

Spring 2018

# Antibacterial properties of novel amphiphiles: Exploring structure-activity relationships

Reafa Hossain  
*James Madison University*

Follow this and additional works at: <https://commons.lib.jmu.edu/honors201019>

 Part of the [Pathogenic Microbiology Commons](#)

---

## Recommended Citation

Hossain, Reafa, "Antibacterial properties of novel amphiphiles: Exploring structure-activity relationships" (2018). *Senior Honors Projects, 2010-current*. 546.  
<https://commons.lib.jmu.edu/honors201019/546>

This Thesis is brought to you for free and open access by the Honors College at JMU Scholarly Commons. It has been accepted for inclusion in Senior Honors Projects, 2010-current by an authorized administrator of JMU Scholarly Commons. For more information, please contact [dc\\_admin@jmu.edu](mailto:dc_admin@jmu.edu).

# Antibacterial Properties of Novel Amphiphiles: Exploring Structure-Activity Relationships

---

An Honors College Project Presented to  
the Faculty of the Undergraduate  
College of Science and Mathematics,  
James Madison University

---

by Reafa Akhter Hossain

May 2018

---

---

Accepted by the faculty of the Department of Biology, James Madison University, in partial fulfillment of the requirements for the Degree of Bachelor of Science.

FACULTY COMMITTEE:

HONORS COLLEGE APPROVAL:

---

Project Advisor: Kyle Seifert, Ph.D.,  
Professor, Department of Biology

---

Bradley R. Newcomer, Ph.D.,  
Director, Honors Program

---

Reader: Pradeep Vasudevan, Ph.D.,  
Assistant Professor, Department of Biology

---

Reader: Kevin L. Caran, Ph.D.,  
Professor, Department of Chemistry and Biochemistry

---

---

## PUBLIC PRESENTATION

This work is accepted for presentation, in part or in full, at James Madison University Biosymposium, on April 13, 2018.

## Table of Contents

List of Figures and Tables.....	3
Acknowledgements.....	4
Abstract.....	5
Introduction.....	6
Materials and Methods.....	11
Results.....	12
Discussion.....	15
References.....	18

## List of Figures and Tables

### Figures

1	Conventional amphiphile containing a singular cationic head and a singular nonpolar tail.....	8
2	Scanning electron microscopy images of <i>E. coli</i> cells.....	8
3	Structures of two headed amphiphiles.....	9
4	Structure of n-bipyridinium-x-bipyridinium-n amphiphile.....	10
5	Antibacterial activity of n-bipyridinium-x-bipyridinium-n compounds against Gram-negative (G-) and Gram-positive (G+) bacteria.....	14

### Tables

1	Minimum inhibitory concentrations of bipyridinium amphiphiles ( $\mu\text{M}$ ) tested against Gram-positive strains.....	13
2	Minimum inhibitory concentrations of bipyridinium amphiphiles ( $\mu\text{M}$ ) tested against Gram-negative strains.....	13

### **Acknowledgements**

I would like to thank Dr. Kyle Seifert for all of the guidance he provided me while going through this process. His insight helped me establish strong relations with my peers and other faculty members. I would also like to thank my committee members, Dr. Pradeep Vasudevan and Dr. Kevin Caran for taking the time out of their schedules to answer my questions. Finally, I would like to thank my family for their support these last four years. Their prayers helped me get through difficult times. Thank you!

## **Abstract**

The increased cases of antibiotic resistance have large implication in hospital settings where infections by antibiotic resistant bacteria are harder to treat resulting in longer stays at the hospital, which drastically increases the costs to patients and hospitals. To address this matter, many research groups are searching for an alternative to antibiotics. One option is the development of amphiphiles, some of which have antibacterial properties. Amphiphiles contain a hydrophilic, polar head group, and a hydrophobic, nonpolar tail, which may intercalate into the cell membrane, resulting in cell lysis. Understanding the impact of amphiphile geometry on antibacterial activity allows for the synthesis of potential novel antimicrobial compounds with a variety of applications. Novel bipyridinium amphiphiles were synthesized and evaluated for antibacterial properties against seven bacterial strains. Amphiphiles contained two pyridinium head groups attached by a carbon chain of varying linker lengths. A 12 carbon tail was also attached to each pyridinium head. A linker length of 12 carbons had optimum antibacterial activity against each strain. Amphiphiles with longer and shorter linker lengths were less effective. The research and development of novel compounds can be used to reduce spread of nosocomial infections and decrease negative impacts of infections caused by antibiotic resistant bacteria.

## Introduction

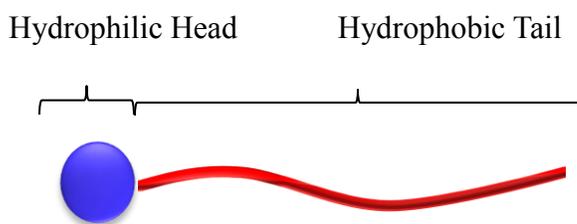
Since the discovery of the first antibiotic in 1926 by Alexander Fleming, over 250 million antibiotics have been prescribed and given to outpatients in community pharmacies (Hicks et al., 2015). The introduction of antibiotics decreased mortality rates associated with pneumonia, tuberculosis, diarrhea, and enteritis, which had previously caused one third of deaths in the United States (Centers for Disease Control and Prevention, 1999). However, mortality rates associated with hospital-acquired infections have remained high. Approximately 1.7 million HAIs occur each year and result in an estimated 99,000 deaths (Klebens *et al.*, 2007). Potential sources of the pathogens include hands of the health care personnel who have encountered contaminated surfaces, direct patient contact with the contaminated surface, and transfer of infected patients to other hospitals (Struelens, 1998). The prevention of HAIs through aseptic practice and methods provided by the U.S. Department of Health and Human Services (HHS) can help save the healthcare system billions of dollars (Kahn et al., 2014).

Another contributor to the costs associated with HAIs is antibiotic resistance. Increased incidences of HAIs have been associated to antibiotic resistant pathogens, increasing the rates of morbidity and mortality (Struelens, 1998). Causes for antibiotic resistance in HAIs include increased antibiotic prescription by hospital doctors and mutation and transfer of resistance genes between bacterial strains (Struelens, 1998). A notable example of an antibiotic resistance pathogen present in areas of healthcare is methicillin resistant *Staphylococcus aureus* (MRSA). Direct correlations have been observed between incidences of MRSA and regulated use of antibiotics (Gardete & Tomasz Alexander, 2014). Glycopeptide antibiotics, such as vancomycin, were used to treat most infections caused by strains of MRSA resistant to antibiotics. However, decreased susceptibility to vancomycin was reported in the U.S. and Japan (Gardete & Tomasz Alexander, 2014).

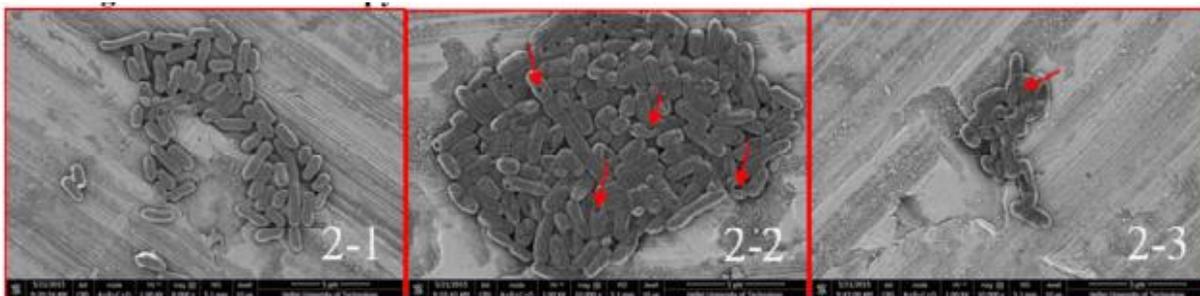
Enterococci, such as *E. faecalis*, have also been observed in increasing amounts as a HAI (Matar, 2017). A 20-fold increase in vancomycin-resistance followed the increased use of the antibiotic in U.S. hospitals. Vancomycin-resistant *E. faecalis* can also be introduced to human flora during consumption. Most strains resistant to vancomycin have also been found to be resistant to other antimicrobials (Struelens, 1998). Antibiotic resistance has been observed among Gram-negative strains including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. Infections caused by these bacteria have resistance to major antibiotics, required extensive antibiotic use during treatment, and caused nosocomial infections that were correlated to increased mortality rates (Struelens, 1998).

Many research groups are searching for alternatives to antibiotics or the development of a new class of antibiotics in order to decrease mortalities associated with antibiotic resistance. One such alternative to antibiotics are amphiphiles. Similar to phospholipids, amphiphiles contain a hydrophilic, polar head group, and a hydrophobic, nonpolar tail (Figure 1). The head groups can also be chemically structured to have a charge. Heads containing a positive charge allows them to interact with the negatively charged bacterial membrane. The hydrophobic tail inserts into the hydrophobic interior of the phospholipid bilayer of the bacterial membrane. Changes in the permeability of the membrane results in a damage to the cell system (Yang *et al.*, 2017). The incorporation of the tail in the membrane of the bacterial cell increases its hydrophobicity, resulting in the formation of transient channels and pores in the membrane (Zhang *et al.*, 2015). This increases the permeability of the cell membranes and results in a leakage of the cell content into the surrounding environment (Zhang *et al.*, 2015). This causes a disruption of the biochemical processes of the cell and eventual cell death. Scanning electron microscopy suggests that amphiphiles cause cell death through lysis (Zhao *et al.*, 2015, Figure 2). Amphiphile

concentrations lower than the minimum inhibitory concentration (MIC) did not show any significant effect on *E. coli* cells (Zhao *et al.*, 2015, Figure. 2). However, some holes are observed indicating disruption of the cell membrane (Zhao *et al.*, 2015, Figure 2). At concentrations above MIC, cells were irregularly shaped and presumed to be dead (Zhao *et al.*, 2015, Figure 2). As a result, it was concluded that disruption of the cell membrane caused leakage of cytoplasmic content, and ultimately cell death.



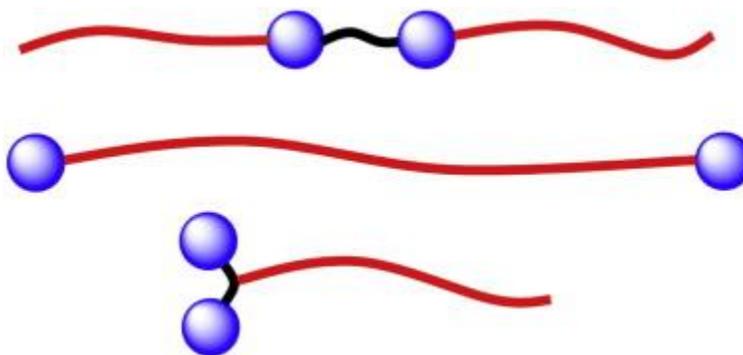
**Figure 1.** Conventional amphiphile containing a singular cationic head and a singular nonpolar tail. The hydrophilic head group typically contains polar bonds and/or ionic charges while the hydrophobic tail is composed of hydrocarbons.



**Figure 2.** Scanning electron microscopy images of *E. coli* cells (Zhao *et al.*, 2015). Cells were untreated (2-1), or treated with 0.3 mg/L of dimeric amphiphiles 12-8-12 (2-2) and 5 mg/L 12-8-12.

A number of studies have previously linked antibacterial activity of amphiphiles to its structure (Marafino *et al.*, 2015). A study of over 200 quaternary ammonium compounds concluded that bis-cationic amphiphiles with alkyl chains greater than 12 carbons were most effective at inhibiting bacterial growth alongside the substitution of trimethylammonium groups

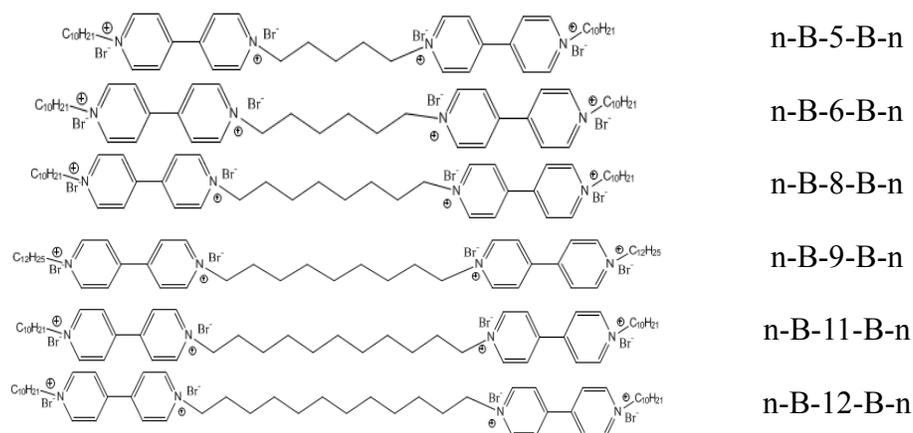
with pyridinium groups (Minbiole *et al.*, 2016). Compounds that were symmetrical had optimum antibacterial activity at chain length of 12 to 14 carbons when compounds were two tailed (Minbiole *et al.*, 2016). Activity decreased when carbon chains were above or below these values. This trend was also observed with cationic amphiphiles. Tails lengths between 12-14 carbons for cationic amphiphiles had the most potent antimicrobial activity. Our lab has previously conducted comparisons between conventional (one cationic head and one nonpolar tail), bicephalic (two heads and one tail), and gemini (two heads and two tails) amphiphiles to determine impact on antimicrobial activity (Ladow *et al.*, 2011, Figure 3). Gemini amphiphiles were found to be the most potent (Ladow *et al.*, 2011).



**Figure 3.** Structures of two headed amphiphiles (Ladow *et al.*, 2011). Blue circles are hydrophilic heads, red lines are hydrophobic tails, and black lines are linkers. Gemini amphiphiles are at the top followed by bolaamphiphiles in the middle, and polycephalic amphiphiles at the bottom.

Amphiphiles previously studied by our lab typically contained 1-2 head groups (Gallagher *et al.*, 2017; Grenier *et al.*, 2012; Ladow *et al.*, 2011; Marafino *et al.*, 2015). The purpose of this research was to assess the antibacterial activity of novel bipyridinium amphiphiles (Figure 4) on various strains of bacteria. The amphiphiles in this study contained four head groups and two hydrophobic tails. The presence of four large arene cationic head groups likely allows the amphiphile to form strong interactions with the anionic bacterial membrane. The relatively large head groups may also allow it to alter the curvature of the

membrane when incorporated, resulting in membrane disruption. By altering the carbon linker length (x) between the bipyridinium groups while keeping tail length (n) consistent, an optimal architecture for antibacterial activity may be determined (Figure 4). Minimum inhibitory concentration (MIC) values were determined for all amphiphile/bacterial strain pairs and evaluated for potential use in synergy assays.



**Figure 4.** Structure of n-bipyridinium-x-bipyridinium-n amphiphiles. Carbon linker lengths (x) range from 5-12 carbons and alkyl tail lengths (n) of 10 or 12 carbons. Bipyridinium groups are denoted by the letter B.

## Materials and Methods

### ***Bacterial Cultures***

Bacterial strains used for testing included Gram-negative *Escherichia coli* ATCC® 25922™, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* strain Boston 41501 ATCC® 27853™. Gram-positive strains included *Staphylococcus aureus* subsp. aureus ATCC® 29213™, *Streptococcus agalactiae* J48, and *Enterococcus faecalis* ATCC® 29212™ (Seifert et al., 2006). The Gram-positive *B. anthracis* Sterne strain was a generous gift from Dr. Louise Temple at James Madison University. Cultures were grown in Mueller-Hinton broth for 24 hours at 37°C.

### ***Minimum Inhibitory Concentration***

Amphiphiles were synthesized by students under the guidance of Dr. Kevin Caran from James Madison University, Department of Chemistry and Biochemistry. A modified broth microdilution method was used to determine Minimum Inhibitory Concentrations (MICs) for amphiphiles (Wayne, 2009). Amphiphiles were warmed at approximately 45°C to increase solubility before being diluted serially using distilled water. Bacteria were diluted to final concentrations of approximately  $5 \times 10^6$  CFU/mL using Mueller-Hinton broth. Bacteria and amphiphile were added to each well in triplicate at final concentrations of 250  $\mu$ M-1  $\mu$ M. Plates were incubated at 37°C for 72 hours before MICs were determined. The lowest dilution of amphiphile with no visible growth in the wells was considered the MIC.

## Results

### *Effect of Spacer Length on Antibacterial Activity*

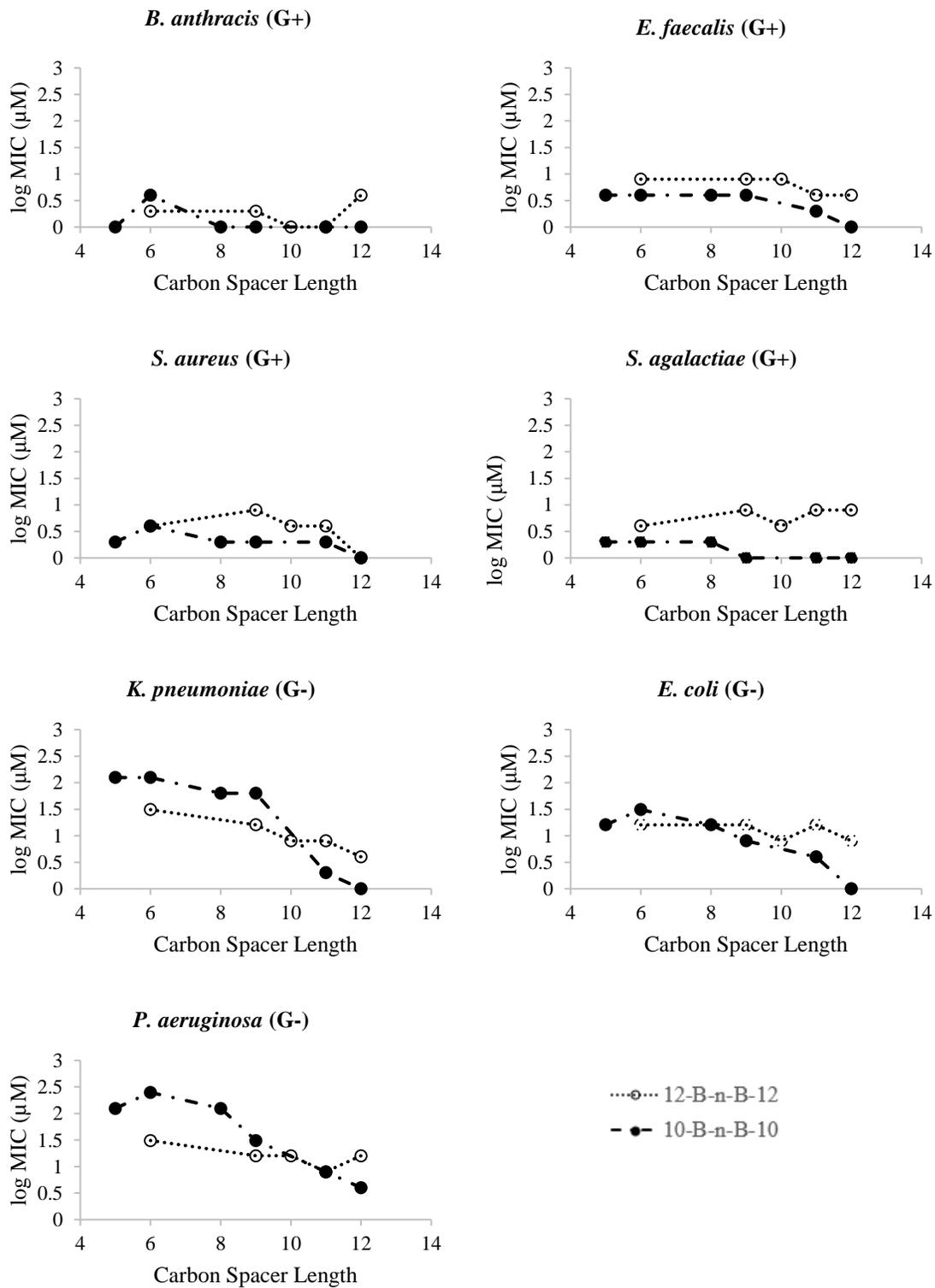
MICs were determined to investigate the effect of spacer length on the antibacterial properties of eleven bipyridinium amphiphiles (Table 1, Table 2, Figure 5). A single amphiphile had the lowest MIC values for all bacterial strains. 10-B-12-B-10 (10 carbon tails and 12 carbon spacer between bipyridinium groups) was effective against all Gram-positive bacteria at MIC values of 1  $\mu\text{M}$  and Gram-negative strains at MIC values of 1  $\mu\text{M}$  excluding *P. aeruginosa* with an MIC value of 4  $\mu\text{M}$  (Table 1, Table 2, Figure 5). The antibacterial activity of the 10-B-12-B-10 against *P. aeruginosa* was best of approximately 80 amphiphiles tested to date (Table 2). Amphiphile 12-B-11-B-12 (12 carbon tails and 11 carbon spacer between bipyridinium groups) was most effective against Gram-positive bacterial strains particularly *B. anthracis* when looking at amphiphiles with 12 carbon tails. However, this trend did not apply to the Gram-negative strains, where the 12 carbon spacer was more effective against *K. pneumoniae* and *E. coli*. A larger concentration of the 12 carbon spacer amphiphile with the 12 carbon tail was required to inhibit growth of *P. aeruginosa* when compared to the 11 carbon spacer (16  $\mu\text{M}$  vs 8  $\mu\text{M}$ ). No direct relation was observed between tail length, spacer length, and MIC values.

**Table 1.** Minimum inhibitory concentrations of bipyridinium amphiphiles ( $\mu\text{M}$ ) tested against Gram-positive strains.

<b>n-B-x-B-n</b>	<i>B. anthracis</i>	<i>E. faecalis</i>	<i>S. aureus</i>	<i>S. agalactiae</i>
10-B-5-B-10	1	4	2	2
10-B-6-B-10	4	4	4	4
10-B-8-B-10	1	4	2	2
10-B-9-B-10	1	4	2	2
10-B-11-B-10	1	2	2	2
10-B-12-B-10	1	1	1	1
12-B-6-B-12	2	8	4	4
12-B-9-B-12	2	8	8	8
12-B-10-B-12	1	8	4	4
12-B-11-B-12	1	4	4	8
12-B-12-B-12	4	4	4	8

**Table 2.** Minimum inhibitory concentrations of bipyridinium amphiphiles ( $\mu\text{M}$ ) tested against Gram-negative strains.

<b>n-B-x-B-n</b>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>P.aeruginosa</i>
10-B-5-B-10	125	16	125
10-B-6-B-10	125	31	250
10-B-8-B-10	63	16	125
10-B-9-B-10	63	8	31
10-B-11-B-10	2	4	8
10-B-12-B-10	1	1	4
12-B-6-B-12	31	16	31
12-B-9-B-12	16	16	16
12-B-10-B-12	8	8	16
12-B-11-B-12	8	16	8
12-B-12-B-12	4	8	16



**Figure 5.** Antibacterial activity of n-bipyridinium-x-bipyridinium-n compounds against Gram-negative (G-) and Gram-positive (G+) bacteria. Filled circles indicated amphiphiles with 12 carbon tails while open circles indicate 10 carbon tails.

## Discussion

Novel bipyridinium amphiphiles were synthesized to determine the impact of linker length and tail length on antimicrobial activity. Amphiphile 10-B-12-B-10 had the lowest MIC for all bacterial strains when compared to the other tested amphiphiles. Amphiphiles with small tail lengths and larger linkers were better than amphiphiles with longer tail lengths and smaller linkers. It is likely that the presence of four large arene cationic head groups allows the amphiphile to form strong interactions with the anionic membrane of the bacterial cell. The relatively large headgroups would also allow it to alter the curvature of the membrane when incorporated resulting in membrane disruption.

All amphiphiles had MIC values at concentrations equal to or below 250  $\mu\text{M}$ . MIC values were higher for Gram-negative strains compared to Gram-positive. This is likely because Gram-negative bacteria have a second, outer membrane, and a densely packed outer membrane composed of lipopolysaccharides, which makes it less permeable. *P. aeruginosa* also expresses selective porins and overproduces drug efflux pumps, hindering the passage of extracellular molecules thus decreasing susceptibility to amphiphiles (Marafino *et al.*, 2015).

*P. aeruginosa* has also been noted for its ability to form biofilms, slime enclosed aggregates that increase bacterial resistance to antibiotics. Many HAIs are also caused by organisms that form biofilms on surfaces such as catheters or medical devices. Antibiotic resistance of organisms in biofilms increases 1000-fold when compared to planktonic cells, and can usually only be eradicated completely from a surface using chemicals and physical methods (Essomba *et al.*, 2013).

Other way to address the problems associated with antibiotic resistance includes the use of combination therapy, using more than a single antimicrobial simultaneously. This option

decreases the probability of an organism is resistant to two or more antimicrobials, especially if the antimicrobials have different mechanisms of action. Synergistic effects may also be observed between two different antimicrobials where the combination of the two antimicrobials are more effective at killing the bacterial together versus alone.

Synergetic effects have been observed between amphiphiles and antibiotics such as tetracyclines. The membrane disrupting ability of amphiphiles have been thought to increase membrane permeability, allowing the antibiotic to enter the cell (Gokel & Negin, 2012). Our lab has conducted preliminary experiments to explore possible synergistic effects between synthesized amphiphiles and tobramycin, oxacillin, sodium metaperiodate, and benzalkonium chloride. While the previously mentioned bipyridium amphiphiles have not been used in synergy assays, previously synthesized amphiphiles (M-DMAP 12,12 and M-IQ 12,12) have been synergistic against *E. coli* and *S. aureus* when used in collaboration with benzalkonium chloride.

Novel antibiotics are required to address combat the increasing rates of mortality and morbidity caused by antibiotic resistant pathogens in healthcare settings. Amphiphiles have shown potential use as an antimicrobial agent. However, elucidation of the structure-function relationship is required to develop potent chemical disinfectants. The investigated amphiphiles have the potential of being used as disinfectants.. The information gathered on the n-bipyridinium-x-bipyridinium-n amphiphiles provides information on the relation between amphiphile tail and linker length, and the impact on bacterial growth. The application of this information has the ability to decrease cost, mortality, and morbidity associated with infections caused by antibiotic resistant bacteria in healthcare settings.

## References

- Centers for Disease Control and Prevention.** (1999). Achievements in Public Health, 1900-1999: Control of Infectious Diseases.
- Essomba, C. N., Leme, L., Esiene, A., Abong, T., Etoundi, O., Gweth, M. N., Abong, T., Bilong, C. F. B.** (2013). Identification and quantification of bacteria associated with indwelling urinary catheterization. *International Journal of Current Microbiology and Applied Sciences*, 2, 168–177.
- Gallagher, T. M., J. N. Marafino, B. K. Wimbish, B. Volkers, G. Fitzgerald, K. Mckenna, J. Floyd, N. T. Minahan, B. Walsh, K. Thompson, D. Bruno, M. Paneru, S. Djikeng, S. Masters, S. Haji, K. Seifert, and K. L. Caran.** 2017. Hydra amphiphiles: Using three heads and one tail to influence aggregate formation and to kill pathogenic bacteria. *Colloids & Surfaces B: Biointerfaces* 157 (09): 440-8
- Gardete, S., & Tomasz Alexander.** (2014). Mechanisms of vancomycin resistance in *Staphylococcus aureus*. *The Journal of Clinical Investigation*, 124(7), 2836–2840.  
<https://doi.org/10.1172/JCI68834>
- Gokel, G. W., & Negin, S.** (2012). Synthetic membrane active amphiphiles. *Advanced Drug Delivery Reviews*. <https://doi.org/10.1016/j.addr.2012.01.011>
- Grenier, M. C., Davis, R. W., Wilson-Henjum, K. L., LaDow, J. E., Black, J. W., Caran, K. L., Seifert, K., Minbiole, K. P. C.** (2012). The antibacterial activity of 4,4'-bipyridinium amphiphiles with conventional, bicephalic and gemini architectures. *Bioorganic & Medicinal Chemistry Letters*, 22(12), 4055–4058.  
<https://doi.org/10.1016/j.bmcl.2012.04.079>
- Hicks, L. A., Bartoces, M. G., Roberts, R. M., Suda, K. J., Hunkler, R. J., Taylor, T. H., &**

- Schrag, S. J. (2015). US outpatient antibiotic prescribing variation according to geography, patient population, and provider specialty in 2011. *Clinical Infectious Diseases*, 60(9), 1308–1316. <https://doi.org/10.1093/cid/civ076>
- Jenkins**, S. G., & Schuetz, A. N. (2012). Current concepts in laboratory testing to guide antimicrobial therapy. In *Mayo Clinic Proceedings* (Vol. 87, pp. 290–308). <https://doi.org/10.1016/j.mayocp.2012.01.007>
- Kahn**, K. L., Mendel, P., Leuschner, K. J., Hiatt, L., Gall, E. M., Siegel, S., & Weinberg, D. A. (2014). The national response for preventing healthcare-associated infections: Research and adoption of prevention practices. *Medical Care*, 52(2 SUPPL.1). <https://doi.org/10.1097/MLR.0000000000000084>
- Klevens**, R. M., Edwards, J. R., Richards, C. L., Horan, T. C., Gaynes, R. P., Pollock, D. A., & Cardo, D. M. (2007). Estimating health care-associated infections and deaths in U.S. Hospitals, 2002. *Public Health Reports*, 122(2), 160–166. <https://doi.org/10.1177/003335490712200205>
- LaDow**, J. E., D. C. Warnock, K. M. Hamill, K. L. Simmons, R. W. Davis, C. R. Schwantes, D. C. Flaherty, J. A. L. Willcox, K. Wilson-Henjum, K. L. Caran, K. P. C. Minbiole, and K. Seifert. 2011. Original article: Bicephalic amphiphile architecture affects antibacterial activity. *European Journal of Medicinal Chemistry* 46:4219-26.
- Marafino**, J. N., Gallagher, T. M., Barragan, J., Volkens, B. L., LaDow, J. E., Bonifer, K., Seifert, K., Caran, K. L. (2015). Colloidal and antibacterial properties of novel triple-headed, double-tailed amphiphiles: exploring structure-activity relationships and synergistic mixtures. *Bioorganic & Medicinal Chemistry*, 23(13), 3566–73. <https://doi.org/10.1016/j.bmc.2015.04.020>

- Matar, G. M.** (2017). Editorial: Emerging Enterobacteriaceae Infections: Antibiotic Resistance and Novel Treatment Options. *Frontiers in Microbiology*, 8, 509.  
<https://doi.org/10.3389/fmicb.2017.00509>
- Minbiole, K. P. C., M. C. Jennings, L. E. Ator, J. W. Black, M. C. Grenier, J. E. LaDow, K. L. Caran, K. Seifert, and W. M. Wuest.** 2016. From antimicrobial activity to mechanism of resistance: The multifaceted role of simple quaternary ammonium compounds in bacterial eradication. *Tetrahedron* 72(25):3559-66.
- Seifert, K. N., Adderson, E. E., Whiting, A. A., Bohnsack, J. F., Crowley, P. J., & Brady, L. J.** (2006). A unique serine-rich repeat protein (Srr-2) and novel surface antigen ( $\epsilon$ ) associated with a virulent lineage of serotype III *Streptococcus agalactiae*. *Microbiology*, 152(4), 1029–1040. <https://doi.org/10.1099/mic.0.28516-0>
- Struelens, M. J.** (1998). The epidemiology of antimicrobial resistance in hospital acquired infections: problems and possible solutions. *BMJ (Clinical Research Ed.)*, 317(7159), 652–654. <https://doi.org/10.1136/bmj.317.7159.652>
- Zhang, S., Ding, S., Yu, J., Chen, X., Lei, Q., & Fang, W.** (2015). Antibacterial Activity, in Vitro Cytotoxicity, and Cell Cycle Arrest of Gemini Quaternary Ammonium Surfactants. *Langmuir : The ACS Journal of Surfaces and Colloids*, 31(44), 12161–9.  
<https://doi.org/10.1021/acs.langmuir.5b01430>
- Zhao, X. F., Li, Y. H., & Yuan, H.** (2015). Structure-antimicrobial activity relationship and action mechanism of dimeric quaternary ammonium amphiphiles. In *Proceedings of the 3rd International Conference on Material, Mechanical and Manufacturing Engineering*. Paris, France: Atlantis Press. <https://doi.org/10.2991/ic3me-15.2015.82>