected to receive either 1.8×10^{10} CFU *B. infantis* EVC001 daily for 21 days (EVC001) or br
using 16S ribosomal RNA, proinflammato
time points: days 6 (Baseline), 40, and 6
RESULTS: Fecal calprotectin concentrati
proinflammatory cytokines correlated wi
abundance. Proinflammatory cytokines w
baseline and compared to control infant
CONCLUSION: Our findings indicate that gut dysbiosis (absence of *B. infantis*) is associated with increased intestinal inflammation. Early addition of EVC001 to diet represents a novel strategy to prevent enteric inflammation during a critical developmental phase.

Pediatric Research (2019) 86:749–757; https://doi.org/10.1038/s41390-019-0533-2

Bifidobacterium longum subspecies *infantis* EVC001 decreases inflammation and mortality in a murine NEC model

Shiloh R. Lueschow1, Steven A. Frese2,3, Bethany M. Henrick2,3, Steven J. McElroy1,4

Preterm infants fed *B. infantis* EVC001 Demonstrate Significant Changes to the Gut Microbiome Composition and Reduction of Intestinal Inflammation

M. Nguyen1, H. Holdbrooks1, P Mishra1, M Abrantes1, S Eskew1, P Roth1, J Garma1, C Oca1; C McGuckin2, C Hein2, S Chew2, R Mitchell2, S Kazi, G Casaburi2, S Frese2,3, and B Henrick2,3

Probiotic Suitable for Premature Infant

- Strain-specific effects
- Other possible effects
- Mechanisms of action
- Safety and regulations

A New Risk Factor for Neonatal Vancomycin-Resistant Enterococcus Colonization: Bacterial Probiotics

Demographic and clinical characteristics of infants.

	VRE (-) (*n*=116)	VRE (+) (*n*=94)	*p*	OR (95% CI)
Gestational age, weeks, mean ± SD	29 ± 2.3	29 ± 2.3	0.738	
Birth weight, g, mean ± SD	1188 ± 265	1190 ± 244	0.270	
Cesarean section, *n* (%)	107 (92)	80 (85)	0.623	
Male/Female	63/53	48/46	0.206	
Respiratory distress, *n* (%)	66 (57)	47 (50)	0.921	
Invasive mechanical ventilation, *n* (%)	67 (57.8)	53 (56.4)	0.889	
Duration, days, median (IQR)	2 (1–7)	3 (1–6)	0.747	
Noninvasive mechanical ventilation, *n* (%)	86 (74)	75 (80)	0.412	
Duration, days, median (IQR)	5.5 (3–14)	8 (4–13)	0.413	
Central venous lines, *n* (%)	55 (47.4)	51 (54.3)	0.335	
PN duration, days, median (IQR)	9 (6–14)	9 (6–14)	0.548	
Antimicrobial treatment, *n* (%)	76 (66)	67 (71.2)	0.655	
Antimicrobial agents, *n* (%)				
Ampicillin ± Gentamycin	30 (26)	14 (15)	0.060	
Vancomycin ± Meropenem	42 (36)	51 (54.3)	0.012	21 (1.2–3.6)
Cefepime	4 (3.4)	2 (2.1)	0.693	
Probiotic, *n* (%)	30 (26)	75 (80)	<0.001	11.3 (6–21.7)
Probiotic + Vancomycin, *n* (%)	13 (11.2)	38 (40.4)	<0.001	5.4 (2.6–11)
Duration of hospitalization, median (IQR)	35.5 (24–54)	37.5 (26–47)	0.894	

CI, confidence interval; IQR, interquartile range; OR, odd's ratio; PN, parenteral nutrition; VRE, vancomycin-resistant enterococcus.

Topcuoglu S, et al. *J Matern Fetal Neonatal Med.* 2015;28(12):1491-1494. (table replicated)

Validating Bifidobacterial Species and Subspecies Identity in Commercial Probiotic Products

	B longum subsp. infantis							*B longum subsp. longum*							*B breve*							*B animalis*							*B bifidum*						
Sample	Label	1st pill	1st lot	2nd pill	1st lot	1st pill	2nd lot	2nd pill	2nd lot	Label	1st pill	1st lot	2nd pill	1st lot	1st pill	2nd lot	2nd pill	2nd lot	Label	1st pill	1st lot	2nd pill	1st lot	1st pill	2nd lot	2nd pill	2nd lot	Label	1st pill	1st lot	2nd pill	1st lot	1st pill	2nd lot	2nd pill	2nd lot
1		–									–								X									X	X	X	X	X	X	X	X
2	X	X		X						X	X		X						X									X	X	X	X	X	X	X	X
3	X									X	X		X		X		X	X	X										X	X	X	X	X	X	X
4		–				–		–		X	X		X		X		X	X		X							–	X	X	X	X	X	X	X	X
5																			X									X	X	X	X	X	X	X	X
6	X									X									X										X	X	X	X	X	X	X
7		–						–		X	X		X						X	–		X						X	X	X	X	X	X	X	–
8	X	X		X		X		X			X				–		–	–	X	X		X		X					X	X	X	X	X	X	X
9	X	–				X		X		X	X		X		X		X	X	X	X		X		X		X		X							
10	X	–								X	X		X						X	X		X		X		X										
11	X	X		X							X									X		X		X		X		X								
12	X	X		X		X		X			–				X		X																			
13	X	X		X		N/A		N/A		X	X		X		N/A		N/A		X	–		X		N/A		N/A								N/A		N/A
14a	X									X																										
14b		X									X								X									X								
15	X	–				–		–			X		X		X		X																			
16	X	X				N/A		N/A		X	X				N/A		N/A		X	X				N/A		N/A		X	X	X		N/A		N/A	

- 16 different probiotics containing bifidobacteria
- Pill-to-pill variability
- Unlisted species
- Only 1 tested matched the species claims on the label

Lewis ZT, et al. *Pediatr Res.* 2016;79, 445-451. (table replicated)

REGULATIONS

- Dietary supplement
  - Center for Food Safety and Applied Nutrition
  - GRAS (Generally Recognized As Safe)

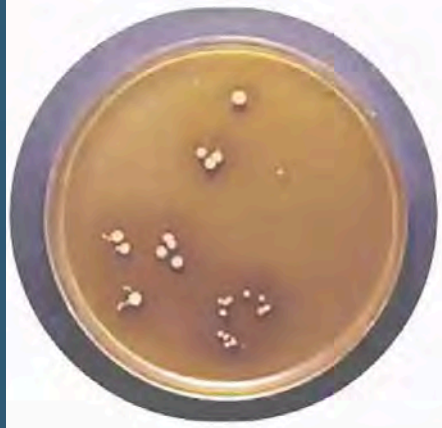
  Most of the products currently available in the United States are categorized as dietary supplements and are not labeled with the number of CFUs for the probiotic strain

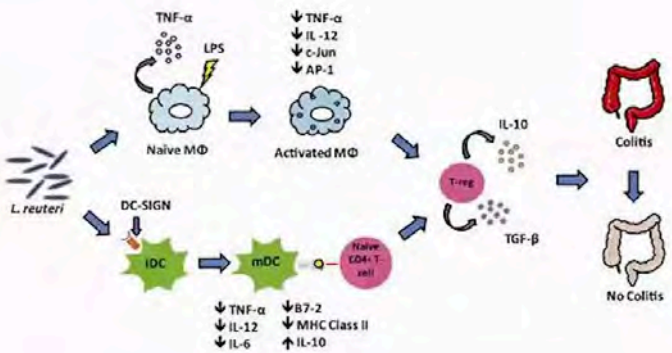
REGULATIONS (continued)

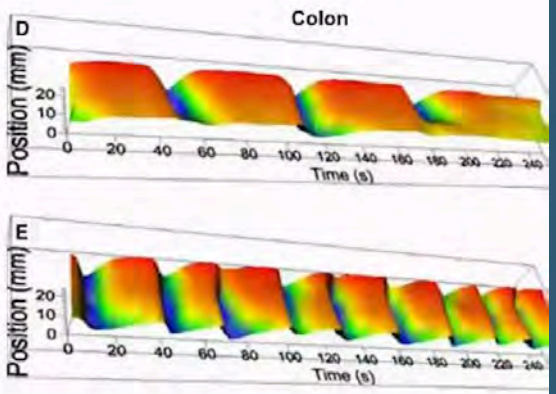
- 2002 International Scientific Association for Probiotics and Prebiotics
  - Defined strain designation
  - Proof of efficacy and effectiveness
  - Safety
- **Live Biotherapeutic (FDA)**
  - A probiotic used to diagnose, cure, treat or prevent diseases is a drug and a biological product
  - The Center for Biologics Evaluation and Research regulates biological products when used for clinical indications
  - IND (US, 21CFR 312)

The Connection Study

Lactobacillus reuteri in IBP-9414







Combats dysbiosis

Reduces gut inflammation

Improves gut motility

A randomized, double blind, parallel-group, placebo controlled study to evaluate the efficacy and safety of IBP-9414 in premature infants 500-1500g birth weight in the prevention of necrotizing enterocolitis – The Connection Study

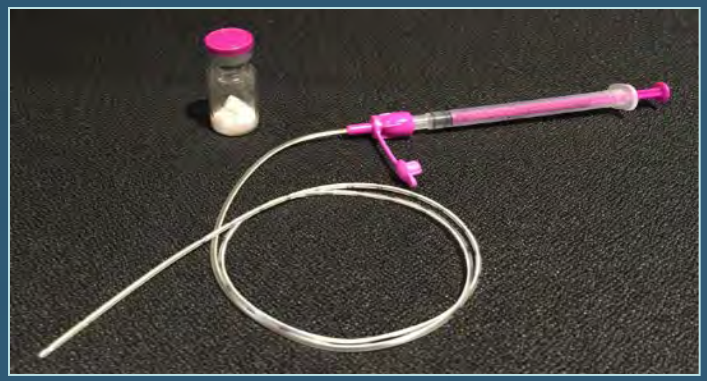
Development of IBP-9414 as a live bacterial therapy for the prevention of NEC.

Under drug manufacture and regulations

IBP-9414 has been approved by the FDA for orphan drug designation for the prevention of NEC.

IBP-9414

- Freeze-dried powder for oral suspension
- Oral-enteral feeding
- Manufacturing process developed to allow opening of IND



A randomized, double blind, parallel-group, dose escalation placebo-controlled multicenter study to investigate the safety and tolerability of IBP-9414 administered in preterm infants

Primary Outcome

	Cohort A: Low dose (n=16)	Cohort A: Placebo (n=13)	Cohort B: High dose (n=16)	Cohort B: Placebo (n=14)	Cohort C: Low dose (n=14)	Cohort C: Placebo (n=16)	Cohort D: High dose (n=15)	Cohort D: Placebo (n=15)
Number of infants with Adverse Events (AEs)	9	11	10	6	13	12	14	14
Total number of AEs	29	30	51	24	51	48	64	58
Number infants with Serious Adverse Events (SAEs)	3	2	2	1	3	2	2	2
Total number of SAEs	6	3	2	3	5	4	2	4
Related AEs	0	0	1	0	3	2	0	2
Related SAEs	0	0	0	0	0	0	0	1
Number infants where AE led to Study Drug withdrawal	0	0	0	0	0	1	0	1
Death	0	0	0	0	0	0	0	0

A randomized, double blind, parallel-group, dose escalation placebo-controlled multicenter study to investigate the safety and tolerability of IBP-9414 administered in preterm infants

Fecal Analysis – Real Time qPCR Analysis

	Cohort A: Low dose	Cohort A: Placebo	Cohort B: High dose	Cohort B: Placebo	Cohort C: Low dose	Cohort C: Placebo	Cohort D: High dose	Cohort D: Placebo
Last day of study treatment	61623* (111110) (n=11)	6 (12) (n=10)	25764* (173111) (n=12)	3 (112) (n=10)	1423^{NS} (10269) (n=5)	7 (874) (n=10)	58251* (311599) (n=8)	40 (75) (n=12)
30 days after last dose	160 (760) (n=7)	297 (371) (n=5)	184 (6437) (n=3)	473 (513) (n=5)	40 (61) (n=9)	59 (184) (n=8)	40 (87) (n=12)	18 (35) (n=12)

*Median (Interquartile range) for bacterial counts per qPCR reaction. * P<0.001 vs placebo and ^{NS} not significant vs placebo.*

- Treatment with IBP-9414 leads to presence of bacterium in the feces on day of last dose: all IBP-treated, 31491 (121875) vs all placebo, 10 (91); P<0.001, Rank sum Wilcoxon
- Cross-contamination did not occur in placebo treated infants
- Smaller infants needed the higher dose to display IBP-9414 in the feces
- 30 days after last dose, the bacteria have been washed out: all IBP-treated, 63 (184) vs all placebo, 42 (290); NS, Rank sum Wilcoxon

Probiotics and Preterm Infants: A Position Paper by the European Society of Paediatric Gastroenterology Hepatology and Nutrition Committee on Nutrition and the European Society for Paediatric Gastroenterology Hepatology and Nutrition Working Group for Probiotics and Prebiotics [1]

- Only products manufactured according to current good manufacturing practices should be used.
- Local laboratories should have the ability to detect probiotic bacteremia.
- The potential risks and benefits are provided to parents of preterm infants.

Use of Probiotics in Preterm Infants [1]

- A pharmaceutical-grade probiotic product is not currently available in the United States.
- Long-term safety remains unknown.
- Current evidence does not support the routine, universal administration of probiotics to preterm infants, particularly those with a birth weight of <1000 g.
- Clinicians must be aware of the lack of regulatory standards for commercially available probiotic preparations manufactured as dietary supplements and the potential for contamination with pathogenic species.

Poindexter B, et al. *Pediatrics.* 2021;147(6):e2021051485.

Probiotics in Perinatology

- Defining the strains or combination of strains that have clinical benefits
- Research to investigate mechanisms of action
- Explore strategies on how and when to deliver probiotic to premature infant