

# Project Notes:

## Project Title:

Name: Emily Wang

**Note Well:** There are NO SHORT-cuts to reading journal articles and taking notes from them. Comprehension is paramount. You will most likely need to read it several times so set aside enough time in your schedule.

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## Knowledge Gaps:

This list provides a brief overview of the major knowledge gaps for this project, how they were resolved and where to find the information.

Knowledge Gap	Resolved By	Information is located	Date resolved
How enzymes break down plastic	[no longer relevant to main project idea]		9/30/22
Health effects of consuming plastic	Journal article	“Health Effects” section of Microplastics in food: scoping review on health effects, occurrence, and human exposure	9/30/22

Toxins absorbed by plastic			
Chemical structure and makeup of plastic/ different types of plastic	Different types of plastic: vocab from "Hitchhikers..."	Different types of plastic: vocab from "Hitchhikers..."	Different types of plastic: 8/27/22
How enzymes can be modified			
What can enzymes be used for			
Common complications/ inconveniences in healing + what has been done to address them	[no longer relevant to main idea]		9/30/22
How to make bandages stick on sweaty skin ex. palms, soles of feet	[no longer relevant to main idea]		
Previous research on how antimicrobial resistance in bacteria can be inhibited			
How to obtain microplastics	Infoemation on how researchers in "Soil plastispheres..." obtained microplastics for their experiment. "Microplastics in food..." also defines microplastics and ways they are produced	"Soil plastispheres as..." and "Microplastics in food..."	9/13/22
What is/what causes antimicrobial resistance in MRSA			

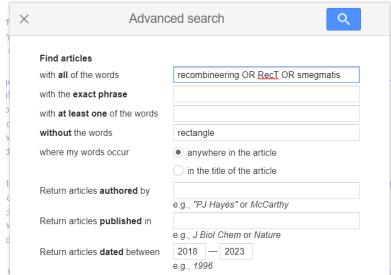
What is/what causes antimicrobial resistance in NRSA			
How to engineer substrates that bind to and inhibit protein pumps (or whatever the inhibitory mechanism of MRSA/NRSA is)			
Prevention measures that must be taken?			

## Literature Search Parameters:

These searches were performed between (Start Date of reading) and XX/XX/2019.

List of keywords and databases used during this project.

Database/search engine	Keywords	Summary of search
Pubmed	microplastic	Found articles 1) <a href="https://pubmed.ncbi.nlm.nih.gov/32800610/">https://pubmed.ncbi.nlm.nih.gov/32800610/</a> 2)
ProQuest	<a href="https://www.proquest.com/biologicalscience1/results/A15ABA85C1E24700PQ/1?accountid=29120#">https://www.proquest.com/biologicalscience1/results/A15ABA85C1E24700PQ/1?accountid=29120#</a>  (Antimicrobial resistance) OR arg AND microplastic	Could not make an account to save my search, so I included a link to it in keywords. I sorted articles by date and if they were peer-reviewed journals. I was hoping to find articles that would help me address my question of what research has been done on addressing ARGs on microplastics before/where I might start my research. I soon found that none of the articles mentioned plastics, so I changed my keywords to just arg AND microplastic. Found articles: 1) <a href="https://www.proquest.com/biologicalscience1/docview/2706241721/109B244270CF49D7PQ/7?accountid=29120">https://www.proquest.com/biologicalscience1/docview/2706241721/109B244270CF49D7PQ/7?accountid=29120</a> : Effects of human activity/microplastics/ oxidative stress... 2) <a href="https://www-proquest-com.ezpv7-web-p-u01.wpi.edu/naturalscience/docview/2692478697/58FB3E27EAF14B56PQ/8?accountid=29120">https://www-proquest-com.ezpv7-web-p-u01.wpi.edu/naturalscience/docview/2692478697/58FB3E27EAF14B56PQ/8?accountid=29120</a> : Microplastics in

		food...
Google Scholar	 <p>Advanced search</p> <p>Find articles with all of the words: recombineering OR RecT OR smegmatis</p> <p>with the exact phrase: rectangle</p> <p>without the words: anywhere in the article</p> <p>Return articles authored by: e.g., "J Hayes" or McCarthy</p> <p>Return articles published in: e.g., J Biol Chem or Nature</p> <p>Return articles dated between: 2018 — 2023 e.g., 1996</p> <p>Key words should include at least one of the following:</p> <ul style="list-style-type: none"> <li>- Recombineering</li> <li>- RecT</li> <li>- Smegmatis</li> </ul> <p>Article should be authors by:</p> <ul style="list-style-type: none"> <li>- Kenan C. Murphy</li> </ul> <p>Article should not include the word</p> <ul style="list-style-type: none"> <li>- Rectangle</li> </ul> <p>Article should not be a</p> <ul style="list-style-type: none"> <li>- Patent</li> <li>- Citation</li> </ul> <p>Article should be published between 2018 and 2023</p>	<p>Found articles:</p> <ul style="list-style-type: none"> <li>- <a href="#"><u>Oligo-Mediated Recombineering and its Use for Making SNPs, Knockouts, Insertions, and Fusions in Mycobacterium tuberculosis</u></a> (do not have access)</li> </ul>

## Article #1 Notes: Title

Article notes should be on separate sheets

**KEEP THIS BLANK AND USE AS A TEMPLATE**

Source Title	
Source citation (APA Format)	
Original URL	
Source type	
Keywords	
Summary of key points + notes (include methodology)	
Research Question/Problem/Need	
Important Figures	
VOCAB: (w/definition)	
Cited references to follow up on	
Follow up Questions	

# Article #1 Notes: Tailor-made Enzymes Poised to Propel Plastic Recycling into a New Era

Article notes should be on separate sheets

Source Title	Tailor-made enzymes poised to propel plastic recycling into a new era
Source citation (APA Format)	Thoden van Velzen, E. U., & Santomasi, G. (2022, April 27). <i>Tailor-made enzymes poised to propel plastic recycling into a new era</i> . Nature News. Retrieved August 10, 2022, from <a href="https://www.nature.com/articles/d41586-022-01075-6">https://www.nature.com/articles/d41586-022-01075-6</a>
Original URL	<a href="https://www.nature.com/articles/d41586-022-01075-6">https://www.nature.com/articles/d41586-022-01075-6</a>
Source type	Website— summer
Keywords	FAST-PETase, recycle, temperature, PET, residue, enzyme, machine learning, closed cycle,
Summary of key points + notes (include methodology)	Though plastic should be recycled to keep it from polluting the environment, most types are ill-suited for recycling because they end up as worse quality material. The aim is closed-cycle recycling, like we have with some polyethylene terephthalate (PET) bottles, where plastic can be broken down into monomers and the monomers can be purified then used to form the same quality plastic again. Most recycling processes require extensive resources, whether it be energy or materials, so they are not very economical. An increasing number of places have instead been collecting and sorting recyclable PET waste for people to take or recycle. Another reason attempts to recycle have been unsuccessful is because many plastics have labels, ink, or other residue on them that forms gels when the plastic is melted down for recycling, preventing further progress in the recycling. Enzymes have been found that break down plastic, but they don't do so completely or quickly and only act on amorphous PET. Some scientists used machine-learning to find a mutated enzyme, which they named FAST-PETase, which can degrade plastic at lower temperatures, so residue on it doesn't interfere, and that can act relatively quickly on multiple types of plastic. Studies have shown that it can produce a closed cycle where most of the plastic can be recycled, so there's hope that it can be utilized to recycle more plastics.
Research Question/Problem/Need	Few plastics can be recycled to produce plastic of equal quality, as most need extreme conditions to melt down, resulting in complications and lower quality products due to residue on their surfaces melting as well.
Important Figures	<ul style="list-style-type: none"> <li>- Standard chemical recycling processes typically involve melting plastics at 195 degrees celsius and require many bases and acids</li> <li>- FAST-PETase depolymerizes PET completely at room temperature within a week or at 50 degrees celsius within a day</li> </ul>
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Amorphous: Not crystalline/ without a defined shape</li> </ul>

	<ul style="list-style-type: none"><li>- Polyethylene terephthalate (PET): Polyester, clear, strong, lightweight, commonly found in packaging, made up of chains of ethylene glycol and terephthalic acid; its raw materials are derived from crude oil and natural gas. Is the most recycled plastic in the world. (Info from <a href="http://www.petresin.org/news_introtoPET.asp">http://www.petresin.org/news_introtoPET.asp</a>)</li><li>- Closed-cycle recycling: Materials can be used, recycled, and made back into the same products.</li><li>- FAST-PETase: mutated enzyme that can degrade multiple types of plastic at lower temperatures and produce a closed cycle</li></ul>
Cited references to follow up on	<input type="checkbox"/> Lu, H. <i>et al. Nature</i> 604, 662–667 (2022).: <a href="https://www.nature.com/articles/s41586-022-04599-z">https://www.nature.com/articles/s41586-022-04599-z</a>
Follow up Questions	<input type="checkbox"/> If FAST-PETase is so efficient and effective, why hasn't it been used/studied more?

# Article #2 Notes: Plastic-eating Bacteria: Genetic Engineering and Environmental Impact

Article notes should be on separate sheets

Source Title	Plastic-eating bacteria: Genetic engineering and environmental impact
Source citation (APA Format)	Dutfield, S. (2022, March 23). <i>Plastic-eating bacteria: Genetic Engineering and Environmental impact</i> . LiveScience. Retrieved July 10, 2022, from <a href="https://www.livescience.com/plastic-eating-bacteria">https://www.livescience.com/plastic-eating-bacteria</a>
Original URL	<a href="https://www.livescience.com/plastic-eating-bacteria">https://www.livescience.com/plastic-eating-bacteria</a>
Source type	Website– summer
Keywords	Microplastics, Ideonella sakaiensis, polyethylene terephthalate (PET), PETase, Mhetase, vanillin, enzyme, bacteria
Summary of key points + notes (include methodology)	The 14 million tons of plastic that enter the oceans every year hurt marine life and become microplastics that soak up toxins, get eaten, and work their way up the food chain to harm humans. In 2016, some Japanese scientists found a bacteria, Ideonella sakaiensis, that was breaking down plastic bottles made of polyethylene terephthalate (PET). The bacteria produced two enzymes which hydrolyzed the long PET chains into monomers, then broke the monomers down again to supplement the bacterias' growth with the energy released. Since the discovery, scientists have genetically engineered E. coli to produce the PETase. They've also combined the DNA of the enzyme with another, Mhetase, that breaks down another type of plastic, to make an enzyme that can eat Polyethylene furanoate (PEF). Other researchers have even used E. coli to convert plastic into vanillin, used to make highly demanded vanilla bean extract. Usually vanillin is made with fossil fuels, so using plastic could be a more eco-friendly solution.
Research Question/Problem/ Need	How can we engineer enzymes to address plastic pollution?
Important Figures	<ul style="list-style-type: none"> <li>● 14 million tons of plastic enter oceans each year</li> <li>● Japanese scientists discovered Ideonella sakaiensis in 2016</li> <li>● Only 16% of plastic is recycled into new plastic</li> </ul>
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Microplastics: plastic less than five millimeters in length = about the size of a sesame seed</li> <li>- Ideonella sakaiensis: A bacteria found by Japanese scientists that produces two enzymes to break down plastic and use it</li> <li>- Hydrolyzed (hydrolysis): any chemical reaction where a molecule of water breaks one</li> </ul>

	or more chemical bonds
Cited references to follow up on	<input type="checkbox"/> Brandon C. Knott, et al, " <a href="#">Characterization and engineering of a two-enzyme system for plastics depolymerization</a> ", PNAS, Volume 117, September 2020. <input type="checkbox"/> University of Edinburgh, " <a href="#">Bacteria: Serving tasty solution to global plastic crisis</a> " ScienceDaily, June 2021. <input type="checkbox"/> Ankita Maurya, " <a href="#">Enzymatic Remediation of Polyethylene Terephthalate (PET)-Based Polymers for Effective Management of Plastic Wastes: An Overview</a> ", frontiers in Bioengineering and Biotechnology, Volume 8, November 2020. <input type="checkbox"/> Rumiana Tenchov, " <a href="#">Can plastic eating super-enzymes solve our destructive plastic problem?</a> ", CAS, March 2021. <input type="checkbox"/>
Follow up Questions	<input type="checkbox"/> What toxins are most often absorbed by the microplastics? <ul style="list-style-type: none"> <li><input type="checkbox"/> How can we prevent them from being absorbed?</li> <li><input type="checkbox"/> How harmful are they to marine/terrestrial life and humans?</li> </ul> <input type="checkbox"/> What are the most common types of plastic found polluting the environment? <input type="checkbox"/> What are the key components of PET? <input type="checkbox"/> How exactly do enzymes convert or break down plastic? <input type="checkbox"/> How can we genetically engineer enzymes to improve them?

# Article #3 Notes: Scientists make plastic more degradable under UV light

Article notes should be on separate sheets

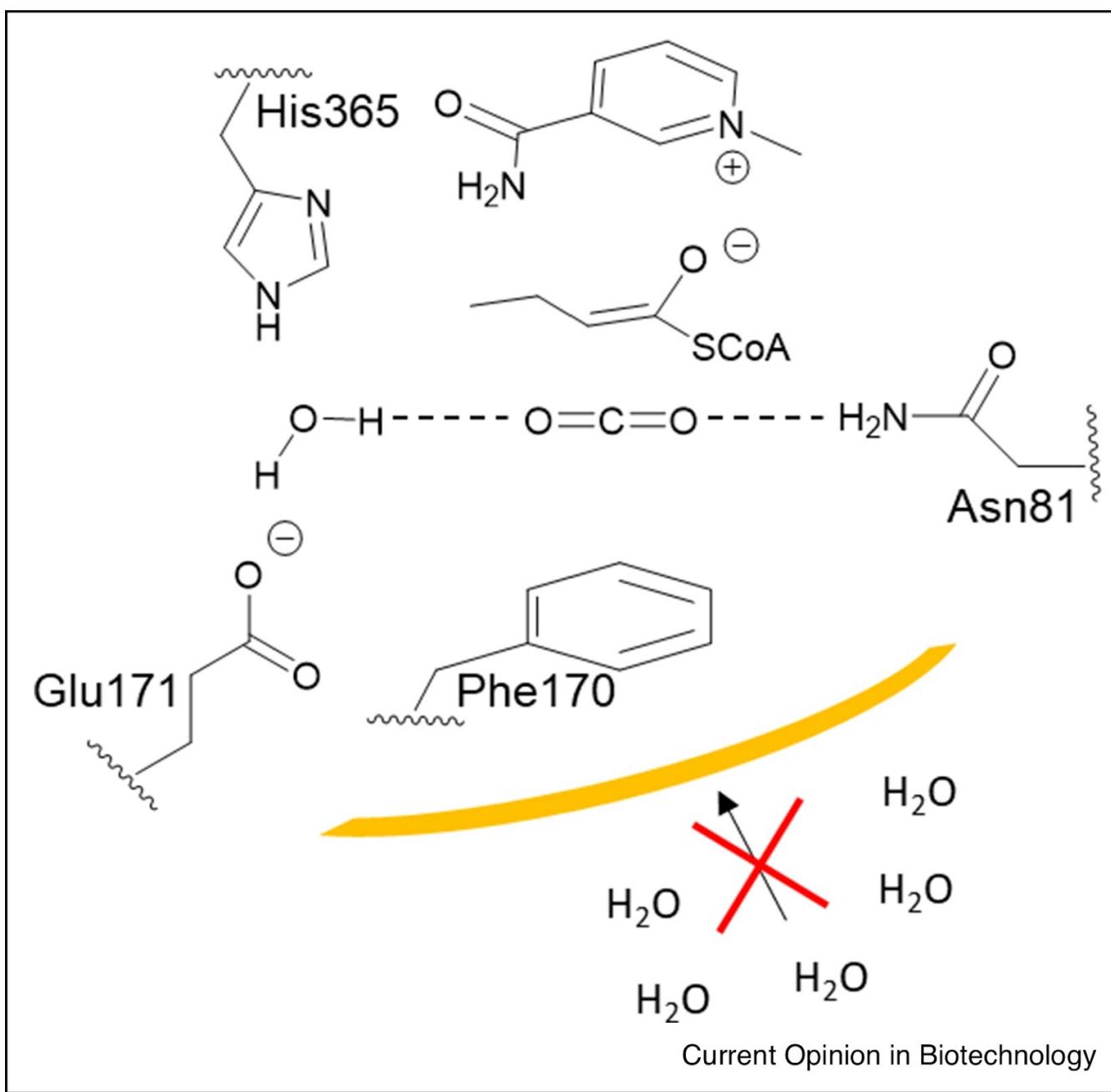
Source Title	Scientists make plastic more degradable under UV light
Source citation (APA Format)	University of Bath. (2022, May 24). <i>Scientists make plastic more degradable under UV light</i> . Phys.org. Retrieved August 20, 2022, from <a href="https://phys.org/news/2022-05-scientists-plastic-degradable-uv.html">https://phys.org/news/2022-05-scientists-plastic-degradable-uv.html</a>
Original URL	<a href="https://phys.org/news/2022-05-scientists-plastic-degradable-uv.html">https://phys.org/news/2022-05-scientists-plastic-degradable-uv.html</a>
Source type	Website— summer (didn't realize it wasn't a journal article because I hadn't read any before)
Keywords	Polylactic acid (PLA), biodegradable, industrial conditions, specific conditions, degrade, UV light, sugar, hydrolysis
Summary of key points + notes (include methodology)	Polylactic acid (PLA) is marketed as biodegradable, but it can only break down easily in specific industrial conditions, not in nature. To address this, scientists used it to create a plastic that could degrade within hours of exposure to UV light. The plastic was made by incorporating as little as three percent of sugar molecules in the long polymer chains of plastics. Since the sugar's bonds could be broken with just UV light, the plastic could be broken into smaller chains more susceptible to hydrolysis. The plastic hasn't been tested with real objects or sunlight yet, but there's hope that it can be introduced into the market for a better biodegradable plastic.
Research Question/Problem/Need	How can we make plastics more biodegradable in nature?
Important Figures	<ul style="list-style-type: none"> <li>- As little as 3% sugar molecules can make the plastic more biodegradable <ul style="list-style-type: none"> <li>- Up to 40% degraded within 6 hours under UV light</li> <li>- Compatible with plastic manufacturing processes= can be used in plastic</li> </ul> </li> </ul>
VOCAB: (w/definition)	Polylactic acid (PLA): biodegradable plastic made of renewable materials
Cited references to follow up on	<input type="checkbox"/> Craig Hardy et al, UV degradation of poly(lactic acid) materials through copolymerisation with a sugar-derived cyclic xanthate, Chemical Communications (2022). DOI: <a href="https://doi.org/10.1039/D2CC01322C">10.1039/D2CC01322C</a>
Follow up Questions	<ol style="list-style-type: none"> <li>1. Why does plastic degrade under UV light?</li> </ol>

- |  |  |
|--|--|
|  | <ul style="list-style-type: none"><li>a. Why did adding sugar molecules to the polymers make the bonds easier to break?</li><li>2. Has any further research about degrading plastics with light been conducted?</li><li>3. Can this technique for making plastics more biodegradable be applied to other/more common plastics (ex. PET)?</li><li>4. To what degree do the plastics degrade/ into what do they degrade?</li></ul> |
|--|--|

## Article #4 Notes: CO<sub>2</sub>-converting enzymes for sustainable biotechnology: from mechanisms to application

Source Title	CO <sub>2</sub> -converting enzymes for sustainable biotechnology: from mechanisms to application
Source citation (APA Format)	Bernhardsgrütter, I., Stoffel, G. M. M., Miller, T. E., & Erb, T. J. (2021). CO <sub>2</sub> -converting enzymes for sustainable biotechnology: From mechanisms to application. <i>Current Opinion in Biotechnology</i> , 67, 80–87. <a href="https://doi.org/10.1016/j.copbio.2021.01.003">https://doi.org/10.1016/j.copbio.2021.01.003</a>
Original URL	<a href="https://www.sciencedirect.com/science/article/pii/S0958166921000082">https://www.sciencedirect.com/science/article/pii/S0958166921000082</a>
Source type	Journal article
Keywords	Carbon dioxide (CO <sub>2</sub> ), carboxylases, enzymes, circular, carbon neutral, sustainable, CO <sub>2</sub> -fixing, mechanisms,
Summary of key points + notes (include methodology)	CO <sub>2</sub> binding and carboxylases have the potential to help us realize a carbon-neutral economy. Different organisms have evolved different carboxylases, which scientists have observed to improve their understanding of the processes, down to the molecular level. With their greater understanding of the limitations and integral elements of carboxylases, they hope to engineer more efficient systems for the capture and conversion of CO <sub>2</sub> .
Research Question/Problem/ Need	<i>How do carboxylases function and how can they help us build a sustainable, carbon-based economy?</i>

## Important Figures



Caption: Occurs at the active site of ECR, in *K. setae*. Three amino acids—His, Glu, and Asn—together with a crystal water, hold a  $\text{CO}_2$  molecule next to the enolate, using hydrogen bonds. A fourth amino acid, either Phe or Tyr, blocks water from the enolate to prevent protonation of the enolate.

- Carboxylases capture and fix carboxylases
- Forming C-C bonds costs about  $-20\text{ kJ/mol}$
- Amino acids work to shield enolates from water= suppress side reactions
- There is a trade-off between  $\text{CO}_2$  specificity and catalytic rate of enzymes, and substrate activation and side reactivity seem directly correlated
  - $\text{CO}_2$  fixing enzymes have low  $K_{\text{cat}}$  values: between  $10\text{s}^{-1}$  (Ribulose-1,5-biphosphate

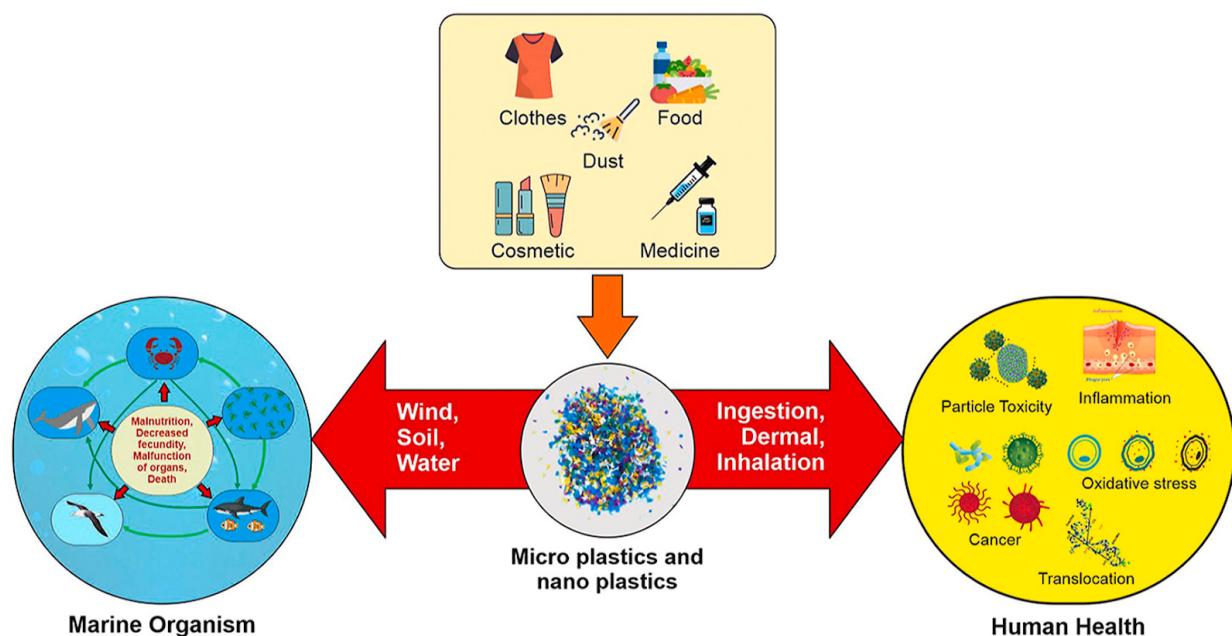
	<p>carboxylase/oxygenase [RubisCO]) and <math>100\text{s}^{-1}</math> (Enoyl-CoA carboxylase/reductase [ECR])</p> <ul style="list-style-type: none"> <li>- Scientists have transplanted CO<sub>2</sub> fixation pathways into heterotrophic organisms, in some (<i>E. coli</i> and <i>Pichia pastoris</i>) achieving full autotrophy</li> <li>- Scientists hope to improve turnover rate or specificity factor in RubisCO</li> </ul>
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Carboxylase: enzyme that captures and converts gaseous CO<sub>3</sub></li> <li>- <i>In vitro</i>: "in a tube/petri dish"</li> <li>- <i>In vivo</i>: "in an organism"</li> <li>- Nucleophile: atom or functional group with lone pair of electrons that can be shared</li> <li>- Nucleophilic attack: proton-rich substance bonds with proton-deficit substance</li> <li>- Enol: Has a hydroxyl group next to the C-C bond</li> <li>- Enolate: has a negative charge on the oxygen atom of the enol</li> <li>- Protonation: Adding a proton = a hydrogen atom</li> <li>- <i>In situ</i>: "in its original place"</li> <li>- Electrophilicity: affinity for electrons</li> <li>- Specificity: ability to bind to specific ligands</li> <li>- Ligand: ion/molecule</li> <li>- K<sub>cat</sub> value: (number of times an enzyme site converts substrate to product) per (unit of time)</li> <li>- Catalytic efficiency: enzyme works well without much substrate = high K<sub>cat</sub> value</li> <li>- CO<sub>2</sub> fixation: process by which plants fix CO<sub>2</sub> into organic compounds</li> <li>- Reductive (adj.): the one that gains electrons</li> <li>- <i>De novo</i>: "from the beginning; anew"</li> <li>- Mutagenesis: process of gene mutation</li> <li>- Microfluidics: use of a pump and chip to move microliters (μL) of liquid</li> <li>- High-throughput screens: use of automated equipment to rapidly test thousands to millions of samples for biological activity</li> <li>- Biotin: a B-vitamin</li> <li>- Decarboxylase: remove carboxyl groups from acidic substrates; usually release CO<sub>2</sub></li> </ul>
Cited references to follow up on	<p><input type="checkbox"/> M. Scheffen, D.G. Marchal, T. Beneyton, S.K. Schuller, M. Klose, C. Diehl, J. Lehmann, P. Pfister, M. Carillo, S. Aslan, <i>et al.</i> A new-to-nature carboxylation module to improve natural and synthetic CO<sub>2</sub> fixation Nat Catal (2021), <a href="https://doi.org/10.1038/s41929-020-00557-y">10.1038/s41929-020-00557-y</a> in press <a href="https://www.nature.com/articles/s41929-020-00557-y">https://www.nature.com/articles/s41929-020-00557-y</a></p> <p><input type="checkbox"/> R.K. Singh, R. Singh, D. Sivakumar, S. Kondaveeti, T. Kim, J. Li, B.H. Sung, B.-K. Cho, D.R. Kim, S.C. Kim, <i>et al.</i> Insights into cell-free conversion of CO<sub>2</sub> to chemicals by a multienzyme cascade reaction ACS Catal, 8 (2018), pp. 11085-11093 <a href="https://pubs.acs.org/doi/10.1021/acscatal.8b02646">https://pubs.acs.org/doi/10.1021/acscatal.8b02646</a></p> <p><input type="checkbox"/> T.E. Miller, T. Beneyton, T. Schwander, C. Diehl, M. Girault, R. McLean, T. Chotel, P. Claus, N.S. Cortina, J.-C. Baret Light-powered CO<sub>2</sub> fixation in a chloroplast mimic with natural and synthetic parts Science, 368 (2020), pp. 649-654</p>

	<p>D.L. Trudeau, C. Edlich-Muth, J. Zarzycki, M. Scheffen, M. Goldsmith, O. Khersonsky, Z. Avizemer, S.J. Fleishman, C.A. Cotton, T.J. Erb Design and in vitro realization of carbon-conserving photorespiration <a href="#">Link</a> Proc Natl Acad Sci U S A, 115 (2018), pp. E11455-E11464</p> <p><input type="checkbox"/> N. Antonovsky, S. Gleizer, E. Noor, Y. Zohar, E. Herz, U. Barenholz, L. Zelcbuch, S. Amram, A. Wides, N. Tepper Sugar synthesis from CO<sub>2</sub> in <i>Escherichia coli</i> Cell, 166 (2016), pp. 115-125 <a href="https://www.sciencedirect.com/science/article/pii/S0092867416306687/pdf?md5=5c32331e502754fff15213af59747f1&amp;pid=1-s2.0-S0092867416306687-main.pdf"><u>https://www.sciencedirect.com/science/article/pii/S0092867416306687/pdf?md5=5c32331e502754fff15213af59747f1&amp;pid=1-s2.0-S0092867416306687-main.pdf</u></a></p> <p><input type="checkbox"/></p>
Follow up Questions	<ol style="list-style-type: none"><li>1. Could more efficient carboxylases in small organisms help to address global issues such as pollution, climate change, and ocean acidification?</li><li>2. Do organisms living in carbon-rich/oxygen-deficit environments have better carbon-processing pathways?</li><li>3. How can we inhibit side reactions/ improve the efficiency of enzymes?</li><li>4. Would changing the surrounding environment to reduce inhibitory substances reduce the energy expenditure?</li><li>5. How does the carbon cycle in RubisCO work?</li></ol>

# Article #5 Notes: Source, distribution and emerging threat of micro- and nanoplastics to marine organism and human health: Socio-economic impact and management strategies

Source Title	Source, distribution and emerging threat of micro- and nanoplastics to marine organism and human health: Socio-economic impact and management strategies
Source citation (APA Format)	Mofijur, M., Ahmed, S. F., Rahman, S. M. A., Arafat Siddiki, S. K. Y., Islam, A. B. M. S., Shahabuddin, M., Ong, H. C., Mahlia, T. M. I., Djavanroodi, F., & Show, P. L. (2021). Source, distribution and emerging threat of micro- and nanoplastics to Marine Organism and human health: Socio-Economic Impact and Management Strategies. <i>Environmental Research</i> , 195. <a href="https://doi.org/10.1016/j.envres.2021.110857">https://doi.org/10.1016/j.envres.2021.110857</a>
Original URL	<a href="https://www.sciencedirect.com/science/article/pii/S0013935121001511">https://www.sciencedirect.com/science/article/pii/S0013935121001511</a>
Source type	Scientific journal
Keywords	Microplastic effect, nanoplastic effect, toxins, food chain, manage, sources, address, absorption, toxins, food chain, health, ocean
Summary of key points + notes (include methodology)	Micro- and nanoplastics pose a huge issue, as they absorb toxins, get eaten and travel up food chains, deteriorate health, and suffocate and harden the ocean floor, to name a few of their effects. Most micro- and nanoplastics come from land, from common sources such as clothes, water bottles, city dust, cosmetics, etc. More research has been conducted in recent years in efforts to understand the risks they pose and find solutions to manage them.
Research Question/Problem/ Need	What effect do microplastics and nanoplastics have on the world and how can we address them?

## Important Figures



Caption: graphic showing where microplastics come from, how it gets taken up by marine life and humans, and the effects it can have once it is taken up.

- 335 million tons of plastic are used globally, and use is increasing
- Plastic is very resilient to degradation, and some can be used for up to 50 years. However, its resilience makes it difficult to dispose of, as it can last hundreds of years in the environment, during which it produces micro- and nano particles.
- 4.8–12.7 million tonnes of plastic entered the ocean in 2010 and pollution is projected to be 100–250 million tons by 2025
- Plastics are classified based on size:
  - Macroplastics= > 1 or 2 centimeters
  - Mesoplastics= 1 millimeter to 1 or 2 centimeters
  - Microplastics= 1 micrometer to one millimeter
  - Nanoplastics= < 1 micrometer
- Over 80% of all environmental microplastic pollution is from textiles, tires, and city dust
- One piece of clothing can shed over 1,900 microplastic fibers in one wash.
- In 2018, 91% of bottled water samples were contaminated with microplastics, with bottled water containing twice as many microplastics as tap water
- Nanoparticles can slip through water treatment plants
- 98% of plastics in the ocean are from land-based activities
- Plastics enter the ocean due to weather and through drainage systems
- Plastics absorb toxins in the water, such as those from fertilizer
- Plastic travels up trophic levels, eventually reaching humans
- 

## VOCAB: (w/definition)

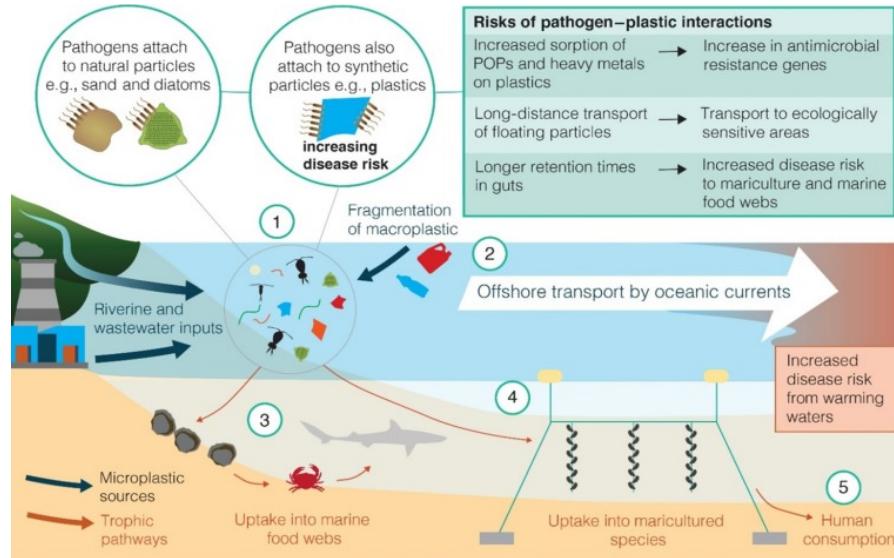
- Macroplastics: plastic > 1 or 2 centimeters
- Mesoplastics: plastic 1 millimeter to 1 or 2 centimeters
- Microplastics: plastic 1 micrometer to one millimeter

	<ul style="list-style-type: none"> <li>- Nanoplastics: plastic &lt; 1 micrometer</li> <li>- Polymer degradation: chemical process through which the structure and properties of the polymer changes. The degradation process differs depending on the chemical structure of the polymer.           <ul style="list-style-type: none"> <li>- Types of polymer degradation:</li> <li>- Hydrolysis: chemical reaction in which a molecule of water breaks one or more chemical bonds</li> <li>- Photodegradation: When the absorption of photons/light/UV light causes molecules to degrade</li> <li>- Thermal degradation: when the polymer changes its properties under the influence of increased temperature.</li> <li>- Thermo oxidative degradation: when polymer chains form radicals (molecules with at least one unpaired electron), by hydrogen abstraction or homolytic scission of a carbon-carbon bond. Can occur during manufacture, processing or during service when exposing the polymer to light or heat.</li> </ul> </li> </ul>
Cited references to follow up on	<p><input type="checkbox"/> <a href="#">Amereh et al., 2020</a>  <u>F. Amereh, et al.</u>  <a href="#">The emerging risk of exposure to nano (micro) plastics on endocrine disturbance and reproductive toxicity: from a hypothetical scenario to a global public health challenge</a>  <u>Environ. Pollut., 261 (2020), p. 114158</u></p> <p><input type="checkbox"/> <a href="#">Dąbrowska et al., 2021</a>  <u>J. Dąbrowska, et al.</u>  <a href="#">Marine waste—sources, fate, risks, challenges and research needs</a>  <u>Int. J. Environ. Res. Publ. Health, 18 (2021), p. 433</u></p> <p><input type="checkbox"/> <a href="#">Huang et al., 2020</a>  <u>W. Huang, et al.</u>  <a href="#">Microplastics and associated contaminants in the aquatic environment: a review on their ecotoxicological effects, trophic transfer, and potential impacts to human health</a>  <u>J. Hazard Mater. (2020), p. 124187</u></p> <p><input type="checkbox"/> <a href="#">Lehner et al., 2019</a>  <u>R. Lehner, et al.</u>  <a href="#">Emergence of nanoplastic in the environment and possible impact on human health</a>  <u>Environ. Sci. Technol., 53 (2019), pp. 1748-1765</u></p>
Follow up Questions	<ol style="list-style-type: none"> <li>1. Is there a way to remove toxins from either plastics or the ocean or prevent absorption?</li> <li>2. In what environment do plastics break down the best in/in what environment does bacteria break down plastic the best in?</li> <li>3. Is there a way to completely break down microplastics instead of simply degrading them into nanoplastics, which are even more harmful?</li> <li>4. How do the toxins found in plastics harm humans/animals? How much plastic must be ingested for it to start impacting our health?</li> </ol>

# Article #6 Notes: Oceanic Hitchhikers - Assessing Pathogen Risks from Marine Microplastic

Source Title	Oceanic Hitchhikers - Assessing Pathogen Risks from Marine Microplastic
Source citation (APA Format)	Bowley, J., Baker-Austin, C., Porter, A., Hartnell, R., & Lewis, C. (2021). Oceanic hitchhikers – assessing pathogen risks from marine microplastic. <i>Trends in Microbiology</i> , 29(2), 107–116. <a href="https://doi.org/10.1016/j.tim.2020.06.011">https://doi.org/10.1016/j.tim.2020.06.011</a>
Original URL	<a href="https://www.cell.com/trends/microbiology/fulltext/S0966-842X(20)30190-6?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0966842X20301906%3Fshowall%3Dtrue">https://www.cell.com/trends/microbiology/fulltext/S0966-842X(20)30190-6?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0966842X20301906%3Fshowall%3Dtrue</a>
Source type	Journal
Keywords	Microplastic, aquaculture, bivalves, food safety, human health, vibrios, plastisphere
Summary of key points + notes (include methodology)	<p><b>Summary:</b>      Pathogens and other microbes have been reported to form plastispheres on microplastics. On the microplastics, they can evolve, for example by obtaining antimicrobial resistance genomes through plasmids. The pathogens' resistance raises global concerns, as does the presence of bacteria among them, such as vibrios, that may impact food safety and human health when microplastic is consumed by marine food sources. More research must be conducted on the transmissibility of their pathogens and their potential impact on aquaculture, among other questions and concerns. As microplastics can travel great distances, the research will be valuable for ensuring food confidence and safety in the future, when the world's expanding population will rely heavily on aquaculture for sustainable food.</p> <p><b>Notes:</b></p> <p>key:      all <b>HIGHLIGHTED</b> are important  <b>PINK</b> is stuff I found particularly interesting  <b>BLUE</b> is areas of future research identified/implied by article</p> <ul style="list-style-type: none"> <li>- It is possible that microplastics could transport pathogens long distances</li> <li>- Few detailed studies have been done to assess effects of pathogens attaching to microplastics</li> <li>- Estimated that 15 to 51 trillion plastic particles float on the surface of oceans = only 1% of the 4.8 to 12.7 million tons thought to enter the oceans each year</li> <li>- Plastic pollution comes from humans, entering the oceans through rivers, eventually sinking due to fouling/flocculation/egestion</li> <li>- Sorption of biological materials to a polymer changes its once smooth and hydrophobic surface to one more molecules/organisms can attach to</li> <li>- Infochemicals/protein signaling molecules can attach and influence interactions with marine ecosystems. (★ - don't know what I'll do with it, but it's very interesting, something I never thought of)</li> </ul>

- Ex. dimethyl sulfide (DMS), infochemical that stimulates feeding from several zooplankton species, can be produced by the fouling communities on microplastic→ increases plastic ingestion by other animals
  - Organisms on microplastics can “disguise” the plastic and even encourage ingestion (wow!)
- In seawater, plastics quickly develop conditioning films then biofilms, many of which, concerningly, house pathogenic microbes (has been found in various ocean regions)
  - Vibrios especially prevalent, particularly in summer– usually rare in ocean, but high numbers reported on microplastic in mid-North Atlantic
  - Vibrios transported through other hosts, too, like wood and birds
- Possible that number of pathogens on plastics similar to number on natural particles
- Want to know if microplastics increase risk of pathogen transfer and disease occurrence instead of just providing more particles to house microbes
  - Additional factors to consider:
    - Attachment process and microbial interactions
    - Rate and distance of transport
    - If the plastisphere changes through different ocean regions
    - Vertical transport process to sea floor, where plastics/microbes are eaten and trophic transfer begins
    - Uptake and retention of plastics/microbes into mariculture organisms
    - Likelihood of disease transfer
    - Risk to human consumers
- Potential risks of plastics housing pathogens:
  - Increases resistance to antimicrobial substances
  - Transports pathogens to vulnerable areas
  - Increases disease risk due to longer retention time in guts



- good visualization of path of pathogens and microplastics: microplastics wash into ocean, pathogens attach, move up trophic levels
- Biofilms formation mostly due to ocean conditions (plastic type not as important)
- Living in a biofilm improves infectiousness, metabolic response, and functional diversity of

### microbes

- Concentrations of antimicrobial resistance bacteria 100-5000 times higher on microplastics and natural aggregates than surrounding water
  - Plastipheres have shown significant improvement in metabolic pathways contributing to diseases
- (above shows microplastic may also worsen pathogens through HGT) ⇔ horizontal gene transfer
- Antimicrobial resistance = critical, global issue
- Microplastics might be important environmental factor in advancement of AMR
- Several studies indicate that microplastic influences evolution of microbial communities, have found that bacteria on microplastic transfer plasmids more frequently
- Understanding role of biofilms and the types of gene exchange on microplastics could lend insight to more effective gene exchange for bacteria – is a promising area of research
- Rivers = major source of microplastics and pathogenesis microbes
  - Many microplastics are from sewage
- Plastics have been linked to transport of invasive species across oceans
- Unknown if/how/in-what-ways plastisphere changes across long distances
  - Current evidence suggests plastisphere shaped by geographical location and environmental conditions of seawater; since conditions change through regions, possible that communities change as transported
    - Has been observed in a system using a river
- Reports on the plastisphere communities not the best:
  - Don't know processes of attachment or ecological succession
  - Reports of pathogens on microplastics in areas where it was hardly reported before
  - Studies that have discovered pathogens only looked at them, not the whole community, so we can't observe the whole plastisphere
  - Needs more organized research
- Filter feeders that feed on the ocean floor, like mussels and oysters, have been demonstrated by many studies to take up microplastic
  - Average microplastic contamination ranges from 1.5 to 7.64 particles per individual, and maximums of up to 178 particles.
  - Size, shape, and polymer type seem to influence uptake, with microfibers most commonly eaten.
  - Difficult to observe exposure because most microplastic gets excreted over time → data is only a sample from a specific point in time (so could conduct more thorough, dedicated observation)
- Aquaculture important for meeting future sustainable seafood demand and is currently the fastest growing food sector
- Filter-feeding bivalves will be important to food production
- Disease poses a major problem to aquaculture industry, with Vibrios causing disease and mass larvae (occasionally adult) mortality in bivalves
  - Disease outbreaks = severe economic losses in aquaculture, so factors that increase them are a serious concern
- Microplastic has often been found in bivalves commercially available for eating.
- There is concerning overlap between areas with many microplastics and areas of high aquaculture production
  - In China, average of 57.18 microplastics per Yesso clam

- Mediterranean also high in microplastic, but particles per mussel relatively low → shows complexity (“spatiotemporal dynamics”) of microplastic pollution
- Studies suggest that microplastics housing pathogens may transfer them to consumers, but have not demonstrated it experimentally.
  - Plastic with pathogens strongly associated with disease in Asian-Pacific corals: disease increased by 85% when the coral region had plastic over it.
  - In one study, scientists tagged the pathogen (*Escherichia coli* = *E. coli*) with GFP after northern star coral ingested it, demonstrating transfer of the coli from the plastic to the gut tissues.
    - = evidence pathogens can transfer to digestive tissue, supporting that microplastic transfers pathogens
  - Needs more research on if it occurs naturally, in other organisms, how it affects infection rates, & how it affects human health
- New technology may help answer outstanding questions:
  - Inexpensive, efficient genome-sequencing
  - Tools to analyze data and details of microbial interactions
  - Nanopore sequencing technologies: quickly and cheaply produce long genome sequences
  - Portable enough to use in field= more data from more locations + more manageable budget
  - Improvements in microscopy: let researchers determine what bacteria is on the plastic
  - Data collected could identify bacteria on microplastics, the pathogens' relative abundances, and different genes

Research Question/Problem/Need	What risk is there to food safety regarding pathogen transport through microplastics?
Important Figures	<p>The diagram illustrates the complex pathways of pathogen and microplastic transport in marine ecosystems. It starts with two circular insets: one showing pathogens attaching to natural particles like sand and diatoms, and another showing them attaching to synthetic particles like plastics, both labeled 'increasing disease risk'. A central circle represents a macroplastic particle containing various microorganisms. Numbered arrows indicate the following pathways:</p> <ol style="list-style-type: none"> <li>1. Fragmentation of macroplastic into smaller microplastic particles.</li> <li>2. Offshore transport by oceanic currents, leading to increased disease risk from warming waters.</li> <li>3. Uptake into marine food webs by organisms like sharks and crabs.</li> <li>4. Uptake into maricultured species like fish.</li> <li>5. Finally, human consumption of these contaminated organisms.</li> </ol> <p>A callout box titled 'Risks of pathogen–plastic interactions' lists three main risks:</p> <ul style="list-style-type: none"> <li>Increased sorption of POPs and heavy metals on plastics → Increase in antimicrobial resistance genes</li> <li>Long-distance transport of floating particles → Transport to ecologically sensitive areas</li> <li>Longer retention times in guts → Increased disease risk to mariculture and marine food webs</li> </ul> <p><small>Trends In Microbiology</small> Figure one: provides good visualization of path of pathogens and microplastics: microplastics wash into ocean, pathogens attach, move up trophic levels</p>

	<ul style="list-style-type: none"> <li>- Estimated that 15 to 51 trillion plastic particles float on the surface of oceans = only 1% of the 4.8 to 12.7 million tons thought to enter the oceans each year</li> <li>- Concentrations of antimicrobial resistance bacteria 100-5000 times higher on microplastics and natural aggregates than surrounding water</li> <li>- Studies show average microplastic contamination in some bivalves ranges from 1.5 to 7.64 particles each, with maximums of up to 178 particles. <ul style="list-style-type: none"> <li>- In China, average of 57.18 microplastics per Yesso clam</li> </ul> </li> <li>- Plastic with pathogens strongly associated with disease in Asian-Pacific corals: disease increased by 85% when the coral region had plastic over it.</li> </ul>
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Anthropogenic: (of pollutants) originating in human activity</li> <li>- Recalcitrant: Obstinately uncooperative</li> <li>- Fouling: contaminants collect on surface/pores of filtration membrane (in this case it's plastic)</li> <li>- Flocculation: a chemical coagulant added to water facilitates bonding between particles to create larger, easier-to-remove clusters</li> <li>- Egestion: expulsion of undigested food</li> <li>- Vibrios: a group of marine bacteria, not all pathogenic, but some causing vibriosis and cholera</li> <li>- Sorb: to gather on a surface by absorption (liquid soaked up into something ex. a sponge), adsorption (individ. molecules/atoms/ions gathering on surfaces), or combination of the two</li> <li>- Ecocorona: formed by sorption of biological materials to a polymer's surface... so a small ecosystem of particles and pathogens?</li> <li>- Plastisphere: ecosystems in plastic</li> <li>- Benthos: the gathering of organisms inhabiting the sea floor</li> <li>- Mariculture: farming of marine organisms for food and other products (types of plastic:)</li> <li>- Aggregate: a material formed from loosely compacted particles</li> <li>- Metagenomic: studying the structure and function of entire nucleotide sequences from all the organisms (usually microbes) in a bulk sample</li> <li>- Vector: organism that transmits pathogen from infected animal to another animal</li> <li>- Microcosm: smaller setting encapsulating characteristics of something much bigger</li> <li>- Conjugation: temporary union of two bacteria/unicellular organisms for genetic exchange</li> <li>- Effluent (not affluent): liquid waste or sewage discharged into body of water</li> <li>- Benthic: anything associated with or occurring at the bottom of a body of water</li> <li>- Bivalve: aquatic mollusk with a compressed body enclosed in a hinged shell</li> <li>- Aquaculture: the production of aquatic organisms under controlled conditions</li> <li>- Spatiotemporal: belonging to space and time</li> <li>- Genome: complete set of genes in organism/cell</li> </ul> <p><b>Abbreviations:</b></p> <ul style="list-style-type: none"> <li>- Plastic: <ul style="list-style-type: none"> <li>- LDPE: low-density polyethylene: soft, flexible, light ex. Plastic bag</li> <li>- PE: polyethylene: ex. packaging film, bags, squeeze bottles</li> <li>- PET: polyethylene: clear, strong, lightweight, recyclable ex. Food packaging</li> <li>- PP: polypropylene: soft, flexible, considered safer than other plastics, has cloudy finish ex. Yogurt cups, water bottles with cloudy finish</li> <li>- PS: polystyrene: hard, solid, ex. Electronics, car parts, equipment, styrofoam</li> <li>- PVC: polyvinyl chloride: versatile, ex. Pipes, flooring, footwear, packaging</li> </ul> </li> </ul>

	<ul style="list-style-type: none"> <li>- HGT: horizontal gene transfer: movement of genetic information between organisms, ex. Antibiotic resistance genes, evolving pathogens</li> <li>- PAIs: pathogenicity islands: DNA segments acquired through HGT that encode non-essential proteins ex. virulence (severity or harmfulness of a disease/poison)</li> <li>- ARB: antimicrobial resistance bacteria</li> <li>- AMR: antimicrobial resistance</li> <li>- AMG: antimicrobial resistance genes</li> <li>- MRG: metal-resistance genes</li> <li>- <i>Et al.</i>: "and others"</li> <li>- GFP: green fluorescent protein</li> </ul>
Cited references to follow up on	<p><input type="checkbox"/> Bucci K. et al. <b>What is known and unknown about the effects of plastic pollution: A meta-analysis and systematic review.</b> <i>Ecol. Appl.</i> 2020; <b>30</b>: 1-16 <a href="https://esajournals.onlinelibrary.wiley.com/doi/abs/10.1002/eap.2044">https://esajournals.onlinelibrary.wiley.com/doi/abs/10.1002/eap.2044</a> Procter J. et al.</p> <p><b>Smells good enough to eat: Dimethyl sulfide (DMS) enhances copepod ingestion of microplastics.</b> <i>Mar. Pollut. Bull.</i> 2019; <b>138</b>: 1-6 <a href="https://pubmed.ncbi.nlm.nih.gov/30660250/">https://pubmed.ncbi.nlm.nih.gov/30660250/</a></p> <p><input type="checkbox"/> Zhang Y. et al. <b>Potential risks of microplastics combined with superbugs: Enrichment of antibiotic resistant bacteria on the surface of microplastics in mariculture system.</b> <i>Ecotoxicol. Environ. Saf.</i> 2020; <b>187</b>: 109852 <a href="https://pubmed.ncbi.nlm.nih.gov/31670243/">https://pubmed.ncbi.nlm.nih.gov/31670243/</a> Oberbeckmann S. Labrenz M.</p> <p><b>Marine microbial assemblages on microplastics: diversity, adaptation, and role in degradation.</b> <i>Annu. Rev. Mar. Sci.</i> 2020; <b>12</b>: 209-232 <a href="https://www.cell.com/servlet/linkout?suffix=e_1_5_1_2_33_2&amp;dbid=8&amp;doi=10.1101/j.tim.2020.06.011&amp;key=31226027&amp;cf=">https://www.cell.com/servlet/linkout?suffix=e_1_5_1_2_33_2&amp;dbid=8&amp;doi=10.1101/j.tim.2020.06.011&amp;key=31226027&amp;cf=</a></p>
Follow up Questions	<ol style="list-style-type: none"> <li>1. Do pathogens need a biofilm in order to be present in the plastisphere?</li> <li>2. How does biofilm formation on plastics differ from its formation on natural particles? / What promotes formation of plastispheres?</li> <li>3. Why is the frequency of plasmid transfer greater on microplastics than on natural particles/ when bacteria don't live on particles?</li> <li>4. Does eating pathogen-contaminated microplastic lead to disease? How much exposure is required to do so?</li> <li>5. Does the transfer of pathogens from ingested microplastics to gut tissue...       <ol style="list-style-type: none"> <li>a. occur naturally?</li> <li>b. occur with organisms other than E. coli and northern star coral?</li> <li>c. how does it affect infection rates?</li> <li>d. how does it affect human health?</li> <li>e. how will it affect the aquaculture economy?</li> </ol> </li> <li>6. What effects might pathogens on microplastic have on bivalve aquaculture?</li> </ol>

## Article #7 Notes: Microplastic removal from urban stormwater: Current treatments and research gaps

Source Title	Microplastic removal from urban stormwater: Current treatments and research gaps
Source citation (APA Format)	Stang, C., Mohamed, B. A., & Li, L. Y. (2022). Microplastic removal from urban stormwater: Current treatments and research gaps. <i>Journal of Environmental Management</i> , 317. <a href="https://doi.org/10.1016/j.jenvman.2022.115510">https://doi.org/10.1016/j.jenvman.2022.115510</a>
Original URL	<a href="https://www.sciencedirect.com/science/article/pii/S0301479722010830">https://www.sciencedirect.com/science/article/pii/S0301479722010830</a>
Source type	Journal
Keywords	Bioretention, Microplastic, Microplastic removal, Treatment technology, Tire-wear particle, Urban stormwater runoff,
Summary of key points + notes (include methodology)	<p>Summary:</p> <p>Microplastics are often deposited in the environment by stormwater runoff. To remove them, there are various techniques and systems. Some systems include wetlands, retention ponds, and flocculation. Different systems can be more effective on certain types of plastic. There isn't as much data on wetlands and retention ponds, so more research must be done on them and other potential systems.</p> <p>Notes:</p> <p>key: all <b>HIGHLIGHTED</b> are important <b>PINK</b> is stuff I found particularly interesting <b>BLUE</b> is areas of future research identified/implied by article</p> <ul style="list-style-type: none"> <li>- Stormwater = major contributor to marine microplastic pollution</li> <li>- Article compares effectiveness of microplastic removal techniques and performance of stormwater treatment systems</li> <li>- Bioretention and filtration systems removed 84-96% of stormwater <ul style="list-style-type: none"> <li>- Did not perform too well removing fibrous microplastics</li> </ul> </li> <li>- Less data on wetlands and retention ponds, but available data indicates 28-55% microplastics removed in wetlands and 85-99% removed in retention ponds <ul style="list-style-type: none"> <li>- Limited data might contribute to inaccurate representation of performances</li> </ul> </li> <li>- More research needed for different treatments, ex. Ballasted and flocculation, flotation, biological degradation</li> <li>- More research needed to determine effectiveness of systems for microplastics &lt; 100 micrometers</li> <li>- </li> </ul>

Research Question/Problem/Need	How effective are different methods of microplastic removal from stormwater?
Important Figures	<p>How effective different strategies are at removing microplastics.</p>
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Bioretention (system): has soil with non-invasive plants. Stormwater runoff is filtered through the soil and the vegetation removes pollutants and runoff before the remaining runoff drains into a drain or soil under the system</li> <li>- Retention pond: a permanent body of water that holds runoff, which can then be treated. The still water and time in the pond lets pollutants settle to the bottom so vegetation and other biological uptake mechanisms can help remove pollutants</li> <li>- Ballasted: lined with gravel or coarse stone</li> <li>- Flocculation: Over time, and through gentle and prolonged agitation of the water, small particles collide to form larger particles</li> <li>- Flotation: separating particles of materials in water through treatment with chemicals which make some adhere to air bubbles and rise while others stay</li> <li>-</li> </ul>
Cited references to follow up on	<p><input type="checkbox"/> <b>Mahara et al., 2022</b> N. Mahara, J. Alava, M. Kowal, E. Grant, J. Boldt, L. Kwong, B. Hunt  Assessing size-based exposure to microplastic particles and ingestion pathways in zooplankton and herring in a coastal pelagic ecosystem of British Columbia, Canada  Mar. Ecol. Prog. Ser., 683 (2022), pp. 139-155, 10.3354/meps13966</p> <p><input type="checkbox"/> <b>Pereira et al., 2022</b> A. Pereira, T. Marinho, M. Ahmed, I. Ahmed, M. Mohammad  Toxicity assessment of polyethylene microplastics in combination with a mix of emerging pollutants on <i>Physalaemus cuvieri</i> tadpoles  J. Environ. Sci. (2022), 10.1016/j.jes.2022.05.013</p>

Follow up Questions	<ul style="list-style-type: none"><li>- What happens to the microplastic after it gets removed by these methods?</li><li>- What happens to the ecosystems as plastic particles build up in places like ponds and wetlands?</li><li>- Has there been a documented increase in toxicity in these ponds?</li><li>- How can we remove the remaining plastics?<ul style="list-style-type: none"><li>- Are they smaller and harder to remove or of various sizes?</li></ul></li></ul>
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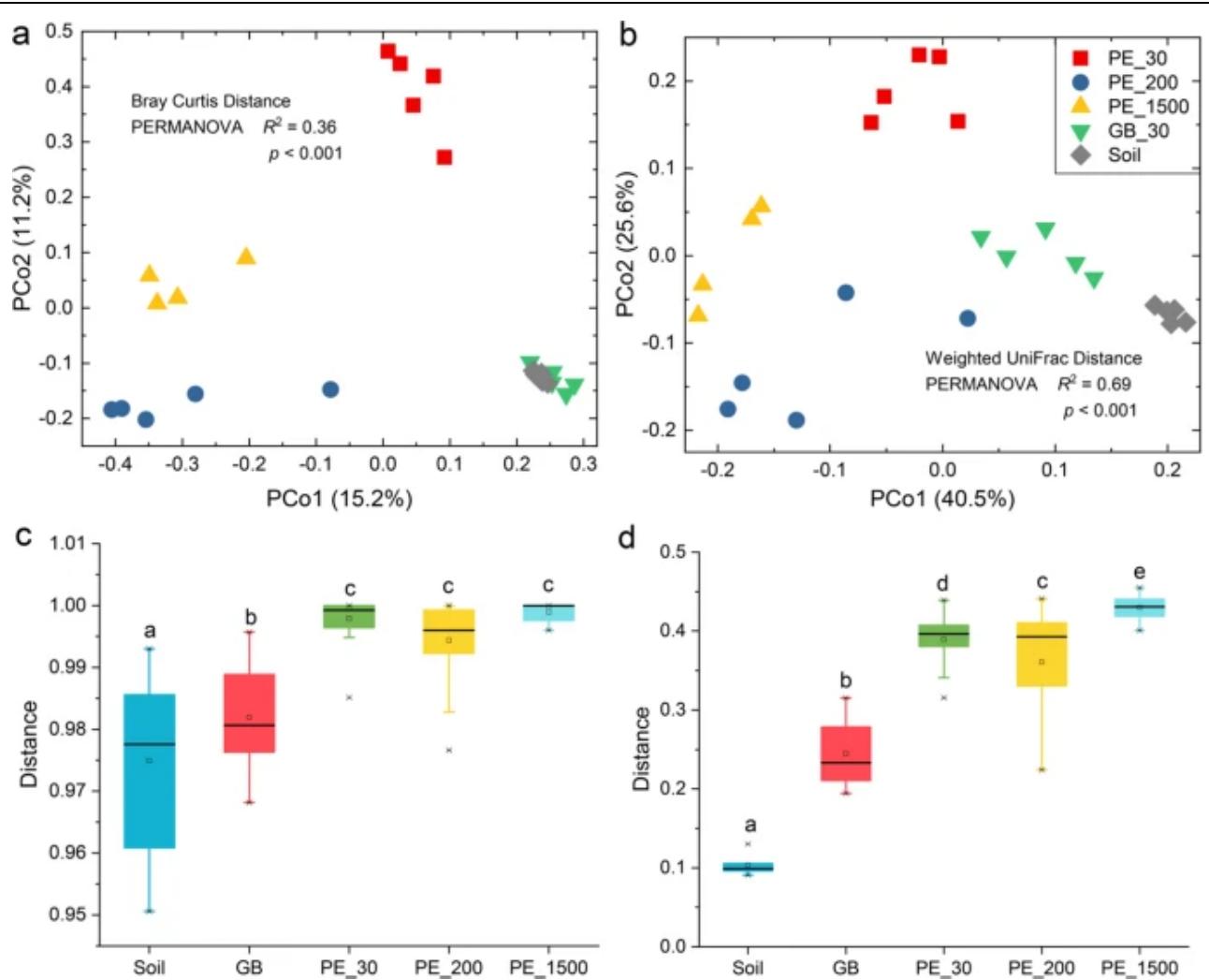
# Article #8 Notes: Soil plastispheres as hotspots of antibiotic resistance genes and potential pathogens

Source Title	Soil plastispheres as hotspots of antibiotic resistance genes and potential pathogens
Source citation (APA Format)	Zhu, D., Ma, J., Li, G., Rillig, M. C., & Zhu, Y.-G. (2021). Correction: Soil plastispheres as hotspots of antibiotic resistance genes and potential pathogens. <i>The ISME Journal</i> , 16(2), 521–532. <a href="https://doi.org/10.1038/s41396-021-01137-z">https://doi.org/10.1038/s41396-021-01137-z</a>
Original URL	<a href="https://www.nature.com/articles/s41396-021-01103-9">https://www.nature.com/articles/s41396-021-01103-9</a>
Source type	Journal Article
Keywords	MPs, microplastics, manure, temperature, moisture, humidity, soil, study, China, glass beads, 30um, plastic beads, plastispheres, ARGs, antimicrobial resistance, microbial communities, microbes, bacteria
Summary of key points + notes (include methodology)	<p>Summary: Not much is known about the microbial communities on plastispheres, and, due to research prioritizing marine microplastics, even less is known about soil plastispheres. This study aimed to answer several questions about soil plastispheres, including how different substrates affect the microbes/pathogens, what microbes/pathogens form and how diverse the community is, and what affect manure, temperature, and soil moisture have on the community. The study's results showed, among other findings, that different MPs select for different communities and that the microbes are involved in various processes in the ecosystem. They also found that plastispheres house many ARGs and potential Pathogens, enhanced by the addition of manure or the increase of temperature or moisture.</p> <p>Notes:</p> <p>key: all HIGHLIGHTED are important PINK is stuff I found particularly interesting BLUE is areas of future research identified/implied by article GREEN is how experiment done</p> <ul style="list-style-type: none"> <li>- Plastic pollution is creating plastispheres</li> <li>- How plastisphere affects ARGs and microbial communities is of global concern</li> <li>- Studies in marine ecosystems, still meager understanding of in soil ecosystems</li> <li>- Study investigates plastispheres of four types of MPS in various soils <ul style="list-style-type: none"> <li>- Shows effects of different environments on them</li> <li>- Showed MPs select for microbial communities in the plastisphere</li> <li>- Showed plastisphere communities involved in various metabolic pathways, could drive ecological processes in soil</li> </ul> </li> <li>- Potential pathogens and ARGs detected in plastisphere– varied between MP and soil types but more than in soil</li> <li>- Manure, increase in soil temp, and moisture ⇒ enhance ARGs</li> </ul>

- Results suggest plastispheres are habitats with increased potential pathogen abundance and increased ARG abundance, provided insight into ecology and plastisphere ARGs
- Agricultural use of plastic film, organic fertilizers, irrigation, atmospheric deposition, etc, cause large amounts of plastic accumulation on land.
- MPs make up majority of plastic debris → some soils are up to 6.7% MPs
- Increasing number of studies show that MPs in soil could negatively impact life in it and change soil properties and biogeochemical cycles
  - Ex. one study showed MPs could affect plant health (lower mass) and soil structure (stability)
- MPs provide new surface for microbiota to colonize=form biofilm ("plastisphere") on
- Plastispheres in marine ecosystems could affect functions like carbon cycling, xenobiotic compound degradation, and gene exchange
- Have learned composition and diversity of plastisphere microbiota, but more so in marine plastispheres– need more about in soil
- One study shows adding MPs could improve microbial turnover and nutrient efficiency in plastisphere
  - Did not study what microbes were in the plastisphere= restricts understanding of roles of plastisphere microbes in soil function
  - Also don't understand how plastisphere communities form from soil community
- MPs may be important factor in spread of ARFGs, could potentially affect disease transmission and treatment
- Lab experiments, though mostly focused on aquatic MPs, indicate plasmids are transferred more frequently between bacteria associated with plastic than free-living ones
- Few studies done to find ARGs on soil microplastics and have only focused on a few types of ARGs/MPs
- Assembly of bacterial community determined by predictable and random processes, is a topic currently being explored in microbiology
- Contribution of microbial communities to variation of ARGs rarely studied
  - Past studies show more ARGs in plastisphere than surrounding environment, indicating predictable process of environmental selection on plastispheres' bacteria
  - Random process could be bacteria, potentially carrying ARGs, randomly joining the plastisphere and transferring genes
- Global changes cause concerning changes in soil ex. In its temperature and moisture
  - Greatly influence its microbial community
  - Also affect ARGs
  - Affect microbiome and antibiotic resistance of building surfaces
  - Most MPs hydrophobic, so plastisphere may be more sensitive to temperature and moisture than if on soil
- Past studies suggest manure increases abundance of ARGs, such as around plants
- Need more studies to determine effects of manure, temperature, and moisture on plastisphere
- Study aims to...
  - Determine the microbes in plastispheres of different MP and soil types
  - Determine the interaction, assembly, and potential uses of plastisphere microbiota
  - Find what bacterial pathogens and ARGs are in plastispheres
  - Explore relationship between bacteria and ARGs
  - Determine effects of manure, temperature, and moisture on microbiome and ARGs

- of plastisphere
- Will try to do so through amplicon and metagenomic sequencing and high-throughput qPCR techniques
- Used 3 types of soil:
  - Red soil, arable, from Yunnan province, China
  - Black soil, arable, from Heilongjiang province, China
  - Yellow brown soil, arable, from forested Jiangsu province, China
- Soil divided in half, one to analyze soil properties, one to incubate MPs after debris >2mm (plants, rocks) removed
- Used 4 types of MPs:
  - All beads purchased from Youngling Electromechanical Technology Co. in Shanghai, China
  - Were PVC, PA, PE, and PS, chosen because some of the most common plastics in soils
  - All about 30 µm (micrometers) (article has charts with more details on each plastic's properties)
- Also used:
  - Glass beads around 30 um (negative control)
  - PE MPs around 30, 200, and 1500 um
- Disinfected microplastics with 1% sodium hypochlorite for 30 minutes then washed 5 times with sterile water. Then incubated.
- Conducted 3 microcosm experiments and 1 field experiment
- Since MPs were incubated in soil, were difficult to collect
- Used nylon mesh bags of 50 um mesh to study them
  - Each bag contained 1 g MPs buried in soil and incubated for 8 weeks
- Microcosm experiment:
  - MPs incubated in glass containers with length .22 m, width .155m, and height .108 m
  - Each container held 2kg dried soils
  - Each treatment replicated 5 times
  - Before incubating MPs, soil moisture adjusted to 75% of field capacity
  - Soils then incubated 2 weeks to activate microbiota
  - While MPs were incubating, sterile water was added twice a week to maintain moisture
- After incubation, samples stored at -80 degrees C
- High-throughput qPCR used to detect ARGs
- Bacterial community analyzed by amplicon sequencing
- Microcosm experiment 1:
  - Made sure the bags wouldn't affect their experiment
  - 30 um glass beads and differently sized PE MPs each put into bags then incubated in yellow brown soil
  - Glass beads used to exclude that any 30um sized particle would lead to similar effects due to the mesh bag
- Microcosm experiment 2:
  - 15 glass containers, with different soils, used to incubate MPs
  - Each MP divided into 15ths, put in 15 sterile nylon bags⇒ 60 bags used
  - Each glass, = the experimental unit, had 4 bags, one of each MP type

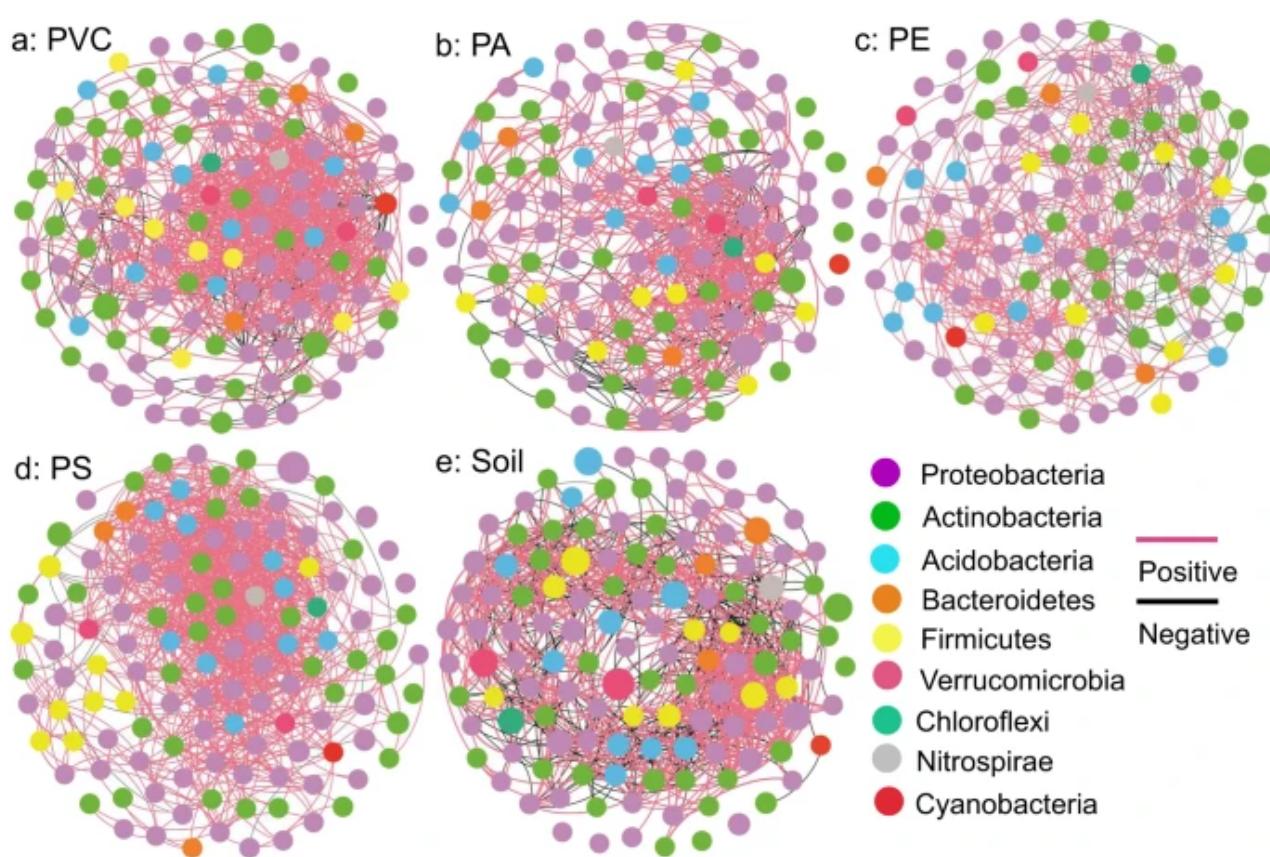
- Microcosm experiment 3:
  - Studied effects of 3 factors, manure; temperature; and moisture, on 30um PE plastispheres
  - Each tested at 2 levels ⇒ 8 treatments total
  - MPs incubated in yellow brown soil
  - Manure from a Bio-fertilizer co. LTD in Zhejiang, China
  - In treatment with added manure, 5g manure was mixed in per 1 kg dry soil
- Field experiment:
  - 30 um glass beads and the 4 MPs were buried in black soil in the field, each in a nylon bag
  - Burial site same site black soil was sourced from
  - Each treatment repeated 3 times
  - After 8 weeks, samples collected and metagenomic sequencing was used to analyze microbial composition, function, and resistome
  - Extracted DNA from .5g of soils, glass beads, and MPs
  - Then used spectrophotometric analysis and gel electrophoresis to check quality and concentration of the isolated DNA
  - Then stored DNA at -80 degrees C
  - 285 ARGs, 10 mobile genetic elements, and 16S rRNA found in each soil and MP sample
  - Gene amplification replicated for total of 3 times each
  - To differentiate samples, used bacterial universal primer to amplify the V4 region of the 16S rRNA gene ⇒ each sample had unique barcode, combined with primer
  - Used tools to check sequences, make libraries, filter through/discard some (ex. ones likely to have error), calculate alpha and beta diversity, and identify potential bacterial pathogens
  - Performed metagenomic sequencing, discarded two of the PVC samples due to insufficient data, and thus obtained total of 16 samples
  - Made a neutral community model to determine how the microbial community assembles
    - Model assumed the zOTUs (closely related individuals) were sampled randomly from a common pool, that death and growth rates of closely related individuals were the same in the plastisphere, and that dispersal rates were the same between plastispheres
    - Assumed variations of microbial communities in the plastisphere were caused by random changes of zOTU abundance
  - In microcosm experiment 1:
    - Obtained 341,304 sequences and 4650 bacterial zOTUs at 100% identity.
    - Found that bacteria in PE plastisphere was clearly different from those on glass and soil
    - Alpha diversity of PE plastisphere was lower than on glass and soil
    - Different sizes of PE plastic had different bacteria and alpha diversities
    - Plastisphere samples clustered based on sample size, were different from glass and soil
    - Bacterial communities between each substrate and soil were clearly different
    - Glass and soil more similar communities than plastisphere and soil
    - Results provide evidence that the microplastic is what selects the bacterial community, and that the properties of the plastic, such as size, impact which are selected



In image, can see how results are grouped depending on plastic size ( $\mu\text{m}$ ) and if it's a plastic, glass, or soil sample

Article's more detailed description: "Principal coordinates analysis (PCoA) presented the distribution of bacterial communities from different substrates (soil, glass bead (GB: 30  $\mu\text{m}$ ) and polyethylene microplastics (PE: 30, 200, and 1500  $\mu\text{m}$ )) based on the Bray Curtis (a) and Weighted Unifrac (b) distances. Different shapes and colors represented different types of samples. The variation explained by the PCoA axes was listed in parentheses. The PERMANOVA was used to test significant difference (significant level  $p < 0.05$ ). Boxplots revealed the distance of bacterial communities between each substrate and soil sample (c: Bray Curtis and d: Weighted Unifrac), which reflected the similarity of bacterial community between each substrate and soil sample. Significance of results was evaluated using pairwise PERMANOVA and labeled using different letters. center line, median; box limits, first and third quartiles; whiskers, 1.5 $\times$  interquartile range."

- Some clustering by MP or soil type also observed; tests showed MP type contributed to 27% of differentiation, and soil type 21%
- In at least 80% of the samples, study found relationships between prevalent bacteria
  - More positive than negative edges detected, most positive edges was PS with 1034, 98%. Lowest number of edges was PE with 557



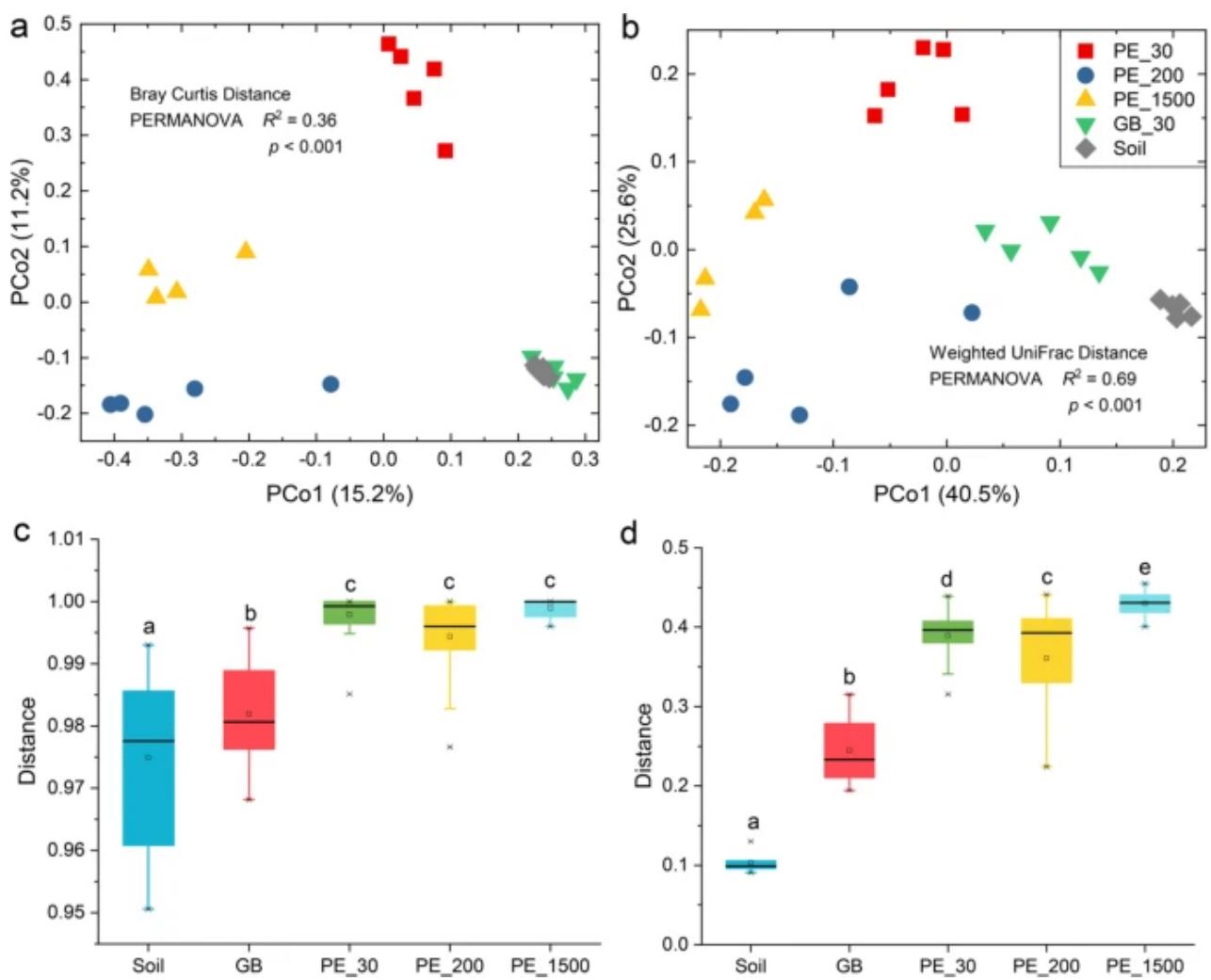
Shows co-occurrence networks in the different environments. Size of circle corresponds to the relative abundance of the bacterial zOTUs.

- A neutral community model was used to see how much random processes contributed to the formation of the microbial communities—84% of the distribution of zOTUs could be predicted by it
  - zOTU of the phylum Patescibacteria had highest ratio, of 34%, falling below the model
- Biological pathways are divided into 3 levels
- Results show plastispheres had 356 metabolic pathways at level 1
- Level 3 metabolic pathways = metabolism, cellular processes, genetic info processing, environmental information processing, and human diseases
- Level 2 metabolic pathways = antimicrobial, including beta-Lactam, drug resistance
  - Had highest abundance of 0.36 in all predicted functional genes
- Overall, ratio of potential bacterial pathogens to total bacteria in plastisphere was 12.4 times higher than in soil
- 73 potential bacterial pathogens were detected in all plastispheres
- Higher bacterial pathogen : bacteria ratio found in all plastispheres compared to soil except for yellow brown soil
  - Yellow brown soil: similar ratio found among PVC, PA, PE, and soil, ratio higher in PS
- Most dominant potential bacterial pathogenic species: *Bordetella avium* (34%), *Pseudomonas fluorescens* (13%), *Pseudomonas protegens* (6.8%), *Burkholderia* sp. (5.2%), and *Pseudomonas syringae* (5.1%).

- Total of 102 ARGs and 3 MGEs detected in all plastiSphere samples, in 9 ARG types
- Relative abundance of plastiSphere ARGs varied across soil and MP types, with MP types having significant, 18%, influence on change of ARGs
- Besides for PVC, more ARGs in plastiSpheres than soils; ARGs on PE significantly more than in soil
- 71% of ARGs shared between plastiSpheres and soil, 11% unique to plastiSphere; for same MPs, 45-51% of ARGs shared when in different soils
- Predictable processes could explain 7.32% of ARGs variation, compared to 3.84% of random processes and 76.9% unexplained. (article has diagram for this)
- Microcosm experiment 3: obtained 3,613,142 sequences, 9116 bacterial zOTUs at 100% identity
- Total of 127 ARGs detected in all soil and plastiSphere samples, in 9 ARGs types
- Addition of manure significantly increased the relative abundance of ARGs, by 179% in plastiSpheres and 480% in soil, compared to control
- Adding manure didn't change the ratio of potential pathogens : bacteria in plastiSpheres
- In control and manure treatments, pathogens was 3.4 times higher than in soil, bacteria 6.7 times higher
- Samples clustered based on if manure was added
- Manure could explain 13% of variation in the bacterial communities and 57% variation in ARGs
- Moisture explained 15% of variation in bacteria, 12% in ARGs
- Temperature explained 7% variation in bacteria, 2% in ARGs
- Increase in soil moisture increased ratio of pathogens:bacteria
  - Without manure, significantly higher ratio of pathogens:bacteria found when soil had 80% moisture vs 60% moisture
  - Increasing temperature increased effect of moisture
- Microbial communities on plastiSpheres obviously different from on glass and soil
- Structure of the microbial community had a strong correlation with the substrate's hydrophobicity
- Two metabolic pathways, xenobiotics degradation and metabolism and antimicrobial drug resistance, enhanced in plastiSphere, especially for PE
- For more detail on results of AMGs found, see paragraph above figure 8
- Found:
  - Alpha diversity of plastiSpheres distinctly lower than in soil
  - Communities different on MPs vs. glass vs. soil
  - Variations in communities strongly linked to substrate hydrophobicity (also demonstrated in a previous aquatic system study)
  - MP types also influence community, possibly due to different plastic properties
  - Plastic hydrophobicity contributes to colonization of microbes
  - More negative correlations between bacteria found in soil than plastiSphere, suggesting may be less competition in the community on MPs, possibly because of limited resources on plastics
    - Degrading MPs difficult → microbes may cooperate in order to use the MPs
      - Stress gradient hypothesis: positive interactions dominate competitive ones in harsher environments
      - Or niche overlap between related bacteria
      - Or mutualistic/facilitative interactions between different taxa

	<ul style="list-style-type: none"> <li>- Could predict distribution of most bacteria→ suggests distribution mainly shaped by passive dispersal and ecological drift           <ul style="list-style-type: none"> <li>- Also neutral process unable to predict→ supports that MPs could act as environmental filter</li> </ul> </li> <li>- Many metabolic pathways found on plastispheres, different from in soil</li> <li>- Another study showed microbial turnover and nutrient efficiency enhanced in plastisphere</li> <li>- Plastisphere communities may be under more environmental stress than in soil (from types of pathways enhanced in plastispheres) (similar results in aquatic)</li> <li>- Multiple metabolic pathways involving antibiotic resistance identified           <ul style="list-style-type: none"> <li>- Could be because MPs absorb compounds in soil due to hydrophobicity, producing more pressure on bacteria, MPs select bacteria</li> </ul> </li> <li>- Findings indicated MPs may accelerate dispersal of ARGs into ecosystem</li> <li>- Surfaces of MPs affect absorption of antibiotics, so assessing risk of ARG spread by MP needs more understanding of MP types</li> <li>- Deterministic processes have greater influence on bacterial communities than stochastic processes</li> <li>- Manure significantly increased ARGs→ suggests ARGs introduced by human activities will accumulate in plastisphere</li> <li>- Increasing moisture, temperature, enhance ARGs⇒ warm and humid environment ideal for increase of ARGs (shown that microbes sensitive to above conditions; microbes correlate with ARGs)</li> </ul>
Research Question/Problem/ Need	Though understanding how the plastisphere affects microbial communities and antimicrobial resistance genes is of global concern, we don't know much about plastispheres in soil. This article studies plastispheres in/on various soils, environments, and microplastics.

## Important Figures



In image, can see how results are grouped depending on plastic size ( $\mu\text{m}$ ) and if it's a plastic, glass, or soil sample

Article's more detailed description: "Principal coordinates analysis (PCoA) presented the distribution of bacterial communities from different substrates (soil, glass bead (GB: 30  $\mu\text{m}$ ) and polyethylene microplastics (PE: 30, 200, and 1500  $\mu\text{m}$ )) based on the Bray Curtis (a) and Weighted Unifrac (b) distances. Different shapes and colors represented different types of samples. The variation explained by the PCoA axes was listed in parentheses. The PERMANOVA was used to test significant difference (significant level  $p < 0.05$ ). Boxplots revealed the distance of bacterial communities between each substrate and soil sample (c: Bray Curtis and d: Weighted Unifrac), which reflected the similarity of bacterial community between each substrate and soil sample. Significance of results was evaluated using pairwise PERMANOVA and labeled using different letters. center line, median; box limits, first and third quartiles; whiskers, 1.5 $\times$  interquartile range."

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- In at least 80% of the samples, study found relationships between prevalent bacteria

	<ul style="list-style-type: none"> <li>- A neutral community model was used to see how much random processes contributed to the formation of the microbial communities— 84% of the distribution of ZoTUs could be predicted by it</li> <li>- Level 2 metabolic pathways = antimicrobial, including beta-Lactam, drug resistance <ul style="list-style-type: none"> <li>- Had highest abundance of 0.36 in all predicted functional genes</li> </ul> </li> <li>- Overall, ratio of potential bacterial pathogens to total bacteria in plastisphere was 12.4 times higher than in soil</li> <li>- 73 potential bacterial pathogens were detected in all plastispheres</li> <li>- Most dominant potential bacterial pathogenic species: <i>Bordetella avium</i> (34%), <i>Pseudomonas fluorescens</i> (13%), <i>Pseudomonas protegens</i> (6.8%), <i>Burkholderia</i> sp. (5.2%), and <i>Pseudomonas syringae</i> (5.1%).</li> <li>- Total of 102 ARGs and 3 MGEs detected in all plastisphere samples, in 9 ARG types</li> <li>- Relative abundance of plastisphere ARGs varied across soil and MP types, with MP types having significant, 18%, influence on change of ARGs</li> <li>- Manure could explain 13% of variation in the bacterial communities and 57% variation in ARGs</li> <li>- Moisture explained 15% of variation in bacteria, 12% in ARGs</li> <li>- Temperature explained 7% variation in bacteria, 2% in ARGs</li> <li>- Without manure, significantly higher ratio of pathogens:bacteria found when soil had 80% moisture vs 60% moisture</li> <li>-</li> </ul>
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Anthropocene: the current geological age, when humans have been the dominant influence on climate and the environment</li> <li>- Zero-radius operational taxonomic units: OTUs= an operational definition used to classify groups of closely related individuals</li> <li>- Resistome: includes all the antibiotic resistance genes</li> <li>- Atmospheric deposition: process through which precipitation, particles, gasses, etc. move from atmosphere to earth's surface</li> <li>- Biota: flora and fauna of a region</li> <li>- Biogeochemical cycle: cycle in which chemical elements and simple substances are transferred between living systems and the environment</li> <li>- Xenobiotic: chemical substances foreign to animal life ex. Plants, chemicals</li> <li>- Stochastic: randomly determined</li> <li>- Rhizosphere: area around the plant root that's colonized by microbes</li> <li>- Phyllosphere: the area of the plant above soil, ex. leaves, that's colonized by microbes</li> <li>- Amplicon: a segment of chromosomal DNA that undergoes amplification and contains replicated genetic material (DNA fragments that are products of a PCR)</li> <li>- Sequencing: determines primary structure/sequence of an unbranched polymer</li> <li>- Amplicon sequencing: highly targeted approach allowing researchers to analyze genetic variation in specific genomic regions</li> <li>- Metagenomic sequencing: Allows researchers to sample all genes in all organisms in a complex sample</li> <li>- High-throughput: Use of automated equipment to rapidly test thousands to millions of samples for biological activity at organism, cellular, pathway, or molecular level</li> <li>- qPCR: quantitative PCR/real-time PCR; determines the amount of PCR product present at a given cycle. Uses fluorescent probe, measures intensity of fluorescence each cycle. <a href="#">Video</a></li> </ul>

- Arable: suitable for crops
- Sodium hypochlorite: is commonly used for bleaching, disinfecting, and water treatment; hypochlorite =  $\text{ClO}^-$ , an anion
- Spectrophotometric analysis: determination of materials in a substance or mixture by measuring the amount of light they absorb in the infrared, visible, or ultraviolet region of the spectrum
- Alpha diversity: the species diversity or species abundance in an ecosystem– measures small scale
- Beta diversity: the species diversity between two ecosystems or communities– measures large scale
- Principal coordinates analysis: a statistical method that converts data on distances between items into map-based visualization of those items, used to better understand which items are more similar to each other and which are more different
- Shannon index: in the Shannon index,  $p$  is the proportion ( $n/N$ ) of individuals of a species found ( $n$ ) divided by the total number of individuals found ( $N$ ). For full formula, search it (don't know how to type sigma notation). Higher value of  $H$  = higher diversity of species in the community;  $H=0$  means only one species
- Modularity: system is modular if it can be divided into multiple sets of strongly interacting parts that are relatively autonomous with respect to each other
- Beta-Lactam: antibiotics including penicillins
- Permutational multivariate ANOVA: used to compare groups of objects and test if the dispersion of the groups is the same or different between groups
- Xenobiotics: substances foreign to the body or to an ecological system

**Abbreviations:**

- ARGs: antibiotic resistance genomes
- MPs: microplastics = plastics < 5mm
- zOTUs: zero-radius operational taxonomic units (see definition above)
- PVC: polyvinyl chloride
- PA: polyamide
- PE: polyethylene
- PS: polystyrene
- MGEs: mobile genetic elements
- 16S rRNA: the gene that encodes the small subunit ribosomal RNA molecules of ribosomes, responsible for converting genetic messages to functional cell parts by translating mRNA to proteins
- PCoA: principal coordinates analysis (defined above)
- PERMANOVA: permutational multivariate ANOVA (defined above)

Cited references to follow up on	<input type="checkbox"/> Zhou J, Gui H, Banfield CC, Wen Y, Zang H, Dippold MA, et al. The microplastisphere: Biodegradable microplastics addition alters soil microbial community structure and function. <i>Soil Biol Biochem.</i> 2021;156:108211. <input type="checkbox"/> Zhu D, Delgado-Baquerizo M, Su J-Q, Ding J, Li H, Gillings MR, et al. Deciphering potential roles of earthworms in mitigation of antibiotic resistance in the soils from diverse ecosystems. <i>Environ Sci Technol.</i> 2021;55:7445–55. <input type="checkbox"/> Hesse E, O'Brien S, Luján AM, Sanders D, Bayer F, van Veen EM, et al. Stress causes interspecific facilitation within a compost community. <i>Ecol Lett.</i> 2021;00:1–9. <input type="checkbox"/>
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Follow up Questions	<ol style="list-style-type: none"><li>1. How do microbial communities first assemble on microplastics in soil?<ol style="list-style-type: none"><li>a. Bacterial communities?</li></ol></li><li>2. At what rate do bacterial communities in soil plastispheres transfer ARGs?<ol style="list-style-type: none"><li>a. How does the type of ARG/MP affect transfer?</li><li>b. (Rate of soil vs. marine vs. free-living bacterial plasmid transfer?)</li></ol></li><li>3. What role do absorbed toxins play in the evolution of ARGs on plastispheres?</li><li>4. How can we remove MPs from soil (either to clean up or to analyze for data)?</li></ol>
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# Article #9 Notes: Microplastics in food: scoping review on health effects, occurrence, and human exposure

Source Title	Microplastics in food: scoping review on health effects, occurrence, and human exposure
Source citation (APA Format)	Udovicki, B., Andjelkovic, M., Cirkovic-Velickovic, T., & Rajkovic, A. (2022). Microplastics in food: Scoping review on health effects, occurrence, and human exposure. <i>International Journal of Food Contamination</i> , 9(1). <a href="https://doi.org/10.1186/s40550-022-00093-6">https://doi.org/10.1186/s40550-022-00093-6</a>
Original URL	<a href="https://www-proquest-com.ezpv7-web-p-u01.wpi.edu/naturalscience/docview/2692478697/58FB3E27EAF14B56PQ/8?accountid=29120">https://www-proquest-com.ezpv7-web-p-u01.wpi.edu/naturalscience/docview/2692478697/58FB3E27EAF14B56PQ/8?accountid=29120</a>
Source type	Journal article
Keywords	Additives; Food; Microorganisms; Shellfish; Contaminants; Monomers; Microplastics; Exposure; Food cans; Ecotoxicology; Plastic debris; Food contamination; Chronic exposure; Contamination; Plastic pollution; Microplastics; Human health; Occurrence; Food; Human exposure
Summary of key points + notes (include methodology)	<p>Summary: The article covered the effects of ingested microplastics. It is an increasingly pressing issue, as we are producing more and more plastic, which breaks down into MPs. We all ingest MPs, as they can be found in both water and food. Plastics may have detrimental health effects, for example on reproductive health and the health of offspring. Plastics also absorb toxins from the environment, which would pose a further health risk. It is still difficult to measure MP presence in food and calculate general intake.</p> <p>Notes:</p> <p><b>key:</b></p> <p>all <b>HIGHLIGHTED</b> are important</p> <p><b>PINK</b> is stuff I found particularly interesting</p> <p><b>BLUE</b> is areas of future research identified/implied by article</p> <ul style="list-style-type: none"> <li>- Microplastics' presence in food and influence on human health= emerging concern <ul style="list-style-type: none"> <li>- Review aimed to inform on possible health effect of ingested MPs, occurrence and levels of MP contamination, and estimated exposure to MPs through food</li> <li>- Toxic consequences from exposure to MPs through food can come from MPs, diffused monomers and additives, and sorbed contaminants/microorganisms that colonize MPs</li> <li>- MPs have contaminated many foods ex. Water and salt</li> <li>- We ingest up to several hundred thousand MPs yearly</li> <li>- Global plastic production reached almost 370 million in 2019 <ul style="list-style-type: none"> <li>- &gt;1/3 in US and Europe used in disposables, made to discard after 3 years</li> </ul> </li> <li>- Estimated&gt; 75% plastic ever produced now waste</li> <li>- MPs pollutant in water, soil, air, food <ul style="list-style-type: none"> <li>- Also in food from release/leakage of packaging, uptake by plants</li> </ul> </li> </ul> </li> </ul>

- Little known about:

- What happens to MPs in digestive tract
  - Absorption uptake kinetics
  - Distribution of MPs in body
  - Exposure to MPs complex issue because plastics, monomers, additives, and various pollutants can all impact health
  - MP particles all different, one food may have many different types
  - May intake MPs through other ways ex. Breathing, touching → difficult to determine effects of just food
  - Review aims to determine possible effects of ingesting MPs, occurrence, level of MP contamination, estimated exposure through food
  - Plastics = polymers made from polymerising monomers through addition of additives
  - Additives catalyze polymerization, give plastic properties ex. Rigidity, UV stability
  - 4% weight of plastics common as MPs = additives
  - 2 main categories of plastics are thermoplastics and thermosetting plastics = thermosets
  - Thermoplastics include PE, PP, PVC, PET, PS, PC, and PA
  - Thermosets include PU, epoxy resins, vinyl esters, and silicones
  - Common MP plastics commonly used= thermoplastics PE, PP, PVC, PS, and PET = >70% of European plastics demand
  - Types of plastics: fragments, fibers, spheroids, granules, pellets, flakes, and beads; are between .1 um and 5 mm
  - NPs= .001 to .01 um; are considered more damaging than microplastics because are small enough to permeate biological membranes
  - \*\* IN THIS PAPER, MPs include NPs
  - 2 categories MPs:
    - Primary: industrially manufactured to be that size, found in textiles, medicines, cosmetics, etc. Enter environment through leakage during manufacture, transport, or use
    - Secondary: more abundant, from breaking down of larger plastics, laundry, agricultural mulch plastics
    - Can be fragmented into NPs through abiotic and biotic processes
    - One MP can form billions of NPs– will become global issue
    - Abiotic degradation before biodegradation, initiated thermally, hydrolytically, or by UV light in the environment
    - Bacteria/microorganisms can biodegrade MPs by intra or extracellular depolymerases
    - Analysis of MPs has two phases:
      - 1) Physical characterization
      - 2) Chemical characterization
      - Microscopy methods, ex. Stereo, fluorescence microscopy, TEM, SEM, used for physical characterization
      - TEM, SEM, fluorescence microscopy used for chem and phys properties of many polymers
      - FTIR, Raman spectroscopy, and thermal techniques used for chemical characterization
      - integrated techniques, ex. Py-GC/MS and TED-GC/MS, more popular because faster, give a lot of useful information
- **HEALTH EFFECTS**
- Health effects can be from physical or chemical impact of MPs
- MPs not chemicals, but may eventually have same effect on immune systems

- “Frustrated phagocytosis”= failure of macrophages to engulf and eliminate target, resulting in long inflammation, potential tissue damage
- Most information on effects of MPs from marine wildlife, lab animals, or in vitro (test tube) studies
- Ingestion by marine animals shown not-lethal effects: reduced reproduction, reduced individual growth, reduced fitness (ex. Because internal damage, gastrointestinal blockage, reduced feeding, inflammation, plastics replacing food)
- Though some unlikely for humans, other problems likely:
- MPs enter tissues⇒ potentially physical stress, damage, apoptosis, necrosis, inflammation, oxidative stress, immune responses
- Important Q: can MPs be absorbed and enter circulation ant tissues or stay in the gut
- Estimated human excretory system removes over 90% MPs
  - Intestinal absorption depends on size of particles, appears low
  - Immune system:
  - MPs <= a few microns may be taken up by cells' phagocytosis and endocytosis mechanisms
  - MPs up to 10 um may be taken up by specialized cells in Peyer's patch
  - Up to 130 um: can enter tissue through transport between cells through persorption
  - To 150 um not absorbed, effects on immune system and fut expected
- Not much info on MP absorption rates and bioavailability
- Plastics around 2um: estimates .absorption rate 0.04 to 0.03%, in nanoscale, absorption rate up to 0.2 and 1.7%
- Absorbed MPs could travel through body, with smallest penetrating tissues and blood-brain barrier
- Experiments on animals/in vitro investigates bioaccumulation, effects of MPs
- Most used pure PS particles, so could assume effect from MPs, not another factor
- Research shown accumulation in liver, kidney, gut of mice
- 5 and 20um MPs given orally, at concentrations of 0.01, 0.1, 0.5 mg per day for 28 days
- Found liver inflammation, presence of lipid droplets, disturbed energy and lipid metabolism, oxidative stress, blood alternations of biomarkers and neurotoxicity (see vocab), reduced weight, decreased colon mucus secretion, etc.
- Various studies done to see effects of ingesting MPs on animals, with various time spans, concentrations, methods
- One study introduced MPs to pregnant mice→ increased risk of metabolic disorder in offspring (F1), with greater effects from group given larger MPs (were .5 and 5um); further research shows can have long-term consequences even in F2 generation
- Ingestion MPs also damage sperm, reduce production, in mice
- NPs could have more serious effects than MPs on microbiota dysbiosis and inflammation in the gut of zebrafish
- Study using various MPs and cell lines reported various levels of uptake and usually only cytotoxicity for high MP levels
- Another concern: toxicity of diffusing monomers and additives, as we have lots of information on their toxicities
  - Low presence in MPs but exposure builds up over time
- Unreacted monomers left over from polymerization reactions can be found in the polymers
- Additives have weak non-covalent bond to the polymer backbone, so they leach quickly into environment
- MPs degrading augments leaching of monomers and additives
- Little known of MP degradation in digestive tract, though know MPs degrade as part of feeding

- and digestion of earthworms, fresh-water amphipods, and Antarctic krill
- 2011 study found that 16/55 monomers used in common plastics are carcinogenic, mutagenic, or toxic for reproduction
    - Of highest concern = bisphenol A (BPA), vinyl chloride, acrylamide, and styrene
    - BPA used for polycarbonate plastics, epoxy resins, an additive in other plastics, found in food contact materials
  - BPA has been shown to disrupt endocrine system, ex. Estrogen receptor, thyroid hormone receptor, etc, resulting in issues for reproductive, nervous systems, metabolic, immune function, and offspring development
  - BPA-free products more common, but substitutes may share BPA's effects
  - Vinyl chloride main component in manufacture of PVC
    - PVC mainly used for plastic piping
    - Vinyl chloride causes angiosarcoma of the liver, hepatocellular carcinoma, is classified by the International Agency for Research on Cancer (IARC) as a group 1 carcinogen
  - Acrylamide=monomer of polyacrylamide, used mostly in water treatment or industry
    - Acrylamide and its metabolite glycidamide are genotoxic and carcinogenic, associated with cancer
  - Styrene one of most important monomers for plastics-used-for-many-things
    - IARC classifies styrene as probably carcinogenic, as styrene is associated with lymphohematopoietic malignancies
  - Hundreds of additives. Most concerning ones:
    - Phthalates
    - Polybrominated diphenyl ethers
    - Heavy metals
  - Phthalates: used to give flexibility and durability to PVC
    - Evidence it can disrupt oestrogenic activity, reproduction, development, cause liver toxicity
  - Polybrominated diphenyl ethers: chemicals added to plastics, foam to make them fire-resistant
  - Related to cancer-causing, disrupting reproductive health/behavior, disruption hormones, neurotoxicity, neurodevelopmental disorders, behavioral deficits in humans, etc.
  - Heavy metals ex. Lead, mercury, chromium, cadmium, antimony most concern, used in fillers, pigments, stabilizers
    - Use regulated, but poor management, disposal of historical plastics→ dispersed in modern goods
  - Observed effects MPs interconnected– hypothesis MPs and plastic additives could play role in obesity pandemic– obesity increased x3 over past 50 years, similar to with plastics
  - There are also organic pollutants in plastics (see page 5 of pdf for names)
    - Concentrations higher than in surroundings, concentration up to millions
    - Ex. PCBs suppress immune system, tumor promoters that enhance effects of other carcinogens
  - Increasing amounts antibiotics also released into environment, contaminating and sorbing to plastics.
  - Microorganisms shown to colonize MPs, though most studies on aquatic environments, need more info on impacts on human health and food
  - Most common on MP surfaces is *Vibrio* Spp, also found *Escherichia coli*, *Pseudomonas*, *Aeromonas*, *Haemophilus*, *Acinetobacter* and bacteria from families *Pseudomonadaceae*, *Proteobacteria*, and *Campulobacteraceae*

- Another issue = increasing antimicrobial resistance
- MPs could act as microcosm for more effective gene exchange between bacteria, as many pathogenic bacteria are close to other microbes and sorbed contaminants, ex. Metals
- Metals often co select for antimicrobial resistance
- Increased frequency of plasmid transfer observed in bacteria on MPs
- Water main source of dietary MPs- drunk, used to make food, exposed to MPs through many ways
- Conventional, optimised wastewater treatment can remove over 90% MPs from wastewater
- MP contamination in bottled water higher than from other sources
- Fishery, aquaculture booming in last 50 years– products = about 17% pf animal protein intake by world
- Plastic, 80% from land, enters aquatic environments
- Levels of MPs in fish and shellfish are good indicators of MP contamination in environments
- Small fishes that are consumed whole= greater risk of eating MPs, since gastrointestinal tract not removed
- Researchers have found MPs in fish and shellfish in the wild and from aquaculture farms/markets
- Over 400 research papers on MPs in fish, 62% marine, 38% freshwater
- Min concentration = 0 to 4 or 5 particles
- Max concentration= 56 particles
- More found in carnivores
- Most common types: PE, PP, PET, PA, cellophane, acrylonitrile
- Up to 297.74 particles/gram found in Atlantic mud crab
- Only a few studies have studied MPs in body other than digestive system, gills, and skin
- Salts, sugars another way to consume MPs– eaten daily, sed as additives, stabilizers, thickeners
- Several studies on MPs in salt, only one on in sugars
- Contamination processed foods, honey with MPs from environmental sources, packaging, materials used during manufacturing
  - MPs in processed foods probably won't be cleaned out like will with fish– are consumed
- Terrestrial plants directly exposed to plastic pollution ex. From fertilizer, sewage, agricultural plastic film, etc.
- 24 studies report plants can take in MPs to a certain degree
  - Ex. one showed wheat, lettuce took up .2um and 2.0 um PS beads– were in xylem sap, showing were transported through roots and stem
- In 1st study, in 2020, about presence of MPs in vegetables and fruits, analyzed the foods using SEM-EDX method
  - High median level of MPs in fruits = 223,000 particles/g; 97,800 in veggies
  - Apples most contaminated fruit, carrots most contaminated vegetable
  - Lower median level was 52,050 particles/g in lettuce
  - MP size between 1.36 and 2.52 um
  - Hypothesized fruit contained more MPs because of high vascularization of fruit pulp and larger, more complex root system and tree age
- Food contact materials could be source of hazards, despite regulations
  - Most MP particles in returnable plastic bottles= 84% PET, 7% PP, = materials of bottle
    - Infant feeding bottles made from PP could shed up to 16,200,000 particles/L
  - In cartons, glass bottles, found PE and biofilms, used as coatings/lubricants
  - Packaged meat: 4 to 18.7 particles/kg
  - 3 to 29 particles per container in takeout
- Increase in data on MPs in food⇒ increased effort on assessing human exposure to MPs, which

- varies depending on person's characteristics, location, lifestyle, etc.
- Only two comprehensive studies on assessing intake of MPs through foods & other sources were done— were based on published data on MP occurrence
  - Based on American diet: estimated annual MP consumption from 39,000 to 52,000 particles
    - Increase to 74,000 and 121,000 when inhalation considered
    - People who meet recommended water intake using bottles sources may ingest additional 90,000 MPs annually
    - Values likely underestimated
  - Another estimated global average ingestion of MPs between .1 and 5g weekly, tap and bottled water being greatest contributors
  - EFSA estimated 900 particles MPs, ~7g plastic, would be consumed with 225g of mussels
  - Human exposure better estimates by body fluids analysis
  - More research needed to understand investigate MPs presence and interaction in human body
  - Presence MPs confirmed in 8 out of 8 tested stool samples
    - Median of 20 MPs, 50 to 500um, per 10g stool
    - Found 9 plastic types; PE, PET most abundant
  - PC, PA, PP found in 11 colectomy samples, with average of 331 particles per sample, suggesting MPs found everywhere in digestive tract
  - MPs also confirmed in the human placenta
    - Total of 12 particles, from 5 to 10 um, in four out of six placentas tested
    - Three were stained PP
    - Other nine, could only identify pigments, all used for coatings.paints/adhesives/cosmetics/etc.
    - Exposure usually measured in quantity, not type plastic, shape, size, though latter are also important for exposure, risk assessment
    - Plastics can pose health risks, house bacteria, spread bacteria
    - Generally believed large plastics not absorbed by intestines due to size, do not cross blood-brain barrier— thus, are excreted
      - Upon entering environment, plastic break down into smaller plastics, which are more concerning
      - Meals that can reach brain and have many neurotoxic effects may have similar effect to plastic particles
      - Studies using animals indicate alterations in gene expression, inflammation of gut, gills, etc, accumulation in various tissues.organs, oxidative damage, disturbed metabolism, alterations in behavior and internal function, increased mortality, etc.
      - Exposure to plastics typically expressed using daily basis, for dietary exposure, and annual, for correlating exposure to annual plastic production
      - Exposure can be modeled using intake and loss processes (ex. excretion)
        - Still difficult to model, since some areas of foods ex. Fish gut inedible, how to rescale plastics, how to estimate multiple (instead of just one) source of plastic intake
      - Detection of MPs has limitations in detection of NPs⇒ levels MPs could be underestimated
      - All plastic ever produced still in environment, degrading, making more MPs for future

	<ul style="list-style-type: none"> <li>- Many possible health effects of MPs/related hazards</li> <li>- Need more research on MP (+ sorbed chemicals') toxicity, pathogenic potential of MPs' organisms/their toxins, role of MPs in spreading antibiotic resistance, realistic models of MP exposure, understanding consequences of MPs in human body</li> </ul>												
Research Question/Problem / Need													
Important Figures	<p><b>Fig. 1</b> Main identification methods for microplastics in food</p> <p>Ratio of main MP identification methods. FTIR and Raman spectroscopy most common.</p> <table border="1"> <thead> <tr> <th>Method</th> <th>Approximate Proportion</th> </tr> </thead> <tbody> <tr> <td>FTIR</td> <td>~45%</td> </tr> <tr> <td>Raman spectroscopy</td> <td>~30%</td> </tr> <tr> <td>SEM-EDX</td> <td>~15%</td> </tr> <tr> <td>Microscopy</td> <td>~10%</td> </tr> <tr> <td>Thermal techniques</td> <td>~5%</td> </tr> </tbody> </table>	Method	Approximate Proportion	FTIR	~45%	Raman spectroscopy	~30%	SEM-EDX	~15%	Microscopy	~10%	Thermal techniques	~5%
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**Table 1** Occurrence and characteristics of microplastics in water, beverages, and alcoholic drinks

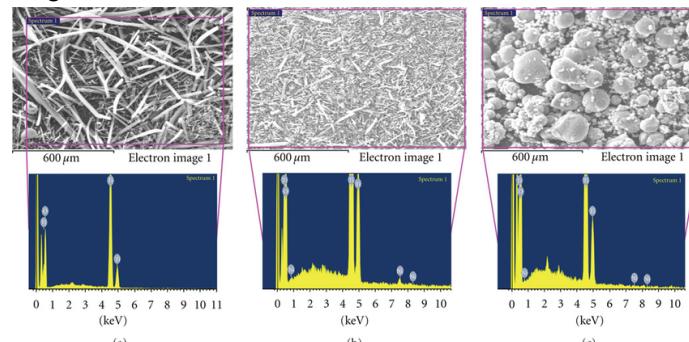
Type of product	Type of polymer	N/Np	Size range ( $\mu\text{m}$ )	Analytical method	Microplastic content range (particles/L) <sup>a</sup>	Sample origin	Reference
Ground and drinking water	PE, PVC, PA, polyester, epoxy resin	24/10	50-150	FTIR microscope	0-7 <sup>b</sup>	Germany	(Mintenig et al. 2019)
Water from three treatment plants	PET, PE, PP, PS, PVC, polyacrylamide, polybutyl-acrylate	3/3	1-100	SEM + FTIR + Raman spectroscopy	338 ± 76-628 ± 28 (treated water)	Czech Republic	(Pivokonsky et al. 2018)
Tap water	nr	110/86	50-4830	Stereo microscope	0-8.605	Hong Kong	(Lam et al. 2020)
Tap water	Anthropogenic debris <sup>c</sup>	159/126	100-5000	Stereo microscope	0-61	Global	(Kosuth et al. 2018)
Bottled water (plastic and glass), beverages, tap water	PE, PP, PS, PET, PA, polymethyl methacrylate	15/15	nr	TED-GC/MS	<0.01 $\mu\text{g}/\text{L}$ – 2 $\mu\text{g}/\text{L}$ (mass content)		(Braun et al. 2021)
Bottled mineral water (PET bottles)	–	10/10	1.28-4.2	SEM-EDX	$3.16 \times 10^7$ - $1.1 \times 10^8$	Italy	(Zuccarello et al. 2019)
Bottled mineral water	PP, PE, PET, PS, PA, polyester	38/38	5-100	Micro-Raman spectroscopy	2-44 (single use) 28-241 (returnable) 5-20 (cartons) 4-156 (glass)	Germany	(Schymanski et al. 2018)
Bottled mineral water	PET, PE, PP styrene-butadiene	32/32	1- > 10	Micro-Raman spectroscopy	4889 ± 5432 (reusable) 2649 ± 2857 (single use) 3074 ± 2531 (glass)	Germany	(Ößmann et al. 2018)
Bottled water	PP, PE, PA	259/242	6.5-5000	Optical microscope+FTIR	0-10,000	Global	(Mason et al. 2018)
Beer	Anthropogenic debris <sup>c</sup>	12/12	100-5000	Stereo microscope	0 <sup>d</sup> -14.3	Laurentian Great Lakes	(Kosuth et al. 2018)
Beer	nr	24/24	nr	Stereo microscope	2-79 (fibres) 12-109 (fragments) 2-66 (granules)	Germany	(Liebezeit and Liebezeit 2014)
Beer and soft drinks	PP, PE, polyacrylamide	29/29	3.5-2224.25	Inverted microscope+FTIR	8-117	Ecuador	(Diaz-Basantes et al. 2020)
Soft and energy drinks, beer, cold tea	PET, PA, polyester, acrylonitrile-butadiene-styrene	57/48	100-3000	Epifluorescence microscope+SEM-EDX	0-7 (soft and energy drinks) 0-28 (beer) 1-6 (cold tea)	Mexico	(Shruti et al. 2020)
White wine	PE	26/24	7-475	Micro-Raman spectroscopy	2563-5857	Italy	(Prata et al. 2020)

<sup>a</sup>N/Np Number of samples/positive samples, nr Not reported<sup>b</sup>Presented as Range, Mean ± SD or Range of Mean ± SD<sup>c</sup>Per cubic meter<sup>d</sup>Not stained by the Rose Bengal dye<sup>d</sup>The three-trial average was less than the number found in the blank

Table  
organizing findings of articles that have found MPs of various size ranges, type, and source.

VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Kinetics: the rates of a chemical or biochemical reaction</li> <li>- Thermosets: thermosetting plastics, can not be remelted</li> <li>- Thermoplastic: plastic that can be remelted</li> <li>- Abiotic: physical rather than biological; not derived from living organisms</li> <li>- Stereo microscopy: Stereo microscope is a type of microscope with magnification from <a href="#">6 to 50x</a></li> <li>- Fluorescence microscopy: imaging technique used in light microscopes that allows excitation of fluorophores and thus detection of the fluorescence signal; benefits: great detection sensitivity, could be used for opaque objects</li> <li>- Transmission electron microscopy: a technique of imaging the internal structure of solids using a beam of high-energy electrons transmitted through the solid</li> <li>- Scanning electron microscopy: Creates an image by using an electron beam then detecting reflected or knocked-off electrons</li> <li>- Mass content: a large, coherent collection of something</li> <li>- Peyer's patch: group of lymphoid follicles in the mucous membrane of the small intestine; used for immune surveillance of digestive system</li> <li>- Endocytosis: Taking in of matter by living cells. Three types = pinocytosis, phagocytosis, receptor-mediated endocytosis</li> </ul>
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- Persorption: The deep penetration of a liquid into a highly porous solid, resulting in a
- Bioavailability: the proportion of a drug or other substance which enters the circulation when introduced into the body and so is able to have an active effect
- Alternation: the repeated occurrence of two things in turn
- Biomarkers: biological molecule found in blood/other body fluids/tissues, that is a sign of a normal or abnormal condition/process
- Neurotoxicity: Occurs when exposure to toxins alters normal activity of nervous system. Can eventually damage/kill neurons
- Microbiota dysbiosis: imbalance in the gut microbial community, associated with disease
- Cytotoxicity: degree to which a substance can damage a cell
- Amphipod: any of a large order (Amphipoda) of small crustaceans (ex. sand flea) with flat body.
- Metabolite: a substance formed in or necessary for metabolism
- Oestrogenic: causing estrus in animals (estrus= heat)
- Macrophyte: plant, especially aquatic one, large enough to be seen with the naked eye
- SEM-EDX method: scanning electron microscopy with energy dispersive X-ray spectroscopy, the best known and most widely-used of the surface analytical techniques. Produce high resolution images of surfaces using electron beam



- Result ex:

[https://www.researchgate.net/figure/SEM-images-with-EDX-analysis-The-EDX-area-of-the-pristine-TiO<sub>2</sub>-nanofibers-and-its\\_fig2\\_259771806](https://www.researchgate.net/figure/SEM-images-with-EDX-analysis-The-EDX-area-of-the-pristine-TiO2-nanofibers-and-its_fig2_259771806)

- Food contact materials: materials and articles intended to come into contact with food at any level of the food chain
- EFSA: European Food Safety Authority
- Ubiquitous: found everywhere
- Colectomy: a surgical procedure to remove all or part of your colon

#### Abbreviations:

- MPs: microplastics; a heterogenous mixture of differently shaped materials (see size above)
- NPs: nano plastics: a material with any external dimension in the nanoscale or internal structure or surface structure in the nanoscale (see size above)
- TEM: transmission electron microscopy
- SEM: scanning electron microscopy
- EDX: energy dispersive X-ray analysis
- FTIR: Fourier-transform infrared spectroscopy
- Py-GC/MS: pyrolysis-gas chromatography-mass spectrometry
- TED-GC/MS: thermal extraction desorption gas chromatography-mass spectrometry
- IARC: International Agency for Research on Cancer
- PCBs: polychlorinated biphenyls, organic pollutants

Cited references to follow up on	<input type="checkbox"/> Health effects from Deng et al. (2017), Lu et al. <input type="checkbox"/> (2018)
Follow up Questions	<ol style="list-style-type: none"><li>1. Is there an easy way (does not require lab) to determine microplastic concentration in food?<ol style="list-style-type: none"><li>a. Make some sort of stain?</li></ol></li><li>2. Health effects over long span of time?</li><li>3. How does presence of microplastics also impact gene expression?</li><li>4. Is there a way to remove microplastics?</li><li>5. More detail on difference between physical and chemical characterization</li><li>6. Why is presence of lipid droplets, disturbed energy, and lipid metabolism a problem?<ol style="list-style-type: none"><li>a. How do MPs cause this?</li></ol></li><li>7. Need more research on ratio of main MP identification methods. FTIR and Raman spectroscopy most common.</li><li>8. Howto check and make sure a plastic is BPA free?</li></ol>

# Article #10 Notes: Strategies to Overcome Antimicrobial Resistance (AMR) Making Use of Non-Essential Target Inhibitors: A Review

Source Title	Strategies to Overcome Antimicrobial Resistance (AMR) Making Use of Non-Essential Target Inhibitors: A Review
Source citation (APA Format)	Annunziato, G. (2019). Strategies to overcome antimicrobial resistance (AMR) making use of non-essential target inhibitors: A Review. <i>International Journal of Molecular Sciences</i> , 20(23). <a href="https://doi.org/10.3390/ijms20235844">https://doi.org/10.3390/ijms20235844</a>
Original URL	<a href="https://www.mdpi.com/1422-0067/20/23/5844/htm">https://www.mdpi.com/1422-0067/20/23/5844/htm</a>
Source type	Journal article/review
Keywords	antibiotic resistance; non-essential targets; antibiotic adjuvant therapies; virulence factors; combination therapy; beta-lactamases inhibitors; efflux pump inhibitors; membrane permeabilizers
Summary of key points + notes (include methodology)	<p><b>Summary:</b>  More bacteria are becoming resistant to antibiotics, so we must identify methods to combat their resistance. This article indicates four ways in which bacteria can become resistant to antibiotics: through using an enzyme to neutralize the antibiotic, using efflux pumps to extrude the antibiotic, making their membranes less permeable to antibiotics, and changing the structure of the target so that the drug binds poorly to it.</p> <p><b>Notes:</b>  key:  all HIGHLIGHTED are important  PINK is stuff I found particularly interesting  BLUE is areas of future research identified/implied by article</p> <ul style="list-style-type: none"> <li>- Possible approach= use antibiotic adjuvants against bacterial non-essential targets</li> <li>- Many antibiotics found in plants/still being found in plants <ul style="list-style-type: none"> <li>- 1950s to 1970s were “golden age” of antibacterial drug discovery</li> <li>- 30 year gap until 2000= no new one found in 2000)</li> </ul> </li> <li>- Resistance has 2 groups: <ul style="list-style-type: none"> <li>- Intrinsic resistant bacteria: resistant b/c not target</li> <li>- Acquired resistance: resistant b/c susceptible bacteria acquire mechanism that allows them to resist</li> </ul> </li> <li>- Acquired resistance from <ul style="list-style-type: none"> <li>- Mutations</li> <li>- Acquiring external genetic material</li> </ul> </li> <li>- Horizontal gene exchange is through plasmids or transposons</li> <li>- 4 ways to become resistant to antibiotics:</li> </ul>

- 1) Enzymatic inactivation: Existing bacterial enzyme modified to interact with an antibiotic to make it inactive towards bacteria, due to transfer of ARG through plasmid
  - Ex. beta-lactamase enzymes which hydrolyze beta-lactams = penicillins, cephalosporins <https://academic.oup.com/femsle/article/151/2/115/487009>
- 2) Drug extrusion by efflux pumps: (efflux pumps=proteins that can extrude many compounds from cell): efflux pumps overexpressed by bacteria to extrude the antibiotic
  - Ex. *P.aeruginosa* and *Acinetobacter* spp.
- 3) Decreased uptake by changes in outer membrane permeability or by presence of porins: interfere with entrance of antibiotics

(difference between porins and efflux pumps: efflux pumps found in many types of organisms ex. both +and- bacteria, eukaryotes, while porins only in gram-negative bacteria, in the outer membrane) (<https://academic.oup.com/jac/article/51/1/9/771243>, <https://academic.oup.com/femsle/article/151/2/115/487009>)

- 4) Modification of the drug target: impedes binding of he antibiotic and limits its potency
- February 2017: an extensive list of AMR bacteria was published by the WHO
  - Classified as critical, high, and medium
  - Classification based on mortality, level of resistance, and treatability
- Highly critical= Gram-negative ESKAPE: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter*, + *Mycobacterium tuberculosis* (causes tuberculosis)
  - Resistant to carbapenems=last resort antibiotics and third generation cephalosporins= most effective antibiotics for treating multi-drug-resistant bacteria
  - Global health priority
  - May cause serious and deadly infections ex. Bloodstream infections, pneumonia infections
- Multi-drug-resistant bacteria cause death of 23,000 people among 2 million infected annually in US
- AMR considered by WHO as one of the three most important public health threats of 21st century
- Recent years, cut in investment by pharmaceutical companies for discovery of new antibiotic drugs
  - Economic reasons: antibiotic therapies short, less curative than drugs targeting chronic diseases, less profitable because ineffective against resistant bacteria
  - Scientific reasons: only two new antibiotic classes introduced to clinic since the “golden age”, developing antibiotics are all derivatives of existing drugs which many bacteria are resistant to
- Need new, more potent anti-infective drugs
- Promising success from targeting non-essential pathways in order to minimize impact of resistance through use of antibiotic “adjuvant” with an antibiotic
- 3 main types of antibiotic adjuvants developed to block mechanisms of antibiotic resistance:
  - 1) beta-lactamase inhibitors, for gram + and -
  - 2) efflux pumps inhibitors, for gram + and gram -]
  - 3) outer membrane permeabilizers

- Can also use antivirulence compounds
- New potential inhibitors designed to target pathogenicity= capacity of bacterium to cause infection
  - Involves identification of proteins, genes, other macromolecules responsible for bacterial virulence, that can be inhibited to reduce bacterial fitness
  - Reduce bacterial fitness⇒ bacterial more susceptible to immune system's attack, to antibiotics
  - As targets aren't critical for survival, less likely to generate mutations
  - To expand drug targets, small molecules that target these non-essential genes can be combined with existing antibiotics
- **Targeting Efflux Pumps:**
  - Resistance mechanism to antibiotics that work inside the cell ex. Macrolides, fluoroquinolones, tetracyclines
  - Efflux systems extrude antibiotics⇒ increase their minimum inhibitory concentration (MIC) or make them ineffective
  - Efflux systems can also extrude other substrates ex. Detergents, heavy metals
  - Are proteins that span membranes, are in almost all living organisms
  - Classified in 5 families based on sequence similarity, substrate specificity, structural fold, and energy source:
    - 1) ATP-binding cassette (ABC) superfamily
    - 2) Resistance-nodulation-division (RND) family
    - 3) Small multi-drug resistance (SMR) family
    - 4) Major facilitator superfamily (MFS)
    - 5) Multi-drug and toxic compound extrusion (MATE) family
  - MFS, ABC, SMR, MATE families expressed in both gram-positive and gram-negative bacteria, RND in gram-negative bacteria
  - ABC family uses ATP hydrolysis as energy source to actively extrude the substrate; RND, SMR, MFS, MATE use proton gradient
  - Widely believed efflux pumps give moderate AMG resistance
  - **Effects of efflux:**
    - 1) Apparent poor antibiotics permeability
      - Ex. in *Pseudomonas aeruginosa*, stopping *mexB* gene⇒ mutants more susceptible to different classes of antibiotic, ex. chloramphenicol, fluoroquinolones, tetracyclines, or beta-lactams
    - 2) Cross-resistance to unrelated antibiotic classes
      - Resistance to an antimicrobial drug also decreases sensitivity to multiple drugs, ex. Due to high exposure to given antibiotic
    - 3) Wide spectrum of resistance
      - Observed in bacteria where efflux functions & other resistance mechanisms work together
      - Ex. in an *E.coli* strain that expresses both beta-lactamases (thus resistant to beta lactams) and efflux pumps: increases resistance to quinolones (another class of antibacterial drugs)
    - 4) There can be more mutations in bacteria overexpressing efflux pumps
      - Bacteria exposed to subinhibitory (insufficient to stop bacteria) concentrations of antibiotics→ mutate to inhibit effect of antibiotics⇒ high-level resistance
  - Active efflux of antibiotics first described by McMurry et al. 30 years ago
    - Was in *E.coli*– plasmid-encoded, could extrude and become resistant to

antibiotic tetracycline

- Could inhibit efflux pumps to inhibit bacteria
  - One type of efflux pump can be used to extrude many different families of antibiotics → inhibiting efflux pumps could help sustain several antimicrobials.
- How to inhibit efflux pumps:
  - 1) Interfere with efflux gene expression
  - 2) Add functional groups to the drug substrate so pumps can't recognise it
  - 3) Interfere with the assembly of channel proteins
  - 4) Develop small molecules as substrate analogues to block the pump activity
  - 5) Disconnect the energy transfer mechanism of the pump (to deprive it of the energy it needs to function, I think)
  - 6) Obstruct the channel
- Inhibiting efflux pumps ⇒ positive results ex. Increasing activity, thus impact, of antibacterial drugs challenged by efflux; keeping the necessary concentration of the drug at acceptable (article said "therapeutic") dosage; shortening treatment time by reducing resistance to several drugs
- Most widely used strategy= development of efflux pump inhibitors= EPIs, to be used together with specific antibiotics
- EPIs:
  - EPIs are small molecules that can bind efflux pumps and block their extrusion activity
  - Not antibacterial alone— used with different concentrations of antibiotics against a single concentration of inhibitor in bacterial strains containing efflux pumps
  - Inhibitors showing 8 times or more reduction in MIC further evaluated:
    - Used the fractional inhibitory concentration (FIC) method
  - An inconvenience of targeting pumps:
    - Inconvenience is linked to the many physiological functions they're involved in— blocking can cause related toxicities, especially for EPIs for drug repurposing
    - Need to use EPIs at high dosages
    - Off and on target side effects
  - Many studies to find compounds that inhibit pumps specific to prokaryotic cells
  - First few EPIs were found by chance, from existing drugs
    - Ex. reserpine, shown to inhibit multi-drug transporters like NorA; phenothiazines, calcium channel antagonists, selective inhibitors of serotonin reuptake, proton pump inhibitors
  - Only documented inhibitor currently is MP-601, for patients with ventilator-associated pneumonia, cystic fibrosis
  - Many strategies to inhibit EPs
  - Some compounds compete with the antibiotics to be extruded
    - Phenylalanine-arginine-β-naphthylamide (PaβN) (is a dipeptide amide): inhibits several RND EPs, thus improving activity of different classes of antibiotics (ex. 4-fluoroquinolones, macrolides, and

chloramphenicol) in many pathogens

- Acts competitively: binds to the target transporters at the same site the antibiotic the EP wants to extrude binds to
  - PaBn and molecules that come from it are too toxic to be used
- Other EPI molecules= phenothiazine derivatives
  - Improve activity of various antibiotic classes (ex. erythromycin, levofloxacin, and azithromycin)
  - Phenothiazine derivatives interfere with the proton gradient at the inner membrane of the bacteria
- Chlorpromazine: inhibits AcrB in salmonella enterica by controlling the expression of the acrB gene
- Quinolines have shown inhibition of efflux in some resistant bacteria
  - Have been shown to work with tetracycline, norfloxacin, and chloramphenicol in gram-negatives, K. pneumoniae, and E. aerogenes
  - Stop activation of AcrAB-ToIC (part of the RND family) efflux transporters
- Another group of EPIs= N-heterocyclic compounds, especially the arylpiperazine derivatives
  - Leading compound of N-heterocyclic compounds= 1-(1-Naphthylmethyl)-piperazine (NMP)
  - Have shown effectiveness against AcrAB and AcrEF EPs in E. coli– makes them susceptible to fluoroquinolones
  - NMP inhibits AcrB EP by stopping the protein's assembly
  - Disadvantage of using aryl piperazines: relatively low potency and similar to serotonin agonists
- Antibiotic's structure can also be modified to reduce affinity for EP
  - Ex. (tetracycline and macrolide class of derivatives: new compounds in glycylglycine and ketolide classes have lower affinities for EPs than parent compounds)
  - Ex. lower amounts of tigecycline extruded by gram negative and gram positive; telithromycin (which I'm assuming is a type of macrolide) shows increased activity in bacteria that usually extrude macrolides
- EPIs also shown to inhibit some M. tuberculosis EPs, in vitro and in vivo
- A study by Pieroni et al, investigated how compounds interfering with bacterial energy metabolism may be used to affect EPs
  - Analyzed how some Thioridazine (TZ) derivatives form, how they act as EPIs with known–anti-tuberculosis–drugs
  - Aim was to modify TZ to develop a compound that improves antibiotic effectiveness while decreasing side effects, especially associated with the central nervous system (CNS)
  - 13 small molecules rationally synthesized, then had their activities compared to that of TZ
    - Found that the 13 were less toxic to human macrophages, but still worked well with first line drugs for tuberculosis (= isoniazid, rifampicin)

- **Targeting B (B as in "beta", not "bee")-Lactamases**

- Bacteria can produce enzymes to deactivate drugs
- B-lactam antibiotics = important class of antibiotics, but many resistant bacterial strains have formed
  - Ex. hydrolysis of the first commercial penicillin by B-lactamases: they destroy the B-lactam ring  $\Rightarrow$  antibiotics with B-lactam ring lose effectiveness
    - B-lactam ring= most important element for antibiotics because it likes to accept electrons– thus it acylates (=adds acyl group) to the penicillin-binding proteins (PBPs)
    - PBPs synthesize peptidoglycan to provide structure to the bacterial cell wall
    - To preserve cell wall, B-lactamases hydrolyze B-lactams-based antibiotics, though extent of hydrolysis depends on type and amount of B-lactamases
    - B-lactamases released in periplasmic space in gram-negative, in extracellular space in gram-positive.
- Hundreds of B-lactamases discovered
  - Most have similar mechanisms, just different b/c have some differing amino acid sequences  $\Rightarrow$  work on different substrates
- B-lactamases classified with 2 methods:
  - 1) Amber classification
    - Based on structure
  - 2) Bush and Jacoby classification
    - Based on function
- Many B-lactams antibiotics used in therapy  $\rightarrow$  has led to a whole class of B-lactamases, called extended-spectrum B-lactamases (ESBL)
  - Ex. in Enterobacteriaceae like E. coli, K. pneumoniae, and P. mirabilis
- Carbapenemases= most adaptable family of B-lactamases– can hydrolyse most hydrolyzable B-lactams, and most are resistant to all commercial B-lactamase inhibitors
- Urgent need for new B-lactamase inhibitors, antibiotics
- 2 strategies to address B-lactamase-mediated resistance:
  - 1) develop antibiotics they don't work on
  - 2) develop B-lactamase inhibitors (BLIs) to use with an antibiotic
    - Complex: need to consider that inhibitors need to protect antibiotic against enzymatic hydrolysis, what dose of inhibitor is needed, and the feasibility of the pairing
- Discovery of clavulanic acid, a Streptomyces clavuligerus secondary metabolite; is a B-lactam able to inactivate most B-lactamases
  - Led to first B-lactam + B-lactamase-inhibitor combination =
    - Augmentin(amoxicillin/clavulanic acid)= great success
  - More inhibitors developed, ex. Sulbactam and tazobactam
    - Similar activity as clavulanic acid (clavulanic = original, able to inactivate most B-lactamases)
    - Sulbactam combined with ampicillin for global use, with cefoperazone for anaerobic bacteria
    - Tazobactam combined with piperacillin, cefoperazone, and ceftolozane for nosocomial infections ex. Those caused by MDR P.

- aeruginosa
- Usually can't be used alone, but just-clavulanic-acid has MIC of 1ug/ml against N. gonorrhoeae, Sulbactam has MIC of <8 ug/mL against wild-type Acinetobacter spp and 10 ug/mL against Burkholderia cepacian, but doesn't inhibit strains with multiple resistance mechanisms
- Class of non-B-lactam and B-lactamase inhibitors, based on diazabicyclooctanes (DBOs), discovered (after 20-year gap following the B-lactam discoveries)
  - Avibactam= first inhibitor of the DBO class– has bigger effective range than clavulanic acid, can be used in therapy with ceftazidime
  - Still working on finding other combos ex. Nacubactam or rebabactam, used with imipenem
    - Relebactam has similar range to avibactam and another DBO, RG6080 (RG6080 also naturally effective against some intestinal bacteria)
  - Another class of non-B-lactam B-lactamase inhibitors are made of boronic acids
    - Ex. RPX7009 used with meropenem to target pathogens that make carbapenemases
  - Gram-negative bacteria spread resistance to B-lactams faster
- **Targeting Outer Membrane:**
- Most antibiotics need to be able to penetrate the bacteria's membrane to reach target inside
  - Gram-negative have another, outer membrane for extra defense against foreign substances– is made of polyanionic lipopolysaccharides and porins
- Most resistant bacteria have developed protein mutations at the outer membrane level
- Antibiotics have two penetration strategies:
  - 1) Hydrophobic compounds– ex. Macrolides, rifampicin– cross through passive transport mechanisms
  - 2) Hydrophilic molecules– ex. B-lactams, fluoroquinolones, phenicol antibiotics– diffuse through active transport mechanisms
  - Can use substances like permeabilizer to increase permeability of membranes
    - Permeabilizers are usually positively charged, amphiphilic molecules or chelators; by interacting with the lipopolysaccharides (in the gram-negative membrane) or by capturing outer layer cations, destabilize membrane wall
    - Examples of permeabilizers: polymyxins, ex. Polymyxin B; colistin; aminoglycosides; cationic peptides; cationic colic acid derivatives; polyamines
    - Still want more small molecules to improve performance of antibiotics
      - Some chemosensitizers (ex. Detergents, surfactants, antimicrobial peptides) able to disrupt membrane protein (ex. porins, membrane channels) activities
  - One study showed that a high concentration of glycine basic peptide (GBP) can damage membrane of E.coli
    - GBP is a cationic peptide that disrupts the membrane barrier and the E. coli ion channel (= works as a permeabilizer)
      - Disruption results in loss of Ca<sup>2+</sup>, K<sup>+</sup>, and Mg<sup>2+</sup> and makes E. coli more

susceptible to erythromycin and rifampin, drugs which were initially unable to penetrate

- Another study on using menadione to affect membrane permeability of MDR strains S. aureus, P. aeruginosa, and E. coli
  - Menadione works with antibiotics in the aminoglycoside family
- Endogenous antimicrobial peptides (AMPs)= molecules secreted by host cells and organs (ex. Exocrine glands)= innate immunity
  - Destabilize outer cell membrane through forming amphipathic a-helix or short B-pleated-sheet– is fast and specific
  - Problem: high production costs, potential side effects, already-documented development of resistance in bacteria (through secretion of proteases)

### **Targeting Anti-Virulence Factors**

- Could instead target alternative cellular pathways
- Pathogens need to mutate metabolic systems because of the challenging conditions inside the host
- Targeting metabolic functions that they need to survive could improve effects of antibiotics
- Antimicrobial drugs kill bacteria or block bacterial growth; meanwhile, antivirulence drugs hit specific targets– called virulence factors– only expressed in bacteria during infection
  - Antivirulence drugs are only needed for creating bacterial infection
  - Antivirulence inhibitors make bacteria unable to infect the host, so immune system can get rid of bacteria
  - Antivirulence inhibitors don't target essential factors for the bacteria's survival, so mutations against them less likely
  - Possible target pathways in bacteria: sulfur assimilation pathway, quorum sensing, and biofilms

### **Targeting Cysteine Biosynthesis**

- Target amino acid synthesis
  - Reason: some pathogens (ex. Macrophages, gastric mucosa) live in harsh conditions where need to adapt, including adaptations with metabolic pathways
    - Interference ⇒ susceptibility to antibiotics
    - Molecules interfering with biosynthetic pathways may meet less resistance from the bacteria than normal antibiotics
  - Can target enzymes involved in cysteine biosynthesis
    - cysteine biosynthetic enzymes dispensable during growth but essential during infection
  - Studies on bacterial response to harsh environments show increased usage of many genes in cysteine region
  - Studied deletion mutations in the cysteine biosynthetic pathway of S. typhimurium, determined that inhibition of cysteine biosynthesis caused (unpaired oxidative stress response,) decrease in antibiotic resistance
  - Most bacteria use the reductive sulfate assimilation pathway (RSAP) to carry out cysteine biosynthesis
    - RSAP= multistep process
      - 1) Bacteria actively transport sulfate from environment through membrane, then reduce the sulfate into bisulfide– costs a lot of energy
      - 2) The bisulfide is incorporates into cysteine by the enzymatic enzyme in the cysteine synthase complex (CSC) family

- Members of CSC are serine acetyl transferase (SAT) and O-acetylserine sulfhydrylase (OASS)
- SAT produces O-acetyl-L-serine (OAS)= a cysteine precursor
  - OAS is unstable– easily converts to N-acetylserine (NAS)= turns on cysteine signaling
  - SAT catalyzes reaction where OAS and CoA are formed
  - C-terminus tail of SAT important for function and regulation
    - Is responsible for inhibition in presence of cysteine by binding to OASS for formation of CSC
  - Last step of cysteine biosynthesis is catalyzed by different sulfhydrylases– have some functional/structural differences
  - First version of OASS, OASS-A, isolated in *Salmonella typhimurium*; later, Two isoforms of the enzyme often found in other pathogens:
    - O-acetylserine sulfhydrylase (OASS-A, encoded by *cysK*), expressed in presence of oxygen
    - O-phosphoserine sulfhydrylase (OASS-B, encoded by *cysM*), expressed in absence of oxygen
- OASS thought to be involved in many functions, ex. Toxin activation in *E. coli* and antibiotic resistance in *S.typhimurium*
- Need to find a small molecule to inhibit both forms of OASS to investigate their role in bacteria's life cycle
- SAT competitively inhibits OASS-A
- Pieroni et.al designed and synthesized a series of 2-phenylcyclopropane carboxylic acid derivatives that could inhibit OASS A and B by competing with SAT
  - Used their previous studies; computational and spectroscopic approaches ex. saturation transfer difference (STD) and nuclear magnetic resonance (NMR)
  - Showed that it's possible to develop small molecules able to inhibit both OASS enzymes, that could use tools to address drug resistance
- Another study by Brunner et al:
  - CysM= an important enzyme in cysteine (amino acid for making protein, metabolic functions– [source](#)) biosynthesis during mycobacterium (a type of bacteria) dormancy
  - Screened 17,312 compounds to identify CysM inhibitors
  - Found two inhibitors that worked in nutrient-starved, dormant *M. tuberculosis*, that didn't hurt mammalian cells
- Monofluoralanine is a weak substrate analogue for OASS-A and B
- **Targeting Quorum Sensing**
- In many bacteria, quorum sensing (QS) controls population growth, bacteria constantly secrete signal molecules called autoinducers, the QS- controlled process is activated when concentration of autoinducers reaches a threshold
- QS influence some virulence→ want to find QS inhibitors
- QS identified in gram + and - bacteria, has also been shown to play a role in biofilm formation
- Many autoinducers similar, so allows bacteria to communicate between species⇒ helps them in coinfections
- Can be multiple QS systems in one species
- In a study, found that azithromycin reduces QS signals in *pseudomonas aeruginosa*→ may help to reduce virulence in infections
- Strategies for inhibiting quorum sensing start by mimicking chemical structure of quorum

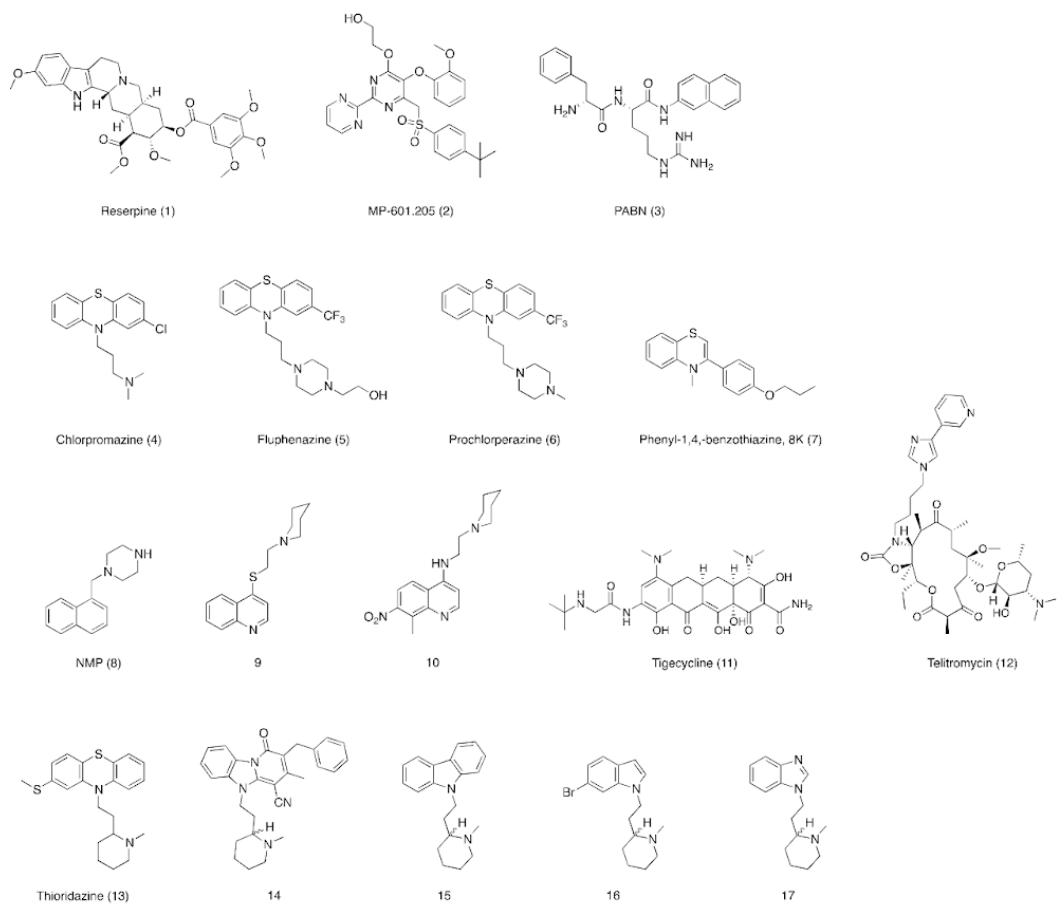
	<p>sensing signal molecules</p> <ul style="list-style-type: none"> <li>- In study, targeted pseudomonas quinolone signal (PQS) system of <i>P. Aeruginosa</i></li> <li>- [more details in article]</li> <li>- Shown that compound 31 reduces biofilm formation, that several compounds inhibited QS system and biofilm formation</li> </ul> <p>- <b>Targeting Biofilms</b></p> <ul style="list-style-type: none"> <li>- Bacterial grow as biofilms in almost 65% of infections; while infections are in growing phase, bacteria become 10-1000 times more resistant to antibiotics</li> <li>- Two groups of current approaches to targeting biofilms:           <ul style="list-style-type: none"> <li>- 1) physical-mechanical approach= disrupting and removing the biofilms</li> <li>- 2) using antibiotics or antimicrobials on the material to prevent biofilm formation</li> </ul> </li> <li>- Biofilm-targeting field growing: includes strategies to interfere with bacterial cell communication</li> <li>- [article lists other sources on targeting biofilms]</li> </ul>
Research Question/Problem/Need	What antibiotics do we currently have and how do they address different mechanisms of antimicrobial resistance?
Important Figures	<p><b>Antibiotic introduced</b></p> <p><b>Antibiotic resistance identified</b></p> <p>Some antibiotics on top + year introduced; antibiotic resistance on bottom + year identified</p>

**Table 1.** Antibiotic adjuvant classes and their inhibitors reported in this review.

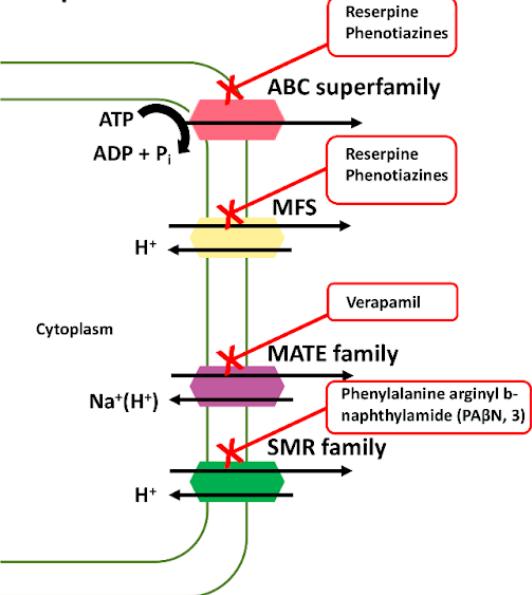
Antibiotic Adjuvant Class	Compound Name	Bacterium	
<i>Efflux Pump Inhibitors</i>	Phenotiazines, Phenylalanine-arginine-β-naphthylamide (PaβN), Arylpiperazine, Quinolines, Thioridazine (TZ) derivatives	Gram-positive Gram-negative	
<i>β-Lactamase inhibitors</i>	Clavulanic acid, Sulbactam, Tazobactam, Diazabicyclooctane (DBO) Boronic acids	Gram-positive Gram-negative	
<i>Membrane Permeabilizers</i>	Polimixyn B Colistin Aminoglycosides Polycationic/cationic antimicrobial peptides Glycine basic peptide (GBP) Caragenins Menadione	Gram-positive Gram-negative	
<i>Antivirulence Compounds</i>	Reductive Sulfur Assimilation pathway Quorum Sensing Biofilm	OASS-inhibitors, SAT-inhibitors, Cys-inhibitors PqsD transition state analogues physical-mechanical approach antibiotics or antimicrobials on a matrix peptide 1018	Gram-positive Gram-negative

Some efflux pump inhibitors: Phenotiazines, Phenylalanine-arginine-β-naphthylamide (PaβN), Arylpiperazine, Quinolines, Thioridazine (TZ) derivatives  
 B-lactamase inhibitors: Clavulanic acid, Sulbactam, Tazobactam, Diazabicyclooctane (DBO) Boronic acids  
 Membrane Permeabilizers: Polimixyn B Colistin, Aminoglycosides, Polycationic/cationic antimicrobial peptides, Glycine basic peptide (GBP), Caragenins, Menadione

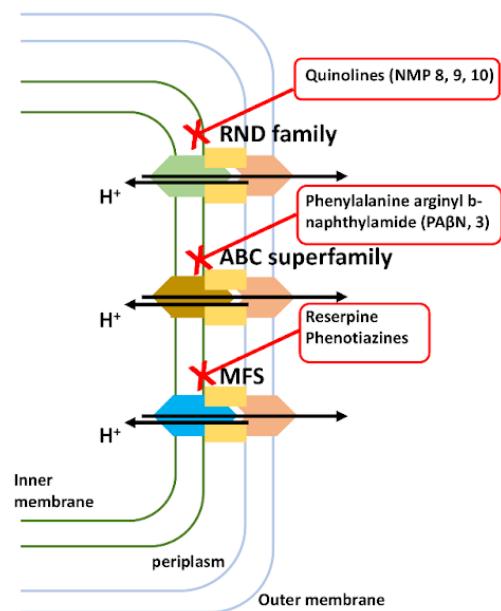
A)



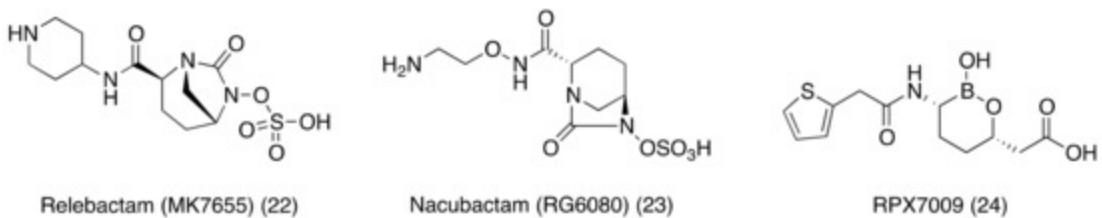
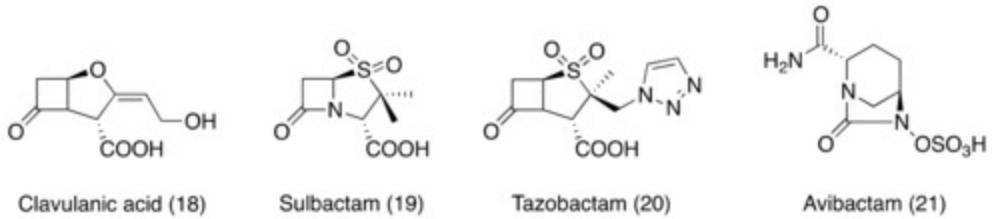
## B) Gram-positives



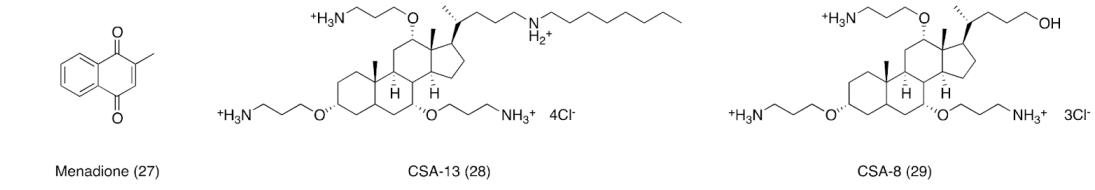
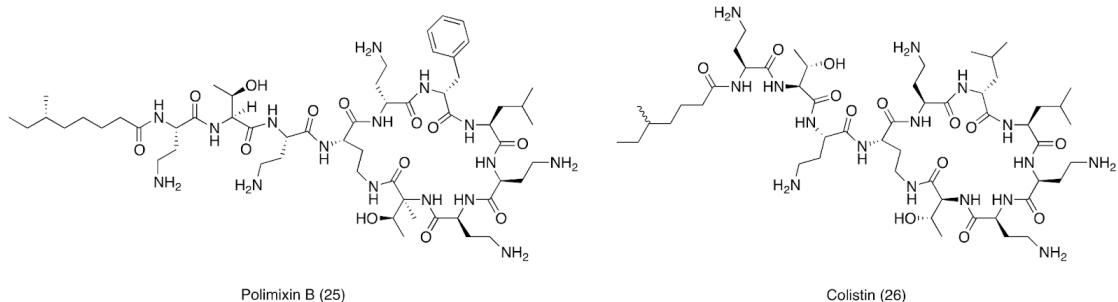
## Gram-negatives



A= Chemical structures of some EPIs. 1st row, 2nd column- MP-601, used for pneumonia/cystic fibrosis; 1st row, 3rd column= PaBN, too toxic to use for therapy; 2nd row= phenothiazine derivatives, B= the EPs in gram- + and gram- – bacteria, 1-(1-Naphthylmethyl)-piperazine= row 3, columns 1-3 and the EPIs of each type of pump, etc.

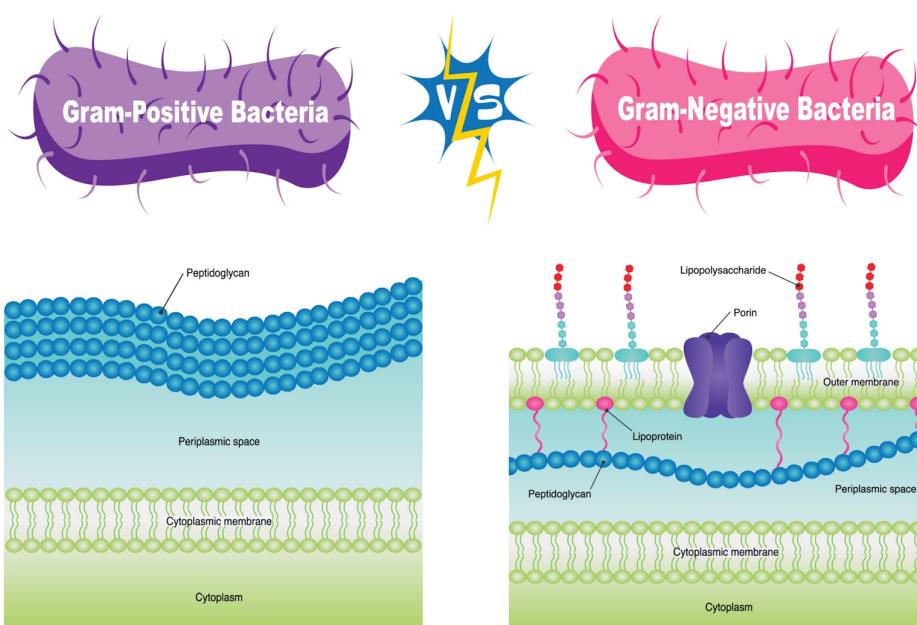


Chemical structures of some B-lactamase inhibitors



- Chemical structures of some membrane permeabilizers

VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Adjuvant: substance which enhances the body's immune response to an antigen</li> <li>- Transposons: transposable elements, DNA sequences that move from one location on the genome to another</li> <li>- Plasmids: small molecule of DNA on which genes are assembled and reorganized</li> <li>- Gram-negative bacteria: surrounded by thin peptidoglycan wall, surrounded by outer membrane containing lipopolysaccharide– gram stain stains pink/red</li> <li>- Gram-positive bacteria: lack outer membrane but have many, much thicker, layers of peptidoglycan than gram-negative– gram stain stains purple</li> </ul>
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visual of - vs +

from

<https://www.vecteezy.com/vector-art/8131530-gram-positive-vs-gram-negative-vector-illustration> (I compared it to other images, and it seems to have the same idea, even if it's from a stock photo site)

- Porins: beta barrel proteins that cross a cellular membrane and act as a pore through which molecules can diffuse
- Carbapenems: last resort antibiotics, inhibit cell wall synthesis
- Third generation cephalosporins: beta-lactam antimicrobials used to manage many gram-positive and gram-negative bacterial infections
- Beta-lactam: act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls, all B-lactam drugs have 3-carbon and 1-nitrogen ring = B-lactam ring that is highly reactive
- Minimum inhibitory concentration: the lowest concentration of a chemical, usually a drug, which prevents visible growth of a bacterium or bacteria. Is also a test to determine lowest concentration of antimicrobial needed to inhibit visible in-vitro growth
- Cross-resistance: tolerance (as of a bacterium) to a usually toxic substance (such as an antibiotic) that is acquired not as a result of direct exposure but by exposure to a related substance.
- Subinhibitory: not producing inhibition of bacterial growth
- Substrate analog: chemical compound with chemical structure similar to the substrate molecule in an enzyme-catalyzed reaction
- Fractional inhibitory concentration method: the measure to determine interaction between two or more drugs intended to be used in combination.
- On-target effects: Exaggerated, adverse effects at intended target
- Off-target effects: Drug binds to unintended target
- Dipeptide: two joined amino acids
- Amide: Organic compound that contains a functional group consisting of an acyl group ( $R-C=O$ ) linked to a nitrogen atom

- Fluoroquinolones: highly effective antibiotics– over 60 ones
- AcrB protein: Acriflavine Resistance Channel Protein B: protein that spans the inner membrane and extrudes into the space between membranes of e. Coli
- Serotonin agonist: bind to and activate receptors in the brain to inhibit release of serotonin to reduce pain, symptoms of migraine, used as antidepressants, etc.
- Macrophage: white blood cell– can be mobile white blood cell or one that stays in place in tissues
- Rational synthesis: Strategic, step-by-step production of a compound through chemical reactions
- First-line drugs: a drug that's the first choice for treating a condition
- Electrophilic: wants to accept electrons
- Acylate (verb): introduce an acyl group
- Acyl group: A group of atoms with a carbonyl group bonded to a carbon group
- Carbonyl group: a carbon atom double-bonded to an oxygen atom
- Hydrolyze: cleavage of chemical bonds by the addition of water
- Amber classification: way to classify B-lactamases based on structure
- Bush and Jacoby classification: way to classify B-lactamases based on function
- Extended-spectrum B-lactamases: synthesized due to many B-lactams used in therapy; are able to hydrolyze most B-lactam antibiotics
- Metabolite: a substance formed in or necessary for metabolism
- Contains two carbon-containing groups linked to a SO<sub>2</sub>
- Enteric: relating to or occurring in the intestines
- Polyanionic: substance that's negatively charged in multiple places– merriam webster
- Propensity: an inclination or natural tendency to behave in a particular way– oxford lang
- Amphiphilic/amphipathic: a molecule, especially a protein, that has both hydrophilic and hydrophobic parts
- Chelate: a water-soluble complex formed between a metal ion and a complexing agent– <https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/protein-biology/protein-purification/chelators#:~:text=A%20chelate%20is%20a%20water,ion%20can%20be%20readily%20exchanged>.
- Chemosensitizer: drug that makes tumor cells more sensitive to the effects of chemotherapy– <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/chemosensitizer>
- Surfactant: a substance that reduces the surface tension of the liquid it's dissolved in– oxford lang
- Virulence: the severity of harmfulness of a disease– oxford lang
- Virulence factors: targets of antivirulence drugs, are only expressed during infections, [see notes for more]-- article
- Oxidative stress: results from imbalance between levels of reactive oxygen species and antioxidants
- Reductive sulfate assimilation pathway: multiple steps to convert sulfate into cysteine
- Saturation transfer difference (STD) nuclear magnetic resonance (NMR) spectroscopy: a method for studying protein-ligand interactions in solution. Can find where they bind. Ligand protons closer to protein are more saturated=stronger STD NMR signals. [More info on process](#)
- Ligand: a molecule that binds to another, usually larger, molecule
- Quorum sensing: the regulation of gene expression in response to changes in cell population– quorum sensing bacteria release chemical signaling molecules called autoinducers that increase with population–

<https://pubmed.ncbi.nlm.nih.gov/11544353/#:~:text=Quorum%20sensing%20is%20the%20regulation,a%20function%20of%20cell%20density>

Sources: the article, oxford languages, google's related search dropdown menus, merriam webster,

### Abbreviations

- WHO: world health organization
- ESKAPE: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, and Enterobacter= bacteria causing highly critical infections– resistant to carbapenems and third generation cephalosporins (Mycobacterium tuberculosis also added to list)
- MIC: Minimum inhibitory concentration
- 5 efflux families:
  - 1) ABC: ATP-binding cassette (superfamily)
  - 2) RND: Resistance-nodulation-division ((family))
  - 3) SMR: Small multidrug resistance (family)
  - 4) MFS: Major facilitator superfamily
  - 5) MATE: Multi-drug and toxic compound extrusion (family)
- EPIs: efflux pump inhibitors
- FIC: fractional inhibitory concentration (method)
- AcrB protein: Acriflavine Resistance Channel Protein B
- CNS: central nervous system
- PBPs: penicillin-binding proteins
- ESBL: extended-spectrum B-lactamases
- BLIs: B-lactamase inhibitors
- GBP: glycine basic peptide
- AMP: antimicrobial peptides
- RSAP: reductive sulfate assimilation pathway
- SAT: serine acetyltransferase
- OASS: O-acetylserine sulfhydrylase
- CoA: coenzyme A, known for its role in the synthesis and oxidation of fatty acids, or oxidation of pyruvate in the citric acid cycle–  
<https://pubchem.ncbi.nlm.nih.gov/compound/CoASH#:~:text=Coenzyme%20A%20>
- QS: quorum sensing

Cited references to follow up on	<ol style="list-style-type: none"> <li>1. Singh, S.B. Confronting the challenges of discovery of novel antibacterial agents. <i>Bioorg. Med. Chem. Lett.</i> <b>2014</b>, 24, 3683–3689. [Google Scholar] [CrossRef] [PubMed]</li> <li>2. Morita, Y.; Tomida, J.; Kawamura, Y. Responses of Pseudomonas aeruginosa to antimicrobials. <i>Front. Microbiol.</i> <b>2014</b>, 4.</li> </ol>
Follow up Questions	<ul style="list-style-type: none"> <li>- More research on targeting peptidoglycan wall, current antibiotics that do it           <ul style="list-style-type: none"> <li>- Could improve an antibiotic that no longer works as well</li> </ul> </li> <li>- What is RSAP? (find a video)</li> </ul>

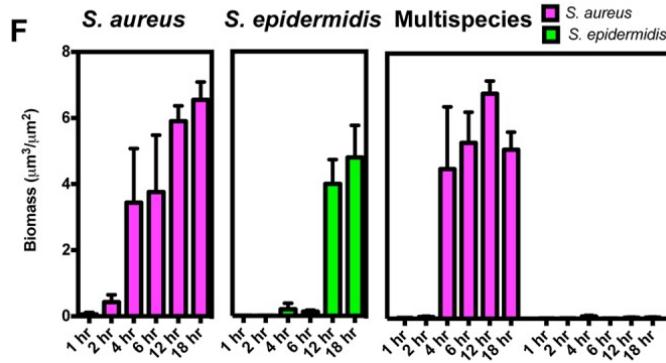
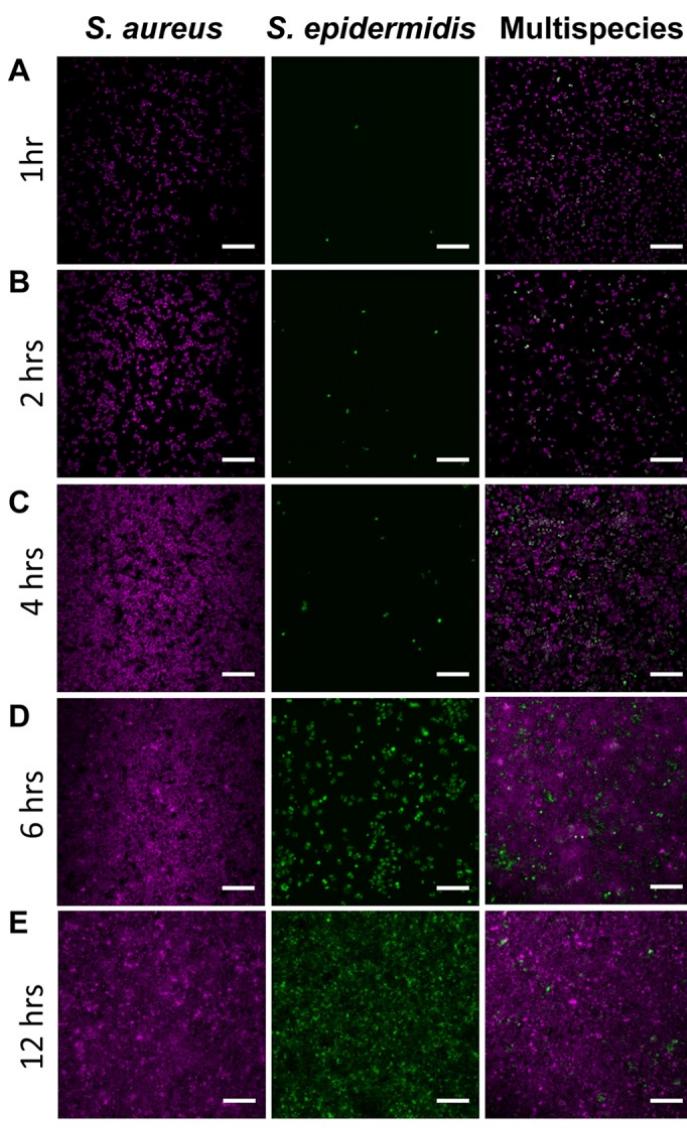
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|--|--|
|  | <ul style="list-style-type: none"> <li>- What role does quorum sensing have on formation of palastispheres?</li> <li>- How do transposons work?</li> </ul> |
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## Article #11 Notes: Effect of Antimicrobial and Physical Treatments on growth of Multispecies Staphylococcal Biofilms

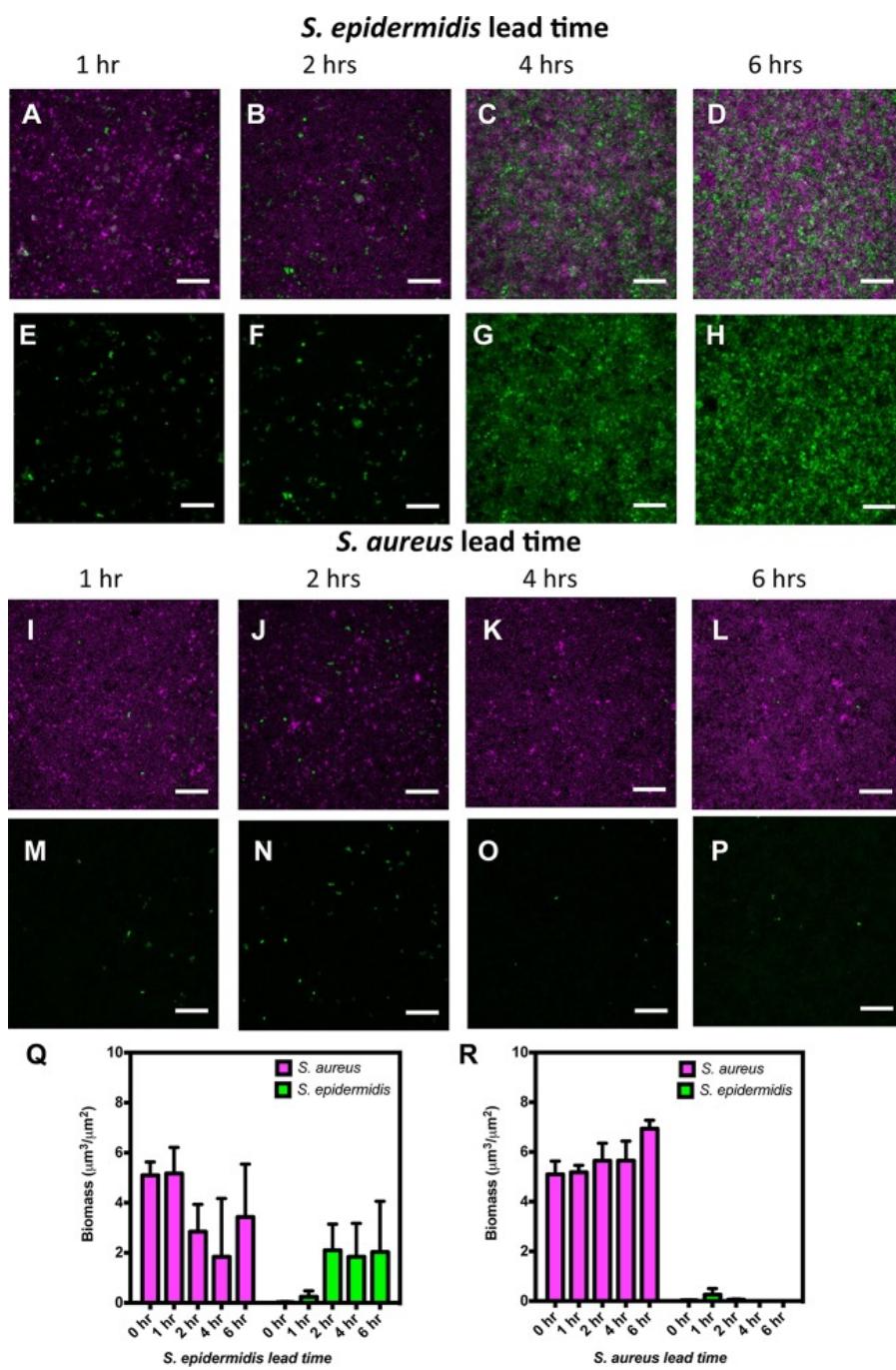
Source Title	Effect of Antimicrobial and Physical Treatments on growth of Multispecies Staphylococcal Biofilms
Source citation (APA Format)	Stewart, E. J., Payne, D. E., Ma, T. M., VanEpps, J. S., Boles, B. R., Younger, J. G., & Solomon, M. J. (2017). Effect of antimicrobial and physical treatments on growth of multispecies staphylococcal biofilms. <i>Applied and Environmental Microbiology</i> , 83(12). <a href="https://doi.org/10.1128/aem.03483-16">https://doi.org/10.1128/aem.03483-16</a>
Original URL	<a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5452825/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5452825/</a>
Source type	Journal article
Keywords	Staphylococcus aureus, Staphylococcus epidermidis, biofilm structure, multispecies biofilms
Summary of key points + notes (include methodology)	<p>Staphylococcus epidermidis (S. epidermidis) and Staphylococcus aureus (S. aureus) are two species of bacteria that often cause infections and form biofilms on medical materials. As they are often present in the nose and at sites of orthopedic implants (/prosthetics), this study aimed to learn more about their growth and behavior. It studied their performance in various potential treatment environments, including when one species was added earlier than the other, when temperature was increased, when pH was increased or decreased, and when different concentrations of vancomycin were added.</p> <p><b>Notes</b></p> <ul style="list-style-type: none"> <li>- Staphylococcus aureus and Staphylococcus epidermidis <ul style="list-style-type: none"> <li>- Nonmotile, gram-positive, Genus = Staphylococcus</li> </ul> </li> <li>- Infect orthopedic implants, commonly form biofilms on medical devices</li> <li>- Aureus more virulent than S. epidermidis, but both difficult to treat because their biofilms are resistant to antimicrobials and other treatment methods</li> <li>- Staph cause 79% (majority) of orthopedic implant-associated infections; infections <ul style="list-style-type: none"> <li>- Aureus = 34%</li> <li>- Epidermidis = 32%</li> </ul> </li> <li>- Bacteria can contact device at different times: initially due to non sterile technique, from the patient's skin, postimplantation through the bloodstream</li> <li>- <b>Infections</b> can be: <ul style="list-style-type: none"> <li>- Early: 29% of prosthetic joint infections; most caused by S. aureus</li> <li>- Delayed: 41% of prosthetic joint infections; most caused by less virulent bacteria ex. S.</li> </ul> </li> </ul>

- epidermidis
- Late: 30% of prosthetic joint infections; mainly caused by hematogenous seeding, often happens in patients with *S. aureus*
- Most prosthetic joint infections are only caused by one organism; 10-16% by multiple
- Both *aureus* and *epidermidis* in human nostrils
  - *Aureus* in noses of 50% of the population— its presence has been linked to *S. aureus* bacteremia
  - There are more *S. aureus* and *S. epidermidis* in the noses of inpatients (compared to healthy people)
- *S. aureus* abundance is negatively correlated with presence of *S. epidermidis*
- Another study indicated that a subset of *S. epidermidis* inhibits colonization of *S. aureus* with the serine protease Esp
- Bacteria more resistant to antimicrobials when part of a biofilm
- Want to research their behavior in multispecies biofilms and in different environments
- 
- Unstressed conditions = 37 degrees C, pH 7
  - *Aureus* grows better
- When addition of *S. aureus* is delayed 6-8 hours, *epidermidis* can grow better
- Vancomycin
- MICs:
  - *Aureus*: ~1.0 ug/mL
  - *Epidermidis*: ~2.0 ug/mL
- At dose of 1.0 ug/mL, more *epidermidis*
- Less of both species at 1.9 ug/mL
- Higher temperature of 45 degrees C results in *aureus* forming porous biofilms
- Importance: understanding structure of the bacterial community at infection sites, how treatments affect their behaviors
- Delayed addition:
- *Aureus* needs about 4 hours to colonize surface, *epidermidis* 6 to 12
- Growth environment = tryptic soy broth with glucose t 17 degrees C, pH7
- *Epidermidis* needs 2 (or a little over 2, it seems) to 6 hours to be the dominant species— more time = better
- pH
- Epidermis grows on skin = pH 4-7
- Epidermidis pH 5-6, more *epidermidis* than at 7
- Higher, similar to multispecies
- *S. epidermidis* 1457/pCM29 (AH2982) used as the *epidermidis* strain
- Used GFP pCM29 (40) with a sarA P1 promoter to transform it
- Grown in tryptic soy broth (TSB) or tryptic soy agar (TSA),
- Supplemented with 10ug/mL chloramphenicol because it's commonly used to grow GFP. *S. epidermidis* 1457
- *S. aureus* SH1000 (BB 386) is a commonly used model strain of *S. aureus*, was used as the *S. aureus* strain
- MIC determined with broth microdilution method, according to Clinical and Laboratory Standards Institute guidelines
  - $5 \times 10^5$  cells grown with increasing vancomycin concentrations on a plate; MIC.
  - Got cyto stock of MRSA from -80 degrees C

	-
Research Question/Problem/ Need	<ul style="list-style-type: none"><li>- How are the structures and growth of single-species biofilms of <i>S. sical</i> strains?</li><li>- Want to understand the effects treatment may have on an infection site in the presence of more than one species</li></ul>
Important Figures	



CLSM imaging and graphs biofilms of aureus (pink), epidermidis(green), and multispecies after a range of hours at neutral environmental conditions. Images taken with CLSM (confocal laser scanning microscopy). S. aureus dominates the multispecies biofilms.



VOCAB: (w/definition )	<p>Video on CLSM: <a href="https://www.youtube.com/watch?v=i37VoiOQrnc">https://www.youtube.com/watch?v=i37VoiOQrnc</a></p> <ul style="list-style-type: none"> <li>- Vancomycin: an antibiotic commonly used to treat staph infections– antibiotic that inhibits the formation of the bacterial cell wall and interferes with peptidoglycan synthesis in Gram-positive bacteria</li> <li>- Minimum inhibitory concentration: lowest concentration of antibiotic requires to inhibit visible bacterial growth after overnight culture</li> <li>- Biofilms: structured communities of bacteria surrounded by polysaccharides, proteins, and DNA</li> <li>- Early infection: when infection occurs less than 3 months after operation</li> </ul>
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	<ul style="list-style-type: none"> <li>- Delayed infection: when infection occurs between 3 months and 2 years after operation</li> <li>- Late infection: when infection occurs more than 2 years after operation</li> <li>- Hematogenous seeding: bacteria are introduced to the infection site via the bloodstream or reoccur due to inadequately treated early infections</li> <li>- Bacteremia: presence of bacteria in the blood <a href="https://www.merckmanuals.com/home/infections/bacteremia-sepsis-and-septic-shock/bacteremia#:~:text=Bacteremia%20is%20the%20presence%20of,common%20causes%20of%20death%20worldwide.">https://www.merckmanuals.com/home/infections/bacteremia-sepsis-and-septic-shock/bacteremia#:~:text=Bacteremia%20is%20the%20presence%20of,common%20causes%20of%20death%20worldwide.</a></li> <li>- Serine protease Esp: enzymes that break peptide bonds with hydrolysis (<a href="https://www.pnas.org/doi/10.1073/pnas.0601910103">https://www.pnas.org/doi/10.1073/pnas.0601910103</a>) Esp= the name of the serine protease</li> <li>-</li> </ul> <p>Abbreviations</p> <ul style="list-style-type: none"> <li>- MIC: minimum inhibitory concentration</li> </ul>
Cited references to follow up on	<p>(not from source but sources Dr. Stewart recommended:</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> Nature Reviews Disease primer on MRSA: <a href="https://www.nature.com/articles/nrdp201833">https://www.nature.com/articles/nrdp201833</a>.</li> <li><input type="checkbox"/> Here is some general information from the CDC: <a href="https://www.cdc.gov/mrsa/index.html">https://www.cdc.gov/mrsa/index.html</a> )</li> </ul> <p>From article:</p> <ul style="list-style-type: none"> <li><input type="checkbox"/> 25. Sturtevant RA, Sharma P, Pavlovsky L, Stewart EJ, Solomon MJ, Younger JG. 2015. Thermal augmentation of vancomycin against staphylococcal biofilms. Shock <b>44</b>:121–127. doi: 10.1097/SHK.0000000000000369. [<a href="#">PMC free article</a>] [<a href="#">PubMed</a>] [<a href="#">CrossRef</a>] [<a href="#">Google Scholar</a>]</li> <li><input type="checkbox"/> 32. Wang G, Hindler JF, Ward KW, Bruckner DA. 2006. Increased vancomycin MICs for <i>Staphylococcus aureus</i> clinical isolates from a university hospital during a 5-year period. J Clin Microbiol <b>44</b>:3883–3886. doi: 10.1128/JCM.01388-06. [<a href="#">PMC free article</a>] [<a href="#">PubMed</a>] [<a href="#">CrossRef</a>] [<a href="#">Google Scholar</a>]</li> </ul>
Follow up Questions	<ul style="list-style-type: none"> <li>- Why are bacteria more resistant to antibiotics as biofilms? Is this true for all/most bacteria or just staph?</li> <li>- Does infectiousness relate to resistance? (<i>S. aureus</i> is more infectious than <i>S. epidermidis</i>, but has a lower MIC— wonder why?)</li> <li>- Could we make <i>S. aureus</i> make a molecule/ spread plasmid that inhibits it and <i>S. epidermidis</i> <ul style="list-style-type: none"> <li>- Could act late</li> </ul> </li> <li>- How do non-motile bacteria spread?</li> <li>- Research MRSA— why resistant? Could we add something make it susceptible to more common antibiotics?</li> <li>- How well does it grow on plastics? Microplastics?</li> <li>- Could we give <i>S. aureus</i> a late-acting/needs-to-be-triggered gene/molecule that will produce something to inhibit both it and <i>epidermidis</i>?</li> <li>- Why is MRSA resistant to so many antibiotics?</li> <li>- One study showed a type of <i>S. epidermidis</i> excreted the serine protease Esp, which inhibited biofilm formation and nasal colonization of <i>E. aureus</i>— look into</li> </ul>

# Article #12 Notes: Methicillin-Resistant Staphylococcus Aureus (MRSA)

Source Title	Methicillin-Resistant Staphylococcus Aureus (MRSA)
Source citation (APA Format)	<p><i>Methicillin-Resistant Staphylococcus Aureus (MRSA)</i>. (n.d.). Baylor College of Medicine.</p> <p>Retrieved October 23, 2022, from</p> <p><a href="https://www.bcm.edu/departments/molecular-virology-and-microbiology/emerging-infections-and-biodefense/specific-agents/mrsa">https://www.bcm.edu/departments/molecular-virology-and-microbiology/emerging-infections-and-biodefense/specific-agents/mrsa</a></p>
Original URL	<a href="https://www.bcm.edu/departments/molecular-virology-and-microbiology/emerging-infections-and-biodefense/specific-agents/mrsa">https://www.bcm.edu/departments/molecular-virology-and-microbiology/emerging-infections-and-biodefense/specific-agents/mrsa</a>
Source type	Web page (not journal article)
Keywords	MRSA, HA-MRSA, CA-MRSA, Staph, Beta-lactam antibiotics, PBP2a, BLIP-II
Summary of key points + notes (include methodology)	<p>There are multiple types of MRSA, all of which have caused an increasing number of infections in recent years. They are an issue because they are resistant to many drugs commonly used to treat infections,</p> <ul style="list-style-type: none"> <li>-</li> <li>- MRSA= Methicillin-Resistant Staphylococcus Aureus</li> <li>- Staph found in 25-30% of healthy people</li> <li>- Infection can be, in rare cases, life-threatening</li> <li>- Some of most common causes of skin infection <ul style="list-style-type: none"> <li>- Minor like pimples to major like bloodstream infections</li> </ul> </li> <li>- HA-MRSA= healthcare-acquired MRSA— since 1960s <ul style="list-style-type: none"> <li>- Number of infections by it increasing</li> </ul> </li> <li>- CA-MRSA= community-acquired MRSA— occurs outside of hospitals— source difficult to identify, but often happens when people are close together for a long time ex. Soldiers, wrestling athletes, sharing of towels</li> <li>- Enter body through cut</li> <li>- First sign of infection looks like a spider bite = red, swollen, potentially pus</li> <li>- HA-MRSA and CA-MRSA different genetically</li> <li>- MRSA causing increasing number of diseases worldwide: 30 years ago, was 2% of Staph infections; 2003, was 64% of Staph infections <ul style="list-style-type: none"> <li>- 2005, over 94,000 people developed life-threatening infections because of it, nearly 19,000 died due to/partly due to it</li> </ul> </li> </ul>

- 85% MRSA cases associated with healthcare facilities, 14% not associated
- Staph continuing to evolve, shows resistance to additional antibiotics
- 2002: first vancomycin-resistant Staph found– still rare, but worried they'll become widespread and further limit what drugs can combat MRSA
- Drug resistance because Staph needs to reproduce– under stress, evolve to overcome the obstacle
- Wish to know more about the genetic changes in MRSA that let it infect otherwise healthy people
- A type of CA-MRSA = USA300– is one of the two that causes the majority of CA-MRSA cases, appears more virulent than other strains
  - Drs. Sarah Highlander and Joseph Petrosino and colleagues at the Baylor Human Genome Sequencing Center sequenced genome of USA300 and a CA, not-methicillin-resistant Staph strain to compare
  - Found that genomes very similar= that increased virulence of USA300 due to subtle genetic changes
  - Found that USA300 picked up a plasmid containing a gene providing resistance to bacitracin= antibiotic commonly found in over-the-counter skin ointments
- Beta-lactam antibiotics = most widely used drugs for treating bacterial infections– include penicillin, methicillin, amoxicillin, etc.
  - Beta-lactam ring part of the antibiotic target the penicillin-binding proteins (PBP) in the bacterial cell membrane
  - PBP needed for synthesis of cell wall
  - Binding of antibiotic to PBP prevents PBP from helping form cell wall⇒ cell dies
- Dr. Timothy Palzkill and research team studying resistance to beta-lactams
  - Gram+ bacteria, including MRSA, acquire resistance to beta-lactams through producing protein PBP2a– is able to avoid inhibitory effects of the antibiotic
  - Protein BLIP-II able to weakly bind to and inhibit PBP2a= make it susceptible to beta-lactams
  - Are continuing research by looking for mutations that increase the affinity of BLIP-II to PB2a
    - Could some other bacteria that's susceptible to drugs but that outcompetes Staph in certain environments be used?

Research Question/Problem / Need	This article gave an overview of MRSA.
Important Figures	N/A (just pictures of a staph infection, no graphs or anything)
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- MRSA= Methicillin-Resistant Staphylococcus Aureus</li> <li>- PBP= Penicillin-binding protein</li> <li>- Beta-lactam antibiotics = most widely used drugs for treating bacterial infections– include penicillin, methicillin, amoxicillin, etc.</li> <li>- HA-MRSA= healthcare-acquired MRSA– since 1960s</li> <li>- CA-MRSA= community-acquired MRSA– occurs outside of hospitals– source difficult to identify, but often happens when people are close together for a long time ex. Soldiers,</li> </ul>

	<p>wrestling athletes, sharing of towels</p> <ul style="list-style-type: none"><li>- USA300– A type of CA-MRSA– is one of the two that causes the majority of CA-MRSA cases, appears more virulent than other strains</li></ul>
Cited references to follow up on	<input type="checkbox"/> <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5641629/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5641629/</a>
Follow up Questions	<ul style="list-style-type: none"><li>- Are there any other substrates that can inhibit PB2a?</li><li>- Any other proteins that give resistance other than PB2a?</li><li>- How can genetic differences help us identify ARGs?</li><li>- How fast does Staph evolve?<ul style="list-style-type: none"><li>- How can we observe/measure it?</li></ul></li></ul>

# Article #13 Notes: Multidrug Efflux Pumps in *Staphylococcus aureus*: an Update

Source Title	Multidrug Efflux Pumps in <i>Staphylococcus aureus</i> : an Update
Source citation (APA Format)	Costa, S. S., Viveiros, M., Amaral, L., & Couto, I. (2013). Multidrug efflux pumps in <i>Staphylococcus aureus</i> : An update. <i>The Open Microbiology Journal</i> , 7(1), 59–71. <a href="https://doi.org/10.2174/1874285801307010059">https://doi.org/10.2174/1874285801307010059</a>
Original URL	<a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3617543/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3617543/</a>
Source type	Journal article
Keywords	Antibiotics, biocides, efflux pumps, multidrug resistance, <i>Staphylococcus aureus</i> .
Summary of key points + notes (include methodology)	<ul style="list-style-type: none"> <li>- Efflux pumps may provide low level resistance to antimicrobials: <ul style="list-style-type: none"> <li>- May be used as first line of defense— extrude drug so it doesn't reach lethal concentrations</li> <li>- Buys time for another resistance mechanism, thus allowing bacteria's survival</li> <li>- Can be chromosomal or plasmid-encoded</li> </ul> </li> <li>- Nowadays, each common antibiotic has at least one mechanism of resistance against it</li> <li>- Many bacteria resistant to many drugs= MDR bacteria= some life-threatening</li> <li>- <i>S. aureus</i>= gram-positive bacteria with many mechanisms of resistance</li> <li>- MRSA= methicillin-resistant, is of major concern</li> <li>- Resistance mechanisms: <ul style="list-style-type: none"> <li>- Degrade antibiotic</li> <li>- Modify antibiotic</li> <li>- Alter target of antibiotic/otherwise protect the target</li> <li>- Reduce intracellular concentration of the antibiotic <ul style="list-style-type: none"> <li>- By decreasing cell wall's permeability</li> <li>- By efflux of antibiotic from cell</li> </ul> </li> </ul> </li> <li>- Efflux pumps haven't had as much attention, but are able to extrude several types of antimicrobial compounds = promotes MDR</li> <li>- Efflux pumps eliminate harmful metabolites, secrete virulence determinants, involved in cell response to stressors— drugs could be “accidental substrates” of the pumps</li> <li>- Efflux pumps can be: <ul style="list-style-type: none"> <li>- Specific=extrude only one antibiotic/class of antibiotics</li> <li>- MDR efflux pumps= can extrude several classes</li> </ul> </li> <li>- MDR efflux systems classified in 5 families depending on energy requirements and structure: <ul style="list-style-type: none"> <li>- The major facilitator superfamily (MFS)</li> </ul> </li> </ul>

- The small multidrug resistance (SMR) family
- The multidrug and toxic compound extrusion (MATE) family
- The resistance-nodulation-cell division (RND) superfamily
- The adenosine-triphosphate (ATP)-binding cassette (ABC) superfamily.
- MFS, SMR, MATE, RND families = secondary transporters, use proton motive force to provide energy to extrude substrates through antiport H<sup>+</sup>
- MATE: can also use sodium membrane gradient as a source of energy
- ABC: primary transporters that use ATP to extrude substrates
- *S. aureus* has over 10 documented efflux pumps
  
- **EFFLUX PUMP NorA:**
  - One of most studied efflux systems in *S. aureus*
  - Coded for by the gene *norA* in chromosomes
  - Three *norA* alleles – up to 10% of nucleotides different
  
  - NorA is a protein that...
    - Has 388 amino acids
    - Has 12 transmembrane segments
    - belongs to the MFS (major facilitator superfamily)
    - 44% similar with multidrug efflux pump Bme from *Bacillus subtilis*
    - 24% similar with tetracycline efflux pump Tet(A) from *E. coli*
  
  - NorA can extrude:
    - various compounds, mostly hydrophilic fluoroquinolones ex. norfloxacin and ciprofloxacin,
    - Dyes ex. Ethidium bromide
    - Biocides ex. Quaternary ammonium compounds
  
  - *norA* is expressed under normal conditions
  - Increased expression of the *norA* gene leads to increased resistance to fluoroquinolones, biocides and dyes through NorA-mediated efflux
  - Increased expression can be because of mutations in the *norA* promoter region or induced by regulatory proteins
  - Mutations:
    - First ones found were punctual mutations 89 bp upstream of the initiation codon & downstream of the -10 motif, in the 5'-UTR region
    - The mutations may increase *norA* mRNA half-life through altering the mRNA's secondary structure, thus making it less susceptible to RNases
    - Other studies instead suggest mutations may increase the rate of *norA* transcription
    - Other studies report deletions and insertions present in region, which may increase *norA* mRNA's stability
    - NO correlation found between antimicrobial resistance and *norA* coding region mutations
  
  - Not clear how production of NorA is regulated, though may be due to changes in the *norA* promoter region or some regulatory systems
  - NorA uses proton motive force for energy to transport antimicrobials across cell membrane, using an H<sup>+</sup> antiport mechanism

- NorA extrudes ethidium bromide and norfloxacin; the efflux can be disrupted by protonophores ex. Carbonyl m-chlorophenylhydrazone (CCCP)
- Nigericin also reduces the membrane proton gradient– observed in (everted) vesicles with a NorA pump in *E. coli*
  - Results in increasing accumulation of norfloxacin (what NorA usually extrudes)
- Valinomycin collapses the electric gradient but doesn't affect accumulation of norfloxacin, as NorA efflux depends on proton gradient (not electric gradient)
- **NorB**
- NorB pump also in *S. aureus*
- NorB is structurally similar to efflux pumps...
  - In *B. subtilis*: Blt– 41% similar and Bmr– 30% similar
  - In *S. aureus*: NorA– 30% similar and QacA– 39% similar
- Part of the MFS (major facilitator superfamily)
- Is proton driven
- Is made up of 463 amino acids, with 12 transmembrane segments
- Cases resistance to:
  - Some NorA substrates including
    - Hydrophilic fluoroquinolones (norfloxacin and ciprofloxacin)
    - Biocides (tetraphenylphosphonium and cetyltrimonium)
    - The dye ethidium bromide
  - Some non-NorA substrates including
    - Hydrophobic fluoroquinolones moxifloxacin and sparfloxacin
    - Tetracycline
  - NorB shown to be important for *S. aureus* fitness
    - Was overexpressed in environments that were more acidic or had less air = may be *aureus*'s response to survive in those conditions
      - Overexpression also increased resistance
  - Regulating NorB involves many pathways
- **NorC**
- 462 amino acids, 12 transmembrane segments, belongs to the MFS
- 61% similar to NorB
- Must have overexpression of norC to affect susceptibility
- When have overexpression, gives low resistance to ...
  - hydrophilic and hydrophobic fluoroquinolones ex. Ciprofloxacin, moxifloxacin, garenoxacin
  - Dye rhodamine
- **MepA**
- MepA coded with gene mepA
- In MATE family, in *S. aureus*
- 452 amino acids, 12 transmembrane segments
- 26% similar to CdeA from *Clostridium difficile* (in MATE family)
- 21% similar to NorM from *Vibrio parahaemolyticus*
- Found to give low resistance to...
- Quaternary ammonium compounds ex. benzalkonium chloride, cetrimide, dequalinium, tetraphenylphosphonium, pentamidine
- The dye ethidium bromide, to chlorhexidine, pentamidine, as well as to

	<ul style="list-style-type: none"> <li>- Tigecycline, an antibiotic from the class glycylcyclines.</li> <li>- Fluoroquinolones ciprofloxacin and norfloxacin weak substrate os MepA</li> <li>- mepA gene is in the mepRAB operon</li> <li>- <b>MdeA</b></li> <li>- 479 amino acids, 14 transmembrane segments, belongs to the MFS</li> <li>- Uses proton motive force to provide energy for transport of substrates</li> <li>- 37% similar to LmrB from <i>B. subtilis</i></li> <li>- 24% similar to EmrB from <i>E. coli</i></li> <li>- 23% similar to QacA from <i>S. aureus</i></li> <li>- Overexpressed mdeA associated with increased resistance to biocides benzalkonium chloride, dequalinium and tetraphenylphosphonium, to the dye ethidium bromide, and to the antibiotics virginiamycin, novobiocin, mupirocin and fusidic acid</li> <li>- Fluoroquinolones norfloxacin and ciprofloxacin are weak substrates of MdeA</li> <li>-</li> </ul>
Research Question/Problem/ Need	What do we know about MDR efflux pumps in <i>S. aureus</i> ?
Important Figures	
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- <i>S. aureus</i>= gram-positive bacteria with many mechanisms of resistance</li> <li>- Endogenous= growing or originating from within an organism, having an internal cause (oxford lang)</li> <li>- Metabolite: any substance produced during metabolism</li> <li>- Virulence determinants: genes and proteins that play key roles in disease development (<a href="https://www.sciencedirect.com/topics/immunology-and-microbiology/virulence">https://www.sciencedirect.com/topics/immunology-and-microbiology/virulence</a>)</li> <li>- Proton motive force: energy generated by transfer of protons or electrons across an energy converting membrane</li> <li>- Antiport: mechanism for the coupled transport of two different molecules or ions through a membrane in opposite directions (dictionary.com)</li> <li>- Basal level gene expression: the degree to which the cell or tissue produces a certain mRNA or protein under normal circumstances; the default expression of the protein/mRNA (<a href="https://homework.study.com/explanation/a-what-is-basal-level-gene-expression-b-why-does-it-happen.html#:~:text=A%20basal%20level%20gene%20expression,or%20protein%20under%20normal%20circumstances.">https://homework.study.com/explanation/a-what-is-basal-level-gene-expression-b-why-does-it-happen.html#:~:text=A%20basal%20level%20gene%20expression,or%20protein%20under%20normal%20circumstances.</a>)</li> <li>- Upstream DNA: the region that lies towards the 5'end of the DNA coding strand; genetic code in the upstream region is marked with negative numbers, contains the regions to promoter and regulate transcription (<a href="https://byjus.com/biology/difference-between-upstream-and-downstream-dna/#:~:text=Upstream%20DNA,is%20marked%20with%20negative%20numbering.">https://byjus.com/biology/difference-between-upstream-and-downstream-dna/#:~:text=Upstream%20DNA,is%20marked%20with%20negative%20numbering.</a>)</li> <li>- Downstream DNA: region of the coding strand towards the three prime end, is</li> </ul>

	<p>marked with positive numbering. Has the protein-coding region that undergoes transcription and the termination site to end transcription</p> <ul style="list-style-type: none"> <li>- (<a href="https://byjus.com/biology/difference-between-upstream-and-downstream-dna/#:~:text=Upstream%20DNA,is%20marked%20with%20negative%20numbering.">https://byjus.com/biology/difference-between-upstream-and-downstream-dna/#:~:text=Upstream%20DNA,is%20marked%20with%20negative%20numbering.</a>)</li> <li>- 3' and 5' ends of DNA: DNA copied from 5' to 3'; 5' end has a free hydroxyl or phosphate on a 5' carbon and the 3' end has a free hydroxyl or phosphate on a 3' carbon—carbon atoms in the sugar ring are numbered from 1' to 5'. <a href="https://medicine.arizona.edu/sites/default/files/common_terms_in_molecular_biology.pdf">https://medicine.arizona.edu/sites/default/files/common_terms_in_molecular_biology.pdf</a></li> <li>- Half-life: the time required for a property to decrease by half</li> <li>- Protonophores: compounds capable of electrogenic transport of protons across membranes</li> <li>- Transmembrane segments: peptides that span the cell membrane. Are hydrophobic.</li> <li>- Operons: clusters of genes that are controlled as a unit. (science direct)</li> </ul> <p>Abbreviations:</p> <ul style="list-style-type: none"> <li>- MDR: multidrug resistant</li> <li>- (MFS): The major facilitator superfamily</li> <li>- (SMR): The small multidrug resistance family</li> <li>- (MATE): The multidrug and toxic compound extrusion family</li> <li>- (RND): The resistance-nodulation-cell division superfamily</li> <li>- (ABC): The adenosine-triphosphate (ATP)-binding cassette superfamily.</li> <li>- <i>norA</i>: (I will write it without the italics) refers to the gene</li> <li>- NorA: refers to the efflux pump= the protein</li> <li>- CCCP: Carbonyl m-chlorophenylhydrazone, a protonophore</li> </ul>
Cited references to follow up on	
Follow up Questions	<ul style="list-style-type: none"> <li>- Can we make substrates to bind to/inhibit these pumps?</li> <li>- Can we mutate the bacteria and make one without NorA that outcompetes another?</li> <li>- Other bacteria with these pumps?</li> </ul>

# Article #14 Notes: Methylsulfonylmethane (msm) for treatment of drug resistant microorganisms

Source Title	Methylsulfonylmethane (msm) for treatment of drug resistant microorganisms
Source citation (APA Format)	<p>Benjamin, R., Varelman, J., &amp; Keller, A. (2018). <i>Methylsulfonylmethane (msm) for treatment of drug resistant microorganisms</i> (Canada Patent No. CA2778142C).</p> <p><a href="https://patents.google.com/patent/CA2778142C/en?q=PBP2a+inhibitor&amp;oq=PBP2a+inhibitor">https://patents.google.com/patent/CA2778142C/en?q=PBP2a+inhibitor&amp;oq=PBP2a+inhibitor</a></p>
Original URL	<a href="https://patents.google.com/patent/CA2778142C/en?q=PBP2a+inhibitor&amp;oq=PBP2a+inhibitor">https://patents.google.com/patent/CA2778142C/en?q=PBP2a+inhibitor&amp;oq=PBP2a+inhibitor</a>
Source type	patent
Keywords	METHYLSULFONYLMETHANE (MSM)
Summary of key points + notes (include methodology)	<p><b>Summary:</b></p> <p>This patent describes methods using Methylsulfonylmethane (MSM) to affect the resistance of MRSA. They used compositions comprising various concentrations of MSM with an antibiotic— either methicillin or oxicillin. They found that, in certain bacteria, MSM had no effect on resistance; in others, MSM increased the formerly resistant bacteria's susceptibility to the antibiotic. In some cases, MSM allowed the antibiotic to inhibit the bacteria. The authors of the patent indicate that its methods and findings could be extended to more bacteria, as well as other organisms and viruses, and other antibiotics. They used several methods of administering the MSM, for example through topical administration and inhalation. To test its effect on living animals, the study used rabbits.</p> <ul style="list-style-type: none"> <li>- Used methylsulfonylmethane (MSM)- containing compositions to inhibit/sensitize antimicrobial resistant bacteria to drugs</li> <li>- The antimicrobial resistant bacteria is Staph aureus in claims 1 and 2 and MRSA in claim 3</li> <li>- [repeats that it used MSM to sensitize/inhibit the resistant bacteria for</li> </ul>

- several paragraphs– probably used different materials/methods]
- “Methylsulfonylmethane (MSM; (CH<sub>3</sub>)<sub>2</sub>SO<sub>2</sub>), also known as dimethyl sulfone”
    - “is an organosulfur compound that is a metabolite of DMSO and certain sulfur-containing amino acids.”
  - Is a dietary supplement
  - MRSA especially problematic in hospitals where patients have open wounds, invasive devices, and weakened immune systems= are more susceptible to infection
  - Was an unexpected discovery
  - Discovered that MSM sensitizes pathogens, including MRSA, to drugs they are usually resistant to
  - Can inhibit or sensitize resistant drug and prevent drug sensitive bacteria from developing resistance
    - 1) select a resistant pathogen
    - 2) introduce effective amount of a composition containing MSM
  - Method has been used with Staph aureus and beta lactams
  - Sometime bacteria was in a subject; other times, was on a surface
  - Effective amount of MSM varies: about 5-20%, 5-16%, 5-10%, 5-8%, 9-16%, 10-15%, etc; percentages represent weight percent of the MSM in the composition
  - Methods of sensitizing/inhibiting pathogens expected to be useful for situations when we want to sensitize/inhibit resistant drugs or prevent sensitive pathogen from developing resistance
  - ATCC strain 43300 Staphylococcus aureus is a MRSA strain resistant to oxacillin and methicillin
  - MSM sensitizes MRSA to oxacillin
    - ATCC strain 43300 Staph aureus tested in presence of MSM, DMSO, and oxacillin
    - Strain was incubated with 5-16% MSM, 61.1 g/m1 oxacillin (“MA”), or 1% DMSO and 61.1 g/mL oxacillin (“DA”) for 48 hours at 25 degrees C
    - 61.1 g/mL oxacillin is the MIC
    - All conditions showed decrease in bacterial colony’s density after 24 hours
    - Most decrease in bacteria with a specific concentration of MSM plus the antibiotic– without DMSO
  - Another experiment showing MSM sensitizes MRSA to oxacillin:
    - Incubated ATCC strain 43300 of staph aureus with 5-16% MSM and 61.1g/ml oxacillin for 24 hours at 25 C, then added another 61.1 g/ml oxacillin
    - Total oxacillin used= 121.1g/ml
    - Total time = 48 hrs
    - Simulates repeated use of antibiotic
    - Repeated experiment with different growth times of 24 hrs, 48 hrs, and 5 days

- Had positive controls showing bacterial growth without MSM
- Also tested with methicillin— had even lower concentrations of bacteria; less with more concentrated MSM
- Different concentrations of Oxacillin:
  - Different concentrations of Oxacillin were tested:
    - Concentrations were 2x, 5x, and 10x MIC
  - Surprisingly, lower concentrations of MSM decreased bacteria concentration better (figure 4)
  - Results still show MSM sensitizes MRSA to oxacillin
- Did similar experiment with similar results as above with Methicillin (though concentrations for bacteria much closer in value) (figure 5)
- Provided MSM “by any effective route” ex. Spraying, wiping, topical administration, inhaling
- MSM can be used to modify many bacteria, but not bacteria that can cause tuberculosis
- DMSO is a nutritional supplement and pharmaceutical agent
- A bacteria can be drug-resistant to one drug and drug-sensitive to another—are not exclusive
- Used the EtestO system to determine antibiotic sensitivity
- Staph aureus capable of aerobic and anaerobic respiration
  - Most strains ferment mannitol anaerobically
  - Form grape-like, golden or white clusters
  - Can be grown on blood agar
  - About 1 p.m in diameter
  - Produce catalase, coagulase, extracellular cell clumping factors, and some strains produce capsules
- Therapeutically effective amount of an agent can be measured in moles per liter or weight per volume
- Methods inpatient could also be used to treat viruses
- Staph aureus can colonize anterior nares (the nostrils; most common), the respiratory tract, opened wounds, intravenous catheters, and urinary tract
- MRSA progresses substantially within 1-2 days of initial symptoms; after 3 days, can take hold in tissues and become resistant to treatment
- There are many strains of MRSA, ex. hospital (healthcare, the original MRSA) associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA)
- About 25% of CA-MRSA has enhanced virulence= spreads rapidly, causes more severe illness than HA-MRSA
  - can affect vital organs and lead to widespread infection (sepsis), toxic shock syndrome and necrotizing ("flesh-eating") pneumonia
  - thought to be due to toxins carried by CA-MRSA strains
- Non-limiting methods of detecting Staph aureus:
  - tube coagulase test
  - slide coagulase test
  - latex agglutination test
  - DNase and heat-stable nuclease tests
  - commercial biochemical tests such as...

- The VITEK 2 system (bioMerieux)
- Staphychrom II (International Microbio, Signes, France)
- the Phoenix system (Becton Dickinson Microbiology Systems, Sparks, MD)
- molecular tests including...
- PCR- and DNA probe-based tests
- see Brown et al., J.
- Most Staph aureus have MIC for oxacillin of 21.1g/m1 or less
- Must make sure subject has MRSA:
  - can either observe subject for signs ex. Beta lactam antibiotics ineffective in treatment, symptoms in human ("initial presentation of MRSA is small red bumps that resemble pimples, spider bites, or boils that may be accompanied by fever and occasionally rashes. Within a few days the bumps become larger, more painful, and eventually open into deep, pus-filled boils.")
  - Or get a sample and detect MRSA in sample (ex. Swab nose, isolate bacteria)
- Compositions that contain MSM are water-based, contain about 0-5% NaCl by weight
- If administered through inhalation, particle size for inhalation should be about 1 p.m or less so it can reach the alveolar region of the lung for absorption
- DMSO, MSM, and Beta-lactam antibiotic/other inhibitor are soluble in water= good for topical administration, as water doesn't cause adverse effects
- Can achieve controlled release of composition, ex. With use of polymers or liposomes
- DMSO and./or MSM improve performance of antibiotics by:
  - Allowing them to reach deeper levels of the infected tissue
  - Lengthen exposure time to the infected tissue
  - Decrease the time needed for the antibiotic to take effect
- Using MSM can reduce/eliminate the odor usually produced by DMSO
  - Using high concentrations of DMSO is often avoided by practitioners because of its smell
- Using MSM and/or DMSO can also treat fungal infections
- Compositions can have DMSO, MSM, and rifampicin, isoniazid, pyrazinamide, or ethambutol
  - Dosages in g per day of rifampicin, isoniazid, pyrazinamide, or ethambutol varied
- If therapeutic agent doesn't dissolve readily in DMSO, can use homogenization, sonication, high shear fluid processing, or other mechanical methods to combine it with DMSO and/or MSM
- EXAMPLE EXPERIMENT showing MSM does not affect MRSA survival (did in other experiments):
  - Materials (and source details):
    - Flake OptiMSMO MSM (lot number 0604751)
    - ATCC strain 43300 Staphylococcus aureus
    - 30 ml Borosilicate glass cultures tubes

	<ul style="list-style-type: none"> <li>- Lactose Broth (LB; Alpha Biosciences; Lot: L07-03)</li> <li>- Modified Lethen Broth (MLB, Alpha Biosciences, lot 108-09)</li> <li>- Tryptic Soy Agar with Lecithin and Tween 80 (TSA; Alpha Biosciences, lot: F08-42)</li> <li>- oxacillin sodium USP grade lot J</li> <li>- methicillin sodium (AS; Cat No. 1410002, lot KOH338)</li> <li>- Mettler Toledo AG245 SN: 1115210833</li> <li>- USP&lt;51&gt; Antimicrobial Effectiveness protocol used as template model</li> <li>- Tested several concentrations of MSM</li> <li>- All concentrations of MSM plated with serial dilution(I think... article said "i07 dilutions", when I googled "serial dilution" came up) to determine concentration of bacteria</li> <li>- Weighed the OptiMSMO MSM (using a Mettler Toledo AG245 SN: 1115210833, which is probably some sort of scale)</li> <li>- MSM then placed in 30 mL borosilicate glass tubes in different concentrations</li> <li>- All tubes given dilutions of MRSA (Log = 5.23)</li> <li>- Tubes incubated at 25 C and mixed periodically</li> <li>- Contents plated at 24 and 48 hours <ul style="list-style-type: none"> <li>- Plated by diluting 1 mL of growth material into 9 mL of MLB diluent broth</li> <li>- Mixture then serial diluted down to 10-7</li> <li>- 1 mL placed in petri dish at each dilution point</li> <li>- 20 mL TSA added to each dilution, swirled, allowed to solidify</li> <li>- All dilution placed in 35C incubator for 24 hours</li> <li>- Colonies on each plate counted, results changed to log format</li> <li>- Determined positive and negative control</li> </ul> </li> <li>- Results: <ul style="list-style-type: none"> <li>- None of concentrations of MSM affected survival of ATCC strain 43300 Staphylococcus aureus at 24 or 48 hours</li> </ul> </li> <li>- [see patent for other experiments] <ul style="list-style-type: none"> <li>- A greater average log reduction in number of cfu/ml will indicate increased MRSA sensitivity</li> <li>- Mueller-Hinton broth with 6 tg/ml-oxacillin used to recover any MRS cells still alive, even if stressed</li> </ul> </li> </ul>
Research Question/Problem/ Need	The methods in the patent determined how different concentrations of MSM and DMSO affected resistance in MRSA.

## Important Figures

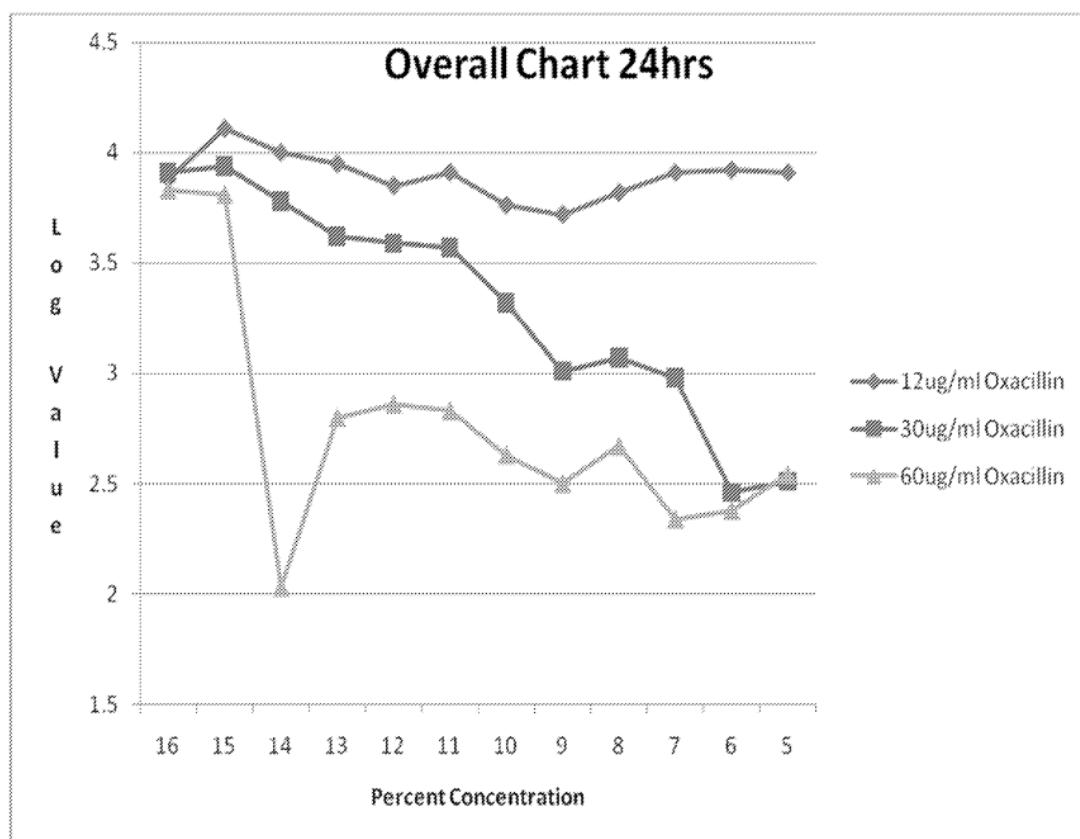


Chart for one experiment in which higher doses of MSM resulted in lower changes to the resistant bacteria's sensitivity, shown by the lower log reduction values. The rest of the charts show a similar trend.

## VOCAB: (w/definition)

- Metabolite: substance made or used when the body breaks down food, drugs, chemicals, or its own tissue.
  - Process= metabolism, which makes energy/materials body needs
  - <https://www.cancer.gov/publications/dictionaries/cancer-terms/def/metabolite>
- Infectious diseases: diseases caused by pathogenic microbial agents ex. Viruses, bacteria, fungi, etc.
- Organosulfur: organic molecules that contain sulfur
- Medicament: substance used for medical treatment–oxford lang
- Inoculation: vaccination
- Comprises: includes
- Agent: Any substance or any combination of substances that is useful for achieving an end or result; for example, a substance or combination of substances useful for inhibiting bacterial growth or survival.
- Beta-lactam antibiotics: A class of antibiotic agents containing a Beta-lactam nucleus in its molecular structure.
- Biological activity: An expression describing the beneficial or adverse effects of a substance on living matter. When the agent is a complex chemical mixture, this activity is exerted by the substance's active ingredient or

- pharmacophore, but can be modified by the other constituents.
- Composition (or formulation): A chemical compound or mixture of compounds capable of inducing a desired effect when properly administered.
- Industrial composition: a chemical compound or composition capable of inducing a desired effect when properly administered to a surface.
- Pharmaceutical composition: a chemical compound or composition capable of inducing a desired effect when properly administered to a subject/cell
- Aprotic: does not contain (or cannot donate) a hydrogen ion (proton).
- Ameliorate: make (something bad or unsatisfactory) better.
- Microorganism=microbial cells=microbes
- Virus: has a core of nucleic acid surrounded by a protein coat; has the ability to replicate only inside a living cell
- Yeast: A eukaryotic microorganism classified in the Kingdom Fungi, with about 1,500 species described. Most reproduce asexually by budding, although a few reproduce by binary fission. Yeasts generally are unicellular, although some species may become multicellular through the formation of a string of connected budding cells known as pseudohyphae, or false hyphae.

#### Abbreviations

- MSM: Methylsulfonylmethane = dimethyl sulfone
- CFU: colony forming units
- DMEM: Dulbecco's modified eagle medium
- DMSO: dimethyl sulfoxide/methylsulfonylmethane/methyl sulfoxide–formula: (CH<sub>3</sub>)<sub>2</sub>SO–
  - It is a colorless liquid, polar, does not have a hydrogen.
  - It dissolves polar and nonpolar compounds.
  - It penetrates skin readily.
  - highly polar solvent and an excellent ligand, with water-like dissolving properties
- DNA: deoxyribonucleic acid
- ELISA: enzyme-linked immunosorbent assay
- IC<sub>50</sub>: inhibitory concentration 50
- LAB: lactic acid bacteria
- MDSA: multidrug resistant *Staphylococcus aureus*
- MDR: multidrug resistant
- MIC: minimum inhibitory concentration
- MRSA: methicillin resistant *Staphylococcus aureus*
  - also called:
  - Multidrug resistant *Staphylococcus aureus* (MDSA)
  - Oxacillin-resistant *Staphylococcus aureus* (ORSA) ("Golden Staph")
- MSM: methylsulfonylmethane
  - Formula: (CH<sub>3</sub>)<sub>2</sub>SO<sub>2</sub>.
  - Chemical structure: 13 -S-C(=O)-CH<sub>2</sub>-CH<sub>3</sub>
  - Also known as DMSO<sub>2</sub>, dimethyl sulfone, methyl sulfone and sulfonylbismethane
  - Is an organosulfur compound

	<ul style="list-style-type: none"> <li>- Less polar and less reactive than DMSO</li> <li>- Is a metabolite of DMSO</li> <li>- OSRA: oxacillin-resistant <i>Staphylococcus aureus</i></li> <li>- PAGE: polyacrylamide-gel electrophoresis</li> <li>- PBP penicillin binding protein</li> <li>- PBS: phosphate buffered saline</li> <li>- PDA: potato dextrose agar</li> <li>- SDS: sodium dodecyl sulfate</li> <li>- TNTC: too numerous to count</li> <li>- TSB: tryptic soy broth</li> <li>- Serial dilution: a series of sequential dilutions that are performed to convert a dense solution into a more usable concentration. - <a href="https://microbenotes.com/serial-dilution/">https://microbenotes.com/serial-dilution/</a> <ul style="list-style-type: none"> <li>- Used to...</li> <li>- Estimate concentration– of subject in unknown sample by finding number of colonies in serial dilutions</li> <li>- Commonly performed to make dilutions with very small volumes of subject(1-10 ul)</li> <li>- Dilutes sample in controlled way = can easily count number of colonies and calculate number of microbes present</li> </ul> </li> </ul>
Cited references to follow up on	<p><input type="checkbox"/> PBP2a is encoded by the <i>mecA</i> gene (see <b>Brown et al., J. Antimicrob. Chemother., 56: 1000-1018, 2005</b>).</p> <p><input type="checkbox"/> Additional genes, which are also found in susceptible isolates, can affect the expression of methicillin resistance in <i>Staphylococcus aureus</i>, resulting in heterogeneity of resistance</p>
Follow up Questions	<ul style="list-style-type: none"> <li>- Why can MSM not be used to affect resistance in tuberculosis-causing bacteria?</li> <li>- How could we improve the effect of experiments where addition of MSM only partially inhibited the resistant bacteria?</li> <li>- How did they discover that MSM inhibited bacteria? <ul style="list-style-type: none"> <li>- To what extent was it luck</li> <li>- Is it possible to have enough information to hypothesize it will work then try it</li> </ul> </li> <li>- Why has this method not been more widely used?</li> </ul>

# Article #15 Notes: Noncanonical Mismatch Repair Protein NucS Modulates the Emergence of Antibiotic Resistance in *Mycobacterium abscessus*

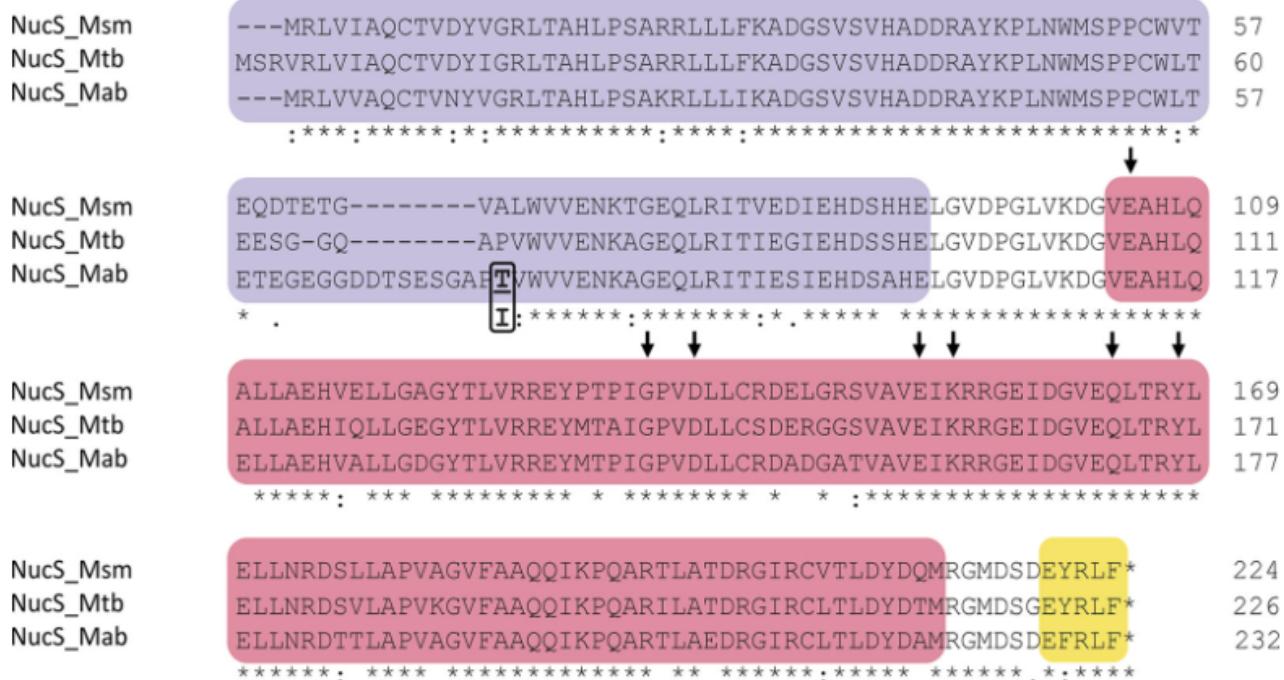
Source Title	Noncanonical Mismatch Repair Protein NucS Modulates the Emergence of Antibiotic Resistance in <i>Mycobacterium abscessus</i>
Source citation (APA Format)	Fressatti Cardoso, R., Martín-Blecua, I., Pietrowski Baldin, V., Meneguello, J. E., Valverde, J. R., Blázquez, J., & Castañeda-García, A. (2022). Noncanonical Mismatch Repair Protein NucS Modulates the Emergence of Antibiotic Resistance in <i>Mycobacterium abscessus</i> . <i>Microbiology Spectrum</i> , 0(0), e02228-22. <a href="https://doi.org/10.1128/spectrum.02228-22">https://doi.org/10.1128/spectrum.02228-22</a>
Original URL	<a href="https://journals.asm.org/doi/10.1128/spectrum.02228-22">https://journals.asm.org/doi/10.1128/spectrum.02228-22</a>
Source type	Journal article
Keywords	MMR, mismatch repair, NucS, <i>M. abscessus</i>
Summary of key points + notes (include methodology)	<p>Summary:</p> <p>Notes:</p> <ul style="list-style-type: none"> <li>- <i>italics/lowercase</i>= gene; <i>capitalized</i>=protein</li> <li>- NucS/EndoMS-dependent noncanonical mismatch repair stabilizes the DNA in mycobacteria <ul style="list-style-type: none"> <li>- Does so through preventing point mutations</li> </ul> </li> <li>- Want to know: <ul style="list-style-type: none"> <li>- Could inactivating noncanonical MMR increase the chance of a bacteria acquiring drug resistance due to gene mutations?</li> </ul> </li> <li>- To answer question... <ul style="list-style-type: none"> <li>- Constructed a strain of <i>Mycobacterium abscessus</i> with deleted nucS gene. <ul style="list-style-type: none"> <li>- Results: increased development of resistance to... <ul style="list-style-type: none"> <li>- macrolides and aminoglycosides= the two main groups of antibacterials for <i>M. abscessus</i> treatment</li> <li>- Second-line drugs ex. Fluoroquinolones</li> </ul> </li> </ul> </li> <li>- Inactivated noncanonical MMR in <i>M. abscessus</i> <ul style="list-style-type: none"> <li>- Results: increased frequency of resistant mutants by 10-22%</li> <li>- Resistant to... <ul style="list-style-type: none"> <li>- Macrolide clarithromycin</li> <li>- Aminoglycosides amikacin, gentamicin, and apramycin</li> <li>- Fluoroquinolone ciprofloxacin</li> </ul> </li> </ul> </li> </ul> </li> </ul>

- **IMPORTANCE:**

- *M. abscessus* causes chronic infections
  - Detrimental to health and economy, especially in patients with cystic fibrosis
  - *M. abscessus* has complex drug resistance mechanisms ex. Through DNA mutation
  - Studies NucS-dependent non canonical MMR= a DNA repair pathway
  - Inactivation of NucS linked to increased mutation enabling drug resistance
  - Study's analysis detected increased mutations resulting in increased resistance to first and second line antibiotics
  - Study could help determine...
    - How *M. abscessus* develops resistance
    - Role of hypermutators in chronic infectious diseases under antibiotic pressure
- *Mycobacterium abscessus* complex is a group of nontuberculous mycobacteria (NTN)
- The most pathogenic and chemotherapy resistant among rapidly growing mycobacteria (RGM)
- NTM can produce serious chronic pulmonary infections
- Recently, there has been a sharp increase of NTM infections, especially in developed countries
- *M. abscessus* responsible for diseases ex...
  - Chronic pulmonary diseases
  - Skin, soft tissue, and bone infections
- *M. abscessus* is the RGM most commonly found in patients with structural lung diseases ex...
  - Cystic fibrosis (CF)
  - Bronchiectasis
  - Chronic obstructive pulmonary disease
- *M. abscessus* leads to severe infections in patients with chronic respiratory impairments
- Promotes lung function decline = is a serious concern for lung transplantation
- Is naturally resistant to many antibiotics ex. First line anti-TB drugs rifampin and isoniazid
- Have limited number of effective drugs and chemotherapy not always successful
- Only a few groups of antibiotics effective; macrolides and aminoglycosides are most effective
- Often recommend a multidrug treatment with an oral macrolide and an intravenous aminoglycoside, sometimes with a second-line antibiotic
  - Outcomes poor in patients with chronic pulmonary infections
    - Infections commonly persist, relapse, or cause death
- One of the main ways of developing resistance is through genomic mutations
  - Macrolides and aminoglycosides target the ribosome
  - Mutation in rRNA gene *rrl*, which encodes the 23S rRNA, confers resistance to macrolides
    - Another study found that 7.3% of *rrl* mutations caused resistance
  - Mutation in rRNA gene *rrs*, which encodes the 16S rRNA, confers resistance to aminoglycosides
    - Another study found that 4.3% of *rrs* mutations caused resistance
    - *M. abscessus* also naturally has enzymes that confer resistance
      - Ex. an Erm methylase encoded by a functional erm(42) gene modifies 23S rRNA⇒ ...
        - resistance to macrolides
        - Chemotherapy failure in some clinical strains of *M. abscessus*
- Hypermutation, mostly due to impaired mismatch repair (MMR) capacity, is often observed in bacteria from patients with chronic pulmonary infections

	<ul style="list-style-type: none"> <li>- Mycobacteria utilize an alternative noncanonical MMR pathway based on the key DNA repair endonuclease NucS/EndoMS protein           <ul style="list-style-type: none"> <li>- Role of the pathway is unknown</li> </ul> </li> <li>- Study investigated the association between NucS/EndoMS and the frequency of antibiotic resistance mutations in <i>M.abscessus</i> <ul style="list-style-type: none"> <li>- Did so by inactivating nucS through exchanging alleles</li> <li>- Analyzed rates of mutations conferring resistance               <ul style="list-style-type: none"> <li>- Did in order to reveal impact of NucS/EndoMS inactivation on drug resistance in <i>M. abscessus</i> and speculate on its role in development of resistance in clinical settings</li> </ul> </li> </ul> </li> <li>- NucS: a DNA repair endonuclease           <ul style="list-style-type: none"> <li>- Can recognise and cleave mismatched DNA substrates</li> <li>- Is an important alternative MMR pathway</li> <li>- Essential to maintain genomic DNA stability               <ul style="list-style-type: none"> <li>- Inactivation of the nucS gene has led to a significant increase of mutations</li> </ul> </li> </ul> </li> <li>- How to identify the nucS gene in <i>M. abscessus</i>:           <ul style="list-style-type: none"> <li>- <i>M. smegmatis</i> NucS protein sequence was compared to proteins encoded by the <i>M. abscessus</i> ATCC19977 genome using BLASTp(NCBI)</li> <li>- MAB_1460 gene was detected as a nucS ortholog               <ul style="list-style-type: none"> <li>- It encoded a protein that was 83% similar to NucS proteins of both <i>M. smegmatis</i> and <i>M. tuberculosis</i></li> <li>- All code essential for DNA sledge was kept</li> </ul> </li> </ul> </li> <li>- To determine the function of <i>M. abscessus</i> NucS...           <ul style="list-style-type: none"> <li>- Deleted the nucS gene through gene replacement to make a nucS-deficient strain</li> <li>- </li> </ul> </li> </ul>
Research Question/Problem/ Need	Could inactivating noncanonical MMR increase the chance of a bacteria acquiring drug resistance due to gene mutations?

## Important Figures

**FIG 1**

**FIG 1** Multiple sequence alignment of the NucS protein sequences in mycobacteria. The alignment shows NucS sequences of *M. smegmatis* mc<sup>2</sup> 155 (NucS\_Msm), *M. tuberculosis* H37Rv (NucS\_Mtb), and *M. abscessus* ATCC 19977 (NucS\_Mab). Colors indicate protein domains according to the NucS structure: DNA-binding domain (purple), catalytic domain (pink), and β-clamp binding sequence (yellow). Symbols beneath the sequences: asterisks indicate identical amino acids, a colon indicates conservation between groups of strongly similar properties, and a period indicates conservation between groups of weakly similar properties. Arrows indicate key catalytic residues required for nuclease activity. The amino acid substitution found in some *M. abscessus* clinical strains by bioinformatics analysis is represented in bold in a square.

Caption: The MAB\_1460 gene in *M. abscessus* codes for a NucS protein 83% similar to NucS proteins in both *M. smegmatis*. The figure shows their amino acid sequences, highlighting sections with important functions.

NucS\_Msm= amino acid sequence of the NucS protein in *M. smegmatis*;

NucS\_Mtb= in *M. tuberculosis*;

NucS\_Mab= in *M. abscessus*

Purple highlight = the protein's DNA-binding domain

Pink highlight= the protein's catalytic domain

Yellow highlight= the B(beta)-clamp binding sequence  
 \* = identical amino acids  
 : = groups of amino acids with strongly similar properties  
 . = groups of amino acids with weakly similar properties  
 Arrows = essential catalytic residues for nuclease activity  
 Bold square: in some *M. abscessus* strains, it was a T(threonine) amino acid; in others, it was an I(isoleucine).

[the below chart of amino acid abbreviations is not from the journal– from

[https://www.researchgate.net/figure/The-amino-acids-and-their-three-letter-and-one-letter-codes\\_tbl1\\_220176841.\]](https://www.researchgate.net/figure/The-amino-acids-and-their-three-letter-and-one-letter-codes_tbl1_220176841.)

Amino acid	Three letter symbol	One letter symbol*
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamic acid	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

VOCAB:  
 (w/definition)

- EndoMS= nucS= “a restriction enzyme that is active on mismatched DNA”--  
<https://www.sciencedirect.com/science/article/pii/S0969212616302945>
- Noncanonical: a pathway with features that aren’t standard –  
<https://www.biosyn.com/faq/What-does-%22canonical%22-mean-in-biology.aspx#:~:text=In%20general%2C%20canonical%20in%20biological,referred%20to%20a%20non%2Dcanonical.>
- Δ: greek letter delta, stands for “deleted” in biology
- Second line drugs: drugs used to treat drug resistant TB
- Ciprofloxacin: a type of fluoroquinolone
- Hypermutation: increased mutation rates
- Confer: grant, bestow (oxford lang)
- Endonuclease: enzyme that cleaves a polynucleotide chain by separating nucleotides other than the two end ones(oxford lang)

	<ul style="list-style-type: none"><li>- Orthologs: genes in different species that have evolved through speciation events only– <a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2845645/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2845645/</a></li><li>-</li></ul> <p>Abbreviations:</p> <ul style="list-style-type: none"><li>- MMR: Mismatch repair</li><li>- NTM: nontuberculous mycobacteria</li><li>- RGM: rapidly growing mycobacteria</li><li>- CF: cystic fibrosis</li></ul>
Cited references to follow up on	
Follow up Questions	<ul style="list-style-type: none"><li>- Is <i>M. smegmatis</i> often used instead of TB?</li><li>- What mutations does NucS fix?</li><li>- How does NucS fix mutations?</li><li>- Mutation rates with vs without NucS?</li></ul>

## Article #16 Notes: Genetically modified strains of mycobacterium smegmatis

Source Title	Genetically modified strains of mycobacterium smegmatis
Source citation (APA Format)	Kana, B. D., & Machowski, E. E. (2020). <i>Genetically modified strains of mycobacterium smegmatis</i> (United States Patent No. US10774391B2).  <a href="https://patents.google.com/patent/US10774391B2/en?q=mycobacterium+smegmatis&amp;oq=mcobacterium+smegmatis">https://patents.google.com/patent/US10774391B2/en?q=mycobacterium+smegmatis&amp;oq=mcobacterium+smegmatis</a>
Original URL	<a href="https://patents.google.com/patent/US10774391B2/en?q=mycobacterium+smegmatis&amp;oq=mycobacterium+smegmatis">https://patents.google.com/patent/US10774391B2/en?q=mycobacterium+smegmatis&amp;oq=mycobacterium+smegmatis</a>
Source type	patent
Keywords	M. smegmatis, assays, MRSA, S. aureus, multidrug resistance, patent, M. tuberculosis, TB
Summary of key points + notes (include methodology)	<p>This patent studied how M. smegmatis could be used to identify multidrug resistance in M. tuberculosis and S. aureus. This is important because multidrug resistant TB and S. aureus are both of high concern to public health. Testing for them is dangerous and requires highly trained equipment and personnel. Transferring nucleotide sequences into M. smegmatis then testing with assays will reduce the risk and cost.</p> <ul style="list-style-type: none"> <li>- Have a recombinant bacterium based on a non-pathogenic one <ul style="list-style-type: none"> <li>- Has a gene containing DNA from a pathogen they want to detect</li> <li>- Can be used to detect M. tuberculosis and S. aureus</li> </ul> </li> <li>- TB is increasingly difficult to treat, increasing drug resistance</li> <li>- NAA diagnostic tests can identify TB and determine resistance to RIF and/or INH <ul style="list-style-type: none"> <li>- Has allowed for better drug treatment plans &amp; restricting spread</li> </ul> </li> <li>- Current treatment regimen: drugs= isoniazid (INH), pyrazinamide (PZA), ethambutol (EMB), and rifampicin (RNAP) <ul style="list-style-type: none"> <li>- RIF: binds to RNA polymerase (RNAP) beta subunit RpoB of transcription complex ⇒ inhibits synthesis of RNA and proteins</li> <li>- Resistant to RIF = RIF<sup>R</sup> <ul style="list-style-type: none"> <li>- Most caused by single nucleotide polymorphism in the 81 bp RIF resistance determining region (RRDR)</li> <li>- RIF= an effective, low-cost first line TB drug = important</li> </ul> </li> </ul> </li> <li>- Multidrug resistant (MDR) strains are treated with EMB and PZA <ul style="list-style-type: none"> <li>- Likely to develop resistance = need to switch drugs to ensure efficacy</li> </ul> </li> <li>- (MRSA believed to have evolved by acquiring a mobile genetic element = the Staphylococcal cassette chromosome (SCC) by horizontal transfer from another species)</li> </ul>

	<ul style="list-style-type: none"> <li>- Carries the <i>mecA</i> gene= confers methicillin resistance</li> <li>- Over 278,000 hospitalized people infected by MRSA annually = 60% of hospital-acquired <i>Staph aureus</i> infections in US</li> <li>- GeneXpert® MRSA assay (Xpert®-MRSA) is used to survey the region where SCCmec integrates into the <i>Staph aureus</i> chromosome</li> <li>- Currently no testing available to test for false +/- results <ul style="list-style-type: none"> <li>- Using live bacteria as positive controls = health risk; killing requires specialized labs &amp; staff</li> </ul> </li> <li>- This patent tests if <i>M. smegmatis</i> can be used to verify results of GeneXpert® and Hain Lifescience® LPA assays</li> <li>- <i>M. smegmatis</i> will contain nucleotide sequences from <i>M. TB</i> or <i>S. aureus</i> that would be used to diagnose them</li> <li>- Example use of process: <ul style="list-style-type: none"> <li>- <i>E. coli</i> with plasmid grown at 37 degrees C (on solid or liquid medium; see patent for exact conditions)</li> <li>- <i>Mycobacterium smegmatis</i> strains were grown at 37° C, (shaking in Middlebrook 7H9 liquid medium (Difco) supplemented with 0.085% NaCl, 0.2% glucose, 0.2% glycerol and 0.05% Tween80, or on Middlebrook 7H10 solid medium (Difco) supplemented with 0.085% NaCl, 0.2% glucose and 0.5% glycerol)</li> <li>- Cells were grown to log phase (OD600 0.5-0.9) and harvested by centrifugation (3 500 rpm, 10 min, 4° C).</li> <li>- Pelleted cells washed x3, resuspended, and used immediately</li> <li>- Electro-competent cells put with plasmid in electroporation cuvettes (Bio-Rad)</li> <li>- Rescued cells plated and incubated</li> <li>- Vectors with sequence of interest combined with a fragment of mycobacteriophage L5 and a resistance marker gene and cloned</li> <li>- Resulting vector capable of plasmid replication and integration into a site in mycobacteriophage L5</li> <li>- Wild type <i>M. smegmatis</i> tested in assays = negative control</li> <li>- Used strains dreem1/dreem2/dreem3/.../dreem6, for positive control at various dilutions</li> <li>- <i>M. smegmatis</i> with dreemX strain grown, tested → result negative, as expected</li> <li>- </li> </ul> </li> </ul>
Research Question/Problem/ Need	Can <i>M. smegmatis</i> be used to identify resistance in <i>M. TB</i> and <i>S. aureus</i> ?s

Important Figures	<p><b>TABLE 1</b></p> <p>Strains used and generated</p> <table border="1"> <thead> <tr> <th>Plasmid</th><th>Genotype</th><th>Source</th></tr> </thead> <tbody> <tr> <td><i>Escherichia.coli</i></td><td>fhuA2 lac(del)U169 phoA glnV44</td><td>Promega</td></tr> <tr> <td>DH5α</td><td>Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17</td><td></td></tr> <tr> <td><i>Mycobacterium smegmatis</i></td><td>ept-1</td><td>Snapper et al 1990</td></tr> <tr> <td>mc<sup>2</sup>155</td><td></td><td></td></tr> <tr> <td>dreem1</td><td>mc<sup>2</sup>155::RRDR-57-attP-int; hyg</td><td>This work</td></tr> <tr> <td>dreem2</td><td>mc<sup>2</sup>155::513-57-attP-int; hyg</td><td>This work</td></tr> <tr> <td>dreem3</td><td>mc<sup>2</sup>155::516-57-attP-int; hyg</td><td>This work</td></tr> <tr> <td>dreem4</td><td>mc<sup>2</sup>155::526-57-attP-int; hyg</td><td>This work</td></tr> <tr> <td>dreem5</td><td>mc<sup>2</sup>155::531-57-attP-int; hyg</td><td>This work</td></tr> <tr> <td>dreem6</td><td>mc<sup>2</sup>155::533-57-attP-int; hyg</td><td>This work</td></tr> <tr> <td>dreemX</td><td>mc<sup>2</sup>155::orfX-SCC junction-attP-int; hyg</td><td>This work</td></tr> </tbody> </table> <p>Table shows strains used in the patent: a plasmid, <i>E. coli</i>, <i>M. smegmatis</i>, and dreem strains 1-6 and x.</p>	Plasmid	Genotype	Source	<i>Escherichia.coli</i>	fhuA2 lac(del)U169 phoA glnV44	Promega	DH5α	Φ80' lacZ(del)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17		<i>Mycobacterium smegmatis</i>	ept-1	Snapper et al 1990	mc <sup>2</sup> 155			dreem1	mc <sup>2</sup> 155::RRDR-57-attP-int; hyg	This work	dreem2	mc <sup>2</sup> 155::513-57-attP-int; hyg	This work	dreem3	mc <sup>2</sup> 155::516-57-attP-int; hyg	This work	dreem4	mc <sup>2</sup> 155::526-57-attP-int; hyg	This work	dreem5	mc <sup>2</sup> 155::531-57-attP-int; hyg	This work	dreem6	mc <sup>2</sup> 155::533-57-attP-int; hyg	This work	dreemX	mc <sup>2</sup> 155::orfX-SCC junction-attP-int; hyg	This work
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VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Nucleotide polymorphism: when 1 nucleotide changes</li> </ul> <p>Abbreviations:</p> <ul style="list-style-type: none"> <li>- TB: tuberculosis</li> <li>- WHO: world health organization</li> <li>- NAA: nucleotide amplification assay (diagnostic tests)</li> <li>- RIF: rifampin</li> <li>- INH: isoniazid</li> <li>- RRDR: RIF resistance determining region</li> <li>- SCCmec: staphylococcal cassette chromosome mec – carries the resistance determinant <i>mecA</i>, which encodes methicillin resistance</li> </ul>																																				
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Follow up Questions	<ul style="list-style-type: none"> <li>- What method can identify deviations from standard nucleotide sequences rather than specific sequences?</li> <li>- How would this method perform using another Staph bacteria? <ul style="list-style-type: none"> <li>- Why did they choose <i>M. smegmatis</i> to test for MRSA?</li> <li>- Why grow <i>E. coli</i>/bacteria on different mediums?</li> </ul> </li> </ul>																																				

# Article #17 Notes: RecA-independent single-stranded DNA oligonucleotide-mediated mutagenesis

Source Title	RecA-independent single-stranded DNA oligonucleotide-mediated mutagenesis
Source citation (APA Format)	Murphy, K. C., & Marinus, M. G. (2010). RecA-independent single-stranded DNA oligonucleotide-mediated mutagenesis. <i>F1000 Biology Reports</i> , 2, 56. <a href="https://doi.org/10.3410/B2-56">https://doi.org/10.3410/B2-56</a>
Original URL	<a href="https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2920528/">https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2920528/</a>
Source type	Journal article
Keywords	Y beta, oligonucleotide, recombineering, SSAP, single stranded annealing protein, mismatch repair, MMR
Summary of key points + notes (include methodology)	<p>Summary: The lambda Beta protein is a single stranded annealing protein in bacteriophage lambda which promotes mutations. When an oligo is introduced into the bacteria, in this case E. coli, the lambda beta protein will help it anneal to ssDNA at the replication fork, most often on the lagging strand. Lambda beta also protects the oligonucleotide from endonucleases, enzymes which would cleave it. Success of mutation is also determined by other factors, such as the type of mismatch.</p> <ul style="list-style-type: none"> <li>- High gene mutation rates correspond to presence of mutations resistant against mismatch repair (MMR) or lack of MMR</li> <li>- (Beta= the single-stranded annealing protein (SSAP) in bacteriophage lambda (I will type lambda as y) in E. coli) = y Beta <ul style="list-style-type: none"> <li>- Promotes gene mutations (though mutations still happen slowly without it)</li> <li>- Anneals oligonucleotides to ssDNA in the replication fork</li> </ul> </li> <li>- 3 proteins (genes have same names) important to Red recombination: <ul style="list-style-type: none"> <li>- 1) Exo: goes from 5' to 3' on dsDNA to produce 3' single strands for bet to bind to</li> <li>- 2) Bet: is a SSAP: promotes recombination &amp; annealing of complementary ssDNA <i>in vitro</i> (structure = oligomers with rings of 12-18 subunits)</li> <li>- 3) Gam: binds to and inhibits RecBCD, an exonuclease that would otherwise inhibit recombination (to get rid of RecBCD, can also delete its genes)</li> </ul> </li> <li>- Genes used in recombineering are 40-50 bp long– obtained from PCR</li> <li>- Oligonucleotides can be used to alter chromosomes: (steps below) <ul style="list-style-type: none"> <li>- 1) Use oligos (are ssDNA) that match up with the bases in the target region on the opposite strand</li> <li>- 2) Use electroporation to allow oligonucleotides into cells expressing only the y Beta protein</li> </ul> </li> </ul>

- (oligonucleotides are single stranded, so  $\gamma$  Exo is not needed)
- 3) Oligos create mutations due to bp changes
- In *E. coli* expressing  $\gamma$  Beta, 0.1% of plated cells with 1-bp changes mutated (a "respectable" rate)
- Method generates mutants, ex. those without antibiotic resistance, thus requiring higher rates of mutagenesis
- Example of mutation of one bp (process also works for 1-4 bp insertions/deletions) using oligos:
  - 1) Oligo is annealed to the chromosome, creating a 1 bp mismatch
  - (The mismatched base pair becomes a substrate for the Dam-directed MMR system of *E. coli*, which tries to fix it)
  - 2) To prevent MMR, can:
    - inhibit proteins used in the process ex. NucS  
or
    - use mismatches that aren't easily recognized by the MMR system ex. C  
or
    - Surround the base pair with 19 additional changes in the sequence
      - Works b/c mismatches of  $\geq 5$  bp aren't easily recognized by MMR system
      - After, use a second electroporation to change all bases, except for the desired base change, back to original sequence
    - 3) mutagenesis frequencies can be as high as 25% out of the survivors of electroporation
  - Mutagenesis frequency depends on:
    - Sequence of base pair change
    - Which strand of DNA is targeted by the oligo
    - How effective the electroporation was at allowing oligos into the cell
    - Mutagenesis rates of oligos complementary to lagging strand are 3 to 50 times higher
      - Can be several thousand times higher in *M. smegmatis* & *P. aeruginosa*
  - Using oligos complementary to lagging-strand, introduce them at replication fork (more ssDNA to target there)
  - Oligos will be annealed to regions between Okazaki fragments/ will displace Okazaki fragments
  - The annealed oligos are connected to the Okazaki fragments
  - It has been shown that it's possible for DNA polymerase to leave gaps in DNA strands (in this case, when they meet the 5' end of the oligo), are likely to also be able to return to the DNA further down the sequence
    - (There are other ways of oligo-mediated recombination: "formation of D-loops, annealing of the oligo to the non-transcribed strand of a transcription bubble, and SSAP-directed template switches")
  - For bacteria ex. *M. smegmatis* not as electrocompetent as *E. coli*, electroporate both the oligo and a plasmid (plasmid will encode gene that lets the bacteria that took it in survive; in this case was *sacB*, which allows it to survive on sucrose)
    - 3-5% of cells that take up the plasmid also contain the mutation from the oligo
    - (research was done by van Kessel and Hatfull, cited source # 23)
  - SSAPs are classified into 3 superfamilies that evolved independent of each other:
    - $\gamma$  Beta/RecT

- Erf
- Rad52
- There could be many  $\gamma$  Beta-like SSAPs in phages that will promote mutagenesis with oligos, without RecA
- One study tries out Beta-like recombinases from other bacteria in E. coli, sees how they promote mutagenesis
  - More distantly related bacteria ex. M. smegmatis gave lower rate of recombination, though still higher than without recombinases. (by Datta et al, source # 36)
- Similar study done in M. smegmatis
  - (Mycobacterial SSAP) Che9c gp61 gave highest mutagenesis rate
  - (Mycobacterial SSAP) Halo phage gp43 and RecT (from E. coli) gave 10 times lower mutagenesis rate
  - Y Bet (from E. coli) didn't work at all
- Though replication fork thought to be target for  $\gamma$  Beta protein, their interactions have yet to be documented
- SSAPs perform worse in bacteria other than the bacteria they're usually in ( $\times 1000$  worse)
- ssDNA annealing ability of Beta is important to the high levels of mutagenesis with oligos
- Another study (#37) showed E.coli without 3' ssDNA exonucleases still had significant levels of oligo-mediated mutagenesis
  - Suggests Beta both anneals the oligos and protects the DNA from degradation caused by exonucleases
  - Further suggests that, even without a SSAP, may be able to mutate a bacteria's DNA with oligos
- Another study (38) showed chromosome of P. syringae cold be modified by ssDNA oligos without an exogenous phage annealing function
  - Added in more oligos without an SSAP – didn't result in as many mutations as with SSAP, but still  $10^6$  times more than mutation rate without oligos
  - More DNA= more substrate to occupy the exonucleases= protects oligo from being broken down
- Both SSAP-independent and -dependent oligo-mediated mutations are better at targeting the lagging strand, favor C-C mismatches, and are independent of RecA, RecB, and RecFOR
  - SSAPs increase rate of mutation by protecting oligo from exonucleases and help the oligo anneal the the DNA
- Differences:
  - In SSAP-independent mutagenesis, difference between rates of mutation with oligos of diff. lengths was small: only up to  $\times 10$  more between different lengths
  - In SSAP-dependent, require minimal length of 50 nucleotides: any less showed a significant drop ex.  $\times 10^4$
- Can use oligo-mediated mutagenesis to precisely manipulate genes

Main things:

Q: Mechanism of oligonucleotide-mediated mutagenesis (focusing on with the SSAP  $\gamma$  beta)

How beta helps oligo anneal

	<p>How beta protects oligo [page 4: mutation w/out exonucleases was lower]</p> <p>4: oligos to occupy– how decide?</p>
Research Question/Problem/Need	How does the single stranded annealing protein lambda beta affect oligonucleotide-mediated mutagenesis in E. coli?
Important Figures	<p>(a) Beta-promoted annealing to the lagging strand template</p> <p>(b) Beta-promoted annealing to the leading strand template</p> <p>Caption: .The figure shows how the oligo (red arrow) becomes incorporated into DNA at the replication fork. The 3' end of strands is shown with an arrowhead. The circle is an oligomer of the <math>\gamma</math> Beta protein, which transports and anneals the oligo to its position in the replication fork. The green cylinder is helicase DnaB.</p> <p>The left side, labeled a, shows the incorporation of the oligo into the lagging strand template.</p> <ul style="list-style-type: none"> <li>- The oligo is annealed between Okazaki fragments or by displacing one</li> <li>- Once the oligo is annealed, some mechanism in the replication fork removes the <math>\gamma</math> Beta protein</li> <li>- DNA polymerase I extends the oligo and the oligo is connected to an Okazaki fragment</li> </ul>

	<p>The right side, labeled b, shows the incorporation of the oligo into the lagging strand template.</p> <ul style="list-style-type: none"> <li>- The oligo is placed in front of where the polymerase is building</li> <li>- The <math>\gamma</math> Beta protein is removed</li> <li>- The polymerase synthesizes up until it meets the oligo, is removed, then starts synthesizing again by using the 3' end of the oligo as a primer</li> </ul>
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Beta= the single-stranded annealing protein in bacteriophage lambda (will type lambda as <math>\gamma</math>) in <i>E. coli</i></li> <li>- Mutagenesis= gene mutation <a href="https://www.ncbi.nlm.nih.gov/books/NBK560519/#:~:text=Mutagenesis%20is%20the%20process%20by.protein%20function%20and%20phenotypic%20changes">https://www.ncbi.nlm.nih.gov/books/NBK560519/#:~:text=Mutagenesis%20is%20the%20process%20by.protein%20function%20and%20phenotypic%20changes</a>.</li> <li>- Recombineering: using</li> <li>- <i>Exo, bet, and gam</i>: Red recombination genes of bacteriophage lambda</li> <li>- Oligonucleotides: usually around 70 nucleotides</li> <li>- Electroporation: uses an electric pulse to create temporary holes in cell membranes for substances ex. DNA to pass through <a href="https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/transfection-basics/transfection-methods/electroporation.html#:~:text=Electroporation%20is%20a%20physical%20transfection.acids%20can%20pass%20into%20cells">https://www.thermofisher.com/us/en/home/references/gibco-cell-culture-basics/transfection-basics/transfection-methods/electroporation.html#:~:text=Electroporation%20is%20a%20physical%20transfection.acids%20can%20pass%20into%20cells</a>.</li> <li>- Abbreviations: <ul style="list-style-type: none"> <li>- SSAP: single-stranded annealing protein</li> <li>- SSO: single-stranded oligonucleotide</li> <li>- MMR: mismatch repair</li> <li>- ssDNA: single-stranded DNA</li> <li>- dsDNA: double-stranded DNA</li> <li>- RecA: enzyme that helps repair DNA</li> <li>- PCR: polymerase chain reaction</li> <li>- bp: base pair(s)</li> </ul> </li> </ul>
Cited references to follow up on	none
Follow up Questions	<p>[page 2]</p> <ol style="list-style-type: none"> <li>1. Why aren't mismatches of 5 bp and greater easily recognized by the MMR system</li> <li>2. They electroporelate in an oligo with the desired 1-base mutation + surrounding mutations so it's not easily recognized then electroporelate again with an oligo that changes bases back- what's the point? Left with one mismatch.. Unless b/c it lets them separate [draw it, see if have right understanding]</li> </ol> <p>[page 3]</p> <ol style="list-style-type: none"> <li>1. Electroporate oligo with a plasmid encoding <i>sacB</i>, a protein that kills the bacteria when grown in sucrose. Can duplicate colonies and test one group of the groups to find the ones that successfully took in the DNA, but isn't this a bit tedious? Any better way?</li> <li>2. (updates on hypothesis that leading strand polymerase can also hop off then back on?)</li> </ol>



# Article #18 Notes: ORBIT: a New Paradigm for Genetic Engineering of Mycobacterial Chromosomes

Source Title	ORBIT: a New Paradigm for Genetic Engineering of Mycobacterial Chromosomes
Source citation (APA Format)	Murphy, K. C., Nelson, S. J., Nambi, S., Papavinasa Sundaram, K., Baer, C. E., & Sassetti, C. M. (2018). ORBIT: A New Paradigm for Genetic Engineering of Mycobacterial Chromosomes. <i>MBio</i> , 9(6), e01467-18. <a href="https://doi.org/10.1128/mBio.01467-18">https://doi.org/10.1128/mBio.01467-18</a>
Original URL	<a href="https://journals.asm.org/doi/full/10.1128/mBio.01467-18">https://journals.asm.org/doi/full/10.1128/mBio.01467-18</a>
Source type	Journal article
Keywords	Mycobacterium smegmatis, bacteriophage genetics, gene replacement, genetic fusions, metabolic engineering, promoter replacements, recombineering, tuberculosis
Summary of key points + notes (include methodology)	<p>Summary: ORBIT, which stands for oligonucleotide-mediated recombineering followed by Bxb1 integrase targeting, is a system created by Murphy et al. in 2018 for transforming bacterial genes with plasmids. To transform the chromosome, ORBIT simultaneously inserts an oligonucleotide (the “targeting oligo”), which contains an <i>attP</i> site, into the chromosome and integrates a plasmid (the “payload plasmid”), which contains an <i>attB</i> site and the desired nucleotides to insert, into the chromosome at the site of the targeting oligo.</p> <p>Notes:</p> <ul style="list-style-type: none"> <li>- Important proteins: <ul style="list-style-type: none"> <li>- Phage Che9c RecT annealase (RecT)</li> <li>- Directional Bxb1 integrase (Int)</li> </ul> </li> <li>- ORBIT= oligonucleotide-mediated recombineering followed by Bxb1 integrase targeting</li> <li>- Increases uses of oligonucleotide-mediated recombineering</li> <li>- Can select for the successful recombinants</li> </ul> <p>What is needed:</p> <ul style="list-style-type: none"> <li>- Only substrate required to be made specifically for the target gene is the oligo, (the “targeting oligo”) <ul style="list-style-type: none"> <li>- Has a Bxb1 phage <i>attP</i> site (48 bases) with 45 to 70 bases on either side that complement the bases on the targeting gene</li> </ul> </li> <li>- Nonreplicating “payload plasmid” <ul style="list-style-type: none"> <li>- Has a Bxb1 phage <i>attB</i> site</li> <li>- Has gene(s) you want to insert</li> </ul> </li> <li>- Host bacteria should be prepared with a plasmid</li> </ul>

- Plasmid should express the Che9c phage RecT annealase
- Plasmid should express the Bxb1 phage integrase
- Electroporate in the oligo and the payload plasmid
- RecT will help transport the oligo to the replication fork and protect it from endonucleases
  - The attP on the oligo will make a “bubble” because it doesn’t pair with the bases on the template strand
- Now one of the four daughter strands (one per bacteria, so  $\frac{1}{4}$  bacteria) has the desired gene integrated (ideally— other functions could mess it up)
- 
- Bacteria will replicate
- Knockouts vs knockdowns:
  - Knockouts: oligo’s attP site will replace the gene (gene is nonessential)
  - Knockdowns/ usually C-terminal tags: inserts attP at the end of the coding sequence (gene is essential)
- Other recombineering methods:
  - Target-specific dsDNA:
    - can make diverse and selectable mutations
    - but need ~ 500 bp flanking for best efficiency = too many for PCR
  - ssDNA oligos:
    - easy to make and can alter some bases
    - But usually aren’t selectable and are difficult to isolate
- Want a method that uses oligos (b/c oligos easy to make and efficient) to make selectable mutations

#### TESTING FEASIBILITY– can an oligo be put into gene w/ RecT?:

- Measure frequency of incorporation of a nucleotide about the size of the 48 bp attP site
- (done in *M. smegmatis*)
- Hygromycin resistance ( $\text{Hyg}^r$ ) gene w/ 60 bp deletion put in the L5 phage attachment site of chromosome
- Oligos with the missing 60 nucleotides and 60 flanking (180-mers) targeting lagging strand electroporated
- Smegmatis already had Che9c RecT annealase
  - Is from the anhydrotetracycline (ATC)-inducible Ptet promoter (pKM402)
- Frequency of incorporation = number of  $\text{Hyg}^r$  transformants among survivors of electroporation
- Number of transformants ranged from 10 to over 300 depending on amount of oligo (1000 ng seems best; more = not much difference)
- When RecT used to change only one base, had a 500x higher frequency of mutation: more successful with fewer base pairs being changed
  - So don’t want to insert an oligo with the gene directly— too long of a sequence

#### TESTING ORBIT– can a plasmid be integrated to make recombination selectable?

- Plasmid pKM444 starts off in cell:
  - Contains Che9c phage RecT annealase
  - Contains Bxb1 phage integrase (Int)
  - Uses a promoter (Ptet) that is inducible by anhydrotetracycline (ATC)
- Plasmid pKM446 = “payload plasmid” is electroporated into cell:

- Does not replicate in mycobacteria
- Contains Hyg<sup>r</sup> marker so it can be selected for
- Contains Bxb1 **attB** site
- Contains Flag tag and Das+4 peptide tag next to attB site
  - Das+4 tag: once SspB adapter protein is expressed, it will bind to Das+4 and bring it to the ClpXP system (a system that degrades proteins; ClpXP is a protease): end up with a hypomorph strain (expresses less of a protein than wild-type) ex. For testing possible drugs
- Targeting oligos electroporated into cell at same time as payload plasmid:
  - Contains Bxb1 **attP** site
  - Made to target the 3' ends (close to stop codon) of 3 genes: *recA*, *divIVa*, and *leuB*
    - *recA*: repairs damage due to UV light
    - *divIVa*: **NOT SURE** but something about helping with the cell membrane
    - *leuB*: for making leucine, an essential protein
- When payload is integrated into the cell, region on left = attL site; on right= attR site
- Tested if recombinant's sequence was as expected (PCR then gel and sequencing)
  - PCR then gel: tested for junction at 5' end and at 3' end of integrated payload.
  - Sequencing: there was a 3' junction for each 5'. 5' junctions were sent for sequencing and confirmed.
    - Sequencing also used to confirm attachment of Das+4 tag to target gene (sequence found in 5' junction)
  - 9/12 of tested candidates were mutated as expected= 75% (though need more trials for more accurate success rate)

#### MAKING KNOCKDOWNS:

- Use the Das+4 tag:
  - If use pGMCgSTetOFF-18 plasmid to transform the Das+4 tagged strains, **adding** Atc will **decrease** the number of target gene proteins
  - If use pGMCgSTetON-18 plasmid to transform the Das+4 tagged strains, **NOT adding** Atc will **decrease** the number of target gene proteins
    - Das+4 is connected to the protein coded by the target gene
    - SspB binds to Das+4 and drags it, along with target protein, to protease ClpXP system→ get degraded
    - SspB's promoter replaced by a Tet promoter = can be activated by anhydrotetracycline (ATc) (gets rid of its repressor), which they add to make SspB
    - Also make a mutated repressor of the Tet promoter so Tet is turned on in the absence of ATc
  - Number of colonies reduced where expected: shows mutations using ORBIT to tag a protein are successful

#### MAKING KNOCKOUTS:

- ORBIT decreases necessary length of the oligo, amounts of oligo and nonreplicating plasmid
- attP put between last codon of target gene and stop codon
- Gentamicin-resistant integrating plasmid (goes into genome by itself) used as a transformation control
  - b/c electroporation rates cold also affect success
  - Compare successful integration of plasmids due to ORBIT vs successful integration of plasmids that can naturally integrate

- Payload plasmid: allows the bacteria to become Hyg<sup>r</sup> by fixing the 60 bp deletion
- All oligos used for C-terminal fusions made w/ attP site inserted right before stop codon– is important
  - All have the 2 bases CG placed between the attB site and the tag
  - Work with the 43 bases in the attL sequence to connect target genes with tag

**Sequence of ORBIT-promoted chromosomal  
C-terminal Flag-DAS4+ fusion**

last codon of target gene	attL (43 bp)	2 bases from plasmid to allow fusion of target to tag
		
NNNGGTTTGCTGGTCAACCACCGCGGTCTCCGTCGTAGGATCATCGGACTACAAG GACGACGACGACAAGGCCCAACGACGAGAACTACTCCGAGAACTACGCGGACGC CAGCTAGTGA-ori-cat-hyg-GGCTTGTGACGACGGCGGTCTCAGTGGTGTACGGTAC AAACC-TGA..... 		
stop codon of target gene	↑	attR (43 bp)

Color scheme is as follows: **attL**, made up of **attP** and **attB** sequences (core sequence underlined); **CG bp to fuse target gene to tag(s)**; **Flag tag**; **DAS+4 tag**; **plasmid-supplied stop codons**; **plasmid sequence**; **attR**, made up of **attB** and **attP** sequences (core sequence underlined).

Supplementary Figure 3

- After plating 25% of overnight growth = 0.5 ml, grew 5-50 colonies, sometimes over 100
  - About 2 to 4 Hyg<sup>r</sup> candidates analyzed with PCR to find one with successfully integrated plasmid (so 50 to 25 % of Hyg<sup>r</sup> successfully mutated with ORBIT; rest could have just been spontaneous mutations, ones not facilitated by ORBIT)
  - Most oligos used had 60 to 70 bases flanking

#### GET RID OF THE REC-T-INT PLASMID:

- Put sacB in plasmid ('461) coding for RecT and int
- Bacteria transformed as normal but with the sacB-containing plasmid instead of '444
- Bacteria grown on plate w/ Hyg and sucrose
  - Ones successfully transformed with payload plasmid now have hyg<sup>r</sup> and will survive
  - Ones that still have the RecT/int plasmid have sacB and will die
- Getting rid of bacteria still with RecT/Int plasmid produces colonies a bit slower but produces plasmid-free mutants quicker

#### ORBIT MODIFICATION STABILITY:

- Found that the integrated payload plasmid was not lost even after >100 generations
  - Ex. used PCR and fluorescence microscopy to check if an integrated GFP tag was still there
  - Investigate if temporary expression of RecT and Bxb1 integrase quill be enough for

### ORBIT recombination:

- Delete oriM from '444
- Follow standard procedure
- Had less recombinants than usual; 8 PCRs were done and all came back negative for the 5' junction of the expected recombinant
- Thus, seems lots of RecT needs to be present before oligo is electroporated

### LIBRARY OF ORBIT MODIFICATIONS:

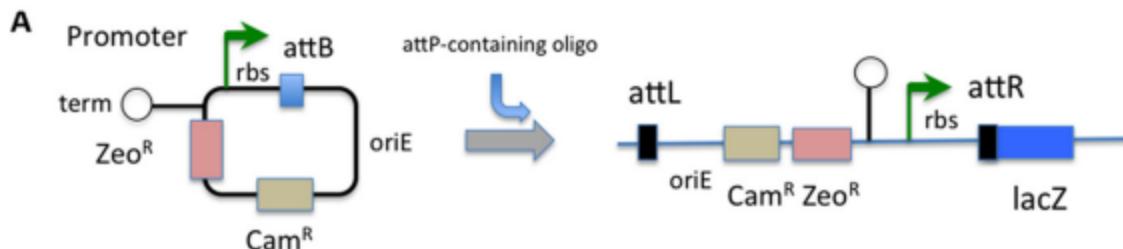
- Made a library of diff. payload plasmids with diff. knockouts/promoters/tags: can be used with an oligo to make different mutations
  - Can put in promoters of different strengths

### GFP FUSIONS:

- Proteins, ex. DnaN, were tagged with GFP to find their locations in the bacteria (see figure w/ squares & GFP)
- ORBIT= efficient, can modify genes without needing to make specific target and modification dsDNA substrates

### PROMOTER REPLACEMENTS:

- Can use ORBIT to change promoters and how the gene is expressed
  - If promoter weaker, less of gene expressed
- Strength of ribosome binding site impacts strength of promoter: weak binding = weak promoter
  - Ribosome binding site strength decided in a sequence after the promoter
- Replaced the promoter with attP
- Integrated in different plasmids with different promoters
- Promoters are going to help express B-galactosidase
- Amount of B-galactosidase among recombinants increased with strength of promoters
- Shows promoters can be replaced to modify expression of chromosomal gene



### MARKERLESS GENE DELETIONS

- Bxb1 phage produces the gp47 protein
  - Gp47 is a recombination directionality factor—works w/ Bxb1 integrase to promote site-specific recombination between attL and R to turn it back to attP
  - A plasmid ('512) made that expresses, under a Ptet promoter, gp47(instead of RecT), integrase, Zeo<sup>r</sup>, and SacB from '461
    - Can select for successfully mutated bacteria by growing it with ATc on plates with zeocin
    - Bacteria mutated with '461 (RecT, int, SacB) will have kanamycin<sup>r</sup>; with '446 (payload) will have hygromycin<sup>r</sup>; with '512 (gp47, int, SacB) will have zeocin<sup>r</sup>
      - Site-specific recombination of attR and attL sites replaces the deleted portion between them (which contains hyg<sup>r</sup> when payload integrated)

	<p>to make the bacteria hgy<sup>A</sup>s again</p> <ul style="list-style-type: none"> <li>- 7/37 colonies transformed with '461, '446, and '512 had hygromycin <b>sensitivity</b>; all of them had the other <b>sensitivities</b> (b/c resistant ones killed b/c had sacB in plasmids)           <ul style="list-style-type: none"> <li>- Not as many Hyg sensitivity b/c needs successful recombination (see above), not just presence of the plasmids</li> </ul> </li> <li>- 4/7 of Hyg<sup>A</sup>s colonies sequenced, confirmed the expected replacement of the deletion with attP</li> <li>-</li> </ul>																
Research Question/Problem/Need	Testing the application and effectiveness of ORBIT.																
Important Figures	<p><b>B</b></p> <table border="1"> <thead> <tr> <th>oligo (ng)</th> <th># of Hyg<sup>R</sup> colonies/ml</th> </tr> </thead> <tbody> <tr> <td>5000 ng</td> <td>~600</td> </tr> <tr> <td>2000 ng</td> <td>~550</td> </tr> <tr> <td>1000 ng</td> <td>~500</td> </tr> <tr> <td>500 ng</td> <td>~250</td> </tr> <tr> <td>100 ng</td> <td>~40</td> </tr> <tr> <td>50 ng</td> <td>~35</td> </tr> <tr> <td>10 ng</td> <td>~8</td> </tr> </tbody> </table>	oligo (ng)	# of Hyg <sup>R</sup> colonies/ml	5000 ng	~600	2000 ng	~550	1000 ng	~500	500 ng	~250	100 ng	~40	50 ng	~35	10 ng	~8
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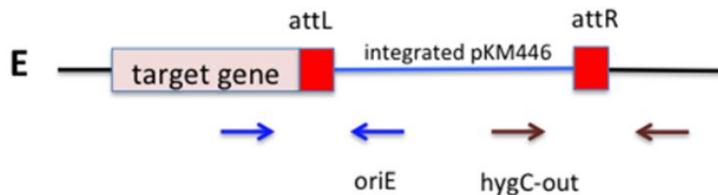
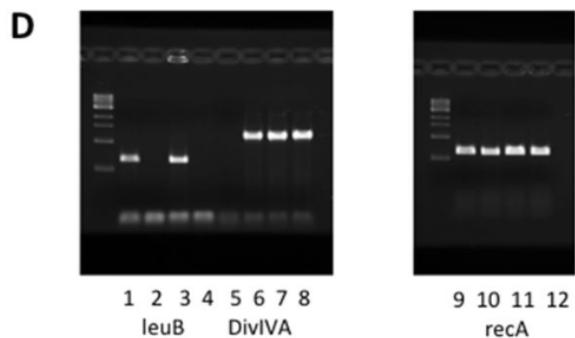
**Caption:** Tests effects of different amounts of oligo on the number of recombinants. Bars show standard errors (no cross= not certain there's no difference; cross= certain there's no difference. Seems to plateau after 1000 ng of oligo = about 350 transformants per ml: not much difference in number of recombinants after. About  $2 \times 10^{-6}$  recombinants per electroporation survivor.

C	Target	# correct/ # tested
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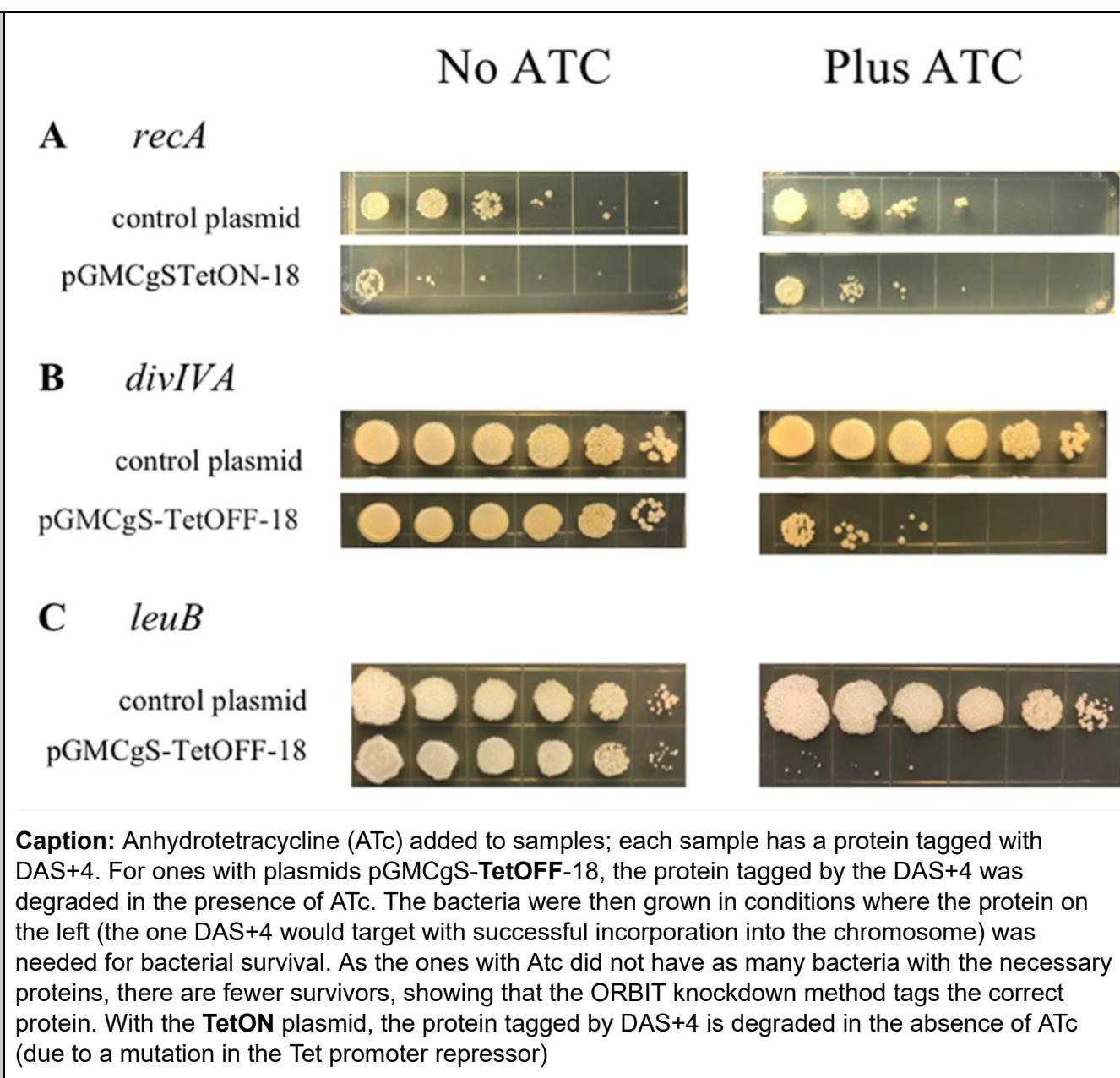
MSMEG\_2379 (leuB) 2/4

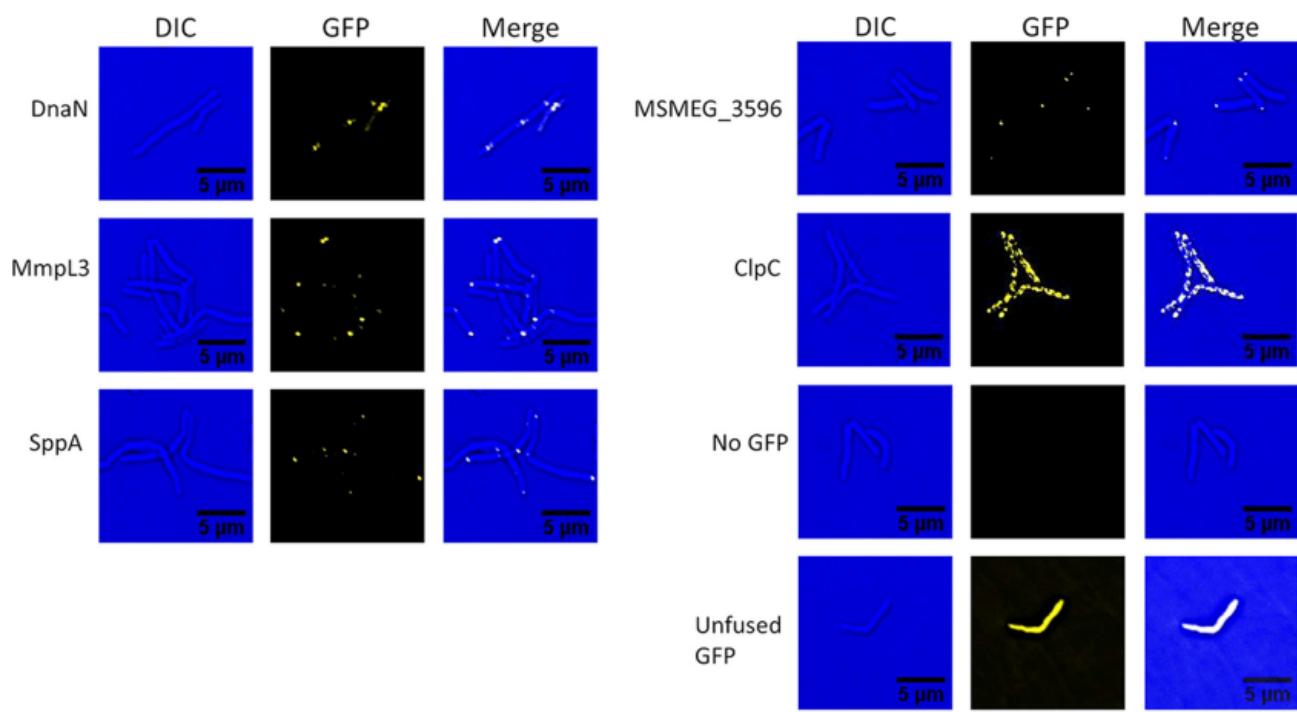
MSMEG\_4217 (DivIVA) 3/4

MSMEG\_2723 (recA) 4/4

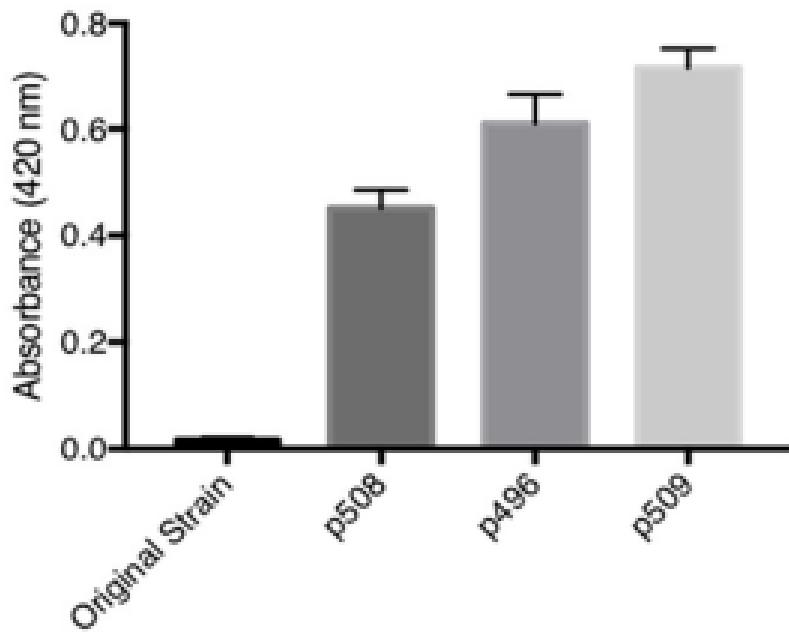


**Caption:** C: how many successful recombinants there were/ how many candidates tested (4) for each gene. E: the region where they integrated the plasmid and the locations of the primers. Blue= for the 5' junction and brown = for the 3' junction. D: the gels to determine the presence of the 5' junctions. For each 5' junction present, there was also a 3' junction present, so the 3' gels are not shown. To make sure the 5' junctions were the expected sequences, they were sent for sequencing and thus confirmed.

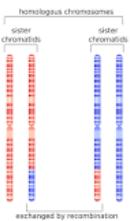




**Caption:** Different proteins in *smegmatis* tagged with eGFP (enhanced GFP). For DnaN, spots are replication forks. MmpL3 was suspected to be at the poles of the cell (which they are in the figure). Other three had unknown distribution.



**Caption:** Promoters are listed (L to R) from weakest expression of gene to strongest expression of gene, likely due to strength of the ribosome-binding site (rbs) following each (see promoter replacement diagram in notes for rbs). Plasmids express lacZ, so beta-galactosidase assays done (x3). Higher absorbance indicates more

	lacZ, as it is white and gives off more light
VOCAB: (w/definition)	<ul style="list-style-type: none"> <li>- Paradigm: a typical example or pattern of something; a model (oxford lang)</li> <li>- Obviates: removes [a need or difficulty] (oxford lang)</li> <li>- Cognate: related, connected (oxford lang)</li> <li>- Homologous recombination: a type of genetic recombination in which nucleotide sequences are exchanged between two similar or identical molecules of DNA.  <a href="https://www.genome.gov/genetics-glossary/homologous-recombination#:~:text=Homologous%20recombination%20is%20a%20type,or%20identical%20molecules%20of%20DNA">https://www.genome.gov/genetics-glossary/homologous-recombination#:~:text=Homologous%20recombination%20is%20a%20type,or%20identical%20molecules%20of%20DNA</a> </li> </ul>  <p>The diagram illustrates two homologous chromosomes. Each chromosome is composed of two sister chromatids. In the first chromosome, the sister chromatids are red. In the second chromosome, the sister chromatids are blue. A vertical line labeled "exchanged by recombination" connects the two chromosomes, indicating that a segment of one chromatid has been replaced by a segment from the other, demonstrating a crossover event.</p> <ul style="list-style-type: none"> <li>- Directional Bxb1 integrase (Int): allows “simultaneous integration of an oligonucleotide and a ‘payload plasmid’ (contains a recombination site and a selectable marker)</li> <li>- Phage integrases: enzymes that allow site-specific recombination between two DNA recognition sequences (identified below) <a href="https://pubmed.ncbi.nlm.nih.gov/14687564/">https://pubmed.ncbi.nlm.nih.gov/14687564/</a> <ul style="list-style-type: none"> <li>- attP: the phage attachment site <a href="https://pubmed.ncbi.nlm.nih.gov/14687564/">https://pubmed.ncbi.nlm.nih.gov/14687564/</a></li> <li>- attB: the bacterial attachment site <a href="https://pubmed.ncbi.nlm.nih.gov/14687564/">https://pubmed.ncbi.nlm.nih.gov/14687564/</a></li> </ul> </li> <li>- Site-specific recombination: DNA molecules are rearranged by breaking and rejoining the strands at specific points:   <p>The diagram shows a double-stranded DNA molecule with two specific sites for recombination, each marked by a yellow box. A recombinase protein (represented by a purple box) binds to these sites and catalyzes the rejoining of the DNA strands in a different orientation, effectively swapping the segments between the sites.</p> </li> <li>- Self-autonomous replication vectors: ex. Plasmids: can copy themselves independent of the bacterial chromosome ⇒ can end up with 100s of plasmid copies within one cell  <a href="https://www.sciencelearn.org.nz/resources/1900-bacterial-dna-the-role-of-plasmids#:~:text=Every%20plasmid%20has%20its%20own,hundreds%20E2%80%93%20within%20one%20bacterial%20cell.">https://www.sciencelearn.org.nz/resources/1900-bacterial-dna-the-role-of-plasmids#:~:text=Every%20plasmid%20has%20its%20own,hundreds%20E2%80%93%20within%20one%20bacterial%20cell.</a> </li> <li>- Cassette: A mutation-containing <u>restriction</u> fragment that can replace the fragment excised from the genome.  <a href="https://www.genscript.com/biology-glossary/433/cassette#:~:text=A%20mutation%2Dcontaining%20restriction%20fragment,Molecular%20Biology">https://www.genscript.com/biology-glossary/433/cassette#:~:text=A%20mutation%2Dcontaining%20restriction%20fragment,Molecular%20Biology</a> </li> <li>- C-terminus: the end of a peptide where an amino acid's carboxyl group is not part of a peptide bond and will thus become part of a carboxylic acid or carboxylate  <a href="http://www.chem.ucla.edu/~harding/IGOC/C/c_terminus.html">http://www.chem.ucla.edu/~harding/IGOC/C/c_terminus.html</a> </li> <li>- Tag: peptide sequences that are attached to proteins to facilitate easy detection and purification of expressed proteins  <a href="https://info.gbiosciences.com/blog/bid/198500/8-protein-tags-explained#:~:text=Basically%2C%20protein%20tags%20are%20peptide,for%20your%20protein%20of%20interest.">https://info.gbiosciences.com/blog/bid/198500/8-protein-tags-explained#:~:text=Basically%2C%20protein%20tags%20are%20peptide,for%20your%20protein%20of%20interest.</a> </li> <li>- assay: determine the biochemical or immunological activity of (a sample) (oxford lang)</li> </ul> <p>Abbreviations:</p> <ul style="list-style-type: none"> <li>- Int: (directional Bxb1) integrase</li> </ul>

	<ul style="list-style-type: none"> <li>- <b>ORBIT</b>: Oligonucleotide-mediated Recombineering followed by <b>Bxb1 Integrase Targeting</b></li> <li>- SSR: site-specific recombination</li> <li>- CRIM: conditional-replication, integration, and modular (plasmids)</li> <li>- HR: homologous recombination</li> <li>- SNPs: single nucleotide polymorphisms</li> <li>- Hyg<sup>r</sup> gene: hygromycin resistance gene</li> <li>- Strep<sup>r</sup>: streptomycin resistance</li> <li>- DAS+4: sequence that promotes degradation of the tagged proteins upon expression of SspB</li> <li>- SspB: Stringent starvation protein B, activated by either Tet-OFF or Tet-ON</li> <li>- Tet-OFF: adding ATc</li> <li>- Tet-ON: removing ATc</li> <li>- ATc: anhydrotetracycline— RecA is degraded in its <u>absence</u></li> <li>- recA: protein used for repair of DNA</li> <li>- HAs: homologous arms: guide sequences to enable specific alignment to the right location in order to replace the DNA there</li> <li>- </li> </ul>
Cited references to follow up on	
Follow up Questions	<ul style="list-style-type: none"> <li>- ORBIT vs CRISPR CAS9?</li> <li>- Has any implementation in other bacteria been tried/yielded results?</li> </ul>