PATHOPHYSIOLOGY AND PREVENTION OF NECROTISING ENTEROCOLITIS

Daynia Ballot

Division of neonatology

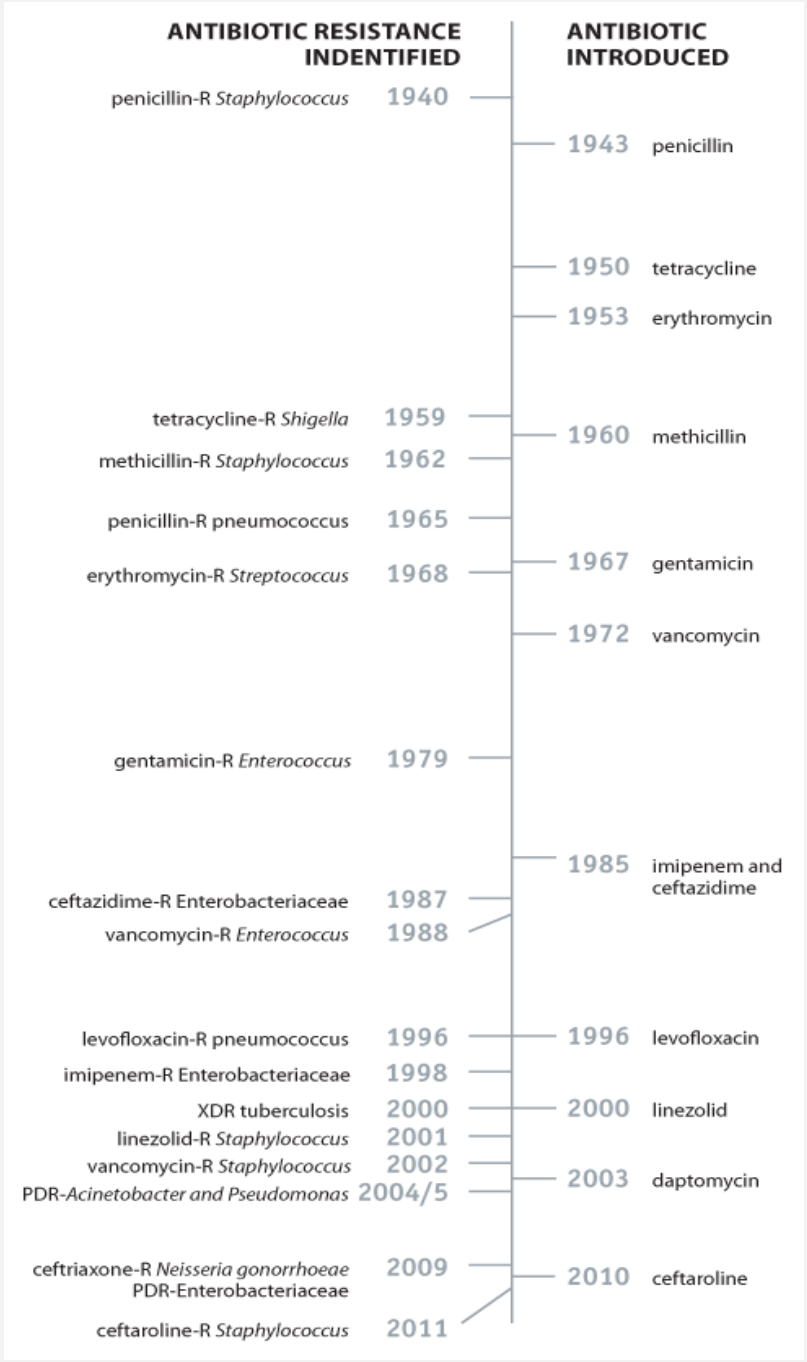
Department of Paediatrics and Child Health

CMJAH.

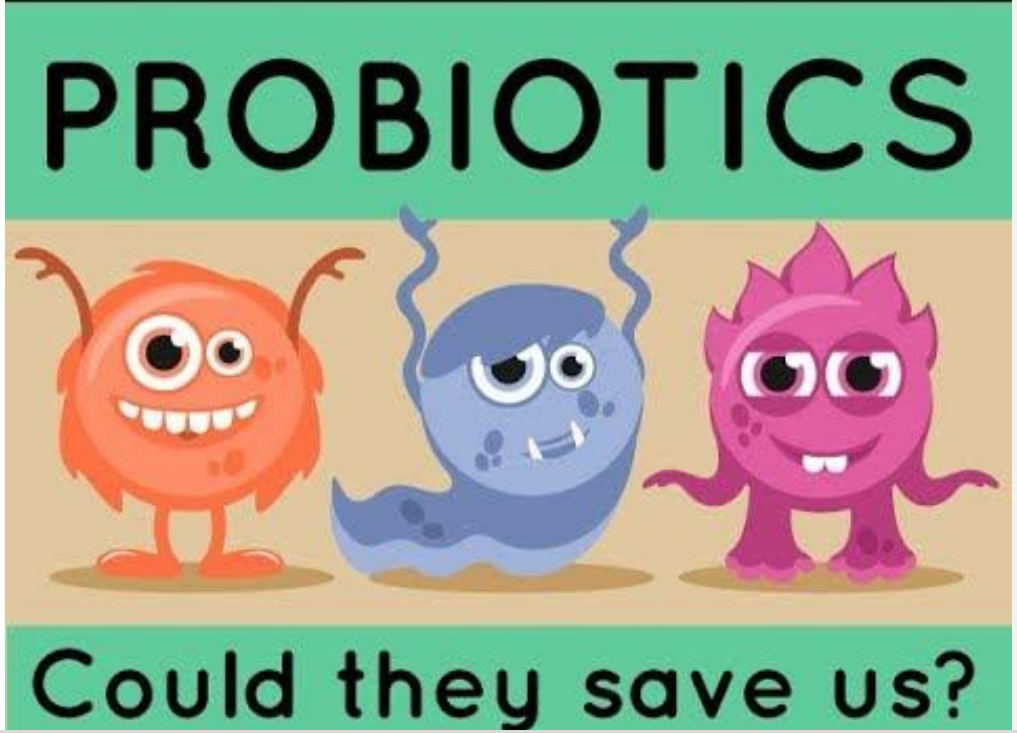
University of the Witwatersrand

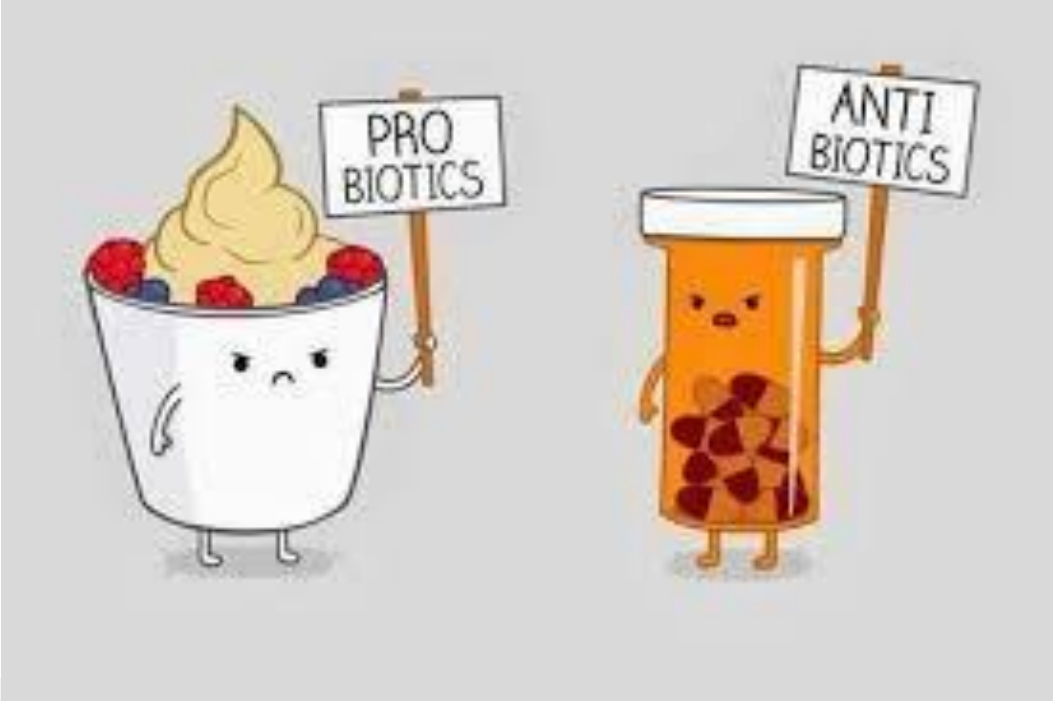
HISTORY LESSON

- 1796 – Edward Jenner vaccinated a milkmaid against smallpox with cowpox
- 1818 – Semmelweiss – handwashing to decrease puerperal fever mortality
- 1861 – Louis Pasteur – Germ theory
- 1928 - Alexander Fleming – Penicillin


ANTIBIOTIC RESISTANCE INDENTIFIED
ANTIBIOTIC INTRODUCED
penicillin-R Staphylococcus 1940
1943 penicillin
1950 tetracycline
1953 erythromycin
tetracycline-R Shigella 1959
1960 methicillin
methicillin-R Staphylococcus 1962
penicillin-R pneumococcus 1965
1967 gentamicin
erythromycin-R Streptococcus 1968
1972 vancomycin
gentamicin-R Enterococcus 1979
1985 imipenem and ceftazidime
ceftazidime-R Enterobacteriaceae 1987
vancomycin-R Enterococcus 1988
levofloxacin-R pneumococcus 1996
1996 levofloxacin
imipenem-R Enterobacteriaceae 1998
XDR tuberculosis 2000
2000 linezolid
linezolid-R Staphylococcus 2001
vancomycin-R Staphylococcus 2002
2003 daptomycin
PDR-Acinetobacter and Pseudomonas 2004/5
ceftriaxone-R Neisseria gonorrhoeae 2009
PDR-Enterobacteriaceae
2010 ceftaroline
ceftaroline-R Staphylococcus 2011


We're not
ALL BAD!!


PROBIOTICS
Could they save us?

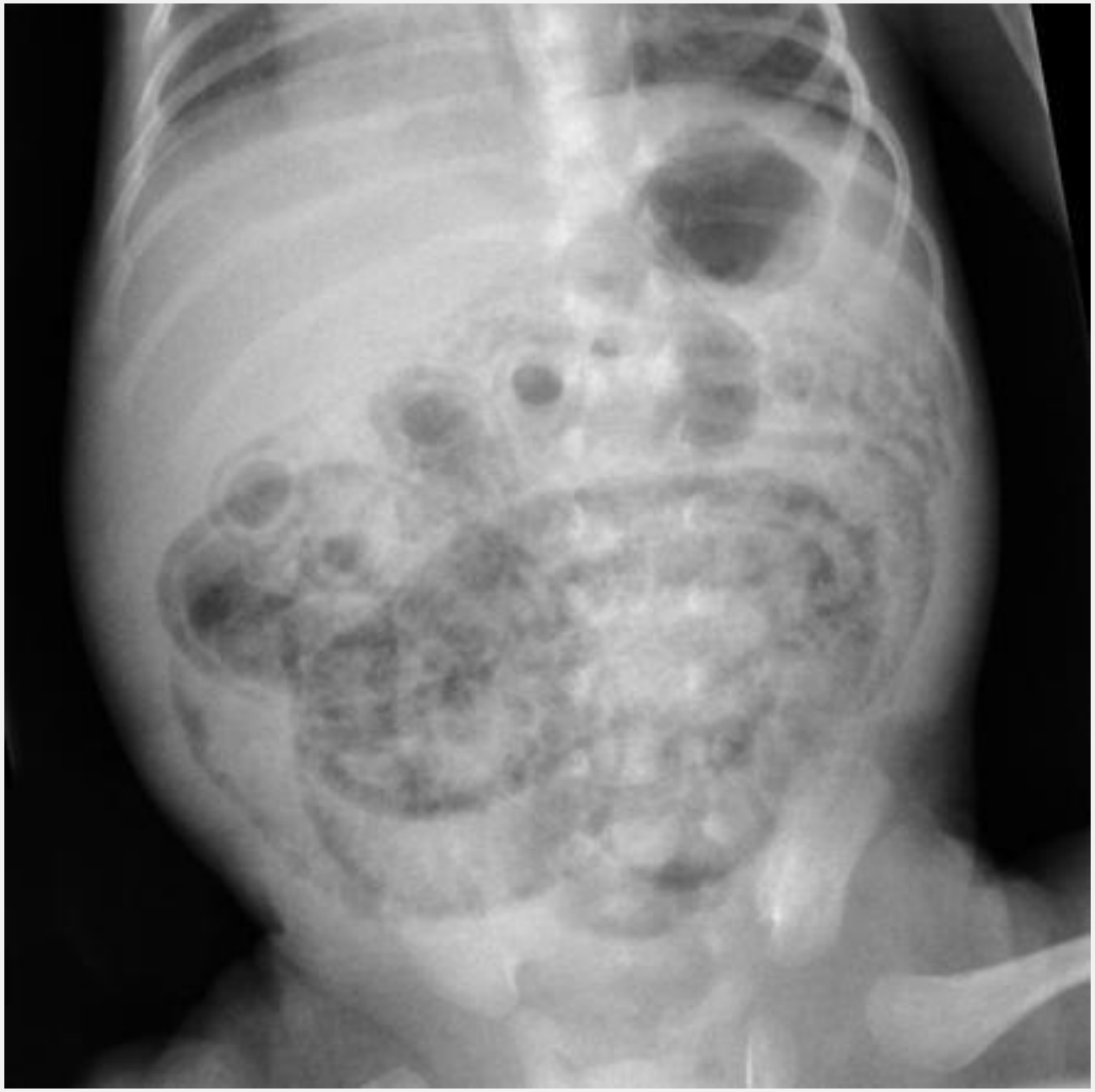

PRO
BIOTICS
ANTI
BIOTICS

INTRODUCTION

- NEC – acute intestinal ischaemia and necrosis
- 5% neonates < 1500 grams, 10% neonates < 1000 grams
- Mortality rate > 20%
- Increased risk of LOS, poor growth, BPD, ROP, prolonged hospitalisation
- Long term morbidity – growth restriction and neurodevelopmental delay

INTRODUCTION

- Pathophysiology of NEC not yet fully understood
- Classic causes - Prematurity, micro-organisms and enteral feeds.
  - NEC cannot be produced in germ free animals
- Immature motility and absorptive function of the GIT
- Innate vs adaptive immunity
- Dysbiosis
- NEC probably not one disease
  - Preterm infants- immaturity
  - Near term/term infants – multi-factorial



FETAL GIT

- Mucosal innate immunity (IgM, B cells) present at about 15 weeks and Peyers patches at 30 weeks gestation
- Epithelial tight junctions in gut present at 10 weeks
- Goblet cells which secrete mucus are also present at 10 weeks
  - Mucus has two distinct layers – the outer layer which prevents bacteria from reaching the mucosal, the inner layer provides scaffolding for antimicrobial peptides and Secretory IgA
- Paneth cells (at the base of intestinal crypts) are present at 13 weeks and produce antimicrobial proteins
  - Enterocytes, macrophages and neutrophils also produce AMPs

MICROBIOME

- Microbial cells outnumber human cells
  - Bacterial genes outnumber human genes 100fold
- Host-microbe interactions – prevention of infection and development of immunity
- Pattern of preterm neonate's microbiome changes with postmenstrual age - increasing anaerobes indicates a mature microbiome
- Disruption of the neonatal microbiome implicated in asthma, celiac disease, type 1 diabetes and obesity

FETAL GIT

- Colonisation of the neonatal gut commences in utero
- Amniotic fluid is not sterile, the fetus is continually exposed to maternal GIT and GUT microbes
  - Fetus bathed in and ingests amniotic fluid
  - Live microbes demonstrated in meconium
- The first 1000 days most important in establishing the gut microbiome, which has lifelong heath implications

GUT IMMUNITY

- At birth, transition from innate to adaptive immunity, both locally in the GIT and systemically
  - Innate immunity – "first responders" – cells and their receptors which respond quickly to microbes
  - Adaptive immune system requires prior antigenic exposure
- Preterm infant has decreased mucin production and "weak" tight junctions which results in invasion of mucosa by organisms, causing an inflammatory response

PRETERM GIT

- Preterm GIT is hyper-inflammatory with an increased risk of NEC
- Maternal conditions that stimulate fetal intestinal inflammatory cascade include:
  - Maternal hypertension
  - Maternal infections
  - Abnormal placental blood flow
  - Substance abuse (cocaine)
  - Choriaomnionitis with vasculitis – 2.5 fold chance of developing NEC
- Role of adaptive immunity in NEC not well understood - ? Role of T regulatory cells in preterm gut
  - Limit intestinal injury and inflammation by enhancing adaptive immunity

TLR4

- In NEC there is impaired signalling in response to colonisation of the neonatal gut
- TLR4 is pro-inflammatory and TLR9 is anti-inflammatory (imbalance in NEC)
  - TLR4 signalling regulates the balance between injury and repair in the newborn intestine
  - Upregulation of TLR4 results in decreased enterocyte proliferation and migration, enterocyte apoptosis and decreased repair of the neonatal intestine

TLR 4

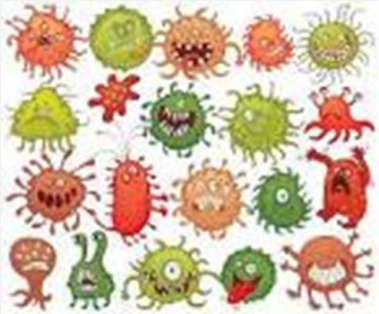
- TLR 4 expression increases with gestational age until term, then decreases significantly and is down regulated at a few weeks post-natally
- Continued TLR4 numbers and expression in preterm infants, which are activated by both commensal and pathogenic organisms, ?major role in NEC
- NEC – failure of downregulation of TLR4 signalling to become tolerant to intestinal microflora
- Inhibition of TLR 4 is a potential pathway for treatment / prevention of NEC
  - TLR4 knockout mice are protected from NEC

EPIDERMAL GROWTH FACTOR

- EGF found in amniotic fluid
- Produced by salivary glands, Brunner's glands in duodenum, Paneth cells and macrophages
- EGF – important for intestinal cell survival, division and migration
- Decreased cord EGF levels predictive for NEC in VLBW infants
- Impaired epithelial healing due to low levels of EGF may be important in NEC

MICROBIAL DYSBIOSIS AND NEC

- Claud and Walker proposed NEC was caused by inappropriate colonisation of the preterm GIT, rather than due to a single pathogen
  - NEC - a result of microbial dysbiosis and an exaggerated secondary inflammatory response
    - Microbe induced neutrophil activation, causes inflammatory mediator release, vasoconstriction and disruption of the intestinal barrier
- GIT of preterm infants – fewer bacterial species, less diversity and increased proportion of pathogens
  - Changes in fecal bacteria prior to onset of NEC
  - Increase in *Enterobacter, E.Coli, Firmicutes, Proteobacteria* with a decrease in *Bifidobacter*



FACTORS AFFECTING THE MICROBIOME

- **Broad spectrum antibiotic use**
  - Decreased microbial diversity
  - Suppressed growth of *Bifidobacter*
  - Negative impact on both innate and adaptive immunity
  - No antibiotics / shorter duration of antibiotics decreases the risk of NEC
- **Probiotics**
  - Meta-analysis of >2000 infants showed that probiotics decreased the risk of NEC by 65% (NNT 25)
  - Use of both *Bifidobacter* and *Lactobacillus* decreased both incidence and mortality due to NEC

FACTORS AFFECTING THE MICROBIOME

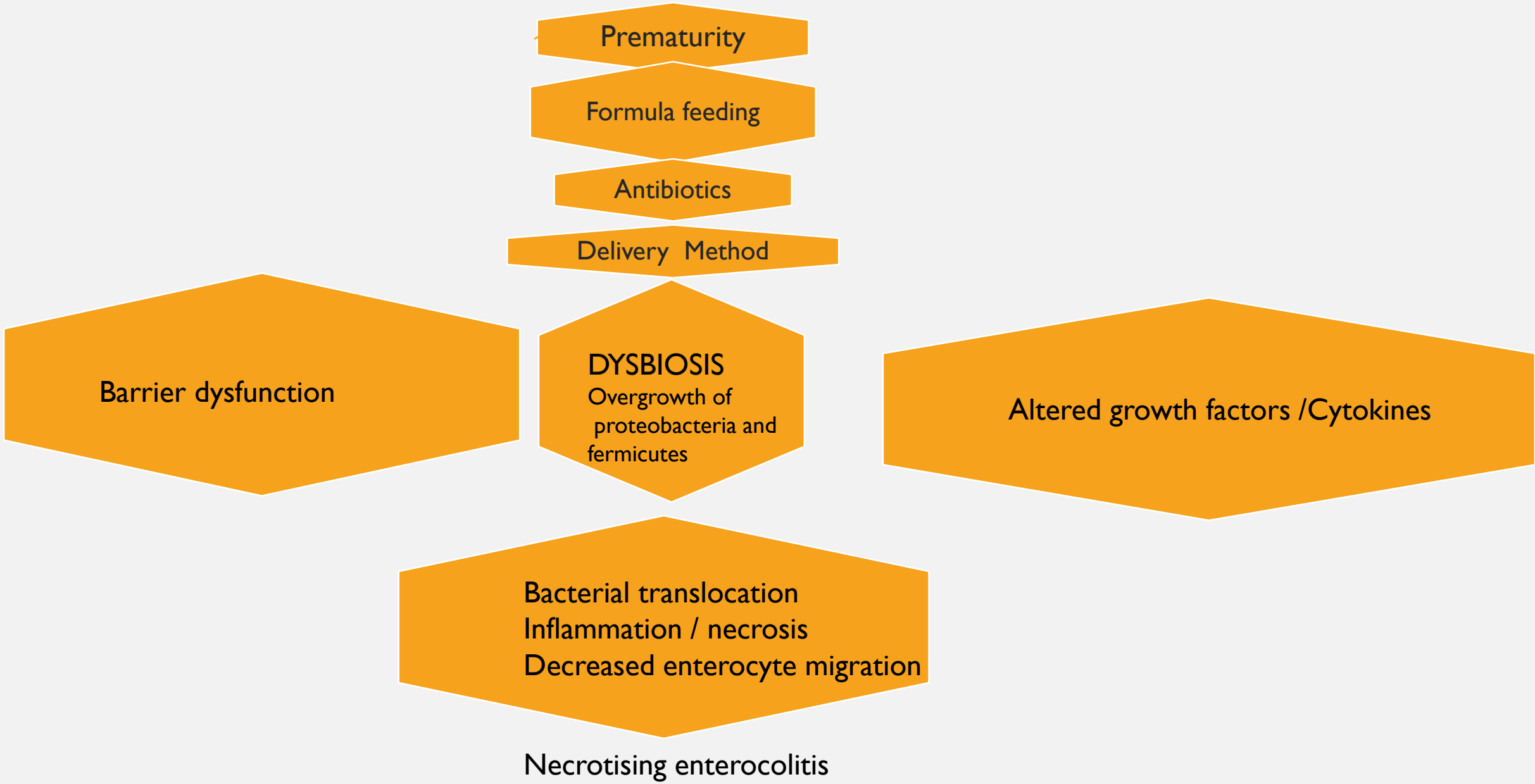
- **Acid base status of the GIT**
  - H2 blockers, antacids, proton pump inhibitors all associated with increased risk of NEC
- **Formula feeding**
- **Caesarean section delivery**
  - No colonisation with maternal GUT organisms
- **Nil per os**
  - Intestinal atrophy, and increased translocation of microbes (risk of NEC and sepsis)

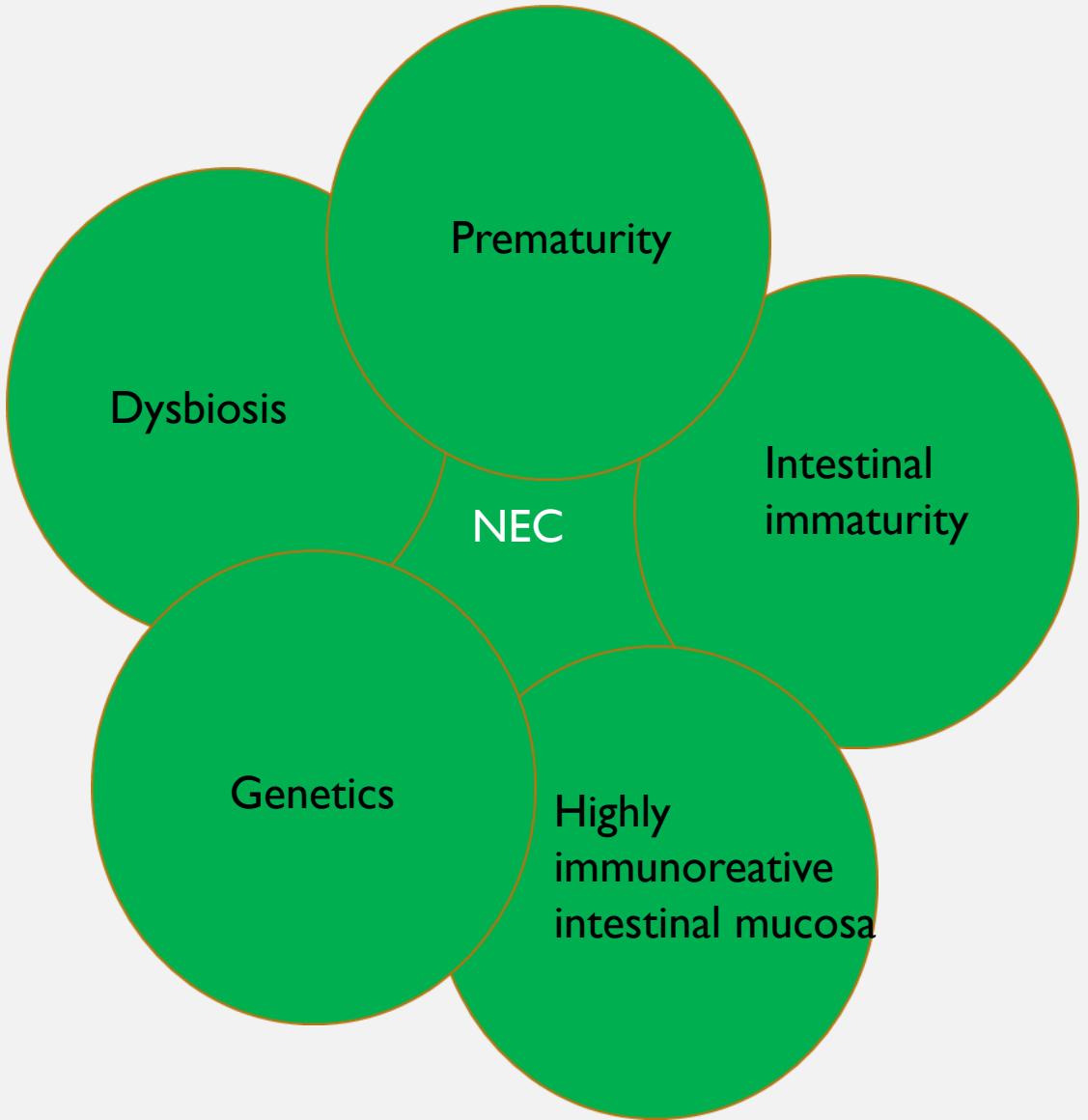
FACTORS AFFECTING THE MICROBIOME: BREASTMILK

- Secretory IgA in breastmilk
  - Reacts with maternal microbiome to prevent adherence and penetration of specific bacteria to neonatal mucosa
  - Influences neonate's intestinal microbiome
  - Prevents NEC
- Growth factors, cytokines, immune modulators (IL6, TNFa. IL10. TGF B, lysozyme) are all found in breastmilk
  - ? Role in preventing intestinal epithelial cell damage in preterm neonates with immature immune system
- "Tailor Made" organisms

OTHER RISK FACTORS

- **Hypoxia –ischaemia**
  - VLBW neonates who require delivery room resuscitation at increased risk of NEC
- **Blood products**
  - Blood transfusion - More frequent in neonates with AB blood group - ? Vulnerable to serum antibodies in blood products
  - High dose immunoglobulin for haemolytic disease
- **Genetic factors**
  - Several genes have been implicated in affecting outcome or severity of disease


Prematurity
Formula feeding
Antibiotics
Delivery Method
Barrier dysfunction
DYSBIOSIS
Overgrowth of proteobacteria and fermicutes
Altered growth factors /Cytokines
Bacterial translocation
Inflammation / necrosis
Decreased enterocyte migration
Necrotising enterocolitis


Prematurity
Dysbiosis
NEC
Intestinal immaturity
Genetics
Highly immunoreative intestinal mucosa

PREVENTION OF NEC

- **Antenatal steroids**
  - NEC decreased by 50%
- **Exclusive breastfeeding** (NNT =10)
  - If mother's milk unavailable, use pasteurised donor milk
  - Preterm formula containing probiotics
  - Trophic feeding – evidence inconclusive, but appears to be safe
    - Start as early as possible
  - **Promoting and preserving healthy gut flora**
    - Probiotics
    - Prebiotics still under investigation

PREVENTION OF NEC

- **Standardized feeding protocol**
  - NEC reduced up to 85% in VLBW neonates with the use of SFP, regardless of the protocol
- **Limit antimicrobial use in the early neonatal period**
  - Positive impact on the neonatal microbiome
  - 40% increased risk of NEC in neonates treated with 7 days vs 2 days of empiric antibiotic therapy for suspected EOS

CONCLUSION

- Dysbiosis and inflammation in the preterm GIT important in the pathogenesis of NEC
- The neonatal microbiome is important
- Prevention
  - Breastfeeding
  - Don’t abuse antibiotics

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- Pathogenesis and prevention of necrotising enterocolitis. JA Morgan, L Young, W McGuire. Current Opinion in Infectious Diseases 2011. 24: 183-189
- Necrotising enterocolitis – some things old, some things new: A comprehensive review. KN Haque. Journal of Clinical Neonatology, Vol 5.2 April-June 2016
- Pathogenesis of necrotising enterocolitis: Modeling the innate immune response, SM Tanner, TF Berryhill, JL Ellenburg, T Jilling, DS Cleveland, RG Lorenz, CA Martin. Am J Pathol 2015; 185: 4-16


Thank you
for listening