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Letter

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Human Milk Oligosaccharides (HMOs) Sensitize Group B *Streptococcus* to Clindamycin, Erythromycin, Gentamicin, and Minocycline on a Strain Specific Basis

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Abstract

Human milk oligosaccharides (HMOs) possess antimicrobial and antibiofilm activity against Group B *Streptococcus* (GBS). HMOs were screened for their ability to potentiate antibiotic activity. We observed that HMOs potentiate the function of aminoglycosides, lincosamides, macrolides, and tetracyclines on a strain specific basis but not β-lactams or glycopeptides that inhibit cell wall synthesis. These findings are notable as GBS has evolved high levels of resistance toward aminoglycosides, macrolides, and tetracyclines. Finally, HMOs potentiate the function of aminoglycosides against both *Staphylococcus aureus* and *Acinetobacter baumannii.* Based on these observations, we hypothesized that HMOs act by increasing membrane permeability. This hypothesis was evaluated using a bacterial membrane permeability assay which revealed that HMOs do increase membrane permeability toward propidium iodide.

Introduction

The development of antibiotics is one of the most important advances in modern medicine. Antibiotics can be classified by the cellular component or system they affect and whether they induce cell death (bactericidal) or inhibit cell growth (bacteriostatic) (Figure 1A). While antibiotics that target cellular viability are effective, these agents impose selective pressures that promote the evolution of resistant phenotypes. This reality, coupled with prevalent antibiotic misuse and overuse, has created a situation wherein bacteria have developed resistance to nearly every antibiotic in clinical use.

Antibiotic combination therapy has emerged as an attractive alternative to address antimicrobial resistance. This approach, which involves co-administration of two or more antibiotics with different modes of action or co-administration of an antibiotic and an adjuvant that potentiates antibiotic function, can improve efficacy and suppress resistance development.[1, 2]

The combination of ampicillin and gentamicin, for example, is a multi-antibiotic therapy that serves as is a front-line treatment for pediatric sepsis.[3-7] Conversely, augmentin is an example of an antibiotic and antibiotic adjuvant therapy. Its formulation features amoxicillin, a β-lactam antibiotic, and potassium clavulanate, a β-lactamase inhibitor.[8, 9]

In a recent series of studies, we assayed heterogeneous HMOs for antimicrobial and antibiofilm activity against *Streptococcus agalactiae* (Group B *Streptococcus*, GBS).[10, 11] GBS is an important bacterial pathogen that can be transmitted from mother to child during labor and delivery and is a leading cause of neonatal morbidity and mortality.[12-15] HMOs were also evaluated against two of the ESKAPE pathogens, *Staphylococcus aureus* and *Acinetobacter baumannii.*[16-18]

Our studies revealed that HMOs possessed narrow-spectrum bacteriostatic and antibiofilm activities against GBS, strong antibiofilm activity against methicillin-resistant *S. aureus* (MRSA), and weak antimicrobial activity against *A. baumannii*, a Gram-negative pathogen. While these results support the therapeutic potential of HMOs in disease intervention, the cellular target(s) remain unknown.[19] Based on our previous studies, we hypothesized that HMOs could sensitize GBS to antibiotics. Testing this hypothesis would enable examination of the therapeutic utility of HMOs in combination therapies as well as assist in deciphering the mechanism(s) underlying HMO antibacterial activity.

Methods and Materials

Materials

Cefazolin sodium salt, 98%; (-)-Erythromycin, 98%; Gentamicin sulfate; Linezolid, 98%; Penicillin G sodium salt, 98%; and Tobramycin, 97% were purchased from Acros Organics. Clindamycin hydrochloride monohydrate and Vancomycin hydrochloride were purchased from

Alfa Aesar. Ampicillin sodium salt and Amikacin were purchased from Fisher BioReagents. Imipenem monohydrate, 98% and Meropenem trihydrate, 97+% were purchased from Ark Pharm Inc. Doripenem hydrate, >99% was purchased from Selleck Chemical LLC. Tigecycline, >99% was purchased from Biotang Inc. Minocycline hydrochloride, potency 849μg/mg was purchased from EMD Millipore Calbiochem. β-galactosidase from *Kluveromyces lactis*, ≥2600 units/g was purchased from Sigma Aldrich.

HMO Isolation

Human milk was obtained from 21 healthy, lactating women between 3 days and 3 months postnatal and stored between -80 and -20°C. De-identified milk was provided by Dr. Jörn-Hendrik Weitkamp from the Vanderbilt Department of Pediatrics, under a collection protocol approved by the Vanderbilt University Institutional Review Board (IRB#100897), and Medolac. Milk samples were thawed then centrifuged for 45 minutes. Following centrifugation, the resultant top lipid layer was removed. The proteins were then removed by diluting the remaining sample with roughly 1:1 v/v 180 or 200 proof ethanol, chilling the sample briefly, and centrifuging for 45 minutes followed by removal of the resulting HMO-containing supernatant. Following concentration of the supernatant *in vacuo*, the HMO-containing extract was dissolved in phosphate buffer (pH 6.5, 0.2 M) and heated to 37°C. β-galactosidase from *Kluveromyces lactis* was added and the reaction was stirred until lactose hydrolysis was complete.[20, 21] The reaction mixture was diluted with roughly 1:0.5 v/v 180 or 200 proof ethanol, chilled briefly, then centrifuged for 30 minutes. The supernatant was removed and concentrated *in vacuo*, and the remaining salts, glucose, and galactose were separated from the oligosaccharides using P-2 Gel (H_2O elutant). The oligosaccharides were then dried by lyopholization.

MS and MS/MS Analysis of HMO Samples

HMOs were analyzed and characterized as previously described.[10, 11] Briefly, dried HMO samples were reconstituted in water to approximately 1 mg/mL as previously described. These solutions were deposited on a matrix-assisted laser desorption/ionization (MALDI) target plate as follows: 1 µL HMO was spotted followed by 0.2 µL 10 mM NaCl and 1 µL DHB matrix (60 mg/mL in 50% methanol). The spots were allowed to air dry then analyzed in positive ion mode on a 9.4T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry (MS) (Bruker Solarix). Mass spectra were acquired in positive ion mode from m/z 300-2500. Sodium ion adducts of HMOs were detected with a mass accuracy of >2 ppm. MS/MS analysis was performed for selected ions with a linear ion trap mass spectrometer equipped with a MALDI source (LTQ XL, Thermo Scientific). Selected sodium adduct ions of interest were isolated with a 1 amu window and fragmented via CID using a collision energy of 35 eV. General HMO composition for donors of varying Lewis blood groups determined using MS fragmentation patterns were previously disclosed.[10, 22]

Bacterial Strains and Culture Conditions

Bacterial strains are shown in Table S1. All strains were grown on tryptic soy agar plates supplemented with 5% sheep blood (blood agar plates) at 37°C in ambient air overnight. All strains were subcultured from blood agar plates into 5 mL of Todd-Hewitt broth (THB) and incubated under shaking conditions at 180 RPM at 37°C overnight. Following overnight incubation, bacterial density was quantified through absorbance readings at 600 nm (OD_{600}) using a Promega GloMax-Multi Detection System plate reader. Bacterial numbers were determined using the predetermined coefficient of 1 OD_{600}= 10^9 CFU/mL.

HMO Bacterial Biofilm Assays

HMO antimicrobial and antibiofilm activities for 3 new donor samples were determined as previously described.[10, 11] Antimicrobial and antibiofilm activity results for the remaining HMO samples were previously disclosed.[10, 11]

Broth Microdilution Method for Determination of Minimum Inhibitory Concentrations

All strains were grown overnight as described above and used to inoculate fresh THB or THB + with 1% glucose to achieve 5 x 10^5 CFU/mL. To 96 well tissue culture treated, sterile polystyrene plates was added the inoculated media in the presence of increasing concentrations of antibiotic or HMO cocktail to achieve a final volume of 100 μL per well. Bacteria grown in media in the absence of any compounds served as the controls. The plates were incubated under static conditions at 37°C in ambient air for 24 h. Bacterial growth was quantified through absorbance readings (OD_{600}). The minimum inhibitory concentrations (MICs) were assigned at the lowest concentration of compound at which no bacterial growth was observed.

Broth Microdilution Method for Antibiotic Sensitization

All strains were grown overnight as described above and the subcultures used to inoculate fresh THB or THB + 1% glucose to achieve 5 x 10^5 CFU/mL. Freshly inoculated media was then supplemented with HMOs. To 96 well tissue culture treated, sterile polystyrene plates was added the inoculated media supplemented with HMOs in the presence of increasing concentrations of antibiotic. Bacteria grown in media in the absence of any compounds served as one control. Bacteria grown in media supplemented with HMOs in the absence of any antibiotic served as a second control. MICs were determined as previously described.

Bacterial Membrane Permeabilization Assay

In order to assess bacterial cell membrane integrity after exposure to HMOs, a LIVE/DEAD™ BacLight™ assay (Invitrogen, ThermoFisher) was employed. All strains were grown overnight

as described above and used to inoculate fresh THB or THB + with 1% glucose to achieve 5 x 10^5 CFU/mL. To 96 well tissue culture treated, sterile polystyrene plates was added the inoculated media in the presence of the following HMO concentrations: 0, 0.32, 0.64, 1.28, 2.56, 5.125, 10.25, 20.5 mg/mL. Following incubation under static conditions at 37°C in ambient air for 24 h, cells were stained with propidium iodide (PI) and SYTO 9 (8 µl/mL) for 15 minutes prior to reading with a Promega Glomax plate reader for excitation/emission 525 nm/580-640 nm (green; SYTO 9) and 625nm/660-720 nm (red, PI). Percent ratio of green to red fluorescence was calculated ($Ratio_{green/red}$ x 100). Three biological replicates were used and statistical significance was calculated using Student's t test comparison to bacteria grown in medium alone (*$P<0.05$).

Statistical Analysis

The data for the HMO antimicrobial and antibiofilm screens represent 3 independent experiments each with 3 technical replicates. Data are expressed as the mean biomass and/or biofilm/biomass ± SEM. Statistical analyses were performed in GraphPad Prism Software v. 7.0c. Statistical significance was determined using one-way ANOVA with *posthoc* Dunnett's multiple comparison test comparing growth and/or biofilm production in the presence of ca. 5 mg/mL HMOs to growth and/or biofilm production in media alone. All antibiotic-only and all antibiotic + HMO antibiotic MIC values against GBS represent at least 3 independent trials each with 3 technical replicates. HMO IC_{50} curves were generated in GraphPad Prism Software v. 7.0c. using an inhibition dose-response nonlinear regression curve fit for log(inhibitor) vs. normalized response with a variable slope. All antibiotic-only MIC values against *S. aureus* and *A. baumannii* represent at least 3 independent trials each with 3 technical replicates. For *S. aureus*, the following antibiotic + HMO antibiotic MIC values represent 1 trial with 3 technical

replicates: cefazolin, vancomycin, clindamycin, erythromycin, and linezolid. The gentamicin + HMO antibiotic MIC value represents at least 3 independent trials each with 3 technical replicates. For *A. baumannii*, the following antibiotic + HMO antibiotic MIC values represent 1 trial with 3 technical replicates: imipenem, meropenem, minocycline, tigecycline, doripenem. The amikacin and tobramycin + HMO antibiotic MIC values represent at least 3 independent trials each with 3 technical replicates.

Results and Discussion

In the present study, we elected to use heterogeneous HMO mixtures as opposed to single compounds as recent work from our laboratory has shown that while there are several pharmacophoric units in human milk, individual HMOs are less effective against bacterial pathogens than heterogeneous mixtures. In a similar vein, studies from the Bode and Chen laboratories have found that while various disialylated HMOs can prevent necrotizing enterocolitis (NEC) in a neonatal rat model, these single compounds are less effective than heterogeneous HMO samples.[23-25]

We screened three strains of GBS of varying serotypes (GB2, GB590, and CNCTC 10/84) to determine whether antibiotic potentiation was strain specific. GBS strains can be divided into 10 distinct serotypes (1a, 1b, II to IX) based on a serological reaction directed against the polysaccharide capsule.[26, 27] GB2, GB590, and CNCTC 10/84 are serotype Ia, III, and V strains respectively. Serotypes Ia, III, and V are currently the most common isolates associated with early-onset disease in the United States as they comprise over 80% of isolates.[28] Type III GBS are the most prevalent isolates associated with neonatal disease in the developed world.[29, 30] We elected to evaluate the following antibiotics: penicillin, ampicillin, cefazolin, vancomycin, clindamycin, gentamicin, erythromycin, linezolid, and minocycline. β-lactams are the

recommended antibiotic for intrapartum antibiotic prophylaxis (IAP) for the prevention of early-onset GBS disease, while clindamycin and vancomycin are used for patients with β-lactam allergies who are at low risk for anaphylaxis.[31] Erythromycin was previously recommended as an alternative antibiotic for women at high risk of anaphylaxis. However, due to the evolution of macrolide resistance, current guidelines no longer recommend erythromycin.[32-34] While aminoglycosides and tetracyclines are not used to treat GBS infection, the prevalence of GBS resistance to these classes made these antibiotics intriguing areas of focus for combination therapies.[35] Their mode of actions could also assist with mechanistic analysis.

In the opening stages of the program, HMOs were isolated from the milk of 21 donors and pooled to create a cocktail. Minimum inhibitory concentrations (MICs) of the cocktail and all antibiotics were determined in both Todd Hewitt broth (THB) and THB supplemented with 1% glucose using a microbroth dilution assay (Tables 1 and 2). In all cases, the MIC of the cocktail was found to be 10.25 mg/mL. Interestingly, at concentrations below 5 mg/mL (low end of physiological concentration), HMOs were generally observed to promote bacterial growth. Strain and media-specific HMO IC_{50} values are shown in Table 3. For combination studies, HMOs were dosed at their IC_{50} values except for treatments against CNCTC 10/84 and GB590 in THB where HMOs were dosed at 5.0 mg/mL. In THB, the HMO IC_{50} curves for CNCTC 10/84 and GB590 were not reflective of the biomass data (see SI). All HMO concentrations used in this study are at the low end of physiological concentrations of 5-25 mg/mL.[36]

While the extent of antibiotic potentiation varied among strains and growth conditions, overarching patterns of activity potentiation did emerge. First, no potentiation was observed against any strain in either growth condition for the β-lactams (including cephalosporins) or vancomycin (glycopeptide) (Tables 1, 2 and SI). Second, aside from linezolid (oxazolidinone)

which saw no significant MIC fold reduction for any strain in either growth condition, all other ribosome-targeting antibiotics saw significant fold reductions against at least one GBS strain. Most notable were gentamicin (aminoglycoside) and erythromycin (macrolide). These antibiotics saw the most consistent activity potentiation and the largest MIC reductions, which reached as high as 32-fold.

Strain-specific GBS susceptibility was found to be dependent on the nutritional content of the growth media. For example, while GB2 was the strain most globally affected by HMO supplementation in THB, in THB + 1% glucose, supplementation had no significant effect on the activity of any antibiotic. While HMOs sensitized CNCTC 10/84 and GB590 to a similar list of antibiotics, the magnitude of MIC fold reductions was highly variable. Perhaps the most striking example of this observation is clindamycin against CNCTC 10/84. In THB, HMO supplementation resulted in only a 2-fold reduction while in THB + 1% glucose, HMO supplementation caused a 16-fold reduction.

Encouraged by these results, we next investigated whether the patterns of antibiotic potentiation observed against GBS were extendable to another Gram-positive pathogen, *S. aureus*. For antibiotic sensitization trials against *S. aureus*, HMOs were dosed at 5.0 mg/mL; the HMO cocktail did not completely inhibit bacterial growth even at 20 mg/mL (high end of physiological concentration) so no IC_{50} concentrations could be determined. Initial screens in THB and THB + 1% glucose revealed that the only significant antibiotic MIC fold reduction was for gentamicin in THB + 1% glucose (Table 4 and SI). Additional trials confirmed an 8-fold MIC reduction for gentamicin when dosed in combination with HMOs in THB + 1% glucose.

As a final point of study, we investigated whether HMOs could sensitize a Gram-negative pathogen, *A. baumannii*, to small molecule antibiotics. The following antibiotics were used in

combination treatments: amikacin, tobramycin, minocycline, tigecycline, and doripenem. An initial screen revealed similar patterns of antibiotic potentiation as were seen with the Gram-positive pathogens. Similar to GBS and *S. aureus*, no antibiotic potentiation was seen for antibiotics that inhibit cell wall synthesis (Table 5 and SI). Furthermore, as with *S. aureus*, the only significant antibiotic MIC fold reductions for *A. baumannii* were seen with the aminoglycosides. Additional trials corroborated 4-fold MIC reductions for both amikacin and tobramycin in THB. No significant fold reductions were seen for any antibiotic in THB + 1% glucose (see SI).

The results presented above parallel a previous experiment from our lab wherein we demonstrated that HMOs could potentiate the activity of polymyxin B against GBS. Polymyxins are used in the treatment of Gram-negative bacterial infections but are generally inactive against Gram-positive species like GBS.[37-40] Mechanistically, polymyxins are believed to target bacterial cellular membranes.[41] In Gram-negative bacteria, the cell membrane is the outer-most layer. In Gram-positive bacteria, however, the cell membrane is protected by a thick peptidoglycan layer. Thus, if HMOs damage the peptidoglycan layer, this action would theoretically provide greater access to the cellular membrane and account for the potentiation of polymyxin B activity. Based on this analysis, we hypothesize that HMOs increase cellular permeability. This mode of activity is characteristic of the role of β-lactams in combination therapies with aminoglycosides.[42]

A recent study from the Bode laboratory provides a premise for this hypothesis.[43] In this study, Bode and coworkers identified a GBS serotype III mutant that exhibited normal growth despite exposure to an HMO mixture. The observed resistance was attributed to inactivation of the gene *gbs0738*, a glycosyltransferase of the carbohydrate active enzymes (CAZY) GT-8

family which is conserved across numerous GBS subspecies of varying serotypes. They hypothesized that this glycosyltransferase could either promiscuously incorporate HMOs into the capsular polysaccharide structure or into the peptidoglycan/glycan-binding proteins of the cell wall. The first of these hypotheses was disproven when they observed that a GBS serotype III capsule-deficient mutant remained susceptible to HMO exposure. In the present study, we aimed to test our central hypothesis that HMOs increase cellular permeability.

To determine if HMO inhibition of bacterial growth and viability is associated with cognate changes in bacterial cell membrane integrity, the LIVE/DEAD™ BacLight™ assay (Invitrogen, ThermoFisher) was used (Figure 2). Briefly, this assay employs two stains, SYTO 9, which passes through intact membranes to stain cells green, and propidium iodide (PI), a larger molecule which can only pass through membranes that have breached integrity to stain cells red (associated with dead cells). Propidium iodide can quench the signal of SYTO 9, thus, a ratio of SYTO 9 to PI signal yields a measurement of live to dead cells or intact to non-intact cell membranes. GB590 grown in THB alone exhibited a LIVE/DEAD cell ratio of 100 +/- SEM 1.3. Interestingly, exposure to 2.56 mg/mL of HMOs resulted in a 33% decrease LIVE/DEAD cell ratio ($P=0.00168$), 5.125 mg/mL of HMO's resulted in a 27% decrease, and both 10.25 mg/mL and 20.5 mg/mL of HMO's resulted in a 28% decrease in LIVE/DEAD cell ratio ($P=0.0011$ and $P=0.00044$, respectively). Similar results were seen with strains GB2 and CNCTC 10/84 as these strains also exhibited significant decreases in membrane integrity at 2.56, 5.125, 10.25, and 20.5 mg/mL of HMOs ($P<0.05$). The addition of glucose to the growth medium inhibited this phenotype at 2.56 mg/mL HMOs in all three strains, but membrane integrity was significantly perturbed in the presence of glucose at HMO concentrations of 10.25 mg/mL and higher ($P<0.05$). These results indicate that the HMOs are in fact altering GBS cell membrane integrity

in a dose-dependent fashion and could be altering downstream processes such as proton motive force.

In this study, we have observed that HMOs potentiate the activity of four classes of antibiotics with intracellular targets (aminoglycosides, lincosamides, macrolides, and tetracyclines) across multiple bacterial strains but do not potentiate the activity of cell wall targeting antibiotics (β-lactams, cephalosporins, glycopeptides, carbapenems). This result is particularly notable as HMOs have been shown to act as bacteriostatic agents, yet bacteriostatic agents are often observed to antagonize the actions of bactericidal antibiotics.[44] Against GBS, HMO combination treatments resulted in up to a 16-fold MIC reduction for clindamycin, a 32-fold reduction for erythromycin, a 16-fold reduction for gentamicin, and a 32-fold reduction for minocycline. Furthermore, HMO supplementation significantly reduced the MIC concentrations of aminoglycosides against 2 of the ESKAPE pathogens. We observed an 8-fold reduction for gentamicin against *S. aureus* and 4-fold reductions for amikacin and tobramycin against *A. baumannii*. The consistent aminoglycoside potentiation across both Gram-positive and Gram-negative species is particularly notable. While aminoglycosides are effective antibiotics, the nephrotoxicity of this class limits their utility.[45, 46] Thus, the ability of HMOs, which are not toxic at any concentration, to lower the effective dosage of aminoglycosides holds real therapeutic promise.

The HMO-fostered activity potentiation observed for clindamycin and erythromycin is particularly promising in the prevention of GBS transmission as these two drugs are still considered to be IAP-recommended antibiotics despite the fact that they are becoming less and less effective due to resistance development. Alarmingly, a recent study by the CDC on antimicrobial susceptibilities among GBS isolates revealed that approximately 25% of isolates

are resistant to clindamycin and nearly 50% of isolates are resistant to erythromycin.[47] Our findings demonstrate the feasibility of sensitizing GBS to antibiotics that have failed or are struggling in the clinic thus offering new insights into the battle against antimicrobial resistance.[35]

A final point of emphasis is that all HMO concentrations used in combination treatments were at the low end of physiological concentrations. Additionally, while the millimolar HMO cocktail IC_{50} values may appear high in comparison to typical micromolar antimicrobial dosages, we remind the reader that HMOs are delivered to the infant in multi-gram doses per day. In this context, the millimolar HMO dosages used in this study are impressive as is the fact that these molecules themselves are bactericidal at the high end of physiological concentrations.

While HMOs generally potentiated clindamycin, gentamicin, erythromycin, and minocycline activity across multiple strains, we highlight that in the context of GBS, activity potentiation is strain specific. This result provides support to a central goal of our program: the development of narrow-spectrum, strain specific chemotherapeutic regimens.

Supporting Information

This material is available free of charge on the ACS Publications website at pubs.acs.org:

- Bacterial strains and sources
- New HMO donor samples; antimicrobial and antibiofilm activity summary tables
- HMO IC_{50} curves against GBS
- HMO antibiotic sensitization data

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Potential conflict of interests

The authors declare no competing financial interest.

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Figure Legends=

Figure 1. A) Antibacterial targets for common classes of antibiotics. B) HMOs potentiate the activity of several ribosome-targeting antibiotics.

Figure 2. LIVE/DEAD™ BacLight™ assay to evaluate bacterial cell membrane integrity reveals that exposure to increasing concentrations of HMOs results in decreased cell integrity as determined by ratio of green fluorescence (SYTO 9 stain of intact cells) to red fluorescence (PI stain of non-intact cells). *$P<0.05$, Student's t test, N=3 replicates.

Figure 1.

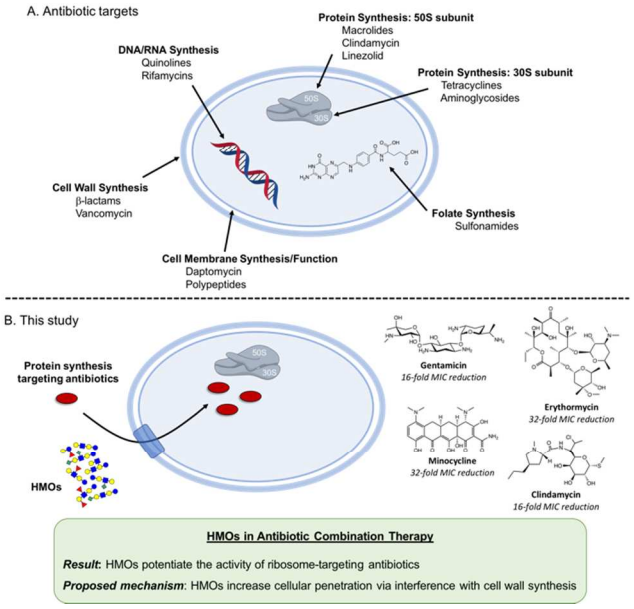


Table 1: Antibiotic sensitization data for HMOs against *S. agalactiae* (GBS) in THB[a,b]

Antibiotic	*S. agalactiae* CNCTC 10/84			*S. agalactiae* GB590			*S. agalactiae* GB2		
	MIC without HMO	MIC with HMO[c]	Fold Reduction	MIC without HMO	MIC with HMO[c]	Fold Reduction	MIC without HMOs	MIC with HMO[c]	Fold Reduction
Penicillin	0.03	0.015	2	0.03	0.03	0	0.03	0.015	2
Ampicillin	0.0625	0.0312	2	0.0625	0.0625	0	0.125	0.0625	2
Cefazolin	0.125	0.0625	2	0.125	0.0625	2	0.125	0.0625	0
Vancomycin	2	1	2	1	0.5	2	1	0.5	2
Clindamycin	0.0325	0.0156	2	0.0312	0.0156	2	0.0312	0.0078	**4**
Gentamycin	16	2	**8**	16	1	**16**	16	2	**8**
Erythromycin	0.0156	0.0019	**8**	0.0312	0.001	**32**	0.0156	0.001	**16**
Linezolid	2	1	2	2	1	2	2	1	2
Minocycline	0.0625	0.0019	**32**	4	0.5	**8**	2	0.25	**8**

[a]All MIC values are given in μg/mL.

[b]Significant MIC fold reductions are bolded.

[c]HMOs were dosed against CNCTC 10/84, GB590, and GB2 at 5.0 mg/mL.

Table 2: Antibiotic sensitization data for HMOs against *S. agalactiae* (GBS) in THB + 1% glucose[a,b]

	S. agalactiae CNCTC 10/84			S. agalactiae GB590			S. agalactiae GB2		
Antibiotic	MIC without HMO	MIC with HMO[c]	Fold Reduction	MIC without HMO	MIC with HMO[c]	Fold Reduction	MIC without HMO	MIC with HMO[b]	Fold Reduction
Penicillin	0.03	0.12	0	0.03	0.06	0	0.03	0.06	0
Ampicillin	0.125	0.125	0	0.0625	0.125	0	0.0625	0.125	0
Cefazolin	0.125	0.125	0	0.125	0.125	0	0.125	0.125	0
Clindamycin	0.0625	0.004	**16**	0.0625	0.0156	**4**	0.0312	0.0156	2
Gentamicin	32	2	**16**	32	4	**8**	32	16	2
Erythromycin	0.0312	0.0078	**4**	0.125	0.0156	**8**	0.0312	0.0156	2
Linezolid	2	1	2	2	1	2	2	2	0
Minocycline	0.0312	0.0156	2	4	1	**4**	0.25	0.125	2

[a]All MIC values are given in μg/mL.

[b]Significant MIC fold reductions are bolded.

[c]HMOs were dosed against CNCTC 10/84, GB590, and GB2 at 5.0 mg/mL.

Table 3. HMO IC_{50} values against 3 strains of *S. agalactiae* (GBS)[a]

	S. agalactiae CNCTC 10/84	*S. agalactiae* GB590	*S. agalactiae* GB2
THB	7.25	7.24	5.04
THB + 1% glc	5.83	5.51	4.45

[a]All IC_{50} values are given in mg/mL.

Table 4. Antibiotic sensitization data for HMOs against *S. aureus* in THB + 1% glucose[a,b]

Antibiotic	MIC without HMOs	MIC with HMOs	Fold Reduction
Cefazolin	8	8	0
Vancomycin	8	8	0
Clindamycin	0.25	0.25	0
Gentamicin	4	0.5	**8**
Erythromycin	32	32	0
Linezolid	1.7	3.4	0

[a]All MIC values are given in μg/mL.

[b]Significant MIC fold reductions are bolded.

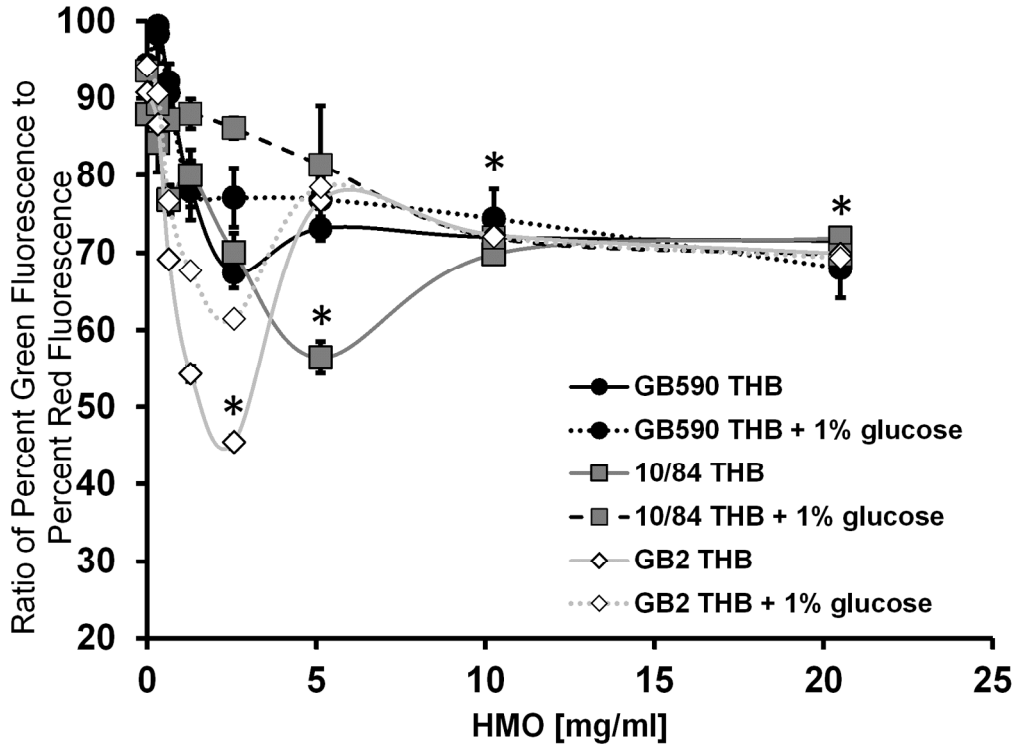
Table 5. Select antibiotic sensitization data for HMOs against *A. baumannii* in THB[a]

Antibiotics	MIC without HMOs	MIC with HMOs	Fold Reduction
Amikacin	16	4	**4**
Tobramycin	8	2	**4**
Imipenem	0.5	1	0
Meropenem	1	1	0
Minocycline	0.31	0.31	0
Tigecycline	0.0625	0.125	0
Doripenem	0.5	1	0

[a]All MIC values are given in μg/mL.

[b]Significant MIC fold reductions are bolded.

Figure 2.


Ratio of Percent Green Fluorescence to Percent Red Fluorescence
100
90
80
70
60
50
40
30
20
0
5
10
15
20
25
HMO [mg/ml]
*
*
*
GB590 THB
GB590 THB + 1% glucose
10/84 THB
10/84 THB + 1% glucose
GB2 THB
GB2 THB + 1% glucose


Important Information
tablet by mouth
(twelve) hours
days.