# Human milk oligosaccharides and antibiotics: a valuable combination to control infectious diseases

## Abstract

Infectious diseases remain a major health challenge and are becoming increasingly difficult to manage due to the spread of antibiotic resistance. A novel approach is to exploit human milk oligosaccharides (HMOs), which are now known to reduce the prevalence of infectious diseases among breastfed infants. HMOs have bacteriostatic or bactericidal activity against a wide range of important pathogens, such as *Streptococcus agalactiae* (group B *Streptococcus*), *Streptococcus pneumoniae*, *Campylobacter jejuni*, *Clostridioides difficile*, *Entamoeba histolytica* and *Candida albicans*. Furthermore, HMOs can act synergistically with antibiotics, reducing the minimum inhibitory concentration for certain antibiotics by up to 32-fold. This could extend the therapeutic window of some antibiotics and also reduce the doses required to treat infectious diseases.

Given the risk posed by the emergence of antibiotic-resistant pathogens, it would be advantageous to further exploit this synergism between antibiotics and HMOs.

Clifford Adams [1]

Bettina Gutiérrez [2*]

[1] ANOZENE Nutritional Sciences, Fruithoflaan 101, bus 14, 2600 Berchem Antwerp, Belgium; Cliff.Adams@anozene.eu

[2*] Jennewein Biotechnologie GmbH, Maarweg 32, D-53619 Rheinbreitbach, Germany

*Corresponding author:
**Bettina.Gutierrez**
Bettina.Gutierrez@jennewein-biotech.de

# Introduction

Infectious diseases remain a major threat to public health and constant vigilance is needed to control them. Antibiotics have clearly had an enormous beneficial effect by allowing the control of infectious diseases.

However, bacterial resistance to antibiotics is becoming more prevalent, and infectious diseases may no longer be readily controlled by these drugs[1].

The development of new antibiotics is a long and difficult process, and so, there is a need for alternative strategies to control infectious diseases. One promising way forward is to exploit the recognized effects of human milk oligosaccharides (HMOs), particularly within the context of infant health. It is now well established that breastfed infants are less likely to suffer from respiratory, urinary tract and ear infections, diarrhoea, necrotizing enterocolitis and sudden infant death syndrome compared to their formula-fed counterparts[2].

HMOs are not digested by the infant and serve as metabolic substrates for certain bacteria such as *Bifidobacteria*, contributing to the development of the infant gastrointestinal microbiome. However, HMOs are also antimicrobials with bacteriostatic or bactericidal activity[3].

They act as soluble decoy receptors that block the attachment of viral, bacterial or protozoan pathogens to glycans on the surface of epithelial cells, thus preventing infections of the gastrointestinal, respiratory and urinary tracts. HMOs have been associated with protection against numerous microbial pathogens that are collectively responsible for many infectious diseases (**Table 1**)[4]. Antibiotics also play a major role in controlling these bacterial pathogens and there is now a possibility to use HMOs early in infant life as a way to reduce the need for antibiotic therapy.

**Table 1** Microbial pathogens inhibited by human milk oligosaccharides

| | |
|---|---|
| *Acinetobacter baumannii* | *Pseudomonas aeruginosa* |
| *Campylobacter jejuni* | *Streptococcus agalactiae* |
| *Candida albicans* | *Streptococcus pneumoniae* |
| *Clostridioides difficile* | *Shigella dysenteriae* |
| *Enterococcus faecium* | *Salmonella fyris* |
| *Escherichia coli* | *Staphylococcus aureus* |
| *Haemophilus influenzae* | *Entamoeba histolytica* |
| *Helicobacter pylori* | Norovirus, Rotavirus |

# Antimicrobial effects of HMOs

## *Streptococcus agalactiae* (group B *Streptococcus*)

Group B *Streptococcus* (GBS) is a leading cause of neonatal sepsis, pneumonia and meningitis. HMOs can modulate both the growth of GBS and its ability to form biofilms[5]. In another study, the HMOs 3-fucosyllactose (3-FL) and lacto-N-difucohexaose I (LNDFHI) inhibited growth of GBS in both infants and breast milk[6].

When GBS was challenged with 5 mg/ml of lacto-N-tetraose (LNT) and lacto-N-fucopentaose I (LNFPI), growth was inhibited by 60–70%[7].

## *Streptococcus pneumoniae*

The prevalence of respiratory infections caused by multidrug-resistant strains of *Streptococcus pneumoniae* is increasing.

Agents that prevent the adhesion of this pathogen to epithelial cells and thus ensure its efficient clearance by mucociliary action are possible alternatives or adjuncts to standard antibiotic therapy.

Sialylated HMOs inhibited the adhesion of nine clinically prevalent *S. pneumoniae* capsular types.

The potency of the HMOs was enhanced 500-fold when the sialylated HMOs were covalently coupled to human serum albumin[8].

## Campylobacter jejuni

Campylobacter jejuni is associated with most cases of bacterial diarrhoea in infants, and leads to high infant mortality in developing countries. However, this type of infection was less frequently seen in infants who received high levels of 2'-fucosyllactose (2'-FL) in their mother's milk [9]. In animal trials, C. jejuni colonization was significantly reduced in mice orally gavaged with pooled HMOs. In addition, intestinal clearance of C. jejuni was significantly faster in mouse pups nursing on transgenic dams that secreted 2'-FL, which is normally not present in mouse milk [9]. These effects occur because 2'-FL serves as a decoy receptor to prevent the attachment of C. jejuni to epithelial cells, inhibiting its ability to colonize the gut and in turn reducing the incidence of gastrointestinal disorders.

## Clostridioides difficile

Clostridioides difficile is responsible for a suite of pathological conditions collectively known as C. difficile-associated disease (CDAD) [10].

C. difficile induces intestinal inflammation and diarrhoea via the action of two protein exotoxins: toxin A (TcdA) and toxin B (TcdB).

The C-terminus of toxin A (TcdA-f2) has a carbohydrate-binding domain which is necessary for infection. HMOs prevented the interactions between TcdA and its cellular receptor, thus preventing the initiation of infection [10].

## Entamoeba histolytica

Entamoeba histolytica uses carbohydrate-binding proteins (lectins) as virulence factors to attach to the intestinal epithelium of the host. E. histolytica causes amoebiasis, the third leading cause of death by a parasitic disease after malaria and schistosomiasis [9].

LNT has been shown to block the attachment of E. histolytica and thus reduce its cytotoxicity at physiologically relevant concentrations [9].

## Candida albicans

Candida species are prominent fungal colonizers of the infant intestine [11]. HMOs protect human premature intestinal epithelial cells (pIECs) from invasion by C. albicans. Treatment with HMOs reduced the invasion of pIECs in a dose-dependent manner by 14–67%. HMOs reduce the virulence of C. albicans and protect the premature infant intestine from invasion and the damage caused by this pathogen [11].

# HMOs potentiate antibiotic effects

The antimicrobial effects of HMOs against a range of important pathogens could help to reduce the use of antibiotics. Antibiotics are still required to control serious infections, but this is becoming more difficult due to the increasing prevalence of antibiotic resistance [1].

A promising response is the use of adjuvants that can potentiate the function of antibiotics where efficacy has been reduced by acquired or intrinsic resistance.

There is substantial evidence that HMOs could be appropriate adjuvants for antibiotics.

For example, multidrug resistance is a common feature of GBS. As discussed above, HMOs have an inhibitory effect on GBS, but they also potentiate the activity of antibiotics to which GBS has evolved resistance. They reduced the minimum inhibitory concentration (MIC) for certain antibiotics by up to 32-fold (**Table 2**)[12].

**Table 2** The potentiation of antibiotic activity against group B Streptococcus by human milk oligosaccharides (HMOs)[12]. MIC = minimum inhibitory concentration

| Antibiotic | MIC (µg/ml) | | Fold reduction |
| --- | --- | --- | --- |
| | Antibiotic alone | Antibiotic + 5.0 mg/ml HMOs | |
| Penicillin | 0.03 | 0.015 | 2 |
| Vancomycin | 2 | 1 | 2 |
| Clindamycin | 0.0312 | 0.0078 | 4 |
| Gentamicin | 16 | 1 | 16 |
| Erythromycin | 0.0312 | 0.001 | 32 |
| Minocycline | 0.0625 | 0.0019 | 32 |

GBS also shows intrinsic resistance to trimethoprim, but HMOs sensitized GBS to this antibiotic, reducing the MIC by up to 512-fold across a diverse panel of isolates [12]. The mode of action of HMOs is based on increasing bacterial membrane permeability to the antibiotic [13]. In further work, HMOs were found to increase membrane permeability by approximately 30% compared to an untreated control [14].

The presence of HMOs dramatically reduced the half-maximal inhibitory concentration (IC50) of both vancomycin (0.25 g/ml alone vs 0.00602 g/ml with HMOs) and ciprofloxacin (1.37 g/ml alone vs 0.0021 g/ml with HMOs) [7]. HMOs were also able to potentiate the activity of aminoglycosides against both *Staphylococcus aureus* and *Acinetobacter baumannii* [13].

# Conclusions

The interactions between HMOs and antibiotics are potentially valuable for two major reasons. First, it is clear that HMOs have inherent bacteriostatic and bactericidal properties.
This explains the recognized beneficial effects of breastfeeding on infant health, and suggests that the supplementation of formula feeds with HMOs could extend those benefits to formula-fed infants. Second, HMOs can potentiate the activity of various antibiotics and thus help to overcome the challenge of antibiotic resistance. This could extend the therapeutic window of some antibiotics and reduce the dose needed to treat infections. Given the severe threat posed by antibiotic resistance in pathogens, it would be advantageous to further exploit this synergism between antibiotics and HMOs.

# References

1. O'Neill J (2016) Tackling drug-resistant infections globally: final report and recommendations. The review on antimicrobial resistance. Wellcome Trust and HM Government, UK. Available from: https://amr-review.org/sites/default/files/160518_Final paper_with cover.pdf

2. Morrow AL, Ruiz-Palacios GM, Jiang X, Newburg DS (2005) Human-milk glycans that inhibit pathogen binding protect breast-feeding infants against infectious diarrhea. J Nutr 135:1304–1307

3. Bode L (2015) The functional biology of human milk oligosaccharides. Early Hum Dev 91:619–622

4. Craft KM, Townsend SD (2018) The human milk glycome as a defense against infectious diseases: rationale, challenges, and opportunities. ACS Infect Dis 4:77–83

5. Ackerman DL, Doster RS, Weitkamp JH, Aronoff DM, Gaddy JA, Townsend SD (2017) Human milk oligosaccharides exhibit antimicrobial and antibiofilm properties against Group B *Streptococcus*. ACS Infect Dis 3:595–605

6. Andreas NJ, Al-Khalid A, Jaiteh M, Clarke E, Hyde MJ, Modi N, Holmes E, Kampmann B, Le Doare KM (2016) Role of human milk oligosaccharides in Group B Streptococcus colonisation. Clin Transl Immunol 5:e99

7. Lin AE, Autran CA, Szyszka A, Escajadillo T, Huang M, Godula K, Prudden AR, Boons GJ, Lewis AL, Doran KS, Nizet V, Bode L (2017) Human milk oligosaccharides inhibit growth of group B Streptococcus. J Biol Chem 292:11243–11249

8. Barthelson R, Mobasseri A, Zopf D, Simon P (1998) Adherence of *Streptococcus pneumoniae* to respiratory epithelial cells is inhibited by sialylated oligosaccharides. Infect Immun 66:1439–1444

9. Bode L, Jantscher-Krenn E (2012) Structure-function relationships of human milk oligosaccharides. Adv Nutr 3:383S–391S

10. Nguyen TTH, Kim JW, Park JS, Hwang KH, Jang TS, Kim CH, Kim D (2016) Identification of oligosaccharides in human milk bound onto the Toxin A carbohydrate binding site of *Clostridium difficile*. J Microbiol Biotechnol 26:659–665

11. Gonia S, Tuepker M, Heisel T, Autran C, Bode L, Gale CA (2015) Human milk oligosaccharides inhibit *Candida albicans* invasion of human premature intestinal epithelial cells. J Nut 145:1992–1998

12. Chambers SA, Moore RE, Craft KM, Thomas HC, Das R, Manning SD, Codreanu SG, Sherrod SD, Aronoff DM, McLean JA, Gaddy JA, Townsend SD (2020) A solution to antifolate resistance in group B *Streptococcus*: untargeted metabolomics identifies human milk oligosaccharide-induced perturbations that result in potentiation of trimethoprim. mBio 11:e00076-20

13. Craft KM, Gaddy JA, Townsend SD (2018) Human milk oligosaccharides (HMOs) sensitize Group B *Streptococcus* to clindamycin, erythromycin, gentamicin, and minocycline on a strain specific basis. ACS Chem Biol 13(8):2020–2026

14. Craft KM, Townsend SD (2019) Mother knows best: deciphering the antibacterial properties of human milk oligosaccharides. Acc Chem Res 52:760–768