This Week in Microbiology

With Vincent Racaniello, Elio Schaecter, Michael Schmidt

Episode 174: A gathering typhoid storm

Aired April 19, 2018

http://www.microbe.tv/twim/twim-174/

Vincent: This week in microbiology is brought to you by the American Society for Microbiology at asm.org/twim.

(music)

Vincent: This is TWIM, This Week in Microbiology, episode 174, recorded on April 12, 2018. I'm Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Small Things Considered, Elio Schaecter.

Elio: Hello there, how are you?

Vincent: Well, and you? Everything well?

Elio: Hanging in there!

Vincent: Hanging in there, that's what they say.

Elio: It's easy to do in San Diego this time of the year.

Vincent: You seen any mushrooms lately?

Elio: Actually, would you believe, we live in a retirement residence and on the front right by the street is a bed of grass and mulch and some morel mushrooms there.

Vincent: Wow.

Elio: Which is not bad.

Vincent: Is this the time of year they would come up?

Elio: Yep, around here, when they come up—it's the place they're getting water. Otherwise in San Diego we have problems with mushrooms.

Vincent: Also joining us from Charleston, South Carolina, Michael Schmidt.

Michael: Hello everyone!

Vincent: Got any mushrooms there, Michael?

Michael: Oh we got loads. It's been raining and they sprout like weeds. We have the scary mushrooms that take out your liver and so…

Elio: Really? The Cortinarius?

Michael: Yes, I remember when I was first here--

Elio: Amanita, I mean, Amanita, don't you?

Michael: Yeah, Amanita, they take out the liver. We had a tragedy where there were a bunch of teenagers who were out in the farm fields near Orangeburg, South Carolina, and they were picking mushrooms off of cow patties and they, unfortunately, the young girl unfortunately took out her liver. But fortunately her mother was able to donate a piece of one of her lobes of her liver and so both the young lady and the mother survived.

Vincent: Wow.

Elio: That's scary.

Michael: It was very scary, and the child was lucky the mother was compatible.

Vincent: That's always an account of alpha amanitin, an inhibitor of RNA polymerase.

Michael: Yes.

Elio: That's right, that's how it works.

Vincent: Today, after our two papers, we are going to give away a copy of Clinical Laboratory Management. We have your emails and we are going to pick one at random. I also want to tell you I have a note from the organizer of the 19th annual microbiology students symposium at the University of California Berkeley. They want you to know about their one day event which will be held on campus on April 28th. This is organized entirely by hardworking and dedicated graduate students and showcases student research through oral and poster presentations. Each year the students vote on two keynote speakers who are leaders in the field of microbial ecology and molecular biology. This year they are hosting doctors Kelly Wrighton from Ohio State University and Melanie Blokesh from the Ecole Polytechnique Federale de Lausanne in Switzerland. The organizers are always growing the symposium, they want to encourage any interested listeners who live in the Bay area to attend. Last year they had attendees representing 19 institutions from the Bay area. The symposium is free and they have a limited number of travel grants for students who need childcare or funding for their trip to Berkeley. Registration and more information can be found at nature.berkeley.edu/calmicro.

Elio: You know, the graduate students in Boston do something very similar and I think it's soon. Unfortunately I don't have contact information but if you, if somebody were to google microbiology Harvard they would find it.

Vincent: Okay, and they organize a similar meeting?

Elio: Yeah.

Michael: Those are generally the best meetings to go to because they are free, you hear the next generation talking about the science that they're pursuing, and often if they have a sufficient budget and they reach out to the name speakers early enough, they can get some really marquee type speakers to come in and they sort of all let their hair down and you hear some of the best talks you will ever hear.

Elio: That's true.

Vincent: Check it out. Alright for you today we have snippet and paper. And Michael Schmidt is going to give us a snippet.

Michael: So today on TWIM we have enteric fever and the gathering storm of whether or not an untreatable typhoid fever is on its way to a country near you. So in the snippet, we are going to talk about two manuscripts, two manuscripts in this snippet. The first is by Levine and Simon from the University of Maryland School of Medicine, and it is a commentary that had appeared in MBIO. And the second is the paper with all the data that also appeared in the February issue of MBIO. So both of these are open source. And when you sit down to read these papers, I would encourage your first if you are unfamiliar with the terror caused by enteric fever, to first read the commentary as it provides a very sobering background that will help put the significance of the content associated with the primary paper into really effectively a shot heard round the world. And that is why I picked these two to talk about today because it is really pretty frightening what's happening. So the commentary is by Myron Levine and Raphael Simon. And it's, as I said, they're from the center of vaccine development at the University of Maryland. And it's specifically comments on the data associated with the second paper, the emergence of an extensively drug resistant Salmonella enterica serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third generation cephalosporins. And this again was published in MBIO on the 20th of February and it is authored by Elizabeth Klemm--

Elio: You don't want to read them all, there's too many.

Michael: Alright, I won't read them all, let's just say Klemm. But its a really internationally recruited group of individuals from the Wellcome trust, the United Kingdom, the Aga Khan University in Karachi, Pakistan. And it's literally the who's who of these nations' infectious disease investigators. So here's the story. Klemm and colleagues conducted a comprehensive antibiotic sensitivity survey in concert with genomic analysis of this particular strain of salmonella that causes typhoid fever. And they did this principally from blood culture isolates during an epidemic of typhoid fever that was occurring in Pakistan at the time. As you might expect from the title, the microbiologic and genomic data suggests that the serovar salmonella responsible has become extensively drug resistant or is now considered an XDR microbe. An XDR is just below the step where you become TDR or totally drug resistant. And so the data with their paper supports--

Elio: It stands for--?

Michael: Extensively drug resistant, XDR. The same abbreviation is used for things like tuberculosis. And so the data within their paper supports this frightening scenario in which we might see the return to the battle days where Salmonella typhi, and I'm just going to truncate it and call it salmonella typhi rather than the god awful taxonomic proper nouns with enterica serovar typhi. The battle days, according to the CDC, typhoid fever is a life threatening illness caused by this bacterium. About 5,700 folks in the US even today will come down with this disease each year, with the majority of the cases being acquired as a consequence of international travel, about 75% of the 5,700 are a consequence of this international travel.

Vincent: The others are locally acquired, is that right?

Michael: Locally acquired, probably case context within the house because again, this disease is fecal oral. And if you are one of the unfortunate ones that becomes chronically infected with this microbe, you can continue to shed this organism fecally, and if you are preparing food for your family or working in a restaurant, and you fail to follow proper hand hygiene, you can actually easily transfer this organism between people.

Vincent: Typhoid Mary.

Elio: That's the story of typhoid Mary, right?

Michael: We are going to talk about typhoid Mary, or we can talk about typhoid Mary now, whichever you prefer, but I'm gonna put typhoid Mary in the context when we get through some of this background. Now the WHO estimates that there are between 130,000 and 160,000 individuals who will actually die each year from typhoid fever. So this is up there with the number of people who die from hospital or healthcare associated infections. Now symptoms include prolonged fever, fatigue, headache, nausea, abdominal pain, and I'm talking significant abdominal pain. And here's the wildcard: constipation or diarrhea. It's either one or theother. About three in ten patients will develop the hallmark rash with the rose spots. And it's one of the classic presentations of rash associated with bacteria. And so I went out and picked up a picture for the show notes so you can see what these hallmark rash with rose spots actually look like, and the severe cases may lead to serious complications.

Now in the battle days, before antimicrobials, typhoid fever had a case fatality rate of 15%. That's effectively only half of the death rate associated with smallpox or in the modern era something like Ebola, where the death rate is 30%. So Levine and Simon in their commentary set the stage of why we need to be worrying about this extensively drug resistant variant of this microbe that has arisen in Pakistan. Now the danger is that most clinicians and clinical microbiologists have forgotten this lethality that typhoid fever exhibited in the pre antibiotic era, 70 years ago effectively. On the surface it comes across as an acute generalized infection of the gut associated lymphoid tissue of our reticuloendothelial system and it can actually infect our gallbladder. When I teach the enteric diseases to the dental students and the medical students, I always put diarrhea into three buckets. The first bucket has the diarrhea in which you can literally ride them out on your toilet. They're typically self limiting, it's an enterototoxic, you get an E. coli that is manifesting a toxin that isn't very significant and you effectively ride it out and you treat yourself.

Elio: Staph too? Staph aureus.

Michael: Staph can do that as well. The second one is where you develop a true infection and that you can note by cramping and the diarrhea is a little bit more severe and you may need antibiotics in order to get rid of it. And the third, this is where Salmonella typhi falls in, is the form of diarrhea that goes systemic. This is where the organism can invade our system and this is effectively what Salmonella typhi does, is it invades through our reticuloendothelium system and can set up housekeeping on our gallbladder and do all sort of bad things. You actually get a bacteremia and in some cases you can become septic. It was so bad that the journal, the American Medical Association or JAMA, would publish yearly updates and 5 year summaries of typhoid disease for the most populous cities in the US and in fact this is the first reference in the Levine paper. And it is an anonymous report, probably from the editorial staff at JAMA in 1914, describing typhoid in the largest cities of the United States.

Now this all miraculously changes in 1948. And this is an interesting story that they again bring to our attention about Theodore Woodward from the University of Maryland School of Medicine who was conducting a clinical trial to determine the efficacy of the antibiotic chloramphenicol to treat scrub typhus. Now typhus and typhoid fever are different. Typhus is caused by an obligate intracellular parasite that is effectively a tick borne disease. And scrub typhus is caused by Orientia tsutsugamushi which is a gram-negative alpha proteobacterium from the family Rickettsia. And what happened is that the people enrolling individuals into the clinical trial for scrub typhus inadvertently enrolled people who had typhoid fever. Today those people, they wouldn't even have talked about those data, they would have been excluded from the data analysis of the clinical trial. But here's where Woodward said never look a gift horse in the mouth, and what he learned from looking at these patients who were actually unknowingly enrolled is they responded well to chloramphenicol, and as they say the rest is history.

Case fatality rates due to typhoid fever crashed from 15% to less than 1. And it was like other diseases successfully treated in our golden era of antimicrobial therapy. It was wondrous, it was a miracle, and it highlighted all good things that were occurring subsequent to the end of the second world war. What we witnessed over the next seventy years is of course how evolution and single drug therapy can lead to the emergence of the resistance of the wonder drugs that we all came up with in the golden era of antibiotic development. So here's where we transition in the Levine and Simon paper, where they describe the resistance patterns in the plasmids. And this is where it gets to inside baseball about resistance plasmids and incompatibility group plasmids.

Now while R factors could be indeed transferred into Salmonella typhi, they could never be stably maintained. And you never use the word never when it comes to microbes, because eventually they figured it out. And that's part of our story here. But for the most part this incompatibility group H1 in the early 1970s finally acquired a resistance trait for chloramphenicol. And it was maintained on the IncH1 plasmid which then resulted in a large epidemic in Mexico, in Vietnam, and then a decade later in Chile and Peru. As this was the golden age of antibiotics, well, resistance to chloramphenicol was devastating to the people afflicted. We soon had another antibiotics that were found and recommended to treat typhoid fever. And these of course were ones that you all likely heard about, ampicillin, amoxicillin, and trimethylprim sulfite. Things again changed in the late 80s and early 90s with the emergence in Southeast Asia that Salmonella typhi is now harboring the heretofore unable to harbor R plasmid. But now this R plasmid is encoding resistance to the most of the first line antimicrobials.

But this was still the era in which antibiotics were being brought to market, and fortunately at that time it was the introduction of the fluoroquinolone class of antibiotics which inhibit another target other than the ones that were being targeted by these early antibiotics. And the target that ciprofloxacin and the other fluoroquinolones is effectively attacking the DNA gyrase. And this, the gyrase class of inhibitors, were found to be this remarkable drug in that they were able to effect high clinical and bacteriological cure rates, and very few of the patients treated with the fluoroquinolones ever became chronic gallbladder carriers, whereas the other drugs, some of those individuals did indeed succumb and become chronic gallbladder carriers.

And so either you had to increase the course of antimicrobials or the therapy at the time was to take someones gallbladder if you couldn't get it out with the antimicrobials. So this was really a wonder drug, and so indeed a four week course of a fluoroquinolone was approximately 90% effective in curing chronic typhoid gallbladder carriers without the cholecystectomy or taking out of their gallbladder. Now like with all antimicrobials, inappropriate treatment courses and the widespread use led to selection in mutation sin the chromosomal gyrase site. So as we approach our millennium, we are faced with multi drug resistant variants of the microbe. So here again an antibiotic against a cell wall target, the cephalosporins came into use, especially ceftriaxone.

So as we are approaching current time, there were occasional reports of patients being infected with ceftriaxone resistant Salmonella typhi, but fortunately none of these resistant strains ever started a large outbreak nor were they endemic in areas where they were found. That's until now. And here is where Klemm and colleagues fire this shot that is heard round the world. First they describe a sizable epidemic of typhoid fever in Pakistan. It's caused by this strain carrying an array of both plasmid borne and chromosomally encoded resistance genes. Not one, but many resistance genes. And they can collectively transfer resistance to the majority of our first line oral antimicrobials used to treat typhoid as well as the IV delivered ceftriaxone. The only remaining reliable oral first line antibiotic left in our kit is azithromycin. Azithromycin that many of us in the US know as the wonder ZPak is this remarkable antibiotic that is acid stable so you can take it orally without any need to protect it from gastric acids, and in fact it works better on an empty stomach. And the remarkable thing about azithromycin is it's very active and accumulates or concentrates into phagocytes.

And if you know anything about the biology of salmonella typhi, it's a facultative intracellular parasite living inside our cells. And it can actually live in phagocytes, so consequently azithromycin is this wonder drug because it can effectively treat the cell in which the organism happens to be hiding in.

Elio: I'm allergic to it as all get out. I hope I don't have to use it. I couldn't use it.

Michael: In spite of Zpaks being our hope in Pakistan, what is most concerning is that we've seen resistance to azithromycin treatment have already been reported in Southeast Asia. So the nuts and bolts of their data is they sequenced and evaluated a large number of the blood culture isolates from the epidemic over this period of time that the epidemic was racing. And what they learned is this strain carries a composite transposon that is integrated into the chromosome and encodes all these multiple resistances to chloramphenicol because you can have more than once gene conferring resistance. So it's multiple resistance to chloramphenicol, amoxicillin, trimethoprim sulfate, and it carries a single mutation in the chromosomal gyrase A gene as found in many other H58 strains circulating in Southeast Asia.

That confers intermediate resistance to ciprofloxacin or Cipro. In addition, this particular H58 clone carries this inkY plasmid. Now the inkY plasmid is a big, big plasmid, it's not like your garden variety cloning plasmid. This is 85 kilobases and this plasmid has got everything in it except instructions on how to build a plane. I mean, it included plasmid quinolone resistance genes, the beta lactamase CTXM15 gene which encodes an extended spectrum beta lactamase conferring resistance to ceftriaxone, and it carries a transfer locus that allows self transmission of this plasmid to other Salmonella typhi and para-typhi lineages. Now if this wasn't bad enough that it had all these resistances, this happens to be the strain that has the ability to displace endemic strains in your community. So if I get on a plane and I am typhoid Mike, and I travel to your city and I make you, the episode shouldn't be called Typhoid Mike.

Vincent: I was thinking about it, it's a good one (laughs)

Michael: I know! If I travel to your city and I am making food for friends at a house and I am actually shedding it it can spread very readily in the community and what this research group learned is from public health service to the United Kingdom, one of these isolates from Pakistan has already made it to Britain.

Vincent: That's surprising, there's not a lot of traffic back and forth between those countries, right?

Michael: No. And you just get the sense, looking at this data, this is not good. And in fact, the collective results and the term they use is panoply of chromosomal and plasmid antimicrobial resistance genes, is that this outbreak in Pakistan with this sort of organism encodes resistance to all main antimicrobials that have been considered first line drugs to treat typhoid fever during the past 70 years. And if when we look at this, you can now see, and this is where I am going to bring in typhoid Mary because typhoid Mary lived between 1869 and 1938. And this is this success story of shoe leather epidemiology.

She was the first person identified as an asymptomatic carrier of this pathogen. She is thought to have infected 51 people who she was cooking for, 3 of whom died over the course of her career, and it was shoe leather epidemiology promulgated by Joel Soper who effectively figured it out. And he ended up incarcerating or quarantining Mary between 1907 and 1910. This woman had committed no crime other than cook, and she transferred this organism to these people. And like some politicians in North Carolina that we talked about once, she didn't understand hand hygiene. Nor would she consent to having her gallbladder removed, and then under New York City code she was quarantined. And thats New York City Code 1169-1170.

And she was literally in isolation on the North Brother island which is next to, it's in the East River, Vincent can probably see it from his office. She was there for three years. Eventually Eugene Porter, who was the New York

public health commissioner for the state, let her out, she swore that she would never cook, she took a job as a laundress, but she lied, changed her name, and she returned to cooking and so then that began the hunt to quarantine her for the second time.

Vincent: Wow.

Michael: Mary is literally a hundred years ago and Klemm and colleagues are saying okay folks, we may not have someone as famous as Typhoid Mary, but Klemm and colleagues employed many of the same techniques in the last century during this outbreak, albeit with very sophisticated molecular tools. And you get the sense of outbreak tracing when you look at their remarkable first figure and they show you the XDR, the extremely drug resistant samples, are separated by 6 single nucleotide polymorphisms that rest on this blue branch, so it's literally tracking where these microbes came from. And it is just absolutely frightening, and they conclude their call to action by matter of factly stating the emergence of spread of XDR typhi in Pakistan and the startling demonstration of how ubiquitous antibiotic resistance plasmids can be acquired by the MDR Salmonella typhi, rendering it extensively drug resistant, narrowing our treatment options, really is a call to action that we really need to think about investing in sanitation, namely sewage treatment plants, as well as water distribution and again reinforcing the importance of hand hygiene in the food preparation areas.

Now, I know there are some individuals out there arguing about small government and we don't need to tell people they need to wash their hands, but we really do. I hate to end on that bad note, but fortunately, there are vaccines available. There are two, there is a heat killed and there is a live attenuated vaccine, and in the show notes I will put the CDC's recommendations on the vaccine and dosings. Unfortunately the vaccines don't seem to be very effective for very long but they will protect you if you are traveling to areas endemic. So I think in addition to the shot heard round the world it is really dropping the gauntlet to really improve the efficacy of the two licensed vaccines that are out there, and I have the greatest hope for the oral typhoid 21A vaccine because I think things are getting much better.

So the bottom line is wash your hands, drink clean water, and hope if you eat out the cooks and food servers are not carriers or even if they are, they wash their hands so as not to contaminate your food. Even if less government types don't think we should have to legislate hand washing. So that's a snippet.

Vincent: (laughs) That's pretty grim.

Elio: Well done.

Vincent: The total drug resistant TB, has that spread globally?

Michael: No, and I think it is because it grows so damn slow.

Vincent: How about this XDR, does this grow slowly, apparently not, right?

Michael: This is a god-fearing enteric. It has generation times measured in minutes.

Vincent: A god fearing enteric, that's great.

Michael: It's out there. And the thing, I think, that is really remarkable is that for many years, salmonella had this incompatibility group plasmid that was keeping the resistance plasmids out of it. But then in the, as we transition towards the beginning of this century, the R plasmid, the resistance plasmids that have these transposons became, figured out how to stay inside the salmonella. They could always get in there but they would be quickly kicked out. But now they figured out how to stay stably maintained and the only thing left that

we have is now we go to the things like the carbapenemases, or some of the more expensive drugs, and those things are not administered orally, they are administered by IV. So even for us folks in the developed world it is not going to be a cheap disease to treat because you are going to need hospitalized or go to an infusion center in order to get the drugs.

Vincent: I think part of the problem is that antimicrobials were just too easy and a lot easier than just cleaning up and not getting fecal contaminated water. That's really the key, you need to clean up and not dump feces into drinking water, it's really hard to do.

Michael: That's what worries me. The clean water act was at the end of the 60s, beginning of the 70s, and that's been 50 years ago that we had a substantial influx of capital to improve our sewage treatment plants in the country, move off of septic, and to make certain that we had good potable water being delivered to every household. So I think our politicians need to think about infrastructure and simple things like water and sewage treatment are big ones that I think we need to worry about again.

Vincent: You better tell the EPA because they don't seem to care.

Michael: (laughs) They will if they get Salmonella typhi because it is not a pleasant disease to have.

Vincent: Have you had it?

Michael: No, but I have diarrheal diseases where I was bent over with gastric pain.

Vincent: I have too, I went to France once and I had something which was horrid. I don't know what it was, but it lasted about a week. Food borne, I think I got it on the plane.

Michael: That was bucket number 2, so those are the ones where it's a self limiting infection.

Vincent: Yeah, it doesn't go systemic. I learned a lot Michael, thank you.

Michael: You're welcome!

Vincent: I love the god fearing enterics, that's great.

Michael: (laughs) Well Typhoid Michael is…

Vincent: We won't use that, but it's a nice phrase. However, you haven't had typhoid, so you're not—Elio, you ever have typhoid?

Elio: I haven't, though I lived in a country that had plenty of it.

Vincent: Alright. Thank you, Michael.

Michael: Thank you.

Vincent: I want to tell you about the upcoming ASM Microbe, the annual meeting of the American Society for Microbiology. Anyway, this year it is in Atlanta, Georgia, and ASM has a special opportunity for our podcast listeners. Get 50 dollars off registration for Microbe 2018, which is June 7-11 in Atlanta using the promo code ASMPOD. ASM Microbe 2018 connects scientists with their science and showcases the best microbial scientists in the world. Delve into your scientific niche in 8 different tracks. Don't miss this opportunity! Visit

asm.org/microbe. That is asm.org/microbe and use the promo code ASMPOD. All one word, for fifty dollars off registration. When you go to register you will see, do you have a promo code? You type in ASMPOD.

Now for something completely different, we are going to talk about amphibians and fungi.

Elio: Oooh.

Vincent: Fungi are one of Elio's favorite things and everyone likes a nice frog, right?

Michael: They're cute!

Vincent: Cute. And this is a paper published in Science in March 2018, it's called "Shifts in disease dynamics in a tropical amphibian assemblage are not due to pathogen attenuation". And the first author is Jamie Voyles and the last author is Corrine Richards. They're from University of Nevada, University of Massachusetts and Boston, the Smithsonian Tropical Research institute in Panama. University of Pittsburgh, UC Berkeley, New Mexico Institute of Mining and Technology, Arizona Game and Fish department, Vanderbilt University, and a few other places in Panama.

Elio: A couple of field stations in Panama.

Vincent: Yeah, a few field stations in Panama, exactly.

Michael: In the canal, probably.

Vincent: No, this map here, I'm not sure where the canal is but there are 3 sites in Panama and this is all about the fungus that is wiping out amphibians globally. It is the Chytrid fungi, chytridiosis, and we've talked about this recently on TWIM but *Batrachochytrium salamandrivorans*, that's the one causing a collapse of populations of salamanders in Europe, and the other one is *Batrachochytrium dendrobatidis*, this is the one causing the lethal skin disease of frogs. It's a fungi. It's a Chytrid fungus, phylum Chytridomycota, there are about a thousand different species, they live in water or moist environments. They are among the oldest kinds of fungi, and as I said they are causing global declines of amphibian species. They are spread by the amphibian trade. You move frogs around the world because you want one and its cute and you introduce the fungus to a population that has never seen it before and the population collapses. And this is a skin disease, and in this paper they are going to use this in particular an outbreak in Panama to understand, how do infectious disease outbreaks end?

Typically you have an outbreak, you have an epidemic or epizootic phase, and then you transition to a phase where the host and the pathogens coexist this is the endemic or enzootic phase. They want to know what are the mechanisms that underlie this transition, and typically this involves not just the pathogen but the host and the environment. It's a little tricky. But this system which they use to study this question is interesting because you have the frogs before and after the epidemic and you have the fungus before and after the epidemic. So you can do all the infections, of course you can't do that with people, and people outbreaks, you cant get people and reinfect them and see what's going on. You have to have animal models like this. So that's a cool part of this study, they are using this decline of the amphibian population to address this question. The story is that over ten years ago there was a series of amphibian declines at three sites at Panama. And these have been well studied. They are El Cope, EL Valle, and Campana. And during these outbreaks they saw increases—what's that?

Elio: El Valle.

Vincent: El Valle, thank you. (laughs) During this outbreak they saw increases, I am going to abbreviate the Chytrid fungus as BD.

Michael: Call it BD, thank you.

Vincent: Beckton Dickinson—BD.

Michael: You're going to get letters for that.

Vincent: So the BD levels went up, the amphibian densities went down, and these three sites have been studied for a long time by field surveys so they have a lot of historical data but between 5 and 13 years between the original outbreak some of the frogs began to recover, but BD was still around. So the idea was it was an epidemic and then there was a shift to an enzootic phase where the frogs and the fungi are coexisting. So they want to know what's going on here. And they say, well here are the possibilities. The pathogen lost virulence, maybe the host resistance increased, or maybe both. So they do a bunch of studies in this paper to try and get at this. And they use, this is a quaint little paper, they call their work garden variety experiments.

Michael: (laughs) I love that.

Vincent: Actually they say garden variety and genome sequencing. So the genome sequencing is not garden variety but everything else is and a lot of the paper is written, if you read the methods it's all we did this we did that. It's very different. They have 12 species that live near the riverbanks and they were nearly wiped out by BD. And they have a graph showing the pre outbreak numbers of these frogs, they have about 12 different species, you can see they went way down during the outbreak and then some of them are coming back again. And what are these, these are interesting frogs like the Harlequin frog, the Rocket frog, these very interesting colorful frogs. And now, the composition of this frog community is sort of what it was before disease broke out.

So that's a good thing because we are afraid that these fungal outbreaks were gonna wipe out amphibians. I think over a hundred species have already been extincted by this. So understanding what is going on is important. So that was one part of the paper, they did a survey of what frogs are still there. The next thing they do is they sampled the frogs, they take skin swabs from these animals because that is where the fungus is, on the skin. And then they look for it by PCR. So they collected 2,035 samples and they found that the prevalence of BD has gone down because they have some samples from pre epidemic phase and during the epidemic which they can compare from the literature. They see that the BD has gone down, just the number of frogs that are infected is much less. So that's part one. Next, how about the pathogenicity of BD as it changed over the years? So they have some isolates from 2004 during the epidemic they call those historic isolates, and then they have isolates of the fungus from 2012 to 2013, this is after the population has recovered. They call this contemporary isolates. They have 3 of each.

They measure the growth of the fungus, the size of the spore case which is the zoosporangium, and the numbers of infectious zoospores, which are motile asexual spores that have flagella. No differences between historic and contemporary samples. Next, a big part of the growth of the fungus is the ability to evade host defense mechanisms. We know that the skin of these frogs have antimicrobial substances that will kill the fungus. So, they ask well is there any difference in the ability of the fungus to grow in the presence of this? So they get skin secretions or the supernatants of 18 different skin bacteria, these are bacteria from the frog skin that are known to inhibit BD, and they add it to BD samples and assay growth. And there is no difference in the inhibition comparing the old to the new ones. So the fungi have not changed in their ability to grow in the presence of these skin antimicrobials.

The fungi also make factors that kill B and T cells, that is a part of the reason they are so pathogenic. So, what they do is they take the supernatants of these fungal strains, they add it to T and B cells and they measure proliferation. No difference. So the old strains of fungi and the new ones, they will both kill B and T cells equally well. So there has been no change there, either. Are we okay so far?

Michael: Yeah, and it's reminiscent of how it is not the same but it is reminiscent of HIV in humans. HIV kills our immune cell and it could potentially be species ending as well if it gets out of control. And so this paper is interesting because it helps you think about what is going to have a long term, going to happen with HIV and the human dynamic and if we were lucky we would be through of HIV but we are not as lucky as the frogs.

Vincent: Well here we can do experiments that we can't do in people, that's the beauty of this. You can try and learn something---

Elio: I was thinking, is it possible to think back historically about diseases that have disappeared, because if this gets, if you carry to a logical conclusion resistance by the host made, may have rendered the pathogen ineffective or get rid of it. So I am wondering, I don't think I know of any cases like that were the disappearance of the disease was not due to immunization or antimicrobials. Can you think of some?

Vincent: That's a good question, I thought the same thing. I can't think of any viruses that have gone away. What about bacteria?

Elio: Yeah, if you were a clinical microbiologist in the year 1000 you would probably be isolating bugs which you had never heard of today.

Vincent: Yes, of course, we don't have that historical data.

Michael: I guess the only thing that would immediately come to my mind was the incidences of scarlet fever because that has waned, but that's been, that comes on a lysogenic phage and that's just phage dynamics, so being excluded from the population and antimicrobials have of course gotten rid of those organisms are quickly depleted them before they could spread.

Elio: The point is that biology being what it is and evolution being what it is some diseases will disappear because the host will adapt to them just like the frogs did. The time frame is enormous and in humans it is very very long but it will happen.

Vincent: I agree but now we have in humans the complication that we intervene, right?

Michael: Yes, we tinker.

Vincent: So it's hard to know what would happen otherwise. But here we have a population where we haven't tinkered yet and we can look and they do rebound in some cases, so that is interesting to look at. But really in the end, as you will see, it is all about immunity. Next experiment, they said has the pathogenicity of BD changed? And they get two different frog species, now this is where I worry a little bit about the results. They get two different frogs that they can buy. One of them is Atelopus varius, which is descended from frogs that are near El Cope which is one of these sites. In 2002, before the emergence of BD. So this is supposed to represent what is there before the outbreak and I guess that is okay, it's the best you can do. And they infect them and they can put them in baths with spores in them and get them infected. And then they compare that to, they compare the old and the new BD isolates and there is really no difference in their ability to infect this frog. The other frog they use is Litoria serulia and this is a common frog, I'm not sure that what, it's a common

green tree frog, I don't know if this frog is present in the area but this is purchased also so I don't know if that is relevant.

Again, there is no difference in the two isolates but I just think it would be better to have frogs completely captured from the site but I suppose that would be hard because they would be BD positive and so forth. And in the transmission experiments, they would infect the frog, put it in a bath with another frog and see transmission. There is no difference between these historic and the contemporary BD isolates. They sequence the genomes of these fungi, the old and the new fungi, whole genome sequencing, they looked at 25 thousand single nucleotide polymorphisms, they say nothing stood out as being really different when you compare the old ones and the new ones. And that's not garden variety by the way, that's deep sequencing. (laughs)

Michael: It was deep.

Vincent: And the last experiment they look to see if there are any differences in the frog's ability to defend themselves against infection. So they take skin secretions and they have post pre and post epidemic and they incubate it with the fungus and they see differences. The first time they see a difference in this paper. There is more, and they have a lovely graph where they, unfortunately you won't be able to see it, but I'll tell you what it says. If you compare pre and post disease frogs, you see that the frogs from, they differ in their ability to inhibit the fungus. So they have this graph showing pre disease and post disease inhibitory effectiveness. So frogs pre disease don't have a lot of ability, their skin secretions do not have a good ability to inhibit the fungus. But frogs post epidemic have a pretty good ability to inhibit the fungal growth.

And in fact, they did a cool experiment where they compare the skin secretions of captive frogs with that of wild frogs and the captive frogs skin secretions don't really inhibit the fungus at all whereas the wild frog's skin secretions do. So they think that there has been some shift in host resistance of the frogs, and that is why they are recovering, because the fungus has not apparently changed, but the frogs are recovering and they think it is because of resistance. Maybe in part from the skin secretions. The skin has antimicrobial peptides that are produced by the microbiome, so it would be interesting to look at that and see what is going on. I suspect there is also something inside the frog that is happening as well, like adaptive immunity that is slightly different. And then the final question is--

Elio: Did I hear you right, you said the antimicrobial peptides are all made by the microbiome? I wasn't aware of that, is that the case?

Vincent: No, most of them are made by bacteria that are on your skin.

Michael: Stuck to the skin.

Vincent: Yeah.

Elio: Defensins and cathelicidins are made by us, not by bugs.

Vincent: Yeah, some are made by us, but some are made by bugs.

Elio: Why?

Michael: The magainins.

Elio: The first one known, gramicidin.

Vincent: Antimicrobial peptides are a part of the innate immune response. Fungi, yes, bacteria, let's see. Some are made by bacteria, I'm pretty sure. Maybe not all of them.

Elio: They are but I think the most important ones in our body are made by us, by the granulocytes for us. Anyhow.

Vincent: Yeah, so anyhow, they were originally called bacteriocins, right?

Michael: Yes.

Elio: That's right.

Vincent: Because they stop, okay. It would be interesting to sort that out. Now here's a good point, remember the frogs were wiped out by the fungus and then they returned. Where did those frogs come from? They didn't just spontaneously generate, right?

Michael: No.

Vincent: So they think that they came from somewhere else, from lowland areas, right? But the thing is they have looked at those frogs and they do not have skin secretions that are resistant to the fungus. So that does not quite make sense. I think, and they suggest this also, that there were a few frogs left. They didn't catch them all, they missed a few.

Michael: The fungus missed a few.

Vincent: Yeah, exactly.

Michael: Because it didn't crash the entire population.

Vincent: Or there were a few resistant frogs that were infected and they were left over and then they reproduced and that is what we are seeing nowadays. Because I'm not sure they could get every last frog, right, during the epidemic? Who knows. Either way it is something to do with immunity which is good because it can tell us if we can figure out what it is we can help some of the populations that are crashing now. And I was looking around the literature and other people have been studying the kinds of immune responses to this fungus. It's more than just a skin secretion, so I think it's more complicated than they make it. So the bottom line is it looks like the frogs have become somewhat resistant to the fungus, the fungus has not changed, and in this particular site the frogs are coming back which is good. So maybe we can learn something and help frogs elsewhere.

Elio: I think it is a great food for thought and one has to think about evolution of infectious diseases in al systems including ourselves. Obviously having an animal with a very short life span, life cycle is easier to handle than humans where the time scale is so much longer.

Vincent: Yes, so this is a ten year evolution, and that would probably be much longer in humans, right. It would be hundreds of years to see a disease disappearing. So that's a good point that you can have a manageable study here. And I should note, funding for this study was provided by several sources, including the Disney World Wide Conservation Fund. You know, Disney loves cute animals and they are supporting research on them. (laughs) Anyway, there you go. Frogs. So that's, we've done a couple of fungus papers lately. Pretty good.

Elio: Nice, good.

Vincent: Alright now I want to end up with giving away a book. We are giving away Management of Clinical Microbiology Laboratories from ASM Press, and I told people to send an email with Micromanagement in the title. And how many people did we get here? We got thirteen emails, we got one from Rachael, from Anthony, from Katie, now Katie says "I love the podcast but as a mycobiologist I feel the fungi are left out." Here today you had a fungus, there you go, Katie. Joseph is a lab manager for Princeton University where he makes plates for the lab, loves to listen. Sabrina is a microbiologist in a clinical lab in a small community hospital. Stephanie has worked in a clinical lab, she loves all the TWIx podcasts. John in a devoted listener, a third year microbiology student who is currently procrastinating by listening to your podcasts.

Michael: (laughs)

Vincent: He worked in a summer clinical microbiology at a university hospital in Linköping, Sweden. He says "while I am writing I might as well mention--"

Elio: Lin-sho-ping.

Vincent: Linshoping? It's not Lin-co-ping, okay.

Michael: It's an umlaut over the o.

Vincent: Okay, thank you. "I want to mention my fascination for the symbiotic relationship between the stingless bee and the fungi you covered in 171." And there is another fungus episode. "My grandfather is a beekeeper and has over the years raised concern about beehives being lost without explanation. It would therefore be very interesting to investigate first if there is similar relationship in honeybees. If that is the case, one could follow up with the effect of various chemicals used in farming on the growth of fungi and consequently the bees." Absolutely.

We got an email from Megan, who works in a microbiology diagnostics lab. One from Nadia, who her future, she said, is clinical laboratory management, that's her chosen career path. She also writes "Michele Swanson and I were at the National Post-Doc conference this weekend in Cleveland and I recognized her voice. I wanted to say hi, met her in Ann Arbor but didn't meet her, and it was great that she came to this post-doc conference. Us post-docs need faculty advocates and I very much appreciate any mentor that makes efforts to improve post-doc training. Aoife writes—Hey Elio how would you pronounce the name Aoife?

Elio: Aoi?

Vincent: Fee.

Elio: I don't know.

Vincent: It's Irish. Aoife? Eef?

Michael: Maybe. Eeefah.

Vincent: Anyway, he or she is in their second year of college studying science in Ireland. And Larry wrote. Amanda is a TWIV listener, she is finishing up a clinical microbiology fellowship in Halifax, Nova Scotia, and then will become the Saskatchewan Provincial Lab Director of Virology and Immunoserology. The book would be a welcome addition. And finally, we have Adam, who is from San Francisco, he is a clinical lab scientist doing microbiome work. And Philip got his BS in Microbiology from the University of Arizona many years ago and has

since moved into clinical lab science. “You all do such a great job explaining the different papers, from the design of the study to the quality of the data, and present complex techniques in such a way that even I understand them. I love that you always include potential applications of all the papers, many of which apply to my field of medical microbiology. We are always looking for ways to identify organisms and antimicrobial resistance faster, so I am always curious what techniques will jump out of research labs and into hospitals. For example, after agar plates dominated clinical lab identification for decades, MALDI-TOF has completely changed hospital lab workflows and I expect further improvements to come at a rapid pace. Thank you all for you time and effort. I haven’t been this excited about reading research papers in a long time, maybe ever, so this means your show is amazing.” Thank you, Phil.

Michael: I figured out how to pronounce Aoife. Eee-fah. That is how you pronounce it. And it means beautiful radiant and joyful thanks to our good friends at Google. And it’s an Irish name, and Irish girl’s name.

Vincent: Thank you, that’s very nice, Michael. An Irish girl’s name. So I just picked a winner, the random number is number 8. That’s Nadia.

Michael: That’s Nadia!

Vincent: So she’s the winner. Nadia, send me your address to twim@microbe.tv and we will ship out this book to you. Congratulations. For those of you who did not win, there will be other opportunities to have books of different sorts. That’ll do it for TWIM 174, you can find it at asm.org/twim. You can find it on your favorite podcast player. Please subscribe, and if you like what we do you can become a patron and donate. Go to microbe.tv/contribute for ways that you can do that. Of course, we love getting your questions and comments. Send them to TWIM at microbe.tv. Elio Schaecter is at the blog Small Things Considered, thanks Elio.

Elio: Thank you, my pleasure.

Vincent: Michael Schmidt is at the Medical University of South Carolina, the harbinger of bad news this week, Michael.

Michael: (laughs) I’m the harbinger of bad news, yes.

Vincent: Thank you, Michael.

Michael: Thank you, Vincent, thanks everyone.

Vincent: I’m Vincent Racaniello, you can find me at virology.ws. I would like the American Society of Microbiology for their support of TWIM, Ray Ortega for technical help, and Ronald Jenkees for his music. Ronaldjenkees.com. Thanks for listening everyone, we will see you next time on This Week in Microbiology.

(MUSIC)

Transcribed by Sarah Morgan.