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Nickel as a Catalytic Driver of Necrotizing Enterocolitis: Dietary Nickel, Microbial Metallomics, and the Activation of Nickel-Dependent Virulence Pathways in the Preterm Gut

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· Necrotizing enterocolitis
· Nickel
· Microbial metallomics
· Infant formula
· Soy-based formula
· Urease
· [NiFe]-hydrogenase
· Glyoxalase I
· Escherichia coli
· Gut dysbiosis
· Nutritional immunity
· Preterm infant microbiome

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Nickel as a Catalytic Driver of Necrotizing Enterocolitis: Dietary Nickel, Microbial Metallomics, and the Activation of Nickel-Dependent Virulence Pathways in the Preterm Gut

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Abstract

Necrotizing enterocolitis (NEC) remains one of the most devastating gastrointestinal diseases of prematurity, characterized by profound microbial dysbiosis, mucosal injury, and high mortality. While formula feeding and Proteobacteria enrichment are well-established risk factors, the mechanistic drivers linking diet to pathogenic microbial dominance remain incompletely explained. Here, we propose a unifying metallomic framework implicating dietary nickel as a critical but overlooked contributor to NEC pathogenesis. We synthesize evidence demonstrating that key taxa enriched in NEC, including Escherichia coli and related Enterobacteriaceae, rely on nickel-dependent enzymes such as urease, [NiFe]-hydrogenases, and glyoxalase I to regulate pH, detoxify metabolic stressors, evade immune killing, and thrive under inflammatory conditions. In parallel, we show that soy-based infant formulas contain substantially higher nickel concentrations than human or bovine milk, potentially delivering a bolus of bioavailable nickel to the immature neonatal gut. We argue that this excess nickel functionally licenses pathogenic microbial metabolism while undermining host nutritional immunity mechanisms such as lactoferrin and calprotectin-mediated nickel sequestration. By integrating microbiome ecology, enzymatic biochemistry, and infant nutrition, this work reframes NEC as, in part, a nickel-enabled disease state driven by mismetallation rather than microbial presence alone. These findings have immediate implications for infant formula composition, NEC risk stratification, and the development of nickel-targeted preventative and therapeutic strategies.

1 | INTRODUCTION

Necrotizing enterocolitis (NEC) is a devastating intestinal disease primarily afflicting premature infants. Despite decades of research, the etiology of NEC remains multifactorial and not fully understood.[1] A consistent observation in NEC is an alteration in the gut microbiome: infants who develop NEC often show markedly reduced microbial diversity along with an overrepresentation of pathogenic Proteobacteria such as *Escherichia coli*, and a reduction in presumably beneficial or benign bacteria.[2]

In one recent metagenomic study, acute-phase NEC patients' stools had elevated *E. coli* and depleted *Staphylococcus haemolyticus, Staphylococcus aureus, Staphylococcus epidermidis, and Lactobacillus paracasei* compared to healthy controls.[2] These shifts suggest a microbial dysbiosis favoring organisms that can thrive in the compromised neonatal gut environment.

A well-known clinical risk factor for NEC is formula feeding as opposed to human breast milk. Preterm infants fed infant formula (especially formulas based on cow's milk) have a significantly higher incidence of NEC compared to those fed human milk.[1] Breast milk is thought to be protective due to its immunologic components and prebiotic human milk oligo-saccharides, whereas formula lacks many of these benefits. However, beyond the absence of protective factors in formula, there may be harmful factors present in infant formula that have been overlooked.

One such factor, we propose, is the trace metal nickel (Ni). Nickel is not recognized as essential for human nutrition, and thus its presence in infant diets has

drawn little attention. Yet nickel is a critical cofactor for several microbial enzymes that could influence gut colonization and virulence. In this paper, we explore the hypothesis that excess dietary nickel – particularly from nickel-rich infant formulas – can drive the growth and pathogenic activity of nickel-dependent bacteria in the infant gut, contributing to NEC.

The pieces of this puzzle – the microbiome changes in NEC, the biochemical needs of those microbes, and the nickel content of infant diets – have largely been studied in isolation. Here, we connect these dots and argue that nickel may be the missing link in NEC pathogenesis.

2 | HUMAN MILK VS. FORMULA

Nickel is ubiquitous in the environment at low levels and can enter the food chain via plant-based ingredients and manufacturing processes. Although infant nutrition guidelines do not list nickel as a required nutrient, measurable amounts of Ni are present in both breast milk and formula. Human breast milk naturally contains very low nickel concentrations, on the order of only 0.005 to 0.016 mg/L (5–16 µg/L).[3]

In contrast, infant formulas, especially those derived from plant sources like soy, contain substantially more nickel. For example, a classic analysis by Biego et al. (1998) found that while cow's milk-based formula contained around 0.03 mg/L of Ni, a soy-based infant formula averaged about 0.45 ± 0.22 mg/L.[3] This means a soy formula may provide an order of magnitude more nickel than breast milk on a volume basis. More recent surveys likewise report elevated Ni in certain formulas; products tested in Malta showed Ni ~0.76–0.82 mg/kg in some infant formula powders.[3] Overall, soy-based formulas have the highest Ni levels among baby foods, whereas breast milk has the lowest.[3]

Several factors contribute to this disparity. Plants naturally accumulate nickel from soil (soybeans being notable Ni accumulators), and manufacturing equipment (stainless steel) can introduce trace nickel into formula. Regardless of source, the result is that formula-fed infants, and particularly those on soy formula, ingest significantly more nickel than breastfed infants.

Notably, preterm infants are often formula-fed (due to maternal milk insufficiency or use of donor milk fortifiers), putting them at risk of higher Ni exposure. The difference is not trivial: A premature baby on soy formula could be receiving a daily nickel dose many times greater than a breastfed infant. We hypothesize that this "nickel bolus" provided by formula could have important biological effects on the infant gut microbiota, effectively supplying certain bacteria with an abundance of a cofactor that is normally scarce in the breastfed gut.

3 | MICROBIAL DYSBIOSIS IN NEC

NEC is characterized by an inflammatory necrosis of the intestine, and infectious or microbial drivers have long been suspected. Culture and sequencing studies have identified various bacteria in NEC lesions and stools, but a single causative microbe has not been confirmed. Instead, an imbalance or dysbiosis is observed. A consistent finding is decreased alpha-diversity (fewer species present) in the gut microbiome prior to or during NEC.[2]

This loss of diversity often coincides with an outgrowth of facultative anaerobic Gram-negative bacteria (like *E. coli*, *Klebsiella*, *Enterobacter*), whereas benign commensals (such as coagulase-negative staphylococci and lactic acid bacteria) diminish. In the acute NEC cases mentioned earlier, *E. coli* dominated while *S. haemolyticus*, *S. aureus*, and *L. paracasei* were depleted.[2] Notably, these latter organisms are not typical "probiotics" (indeed *Staphylococcus* species are often hospital-associated colonizers), yet their presence in healthy controls suggests they may play a role in maintaining balance or at least indicate a less pathological flora within this context.

Why would a bloom of *E. coli* (or other Enterobacteriaceae) be so strongly associated with NEC? And why would *Staphylococcus* and *Lactobacillus* species matter in this context? One clue lies in the functional capacities of these bacteria.

Many of the taxa implicated in NEC, whether as aggressive pathogens **(Table 1)** have metabolic machinery that involves nickel-dependent enzymes.

Table 1: Pathogens Enriched in NEC and Nickel-Dependent Enzymes

Taxon (context)	Enriched/Associated with NEC	Relative abundance NEC vs control	Urease positive	Glyoxalase positive	[NiFe]-hydrogenase
Escherichia coli (Enterobacteriaceae; *e.g.* EHEC O157:H7)	Significantly associated with NEC. Certain *E. coli* strains bloom during NEC. A subset of UPEC (uropathogenic *E. coli*) correlates with higher NEC risk. [t1] Pathogenic *E. coli* (O157:H7) was isolated in a severe NEC case. [t2]	NEC cases: UPEC present in 44% of NEC infants vs 16% of controls.[t1] Often dominates stool at NEC onset, whereas minor in healthy controls.[t1]	**Yes** – *E. coli* O157:H7 carries a urease gene cluster (unlike typical *E. coli*.[t3]	**Yes** – Ni-activated glyoxalase I (GlxI) is present (*E. coli* GlxI is optimally activated by Ni^{2+})[t4].	**Yes** – expresses multiple [NiFe]-hydrogenases for anaerobic H_2 metabolism. [t5]
Klebsiella pneumoniae (and *Klebsiella* spp., Enterobacteriaceae)	Enriched in NEC. Opportunistic *Klebsiella* often overgrows in dysbiotic NEC gut microbiomes. [t12] In one cohort, *Klebsiella* replaced *E. coli* and became dominant in infants who developed NEC.[t6]	Higher in NEC: present in 52% of pre-NEC samples vs 23% of controls;[t7] often comprises a large fraction of gut bacteria in NEC infants.[t8]	**Yes** – *Klebsiella* spp. hydrolyze urea via urease (a common diagnostic trait).[t9], [t17]	**Yes** – possesses Ni^{2+}-dependent GlxI (Ni-activated glyoxalase enzymes occur in bacteria like *Klebsiella*). [t10]	**Yes** – contains [NiFe]-hydrogenases (e.g. formate hydrogenase complex for H_2 uptake/ production under anaerobiosis).[t11], [t17]
Enterobacter spp. (e.g. *E. cloacae*; Enterobacteriaceae)	Enriched in NEC. *Enterobacter* tends to proliferate during NEC episodes as overall microbiota diversity drops. Genus *Enterobacter* has been identified as more abundant in NEC cases vs controls in some 16S analyses.[t8] Also implicated in NICU clusters of NEC.	*Quantitative:* Often noted as part of elevated Enterobacteriaceae in NEC.[t8] (no specific % reported). Outbreak example: 12 NEC cases (vs 0 controls) were linked to *Enterobacter* (Cronobacter) contamination of formula.[t13]	**Variable** – some *Enterobacter* (e.g. certain *E. cloacae* strains) are urease-positive. [t16]	**Yes** – review on nickel in microbial pathogenesis explains that members of the Enterobacteriaceae family are all expected to possess Ni-glyoxalase I (GloI) enzymes [t16]	**Yes** – presumed to carry [NiFe]-hydrogenases for anaerobic respiration (common among Enterobacteriaceae family). [t16]
Citrobacter spp. (e.g. *C. koseri*; Enterobacteriaceae)	Enriched in NEC. *Citrobacter* has been found at high levels in NEC infants. For instance, *C. koseri* was a dominating taxon in some NEC cases (while low/ absent in healthy controls).[t8]	NEC cases: *C. koseri* comprised ~15% of gut bacteria in one NEC cohort vs ~0% in controls. [t8] Identified as a significant NEC-associated taxon (biomarker of NEC risk) in a preterm study.[t8]	**Yes** – many *Citrobacter* strains (including *C. koseri*) produce urease (urea hydrolysis positive).[t15]	**Yes** – contains Ni-dependent GlxI for methylglyoxal detoxification (inferred from close relatives; Ni–GlxI enzymes widespread in bacteria) [t10]	**Yes** – expected to encode [NiFe]-hydrogenases (assumed similar to other Enterobacteriaceae, though not often highlighted in literature due to methodological concerns..[t15]
Ureaplasma spp. (Mollicutes; perinatal/ respiratory colonizer)	Higher NEC incidence when colonized. While not a typical gut commensal, preterm infants colonized by *Ureaplasma* (in the airway) have a significantly higher risk of developing NEC[t18].	*(No fecal abundance data)* – In a cohort of <33 wk gestation infants, NEC incidence 12.3% vs 5.5% in those colonized with *Ureaplasma* (odds ratio ~2.43). [t18],[t19]	**Yes** – *Ureaplasma* relies on(Ni-dependent) urease, because its activity is the sole source of ATP production. [t16], [t19]	**Yes** – but *not prominent* – Ni-dependent glyoxalase is not noted as a key factor in *Ureaplasma* (the organism's energy focus is on urease).[t16]	**No** – *Ureaplasma* does not feature [NiFe]-hydrogenases as a notable trait.[t16]

The *Proteobacteria* that tend to overgrow (e.g. *E. coli*, *Klebsiella*, *Citrobacter*) often express [NiFe]-hydrogenases, Ni-dependent glyoxalase, or (in some cases) urease.

On the other hand, some of the bacteria that decline in NEC, like *Staphylococcus* and *Lactobacillus*, either possess Ni-requiring enzymes (e.g. staphylococcal urease) or thrive in conditions that nickel-utilizing competitors would disrupt. This raises the intriguing possibility that an increased availability of nickel in the gut could selectively favor Ni-dependent microbial metabolism, tipping the balance toward a pathogenic community.

Before exploring that mechanism, it is worth emphasizing how the normal breastfed infant gut environment differs from that of a formula-fed infant in terms of microbiota and chemistry. Breastfed babies typically harbor Bifidobacterium-dominated flora that produce abundant lactate and acetate, yielding a colonic pH that is significantly lower than in formula-fed infants.[4]

The acidic milieu in breastfed guts (fecal pH in breastfed infants is often <5.5, versus ~6–7 in formula-fed infants) [5] is hostile to many pathogens and is considered a protective factor. Formula feeding, by reducing Bifidobacteria and raising gut pH, may remove this inhibition, allowing organisms like *E. coli* to proliferate. If in addition formula supplies extra nickel, we have a scenario where nickel-enabled pathogens can overcome host defenses even more effectively, for example, by using urease to neutralize acid or by metabolizing nutrients that others cannot.

4 | NICKEL-DEPENDENT ENZYMES

Nickel is a transition metal that microorganisms utilize as a cofactor in a limited but critical set of enzymes. To date, only about ten Ni-dependent enzymes are known in biology[6] but several of these are directly linked to microbial survival in stressful environments and virulence in host infections.[6] The most prominent Ni-enzymes relevant to gut bacteria are urease, ,[NiFe]-hydrogenase and glyoxalase I (also called lactoylglutathione lyase).

Urease catalyzes the hydrolysis of urea to ammonia and carbon dioxide, a reaction that raises local pH. [NiFe]-hydrogenases allow bacteria to use hydrogen gas (H_2) as an energy source or to dispose of reducing equivalents, which can be crucial in anaerobic or nutrient-deplete conditions. Glyoxalase I detoxifies methylglyoxal (a reactive toxic byproduct of glycolysis) by converting it to harmless lactate, in a glutathione-dependent reaction. In some bacteria, the active form of glyoxalase I specifically requires Ni^{2+}. Let us examine how each of these Ni-dependent functions could benefit a pathogen in the infant gut:

Urease (Ni-dependent): Urease is a well-known virulence factor in certain pathogens (classic example: *Helicobacter pylori* uses urease to survive stomach acid). Urease requires two Ni^{2+} ions in its active site to function. By generating ammonia, urease can neutralize acidic environments, which helps bacteria survive acid stress and also damages host tissues. In the urinary tract, urease-producers like *Proteus* and *Klebsiella* create alkaline conditions that lead to crystal formation and biofilms.[6] In the gut of a preterm infant, a urease-expressing bacterium could similarly raise the pH of its surroundings, counteracting the acidification normally caused by lactic acid bacteria. This pH shift might both enhance the urease-producer's growth and impair competitors (for instance, acidic pH is needed for optimal growth of *Lactobacillus/Bifidobacterium*).

Moreover, ammonia produced by urease provides nitrogen for bacterial growth and can be directly toxic to cells. *Staphylococcus aureus* is noteworthy here – although typically thought of as a skin commensal/pathogen, *S. aureus* can colonize the infant gut and has a urease gene cluster. In fact, *S. aureus* urease has been shown to be essential for persistence in infection (e.g. in a mouse kidney infection model) and part of its acid-response survival strategy[7].[8]

S. aureus and related Coagulase-negative staph (like *S. haemolyticus*) thus rely on Ni-dependent urease to withstand acidic and other hostile conditions. If nickel is abundant, these bacteria can effectively deploy urease; if nickel is withheld, urease remains inactive. The human body exploits this. Lactoferrin and calprotectin, immune proteins released by neutrophils, avidly chelate Ni^{2+} to inhibit bacterial urease activity.[6]

This is a key point: the innate immune system specifically tries to starve bacteria of nickel (analogous to how it sequesters iron and zinc) because without nickel, urease cannot function and certain pathogens are disarmed.[6]

[NiFe]-Hydrogenase (Ni, Fe-dependent): Hydrogen gas is produced in the gut by fermentative microbes, and it can serve as an energy-rich substrate for any bacterium equipped to oxidize it. NiFe-hydrogenases contain Ni in their active site and are present in many Enterobacteriaceae (including *E. coli*, *Salmonella*, *Klebsiella*) as well as some other gut bacteria. Utilizing H_2 can give pathogens a competitive edge in anaerobic environments.

For example, *Salmonella enterica* serovar Typhimurium requires hydrogenase activity (hence Ni) to efficiently colonize and invade the gut; it literally lives off hydrogen produced by the microbiota.[6] In mouse models, Salmonella mutants unable to use H_2 have attenuated virulence, illustrating that hydrogen-fueled energy production promotes invasion of the gut ecosystem.[6] *E. coli* strains can similarly harbor multiple hydrogenases. In the inflamed preterm gut, an organism that can consume hydrogen might thrive when fermentative commensals produce excess H_2. But again, active Ni-Fe enzymes require nickel availability.

If an infant's diet or physiology severely limits Ni, hydrogenase activity would be low; conversely, a nickel-rich milieu could turbo-charge any H_2-utilizing bacteria. Notably, Ni-dependent hydrogenases also can help bacteria resist redox stress. *Klebsiella pneumoniae*, for instance, uses one of its hydrogenases to consume protons and mitigate oxidative damage.[6] Thus, Ni-dependent hydrogenases contribute to both metabolism and stress resistance, enhancing bacterial persistence in the gut under inflammatory conditions.

Glyoxalase I (Ni-dependent): Methylglyoxal is a cytotoxic byproduct that arises from glycolysis (via glyceraldehyde-3-phosphate diversion) especially under high flux or nutrient imbalance. Bacteria accumulate methylglyoxal under stress (e.g. osmotic or when too much sugar is present) and need to detoxify it to prevent growth arrest or DNA damage.

The glyoxalase system (GlxI and GlxII enzymes) perform this detoxification. In *E. coli* and some other bacteria, glyoxalase I is "Ni-activated", because it requires Ni^{2+} for activity.[9]

E. coli glyoxalase I, with Ni bound, efficiently converts methylglyoxal (plus glutathione) to lactate.[9] Without Ni, the enzyme may remain inactive or use a less efficient metal like Zn or Cd with poor efficiency.[9] The implication is that an *E. coli* in a Ni-rich environment can quickly neutralize methylglyoxal and maintain rapid glycolysis and growth, whereas in Ni-poor conditions it might be poisoned by its own metabolism.

In a high-carbohydrate diet scenario (infant formula is typically rich in lactose or glucose polymers), the ability to remove methylglyoxal could differentiate a fast-growing bacterium from one that stalls. Additionally, methylglyoxal itself has antimicrobial properties; commensal bacteria that cannot eliminate it might suffer, while Ni-equipped bacteria thrive.

Beyond these enzymes, there are other Ni-containing proteins in certain microbes (for example, *Helicobacter* and *Methanogens* have Ni enzymes for other pathways), but urease, hydrogenases, and glyoxalase are the major ones likely at play in the neonate gut.

It cannot be overstated that nickel is absolutely required for the activity of these enzymes. If the apo-enzyme does not get its Ni cofactor, the catalytic activity is essentially zero. Bacteria have dedicated Ni transport systems (such as the ABC-type *Nik* transporter) to scavenge nickel from the environment,[6] underlining how crucial Ni is despite being needed in tiny amounts. Many pathogens even secrete metallophores or use host amino acids (like histidine) to chelate and import Ni.[6]

In fact, a recent study showed that interfering with bacterial Ni uptake (using a chelator compound) could suppress multiple Ni-enzymes simultaneously and attenuate pathogen virulence, affecting ureolysis, H_2 metabolism, and biofilm formation.[6] This strongly supports the idea that microbial virulence in vivo often hinges on obtaining enough nickel to power these key enzymes.[6] Conversely, if we unwittingly *supply* excess nickel, we may be boosting those virulence factors.

5 | DIETARY NICKEL AS A CATALYST FOR NEC PATHOGENESIS

Bringing together the above considerations, we propose the following model: An infant diet high in nickel (such as a nickel-rich formula, especially soy-based) creates a gut environment that selectively favors Ni-dependent pathogenic bacteria and their enzymatic activities, contributing to the development of NEC. In essence, dietary Ni acts as a fertilizer for the wrong microbes at the wrong time. This concept may explain the otherwise puzzling microbial pattern seen in NEC (the bloom of *E. coli* and loss of others) and link it to the known risk factor of formula feeding.

Several lines of circumstantial evidence support this hypothesis:

Formula vs. Breast Milk Outcomes: As noted, formula feeding increases NEC risk dramatically.[1] While lack of human milk's protective components is one explanation, it is rarely asked if formula might contain a harmful element. Nickel stands out as one such element because it is much higher in formula than in breast milk.[3]

The magnitude of difference (10-fold or more in the case of soy formula)[3] means the gut of a formula-fed preemie is exposed to trace metal levels that an exclusively breastfed infant would never encounter. It is provocative to consider that, by fortuitous evolutionary design, human breast milk is nickel-poor, potentially limiting the proliferation of Ni-hungry microbes in the vulnerable neonatal gut. (Human milk is rich in other trace elements like zinc and copper that babies need, but not nickel, which humans have no known nutritional use for.) On the other hand, cow's milk and soy – not evolved for human infants – contain more nickel, and industrial processing may introduce still more.[3]

Thus, formula could be delivering to infants the exact metal cofactor that pathogenic gut bacteria require. The "writing on the wall" is that breastfeeding's protective effect in NEC may derive in part from its stringent limitation of available nickel in the gut, starving Ni-dependent enzymes of their fuel.

Microbiome Dynamics in NICU Infants: Preterm infants often receive antibiotics and have delayed colonization by maternal microbes. Their gut can become a battleground of hospital-associated organisms. In many NICUs, *Enterobacteriaceae* (like *E. coli*, *Klebsiella*) and *Enterococcus* or *Staphylococcus* are among early colonizers.

If an infant is on parenteral nutrition or minimal feeds, these bacteria persist at low levels. But once feeds start (especially formula), there is a sudden influx of nutrients – and possibly metals like Ni. We posit that Ni-rich feeds could tip the balance, enabling *E. coli* or other gram-negatives with Ni enzymes to outgrow the others. For instance, an *E. coli* that can use a Ni-hydrogenase to tap energy from intestinal hydrogen, and a Ni-glyoxalase to detoxify metabolic byproducts, will have a growth advantage. It could also use any urea present (infant formulas contain significant protein and urea can diffuse from blood to gut) to generate ammonia via urease, raising the local pH.

This rise in pH would further inhibit acid-producing commensals like *Lactobacillus* and reduce short-chain fatty acid levels, disrupting the protective acidic barrier.[4] In essence, a positive feedback loop is established: Ni-fueled urease neutralizes gut pH,[6] making the environment more favorable for pathogens (and more damaging to the gut mucosa), while Ni-fueled metabolism drives faster pathogen growth. Beneficial microbes that lack these Ni-dependent tools cannot compete and die off, leading to the low diversity and dominance of *Proteobacteria* observed in NEC. Importantly, a healthy breastfed gut likely never enters this loop – *Proteobacteria* remain in check partly because they are kept Ni-starved and acid-challenged

Virulence and Immune Evasion: The pathogens implicated in NEC are not merely overabundant; they are also invasive or injurious to the host. Ni-dependent enzymes directly contribute to virulence mechanisms: urease-generated ammonia can damage intestinal epithelial cells and promote translocation of bacteria (by injuring the mucosal barrier). Hydrogenase allows pathogens to thrive in the inflammatory, nitrate-rich gut environment that accompanies NEC, as demonstrated with Salmonella models.[6]

And effective glyoxalase might help *E. coli* withstand the oxidative burst of immune cells. Neutrophils produce reactive species that can lead to methylglyoxal formation; if *E. coli* detoxifies it efficiently (thanks to Ni), it may better survive phagocytic killing.

Furthermore, consider the host's attempt to use calprotectin and lactoferrin to sequester Ni and suppress bacterial urease.[6] If an infant's diet is delivering excess Ni, we might overwhelm this defense: bacteria can grab the surplus nickel faster than calprotectin and lactoferrin can bind it, or simply there is too much Ni for complete sequestration. Thus, dietary nickel could allow pathogens to evade a key innate immune strategy, essentially nullifying the calprotectin and lactoferrin-mediated metal starvation that would normally attenuate invaders' urease and other Ni enzymes[6]

In short, a nickel-replete gut is a playground for urease-positive, hydrogenase-positive microbes to execute their virulence programs unabated, whereas a nickel-scarce (breastfed) gut keeps them partly disarmed.

Soy Formula and Severe NEC: It is noteworthy that soy-based formulas, which have the highest nickel content, have historically been implicated in feeding intolerance and are generally *not* recommended for preterm infants. There have been reports (separate from heavy metal concerns) that soy formula can predispose to gastrointestinal issues in preemies, possibly due to phytates or phytoestrogens.

Our hypothesis provides a completely new angle: an infant on soy formula might be at particularly high risk for Ni-driven dysbiosis and NEC. The bolus of nickel in soy formula[3] could supercharge urease/hydrogenase activity in gut bacteria to an even greater extent than standard formula. While clinical data on soy formula and NEC are limited (because its use in NICUs is limited), this is a question worth investigating. It also serves as a stark example: if our hypothesis is correct, then something as simple as the choice of formula (cow vs soy vs human milk) – through its nickel content – could sway which microbes dominate the preterm gut and whether NEC ensues.

6 | IMPLICATIONS AND FUTURE DIRECTIONS

If nickel is indeed the "secret ingredient" fostering NEC-associated pathogens, this insight carries several important implications:

Re-evaluating Infant Formula Composition: Thus far, regulatory focus for infant formula has been on ensuring adequate nutrients and minimizing known toxins (e.g. lead, cadmium, arsenic). Nickel has not been on the radar as a risk factor in infant feeding, especially since it isn't considered essential or highly toxic at the levels present. Our findings suggest that the upper limits for nickel in infant formula may need revisiting. It may be prudent to set tighter limits on Ni content, particularly for specialty formulas like soy.

Manufacturers might need to modify processes (e.g. use low-Ni source ingredients, avoid excessive contact with Ni-alloy equipment, etc.) to reduce nickel levels. Given that some formulas have Ni nearly 100× higher than breast milk,[3] there is clear room for reduction. In parallel, clinicians and caregivers might consider preferring human milk or low-Ni feeding options for infants at high risk of NEC (e.g. very low birth weight preemies). The push for human milk use in NICUs (mother's milk or donor milk) aligns well with minimizing unnecessary nickel exposure.

Therapeutic Targeting of Nickel in the Gut: An exciting implication is the potential for anti-virulence therapies that chelate nickel in the gut lumen. Since nickel itself is not needed by the infant, a drug or supplement that binds Ni^{2+} could deprive pathogens of this cofactor without harming the baby. For example, a resin or nanoparticle that sequesters dietary nickel might be added to formula or given orally to at-risk infants. Interestingly, the Nature Communications study we cited demonstrated a fungal metallophore (aspergillomarasmine A) that can capture Ni and inhibit bacterial nickel enzymes.[6]

While not ready for clinical use, it shows proof-of-concept that stopping Ni uptake starves pathogens of urease and hydrogenase activity. In the context of NEC, one could imagine supplementing feeds with a safe Ni-

binding agent to mimic the effect of lactoferrin and calprotectin, effectively keeping the gut nickel-poor even if the formula is nickel-rich. This strategy would neutralize a potential advantage of pathogens, ideally preventing the bloom of urease/hydrogenase-positive bacteria in the first place. It's a novel *anti*-virulence approach that doesn't kill bacteria (and thus might not disrupt the microbiome as antibiotics do), but instead disarms them by removing their metallic weaponry.

Biomarkers and Risk Assessment: If our hypothesis holds, we might expect to see correlations in human infants between nickel exposure and microbiome or NEC outcomes. It raises some testable questions: Do infants who develop NEC have higher nickel levels in their blood, urine, or tissues compared to matched controls? Was there a higher proportion of them on soy formula or formulas from certain regions (with higher Ni content)?

Modern metabolomic and metallomic analysis of stool could be insightful. Perhaps NEC stools have higher nickel content, reflecting the dietary intake and bacterial accumulation. Additionally, one could look at expression of Ni-enzymes in fecal bacteria; e.g., *E. coli* isolates from NEC cases might show upregulated urease or hydrogenase genes (if present) when nickel is abundant. A particularly intriguing biomarker could be fecal urease activity or ammonia levels: if nickel-driven urease is at work, stool from infants headed towards NEC might have higher ammonia (alkaline pH) and detectable urease enzyme activity. This could potentially be an early warning sign of the pathogenic shift.

Probiotics and Microbiome Management: Probiotic strategies for NEC prevention have had mixed success, but many involve supplementing *Bifidobacterium* or *Lactobacillus*. These organisms do not rely on nickel-based enzymes; they typically acidify their environment and can outcompete *Proteobacteria* if established early. Our analysis reinforces the importance of those beneficial microbes: by maintaining low pH and perhaps even producing metal-chelating metabolites, they create a nickel-hostile environment for pathogens.

Certain lactic acid bacteria might even bind metals like nickel onto their cell surface or in biofilms, reducing availability to pathogens. Thus, combining probiotics with the concept of nickel limitation might yield synergistic effects.

For instance, ensuring the infant gut is quickly colonized with lactate-producing, Ni-independent flora could prevent Ni from being monopolized by pathogens. Future studies might explore if probiotic strains can be selected or engineered to sequester nickel or to flourish in low-nickel conditions that suppress *E. coli*. On the flip side, if an infant lacks those commensals and is fed high-Ni formula, it sets the stage for the wrong microbes to dominate. This underscores that microbiome-focused NEC interventions should consider the trace metal landscape as well.

7 | CONCLUSION

The pieces of the NEC puzzle – feeding type, microbiome shifts, and bacterial virulence factors – point toward a previously unrecognized connector: nickel bioavailability in the infant gut. The evidence compiled here suggests that what infants are fed can influence not only which microbes grow, but also which microbial enzymes are activated, by virtue of trace nickel content. *E. coli* and other pathogens exploit nickel-dependent urease, hydrogenase, and glyoxalase to survive and overgrow in the vulnerable neonatal intestine, contributing to the epithelial damage and inflammation that define NEC. Human milk, naturally low in nickel, may protect infants in part by denying pathogens this crucial cofactor, whereas some formulas unwittingly provide a surplus of it, potentially fueling the disease process.

This hypothesis, if confirmed, has a certain "hiding in plain sight" quality – a realization that the role of nickel in NEC may have been evident all along, yet overlooked. All the individual facts were known: the microbiology of NEC, the nickel content of formula, the nickel dependence of bacterial virulence enzymes. Only by synthesizing across disciplines (neonatology, microbiology, and metallomics) do we arrive at this perspective that nickel is a critical factor at the interface of diet, microbe, and host in NEC.

We hope this work will inspire targeted research to verify the role of dietary nickel, such as controlled studies of nickel intake and NEC incidence or experiments in animal models where nickel levels can be manipulated. If the hypothesis holds, it opens new avenues for prevention – from reformulating infant diets to developing nickel-chelating therapies – ultimately aiming to reduce the burden of NEC.

The potential to save fragile infants by simply adjusting trace metal exposure is an exciting and novel paradigm. At the very least, our proposal expands the conversation about NEC pathogenesis and exemplifies the importance of considering trace nutrients and metals in the delicate choreography between infants and their newly establishing microbiomes. The hope is that this line of inquiry will lead to that "wow" moment for clinicians and researchers alike: a realization that by paying attention to an overlooked detail like nickel, we might unravel a key to preventing one of neonatal medicine's most challenging conditions.

8 | REFERENCES

[1] Shulhan J, Dicken B, Hartling L, Larsen BM. Current Knowledge of Necrotizing Enterocolitis in Preterm Infants and the Impact of Different Types of Enteral Nutrition Products. Adv Nutr. 2017 Jan 17;8(1):80-91. doi: 10.3945/an.116.013193.

[2] Lin ZY, He SS, Mo ZT, Liao XT, Feng ZS, Kong J, Zhu L, Li Y, Tan HY, Su ZW, Jia CH, Wu F. Integrated analysis of serum metabolomics and fecal microbiome in infants with necrotizing enterocolitis. Front Microbiol. 2025 Jun 5;16:1584041. doi: 10.3389/fmicb.2025.1584041.

[3] Małgorzata, Dobrzyńska & Kaczmarek, Katarzyna & Wozniak, Dagmara & Przysławski, Juliusz & Drzymała-Czyż, Sławomira. (2025). Nickel exposure from food and levels in children's blood and tissues: health implications -a narrative literature review. Acta Scientiarum Polonorum, Technologia Alimentaria. 24. 315-331. doi: 10.17306/J.AFS.001332.

[4] O'Sullivan A, Farver M, Smilowitz JT. The Influence of Early Infant-Feeding Practices on the Intestinal Microbiome and Body Composition in Infants. Nutr Metab Insights. 2015 Dec 16;8(Suppl 1):1-9. doi: 10.4137/NMI.S29530. Erratum in: Nutr Metab Insights. 2016 Oct 27;8(Suppl 1):87. doi: 10.4137/NMI.S41125.

[5] Rubaltelli FF, Biadaioli R, Pecile P, Nicoletti P. Intestinal flora in breast- and bottle-fed infants. J Perinat Med. 1998;26(3):186-91. doi: 10.1515/jpme.1998.26.3.186.

[6] Sychantha, D., Chen, X., Koteva, K. et al. Targeting bacterial nickel transport with aspergillomarasmine A suppresses virulence-associated Ni-dependent enzymes. Nat Commun 15, 4036 (2024). https://doi.org/10.1038/s41467-024-48232-1

[7] Costa FG, Horswill AR. 2025. Urease promotes pH homeostasis and growth of *Staphylococcus aureus* in skin-like conditions. J Bacteriol 207:e00208-25. https://doi.org/10.1128/jb.00208-25

[8] Zhou C, Fey PD. The acid response network of Staphylococcus aureus. Curr Opin Microbiol. 2020 Jun;55:67-73. doi: 10.1016/j.mib.2020.03.006.

[9] Mullings KY, Sukdeo N, Suttisansanee U, Ran Y, Honek JF. Ni2+-activated glyoxalase I from Escherichia coli: substrate specificity, kinetic isotope effects and evolution within the βαβββ superfamily. J Inorg Biochem. 2012 Mar;108:133-40. doi: 10.1016/j.jinorgbio.2011.11.008.

PREPRINT

Pandelova, M., Lopez, W. L., Michalke, B., & Schramm, K.-W. (2012). Ca, Cd, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn contents in baby foods from the EU market: Comparison of assessed infant intakes with the present safety limits for minerals and trace elements. Journal of Food Composition and Analysis, 27(2), 120–127. https://doi.org/10.1016/j.jfca.2012.04.011

TABLE 1 REFERENCES

[t1] Ward DV, Scholz M, Zolfo M, Taft DH, Schibler KR, Tett A, Segata N, Morrow AL. Metagenomic Sequencing with Strain-Level Resolution Implicates Uropathogenic E. coli in Necrotizing Enterocolitis and Mortality in Preterm Infants. Cell Rep. 2016 Mar 29;14(12):2912-24. doi: 10.1016/j.celrep.2016.03.015

[t2] Guner, Y.S., Malhotra, A., Ford, H.R. et al. Association of Escherichia coli O157:H7 with necrotizing enterocolitis in a full-term infant. Pediatr Surg Int 25, 459–463 (2009). https://doi.org/10.1007/s00383-009-2365-3

[t3] Friedrich AW, Köck R, Bielaszewska M, Zhang W, Karch H, Mathys W. Distribution of the urease gene cluster among and urease activities of enterohemorrhagic Escherichia coli O157 isolates from humans. J Clin Microbiol. 2005 Feb;43(2):546-50. doi: 10.1128/JCM.43.2.546-550.2005

[t4] Susan L. CLUGSTON, Rieko YAJIMA, John F. HONEK; Investigation of metal binding and activation of Escherichia coli glyoxalase I: kinetic, thermodynamic and mutagenesis studies. Biochem J 15 January 2004; 377 (2): 309–316. doi: https://doi.org/10.1042/bj20030271

[t5] Beaton SE, Evans RM, Finney AJ, Lamont CM, Armstrong FA, Sargent F, Carr SB. The structure of hydrogenase-2 from Escherichia coli: implications for H2-driven proton pumping. Biochem J. 2018 Apr 16;475(7):1353-1370. doi: 10.1042/BCJ20180053.

[t6] Coleman S, Unterhauser K, Rezaul K, Ledala N, Lesmes S, Caimano MJ, Zhou Y, Jackson E, Gratalo D, Driscoll MD, Matson AP. High-resolution microbiome analysis reveals exclusionary Klebsiella species competition in preterm infants at risk for necrotizing enterocolitis. Sci Rep. 2023 May 16;13(1):7893. doi: 10.1038/s41598-023-34735-2.

[t7]McCartney AL, Hoyles L. The role of Klebsiella populations in preterm infants. Biochem Soc Trans. 2023 Apr 26;51(2):887-896. doi: 10.1042/BST20200325.

[t8] Dobbler PT, Procianoy RS, Mai V, Silveira RC, Corso AL, Rojas BS, Roesch LFW. Low Microbial Diversity and Abnormal Microbial Succession Is Associated with Necrotizing Enterocolitis in Preterm Infants. Front Microbiol. 2017 Nov 15;8:2243. doi: 10.3389/fmicb.2017.02243

[t9] Cabanel N, Rosinski-Chupin I, Chiarelli A, Botin T, Tato M, Canton R, Glaser P. Evolution of VIM-1-Producing Klebsiella pneumoniae Isolates from a Hospital Outbreak Reveals the Genetic Bases of the Loss of the Urease-Positive Identification Character. mSystems. 2021 Jun 29;6(3):e0024421. doi: 10.1128/mSystems.00244-21.

[t10] J. F. Honek, in The Biological Chemistry of Nickel, ed. D. Zamble, M. Rowińska-Żyrek, and H. Kozlowski, The Royal Society of Chemistry, 2017, pp. 200-219.

[t11]Xuan, J., He, L., Wen, W., & Feng, Y. (2023). Hydrogenase and Nitrogenase: Key Catalysts in Biohydrogen Production. Molecules, 28(3), 1392. https://doi.org/10.3390/molecules28031392

[t12] Olm MR, Bhattacharya N, Crits-Christoph A, Firek BA, Baker R, Song YS, Morowitz MJ, Banfield JF. Necrotizing enterocolitis is preceded by increased gut bacterial replication, Klebsiella, and fimbriae-encoding bacteria. Sci Adv. 2019 Dec 11;5(12):eaax5727. doi: 10.1126/sciadv.aax5727.

[t13] van Acker J, de Smet F, Muyldermans G, Bougatef A, Naessens A, Lauwers S. Outbreak of necrotizing enterocolitis associated with Enterobacter sakazakii in powdered milk formula. J Clin Microbiol. 2001 Jan;39(1):293-7. doi: 10.1128/JCM.39.1.293-297.2001.

[t14] Urease Test. UK Standards for Microbiology Investigations. UK Health Security Agency. https://www.rcpath.org/static/dd5548d3-f05f-4a1f-a74d4e2cd4f45f2f/uk-smi-tp-36i4-1-urease-test-march-2025-pdf.pdf

[t15] Khan, F., Rizvi, M., Shukla, I., & Malik, A. (2011). A novel approach for identification of members of Enterobacteriaceae isolated from clinical samples. Biology and Medicine, 3(2 SPECIALISSUE), 313-319.

[t16] Maier, R. J., & Benoit, S. L. (2019). Role of Nickel in Microbial Pathogenesis. Inorganics, 7(7), 80. https://doi.org/10.3390/inorganics7070080

[t17] Benoit, S.L., Schmalstig, A.A., Glushka, J. et al. Nickel chelation therapy as an approach to combat multi-drug resistant enteric pathogens. Sci Rep 9, 13851 (2019). https://doi.org/10.1038/s41598-019-50027-0

[t18] Okogbule-Wonodi AC, Gross GW, Sun CC, Agthe AG, Xiao L, Waites KB, Viscardi RM. Necrotizing enterocolitis is associated with ureaplasma colonization in preterm infants. Pediatr Res. 2011 May;69(5 Pt 1):442-7. doi: 10.1203/PDR.0b013e3182111827.

[t19] Wu HN, Fujita J, Nakura Y, Inoue M, Suzuki K, Ekimoto T, Yin B, Fukuda Y, Harada K, Inoue T, Ikeguchi M, Namba K, Yanagihara I. Structural Analysis and Molecular Dynamics Simulations of Urease From Ureaplasma parvum. J Mol Biol. 2025 Nov 1;437(21):169368. doi: 10.1016/j.jmb.2025.169368.

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