This Week in Microbiology

With Vincent Racaniello and Michael Schmidt

Episode 188: Turducken antibiotics

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Vincent: This is TWIM, This Week in Microbiology, episode 188 recorded on October 25, 2018. I'm Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Charleston, South Carolina, Michael Schmidt.

Michael: Hello, everyone!

Vincent: How's your weather, you got a hurricane?

Michael: No, it's from the west coast blowing across and they are predicting rain all weekend, we haven't seen a drop. So my guess is it is gonna rain all weekend.

Vincent: What's your temps down there, roughly, is it pretty warm still?

Michael: No, it cooled off, mercifully, it cooled off and right now, I'll check the weather app like they do on TWIV, right now it is 68 degrees which of course is 20 degrees Celsius. I think I'm doing my conversions right in my head. Tomorrow is gonna be 78 for a tie, and Saturday is going to be 70 with clouds and Sunday we get back the sun and 72. It's finally cooled off. Last week we had 90 and I went out to San Francisco, Sacramento area, and gave two talks at two different VAs, and California weather is just spectacularly gorgeous and fortunately, I came home to gorgeous weather here in Charleston.

Vincent: Speaking of VAs and hospitals, I have to tell you this, a Columbia student came by, we were chatting the other day, and there is a company here in New York, I think it was formed by former Columbia people, but I might be wrong. It's called Kinnos, and what they have developed is a product for hospitals, instead of refitting with copper, they have these wipes that have a blue color that it's transferred to whatever you're wiping and it goes away in 5 minutes. And so that's long enough to disinfect and so you don't have to tell people, wait five minutes before you wipe off the stuff, because the color simply goes away. Isn't that cool?

Michael: Wow, that is very cool. What's the active ingredient?

Vincent: I don't know, look into the company, maybe we can get them on TWIM to talk about it.

Michael: Yeah, let's bring em on because I tell ya, I was out at Infection Control Week at these two particular VAs in California and they had a number of different vendors who were there and some of the vendors were promoting new products, but what I liked is a product that I hadn't heard about nor

have I seen from Kinnos, but one of the cool things they had was a wearable device that actually had the equivalent of gas chromatograph in this badge that you wore and if you zapped your hands with alcohol, then you wiped your hands and you literally scanned your hand across this badge, the badge went to a green light.

So the patient would know that you have washed your hands with an alcohol sanitizer. If they were in an isolation room where you are supposed to use soap and water, it would have a different detector that would smell the soap and the water and it would change to a pleasant blue color. So the patient would always know if the clinician was following the hand hygiene guidelines, and it was a much more efficient. And so this product that you just talked about actually reinforces that behavior because by the time the color disappears, it's ready to go. Very cool.

Vincent: The thing is, you still have to wipe the surfaces quite frequently, right?

Michael: Yes, yes. The bacteria literally come out of the woodwork, so to speak, within typically 4-6 hours, depending upon how well the biofilm has established itself in there. And that's been in the news, especially in New Jersey now, with this deadly outbreak of Adenovirus, last night I saw on the news that 8 children had passed from this outbreak and it is principally due to failure of hand hygiene. And the news was very much concerned about Adenovirus killing children, but the one thing that they failed to articulate in their story is that the hospital that this was occurring at was one that treated immune compromised children.

Vincent: Exactly, exactly.

Michael: So the Adenovirus is not any more aggressive than probably the Adenovirus running around your child's school or your child's daycare center. It just so happens that the kids that were in this long term care facility happened to be immune compromised. So I don't think we are gonna see a widespread epidemic of lethal Adenovirus. I would be interested to hear your thoughts.

Vincent: That's exactly right. It's a long term care facility, the kids are immunocompromised, the viruses circulate all the time everywhere and most people wouldn't know the different between a cold caused by rhino or adenovirus, so in this facility, though, these kids have problems clearing the infection, so they get more serious infections. As you say, they have bad hand hygiene, but also in a closed facility the kids are more likely to pass it to each other. So what happened is someone walked in with it, they were probably sick but had either no symptoms or very mild. They exhaled it, or they got mucous somewhere, and that started the chain of infection.

Michael: Probably on their toys.

Vincent: Yeah. So the rest of us are not at any risk, but these are some of the subtleties that they get lost. Although one article I did see mentioned in the body of the article this immunosuppression. But not much of the people would understand that, you know, these kids are… I'm not sure, the cause of the immunosuppression could be genetic, of course…

Michael: Cancer therapy.

Vincent: Cancer therapy, right. And some certain kinds of therapies will immunosuppress you. Of course, other virus infections will immunosuppress you, like measles and HIV1. But it destroys your ability to respond to other infections. And you could have inborn errors that immunosuppress you. I was just looking at a paper the other day where they studied a child that had recurrent serious rhinovirus infections. They sequenced the genome and had a mutation in the interferon pathway. That confers the inability to deal with infection. So with genome sequencing we are learning more and more the basis for some people getting serious respiratory infections.

Michael: And with the cost of sequencing coming down, I think it is one of the most important things we can fund in every public health department to know and understand what viruses and bacteria are running loose, and to actually begin to sequence those and to have, if you will, a database of the usual suspects akin to what the FBI does.

Vincent: Totally, totally. Everybody has to get their genome sequenced at birth, it goes into your medical record, and then researchers can mine it. It could be a gold mine of information on what's going on in our genome. You could look at all these interferon pathway genes, but wow, look at all these new mutations we hadn't seen before! What have we sequenced, less than 10,000 human genomes, right?

Michael: Yeah.

Vincent: That's nothing. It's nothing in the big scheme of things.

Michael: Well, if you have your genome done by 23 and Me and you buy the more expensive version, the medical variant, to see in addition to figuring out which country your ancestors came from, you figure out what you may, what illnesses may be in your genome. You can actually download your own DNA because they, your own DNA sequence, because they sequence it. So I, this past summer when Amazon was having its Amazon Prime sale, I did my genome and figured out what I already knew from my relatives and I figured out that I wasn't adopted because my parents were true in telling me where my grandparents came from, and I then downloaded my genome, and it is sitting on my computer.

Vincent: Yeah. But I want to know if 23 does the sequence, I think they do just micro arrays.

Michael: I got the whole kit and caboodle.

Vincent: They do exomes, really?

Michael: Yeah, I got the whole kit and caboodle. I got to go back and see if there were exomes, but it was a big, big file.

Vincent: That's interesting. Our son, one of our sons had his exome sequenced here at Columbia for medical reasons. That (laughs) I think the bill for that was 25 grand.

Michael: Holy moly!

Vincent: Of which I paid 500 bucks, because…

Michael: The deductible.

Vincent: I mean, yeah, the insurance company paid for it, but that's ridiculous to pay that much money for something that is far less expensive, right. I'd like to get a hold of it to take a look at it, though. But yeah, we were talking about this the other day, it is a gold mine of information depending on what you are looking for. You can look at mobile elements in the genome, how it differs among populations, all sorts of mutations and so forth, so I'm hoping one day that happens, yeah. Now we have one letter. As listeners may have gathered, there's no one else here but Michael and me. It's just us.

Michael: Michele is fulfilling her presidential responsibilities for ASM attending the Board of Directors meeting, and Elio's fulfilling his community responsibilities in attending what was it, something about fungi.

Vincent: Mushroom something, mushroom club or something.

Michael: It's always about mushrooms with Elio.

Vincent: So it's Michael and I, so we're just gonna talk for hours, right Michael?

Michael: To basically fill (laughter) we're gonna get in trouble.

Vincent: Before we get to some science, we have a follow up from James who writes, this with respect to the last episode, I think:

The quote that Michael was trying to say and Vincent got correct was: "Many Bothans died to bring us this information." I believe that was in Return of the Jedi and was a quote from Mon Mothma. The quote Michael was thinking of was: "These are not the droids you are looking for." Of course that was Obi Wan when he and Luke and C3PO and R2D2 were entering Mos Eisley Space Port. LOL! Yeah, I have watched all the Star Wars movies way too many times.

Thanks for the great podcasts! I am a regular listener to all the TWIX, plus Immune podcasts you do. Long time past Biology and Chemistry double major and 25 years in the Pharma industry having sold many antibiotics and antivirals really makes me love and appreciate all the info you guys share!

Also love the Ronald Jenkees music you use on all the shows! Have downloaded several of his tracks!

All the best, James Morrison

James is from Austin, Texas. James, we did TWIV 500 in Austin this summer! You should have come!

Michael: Maybe he was there.

Vincent: Speaking of antivirals, just this week, the FDA approved a brand new one for influenza. Did you know that Michael?

Michael: I saw that, that made our actual paper this morning, and it is, I guess similar to Tamiflu except you only need to take one pill, from what I understand.

Vincent: It's totally different, it's a different mechanism of action.

Michael: Oh, that was not in our paper this morning. So how does it work?

Vincent: It's called Xofluza, and it is an inhibitor of the viral endonuclease.

Michael: Ooh, that's new!

Vincent: It's the first new flu drug in 20 years, brand new mechanism of action. So the endonuclease is part of the RNA polymerase of the virus and it is needed to cut the caps off of cellular MRNAs so they can be used as primers for the viral polymerase. So this binds to the endonuclease, this drug, inhibits it and blocks virus production. So it went through Phase I, Phase II, and Phase III, shown to be effective and patients with uncomplicated influenza, a little bit better than Tamiflu, which they compared it with. But again, you need to take it within 48 hours of onset of symptoms.

Michael: Otherwise there are too many viruses wandering around the neighborhood and it makes perfect sense.

Vincent: But it's good because if you have a resistance to some other flu drug you could try this. What I think would be the most interesting would be if they tried a combination, a dual drug therapy with this drug and say Tamiflu, of course they have to do a clinical trial to test that, but two drug therapy could solve the problem of resistance for Hep C, so it could for the flu, as well.

Michael: And that would be very, very big.

Vincent: Yeah, for sure. So that's good, that is cool news. And James, you probably know about that, since you're in pharma. Alright. Let us, we do have some more letters, we'll read them later, but let's move on and Michael has a snippet for us.

Michael: I'll start with the first snippet. This is entitled "The synthetic dual drug Sideromycin induces gram negative bacteria to commit suicide with a Gram positive antibiotic." And this appeared in the Journal of Medicinal Chemistry and it comes to us from a laboratory of Rui Liu, Patricia Miller, Sergei Vakulenko, Nichole Stewart, William Boggess, and Marvin Miller, all in the Department of Chemistry and Biochemistry at the University of Notre Dame and South Bend, Indiana.

There are a couple of facts that we need to know before we start this. First, iron is an essential micronutrient. It is required in micromolar concentrations by bacteria in order to sustain infections. It is needed for DNA replications involved in the iron-sulfur centers, in the membrane to move electrons, so it is an essential element that all cells need. At the concentration of free iron in infected tissues is very low because we of course have the remarkable thing called transferrin. And in a typical aerobic neutral pH environment, its concentration of iron is at 10^-18 molar, or about 10^-12 micromolar. So you see bacteria are continuously starved for iron. So that's fact 1 that you gotta remember.

Fact 2 is Gram positive bacteria are very different than Gram negative bacteria. Gram negatives of course have this second membrane, or outer membrane, that can effectively exclude antibiotics, limiting their effectiveness, or should they get across that outer membrane and present themselves to the cell, the cell then has efflux mechanisms where even if the antibiotic gets in, it gets pumped out before it can actually reach its target.

The other thing is Gram negatives have a much thinner cell wall, the peptidoglycan is only a bimolecular layer of peptidoglycan, so they don't have as many cross links facilitating the holding together of that molecular corset that contains the bacterial cytoplasm. Gram positives alternatively have a very thick peptidoglycan layer with many crosslinks associated with it. Recall that bacteria are under tremendous pressure just like graduate students, and they are at about 80 pounds per square inch. So any antibiotic that interferes with crosslinking can make the organism lyse simply because as they grow, that pressure results in the fracturing if the crosslinking doesn't take place, and that's what the beta lactam class of antibiotics do, is they prevent crosslinking, so as the cell grows those crosslinks that are not present, it effectively allows the cell to explode. And we've all seen the movie on YouTube complete with the sound effects.

So here's the story. They hypothesize that all bacteria are in a desperate need for iron. And through clever biochemical synthesis, they constructed a turducken. (laughter) And this is a turducken of an antibiotic. And they also called it formally a sideromycin. If you have not heard of a turducken you probably will if you watch food network because this is the time of year turduckens become possible. But this concept really helps you visualize the wonder of this sideromycin. So a turducken is a turkey with which a duck has been stuffed inside the turkey, in which a chicken had already been stuffed into the duck.

So the turkey portion of our turducken is where the asiderophore is. An asiderophore of course is a public good whose job it is is to harvest iron from the medium or steal it from our cells simply by grabbing it away from things like transferrin. And then that siderophore is actively transported and this is important for Gram negatives, it is actively transported across that membrane that normally would exclude antibiotics from entering and getting access to the cell and being internalized in the cell. The second piece of the turducken or the duck portion is a cephalosporin and it may be active against the cell wall of a bacterium. However, should the microbe possess a potent beta lactamase, and here is another fact that you probably already know, but you should have in the back of your mind as we think about this, is the beta lactamase is a soluble protein that bacteria export to destroy the family of beta lactam antibiotics.

Some bacteria produce more, some bacteria produce less. If you have the beta lactamase on a plasmid it will produce much more because you make more message, you make more protein off of the plasmid than you do off of the chromosome. The third piece of the turducken or the chicken piece is an antibiotic that traditionally only is targeted against gram positive organisms and it is a family of antibiotics in the one that they used in this particular case is oxazolidinones, which is a class of antiribosomal antibiotics that are very effective against Gram positives but really have no effect against Gram negatives secondary to them being effluxed away before they can knock down protein synthesis because of their interference with the ribosome.

So the first part of their very detailed manuscript goes into the elegant synthesis of how to construct a turducken, or in this case they refer to it as a dual Trojan horse with the siderophore being the Trojan horse about it and the two invading armies inside the horse are a cephalosporin and an oxazolidinone The concept of coupling siderophores to an antibiotic is not new, and in fact, Mother nature figured this out long ago. There are even natural variants out there in Mother Nature. Synthetic variants have also been developed so this is not a new concept, but it is one that I think bears talking about because the danger of Gram negatives is that we are not having very much luck developing new classes of antibiotics that can inactivate the Gram negatives.

So if we can figure out how to trick them into bringing in an antibiotic for which they were naturally resistant, why not go ahead and try? And this siderophore complex can also been shown to actually inhibit antibiotic inactivation, and in fact, one of the interesting things they volunteer in their introduction is that this strategy of coupling the siderophore to an antibiotic enables the delivery of an antibiotic regardless of its size or charge. Recall that if you have ever looked at structures of antibiotics they often are very much positively charged and so it is hard to get in across that negative membrane. And they provide examples in their manuscripts, and one that was most curious to me is that they talk about an antimalarial drug that really has no activity against bacteria. And in their example they talk about this antimalarial drug being active against mycobacterium tuberculosis. Well, we know that mycobacterium tuberculosis has its own very special iron siderophore called mycobactin. And so when it is in the literature, when they couple mycobactin to this antimalarial drug, then the antimalarial drug now works on TB and only TB.

Vincent: Do these transporters, these siderophore transporters, do they have a size limit of what they can bring in?

Michael: It doesn't appear… the antibiotics are relatively small, but it appears that they are getting in. I think it is more of charge, and it is through the active transport system.

Vincent: Because these molecules they make are all organic molecules, they aren't proteins or anything.

Michael: No no no, they are all very complicated, it looks like a schizophrenic organic chemist actually synthesized these.

Vincent: What Dickson would call it is dihydroxychicken wire.

Michael: Dihidroxy chicken wire, that's exactly it! I was trying to remember his expression, but now that you mention it.

Vincent: Isn't that great?

Michael: Dihidroxychicken wire. And it fits with my metaphor of a turducken!

Vincent: Yep! Turducken is awesome.

Michael: It is actually what it's doing. Now secondly, the elegance of their system is that it anticipates or requires resistance to be held by the organism they are attacking. And so what happens is as that beta lactamase made by the bacterium to confer resistance against a cephalosporin acts, it actually releases then the oxazolidinone, except now the oxazolidinone is close enough to get into the cell. This is cool! It is truly the Trojan horse metaphor, but they built this really cool thing and the chemistry is this elegant, they produce spectra showing us how to do it, but I was interested in the microbiology. So again, their foundational hypothesis was that the siderophore would transmit this siderophore sideromycin antibiotic across the outer membrane.

And with the beta lactamase cleaving the cephalosporin, this would then expose the oxazolidinone to the cell where it would transit into the cell, affecting its ribosome target. Now they tested this construct or turducken against a strain of acineto baumannii. Acinetobacter baumannii has a very potent chromosomal beta lactamase. And as expected, in their experiment they do all of the appropriate controls. They have a non conjugated oxazolidinone through which it had no effect on the acinetobacter. They have cephalosporin by itself and they mix and match and all of their mixing and matching of the appropriate controls had no effect whatsoever.

They then conjugated the siderophore to the oxazolidinone alone and the problem with that is the siderophore oxazolidinone, the siderophore got across the outer membrane but when it got inside the cell it really didn't have the activity that they anticipated. And they hypothesized that it was either unable to reach the target of the ribosome or it was somehow sterically excluded from affecting its antimicrobial effect in protein synthesis. But the combination or the intact turducken worked beautifully. You dropped the MIC from 50 micrograms or, excuse me, 50 micromolar I believe is how they present it as all the way down to 0.8 or excuse me, 0.4 micromolar of the compound tested. So is is really showing you how well this hybrid molecule actually delivers the drug to the right target. They next tested their turducken against four clinical isolates of Acinetobacter baumannii and all four were resistant again to the control compounds tested, which was the oxazolidinone, the cephalosporin, the siderophore across the oxazolidinone, and then the intact complex. And then the intact complex was very sensitive, acutely sensitive, to the tribred molecule.

But then they did a riff on the experiment. They had an acinetobacter that had a plasmid that had a very very potent beta lactamase on the plasmid. So obviously it is going to increase the concentration of beta lactam cleavage. And when they looked at that number, the MIC 0.4 micromolar up to 6 micromolar, which makes sense because in the past you had two antibiotics that could do the dirty deed and now you only really had one however much of the hybrid molecule was getting cleaved. So it is absolutely fascinating how this compound behaved when they were testing these compounds.

Vincent: Michael, can I ask you a question?

Michael: Please!

Vincent: When you get through the outer membrane, then you're in the periplasm, right? And there is the beta lactamase, is that right?

Michael: The beta lactamase is there.

Vincent: Cleaves it off, and now how does the oxa get through the inner membrane?

Michael: Through normal membrane transport.

Vincent: I see.

Michael: It just comes in via active transport because it has got over the barrier of the outer membrane.

Vincent: I notice also the siderophore plus ceph is also not bad.

Michael: No, that's good too, because what happens there is the siderophore brings it in and puts it in the protective environment where, because they are constantly exporting beta lactamase, and so they really don't go into the concentration of the beta lactamase enzyme in the periplasm versus the outside, and you know, it's just an elegant experiment.

Vincent: And so the cleavage of the ceph off the siderophore doesn't inactivate the ceph, right? It just allows it to be separated and that can go in. And then finally these numbers, 0.8 micromolar, is that a therapeutically useful concentration?

Michael: Now we're going down the rabbit hole of MIC. The answer is no. Because MICs are, they are sort of related to the therapeutic level. But these MICs are in the therapeutic range. They would be considered therapeutic.

Vincent: Would you have to tweak this to get it better or is this, do you think they would try treating patients with these things?

Michael: I think they would try treating patients with this. The only danger I would say is you are effectively given a pathogen iron, and often that is one of the principle virulence factors. So it is not gonna be without risk because if your organism that you are testing happens to be resistant to the oxazolidinone, in addition to being resistant to cephalosporin, you have now given the pathogen iron, which will make it all that much better.

Vincent: Yeah. Since you don't treat these infections with these antimicrobials, you probably don't have as much resistance, right?

Michael: Inherent resistance, that is indeed correct.

Vincent: These baumannii strains are terribly resistant, right?

Michael: Oh yes, this is the scourge that, this is what when I was working on my DOD project, this is what the colonels referred to aracobacter. It's one of these agents because it is a common soil organism, and when IEDs would go off they would get driven into the wound of the poor trooper and it would effectively result in a very bad infection. And they really had nothing to treat them because if it was plasmid borne it would just trash the cephalosporins. The antibiotic industry has gone to great lengths to develop antibiotics that can get into Gram negatives, can target Gram positives, and the

oxazolidinone is that class of antibiotics. It has principally been used to go after Staph aureus, MRSA, and enterococci, Strep pneumo, it is really against Gram positives.

So I think the take home for all of this is that this turducken concept facilitates the entry of an antibiotic to an appropriate space and thus avails formally an untapped class of antibiotics for use in Gram negative microbes. And as they went through the synthesis it seemed like a relatively straightforward synthetic process to make their turducken. It didn't seem any more complicated, though I'm sure we will get letters from chemists about how complicated the chemistry is. But if you've ever made a turducken, it is complicated too (laughs).

So this was really pretty neat and the fact that they were banking on the cephalosporins needing to be cleaved and one of the experiments that they showed is that if you fail to cleave that linkage molecule, you just didn't have as much activity. And that was one of their control molecules where the oxazolidinone was coupled to the siderophore. So I think based on the background that they presented and the evidence for this, there may be hope that we can get these antibiotics into the clinics sooner because oxzaxolidinone are already approved, cephalosporins are already approved, and it is just the coupling of the siderophore to this whole mixture that may make pharma say we can take the risk on this and do a trial and it will be the inferiority trials that, you know, is it better or worse than what we are currently using.

Vincent: So on TWIV this summer we were at Texas A&M and they have a phage therapy group there. They told us the story of a gentleman in the San Diego area who had come back from the Middle East with an A. baumannii infection, untreatable, was in a coma, was gonna die, and his wife looked up phage therapy, found Texas A&M, and to make a long story short they came up I think they got a phage from the Navy that was specific.

Michael: From Meryl, they got it from my good friend Meryl.

Vincent: Yeah, and they gave it to him, IV for 2 weeks, and he's fine now. He's walking around. Isn't that amazing? (laughs)

Michael: I tell ya, when I was working on phage therapy we could literally cure a mouse of a full blown systemic infection of Pseudomonas aeruginosa that was growing like gang busters. And we just inject the Pseudomonas aeruginosa IP under the mouse's skin and the phage homed to the location in contrast to the phage that was given to the person in San Diego, ours didn't exponentially expand like true phage therapy does. Ours was targeted with one of these toxin mediated genes, and so we actually put in a defined dose, hit it, and it works. So phage therapy is possible but again, the organisms are often lysogenic to the phage which is why you need a cocktail to hope that it doesn't have the repressor proteins in. So if you have never read Mark Ptashne's book The Genetic Switch it will give you a sort of an overview of the limitations that phage therapy may encounter, because of the whole issue of lysogeny and what triggers lysogeny and all of the repressor proteins. It can work and it has worked.

Vincent: Well that group there has Rie Young, he's at the head, and he is very circumspect. He said we're not ready yet, I get calls every day to cure someone but we're not ready. People understandably want to have it if someone they know is sick, but. It's pretty amazing. Alright, thank you, Michael.

Michael: Thank you.

Vincent: That's very cool. Turducken. I think we'll call this episode Turducken Antibiotics.

Michael: Or it could be pigs get fat and hogs get butchered.

Vincent: What does that mean, I don't get that.

Michael: That's a South Carolinian expression. Pigs continue to eat, and that's the case of the Gram negatives eating the siderophore to get themselves the precious iron. But if they eat too much, they're gonna bring in more of the antibiotic and so they're gonna be a hog and then they'll end up getting butchered by the antibiotic. But I like your turducken one, too.

Vincent: Those are both good. Alright. We'll have to think about that. Okay, I have a paper for you. It is published in the Journal of the American Chemical Society. You know, the paper we just did was in another kind of journal.

Michael: Journal of Medicinal Chemistry, also an ACS journal.

Vincent: Two ACS journals, how about that. This one is called "Bacterial genome containing chimeric DNA RNA sequences." And we have as first author, Mehta A, Wang Y, Reed, Supekova, Javahishvili, Chaput, and Schultz. They're coming from the Scripps Reasearch Institute in La Jolla, the Bay Area Innovations Center, which is in Hayward, California, and University of California Irvine. This is a curious paper, and I'm not quite sure what to make of it but I thought we would just toss it out there. It's under the heading, yeah, I don't quite know what to make of this.

Michael: This was in our, this was our week for supporting ACS journals.

Vincent: That's right, we wanted to support them. We don't read their papers very often here on TWIM. This is all about the idea that at one time, the world was an RNA world. Organisms had RNA genomes and they slowly became DNA for a variety of reasons. DNA is more stable, and DNA genomes got much bigger than RNA genomes, as far as we know. And this paper says, well, I should say the authors say, they probably didn't go immediately from RNA to DNA. There was some kind of a continuum phase and so can we find an organism with a hybrid DNA RNA genome?

So they are using E. coli and they want to engineer it so that you get some RNA in the DNA. And this is not something that hasn't happened before. You can find ribonucleotides in the genomes of both bacteria and eukaryotes. And of course there are viruses with RNA genomes as well. So that's the idea. They want to see, could we make a hybrid bacterial genome? So they start with E. coli and they use what they call metabolic engineering to try and reduce the overall strategy is to reduce the amount of deoxy CTP in the cytoplasm. So of course dCTP is one of the four building blocks of DNA. We have C, G, A and T. And eventually these would be phosphorylated because you need a triphosphate to incorporate into DNA as it is being replicated. But they say if we lower cytoplasmic dCTP, can we encourage the incorporation of RNA into the genome?

Michael: Or the R version, the ribosugar version, instead of the deoxyribo, it's the ribo version.

Vincent: Yeah. So the ribo version has two hydroxyls, right? And the deoxy has just one. You need at least one at the 3' position of the sugar so that you can attach the next base, but the RNA has two of them. So the way they do that if you want to know how they do it, so here's a little detail, they introduce a number of genes into E. coli to do this. The first is a, and these are T4 bacteriophage genes. One is gene 56, and in the old days the phage people used to just number their genes. There's a lot of famous ones like gene 32, I remember in graduate school there was a DNA binding protein, I think. Alright, gene 56 is a phosphatase that removes phosphate from CTP and CDP and UND. So that removes it from the precursor pool. You need the phosphate on in order for it to be incorporated. Gene 42 is a DCMP hydroxy methylase. It will cut methyl off of DCMP. Gene 1 is a deoxyribonucleoside monophosphate kinase that will phosphorylate deoxyTMP, deoxyGMP, 5-hydroxy methyl CMP but not DCMP and not DAMP. And finally, gene CD is deoxycytyldylate deaminase.

Now, the result of all this is that you are going to decrease the DCTP pool. And the paper, I don't know if it is open access or not, I can't remember, but there is a lovely figure in the beginning which shows you what happens with all of these enzymes present in E. coli. And you will basically be lowering the DCTP pool. And when they grow E. coli with these extra genes, which they've introduced in 2 plasmids, when you grow the E. coli you can see that about 60% of the genome has 5 hydroxy methyl C substituted for DC basically. Which is what their strategy was meant to do. They're reducing the DC and they are introducing genes that will hydroxymethylate or produce a hydroxymethylated form. And in fact, you get 60% of the genome substituted with that. And they know this because they do a lot of mass spectrometry to figure out composition.

Michael: Pool size.

Vincent: Yeah. Now, that's just step 1. Because that's not RNA, that's just a modified C base. The principle is there, you could get a different base in if you reduce the DCTP concentration. Then what they do is they mutagenize these E. coli that contain their plasmids that have these phage genes on them that I just mentioned. They use nitrosoguanidine, which Michael will remember because, I don't know if anyone uses this anymore.

Michael: It's a cancer causing agent, these have all been drummed out of everyone's lab.

Vincent: People used to use it to introduce mutations randomly into the genome, right?

Michael: Absolutely.

Vincent: And they do that and they get weird colonies. They call them morphologically distinct colonies.

Michael: And they show you them.

Vincent: They show you the colonies and they say the cells if you look under the microscope are spherical. And as everyone should know, E. coli is not spherical. It's a rod shaped cell. So now they have mutagenized this E. coli containing these plasmids and you get weird colonies and spherical cells

and they sequenced the 16S ribosomal DNA, it's E. coli, it's not a contaminant. They were pretty careful to do that, because that's I think the first thing you would think, oh, we got a contaminant in here. And when they analyzed the genome of this weird isolate, they see ribonucleotides. And they can pick a colony and grow it up again and the ribonucleotides increase with these two plasmids that are remember, reducing DCTP concentrations in the genome.

Michael: It's just basically draining the pool of water. So if you have no water in the pool you are not going to be able to swim. By virtue of the fact that you are increasing the ribovariant of this what all cells must do is make DNA in order to divide. Recall that that is what triggers cell cycle division is another copy of the genome. That's the only way it is gonna actually happen. So it is a forcing function. The cell is forced to use the pool of the ribovariant.

Vincent: But remember that they mutagenize and that gives them RNA. The original variant still has a deoxy C. It's a variant hydroxymethyl, but when they mutagenize, then they get RNA incorporated into the genome. So something has been changed, they don't know what it is, that is causing incorporation of RNA. And that only happened after nitrosoguanine mutagenesis.

Michael: My speculation is they changed the specificity of the substrate of the enzyme that is calling for DCTP to be less picky about what it incorporates into the growing nucleic acid.

Vincent: Could be the polymerase, right.

Michael: Could be the polymerase, could be any number of things that is bringing the C to the table.

Vincent: So they purified DNA from this strain. They say the yield is really low. That could be because it is breaking up, right, the treatment condition is to purify DNA might be breaking it up. In fact there's an RNAse treatment during isolation, so yeah (laughs) that would probably do it.

Michael: That would ruin your day.

Vincent: That would. And then they digest the DNA into single nucleosides and they do mass spec and they can actually quantify the levels of ribonucleosides. You get about a C, which is a mixture of RC and DC is about 4%, the RG plus DG is 53%, the RA, these are ratios, I'm sorry. The RC/ RC+DC is 4%. The RG/ RG+DG is 53%. So 53% RG and 57% RA. So they're getting incorporation of RNA into this DNA. They did a bunch of experiments to rule out RNA contamination. They spiked in ribonucleosides and ribonucleotides into the genomic lysates and they show that they are still getting this RNA into their DNA.

So is this trait heritable? They grow the cultures on plates, they pick individual colonies, they isolate DNA and they show this RNA and the DNA as well. These strains grow significantly slower compared to the parental strain. So something is wrong with them. So the question is, is this RNA on one strand only, so you could imagine a DNA RNA hybrid, or is it a chimera where you have DNA linked to RNA on the same strand? And they isolate the DNA and they digest it to oligonucleotides, dinucleotides and trinucleotides, you don't want to digest it to mononucleotides because then you wouldn't know what the neighbor is. So di and tri and they analyze that by mass spec. And they can see the D linked to the

R nucleotides. Wild type is only DCDG, DG DG etcetera, but the mutant strain you get RGDG and DCRG etcetera. So you get chimeric dinucleotides.

Michael: In the same strand.

Vincent: The same strands. So this is a chimeric genome. It's not a hybrid DNA strand RNA strand, but it's a chimeric DNA covalently linked to RNA.

Michael: So this then has to change the topology of the helix, and as you think about why it is impacting growth, because RNA DNA hybrids are much more stable so they will melt much more slowly. So transcription off of the chimeric molecule will likely take longer because of the melting function in order to read the coding strand to make your transcript that will then get converted into protein. And that fact that it is still growing slow also helped to convince me that they were truly getting a chimeric strand where you had the deoxy and the ribo in the same strand.

Vincent: So their hypothesis is they have lowered the CMP pools by their additions of these genes. And that together with some mutation in the DNA machinery of some kind gives you RNA nucleotides in the genome. And so they look at the pools of free deoxyribonucleosides, they lyse E. coli, they do mass spec on that. And they can see it drop in the DCDA and DG, but not T in this mutant strain. And so this is consistent with what they think is going on. The next experiment is whether other polymerases will incorporate RNAs into DNAs. And they take the venerable Klenow fragment of E. coli DNA polymerase 1. Now Klenow fragment lacks the exon, right?

Michael: It does, indeed.

Vincent: The exonuclease. We used to use this for cloning years ago because it wouldn't chew up what it made.

Michael: I was gonna ask you, when was the last time you heard the enzyme Klenow and when was the last time you saw P32 in a paper?

Vincent: Yeah, these things, people don't use anymore, exactly. I used to use this as a post doc. You probably did, right.

Michael: Oh, yes. By the gallons.

Vincent: We don't use P32 anymore, people have figured out safer assays.

Michael: And they used it at 250 microcuries of raw phosphate, so these were glow in the dark bacteria.

Vincent: Yeah, they do a template and they add this polymerase and they add either DNTPS or RNTPS and in fact Klenow polymerase will incorporate ribonucleotides if you don't give them the DNTPS. So that's very interesting. This is a wild type enzyme. It'll incorporate RNTPs if you just take away. Now this is a short little synthesis they do, they use two oligonucleotide with a gap and they show that it

fills it in but they can make longer DNA. So here is another one, Michael, when's the last time you heard M13?

Michael: Oh, God. 30 years?

Vincent: So M13 is a single stranded DNA phage that we used to use for sequencing, it had a double stranded DNA replicative form, you could clone inserts into it. We used to do shotgun cloning, shotgun massive collections of restriction fragments into the replicative form, we put it in, we transfer it into E. coli, get phages out, pick plaques, and use sequence on each single stranded phage because it's single strand, you could anneal an oligo to it. We used to crank out so much sequence. Of course this is all for the museum now, as someone told me.

Michael: All for the museum. I used to do mutagenesis with M13. I put in the mutant variant but you know M13 is a very smart phage, it would actually go to the chromosome, get the right copy that behaved better, and get rid of my mutant variant after.

Vincent: We used to use it for that, too. The beauty of it is you could pick a plaque and you just lyse it and you have single stranded DNA for hybridizing an oligo for mutagenesis with the sequencing. But here they ask if we now put a primer on M13, can we make a big product? What is the length of M13? It's at least, 8, 7, 6 KB.

Michael: It's your garden variety high copy plasmid.

Vincent: And they say you can make full length M13 which has RNA in it. It is sensitive to RNAse H, RNAse H is an enzyme that will cleave an RNA part of an RNA-DNA hybrid. And this happens again, if you leave out a DNTP and put RNTP in instead it will, this enzyme will incorporate it into the M13 template. So I'm not sure if this is covalent or strand specific, they didn't do the same kinds of experiment, but the polymerase is definitely incorporating RNTPs into it. And that's interesting because that's the Klenow, that's the wild type. Now the next thing they want to do is say, what's with this strain of E. coli that we mutagenized, that is able to incorporate RNTPs into DNA? Well, they have some problem isolating and sequencing the DNA, so they can't actually sequence the DNA of this. So they have to do proteomic analysis, and I'm jumping ahead here, but I'll go back to the other. They look at the proteome of all the proteins, because they can't sequence the DNA, it doesn't work in their Illumina sequencing, probably because there is RNA in it, I guess.

Michael: It's RNA, remember Illumina works by chain termination.

Vincent: Yeah, that didn't work on this. What they should have done was digest away the DNA and take the RNA and sequence that by RNAseq. They could have converted it to DNA.

Michael: Or they could have done Maxim Gilbert sequencing which is truly--

Vincent: It's truly, yeah, it's what I used to sequence polio.

Michael: That would have worked.

Vincent: So they have no genome sequence so instead they looked at the proteins and they do 2 dimensional protein gel electrophoresis and they say wow, this strain is really weird and they say it has got 1 to 3 mutations per peptide that they get here. They say this is an unprecedented amount of mutagenesis and so maybe this strain has a lot of mutations in it because it is was transcribing an RNA DNA template. Maybe that's not good.

Michael: Well it is probably getting slop in the base pairs. And it depends on how it lines up and whether or not the polymerase that is either reading the DNA or the ribosome that is reading the transcript maybe stuttering.

Vincent: Right. So they were able to make similar strains by a variety of manipulations independent of this one, and I won't go into that, but in the end they get RNA incorporated into the DNA. They use another technique called post field gel electrophoresis. Now this was a technique where you can run on a gel very very large chromosome size fragments of DNA which you can't usually do on a normal kind of gel electrophoresis. So they can identify big pieces of E. coli DNA and show that they have RNA in them. They are sensitive to RNAse.

Michael: Which will change their mobility in the gel because you add the RNAse to it, it degrades it, and then it changes the size of the fragment.

Vincent: Exactly. And finally, they look at some RNAse H mutants of E. coli. A lot of noise outside my window, here.

Michael: But you're high up, aren't you?

Vincent: You know, we have an air force base up the Hudson river and often the fighter jets fly by. They're pretty noisy, you know.

Michael: I do indeed, I have them fly over my house often.

Vincent: They don't make an effort to make them quiet. They don't have to, I guess. So RNASE H is an enzyme that will cleave the RNA portion of an RNA DNA hybrid, and E. coli has RNAse H. So they make some RNAse H mutants, they delete, there are too alleles for RNAse H in the genome and they delete them independently. They see an increase in the amount of ribonucleotides in the genomes and they say, well, when you get DNA synthesis they are primed with Okazaki primers and part of that is an RNA.

So maybe you're not excising those RNAs and that's what is causing the presence of ribonucleotides in those genomes. Now, when they take their original strain which has these metabolic engineering plasmids in it and they put that together with an RNAse H deletion these strains are really messed up and they say, this probably crosses the amount of RNA that the genome can tolerate. So that is the story. We have a strain of E. coli where a good fraction of the genome, 60%, is RNA. It seems to be linked to DNA. Some of the questions I have are where is this RNA in the genome? Now, it could be just random throughout the genome or it, Michael, in the E. coli genome, is there a lot of space in between genes?

Michael: No.

Vincent: Not a lot, huh.

Michael: No, it's pretty tight because remember, prokaryotes have effectively truncated their genome size for efficiency in replication. They want to be as small and tight as possible. It's a rare event that you have much spacing between it, and again, we have to consider the selection pressure is going to be where you can actually either have a tight hybrid where the juggernauting RNA polymerase will effectively open up, they have sufficient energy and mass to plow through that much stronger hybrid that is taking place in its replication. It is probably not going to be near the insertion site or the operator region of genes because the RNA polymerase effectively has to dissolve the DNA complex in order to get in.

So that's going to be minus 50 to 200 bases upstream depending upon whether or not there are any activators. And also, it's going to be unlikely around the repressor that effectively is binding double stranded DNA. And I wonder, for repressors, how repressors interact with RNA. It would be interesting to take one of these hybrids out, engineer it so that it looks like pick your favorite promoter and hook it up to a beta galgene or GFP gene and ask what it does to repressor release and in read through. That will probably give you an idea of why these things are growing slower or why the mutation rate is as high as it is. It was a fascinating story as to how, or the driving force of why pick one form of nucleic acid over the other and why the chimera is really a no go.

Vincent: Well, in the end they say this shows that you can get chimeric RNA DNA in E. coli, so maybe it tells us that in the old days from the RNA to the DNA world there were such intermediates, right? I think they need to study where exactly this RNA is, I think it will be quite interesting. And like you said, is it avoiding control regions of genes or what? It should be interesting to look at.

Michael: Is it around the origin of replication, I would bet not.

Vincent: Probably not. So those strains dropped out, we lost them because they weren't infectious or the weren't able to grow. And what we have left, and they say, you know, the population is much smaller and these are the ones that are able to survive, so. I guess we'll be hearing more about this. I think it is okay. It seems good to me, it doesn't seem like arsenic, right? (laughs)

Michael: No, doesn't seem like arsenic. They did the right controls with the Klenow fragment. I don't think if... the arsenic story had the Klenow fragment with the appropriate arsenate base nucleotides, then I would have been much more comfortable with the arsenic story. I mean, we wanted the centrifuge that showed that it was arsenic versus phosphate.

Vincent: Alright, there you have it. Let's do a couple of emails. We have one from Mike who writes:

This is in response to a letter read from Jonathan on TWIM 183 regarding viable but non culturable cells in chlorinated effluent of municipal wastewater treatment plants.

Hello All,

This is a response to a letter read (from Jonathan) on TWiM 183 regarding viable but non-culturable cells in chlorinated effluent of municipal wastewater treatment plants. During my undergraduate studies I interned with a very supportive laboratory manager at such a facility. The lab also used IDEXX and we were curious if the chlorination levels were inducing a VBNC state. With resuscitation methods using amino acid supplemented media and extended incubation times, we obtained isolates that were confirmed as E. coli and would have otherwise been uncounted. As Michael mentioned, any oxidative damage would push some cells into the VBNC state. So what we saw was not very surprising. What is interesting to think about though, is that as operators, we try to be as cost effective as possible. We chlorinate just enough to keep bacterial counts low to meet permit requirements, but anything more than that would generally be considered wasteful and expensive. I also wonder if UV disinfecting systems would induce similar VBNC states, and if the cells would resuscitate or regain virulence after entering the receiving waters (and sunlight).

Michael: Well sunlight would give them more UV depending on how high they were in the water column, and it is a good question, and UV light does indeed induce VBNC states if you don't completely kill them all.

Vincent: Yeah as you said it depends on how close to the surface.

Michael: It's all about the photons that hit the nucleic acid, and how much water the water molecules inside the cell have to generate the free radicals that will then transition into the VBNC state.

Vincent: Yeah. This is the second or third email about this, very interesting issue. Mike continues:

I am a new listener, and first time writer. After the internship I obtained my MS while surveying bacterial insertion sequences. I now manage and operate a small laboratory at a wastewater treatment facility, but am gearing up to submit applications and return for more school.

Thank you so much for your podcasts! They're wonderful to listen to in the lab and keep me pleasantly distracted on the treadmill. I especially enjoyed the discussion of antarctic soil bacteria surviving on atmospheric gases in episode 169 and reading the paper afterwards.

I write from Seattle, where it is currently blue bird skies and in the 50's.

Hoping you all the best, Mike.

Michael: That was really nice, thank you!

Vincent: I guess additional education is PhD, right?

Michael: Yes.

Vincent: Good luck with that, Mike. Michael, can you take the next one?

Michael: Rajesh writes:

Hi Dr. Vincent,

Love your podcast.

Thank you so much for such amazing, rich and extensive discussions every couple of weeks.

I am an avid listener and have listened to you all a multiple times.

Thanks in advance for the book on Antibiotics as well.

Thanks again.

I don't know if he won the book or not.

Vincent: I don't think so. I think these are leftovers, yeah.

Michael: And then Kara writes:

Greetings TWIM-ers!

Long-time listener, first time writer here. I stumbled upon your podcast when I was taking general microbiology and wanted something to listen to between studying (I frequently knit when I listen to your podcasts!). I had the pleasure of taking general microbiology not just once, but twice: first as a part of my undergraduate degree in biology and second, more recently, as a prerequisite for nursing school, as schools require classes be taken within 10 years of applying. Not to my surprise, little had changed in the world of general microbiology; gram stains are still gram stains and flagella still wiggle. That is why I am thankful for your podcast as it delivers thorough and entertaining insight into some of the latest research in the field. As it turns out, I am starting a direct-entry nurse practitioner program this fall and the Antibiotics text would be a welcome addition to my growing library.

Thank you for all that you do and keep up the good work!

Sincerely,

-Kara

P.S. It was 84F, clear, and sunny today in Boston. Anything (including locusts) is better than snow!!

Vincent: Locusts! Well we will have more books to give away in the next couple of weeks. Kim writes:

Greetings TWIM crew,

I have been aware of the existence TWIM since 2016 shortly after I graduated from high school and wanted to enlighten myself about the noble field of microbiology. It was not until early this year though, that I started to listen more actively to TWIM and the other TWIX podcasts. While listening to these podcasts I have also become aware of how important science communication is in helping

people understand how important science is and how it works. Additionally, after listen more frequently I feel like the passion you put into these topics have passed on to me, so much so that I have considered careers in virology, mycology and molecular biology although I won't have to choose in a long time. I am currently starting my undergraduate studies in biology on the 3rd of September and would appreciate a free book on antibiotics as it might be useful during my studies and sounds interesting. Thank you for all the time and work you put into these podcasts, I feel podcasts like these are necessary for showing people that science isn't scary, unnatural or something only scientists can understand or do but wonderful, logical and something anyone can understand or do.

It is a partly cloudy Monday morning here in Jyväskylä, Finland with 15°C, 77% humidity and 3m/s windspeed.

Kim Kreuze

P.S. Don´t worry if you butcher the name of the city, it´s hard to pronounce if you are not Finnish.

Well, I tried. Let me know how I did, Kim.

Michael: Too many umulauts.

Vincent: You can take the next one.

Michael: Rebekka writes:

Hello TWIM team,

I have been a long time listener, you've helped me through some rough times as silly as that sounds. Even before getting into the sciences academically (and while agoraphobic) I was able to learn through the podcast. Struggle through papers, constantly grabbing a dictionary, making notes on the important facts and concepts.

You won't read this email but I felt like saying: thank you so much for everything. You're doing a lot of good in this world.

Sincerely,

Rebekka

Well we did read it, Rebekka!

Vincent: Thank you, that's very nice.

Michael: Yes.

Vincent: Justin, go ahead Michael, that's your topic.

Michael: Justin writes, regarding TWIM 183, antibiotic sensitivity in less than 30 minutes,

Wouldn't using a personal cell phone with PHI violate HIPAA even if it was just to use as a camera for a microfluidics device? I know it made me really uncomfortable when I was with my mom in the hospital (on Long Island but I won't name names) and the Doctor was showing her pictures of her own x-rays on his phone and her name was visible on the screen and he acknowledged that his phone syncs pictures to his iCloud account. I mean it was only for a broken wrist but still, didn't seem right.

Don't really want people passing around medical information like it's a common picture. And that's actually true, that physician who we won't name names is indeed violating HIPAA if it is going up to his iCloud account. We frown on that here, you go to HIPAA jail here and you get sent to mandatory school and all of our images are locked down and they are not to be on your own personal device. So that is against the rules and I am sure that physician will likely be encouraged to go to HIPAA school to learn what he can and cannot do or what she can or cannot do. So thanks for the note Justin, it's good to reinforce the HIPAA requirements and not sharing people's personal information in things like iCloud or Amazon cloud or things along those lines.

Vincent: Totally right, you can't do that, it has to be on an encrypted computer, all patient information, personal health information, it has to be on an encrypted computer that is approved by the university. So you can't use any dropbox, you have to use the specific one that they approve. I'm sure iCloud isn't part of it. Any computer here at the medical center that has patient information or that has access to a computer with patient information has to be secured, password locked and encrypted. And if we send email that has patient information in it to someone else it has to be encrypted. And you can do that by putting an encrypt code in the subject line, it will automatically encrypt it. But yeah, that's totally wrong. Can't do that and that physician needs to go, as Michael said, to HIPAA school. And we have to do HIPAA training every year here.

Michael: We do indeed, and we have to achieve a level known as competency where you take an exam and you demonstrate that you know the rules of the road as they say and you don't want to violate this. And there is a lot of information out there.

Vincent: Alright. Hannah writes:

Dear TWiMicrobiologists,

This paper just came across my news feed, and my goodness, just look at those figures!

Please show it to Dickson too: it's such a visual paper that I'm sure he'd love it 🙂

Cheers,

Hannah

And the paper is "Visualization of the Type III secretion mediated Salmonella host cell interface using cryo-electron tomography." And this is published in eLife so it's open access. It is amazing, the pictures of this type III secretion structure which I think we've never seen before in this kind of detail. You can

exactly see this injection apparatus. The outer and inner membranes, and they have a model of it. They're beautiful, they're just beautiful.

Michael: And you can see the needle tracks that the needle leaves behind in, if you will, the patient that is the bacterium, or the eukaryotic cell that is being injected by the salmonella.

Vincent: Very cool.

Michael: And they have some beautiful colorized images that really show you how elegant the system truly is.

Vincent: Nice figures, as well, putting all the structure together. Figure 5 is really cool. The injectosome contracting with the host cell membrane. So if you ever wondered exactly what it looked like, this is it. Open access, check it out, thank you Hannah. Please show it to Dickson, too, it's such a visual paper, I'm sure he'd love it. I will, he's not here today, I'll show it to him tomorrow. Alright, take that last one, Michael.

Michael: Alright, so Joyce writes:

Dear twim rock stars,

Great podcasts! I love them and learn so much valuable information.

I am trying to listen to all the past episodes of all of the podcasts and I was just listening to twim79 and the discussion of the coral reefs and the effects of climate change.

And I too find a lot of what is going on with regard to climate change ti be depressing, but since I have joined citizens climate lobby I have so much more hope. I hope you will look at their website and see how fast they are growing and all they have accomplished in a short time and will mention this on your Podcast.

We, as citizens, can solve this problem, we just have to take action and this organization tells you how you can help accomplish the goal of slowing and then reversing climate change. Even if you don't have that much time, there is a lot you can do.

See https://citizensclimatelobby.org

There are a lot of great organizations out there working on this issue, but I believe this one has the most practical and focused approach. Their efforts have already built a bipartisan climate caucus in Congress that continues to grow. Their philosophy is that climate change is not and should not be a political issue, both sides need to work together to solve this problem.

Thanks for all you do,

Joyce Waterhouse, PhD

Thank you, Joyce.

Vincent: That should be, not be a political issue. Absolutely. In fact, science should not be a political issue.

Michael: I mean, science has given us such great things like iPhones, and hot running water, and cold water, and potable water. I just came from the ID, infectious disease grand rounds, where they were talking about the new hepatitis B vaccine, and Julie Westering who was giving grand rounds today said that the two greatest things of the 20th century were vaccines and clean water. And I think she's right, a clean and available water has really done a lot to address the issues of public health, and vaccines have indeed done a lot in the 20th century. It's truly one of our modern miracles of the last century.

Vincent: Dickson would add to that the toilet, another great invention.

Michael: Oh, that's true too, because you didn't have to carry the stuff. I mean, if you think about it, by effectively moving the chamberpot out of your bedroom, if you had a chamberpot, or going to the outhouse where the fecal material and urine would effectively hit groundwater, it really did a lot by creating a sanitary sewage system and sewage treatment plants. All of which are going to be infrastructure that we desperately have to replace as the United States is aging its infrastructure pretty quickly now.

Vincent: People used to take those chamberpots and throw them out in the streets, right?

Michael: That's right! Out the window!

Vincent: That's why everyone got polio at an early age.

Michael: That's right.

Vincent: When they were still protected by their mother's antibodies, but then we had developed toilets and we delayed the infection and that started epidemics of polio. Interesting, isn't it?

Michael: It's absolutely fascinating, that and the helicobacter loss in the developed world, which started at the turn of the 19th to the 20th century along with the introduction of indoor plumbing.

Vincent: So that loss gave us ulcers.

Michael: Gave us ulcers and more importantly, asthma.

Vincent: That's right, asthma.

Michael: Because the virulent form of helicobacter is also been implicated that you need it when you are a toddler to prevent the onset of asthma, and eventually through selection you get rid of it by the time you are a teenager, unless you don't get rid of it. Then you will develop your ulcer in your 20s and

30s and then unfortunately, if you don't delete it by then then of course it will go into a carcinoma of the stomach.

Vincent: But if you catch it early enough you can treat it and get rid of it.

Michael: Well no, you eliminate the risk of asthma or you greatly reduce the risk of asthma, and it gets selected out as your gut matures.

Vincent: Right, but if you have a person that doesn't lose the helicobacter and develops ulcers, I remember growing up and everyone had their ulcers cut out by surgery.

Michael: And we put them out of business.

Vincent: Right, we put them out of business because now you just take an antibiotic, right?

Michael: An antibiotic, and a proton pump inhibitor together.

Vincent: Amazing, amazing. I'm telling you, my grandfather and my grandmother both had surgery, although my grandfather took tagemetol all his life.

Michael: Oh my.

Vincent: That's a proton pump inhibitor, right? But no antibiotics because they didn't know about it.

Michael: They didn't know until the mid 1980s when the Australian pathologists fulfilled Koch's postulates.

Vincent: Yes! Didn't he get a Nobel prize for that?

Michael: He got a Nobel prize and he would be sent to IRB jail today.

Vincent: He would, yes, you can't do that anymore.

Michael: I tell ya, this is why science is so important. And why experiments are often good.

Vincent: For sure. And that's why we do TWIM and all the other podcasts, to try and share our excitement and knowledge about science with all of you out there, so tell everyone about it. And if you like what we do, consider supporting us at microbe.tv/contribute, there are a number of ways you can do that. You can find TWIM on any podcast player, and usually you can find it by searching and subscribe, please, so that you get every episode, and if there is a way of favoriting a podcast on your podcast player please do that, that helps us as well. I'd also like to ask you if you could subscribe to our YouTube channel, youtube.com/profvrr because YouTube is giving grants for educational science videos and you need 25,000 subscribers to be able to apply, and microbe.tv has 16,000 as of this writing and I'm making an appeal. So just go over there, youtube.com/profvrr, subscribe, it's free, and it will help us to be able to apply for those grants. Of course if you have questions and comments you

can send them to twim@microbe.tv. My colleague today has been Michael Schmidt, Medical University of South Carolina, thank you Michael.

Michael: Thank you, Vincent, and I just clicked the subscribe button to your YouTube channel.

Vincent: Thank you Michael, I appreciate it very much. If we hit 25,000 I think we are around 16,000 something.

Michael: You're 16,140.

Vincent: That's good because yesterday when I started the appeal on Twitter we were just over 15,000. So it has helped, although we do have many thousands to go before I sleep. (laughs)

Michael: Good grief.

Vincent: Youtube.com/profvrr. I'm Vincent Racaniello, you can find me at virology.ws. I want to thank ASM for their support of TWIM. This episode of TWIM was edited by Ray Ortega, of ASM. I also want to thank Ronald Jenkees for his music. Thanks for listening everyone, we'll see you next time on This Week in Microbiology.

(music)

Transcribed by Sarah Morgan