From Probiotics to biotherapeutics in the NICU

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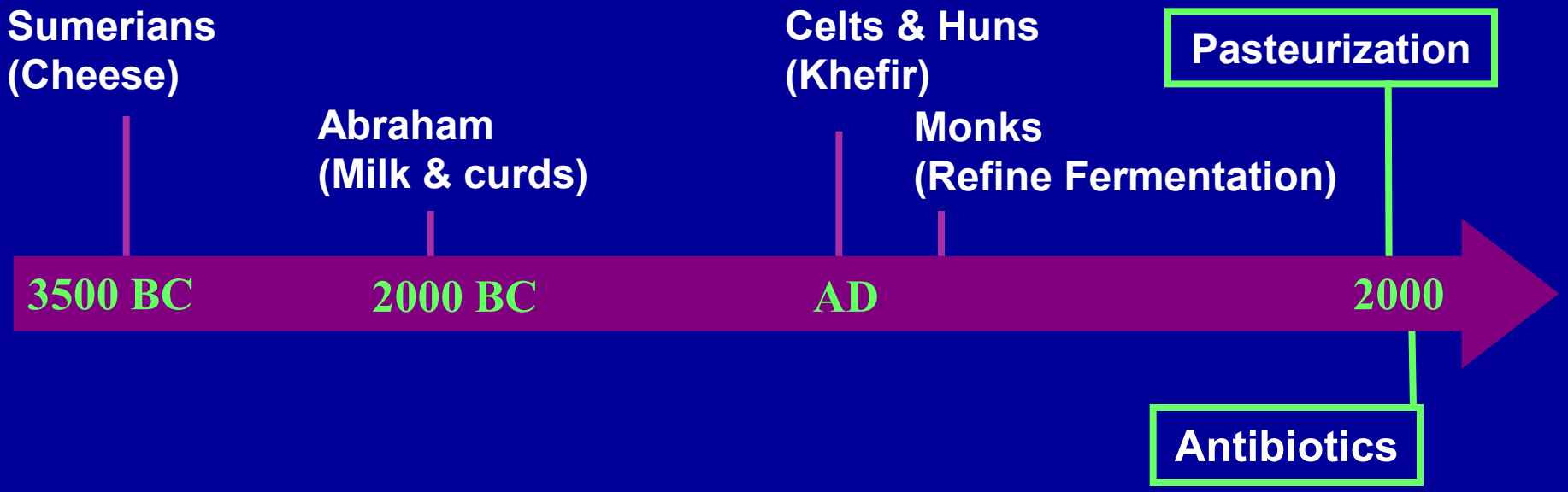
Disclosure

Advisory board

-Mead Johnson

-IBT

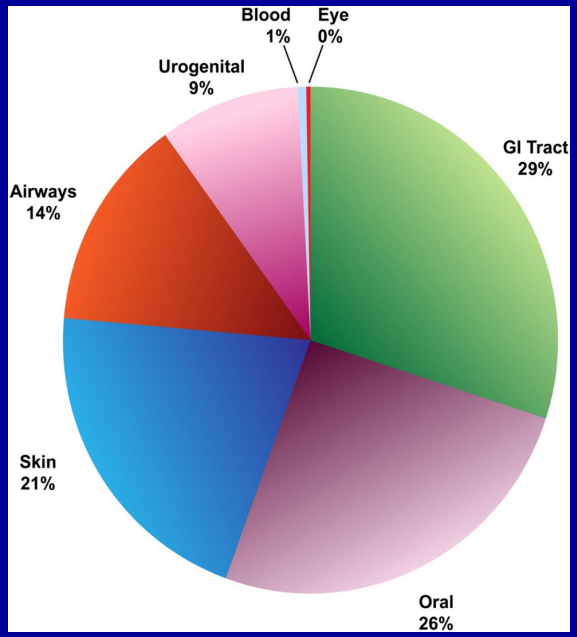
Ingestion of Bacteria



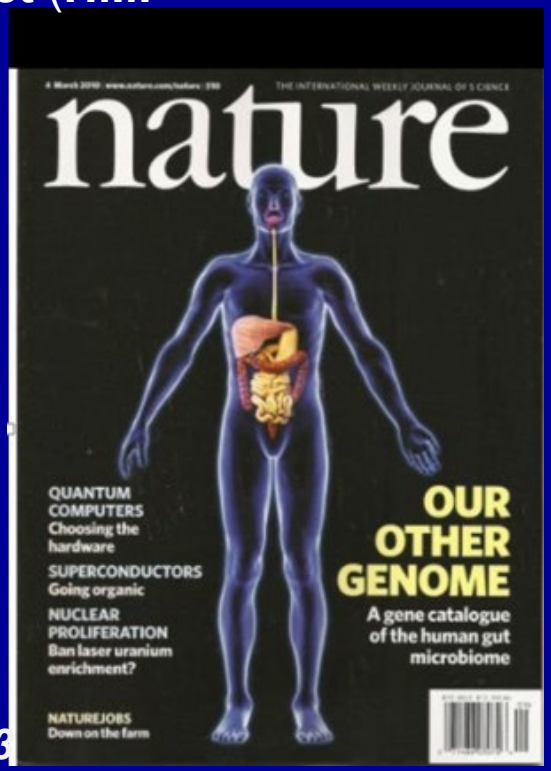
In the last 100 years, we drastically changed our ingestion of microbes and our microbial environment.

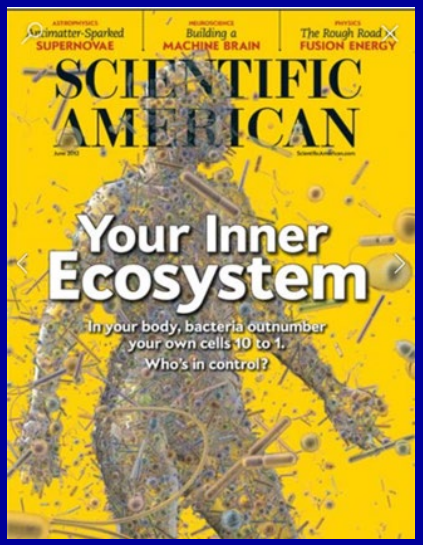
Probiotics: 100 years after Elie Metchnikoff's observation

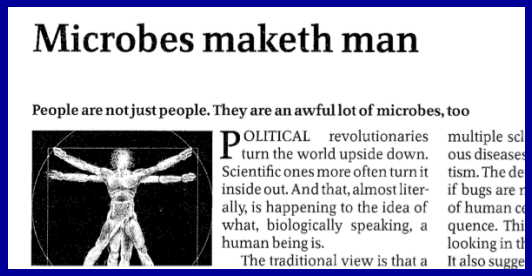
Human Microbiome Project (HMP



Genome Res. 2009;19:2317-2323






Microbes maketh man

People are not just people. They are an awful lot of microbes, too

POLITICAL revolutionaries turn the world upside down. Scientific ones more often turn it inside out. And that, almost literally, is happening to the idea of what, biologically speaking, a human being is.

The traditional view is that a

multiple scl
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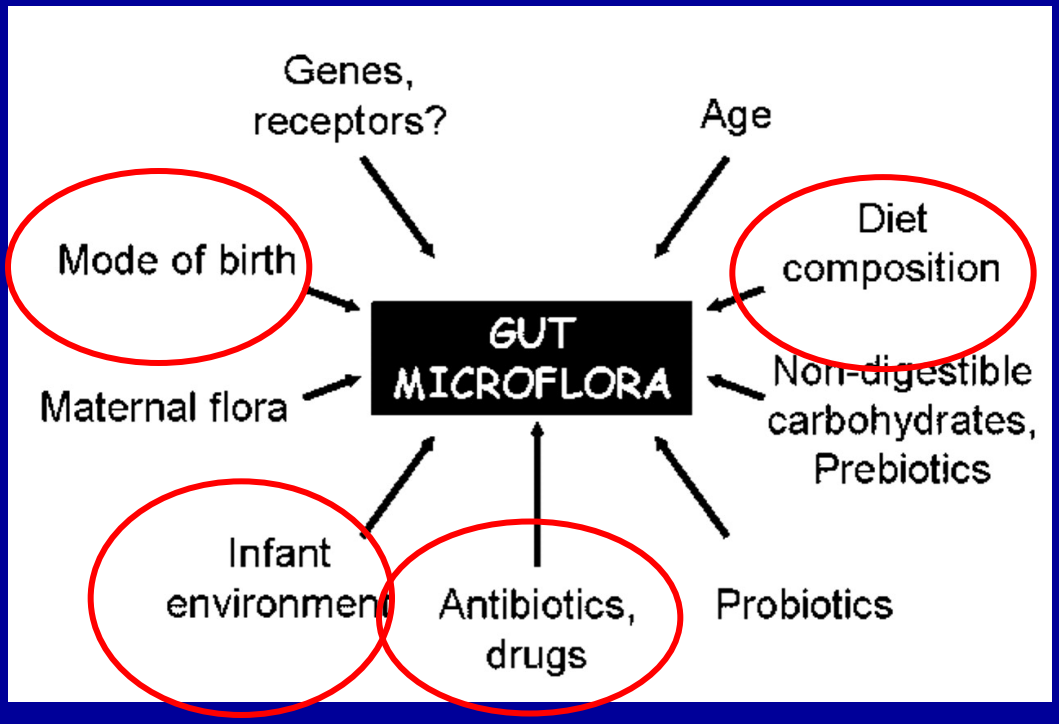
Causes of dysbiosis in premature infants

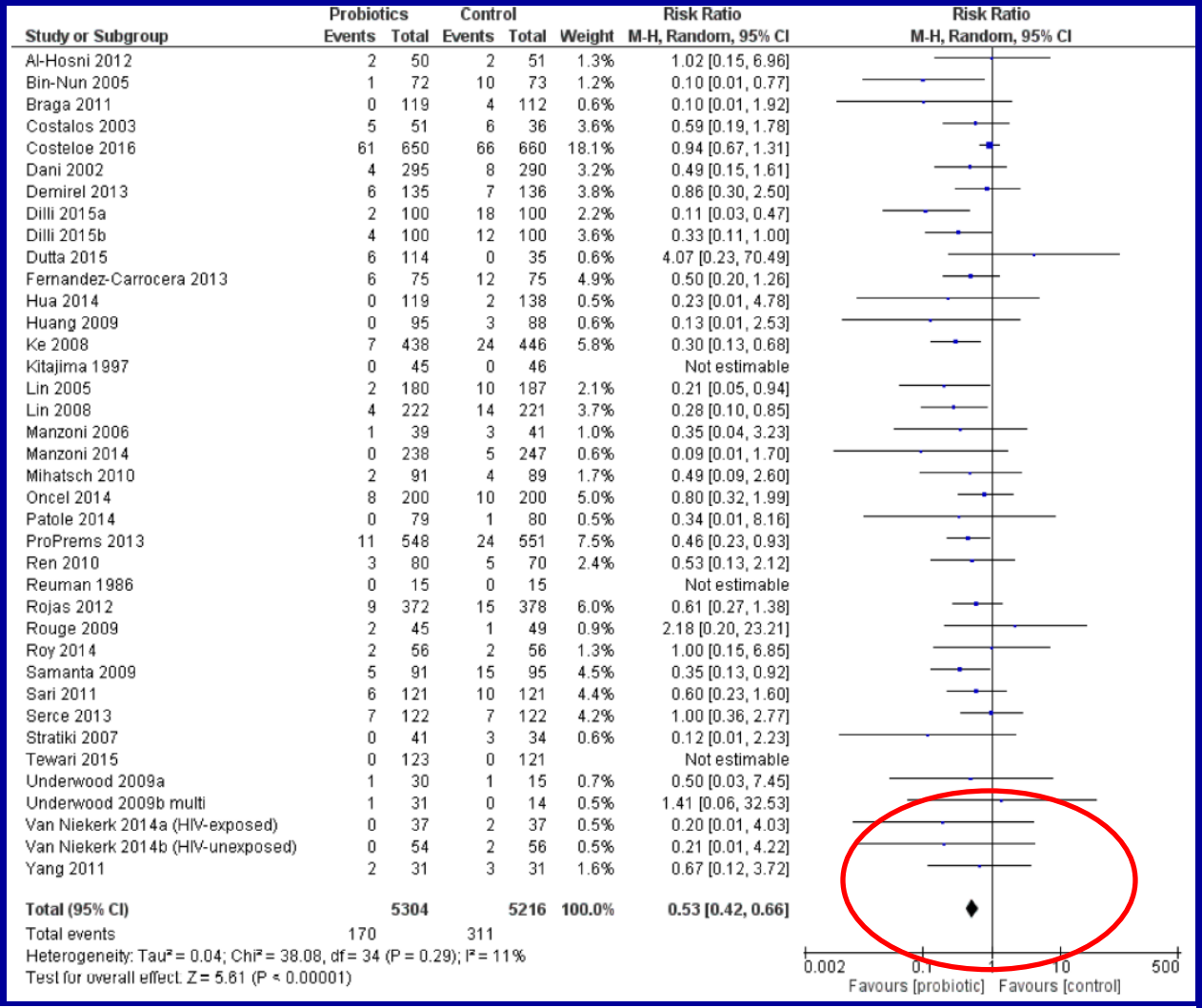


Dysbiosis is prevalent in premature infants

Premature infants is one of the populations that can benefit the most from restauration of microbiota

What is the Evidence?

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 = 5.61 (P < 0.00001)

Risk Ratio M-H, Random, 95% CI

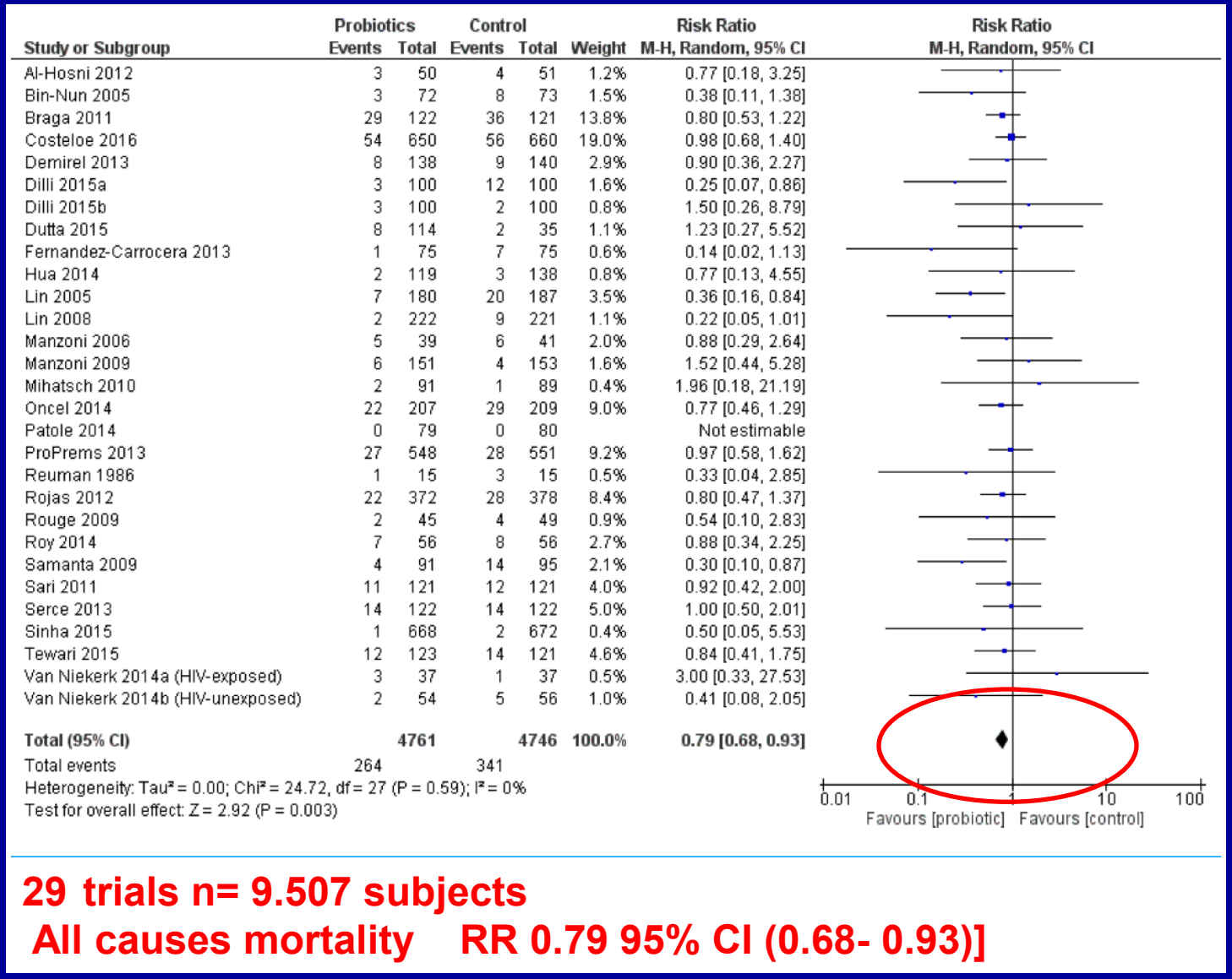
0.002 0.1 1 10 500
Favours [probiotic] Favours [control]

38 trials n = 10,520 subjects

Severe NEC in all infants. RR 0.53 95% CI (0.42-0.66)

Sawh et al. (2016), PeerJ, DOI

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	3	50	4	51	1.2%	0.77 [0.18, 3.25]
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			

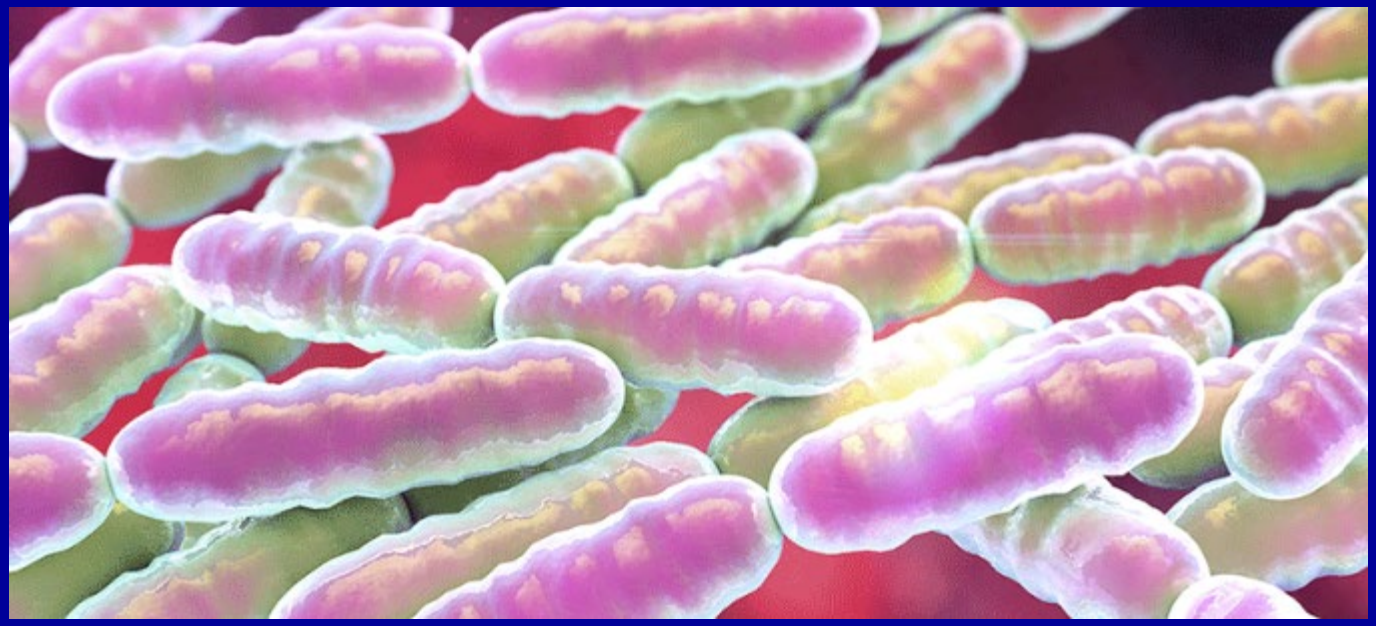
Heterogeneity: Tau² = 0.00; Chi² = 24.72, df = 27 (P = 0.59); I² = 0%
Test for overall effect: Z = 2.92 (P = 0.003)

29 trials n= 9.507 subjects

All causes mortality RR 0.79 95% CI (0.68- 0.93)]

Sawh et al. (2016), PeerJ, DOI 10.7717/peerj.2429

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



Millar M, Wilks M, Fleming P, et al. Arch Dis Child Fetal Neonatal Ed (Sep 2010).

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* *Bifidobacterium* *Streptococcus thermophilus*	7%
Risaquad	*L acidophilus*	6%
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

Viswanatham et al Journal of Perinatology 2016

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

Saccharomyces boulardii

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

L. rhamnosus GG (Dicoflor®)

S. boulardii (Reflor®)

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®)

lobacterium adolescentis

fantis—0.35 billion

fidus—0.35 billion

ermophilus—0.35 billion
C Dophilus®)

rococcus faecalis—1 billion

ngum—1 billion

idophilus—1 billion
co®)

reve YIT4010
ult® Honsva Co. Ltd.,

B. bre

L. aci
mg ca

B. inf
cap (I
Farm

L. aci
mg ca

B. bif
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. aci
Labo
WI)

L. reu
(Biog

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

tis—1 billion/g
vel®)

antis—2.5 billion
idum—2.5 billion
gum—2.5 billion
dophilus—2.5 billion

us coagulans
obacillus sporogenes)
G ITALIA SRL®, Rome,

S. boulardii (Reflor®)

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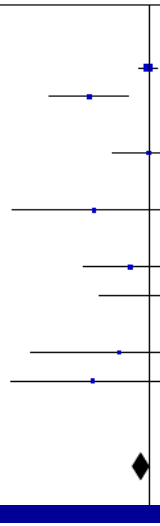
Probiotics to prevent necrotizing enterocolitis in very preterm or very low birth weight infants (Review)

Bifidobacterium spp.

	Probiotics		Control			Risk Ratio	Risk Ratio
	nts	Total	nts	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	18		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 estimable	
Wang 2007	0	22	0	22		Not estimable	
Subtotal (95% CI)		**1559**		**1429**	**31.2%**	**0.72 [0.54 , 0.96]**	
Total events:	75		99				

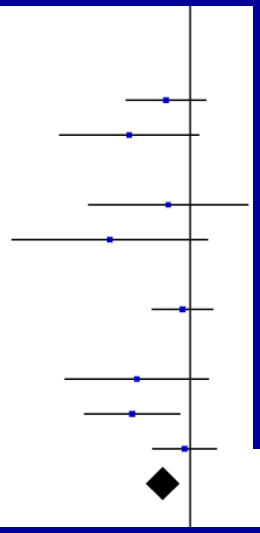


0.72 (0.54-0.96)

Risk of NEC

Lactobacillus spp.

Chrzanowska-Liszewska 2012	0	21	0	26		Not estimable
Dani 2002	4	295	8	290	2.5%	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			

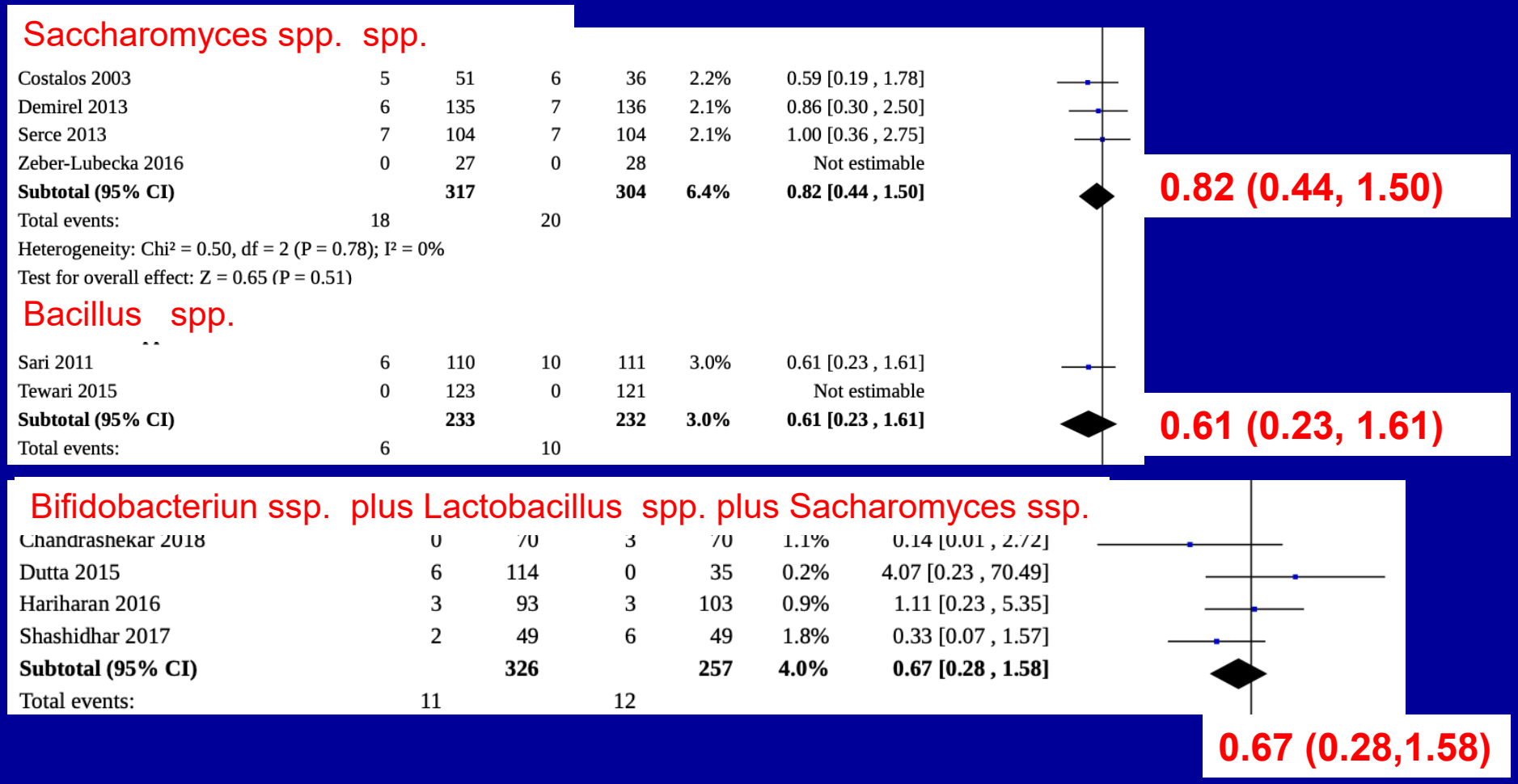


0.45 (0.28-0.71)

Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W 2020

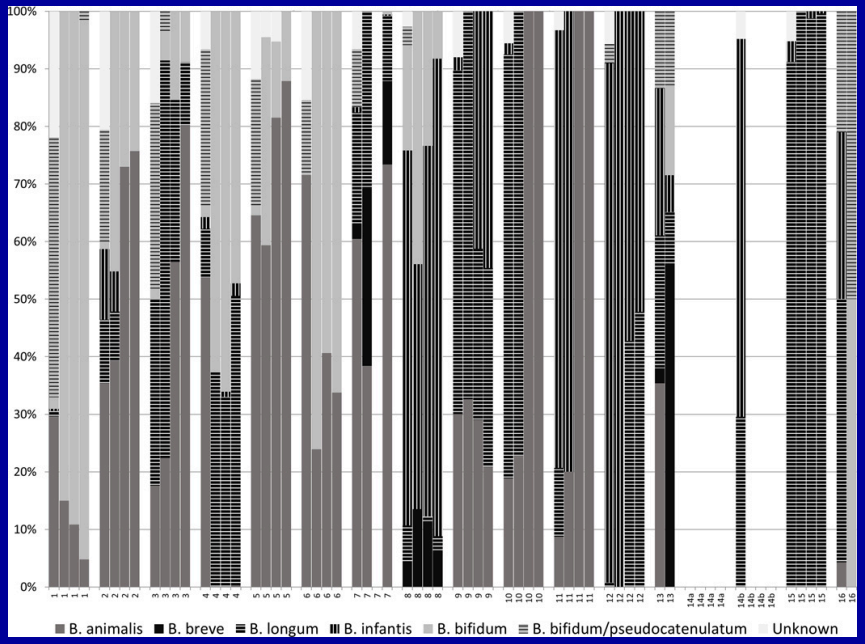
Probiotics to prevent necrotizing enterocolitis in very preterm or very low birth weight infants (Review)

Risk of NEC



Sharif S, Meader N, Oddie SJ, Rojas-Reyes MX, McGuire W 2020

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	2nd pill	2nd lot	Label	1st pill	1st lot	2nd pill	1st lot	2nd pill	2nd lot	Label	1st pill	1st lot	2nd pill	1st lot	2nd lot	Label	1st pill	1st lot	2nd pill	1st lot	2nd lot	Label	1st pill	1st lot	2nd pill	1st lot	2nd lot
1		~							~						X						X	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	X
3	X							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
5																					X	X	X	X	X		X	X	X	X	X
6	X							X							X							X	X	X	X			X	X	X	X
7		~					~	X	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						
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			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			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 16
Unlisted species
Only one tested matched the species claimed on the label

Lewis et al: Ped Res 79, 445-451. 2016

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Factors to consider when establishing the efficacy of probiotics administration include:

- accurate identification and labelling of strain used
- the viability of the organism
- consistency of product formulation over the course of the study

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

Only products manufactured according to current good manufacturing practices should be used

van der Akker et al 2020

Use of Probiotics in Preterm Infants

Brenda Poindexter, MD, MS, FAAP, COMMITTEE ON FETUS AND NEWBORN

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.**

Poindexter B et al, Pediatrics AAP 2021

REGULATIONS

Dietary supplement

- Center for Food Safety and Applied Nutrition
- GRAS (Generally Recognized As Save)

Live Biotherapeutic (FDA)

- **A probiotic used to diagnosis, 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)

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

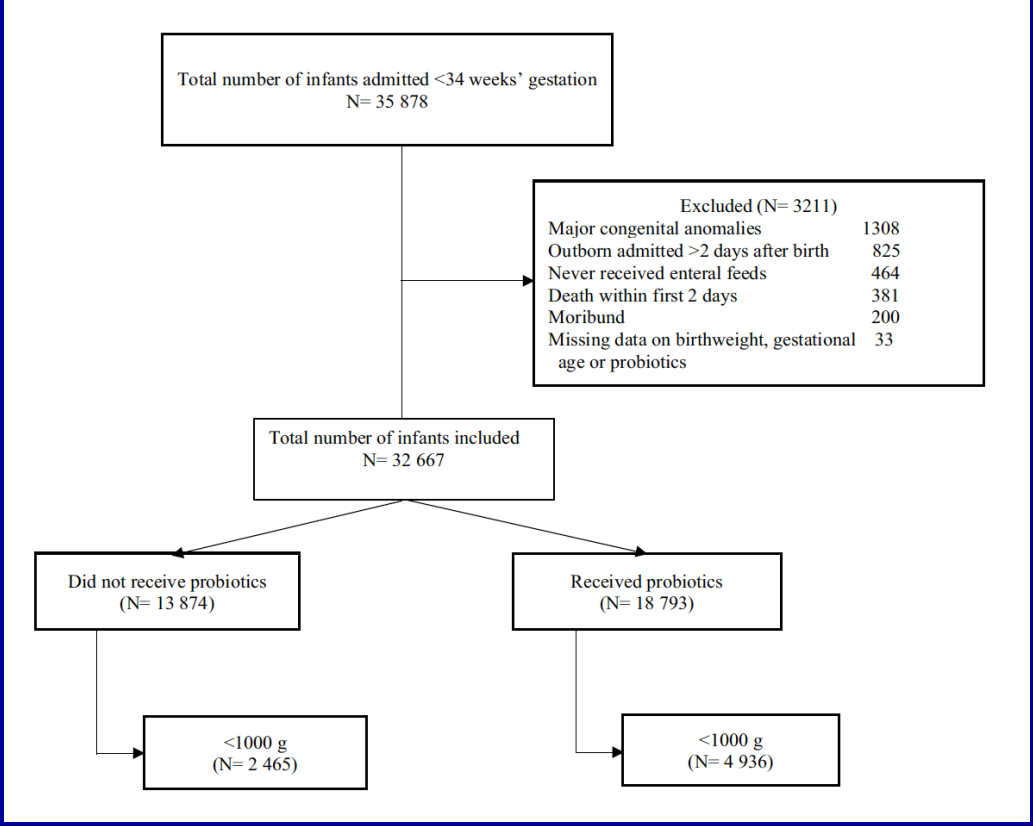
Dietary supplements (Probiotics) vs Biotherapeutic

Supplements: Considered safe until proven unsafe vs
Prescription Medicines: Considered unsafe until proven safe and effective

	Suplements	Biotherapeutic
FDA Classification	Dietary Supplements to Improve Health	Live Biotherapeutic Product
Proof of Safety	Not Required	Required
Proof of Effectiveness	Not Required	Required
Post-Marketing Surveillance	Not Required	Required
Good Manufacturing Practices (GMPs)	Food GMPs	Pharmaceutical GMPs
Reimbursable by Insurance	No	Yes
Disease Treatment Claims	Not Allowed	Allowed

Effectiveness and Risks of Probiotics in Preterm Infants

Alshaikh et al for the Canadian Neonatal Network .Pediatrics Feb 2025



- 4 Bifidobacterium strains (*Bifidobacterium breve, Bifidobacterium bifidum, Bifidobacterium longum sub sp, and Bifidobacterium neonateis*) and *Lactobacillus rhamnosus.*
- Single-strain probiotic *Lactobacillus reuteri.*

Effectiveness and Risks of Probiotics in Preterm Infants

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TABLE 2. Neonatal Outcomes by Probiotic Exposure

Outcomes	<34 Weeks' GA			<1000-g Birth Weight		
	No Probiotics (N = 13 874)	Probiotics (N = 18 793)	*P* Value[a]	No Probiotics (N = 2465)	Probiotics (N = 4936)	*P* Value[a]
Primary, % (n/N)						
NEC (medical or surgical)	2.63 (364/13 862)	3.63 (683/18 792)	<.001	8.78 (216/2460)	9.91 (489/4935)	.12
All-cause death before discharge	3.55 (492/13 874)	3.74 (702/18 793)	.37	15.17 (374/2465)	11.93 (589/4936)	<.001
Late-onset sepsis	6.77 (940/13 874)	9.27 (1742/18 793)	<.001	25.56 (630/2465)	25.75 (1271/4936)	.86

NEC

< 34 weeks 2.6% vs 3.6%, p <0.001

<1000 g, no difference

Mortality

< 34 weeks, no difference

<1000 g 15.7% vs 11.9 %, p <0.001

Late onset sepsis

<34 weeks 6.7% vs 9.2%, p<0.001

< 1000 g, no difference

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Effectiveness and Risks of Probiotics in Preterm Infants

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TABLE 4. Characteristics of Infants With Probiotic-Associated Sepsis

Characteristics	<34 Weeks' GA[a]			<1000-g Birth Weight[a]		
	No Probiotic Sepsis (n = 18 760)	Probiotic Sepsis (n = 27)	*P* Value	No Probiotic Sepsis (n = 4912)	Probiotic Sepsis (n = 20)	*P* Value
GA, median (IQR), wk	29 (27–31)	25 (24–27)	<.001	26 (24–27)	25 (23.5–26)	.02
GA 22–28 wk, % (n/N)	38 (7145/18 760)	85 (23/27)	<.001	89 (4350/4912)	100 (20/20)	.16
GA 29–33 wk, % (n/N)	62 (11 615/18 760)	15 (4/27)		11 (562/4912)	0 (0/20)	
Birth weight, median (IQR), g	1307 (980–1630)	680 (620–1000)	<.001	790 (670–895)	632 (605–778)	.001
Antenatal steroids, % (n/N)	91 (16 828/18 594)	89 (24/27)	.74	92 (4474/4880)	90 (18/20)	.69
Cesarean birth, % (n/N)	64 (12 029/18 750)	59 (16/27)	.60	67 (3315/4912)	60 (12/20)	.48
Male sex, % (n/N)	55 (10 220/18 749)	67 (18/27)	.20	51 (2487/4907)	70 (14/20)	.08
Small for GA, % (n/N)	12 (2201/18 749)	22 (6/27)	.12	23 (1107/4907)	30 (6/20)	.42
SNAP-II >20, % (n/N)	12 (2267/18 703)	37 (10/27)	<.001	30 (1446/4899)	50 (10/20)	.045
Age of first feed, median (IQR), d	2 (1–2)	2 (1–2)	.23	2 (1–2)	2 (1–2.5)	.62

Abbreviations: GA, gestational age; SNAP-II, Score for Neonatal Acute Physiology version II.

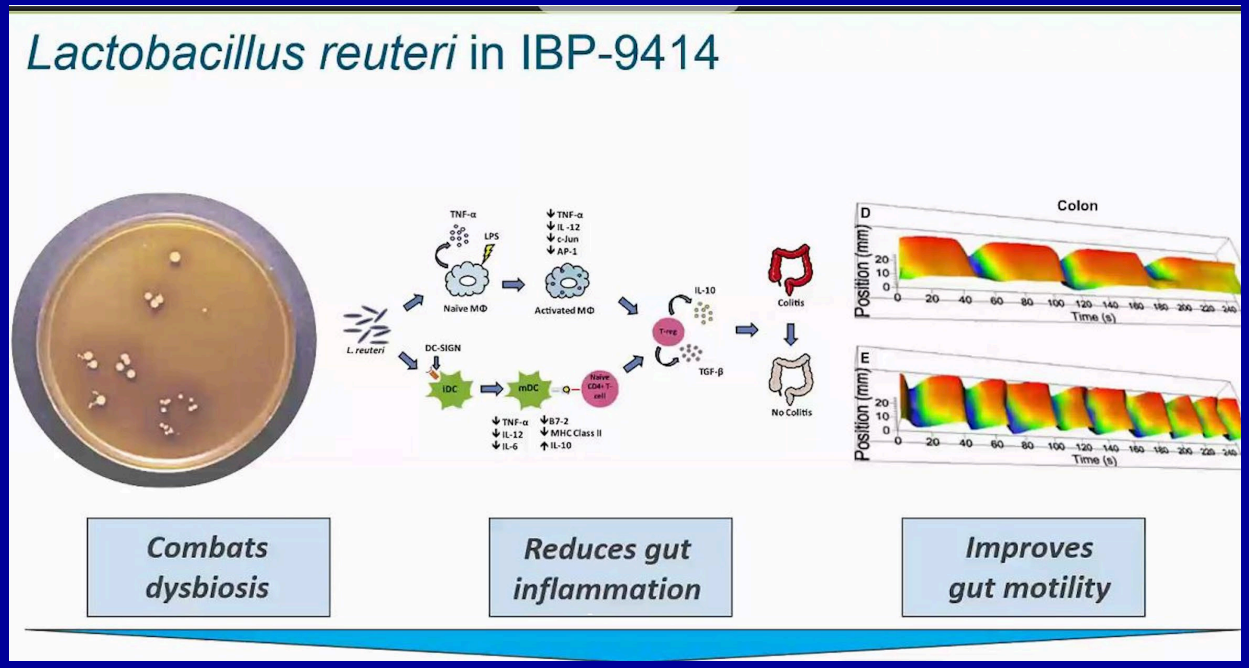
[a] Analysis is restricted to infants who received probiotics.

< 34 weeks: 27 infants had probiotic sepsis / 11 (41%) NEC or spontaneous intestinal perforation 7 days before or after probiotic sepsis

< 1000 g: 24 infants had probiotic sepsis / 9 (45%) had NEC or intestinal perforation 7 days before and after probiotic sepsis. 3 died within 7 days of probiotic sepsis

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

Multicenter study in USA and Europe FDA and EMA regulated



Drug Product IBP-9414

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 to 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

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Neu et al: Presented at Hot Topics 2017

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

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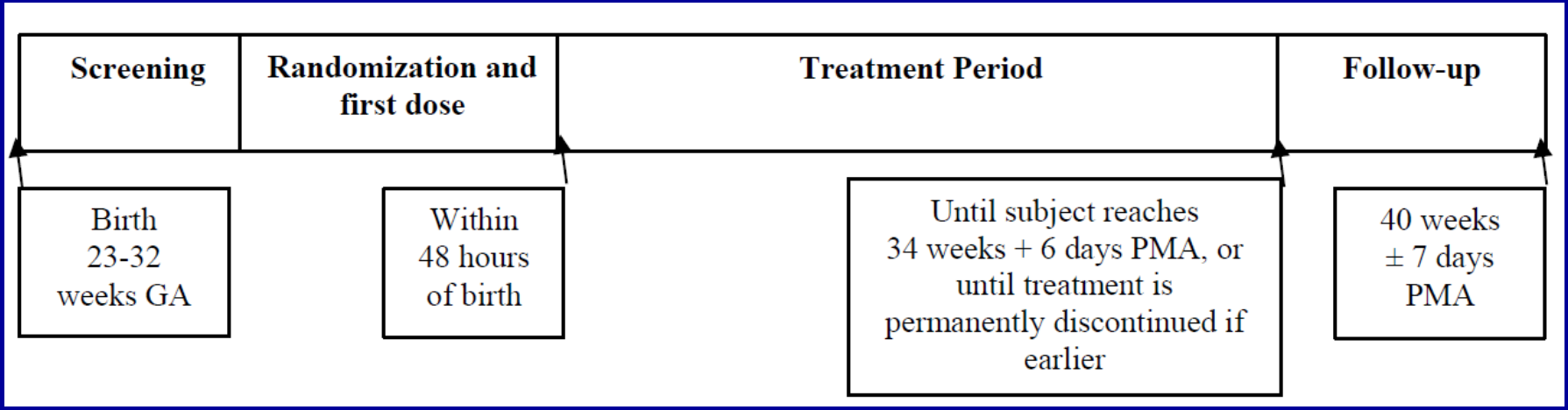
Neu et al: Presented at Hot Topics 2017

Primary efficacy endpoints

- Prevention of NEC
- Time to sustained feeding tolerance

Secondary endpoints,

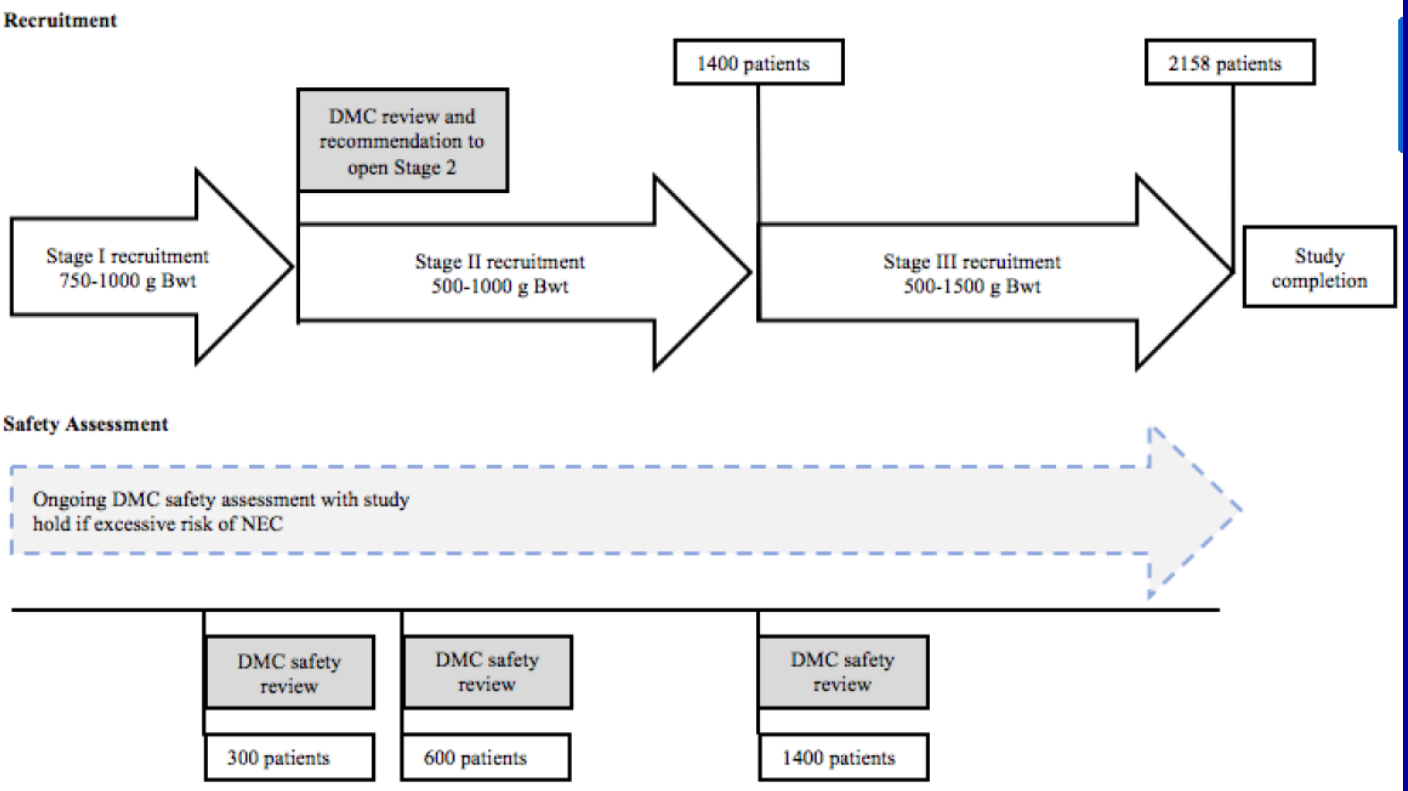
- NEC confirmed at laparotomy or autopsy,
- all-cause mortality,
- total weight gain,
- duration of hospitalization



J.Neu, et al ,submitted for publication

- Birth weight of 500-1500g
- Gestational age 23 weeks+0 days - 32 weeks+0 days at birth,
- ≤48 hours of age

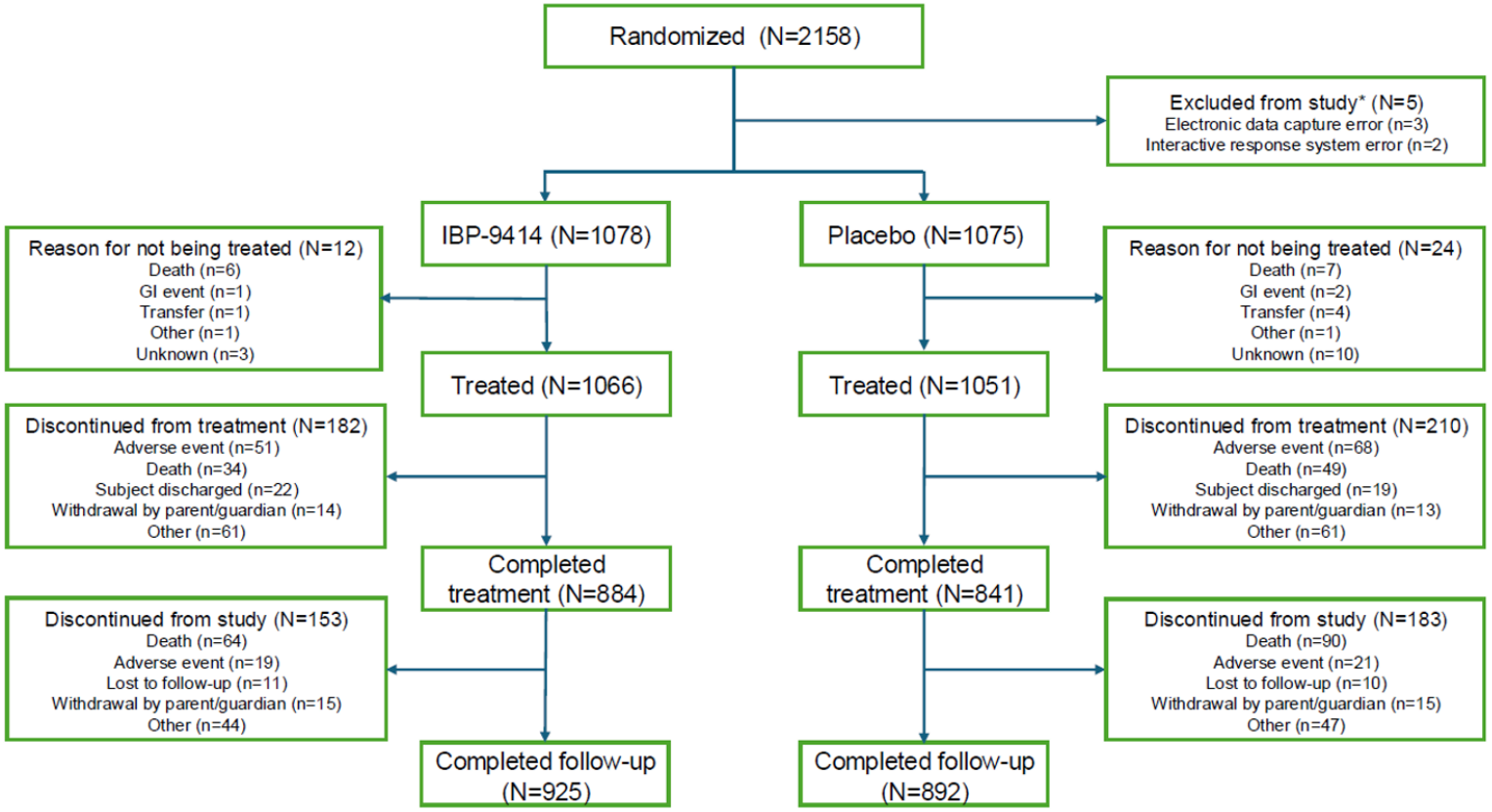
Figure 4.1. Design of Phase 3 Efficacy and Safety Trial



J.Neu, et al ,submitted for publication

Effects of IBP-9414, a Live Biotherapeutic Product on necrotizing enterocolitis, time to full enteral feeding, and mortality in very low birth weight infants: Results from the "Connection Study

CONSORT diagram. Enrollment was conducted from July 2019-April 2024, with final follow-up in July 2024.



J. Neu, et al ,submitted for publication

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Effects of IBP-9414, a Live Biotherapeutic Product on necrotizing enterocolitis, time to full enteral feeding, and mortality in very low birth weight infants: Results from the "Connection Study".

Necrotizing enterocolitis	N	IBT Events (%)	N	Placebo Events (%)	p value
Total	1066	93 (8.7%)	1051	107 (10.2%)	0.24
Post 14 days	1032	59 (5.7%)	1023	79 (7.7%)	0.067
Surgical NEC					
Total	1066	8 (0.8%)	1051	15 (1.4%)	0.128
Post 14 días	1061	3 (0.3%)	1046	10 (1.0%)	0.045
NEC Surgical / Death					
Total	1066	15 (1.4)	1051	23 (2.3%)	0.124
Post 14 días	1057	6 (0.6)	1044	17 (1.6%)	0.018

IBP-9414 decreased the risk of severe NEC (surgical or post morthen path) after 14 days

J.Neu, T del Moral, S.Guthrie, M Hudak, , F.Indrio, J.Kim, A.Kronström, C.Martin, N.Modi, J.Rastad, R. Singh, S.Strömberg, H.Szajewska, M. Thuresson, M.Caplan

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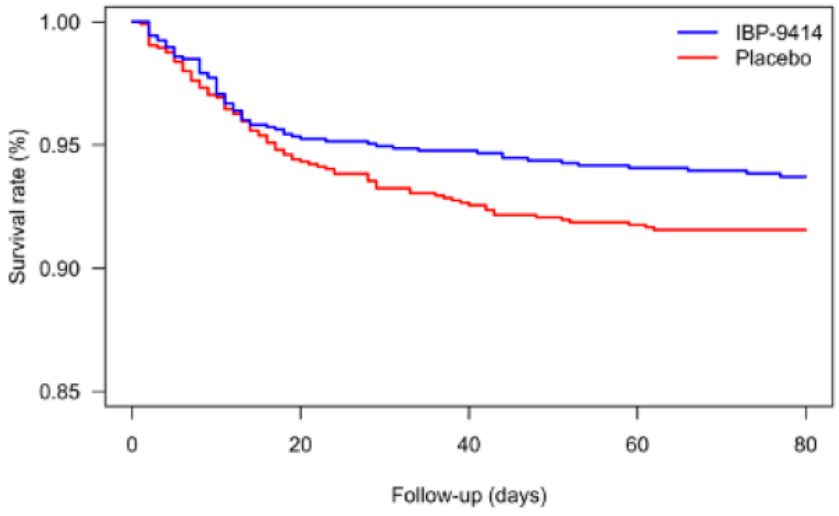

Fig.1. Kaplan-Meier survival curves for IBP-9414 and placebo treated infants.

	IBP- 9414	Placebo	RR (95% CI)	P
Mortality	66 (6.2%)	89 (8.5%)	0.73 (0.54 - 0.98)	0.036
Mortality after 14 days	22 (2.2%)	42 (4.2%)	0.52 (0.31 - 0.85)	0.007

27% MORTALITY DECREASE

J.Neu, T del Moral, S.Guthrie, M Hudak, , F.Indrio, J.Kim, A.Kronström, C.Martin, N.Modi, J.Rastad, R. Singh, S.Strömberg, H.Szajewska, M. Thuresson, M.Caplan

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Serious adverse event, n (%)	IBP-9414 (N=1084)	Placebo (N=1033)
Infants with ≥1 serious adverse event	244 (22.5)	270 (26.1)
Infections and infestations		
Sepsis	42 (3.9)	54 (5.2)
Pneumonia	6 (0.6)	13 (1.3)
Respiratory, thoracic, and mediastinal disorders		
Bronchopulmonary dysplasia	36 (3.3)	31 (3.0)
Respiratory failure	23 (2.1)	30 (2.9)
Pulmonary hemorrhage	13 (1.2)	16 (1.5)
Apnea	4 (0.4)	12 (1.2)
Gastrointestinal disorders		
Necrotizing (entero)colitis	49 (4.5)	61 (5.9)
Intestinal perforation	18 (1.7)	26 (2.5)
Nervous system disorders		
Intraventricular hemorrhage	26 (2.4)	45 (4.4)
Congenital, familial, and genetic disorders		
Patent ductus arteriosus	19 (1.8)	18 (1.7)
Vascular disorders		
Hypotension	10 (0.9)	16 (1.5)

Probiotic supplement reduces risk of necrotizing enterocolitis and mortality in preterm very low-birth-weight infants: an updated meta-analysis of 20 randomized, controlled trial (Wang 2012)

Table 3 Summary of pooled RR with 95% CI in the subgroup analyses

Subgroup analyses	Studies (no. in probiotics group/no. in placebo group)	RR				
		RR (95%CI)	P_{RR}	$I^2_{Heterogeneity}$	$P_{Heterogeneity}$	Model
Bifidobacteria						
NEC	8 (509/467)	0.30 (0.16-0.58)	.0003	0	.64	Fixed
Mortality	3 (174/166)	0.74 (0.18-2.97)	.67	0	.51	Fixed
Sepsis	3 (174/166)	0.84 (0.29-2.41)	.74	0.21	.28	Fixed
Lactobacillus and Bifidobacteria						
NEC	6 (714/689)	0.33 (0.19-0.58)	.0001	0	.51	Fixed
Mortality	5 (653/660)	0.47 (0.26-0.87)	.02	49	.09	Random
Sepsis	5 (653/660)	0.90 (0.60-1.36)	.62	71	.007	Random
Lactobacillus						
NEC	4 (595/610)	0.37 (0.19-0.73)	.004	0	.40	Fixed
Mortality	4 (595/610)	0.61 (0.38-0.97)	.04	0	.88	Fixed
Sepsis	4 (595/610)	0.79 (0.46-1.36)	.40	71	.01	Random

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

PROBIOTICOS IN PERINATOLOGY

- Research to investigate specific mechanisms of action
- Defining the strains or combination of strains that have clinical benefits
- Regulations that guarantee the product composition and safety
- Explore strategies on how to prevent dysbiosis in premature infant

Thank you

Picasso