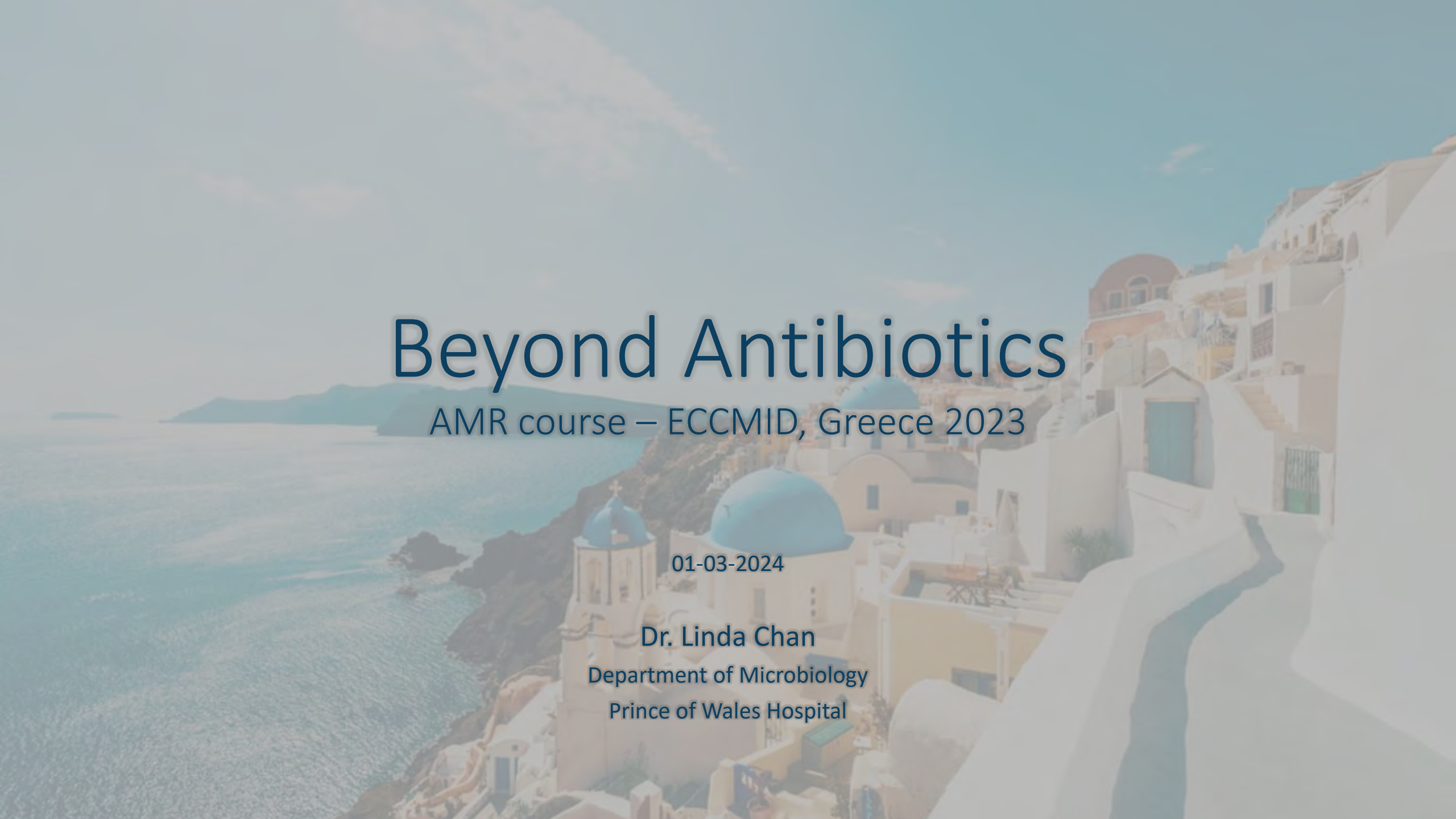
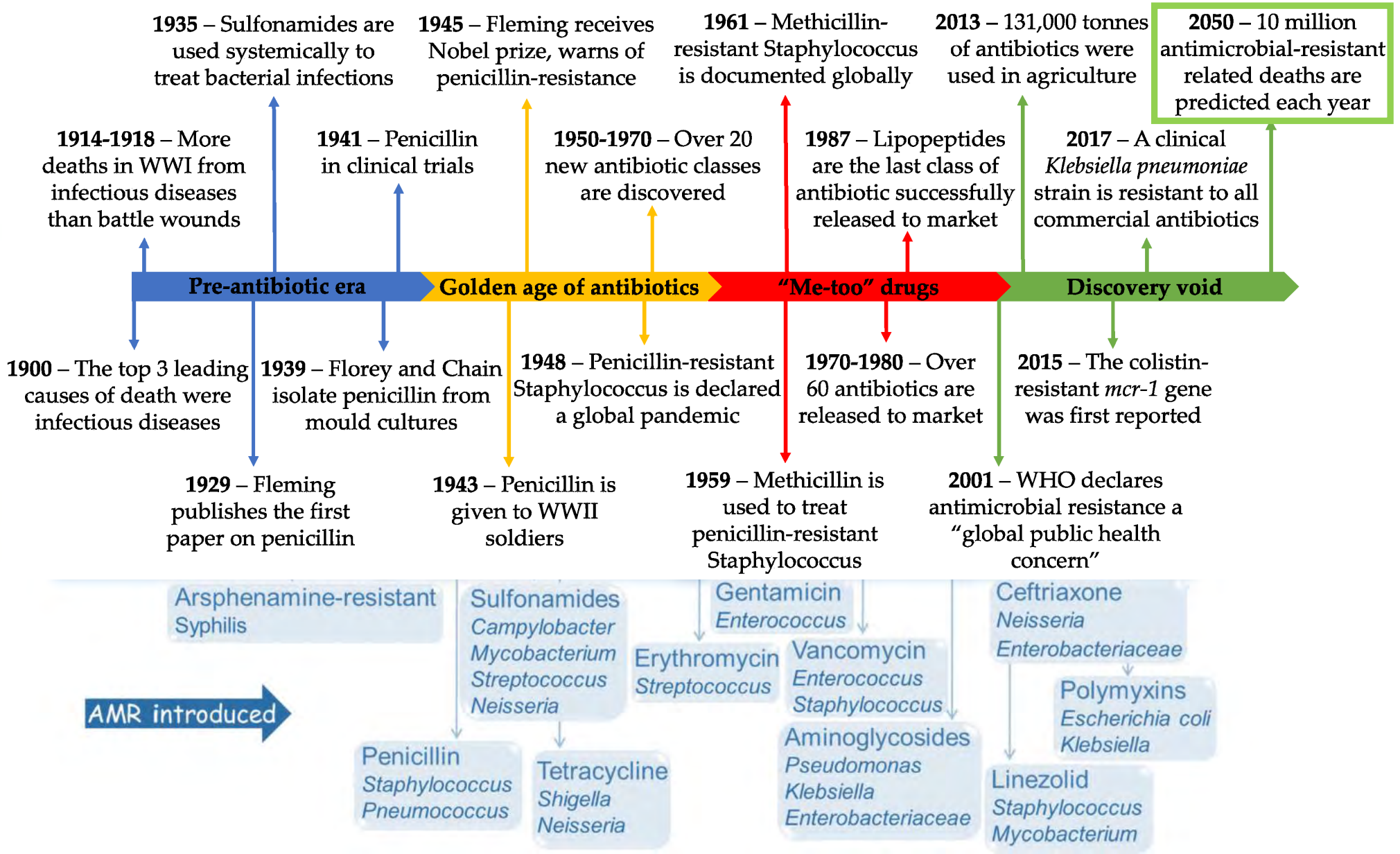


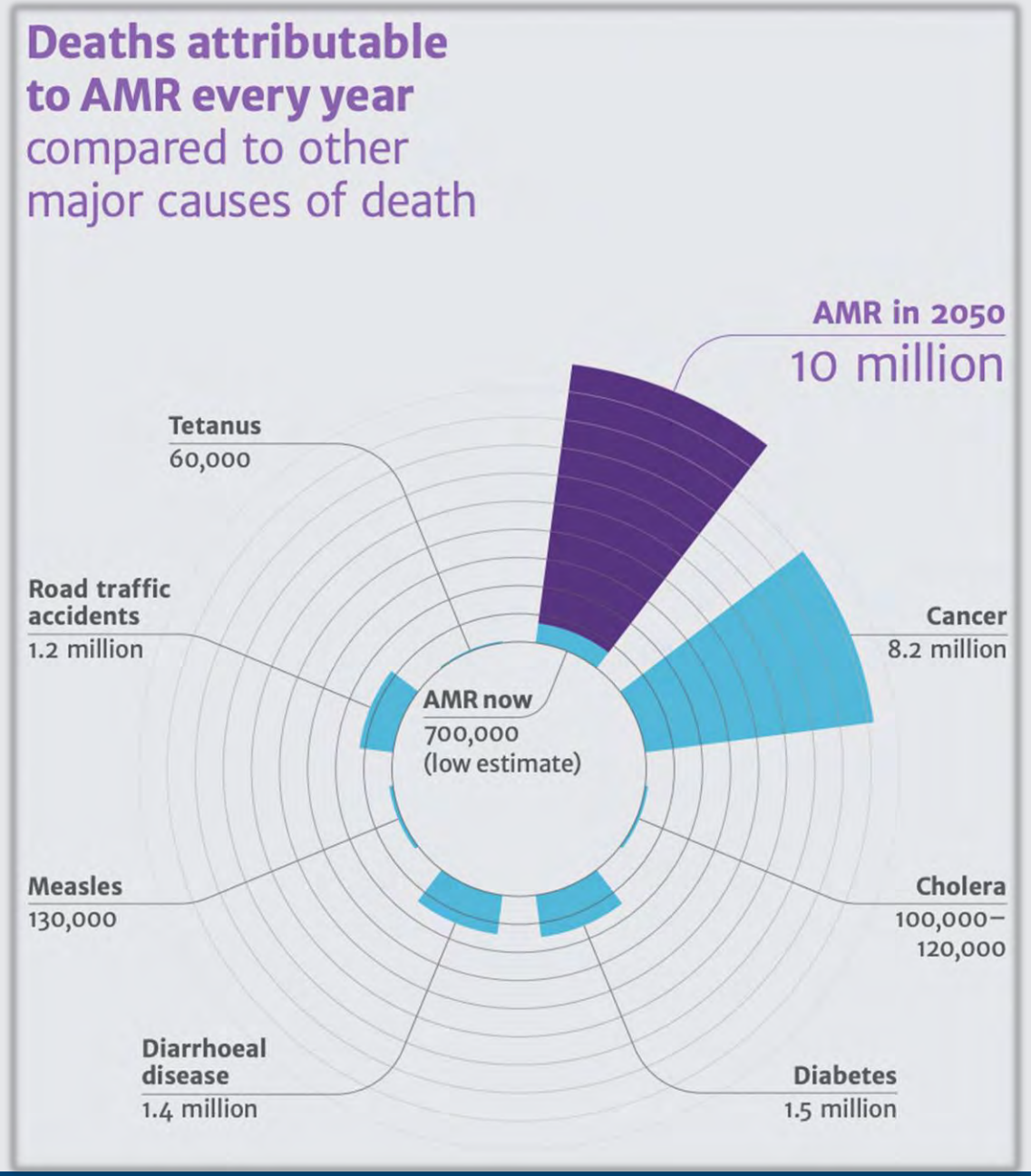
Beyond Antibiotics

AMR course – ECCMID, Greece 2023

01-03-2024

Dr. Linda Chan
Department of Microbiology
Prince of Wales Hospital


1935 – Sulfonamides are used systemically to treat bacterial infections
1945 – Fleming receives Nobel prize, warns of penicillin-resistance
1961 – Methicillin-resistant Staphylococcus is documented globally
2013 – 131,000 tonnes of antibiotics were used in agriculture
2050 – 10 million antimicrobial-resistant related deaths are predicted each year
1914-1918 – More deaths in WWI from infectious diseases than battle wounds
1941 – Penicillin in clinical trials
1950-1970 – Over 20 new antibiotic classes are discovered
1987 – Lipopeptides are the last class of antibiotic successfully released to market
2017 – A clinical *Klebsiella pneumoniae* strain is resistant to all commercial antibiotics
Pre-antibiotic era
Golden age of antibiotics
"Me-too" drugs
Discovery void
1900 – The top 3 leading causes of death were infectious diseases
1939 – Florey and Chain isolate penicillin from mould cultures
1948 – Penicillin-resistant Staphylococcus is declared a global pandemic
1970-1980 – Over 60 antibiotics are released to market
2015 – The colistin-resistant *mcr-1* gene was first reported
1929 – Fleming publishes the first paper on penicillin
1943 – Penicillin is given to WWII soldiers
1959 – Methicillin is used to treat penicillin-resistant Staphylococcus
2001 – WHO declares antimicrobial resistance a "global public health concern"
AMR introduced
Arsphenamine-resistant Syphilis
Sulfonamides Campylobacter Mycobacterium Streptococcus Neisseria
Gentamicin Enterococcus
Erythromycin Streptococcus
Vancomycin Enterococcus Staphylococcus
Ceftriaxone Neisseria Enterobacteriaceae
Polymyxins Escherichia coli Klebsiella
Penicillin Staphylococcus Pneumococcus
Tetracycline Shigella Neisseria
Aminoglycosides Pseudomonas Klebsiella Enterobacteriaceae
Linezolid Staphylococcus Mycobacterium


Deaths attributable to AMR every year
compared to other major causes of death
AMR in 2050
10 million
Tetanus
60,000
Road traffic accidents
1.2 million
AMR now
700,000
(low estimate)
Cancer
8.2 million
Measles
130,000
Cholera
100,000–120,000
Diarrhoeal disease
1.4 million
Diabetes
1.5 million

Beyond antibiotics-outline

1. Probiotics, prebiotics, synbiotics
2. Antimicrobial peptides
3. Bacteriophages
4. Predatory bacteria
5. Fecal microbiota transplant (FMT) therapy
6. Immunological compounds (serum/antiserum & antibodies)
7. Vaccines
8. Nanotechnology

- Substrates for growth of probiotics
- Non-digestable food ingredients in whole grains, bananas, rice, onions etc – substrates for growth of probiotics

- Living non-pathogenic microorganisms (yeast or bacteria)

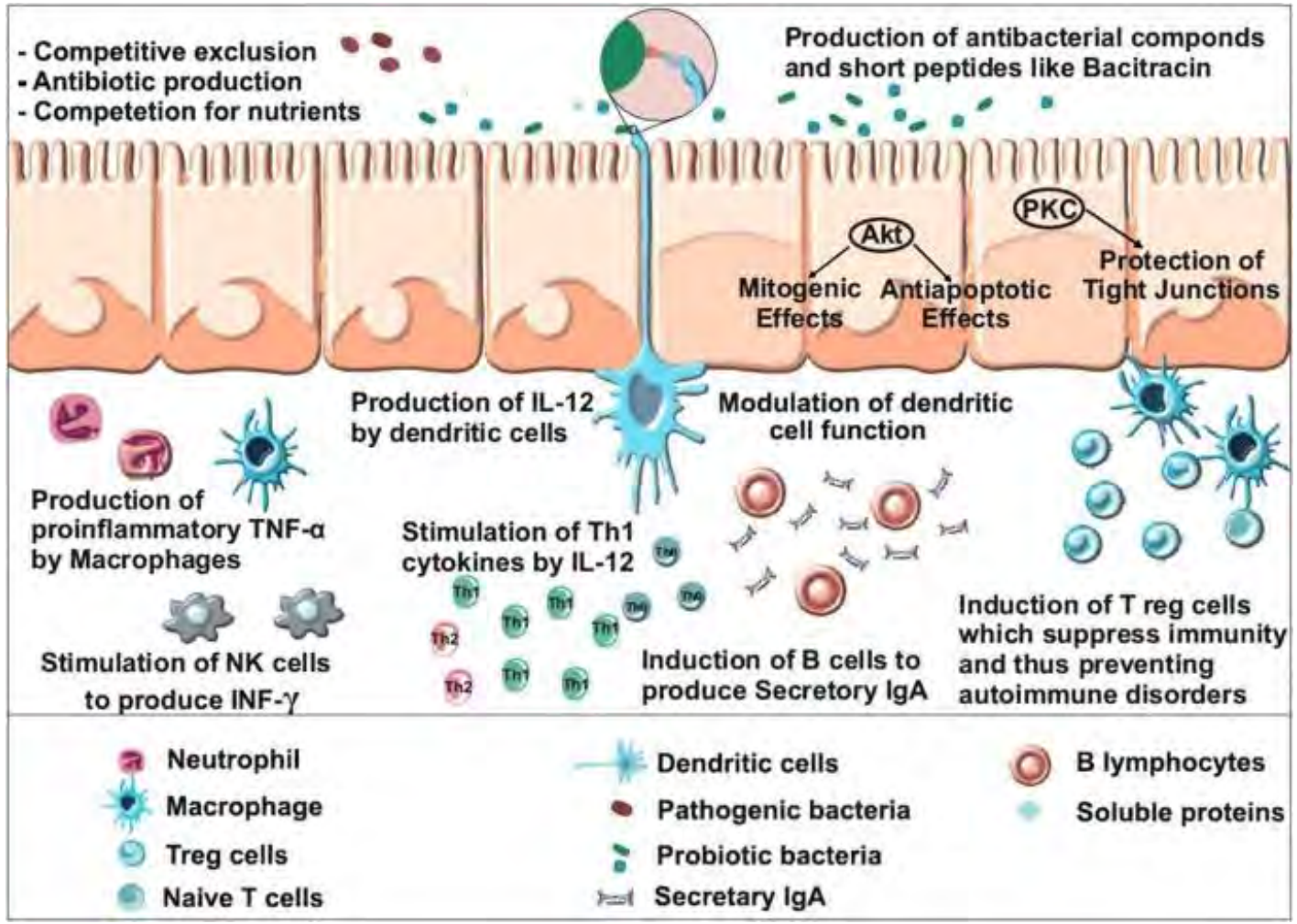
Examples:

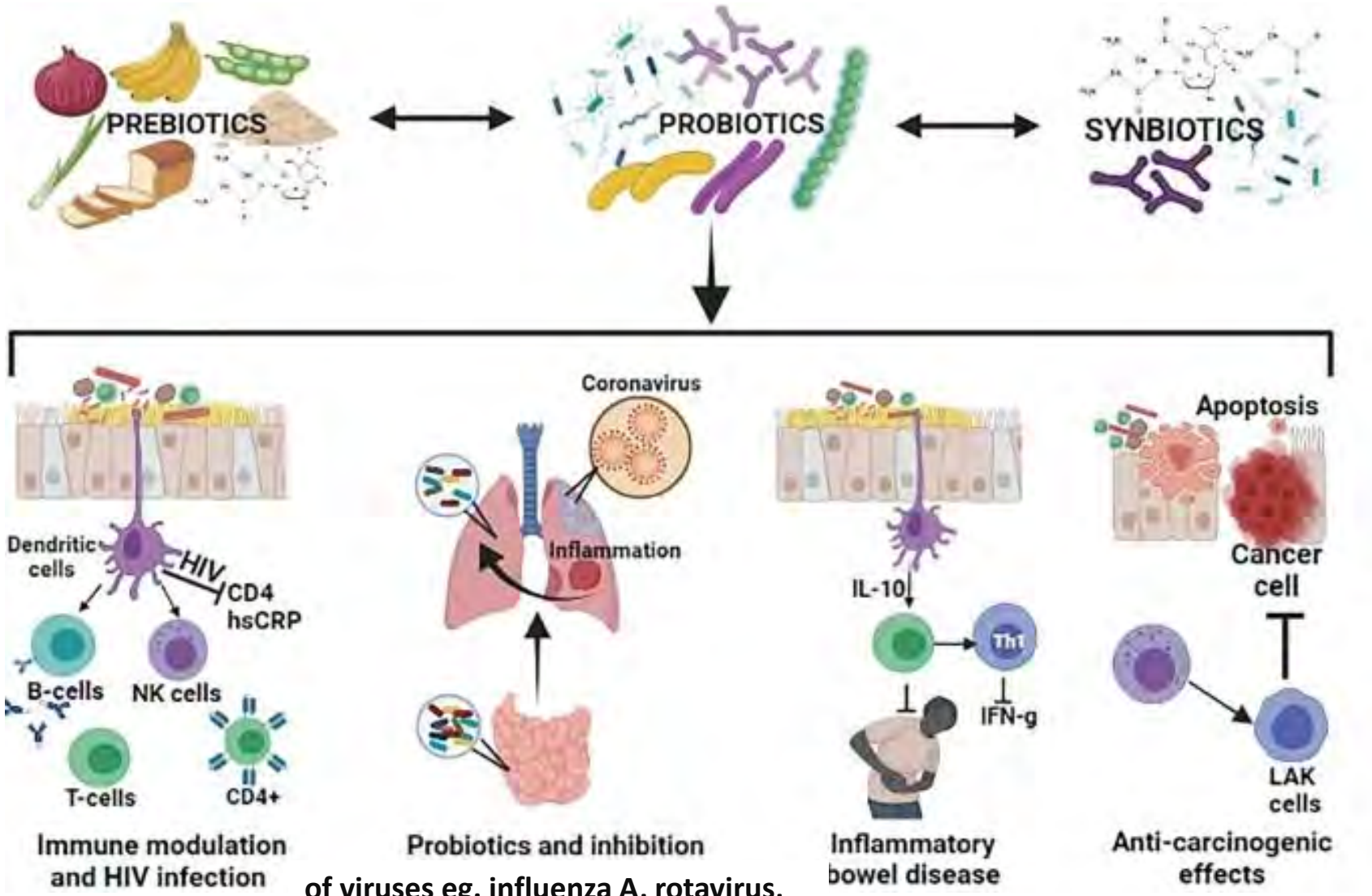
- *Lactobacillus acidophilus*
- *Lactobacillus rhamnosus*
- *Lactobacillus plantarum*
- *Bifidobacterium bifidum*
- *Bifidobacterium lactis*
- *Bifidobacterium subtilis*
- *Saccharomyces boulardii*
- *Streptococcus thermophilus*

etc…

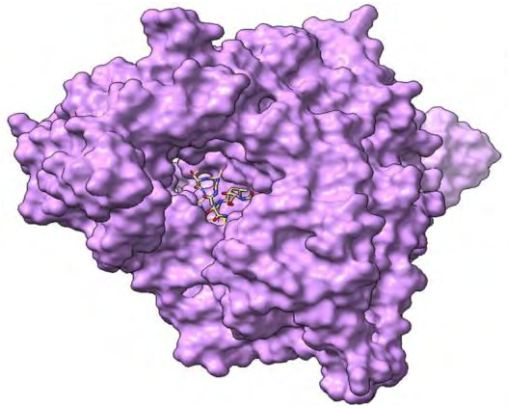
- Mixtures of probiotics and prebiotics

Probiotics - mechanisms of action




PREBIOTICS
PROBIOTICS
SYNBIOTICS
Dendritic cells
HIV
CD4
hsCRP
B-cells
NK cells
T-cells
CD4+
Immune modulation and HIV infection
Coronavirus
Inflammation
Probiotics and inhibition
of viruses eg. influenza A, rotavirus, enterovirus, herpes simplex virus
IL-10
Th1
IFN-g
Inflammatory bowel disease
Apoptosis
Cancer cell
LAK cells
Anti-carcinogenic effects

Development of probiotics for SARS-CoV-2



(Example) Structural modelling of Plantaricin W blocking activity site of RdRp.

Docking scores of *Lactobacillus plantarum* metabolites (Plantaricins) with targets RdRp, RBD, ACE2

Molecule	PubChem CID	RdRp		RBD		ACE2	
		S (kcal/mol)	RMSD(Å)	S (kcal/mol)	RMSD(Å)	S (kcal/mol)	RMSD(Å)
Plantaricin W	139586573	−14.7	3.87	−11.1	2.5	−12.7	4.3
Plantaricin JLA-9	132535900	−11.4	4.1	−8.0	1.3	−9.1	2.6
Plantaricin D	139586697	−10.1	1.9	−8.6	1.9	−8.5	3.2
Plantaricin BN	380907	−6.4	1.8	−5.4	2.2	−6.0	1.47

Strong affinity with low binding energies between *Lactobacillus plantarum* metabolites (Plantaricin W, Plantaricin D, Plantaricin JLA-9) and RNA-dependent RNA polymerase (RdRp), residual binding protein (RBP) on spike protein (S), and human Angiotensin-Converting Enzyme 2 (ACE2) receptor proteins.

Probiotic and synbiotic therapy in critical illness: a systematic review and meta-analysis

William Manzanares[1], Margot Lemieux[2], Pascal L. Langlois[3] and Paul E. Wischmeyer[4*]

Abstract

Background: Critical illness is characterized by a loss of commensal flora and an overgrowth of potentially pathogenic bacteria, leading to a high susceptibility to nosocomial infections. Probiotics are living non-pathogenic microorganisms, which may protect the gut barrier, attenuate pathogen overgrowth, decrease bacterial translocation and prevent infection. The purpose of this updated systematic review is to evaluate the overall efficacy of probiotics and synbiotic mixtures on clinical outcomes in critical illness.

Methods: Computerized databases from 1980 to 2016 were searched. Randomized controlled trials (RCT) evaluating clinical outcomes associated with probiotic therapy as a single strategy or in combination with prebiotic fiber (synbiotics). Overall number of new infections was the primary outcome; secondary outcomes included mortality, ICU and hospital length of stay (LOS), and diarrhea. Subgroup analyses were performed to elucidate the role of other key factors such as probiotic type and patient mortality risk on the effect of probiotics on outcomes.

Results: Thirty trials that enrolled 2972 patients were identified for analysis. Probiotics were associated with a significant reduction in infections (risk ratio 0.80, 95 % confidence interval (CI) 0.68, 0.95, $P = 0.009$; heterogeneity $I^2 = 36$ %, $P = 0.09$). Further, a significant reduction in the incidence of ventilator-associated pneumonia (VAP) was found (risk ratio 0.74, 95 % CI 0.61, 0. 90, $P = 0.002$; $I^2 = 19$ %). No effect on mortality, LOS or diarrhea was observed. Subgroup analysis indicated that the greatest improvement in the outcome of infections was in critically ill patients receiving probiotics alone versus synbiotic mixtures, although limited synbiotic trial data currently exists.

Conclusion: Probiotics show promise in reducing infections, including VAP in critical illness. Currently, clinical heterogeneity and potential publication bias reduce strong clinical recommendations and indicate further high quality clinical trials are needed to conclusively prove these benefits.

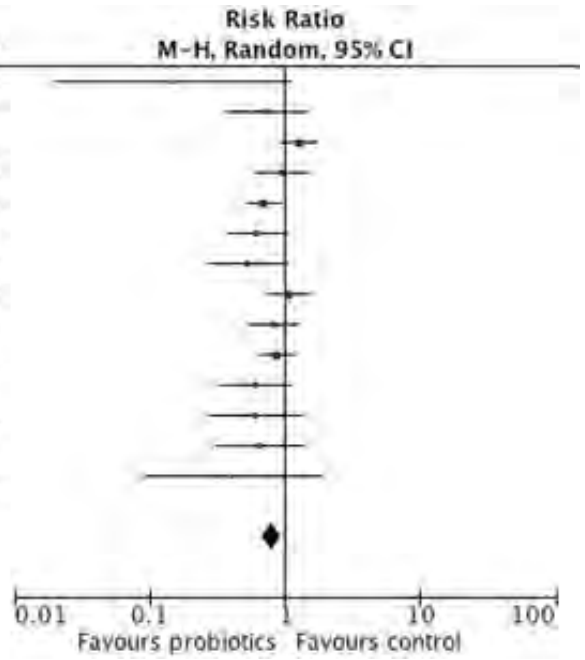
Keywords: Probiotics, Synbiotics, Critical care, Infections, Ventilator-associated pneumonia, Systematic review

Effect of probiotics on overall infections in critically ill patients

Study or Subgroup	Probiotics Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Kecskes 2003	1	22	7	23	0.7%	0.15 [0.02, 1.12]	2003
Lu 2004	8	20	11	20	4.8%	0.73 [0.37, 1.42]	2004
Jain 2004	33	45	26	45	12.8%	1.27 [0.93, 1.72]	2004
McNaught 2005	21	52	22	51	8.3%	0.94 [0.59, 1.48]	2005
Kotzampassi 2006	22	35	27	30	13.7%	0.70 [0.53, 0.93]	2006
Li 2007	8	14	10	11	7.6%	0.63 [0.38, 1.03]	2007
Olah 2007	9	33	15	29	4.9%	0.53 [0.27, 1.02]	2007
Besselink 2008	46	152	41	144	11.1%	1.06 [0.75, 1.51]	2008
Barraud 2010	26	87	29	80	8.9%	0.82 [0.53, 1.27]	2010
Ferrie 2011	14	18	16	18	13.1%	0.88 [0.65, 1.18]	2011
Tan 2011	9	26	15	26	5.4%	0.60 [0.32, 1.12]	2011
Wang 2013	8	62	13	61	3.5%	0.61 [0.27, 1.36]	2013
Lopez de Toro 2014	9	46	13	43	4.1%	0.65 [0.31, 1.36]	2014
Sanaie 2014	2	20	5	20	1.1%	0.40 [0.09, 1.83]	2014
Total (95% CI)		**632**		**601**	**100.0%**	**0.80 [0.68, 0.95]**	
Total events	216		250				

Heterogeneity: $Tau^2 = 0.03$; $Chi^2 = 20.22$, df = 13 (P = 0.09); $I^2 = 36\%$

Test for overall effect: Z = 2.61 (P = 0.009)



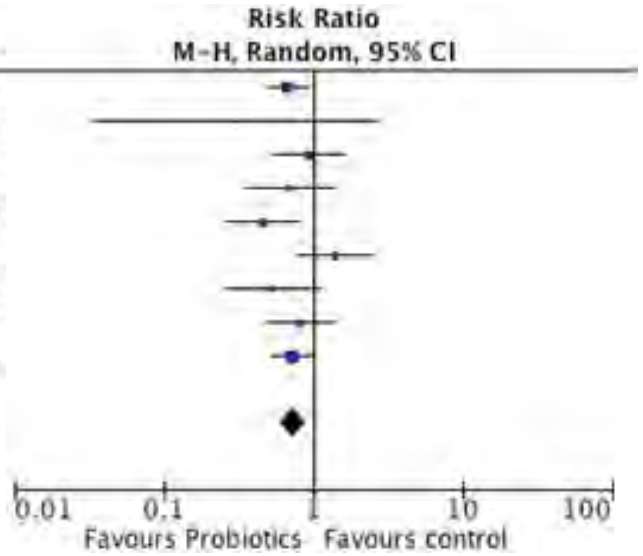
Significant reduction in overall infections with probiotics
RR 0.80, 95 % CI 0.68, 0.95, $P = 0.009$; heterogeneity $I^2 = 36$ %, $P = 0.09$

Overall effect of probiotics on Ventilator associated pneumonia (VAP)

Study or Subgroup	Probiotics Events	Total	Control Events	Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
Kotzampassi 2006	19	35	24	30	20.4%	0.68 [0.48, 0.97]	2006
Klarin 2008	1	23	3	21	0.8%	0.30 [0.03, 2.70]	2008
Forestier 2008	19	102	21	106	10.2%	0.94 [0.54, 1.64]	2008
Knight 2009	12	130	17	129	6.9%	0.70 [0.35, 1.41]	2009
Morrow 2010	13	73	28	73	9.8%	0.46 [0.26, 0.82]	2010
Barraud 2010	23	87	15	80	9.7%	1.41 [0.79, 2.51]	2010
Tan 2011	7	26	13	26	6.2%	0.54 [0.26, 1.13]	2011
Rongrungruang 2015	18	75	22	75	10.9%	0.82 [0.48, 1.40]	2015
Zeng 2016	43	118	59	117	25.1%	0.72 [0.54, 0.97]	2016
Total (95% CI)		**669**		**657**	**100.0%**	**0.74 [0.61, 0.90]**	
Total events	155		202				

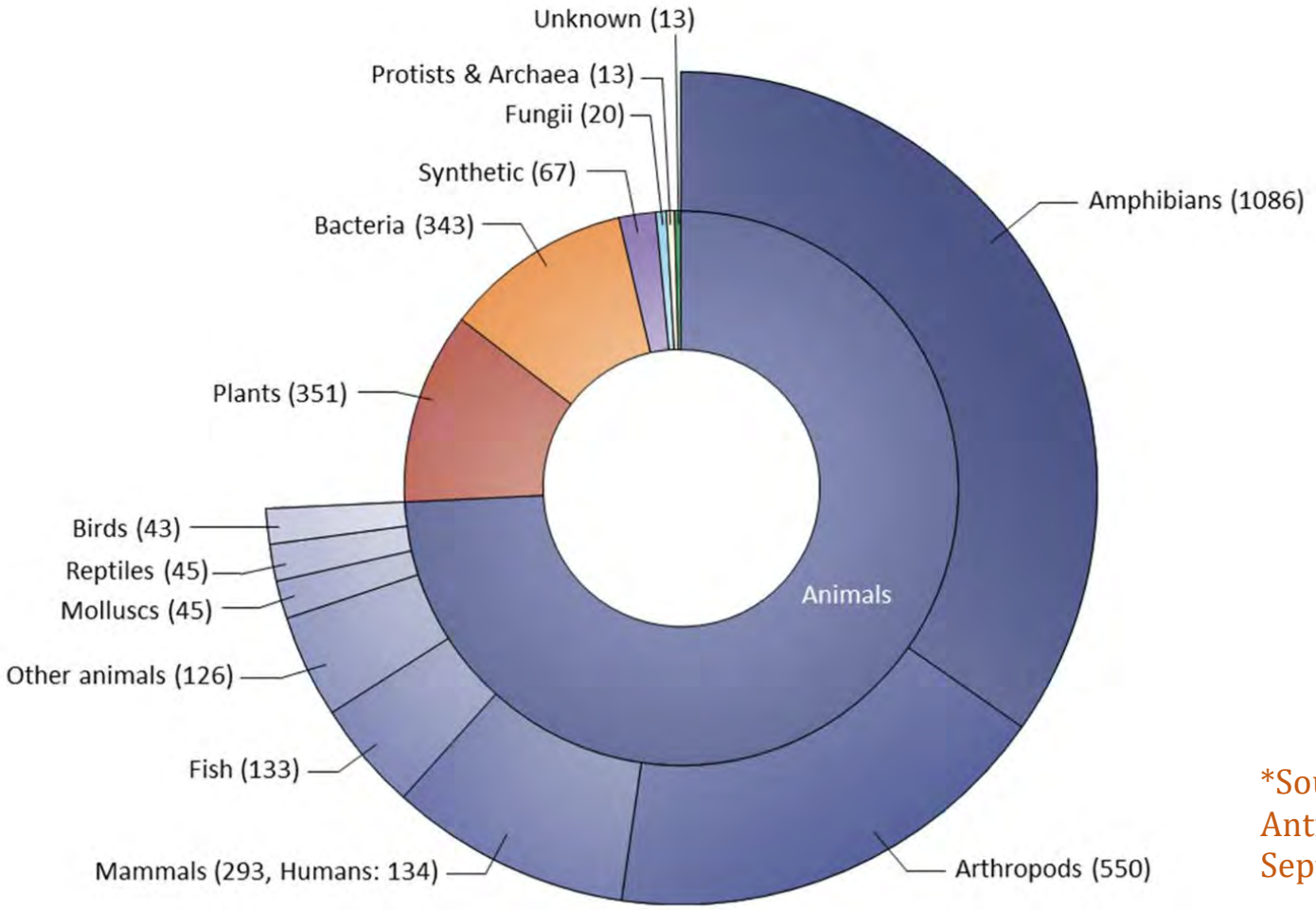
Heterogeneity: $Tau^2 = 0.02$; $Chi^2 = 9.86$, $df = 8$ ($P = 0.27$); $I^2 = 19\%$

Test for overall effect: $Z = 3.04$ ($P = 0.002$)



Significant reduction in the incidence of VAP
RR 0.74, 95 % CI 0.61, 0.90, $P = 0.002$; $I^2 = 19$ %, $P = 0.27$

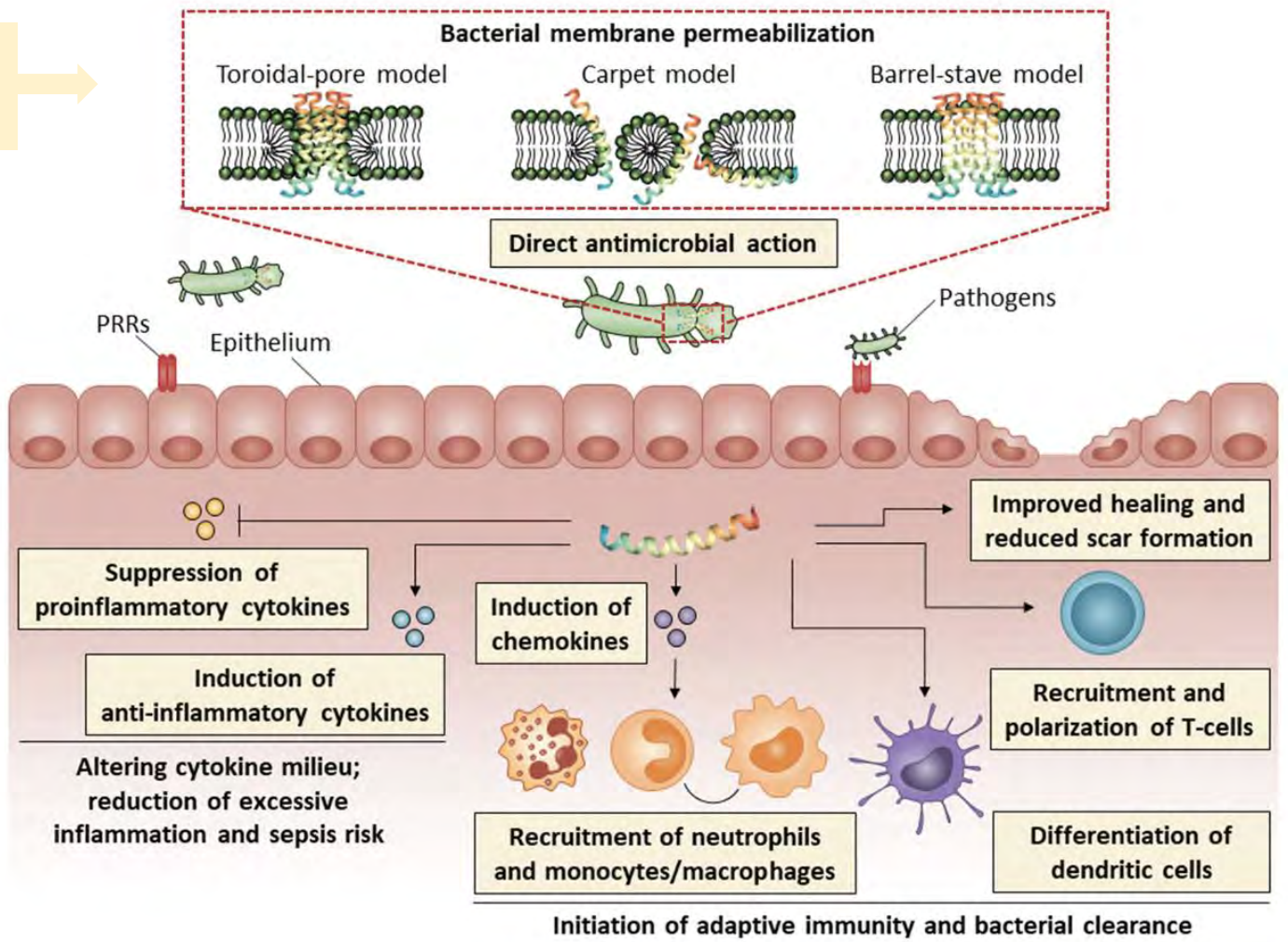
Antimicrobial peptides (AMP)



*Sources of AMP registered in Antimicrobial Database (accessed September 2019)

Antimicrobial peptides (AMP) – mechanism of action

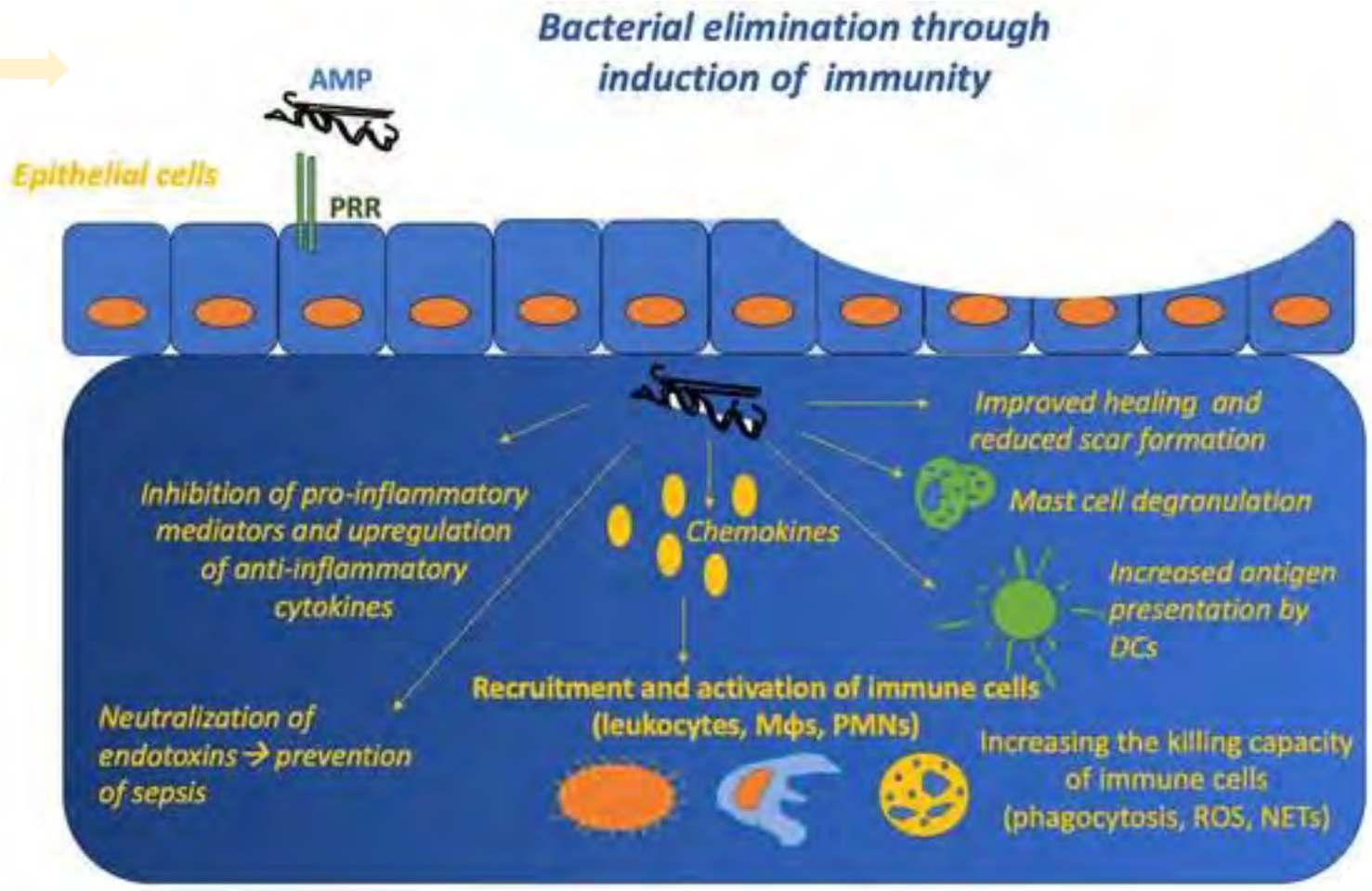
Direct killing by membrane disruption



Mahlapuu et al., Critical Reviews in Biotechnology, 2020.

Antimicrobial peptides (AMP) – mechanism of action

Immunomodulatory function



Examples of AMPs in nature

Name	Source	Mechanism of Action
Abaecin	Bumblebee	Inhibits DnaK
Alamethicin	Fungus *Trichoderma viride*	Barrel-stave transmembrane pore model
Apidaecin Hb1a	Honeybee	Inhibits protein biosynthesis by targeting ribosomes, inhibits DnaK and GroEL, inhibits ABC transport system, binds LPS
Buforin II	Enzymatic cleavage of buforin I (from Asian toad Bufo bufo gargarizans)	Inhibits DNA, inhibits RNA
Cecropin P1	Pig intestine	Carpet model/Detergent-like mode non-membrane pore model
Diptericin	Hemolymph of injured Sarcophaga peregrine larva	Inhibits septation
HD5 (oxidized)	Human small intestine	Inhibits cell division
Histatin-5	Human saliva	Inhibits proteases, inhibits MMP-2 and MMP-9, inhibits generation of ROS
Lacticin Q	*Lactococcus lactis* QU 5	Toroidal transmembrane pore model
Magainin 1	African clawed frog Xenopus laevis	Inhibits energy metabolism proteins, inhibits amino-acid metabolism
MBI-27	Derived from part of silk moth cecropin and bee melittin peptides	Inhibits LPS
Nisin	Lactococcus lactis	Inhibits lipid II in peptidoglycan biosynthesis
Ostricacin-1	Ostrich defensin	Inhibits DNA
Tachyplesin	Horseshoe crab hemocytes	Binds DNA minor groove
Thanatin	*Podisus maculiventris*	Agglutination non-membrane pore model
tPMP-1	Platelet granules	Activation of autolytic enzyme

Antimicrobial peptides (AMP)

Advantages

- Multiple approved uses ; mainly in food industry
- Resistance less likely to occur
- Less susceptible to mutations
- Sepsis attenuation by neutralizing endotoxins
- Multiple targets
- Good thermal stability
- Good water solubility
- Can be used in combination with antibiotoics

Disadvantages

- High extraction costs
- Poor bioavailability
- Short half-lives
- Lack of target specificity
- Cytotoxicity
- Instability – degradable by proteases

AMP - synergy with antibiotics

AMP	Antibiotic	Target
Tridecaptin M	Rifampicin, vancomycin, ceftazidime	*A. baumannii*
Lactoferricin	Ciprofloxacin, ceftazidime	*P. aeruginosa*
LL-37, HBD3	Tigecycline, moxifloxacin, piperacillin/tazobactam, meropenem	*C. difficile*
P10	Ceftazidime, doripenem	*A. baumannii* and *P. aeruginosa*
Gad-1	Kanamycin, ciprofloxacin	*P. aeruginosa*
Nisin	Penicillin, chloramphenicol, ciprofloxacin, indolicidin, or azithromycin	*S. aureus*
SAAP-148	Demeclocycline hydrochloride (DMCT)	*P. aeruginosa*
(SLAP)-S25	Cefepime, colistin, ofloxacin, rifampicin, tetracycline, and vancomycin	multidrug-resistant Gram-negative pathogens
Colistin	tigecycline, carbapenem, gentamicin	*Klebsiella* KPC
Octaarginine	Vancomycin	biofilms of *S. aureus*
Sphistin, Sph12−38	Rifampicin, azithromycin	*P. aeruginosa*
DP7	Azithromycin, vancomycin	*S. aureus*, *P. aeruginosa*, *A. baumannii*, *E. coli*
P10	Ceftazidime, doripenem	*A. baumannii*, colistin-resistant *P. aeruginosa*
Melittin	Doripenem and ceftazidime	*A. baumannii* and *P. aeruginosa*
LL 17–29	Chloramphenicol	*S. aureus*, *P. aeruginosa*
Nisin Z, pediocin, or colistin	Penicillin, ampicillin, or rifampicin	*P. fluorescens*
Melamine	Ciprofloxacin, fluoroquinolone	*P. aeruginosa*
Indolicidin, polymyxin B	Tobramycin, gentamycin, and amikacin	*P. aeruginosa*
Arenicin-1	Ampicillin, erythromycin, and chloramphenicol	*S. aureus*, *S. epidermis*, *P. aeruginosa*, and *E. coli*

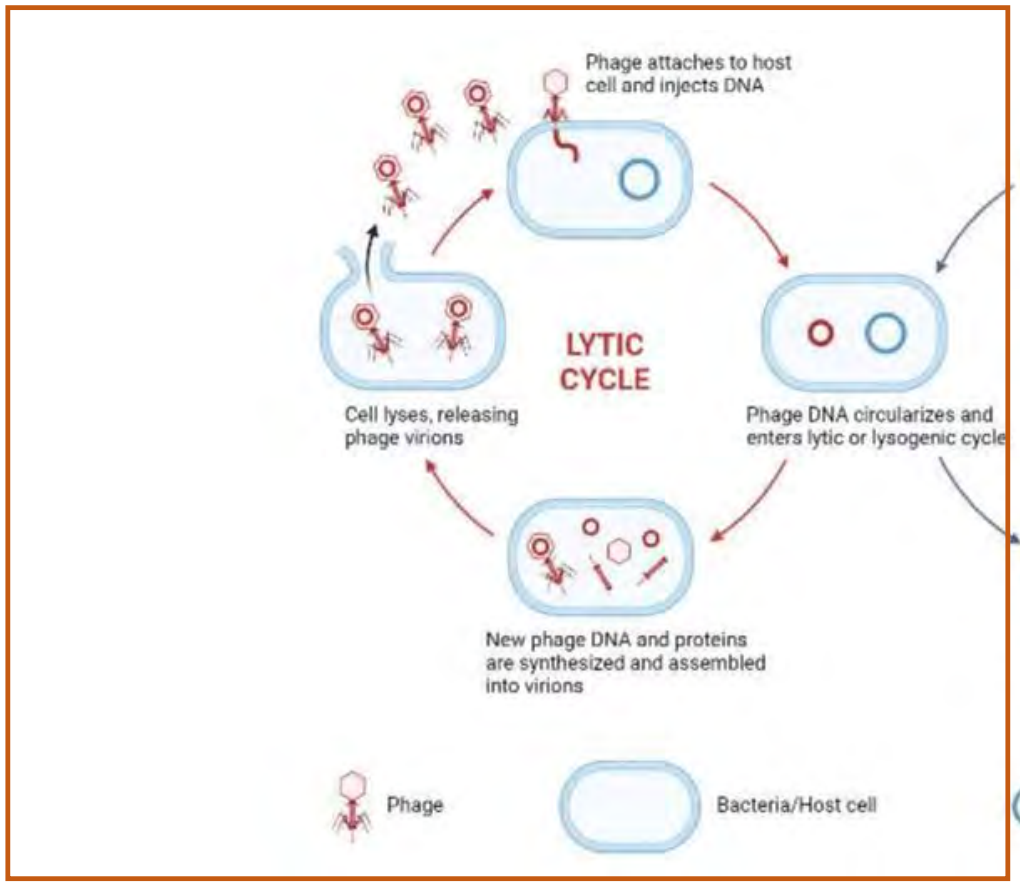
Examples of AMPs in human clinical trials

Study	Population	Intervention	Comparator	Outcome
Fowler et al., 2006 [275]—Phase III RCT	Patients with *S. aureus* bacteremia with or without endocarditis	IV treatment with the AMP daptomycin	IV treatment with low-dose gentamicin plus either an antistaphylococcal penicillin or vancomycin	Daptomycin was non-inferior to standard-of-care. Treatment success rates were similar in subgroups of patients with complicated bacteremia, right-sided endocarditis, and methicillin-resistant S. aureus
Miller et al., 1948 [276]—non-randomized study	130 patients with superficial infections of the skin and 35 patients with secondary skin infections	Bacitracin applied locally	None	Cure rate higher than 50% in superficial skin infections and 100% in secondary skin infections
NCT05340790—Phase I RCT	Healthy female volunteers	Dose 1 to 5 of AMP PL-18 vaginal suppositories	Placebo doses 1 to 5 of vaginal suppositories	Safety assessment (recruiting)
Gronberg et al., 2014 [277]—RCT	Adult patients with hard-to-heal venous leg ulcers	Repeated doses of LL-37 applied locally	Repeated doses of placebo applied locally	Safe and well tolerated. Significant early healing of ulcers
Daley et al., 2017 [278]—Phase III RCT	Adult patients with *Clostridioides difficile* infection	AMP surotomycin orally	Vancomycin orally	Non-inferior but non-superior to vancomycin for clinical response
Lipsky et al., 2008 [192]—Phase III RCT	Adult diabetic patients with infected wounds at the lower extremities	AMP pexiganan locally and oral placebo	Local placebo and oral ofloxacin	Pexiganan is comparable to oral ofloxacin for mildly infected diabetic ulcers

Examples of AMPs in human clinical trials

Study	Population	Intervention	Comparator	Outcome
Peek et al., 2020 [260]—Phase II RCT	Adults with chronic suppurative otitis media resistant to antibiotic therapy	AMP P60.4Ac locally with ear drops	Vehicle locally with ear drops (placebo)	Safe and well-tolerated treatment. Significantly higher treatment success than placebo
NCT00231153, Phase III RCT	Patients with central venous catheters	AMP Omiganan 1% gel local application at the catheter insertion site	Povidone-Iodine 10% local application at the catheter insertion site	Failed to show adequate efficacy in catheter-associated infections
NCT04767321, Phase I/II RCT	Adults 18–65 years old with persistent carriage of *S. aureus*	Nasal application of the AMP LTX-109	Nasal application of placebo	Safety, tolerability, and microbial eradication—recruitment completed
Mercer et al., 2020 [281]—Phase I and Phase II RCTs	12, 48, and 47 patients with onychomycosis of the toenail	Local application of NP213	Local application of placebo	NP213 clinical safety profile. Positive patient-reported outcomes
Mullane et al., 2015 [282]—Phase II RCT	72 patients with *Clostridioides difficile* infection	LFF571 orally	Vancomycin orally	The rate of clinical cure was non-inferior to that of vancomycin. Similar 30-day sustained cure rates. More adverse events for LFF571.
Corey et al., 2014 [283]—Phase IIa RCT	84 adult patients with ABSSSI	GSK1322322 orally	Linezolid orally	Clinical success in the ITT population and the per-protocol population were 67 and 91% in the GSK1322322-treated group and 89 and 100% in the linezolid-treated group

Bacteriophage - Lifecycle



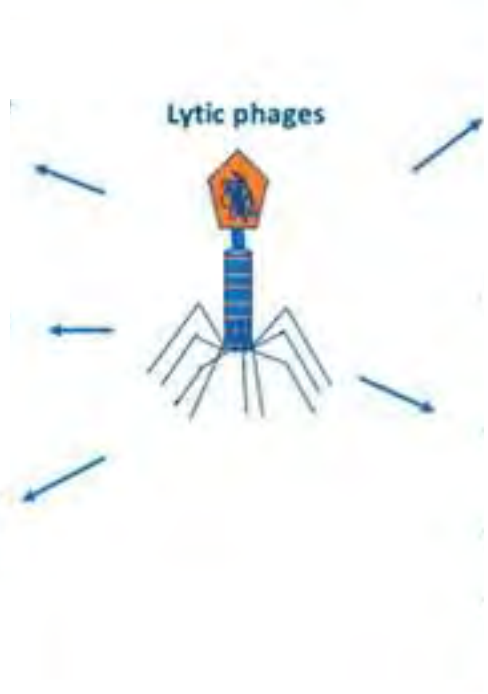
Lytic cycle also known as infectious or virulence cycle.
Ends with lysis of host cells.

Bacteriophage – applications

Food safety
- Reduce entry of pathogenic bacteria responsible for foodborne disease
- Not infecting human cells
- Not altering food
- Prevent biofilm formation

Agriculture
- Reduce possibility of plant diseases due to pathogens

Aquaculture
- Control of bacterial disease leading to optimization of industrial production



Wastewater plant treatment
- Can be specific against different bacteria, and can act as tracers of pathogens, monitoring wastewater

Hospital environment sanitizers
- Can eliminate pathogens that could be isolated in hospital surfaces such as *Escherichia coli, Salmonella spp. MRSA, Acinetobacter baumanii*
- Can amplify decontamination of hospital surfaces with respect to bacteria that are protective
- Can eradicate XDR and PDR pathogens in ICUs
- Can combine with PCHS, targeting bacteria that are resistant to disinfectants

Abbreviations: ICU, intensive care unit; MRSA, methicillin-resistant *Staphylococcus aureus*; PCHS, probiotic cleaning hygiene system; PDR, pan-drug-resistant; XDR, extensively drug-resistant.

Bacteriophage therapy

Advantages

- Works against AMR pathogens
- Can be used in combination with antibiotics
- Few doses needed
- Target bacteria , not normal flora
- Natural and easy to find
- No adverse events
- Not toxic to the environment

Disadvantages

- Difficult to prepare
- Pharmaceutical issues - dose, PK/PD, phage species
- In vitro and in vivo correlation unclear
- Bacterial resistance in long-term
- Immune overreaction after massive bacterial lysis
- Immune system may deactivate phages
- No FDA approval (yet)

Studies evaluating clinical use of phages

Study	Population	Intervention	Comparator	Outcome
Jault et al., 2019 [177] (PhagoBurn) —Phase I/II trial	Adult patients with burns infected by *P. aeruginosa*	Cocktail by 12 anti-*P.-aeruginosa* phages (PP1131)	Standard of care (1% sulfadiazine silver emulsion cream)	Phage cocktail reduced bacterial burden more slowly than the standard of care
Ooi et al., 2019 [159]—Phase I trial	Nine patients with recalcitrant chronic rhinosinusitis (18–70 years old) with failure of surgical and medical treatment and positive cultures for *S. aureus* sensitive to investigational phage cocktail AB-SA01	Serial doses of twice-daily intranasal irrigations with AB-SA01	None	Intranasal irrigation with AB-SA01 was safe and well tolerated
Wright et al., 2009 [178] —Phase I/II trial	24 patients with chronic otitis with positive culture for antibiotic-resistant *P. aeruginosa* sensitive to Biophage-PA	A single dose of Biophage-PA (10^3 directly in the ear) after randomization	Placebo	Poled patient- and physician-reported clinical indicators improved for the phage-treated group relative to the placebo group. No treatment-related adverse event was reported
Sarker et al., 2016 [179]—Double-blind, placebo-controlled	Bangladeshi children hospitalized with acute bacterial diarrhea	40 individuals received phage cocktail M, and 39 individuals received phage cocktail T orally three times daily in oral rehydration solution over 4 days	Placebo (oral rehydration solution)	No significant difference between the group treated with phages and the placebo group was noted
Leitner et al., 2021 [180]—Randomized, placebo-controlled trial	Adult males scheduled for TURP, with complicated UTI or recurrent uncomplicated UTIs	28 patients received at least one intravesical dose of Pyophage after randomization (the planned dose was twice daily for seven days)	32 patients received a placebo and received 37 systematic antibiotics after randomization	Intravesical bacteriophage therapy was non-inferior to standard-of-care antibiotic treatment but was not superior to placebo bladder irrigation in terms of efficacy or safety

Studies evaluating clinical use of phages

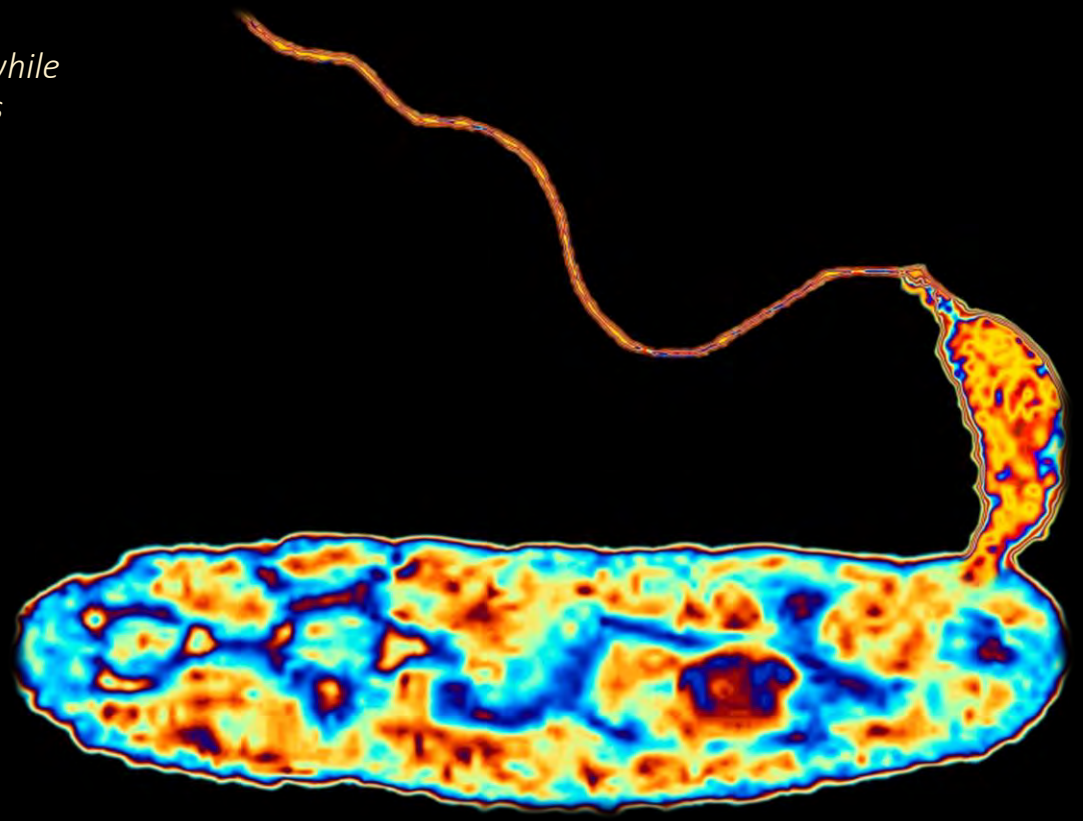
Study	Population	Intervention	Comparator	Outcome
Rhoads et al., 2009 [181]—Phase I trial	42 patients with chronic venous leg ulcers	The ulcers were treated for 12 weeks with bacteriophages targeted against *P. aeruginosa*, *S. aureus*, and *E. coli*	Saline control	No adverse events due to phages. No significant difference for frequency of adverse events, rate of healing, or in the frequency of healing
Samaee et al., 2023 [182]—Double-blind, placebo-controlled, randomized study	60 patients with moderate-to-severe COVID-19	For the intervention group, 10 mL of phage cocktail with a titer of 10^{12} PFU/mL was given with a mesh nebulizer	The control group received 10 mL of phage-free suspension (placebo) every 12 h with a mesh nebulizer	Inhalation phage therapy may have a potential effect on secondary infection and on the outcome of COVID-19 patients
Fedorov et al., 2023 [183]—Non-randomized, open-label, with historical control study	Adult patients with deep PJI of the hip with a 12-month follow-up after one-stage revision surgery	23 patients were treated with specific phage preparation and etiotropic antibiotics	22 patients from a retrospective historical control group received antibiotics only	PJI relapses in the intervention group were eight times lower. The response rate to treatment was 95.5% in the intervention and only 63.6% in the control
Petrovic Fabijan et al., 2020 [184]—Single-arm, non-comparative trial	Adult patients with two consecutive days of *S. aureus* bacteremia	13 patients were administered adjunctive AB-SA01 intravenously	None	No adverse reactions were reported, and AB-SA01 appeared to be safe in severe *S. aureus* infections, including septic shock and infective endocarditis

Studies evaluating clinical use of phages

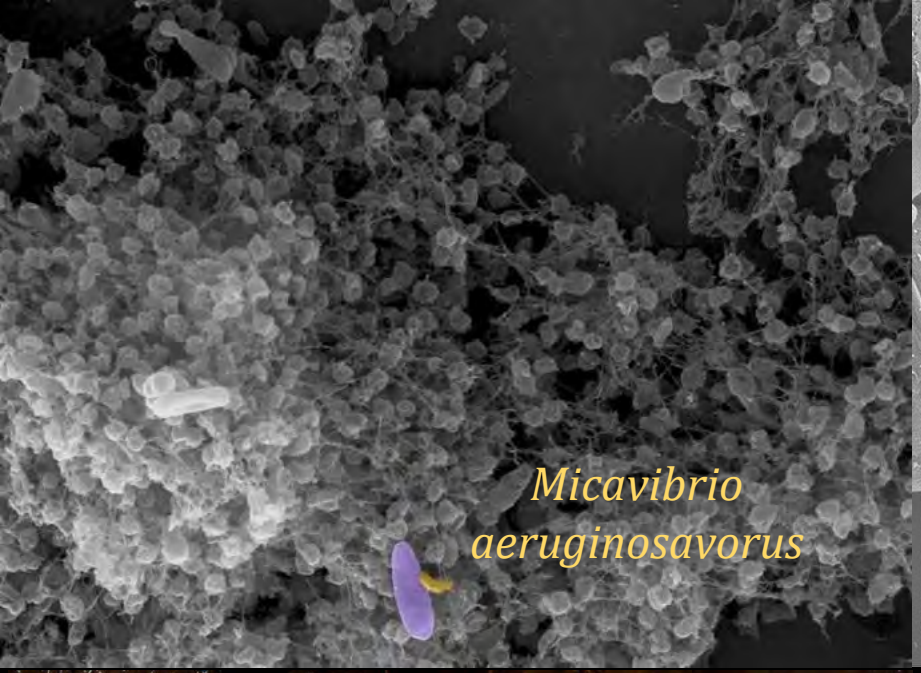
Study	Population	Intervention	Comparator	Outcome
Chan et al., [175]—Case report	A 76-year-old patient with infected aortic graft due to *P. aeruginosa* and complicated by aorto-cutaneous fistula with purulent discharge	A phage active against *P. aeruginosa* that had synergy with ceftazidime was applied locally in the exit point of the fistula, along with systematic administration of ceftazidime. Partial graft excision and replacement took place	None	Cultures were sterile one month later. Two years later, the infection had not relapsed in the absence of antimicrobial treatment
Khawaldeh et al., 2011 [185]—Case report	A 67-year-old woman with extensive intra-abdominal resections and pelvic irradiation for adenocarcinoma, bilateral ureteric stent placement for obstruction complicated by *P. aeruginosa* infection, and with multiple courses of antibiotics and two stent replacements	2×10^7 PFU of a lytic phage active against the infecting strain was directly instilled into the bladder every 12 h for 10 days (antibiotics also started on day 6)	None	Urine cultures were sterile after phage therapy and a 30-day course of meropenem
LaVergne et al., 2018 [186]—Case report	A 77-year-old man with traumatic brain injury who underwent craniectomy and was complicated by postoperative infection by XDR *A. baumannii*	8.56×10^7 PFU of active phage for that bacterial strain administered intravenously every 2 h for 8 days	None	Initial patient improvement was observed, and craniotomy site and skin flap healed well, but fevers and leukocytosis persisted. The patient died after care withdrawal
Schooley et al., 2017 [187]—Case report	68-year-old diabetic man with necrotizing pancreatitis complicated by an MDR *A. baumannii*-infected pseudocyst	5×10^9 PFU administered intravenously every 6 h for 84 days, with minocycline being added on day two	None	The patient improved clinically and the infection resolved

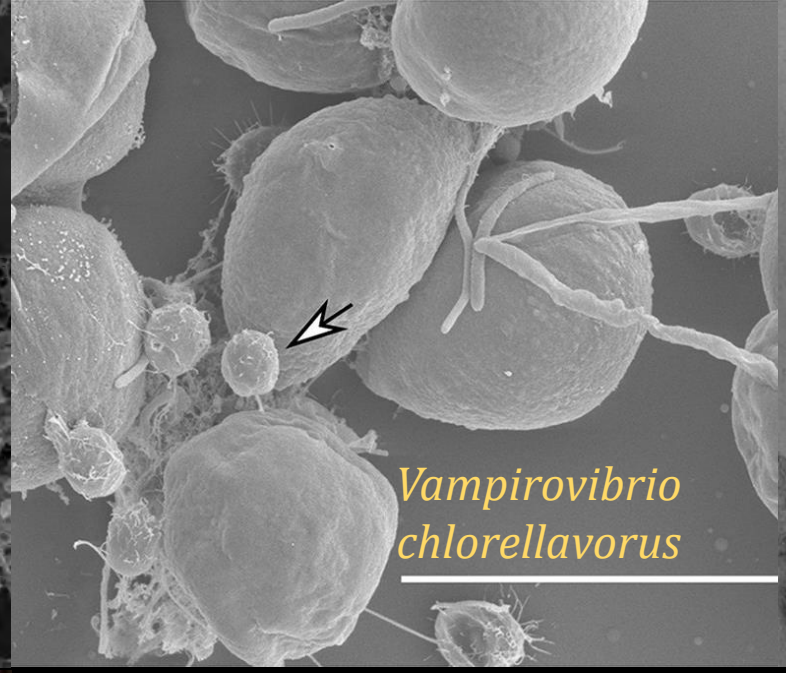
Predatory bacteria

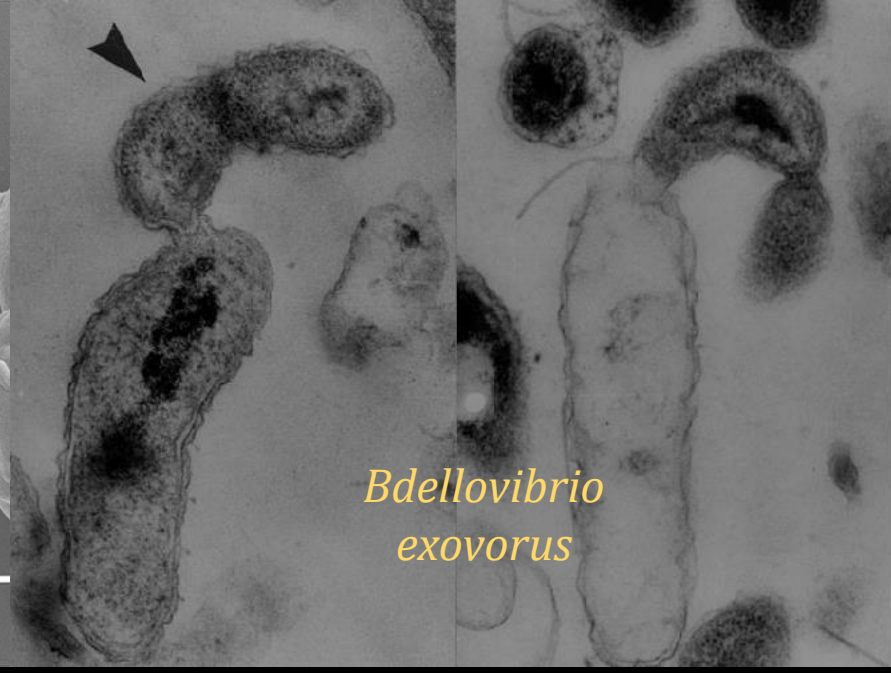
- *First discovered accidentally in 1960 while scientists searched for bacteriophages*
- *Found in soil and water*
- *Harmless to humans*
- *Prey on gram-negative bacteria*



Bacteria Bdellovibrio spp.
—false-color transmission electron microscopy image at 50,000× magnification





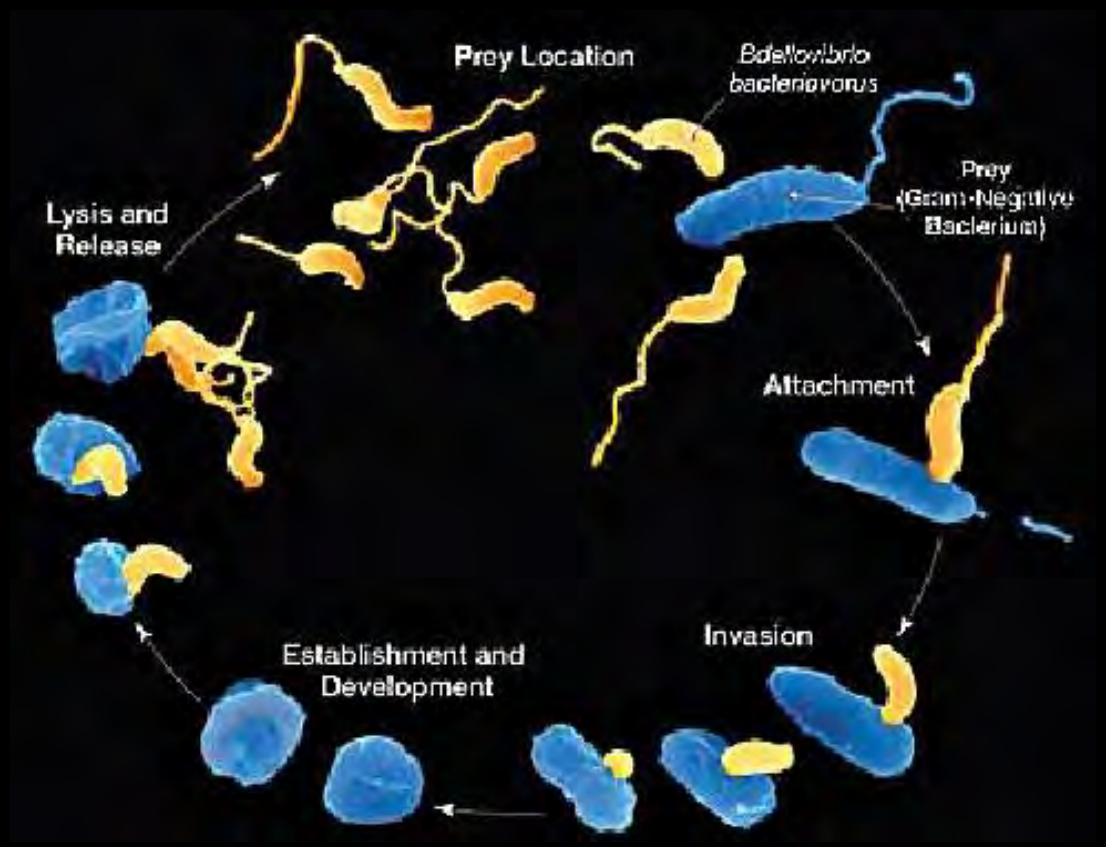


-'Vampire bacteria' are predatory against gram-negative bacteria

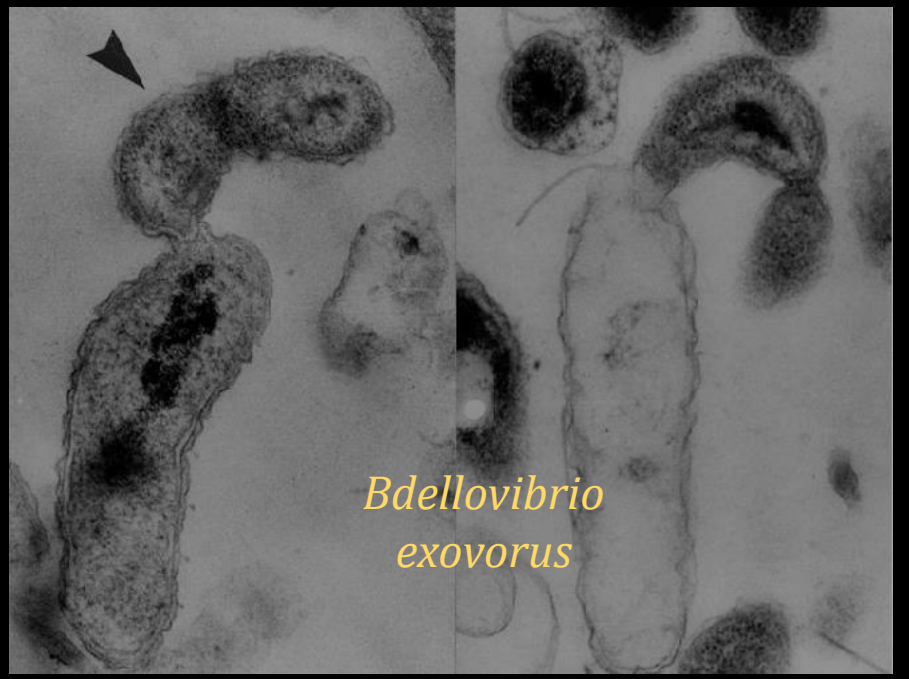
-*Myxobacteria* spp. can kill a broader range of bacteria

-Potential targets include:

Acinetobacter, Aeromonas, Bordetlla, Burkholderia, Citrobacter, Enterobacter, Escherichia, Klebsiella, Listeonella, Morganella, Proteus, Salmonella, Serratia, Shigella, Vibrio, Yersinia, H.pylori, Legionella



Life cycle of *Bdellovibrio bacteriovorus*



Bdellovibrio spp.

Active against many bacteria
May persist non-pathogenically in humans
Not highly immune-stimulatory
Minimal adverse events to microbiome
No resistance development by targets
No incorporation of prey genetic material

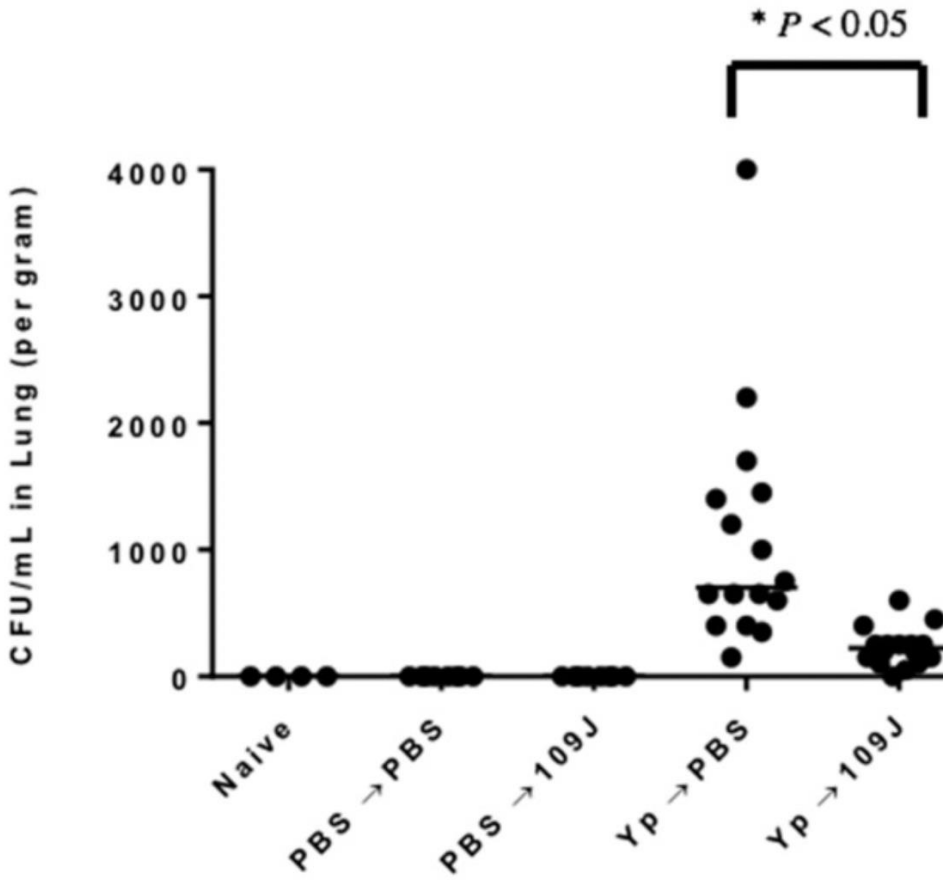
Susceptibility of Virulent *Yersinia pestis* Bacteria to Predator Bacteria in the Lungs of Mice

Riccardo Russo [1], Irina Kolesnikova [1], Thomas Kim [1], Shilpi Gupta [2], Androulla Pericleous [2], Daniel E. Kadouri [2] and Nancy D. Connell [1,3,*]

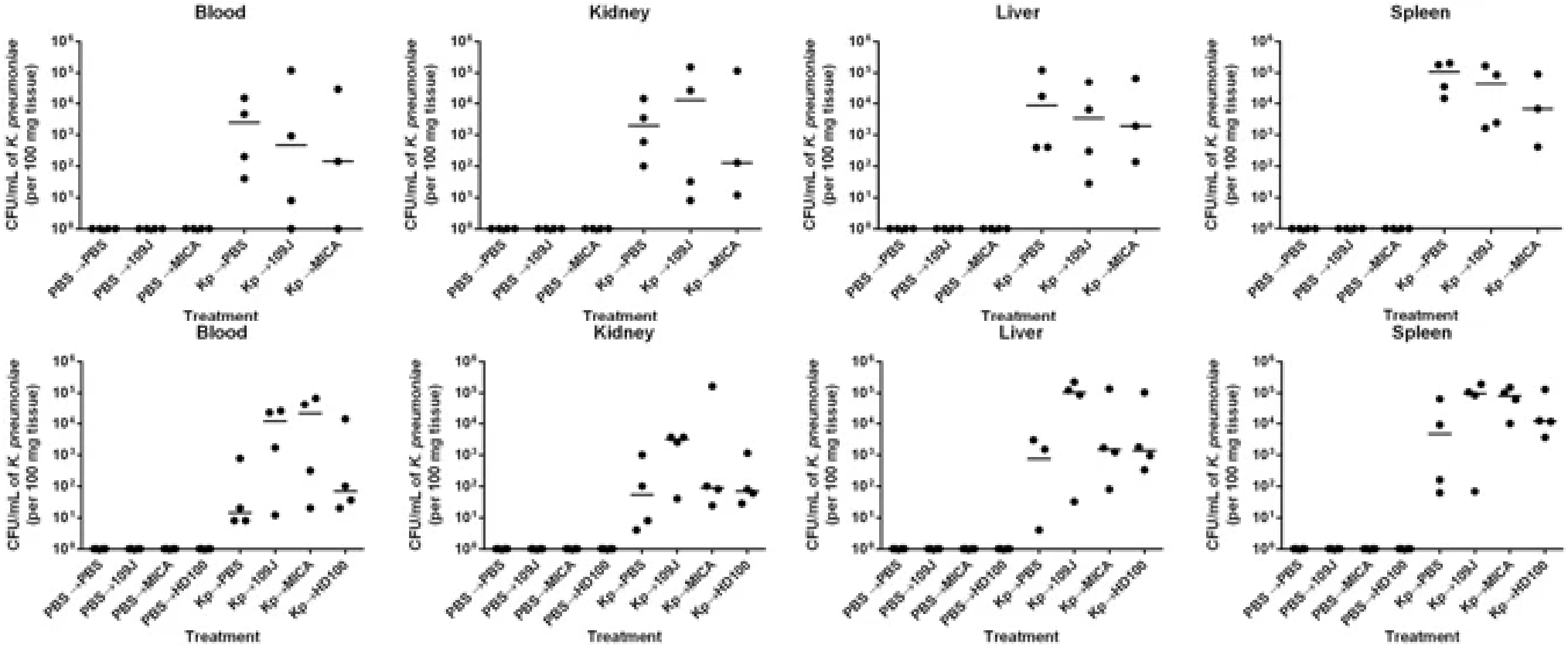
1 Department of Medicine and the Center for Emerging Pathogens, Rutgers, New Jersey Medical School, Newark, NJ 07101, USA; russori@njms.rutgers.edu (R.R.); kolesnir@njms.rutgers.edu (I.K.); tk473@njms.rutgers.edu (T.K.)
2 Department of Oral Biology, Rutgers School of Dental Medicine, Newark, NJ 07101, USA; sg1135@sdm.rutgers.edu (S.G.); andrea83@gsbs.rutgers.edu (A.P.); kadourde@sdm.rutgers.edu (D.E.K.)
3 Center for Health Security, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD 20102, USA
* Correspondence: NancyConnell@jhu.edu

B.Bacteriovorus (109J) reduce bacteria *Yersinia pestis* in mice lungs by 86% within a day of infection as compared with control (PBS).

n=16 in treatment group
Significant differences between treatment groups : p<0.05

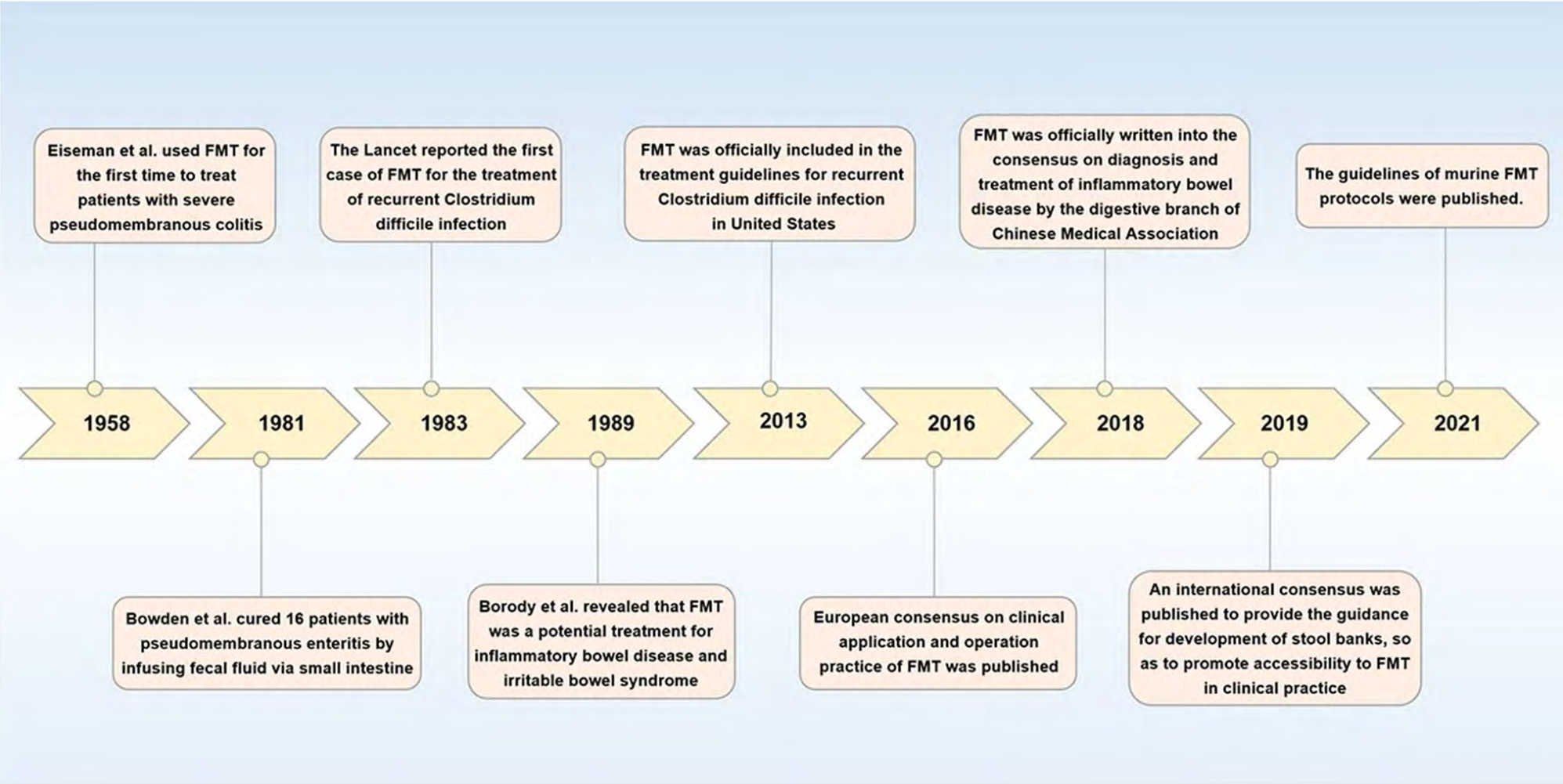


Klebsiella pneumoniae burden after treatment with predatory bacteria

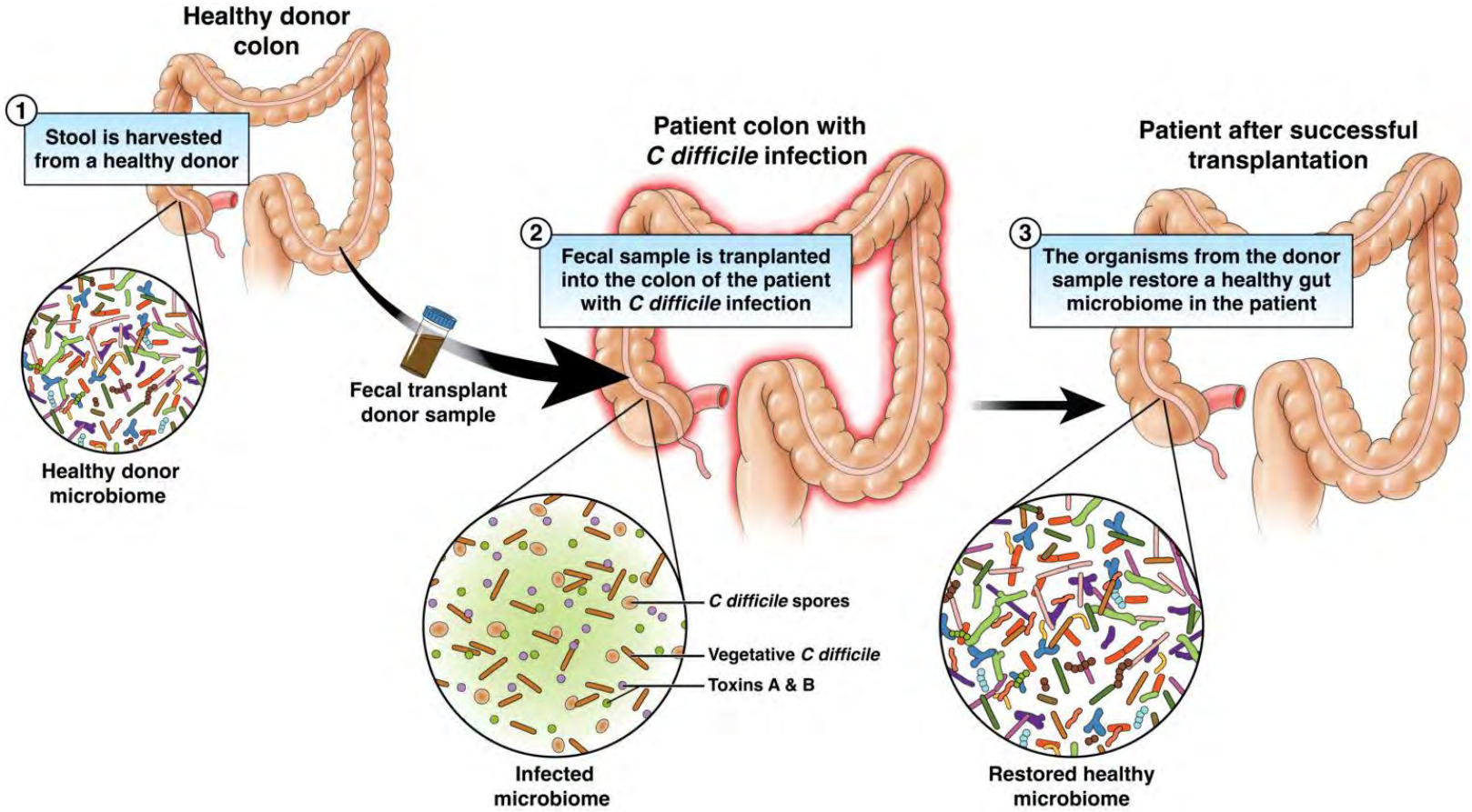


No significant difference in reducing bacterial *(K.pneumoniae)* burden after treatment with predatory bacteria *; B.bacteriovorus (109J, HD100) or M.aeruginosavorus(MICA) as compared with control (PBS) within 24 hours for acute bloodstream infection.*

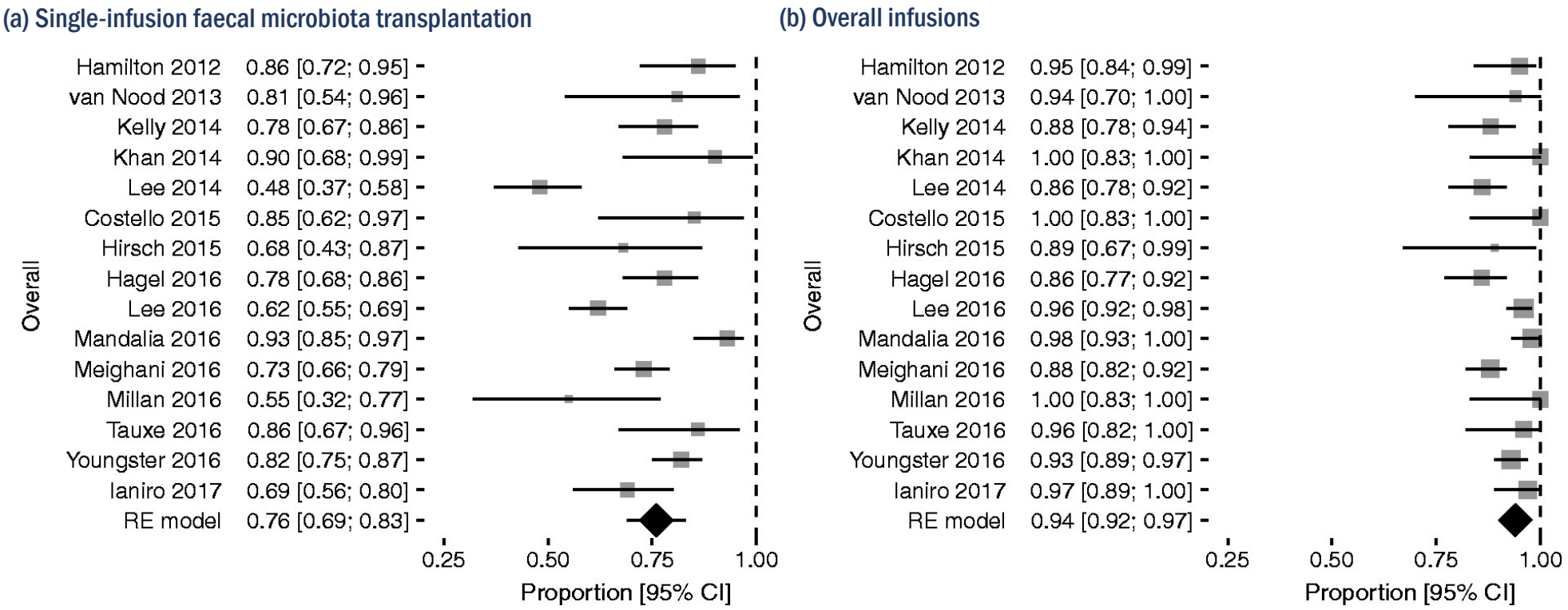
Fecal microbiota transplantation (FMT)



Fecal microbiota transplantation (FMT)

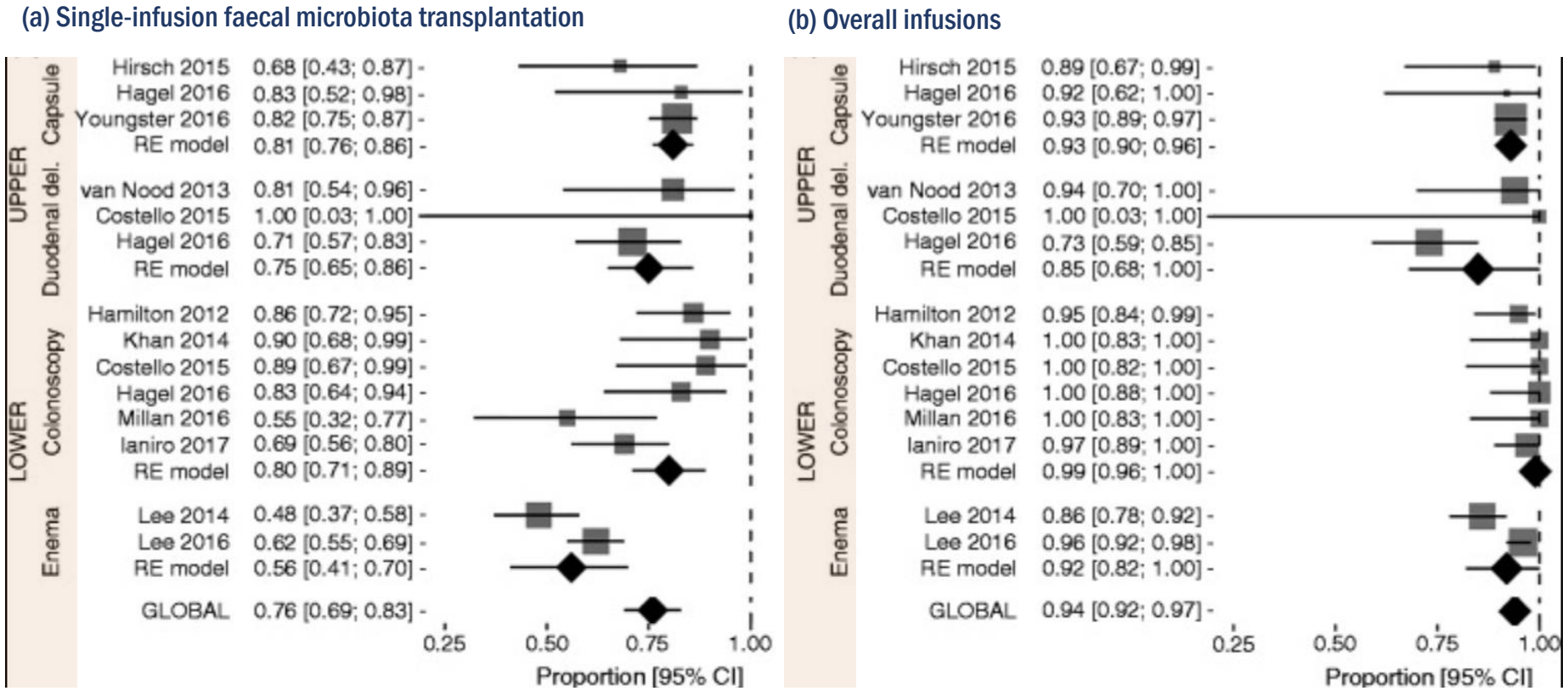


Systematic reviews of FMT



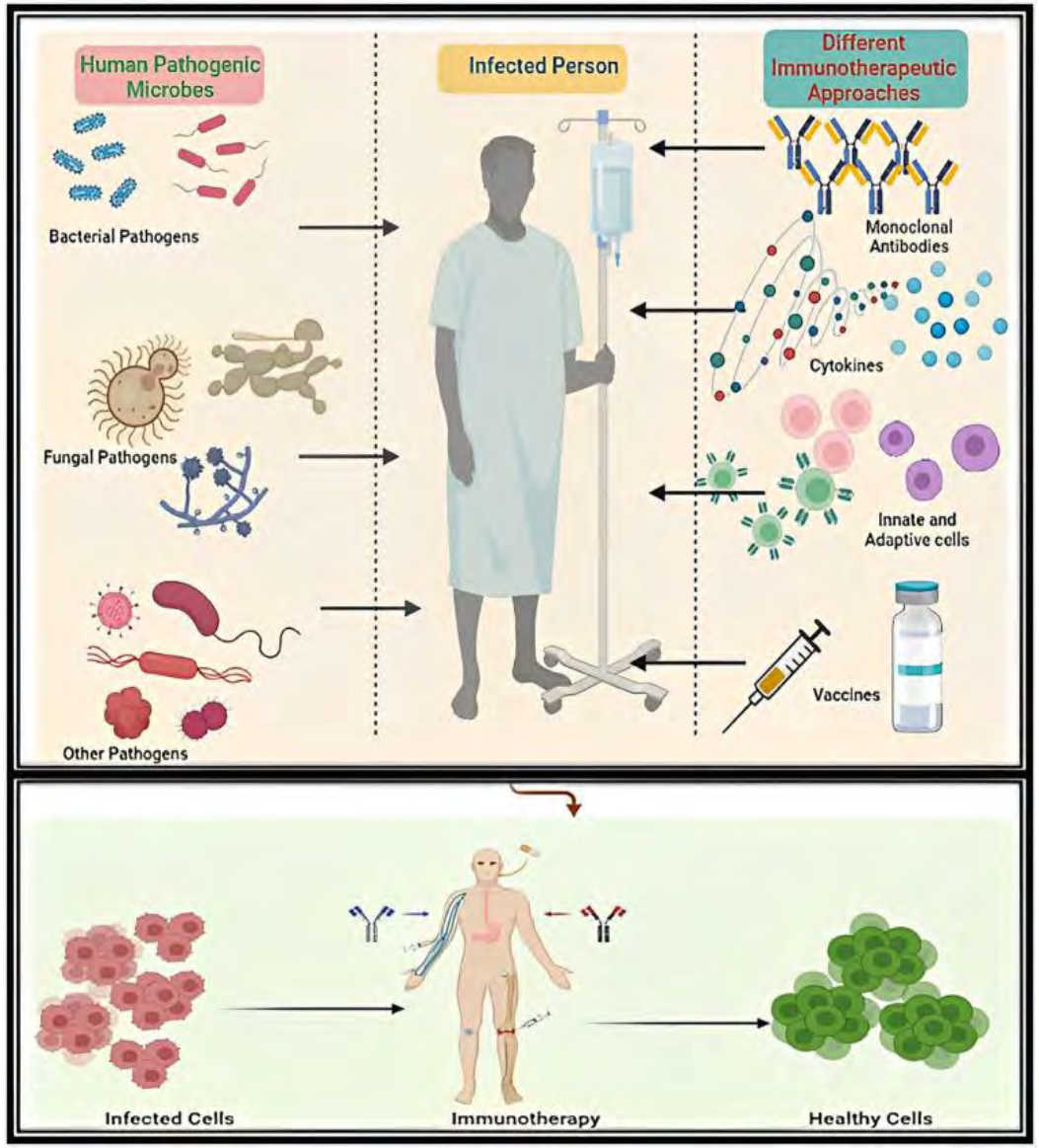
Resolution of *C.difficile* infection is higher with two doses (99%) compared to one dose (55%)

Systematic reviews – route of delivery



Resolution of *C.difficile* infection differs with different routes of delivery

Therapeutic potential of different types of immunotherapies



Monoclonal Abs for human infections

For P.aeruginosa pneumonia

MAb - bispecific IgG1 antibody
Targets:
1. Pseudomonas Pcvr protein
2. Pslexopolysaccharide

Name	Bacterial Species Targeted	Company	Development Phase
AR301	*Staphylococcus aureus*	Aridis Pharmaceuticals	Phase 2 Complete Ongoing Phase 3
MEDI4893	*Staphylococcus aureus*	Medimmune	Phase 2 Complete
MEDI3902	*Pseudomonas aeruginosa*	Medimmune	Phase 1 Complete Ongoing Phase 2
AR101	*Pseudomonas aeruginosa*	Aridis Pharmaceuticals	Phase 1 Complete Ongoing Phase 2
514G3	*Staphylococcus aureus*	XBiotech	Phase 2
ARN-100	*Staphylococcus aureus*	Arsansis	Phase 2 Halted
PolyCAb	*Clostridium difficile*	MicroPharm	Phase 1
RG7861	*Staphylococcus aureus*	Roche	Phase 1
TRL1068	Biofilm—multiple species	Trellis Bioscience	Preclinical Entering Phase 1
AR401-mAb	*Acinetobacter baumannii*	Aridis Pharmaceuticals	Preclinical
VXD-003	*Acinetobacter baumannii*	VaxDyn	Preclinical
Cd-ISTAb	*Clostridium difficile*	Integrated BioTherapeutics	Preclinical
ASN-4	*Escherichia coli* (ST131)	Arsansis—Outlicensed to BB100	Preclinical
ASN-5	*K. pneumoniae*	Arsansis—Outlicensed to BB200	Preclinical

Companies currently pursuing Hu-mAb therapy for bacterial infections caused by ESKAPEE pathogens and *Clostridum difficile*—products and stage of development.

Monoclonal Abs for human infections

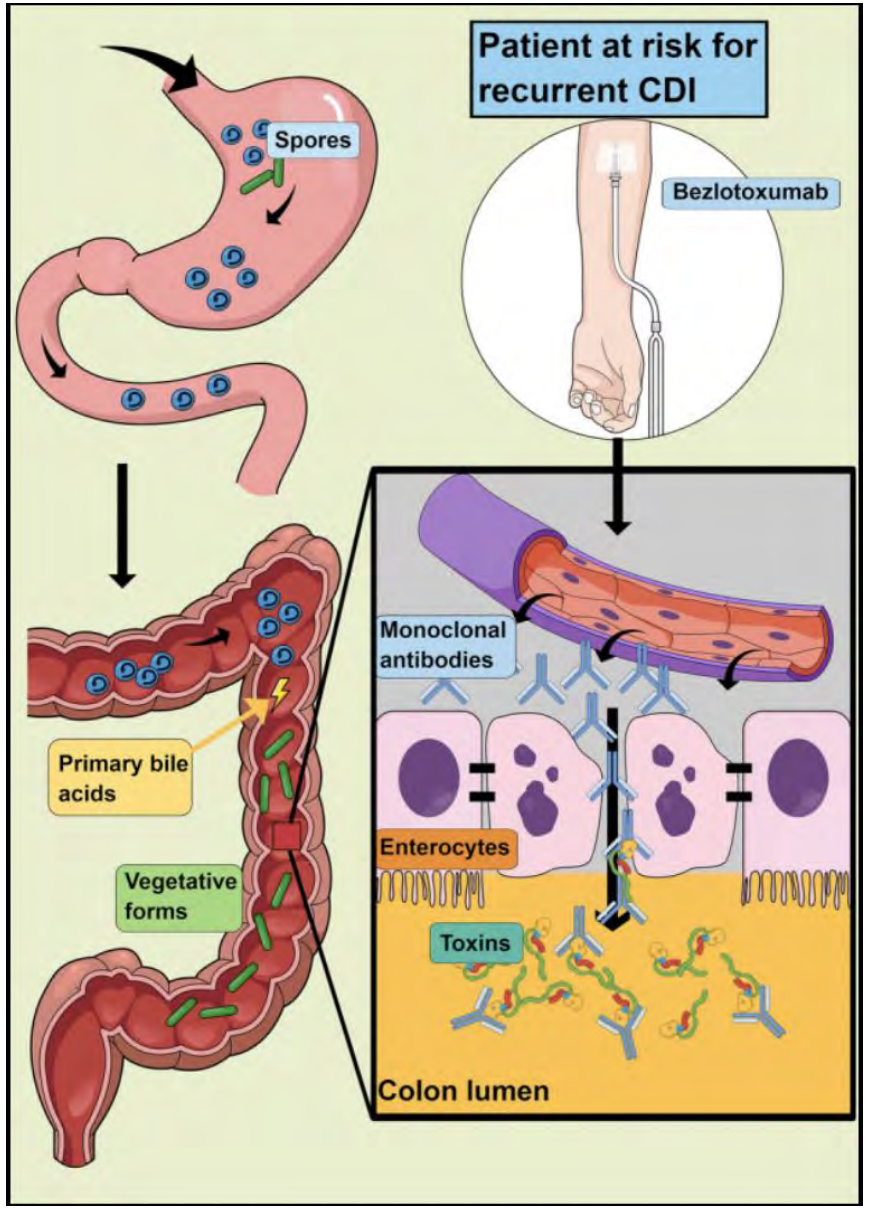
For MRSA pneumonia
MAb with alpha-toxin neutralizing capabilities

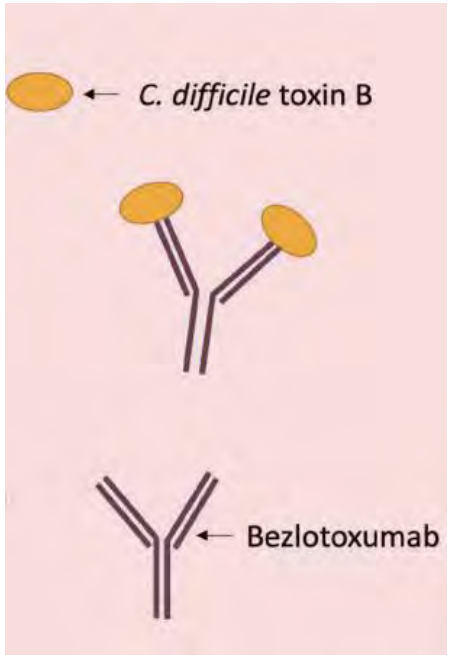
For S.aureus infection
Target: alpha-toxin

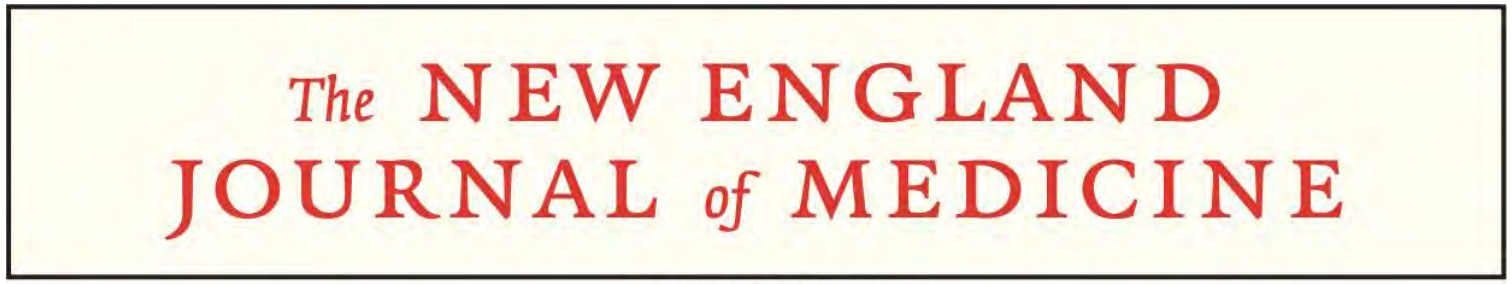
Name	Bacterial Species Targeted	Company	Development Phase
AR301	*Staphylococcus aureus*	Aridis Pharmaceuticals	Phase 2 Complete Ongoing Phase 3
MEDI4893	*Staphylococcus aureus*	Medimmune	Phase 2 Complete
MEDI3902	*Pseudomonas aeruginosa*	Medimmune	Phase 1 Complete Ongoing Phase 2
AR101	*Pseudomonas aeruginosa*	Aridis Pharmaceuticals	Phase 1 Complete Ongoing Phase 2
514G3	*Staphylococcus aureus*	XBiotech	Phase 2
ARN-100	*Staphylococcus aureus*	Arsansis	Phase 2 Halted
PolyCAb	*Clostridium difficile*	MicroPharm	Phase 1
RG7861	*Staphylococcus aureus*	Roche	Phase 1
TRL1068	Biofilm—multiple species	Trellis Bioscience	Preclinical Entering Phase 1
AR401-mAb	*Acinetobacter baumannii*	Aridis Pharmaceuticals	Preclinical
VXD-003	*Acinetobacter baumannii*	VaxDyn	Preclinical
Cd-ISTAb	*Clostridium difficile*	Integrated BioTherapeutics	Preclinical
ASN-4	*Escherichia coli* (ST131)	Arsansis—Outlicensed to BB100	Preclinical
ASN-5	*K. pneumoniae*	Arsansis—Outlicensed to BB200	Preclinical

Companies currently pursuing Hu-mAb therapy for bacterial infections caused by ESKAPEE pathogens and *Clostridum difficile*—products and stage of development.

Bezlotoxumab for C.difficile infection (CDI)



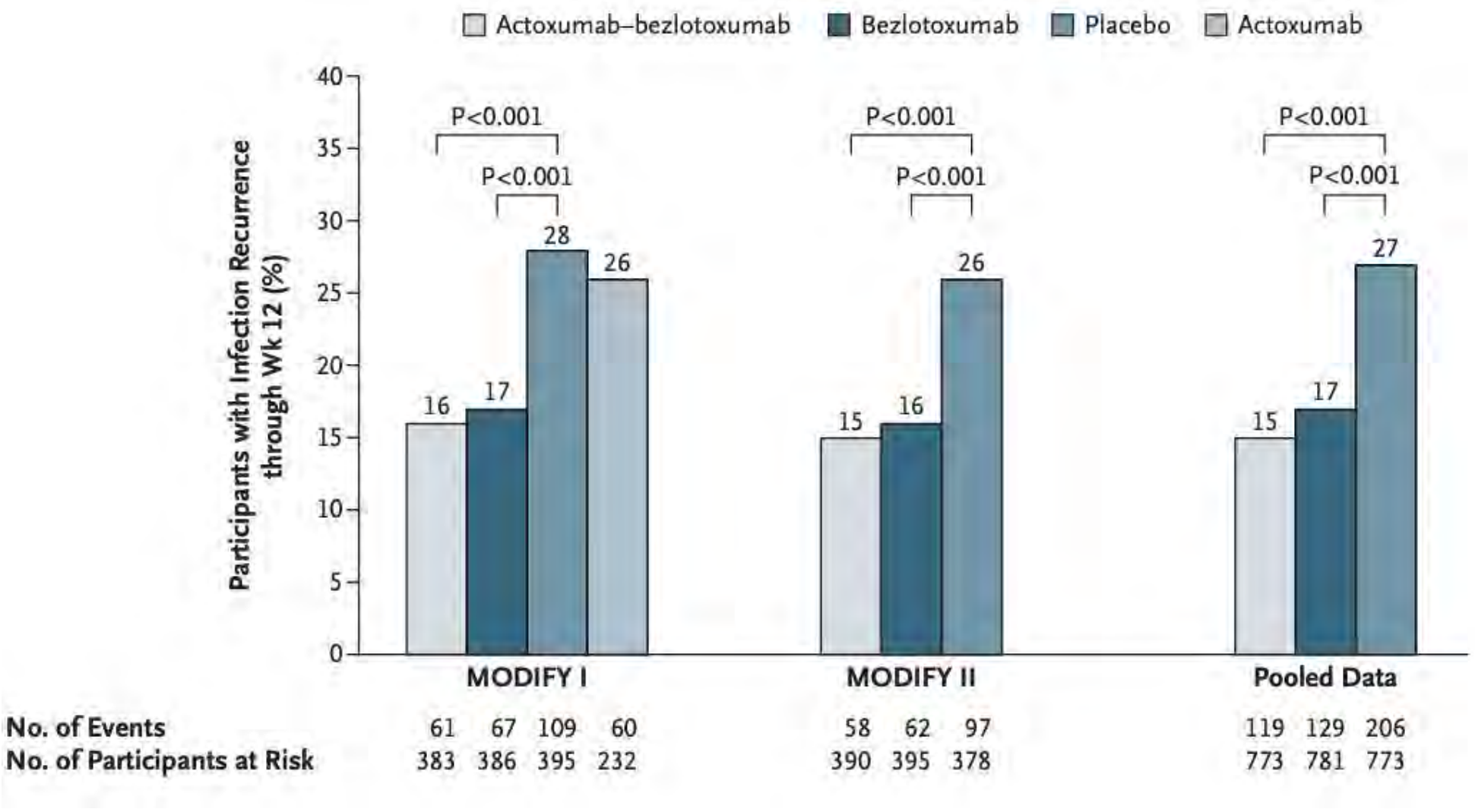




Bezlotoxumab for Prevention of Recurrent *Clostridium difficile* Infection

M.H. Wilcox, D.N. Gerding, I.R. Poxton, C. Kelly, R. Nathan, T. Birch, O.A. Cornely, G. Rahav, E. Bouza, C. Lee, G. Jenkin, W. Jensen, Y.-S. Kim, J. Yoshida, L. Gabryelski, A. Pedley, K. Eves, R. Tipping, D. Guris, N. Kartsonis, and M.-B. Dorr, for the MODIFY I and MODIFY II Investigators*

- Two double-blind, randomized, placebo-controlled, phase III trials - MODIFY I and MODIFY II
- 2655 adults receiving oral standard-of-care (SOC) antibiotics for primary or recurrent C.difficile infection (CDI)
- Randomized into four arms - Bezlotoxumab , Actoxumab + Bezlotoxumab, Actoxumab, placebo (0.9% saline)
- Primary end point – recurrent infection within 12 weeks after infusion in mITT group

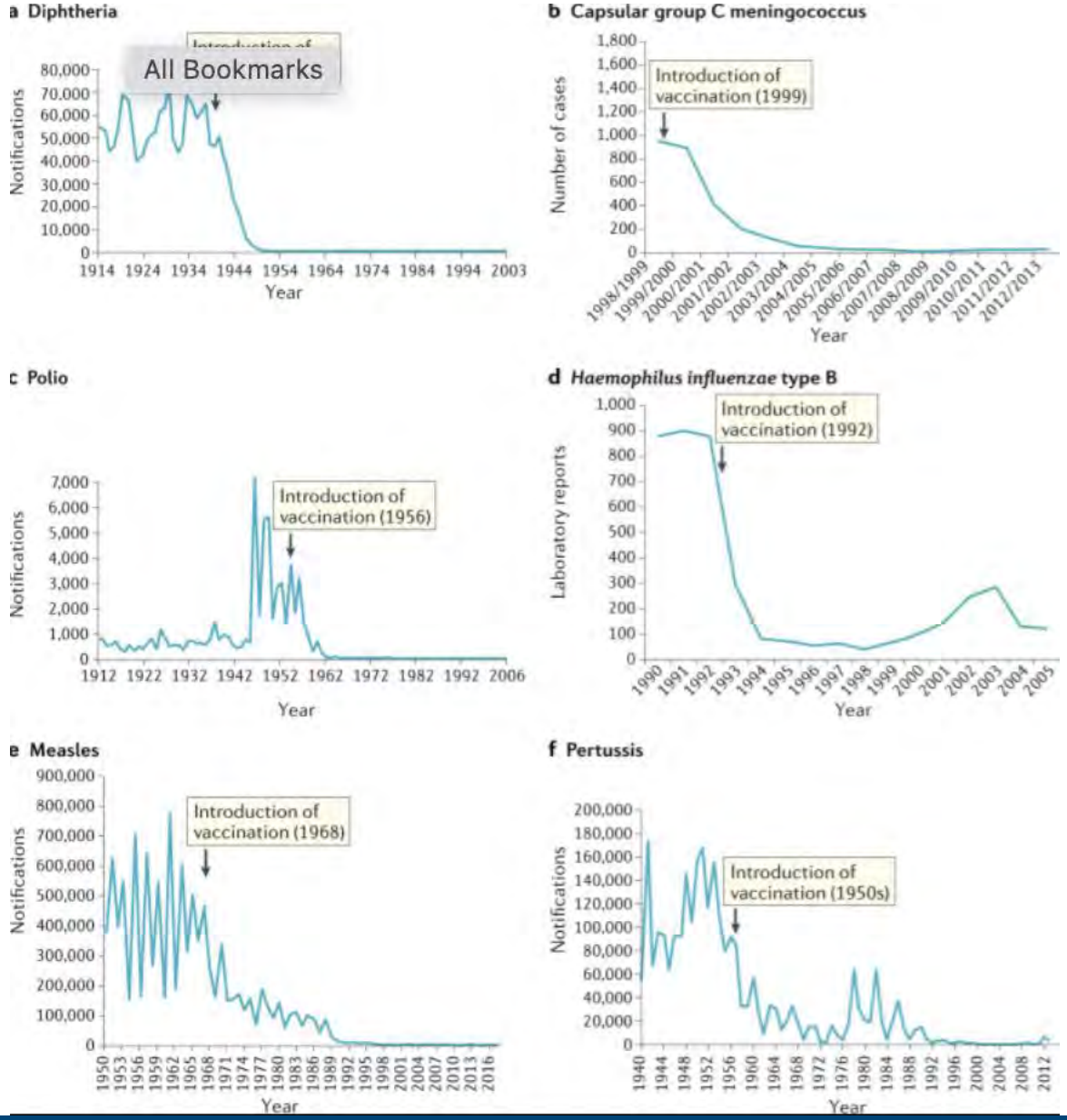


CDI significantly lower with Bezlotoxumab alone vs placebo (MODIFY I: 17%, MODIFY II: 16%), p<0.001

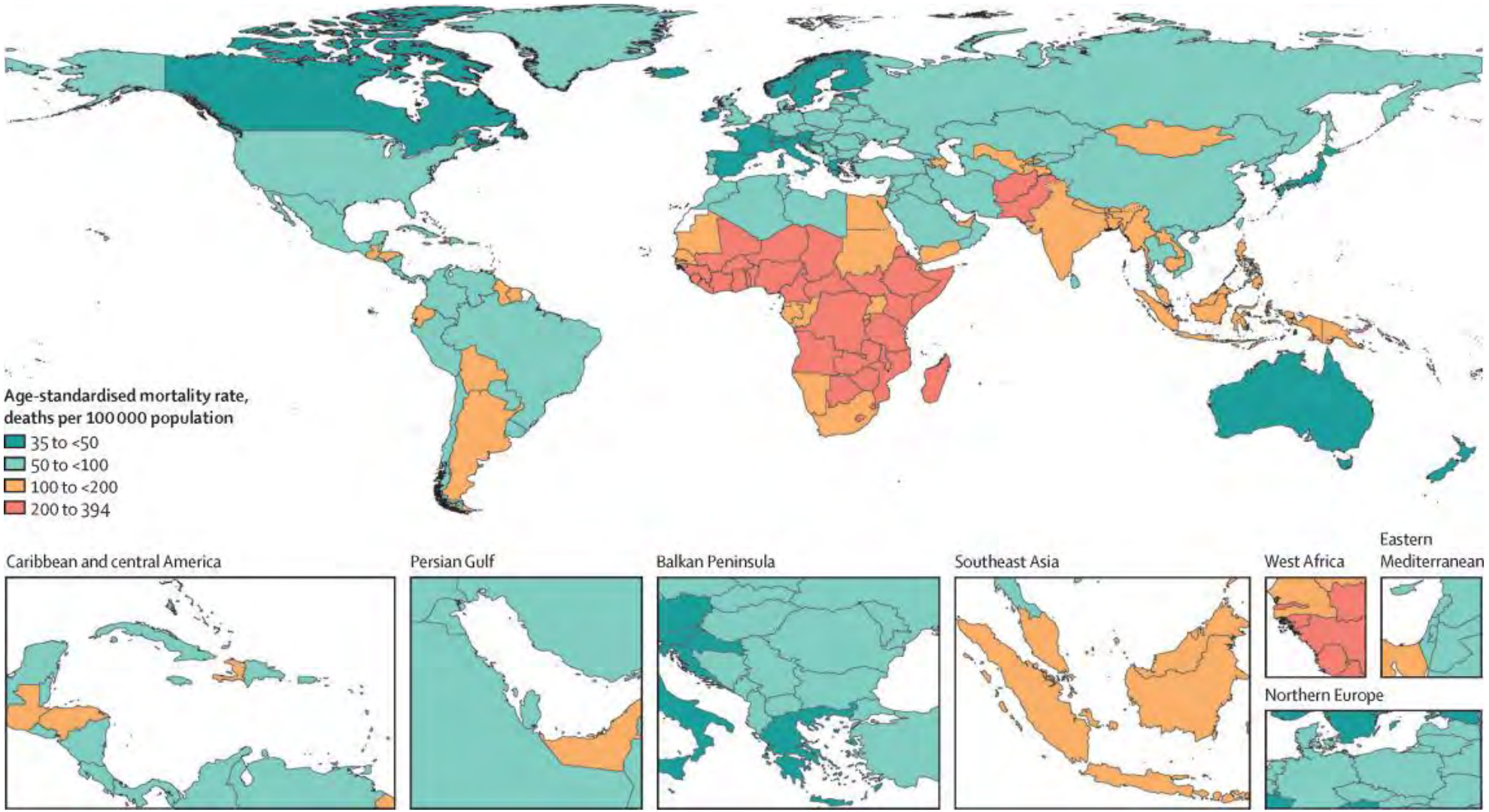
Guidelines comparison for CDI management

	2021 ACG	Strength/Quality	2021 IDSA/SHEA	Strength/Quality	2021 ESCMID	Strength/Quality
Initial Occurrence (non-severe)	Vancomycin preferred	Strong/Low	Fidaxomicin preferred	Conditional/Moderate	Fidaxomicin preferred	Strong/Moderate
	Fidaxomicin preferred	Strong/Moderate	Vancomycin alternative	Strong/High ¥	Vancomycin alternative	Strong/High
	Metronidazole alternative	Strong/Moderate	Metronidazole alternative	Weak/High ¥	Metronidazole alternative	Strong/High
Initial Occurrence (severe)	Vancomycin preferred	Strong/Low	Fidaxomicin preferred	Conditional/Moderate	Vancomycin or Fidaxomicin equal	Good Practice Statement
	Fidaxomicin preferred	Conditional/Very Low	Vancomycin alternative	Strong/High ¥		
First Recurrence	Vancomycin preferred	Strong/Very Low	Fidaxomicin preferred	Conditional/Moderate	Fidaxomicin preferred *	Strong/Low
	Fidaxomicin preferred *	Conditional/Moderate	Vancomycin alternative	Weak/Low ¥	Vancomycin + BEZ Fidaxomicin + BEZ	Weak/Moderate Good Practice Statement
Subsequent Recurrence(s)	FMT preferred **	Strong/Moderate	Fidaxomicin preferred	Conditional/Low	FMT preferred	Weak/Moderate
			Vancomycin alternative	Weak/Low ¥	Vancomycin + BEZ Fidaxomicin + BEZ	Weak/Low
Role of BEZ	High risk only	Conditional/Moderate	Recurrence within 6 months	Conditional/Very Low	High risk initial episode with vancomycin	Weak/Moderate
					Recurrences	Weak/Low
Role of Fecal Microbiota Transplant (FMT)	3+ total CDI	Strong/Moderate	3+ total CDI	Strong/Moderate ¥	3+ total CDI	Weak/Moderate
Role of Probiotics	Recommend against	Conditional/Moderate	No recommendation	-	Recommend Against	Strong/Low

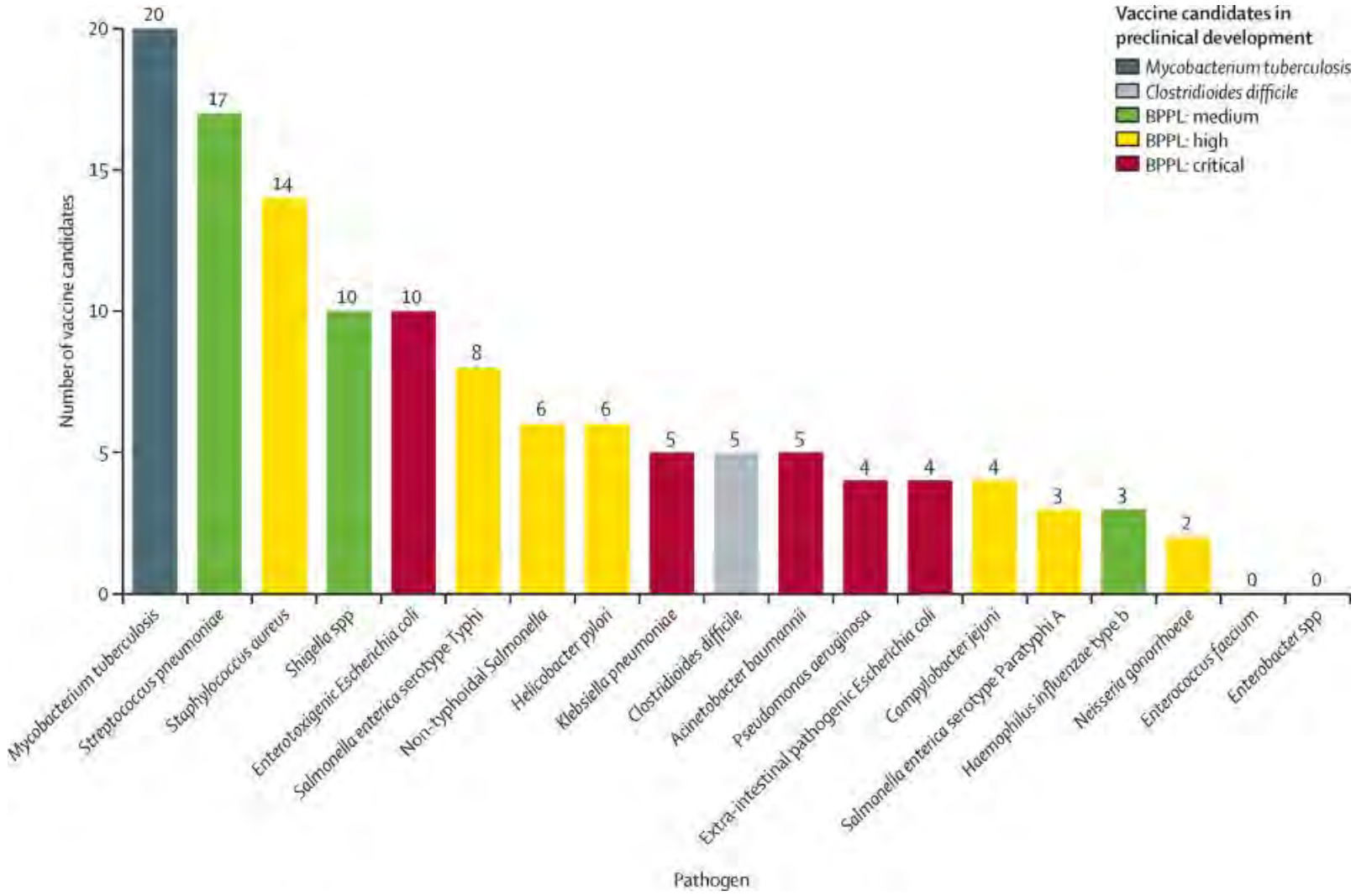
Vaccination impact (UK)



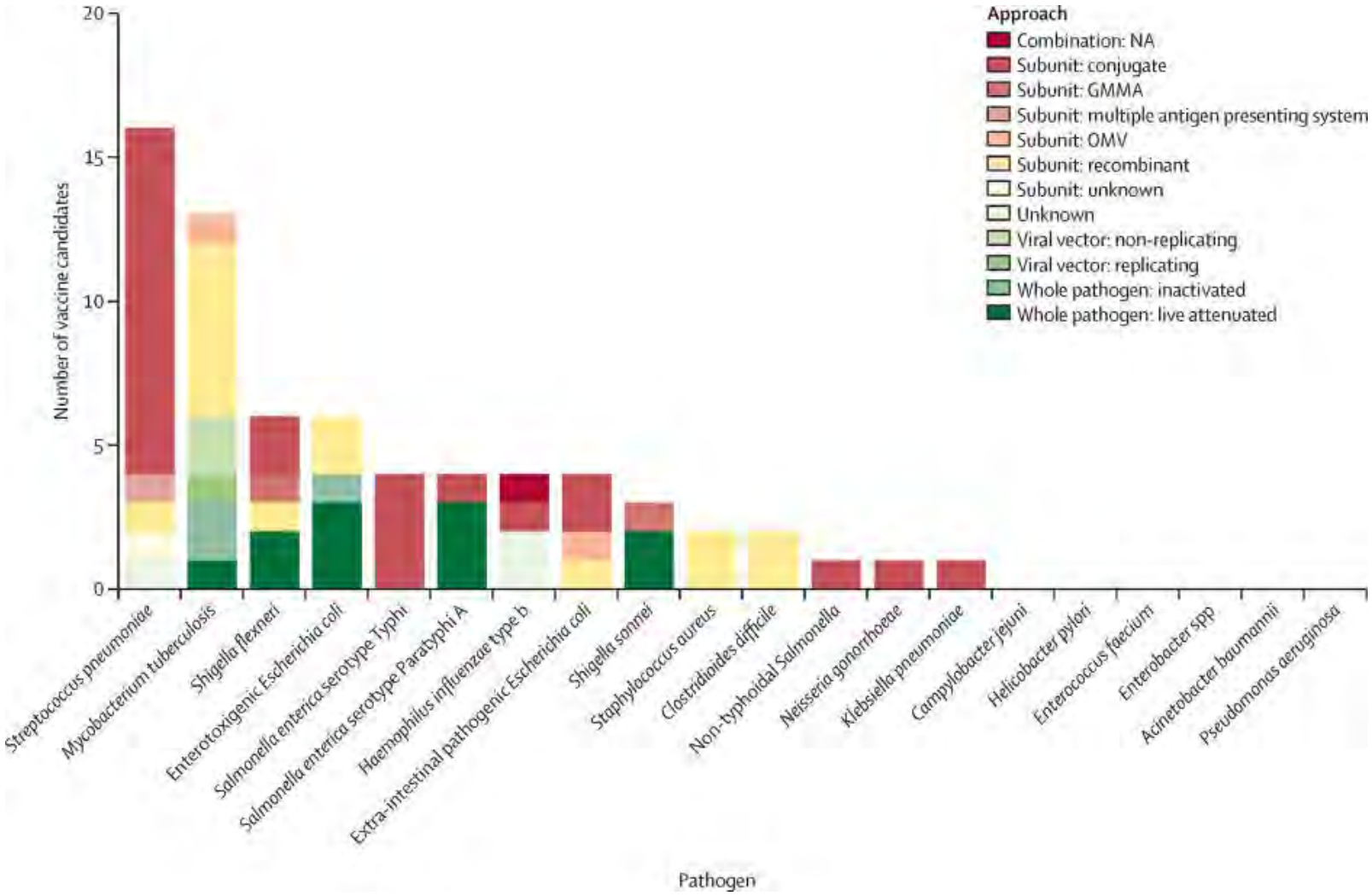
Overall age-standardized mortality rate per 100,000 population



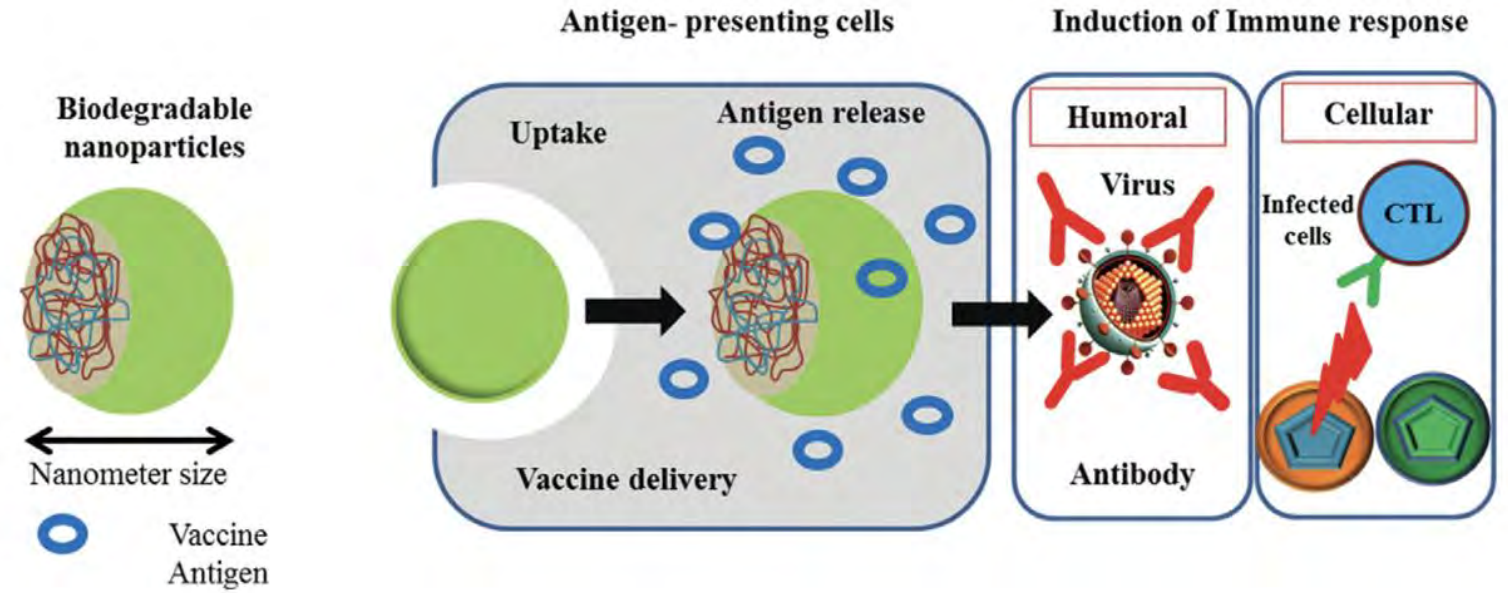
Vaccine candidates in preclinical development



Vaccine candidates in active clinical development



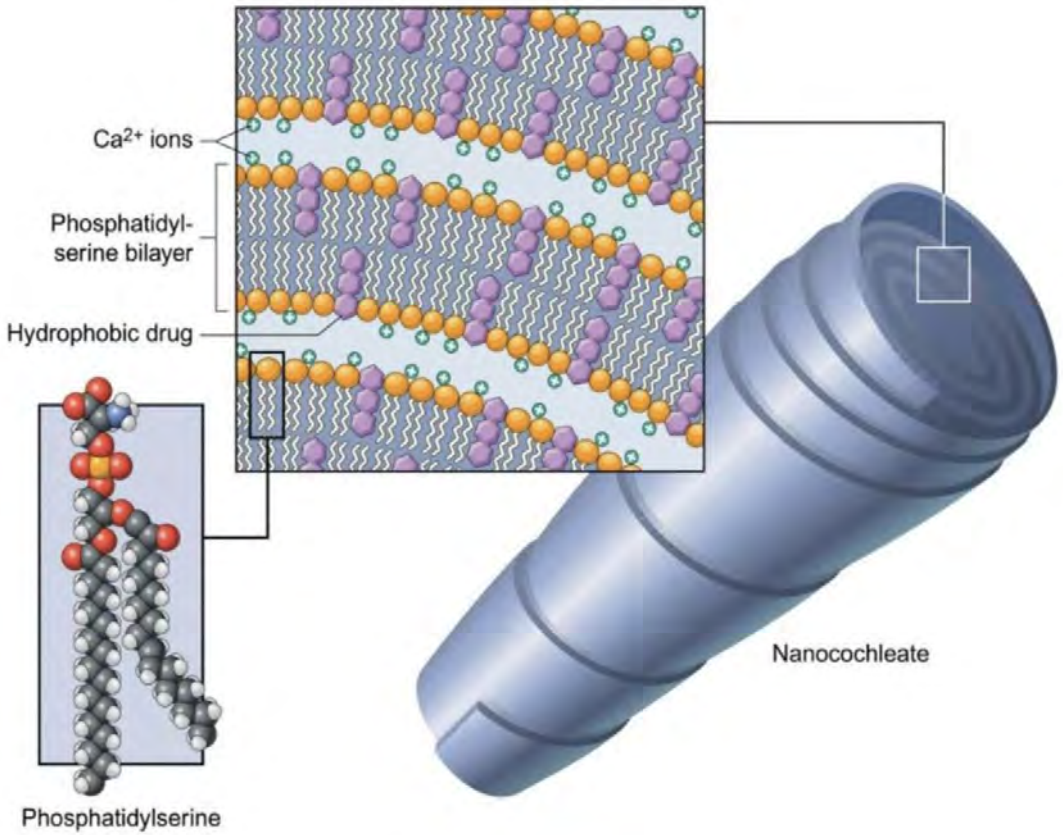
Induction of immune responses by Nanoparticle-based vaccine

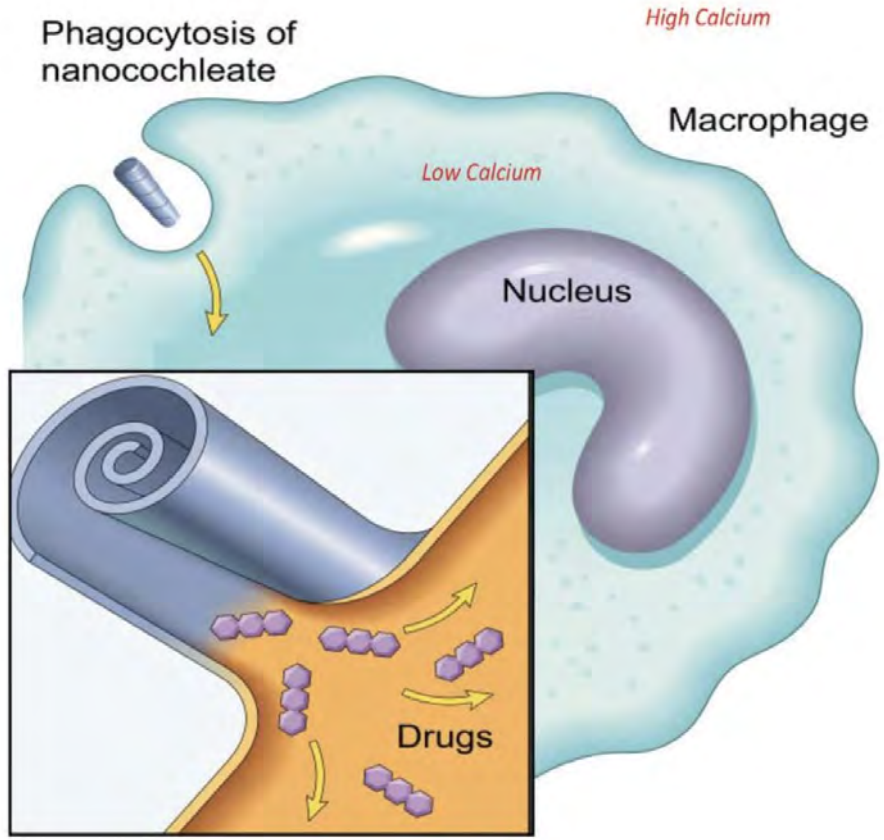


Nanomaterial-based vaccines against bacterial infections

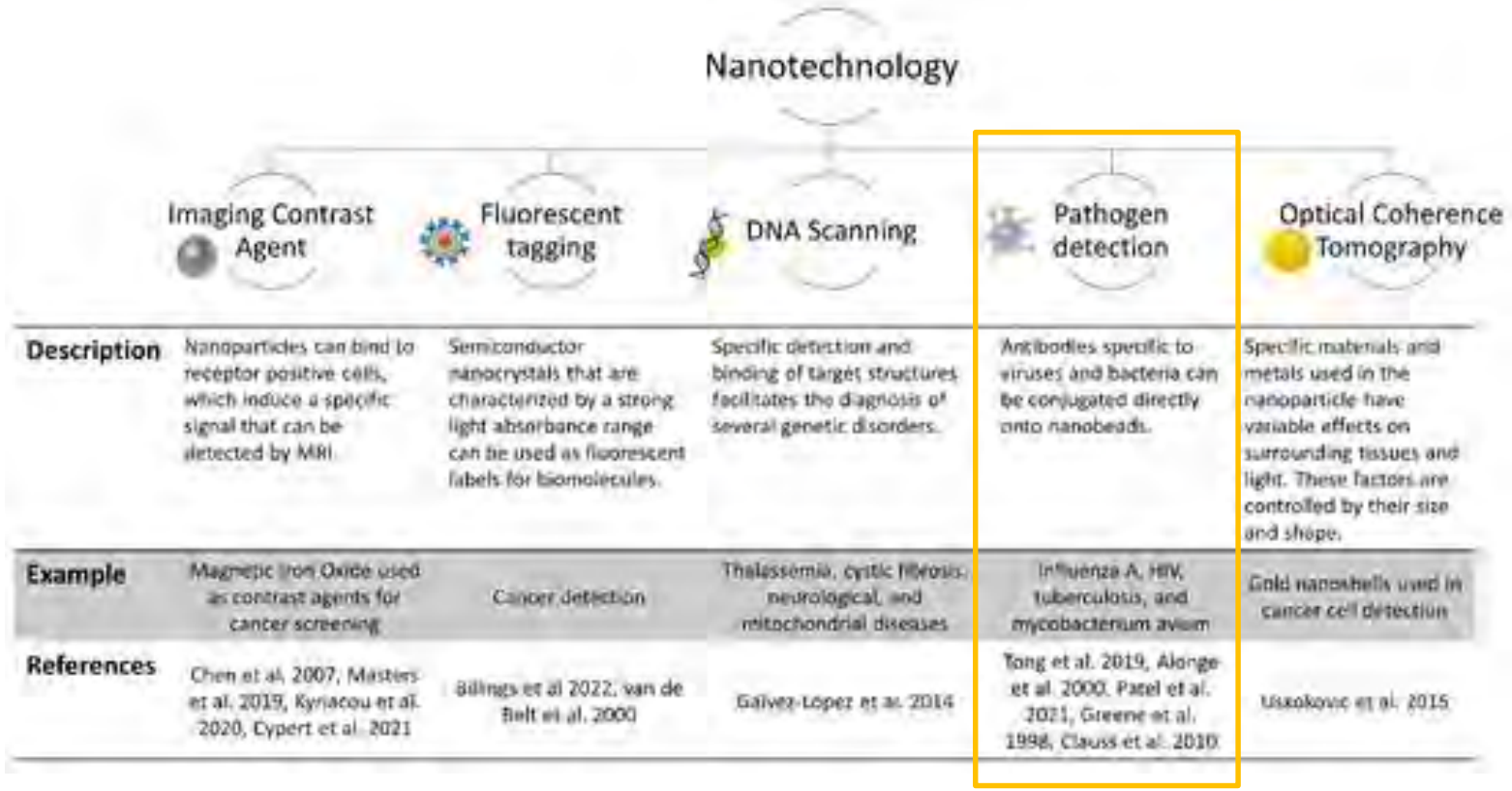
Antigen	Nanocarrier used	Disease
Antigenic protein	PLGA nanospheres	Anthrax
DNA encoding T cell epitopes of Esat-6 and FL	Chitosan nanoparticle	Tuberculosis
Mycobacterium lipids	Chitosan nanoparticle	Tuberculosis
Polysaccharides	Liposomes	Pneumonia
Bacterial toxic and parasitic protein	Liposomes	Cholera and malaria
Fusion protein	Liposomes	*H. pylori*
Antigenic protein	Nano-emulsion	Cystic fibrosis, Anthrax
Mycobacterium fusion protein	Liposome	Tuberculosis
Flagellin protein	AuNPs	*Yersinia pestis*, *S. pneumoniae*
Antigenic protein	Cationic liposome-based, stabilized with synthetic glycolipid (CAF01)	Tuberculosis
Plasmid DNA encoding BoNT heavy chain (Hc)	PLGA	*Clostridium botulinum*
Capsular polysaccharide serotype 14 and T-helper peptide, ovalbumin 323–339 peptide	AuNPs with branched tetra-saccharide unit b-D-Galp-(1–4)-b-D-Glcp-(1–6)-[b-D-Galp-(1–4)-] b-D-GlcpNAc-(1–)	*S. pneumonia*
LomW and EscC	AuNPs	*E. coli (EHEC)*
Listeriolysin O (91–99) and glyceraldehyde-3-phosphate-dehydrogenase (1–22) peptide	AuNPs	*Listeria monocytogenes*
Hemagglutinin and FlgL	AuNP coated with antigenic capsular LPS	*Burkholderia pseudomallei*
N-terminal domains flagellin (1–161)	AuNPs	*P. aeruginosa*
Monosialotetrahexosylganglioside (GM1), host receptor for cholera toxin	PLGA	*Vibrio cholerae*
Serogroup B	OMV-based vaccine	*N. meningitidis*
Membrane proteins	Double-layered membrane vesicles	*P. aeruginosa*
Immunodominant antigens (Ag85A & ESAT-6) and IL-21	Fe_2O_3 coated plasmid DNA TB vaccine	*M. tuberculosis*
Recombinant fusion protein (M72)	Liposomes	*M. tuberculosis*
Heat-induced OMV from enterotoxigenic *E. coli*	Poly(anhydride) NPs	*E. coli*

Nanoparticle-based Encochelated Amphotericin B



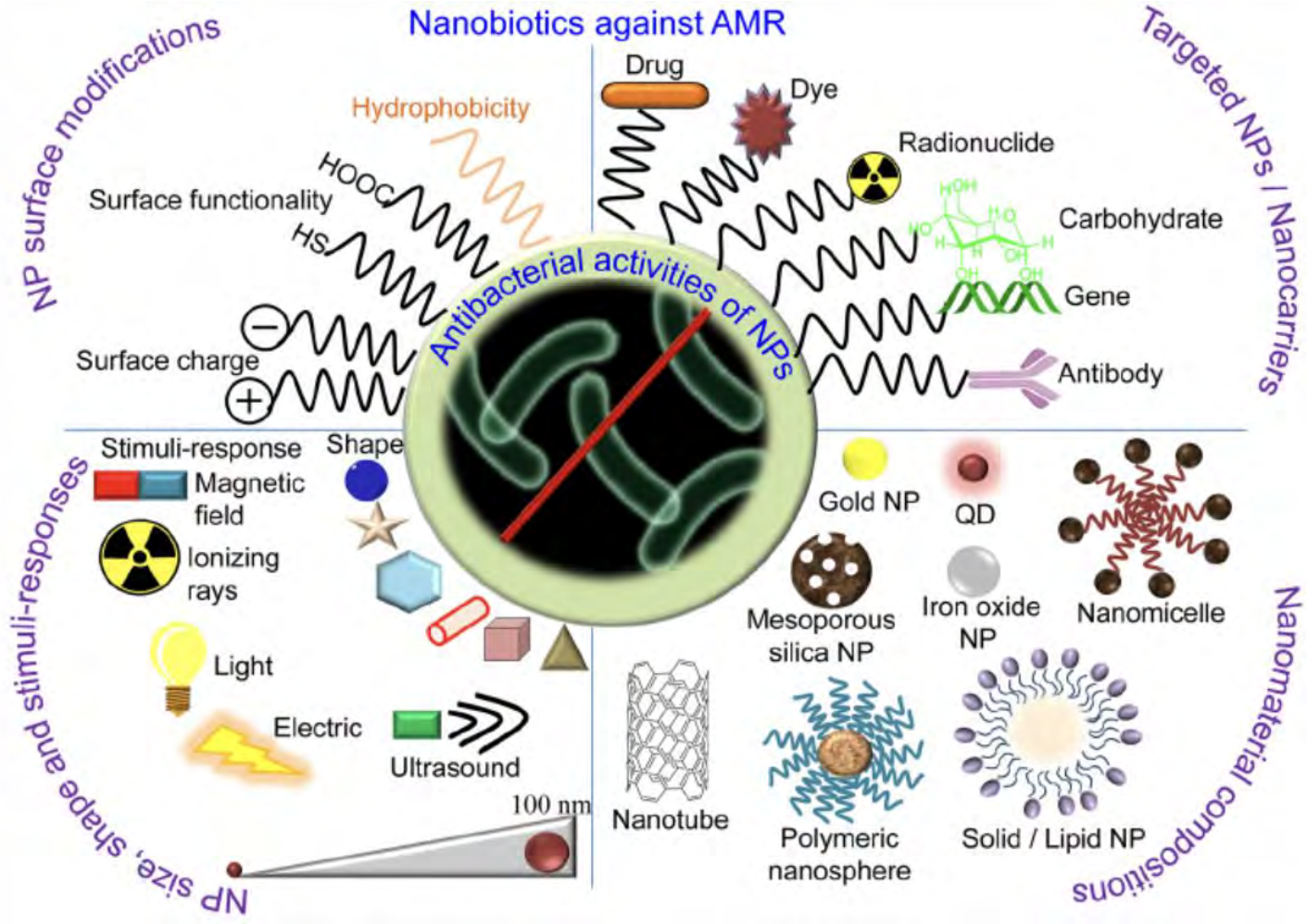


Nanotechnology - diagnostics

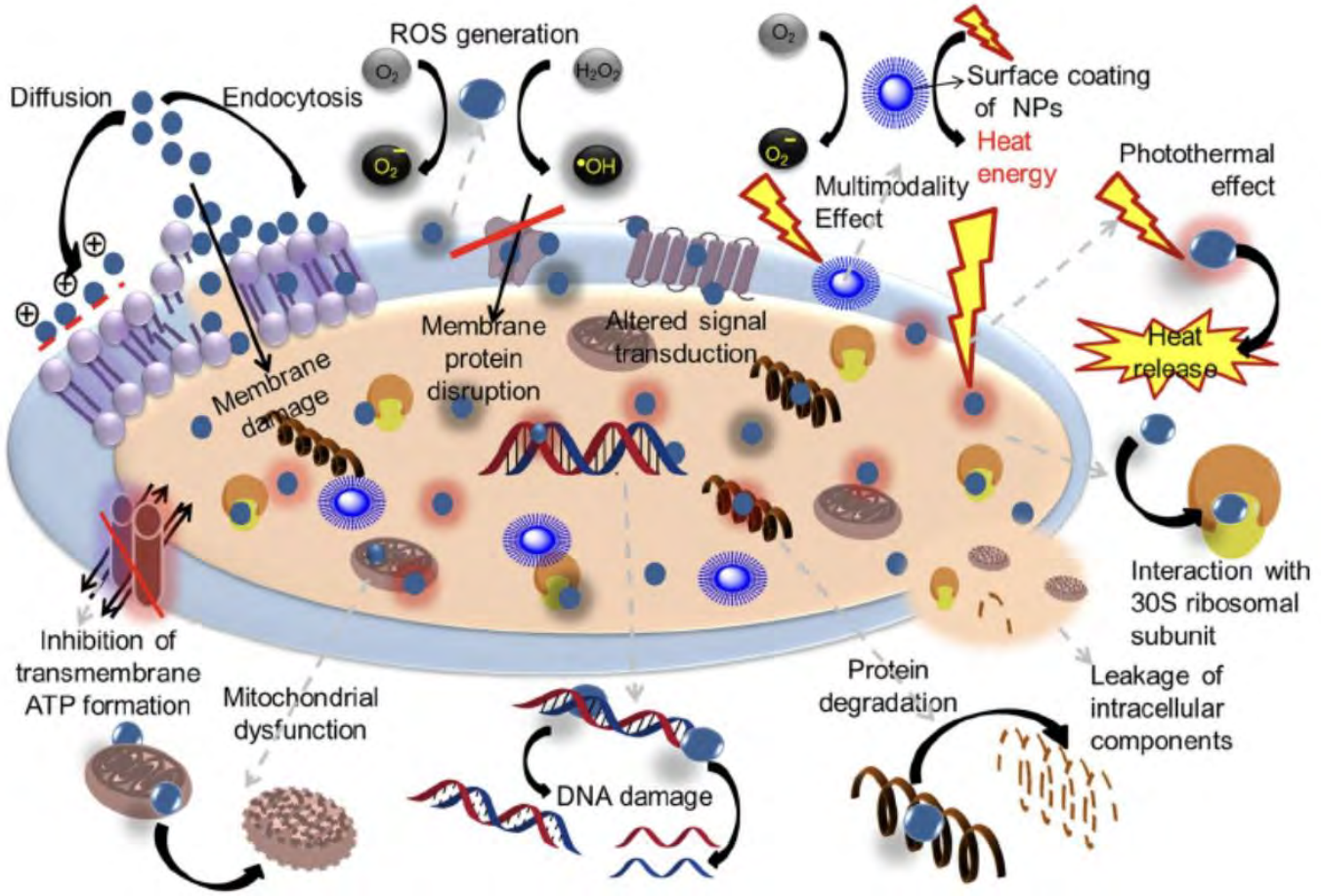


	Imaging Contrast Agent	Fluorescent tagging	DNA Scanning	Pathogen detection	Optical Coherence Tomography
Description	Nanoparticles can bind to receptor positive cells, which induce a specific signal that can be detected by MRI.	Semiconductor nanocrystals that are characterized by a strong light absorbance range can be used as fluorescent labels for biomolecules.	Specific detection and binding of target structures facilitates the diagnosis of several genetic disorders.	Antibodies specific to viruses and bacteria can be conjugated directly onto nanobeads.	Specific materials and metals used in the nanoparticle have variable effects on surrounding tissues and light. These factors are controlled by their size and shape.
Example	Magnetic Iron Oxide used as contrast agents for cancer screening	Cancer detection	Thalassemia, cystic fibrosis, neurological, and mitochondrial diseases	Influenza A, HIV, tuberculosis, and mycobacterium avium	Gold nanoshells used in cancer cell detection
References	Chen et al. 2007, Masters et al. 2019, Kyriacou et al. 2020, Cypert et al. 2021	Billings et al 2022, van de Belt et al. 2000	Galvez-Lopez et al. 2014	Tong et al. 2019, Alonge et al. 2000, Patel et al. 2021, Greene et al. 1998, Clauss et al. 2010	Uskokovic et al. 2015

Nanobiotic properties against AMR



Nanobiotic properties against AMR



Summary

1. Probiotics– shows potential, currently not recommended for CDI
2. Antimicrobial peptides – some currently used as antibiotics with more in development
3. Bacteriophages – shows potential , currently not FDA approved for humans
4. Predatory bacteria – ambiguous results and more studies needed
5. Fecal microbiota transplant - currently useful for CDI
6. Immunological compounds – successful old school
7. Vaccines – have changed the route of human history
8. Nanotechnology – shows great potential

References

1. Alsobhi, G. (2021). Bacteriophages as affordable solution for treatment of multidrug resistant bacteria, and their recent potential applications. *Novel Research in Microbiology Journal*, *5*, 1405–1414.
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