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Alginate Oligomers Inhibit Growth of Bacteria Causing Bovine Mastitis and Potentiate the Activity of Antibiotics Commonly Used For Treatment of the Disease

Anne Tøndervik[1*], Geir Klinkenberg[1], Randi Aune[1], Philip D Rye[2] and Håvard Sletta[1]

[1]*SINTEF Materials and Chemistry, Department of Biotechnology and Nanomedicine, Sem Sælands veg 2A, 7034 Trondheim, Norway*

[2]*AlgiPharma AS, Industriveien 33, 1337 Sandvika, Norway*

[*]**Corresponding author:** Anne Tøndervik, SINTEF Materials and Chemistry, Department of Biotechnology and Nanomedicine, Sem Sælands veg 2A, 7034 Trondheim, Norway, Tel: +47 45054343; E-mail: anne.tondervik@sintef.no

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Abstract

Alginate oligomers have been shown to disrupt microbial biofilms and reduce minimum inhibitory concentrations (MIC) for many clinically relevant antibiotics to a range of human pathogens. The antibiofilm and potentiating effects of alginate oligomers has previously been focused on bacterial and fungal strains that play significant roles in chronic human infections. This study on the effects of an alginate oligomer (OligoG CF-5/20) on bacteria that cause bovine mastitis is the first report that investigates a significant disease in animal health. The effect of OligoG CF-5/20 on the inhibitory concentrations of ampicillin, erythromycin, cephalothin and lincomycin to a test panel of nine bacterial strains associated with bovine mastitis were investigated. Two different media were used for cultivation, the standard Mueller-Hinton normally used for bacterial MIC determination, and a skimmed milk medium to mimic the native growth conditions for mastitis bacteria. OligoG CF-5/20 was shown to inhibit growth of all strains tested, and demonstrated a 2 to 8 fold reduction in MICs for erythromycin, cephalothin and lincomycin. The data highlights a potential role for alginate oligomers in potentiating the efficacy of antibiotics, and thereby potentially reducing antibiotic use, in the treatment of mastitis in dairy cattle.

Keywords: Alginate oligomers; Bovine mastitis; Antibiotic potentiation; Biofilm; Reduction of MIC

Introduction

OligoG CF-5/20 is a low molecular weight alginate oligomer (DPn 16, 3200 g/mol) derived from brown seaweed and composed of α-L-guluronic acid (≥ 85%) and β-D-mannuronic acid (≤ 15%). OligoG CF-5/20 has been shown to modulate the properties of mucus in human cystic fibrosis (CF) sputum [1] and intestinal mucus of CF mice [1,2]. It has also been shown to disrupt established *Pseudomonas aeruginosa* biofilms [3,4], and to potentiate the action of antibiotics from several classes against a wide range bacteria and fungi [5,6]. Alginate oligomers are currently being investigated in the treatment of chronic respiratory diseases like CF and chronic obstructive pulmonary disease (COPD). OligoG CF-5/20 is currently in phase 2b clinical trials for the treatment of CF. The properties of alginate oligomers suggest they could also have value in the treatment of biofilm infections such as bovine mastitis (BM). BM is the most significant disease of dairy cattle, and affects farm economics by reducing milk production and increasing treatment costs [7]. It represents one of the most difficult veterinary diseases to control. Bacterial biofilms are presumed to be responsible for conferring a selective advantage on the causative agents resulting in persistent and recurring infections and reduced efficiency of antibiotic therapy [8]. The main pathogens causing BM are *Staphylococcus aureus*, *Escherichia coli, Enterococcus faecalis, Streptococcus uberis, Streptoccus dysagalactiae* and *Streptococcus agalactiae*, all of which are capable of forming biofilms [8]. In the present study we wanted to explore if alginate oligomers exerted a growth inhibiting effect on these pathogens, as well as investigate whether oligomers have a potentiating effect on antibiotics commonly used for treatment of mastitis. The test strains selected were from commercial strain collections and strains isolated from veterinary BM samples. The antibiotics tested, ampicillin, erythromycin, cephalothin and lincomycin, are drug class representatives for antimicrobials used to treat BM.

Materials and Methods

Bacterial strains, antibiotics and alginate oligomers

The bacterial strains used in this study are listed below and represents culture collection strains (SC) and bovine clinical isolates (CI): *Staphylococcus aureus* ATCC 25923 (SC), *Staphylococcus aureus* ATCC 29213 (SC), *Staphylococcus aureus* CCUG 115915 (SC), *Streptococcus uberis* (CI), *Streptococcus agalactiae* I (CI), *Streptococcus agalactiae* II (CI), *Streptococcus dysagalactiae* 15-77-1-4 (CI), *Streptococcus dysagalactiae* (CI), *Escherichia coli* 1580-1-3 (CI). The clinical isolates were kindly provided by the Trondheim department of Norwegian Veterinary Institute (NVI). The strain designations for the clinical isolates used in this paper originates from NVI. The antibiotics used were pharmaceutical grade obtained from Sigma. The alginate oligomers used were AlgiPharma's active pharmaceutical ingredient (API) OligoG CF-5/20 produced and purified for AlgiPharma by NovaMatrix, a business unit of FMC Biopolymer.

Growth characterization of bacterial strains in presence of OligoG CF-5/20

OligoG CF-5/20 was dissolved in Mueller-Hinton (MH) broth (Lab M limited, LAB114 Mueller-Hinton broth) to 1.11 times the desired assay concentration (0.1, 0.5, 1, 2 and 6%). Bacterial cultures were inoculated from frozen stocks and grown overnight in tryptone soya broth (TSB, Oxoid, CM0129) 6 ml in 50 ml tube tilted to 45-degrees angle, 200 rpm, 2.5 cm amplitude, 37°C. The overnight cultures were diluted in TSB to OD_{600} of 0.10 and then further 1:10 in MH broth. 12.5 µl culture was then inoculated to 112.5 µl MH with the different OligoG CF-5/20 concentrations in 96-well plates (four replicates for each combination). The plates were incubated at 37°C without shaking and OD_{600} readings were made at regular intervals for 45 hour after inoculation.

Determination of minimum inhibitory concentration (MIC) for the bacterial strains

Robotic high throughput MIC assays were performed mainly as described previously [5,6]. Antibiotics were dissolved in MH broth with the highest concentration being 200 µg/ml for each. Threefold serial dilutions were made in MH broth, and 120 µl of the medium was placed in 96-well plates (i.e. lowest concentration used was 0.0034 µg/ml). Inoculums of the bacterial strains were prepared as described above and 6 µl were inoculated to the medium containing wells. Duplicate wells of the same antibiotic concentration were included for each strain. A group of 8 wells with no addition of antibiotics was included on each micro plate as growth reference. The microplates were placed in plastic bags and incubated without shaking at 37°C. OD_{600} was measured after 19 hours of incubation, and the relative cell density in each well was calculated based on the cell density in the reference groups. The MIC value was set to the highest concentration giving less than 30% growth compared to the reference.

Susceptibility testing with OligoG CF-5/20 in combination with antibiotics

OligoG CF-5/20 was dissolved in MH broth to 1.25 times the desired assay concentration (0.5, 2 and 4%). The antibiotics were dissolved in MH broth and MH broth with OligoG CF-5/20 at a concentration of 1.25 times the highest desired assay concentrations. The highest concentration used for all antibiotics in the MIC assay was 10 µg/ml. Two-fold serial dilutions of the antibiotics were made in MH broth with different concentrations of OligoG CF-5/20, and the solutions were placed in four parallel wells in Nunc 384-well micro plates (30 µl per well in Nunc 242757 microplates). A group of 8 wells with no addition of antibiotics for each OligoG CF-5/20 concentration was included on each micro plate as growth reference. Inoculums were prepared as described above except that cultures were diluted 1:40 in MH medium before adding 7.5 µl to the 384-well assay plates. The microplates were placed in plastic bags and incubated without shaking at 37°C. The OD_{600} nm was measured after 19 hours of incubation, and the relative cell density in each well was calculated based on the cell density in the reference groups. The MIC value was set to the highest concentration giving less than 30% growth in all 4 parallel wells within the sample groups.

When cultures were assayed in a skimmed milk medium (Oxoid, LP0031), the procedure was as described above until the OD_{600} measurement following the incubation period of 19 hours. After this incubation, all cultures were inoculated (1%) to fresh MH medium in 384-well plates, incubated at 37°C with shaking for 9-10 hours and then OD_{600} was measured. The inhibitory concentration was defined as the lowest concentration where the cell density in all four wells was less than 30% of the average cell density in those wells inoculated in the skimmed milk medium with the same concentration of OligoG CF-5/20 but without antibiotics. The extra cultivation step was required since the OD_{600} cannot be measured in the skimmed milk medium. Therefore, we designated the values obtained in this assay as inhibitory concentrations (not MIC values) since they were not directly comparable to the absolute values obtained in the MH medium. The growth period in MH was 9-10 hours since most of the cultures were found to be in the middle or late growth phase at that time point based on the growth data obtained in initial experiments.

Results and Discussion

OligoG CF-5/20 reduces growth of bacterial strains

The effect of OligoG CF-5/20 on the growth of the selected BM bacterial strains were explored in MH medium with increasing concentrations of OligoG CF-5/20 (0, 0.1, 0.5, 1, 2 and 6%). Growth was recorded as OD_{600} and followed for 45 h after inoculation. The growth yield for each strain at the time point where the control culture (without OligoG CF-5/20) entered the stationary phase is shown in Figure 1.

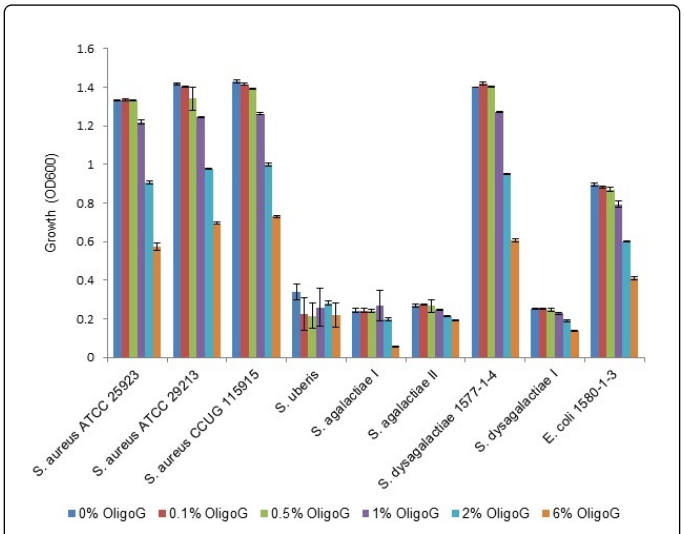

Figure 1: Effect of increasing concentrations of OligoG CF-5/20 (0.1, 0.5, 1, 2 and 6%) on cell densities after cultivation at 37°C in Mueller-Hinton medium. The OD_{600} values given for each strain is recorded at the time point were the control culture (without OligoG CF-5/20) entered the stationary growth phase; 10 hours for *S. agalactiae* I, *S. agalactiae* II and *E. coli* 1580-1-3, 15 hours for S. dysagalactiae I and 19 hours for the rest of the strains. Standard deviation from the mean of the OD measurements is shown as error bars (four replicates).

OligoG CF-5/20 was found to reduce bacterial growth in a dose dependent manner for the majority of the strains. For all strains there was a reduction in growth yield by the addition of 2% and 6% OligoG CF-5/20, and for some of the strains a reduction was also observed at the lowest concentrations used (0.1% and 0.5%). Reduction in growth

at such low concentrations of OligoG CF-5/20 has also been observed previously for *P. aeruginosa* and *Acinetobacter baumanni* strains (unpublished data). *S. uberis*, *S. agalactiae* I and II and *S. dysagalactiae* I displayed low growth yield in the MH medium resulting in OD_{600} below 0.4. The choice of MH for cultivation is however based on this medium being the preferred standard medium for susceptibility testing and thus enable comparing the data obtained with data from the literature. Due to the low growth displayed by these strains, no significant differences were observed at the lowest concentrations of OligoG CF-5/20. Nevertheless, also for these strains there is a significant reduction in growth at 2 and 6% OligoG CF-5/20.

Antibiotic susceptibility testing of bacterial strains

The BM clinical isolates used in this study had not previously been characterised in detail, and an initial screening to determine the minimum inhibitory concentrations (MIC) for the antibiotics was performed. Strains were grown in MH medium (as recommended by CSLI guidelines) with a broad concentration gradient of antibiotics, and the MIC determination was performed after 19 hours incubation (Table 1). The clinical *E. coli* isolate appeared resistant to lincomycin (67 µg/ml) and intermediately resistant to the other antibiotics (2.5-7.4 µg/ml). MIC values for 101 *S. aureus* strains isolated from cases of bovine mastitis in Norway have been reported [9] for ampicillin (≤ 0.06 to 4.0 µg/ml), cephalothin (≤ 0.06 to 8.0 µg/ml), lincomycin (0.06 to 32 µg/ml) and erythromycin (0.25 to 4.0 µg/ml). The *S. aureus* test strains used in the present study are from strain collections, however the MIC values obtained for the strains are all within, or close to the range of the reported values for clinical isolates.

Strain	Ampicillin	Erythromycin	Cephalothin	Lincomycin
Staphylococcus aureus ATCC 25923 (SC)	0,031	0,82	0,031	2,5
Staphylococcus aureus ATCC 29213 (SC)	0,82	0,82	0,27	2,5
Staphylococcus aureus CCUG 115915 (SC)	0,82	0,82	0,27	2,5
Streptococcus uberis (CI)	0,031	0,031	0,091	0,091
Streptococcus agalactiae No. I (CI)	0,091	0,031	0,091	0,27
Streptococcus agalactiae No. II (CI)	0,031	0,031	0,091	0,27
Streptococcus dysagalactiae 1577-1-4 (CI)	0,031	0,82	0,031	2,5
Streptococcus dysagalactiae (CI)	0,010	0,031	0,091	0,091
Escherichia coli 1580-1-3 (CI)	2,5	7,4	7,4	67

Table 1: Minimum inhibitory concentrations (µg/ml) obtained for the selected antibiotics on the test strains grown in Mueller-Hinton medium. SC; strain collection, CI; clinical isolate.

OligoG CF-5/20 is able to potentiate the effect of antibiotics on selected test strains

The ability of OligoG CF-5/20 to potentiate the effect of ampicillin, erythromycin, cephalothin and lincomycin was explored by performing MIC assays in MH with increasing concentrations of OligoG CF-5/20 (0.5%, 2% and 4%). The MIC values (µg/ml) obtained for each combination of antibiotic and OligoG CF-5/20 for the bacterial strains is shown in Table 2.

In general, treatment with OligoG CF-5/20 reduced MICs for erythromycin, chepalothin and lincomycin by 2 to 8 fold, e.g. MIC for erythromycin on *S. dysgalactiae* 1577-1-4 was reduced from 0.313 to 0.039 µg/ml by the addition of 4% OligoG CF-5/20 to the medium. The combination of OligoG CF-5/20 with ampicillin appeared less effective, leading to a reduction in MIC for only three of the strains (2 fold).

To mimic the conditions during a mastitis infection in a bovine mammary gland, MIC assays with the same test strains and antibiotics were also performed using a growth medium based on skimmed milk powder. Initially it was found that concentrations of OligoG CF-5/20 above 4% in this medium could not be used due to aggregation of the oligosaccharides, and therefore the highest concentration used was 4% in both media (see above). Growth of the bacterial strains in the skimmed milk medium could not be detected by OD_{600} measurements. MIC values that are directly comparable to that obtained in MH medium could therefore not be obtained, so inhibitory concentrations were derived by an alternative procedure. Briefly, after the standard incubation period of 19 hours for the bacteria in the growth medium with combinations of antibiotics and OligoG CF-5/20, the cultures were inoculated (1%) to MH without any additions. The cultures were then incubated with shaking for 9 hours before reading of OD_{600}. Inhibitory concentrations were defined as the lowest concentration of an antibiotic giving less than 30% growth compared to the growth in the reference culture, i.e. cultures inoculated from medium with the same concentration of OligoG CF-5/20 without antibiotics. The inhibitory concentrations obtained for the test strains are shown in Table 3. The results in skimmed milk medium were comparable to results obtained in the standard MH-medium. OligoG CF-5/20 reduced the inhibitory concentration (up to 16 fold) for erythromycin, cephalothin and lincomycin for the majority of the test strains, whereas the effect with ampicillin was apparent only for *S. dysgalactiae*. The results obtained for ampicillin indicate that for this antibiotic there is not a general potentiating effect of OligoG CF-5/20. The reasons for this might be complex and presumably related to the mechanisms of action of OligoG CF-5/20 which is not yet fully elucidated (see discussion below).

	Antibiotic MIC (µg/ml) at indicated OligoG CF-5/20 concn (%)															
	Ampicillin				Erythromycin				Cephalothin				Lincomycin			
Strain	0	0.50%	2%	4%	0	0.50%	2%	4%	0	0.50%	2%	4%	0	0.50%	2%	4%
S. aureus ATCC 25923	*0.02*	*0.02*	*0.02*	*<0.01*	*0.156*	*0.156*	*0.078*	*0.039*	0.039	0.039	0.039	0.039	*0.625*	*0.625*	*0.313*	*0.313*
S. aureus ATCC 29213	0.313	0.313	0.625	0.625	*0.313*	*0.313*	*0.156*	*0.078*	*0.156*	*0.156*	*0.156*	*0.156*	*1.25*	*0.625*	*0.625*	*0.313*
S. aureus CCUG 115915	0.313	0.313	0.313	0.625	*0.313*	*0.313*	*0.313*	*0.078*	*0.156*	*0.156*	*0.156*	*0.078*	*1.25*	*0.625*	*0.625*	*0.313*
S. uberis	0.625	0.313	0.625	0.313	*0.313*	*0.313*	*0.156*	*0.078*	*0.156*	*0.156*	*0.156*	*0.078*	*0.625*	*0.625*	*0.625*	*0.313*
S. agalactiae I	0.039	0.039	0.039	0.039	*0.02*	*<0.01*	*<0.01*	*<0.01*	*0.156*	*0.156*	*0.156*	*0.078*	0.039	0.078	0.039	0.039
S. agalactiae II	0.039	0.039	0.039	0.039	*0.02*	*<0.01*	*<0.01*	*<0.01*	*0.156*	*0.156*	*0.078*	*0.078*	*0.078*	*0.078*	*0.039*	*0.039*
S. dysagalactiae 1577-1-4	*0.02*	*0.02*	*0.02*	*<0.01*	*0.313*	*0.156*	*0.078*	*0.039*	*0.039*	*0.039*	*0.039*	*0.02*	*0.625*	*0.625*	*0.313*	*0.156*
S. dysagalactiae	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.078	0.078	0.039	0.039	*0.02*	*0.02*	*0.02*	*<0.01*
E. coli 1580-1-3	*2.5*	*2.5*	*2.5*	*1.25*	>10	10	10	10	5	5	5	5	>10	>10	>10	>10
Italics represents potentiation of antibiotic with increasing OligoG CF-5/20 concentration.																

Table 2: MICs of antibiotics alone and with increasing concentrations of OligoG (0.5, 2, 4%) for the bacterial test strains.

	Inhibitory antibiotic concn (µg/ml) at indicated OligoG CF-5/20 concn (%)															
	Ampicillin				Erythromycin				Cephalothin				Lincomycin			
Strain	0	0.50%	2%	4%	0	0.50%	2%	4%	0	0.50%	2%	4%	0	0.50%	2%	4%
S. aureus ATCC 25923	0.02	0.039	0.039	<0.01	*1.25*	*0.625*	*0.625*	*0.313*	0.039	0.078	0.078	0.039	5	2.5	2.5	5
S. aureus ATCC 29213	0.625	0.625	0.625	0.625	*2.5*	*2.5*	*1.25*	*0.313*	0.078	0.156	0.078	0.039	*5*	*2.5*	*2.5*	*1.25*
S. aureus CCUG 115915	0.625	0.625	0.625	0.625	*2.5*	*1.25*	*1.25*	*0.313*	0.078	0.156	0.156	0.039	*5*	*2.5*	*2.5*	*1.25*
S. uberis	1.25	1.25	2.5	5	1.25	1.25	1.25	2.5	*0.156*	*0.078*	*0.078*	*0.039*	*5*	*2.5*	*2.5*	*1.25*
S. agalactiae I	<0.01	<0.01	0.01	<0.01	*0.156*	*0.156*	*0.078*	*<0.01*	0.02	0.156	0.039	<0.01	*0.625*	*0.313*	*0.313*	*0.039*
S. agalactiae II	0.02	0.039	0.078	<0.01	*0.156*	*0.078*	*0.078*	*<0.01*	*0.156*	*0.156*	*0.156*	*<0.01*	*0.625*	*0.313*	*0.156*	*0.039*
S. dysagalactiae 1577-1-4	0.02	0.039	0.078	<0.01	*1.25*	*0.625*	*0.625*	*0.078*	*0.078*	*0.078*	*0.078*	*<0.01*	*5*	*2.5*	*2.5*	*0.625*
S. dysagalactiae	*0.02*	*0.02*	*0.02*	*<0.01*	*0.039*	*0.039*	*0.02*	*<0.01*	*0.156*	*0.156*	*0.078*	*0.078*	0.078	0.078	0.078	0.078
E. coli 1580-1-3	1.25	2.5	2.5	2.5	>10	>10	>10	>10	5	5	5	10	>10	>10	>10	>10
Italics represents potentiation of antibiotic with increasing OligoG CF-5/20 concentration.																

Table 3: Inhibitory concentrations (µg/ml) of antibiotics alone and with increasing concentrations of OligoG (0.5, 2, 4%) for the bacterial test strains.

The aim of this study was to determine the effect of the alginate oligomer OligoG CF-5/20 on bacterial pathogens known to be involved in bovine mastitis. Both the direct effect on growth of the strains and the indirect effect on the strains susceptibility towards antibiotics were investigated. The antibiotics chosen were from different chemical classes; ampicillin (β-lactam), erythromycin (macrolid), cephalothin (1st generation cephalosporin) and lincomycin (lincosamid). The results clearly show that OligoG CF-5/20 reduce the growth of the test

strains in a dose dependent manner. This is in line with previously reported data on human pathogens associated with respiratory infections in CF and COPD [6] and for fungal strains [5]. The mechanism for the bacteriostatic effect of OligoG CF-5/20 is not yet elucidated, however several properties of OligoG CF-5/20 might contribute to the overall observed effects. For PAO1, it has been shown that OligoG CF-5/20 binds strongly to the surface of the cells and that this causes decreased motility and swarming, a more negatively charged surface and increased aggregation of the bacteria [10]. Furthermore, OligoG CF-5/20 chelates divalent ions with varying efficiency, Ca^{2+} binding most efficiently [11]. The ion modulating property of OligoG CF-5/20 could also presumably influence growth of those bacterial strains dependent on divalent ions such as Mg^{2+} and Fe^{2+}.

OligoG CF-5/20 was able to reduce the MIC values and inhibitory concentrations 2 to 8 fold for three of the four antibiotics for most strains. These data are in accordance with previous reports showing that OligoG is effective with antibiotics from different structural and functional classes and the effect is evident for different bacterial strains. These features indicate a general mechanism of action to account for the potentiating effect, and might be related to the biofilm disruption/inhibition properties of OligoG CF-5/20. The surface changes mediated by the binding of OligoG CF-5/20 to the bacterial cells may not only influence the adhesion of cells to physical surfaces but also destabilize the structure and assembly of the biofilm itself. Biofilm in the presence of OligoG CF-5/20 might therefore release more cells to the planktonic phase where they are more susceptible to antibiotic treatment and this may partly explain the observed reduction in MIC values.

Conclusions

Results obtained in this study show that co-administration of OligoG CF-5/20 with ampicillin, erythromycin, cephalothin and lincomycin can potentiate the effect of the antibiotics against several bacterial strains that are involved in bovine mastitis infections. Specifically, OligoG CF-5/20 was found to potentiate the effect of all tested antibiotics towards up to eight of the nine test strains used including clinical isolates from infected animals. OligoG CF-5/20 is composed of non-toxic, highly water-soluble alginate oligomers. Since these oligomers therefore in principle could be administered in relatively high doses, the findings presented here not only indicate a potential use of alginate oligomers in the treatment of BM, but also represent a promising opportunity to facilitate a reduction in the use of antibiotics and their subsequent impact on drug resistance.

Conflict of Interest

P.D.R. is a director/owner at AlgiPharma AS.

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References

1. Pritchard MF, Powell LC, Menzies GE, Lewis PD, Hawkins K, et al. (2016) A New Class of Safe Oligosaccharide Polymer Therapy To Modify the Mucus Barrier of Chronic Respiratory Disease. Mol Pharm 13: 863-872.
2. Vitko M, Valerio DM, Rye PD, Onsøyen E, Myrset AH, et al. (2016) A novel guluronate oligomer improves intestinal transit and surviaval in cystic fibrosis mice. J Cyst Fibros 15: 745-751.
3. Wang HZ, Song ZJ, Ciofu O, Onsoyen E, Rye PD, et al. (2016) OligoG CF-5/20 Disruption of Mucoid Pseudomonas aeruginosa Biofilm in a Murine Lung Infection Model. Antimicrob Agents Chemother 60: 2620-2626.
4. Powell LC, Sowedan A, Khan S, Wright CJ, Hawkins K, et al. (2013) The effect of alginate oligosaccharides on the mechanical properties of Gram-negative biofilms. Biofouling 29: 413-421.
5. Tøndervik A, Sletta H, Klinkenberg G, Emanuel C, Powell LC, et al. (2014) Alginate Oligosaccharides Inhibit Fungal Cell Growth and Potentiate the Activity of Antifungals against Candida and Aspergillus spp. Plos One 9: e112518.
6. Khan S, Tøndervik A, Sletta H, Klinkenberg G, Emanuel C, et al. (2012) Overcoming Drug Resistance with Alginate Oligosaccharides Able To Potentiate the Action of Selected Antibiotics. Antimicrob Agents Chemother 56: 5134-5141.
7. Viguier C, Arora S, Gilmartin N, Welbeck K, O'Kennedy R (2009) Mastitis detection: current trends and future perspectives. Trends Biotechnol 27: 486-493.
8. Gomes F, Saavedra MJ, Henriques M (2016) Bovine mastitis disease/pathogenicity: evidence of the potential role of microbial biofilms. Pathog Dis 74.
9. De Oliveira AP, Watts JL, Salmon SA, Aerestrup FM (2000) Antimicrobial susceptibility of Staphylococcus aureus isolated from bovine mastitis in Europe and the United States. J Dairy Sci 83: 855-862.
10. Powell LC, Pritchard MF, Emanuel C, Onsøyen E, Rye PD, et al. (2014) A Nanoscale Characterization of the Interaction of a Novel Alginate Oligomer with the Cell Surface and Motility of Pseudomonas aeruginosa. Am J Respir Cell Mol Biol 50: 483-492.
11. Smidsrød O, Haug A (1965) The effect of divalent metals on the properties of alginate in solution. I. Calcium ions. Acta Chemica Scandinavica 19: 329-340.