This Week in Microbiology

With Vincent Racaniello, Michael Schmidt, Elio Schaechter, and Michele Swanson

Episode 185: There's no moa Moa

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Vincent: This is TWIM, This Week in Microbiology, episode 185, recorded on September 6th 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 Schaechter.

Elio: Well hello there, how are you doing?

Vincent: I'm well, and you?

Elio: Okay. Okay. Coming along.

Vincent: On the opposite coast.

Elio: I'm getting younger by the minute. (laughter)

Vincent: There's nothing like it. Also joining us from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello! You're sounding especially enthusiastic today, Vincent.

Vincent: I shouldn't be because I had a horrible morning (laughter) maybe I'm just compensating for it.

Michele: You're happy to be back among your microbiology friends.

Vincent: I just said, people that were in my office, I said, get out, I wanna do something I enjoy, I wanna podcast. (laughter) And that's what I love doing it, you're absolutely right, no matter what happened. Also joining us from Charleston, South Carolina, Michael Schmidt.

Michael: Hello, everyone!

Vincent: How are you down there?

Michael: I am well. We avoided a hurricane, it went quickly across the Gulf of Mexico and it is now raining in the Midwest, hopefully not too much. But any time we dodge a hurricane they say there is another one in the pipes, so. This is what we were affectionately referring to as bowling season where

the storms come off of the African coast like bowling balls. Unfortunately the East coast of the US is where the pins are. Everybody is hoping not for a strike but for a gutter ball.

Vincent: Well we are in September, we are out of the summer of meetings and travel and now back to school. (laughs)

Michele: The classroom.

Vincent: Back to the classroom. You know, here our local school had a half day because it is so hot.

Michael: Really?

Vincent: Can you imagine? They just went back to school this week. Yeah, it's in the mid 90s or something and I don't know if every room is air conditioned, so they sent. Not that I have any kids in middle or high school anymore, but I still get the notices. Here we just continue, our building is too cold, actually. What can you do. Today we have a special treat. We have two special treats for you. We are gonna first get your hands dirty and then we are gonna teach you how to wash them (laughs)

Michael: And that's why we picked the order that we picked.

Vincent: That's why we picked the order. The first paper, Elio's gonna tell us all about coprolites.

Elio: I'll tell you all about coprolites. The paper I am referring to is called "Coprolites reveal ecological interactions lost after the extinction of New Zealand birds." That's a mouthful but I'll explain it. And the authors Alexander Boast, Laura Weyrich, Jamie Wood, Jessica Metcalf, Rob Knight, and Alan Cooper, they are from Australia, New Zealand, and in the case of Rob Knight, San Diego.

So now what are coprolites? Coprolites are fossil feces. And they are nothing short of a marvel. They are a message from the past. They divulge important information about what their makers lived on. And even about how they lived. They were deposited in ecologically relevant sites and in a coprolite, which is a piece of stone, you find remnants of undigested food such as seeds, bits of plant tissue, pollen, starch granules, and of course, DNA! So there is a lot of information contained in this fecal material.

Michele: I would not have said of course DNA. I think it's amazing that the DNA is still there and accessible.

Elio: Yeah, well, accessible, we don't really know to what extent. There is a lot of studies done like this one but just how deep you can sequence, etc, is not quite clear. But there is enough DNA left. And why shouldn't there be, it is protected, after all, in this rock. Now coprolites figure prominently in paleontological studies of all kinds, including humans. And this is what I read, they offer evidence of things like food predilection, predation, cannibalism, infestation by animal parasites, just as an aside there are tapeworm eggs in coprolites from 270 million year old sharks. (laughter)

Michael: I mean, this is absolutely amazing because if you think about it, if we became fossils, what would Elio be saying about us? (laughter) 50,000 years in the future.

Elio: Well, tune in and--

Michael: Tune in?! You're the only one who is gonna be around, so.

Elio: (laughs) Anyhow, the study of coprolites has been crucial in such key questions such as the pattern of colonizing of the Americas and the diets of Neanderthals and the domestication of animals. So they are really a big deal in paleontology. A few facts about coprolites. The earliest come from the Cambrian, which is about 500 million years ago, and the coprolites favor carnivore dung. They are more likely to get fossilized because of the calcium rich bones and teeth decompose more slowly. I found that the longest known coprolite measures 40 cm in length. Obviously from a large animal. And it was sold at an auction for $8,000.

Vincent: Do we know what kind of animal?

Elio: No, I don't think they know what it is. It's just, it was work $8,000 to someone.

Vincent: Do you know how old?

Michael: It was from the Cambrian, I think he said.

Elio: Cambrian, yeah. I don't know, there's no information about it that I could see. But anyhow, there are lots of coprolites around, they have been made into jewelry.

Vincent: Oh my gosh.

Elio: That should prompt you to do some interesting comments with four letter words. Anyhow. How can you tell a coprolite from something which is similar in shape? Turns out that not every turd shaped rock is a coprolite. Far from it! Most of all you have to be able to tell. So you tell it with chemistry because there is a lot of biomarkers like steroids and bile salts, bile acids, which are decomposed very slowly, they last forever, and they are specific to certain animal groups. And of course there is DNA. But you have to know what you are doing. In other words, you have to section them, you have to know, in other words, coproetiology, that's a term I just coined. Okay. Coproetiology, the study of coprolites, is not for the faint of heart.

Vincent: Elio, what would you call someone who studies coprolites?

Elio: Coprolitologist.

Vincent: A coprolitologist.

Michele: (laughs)

Elio: That's right.

Vincent: Excellent.

Michael: And you also need to be doing this in a clean room because any DNA that should fall in from the sky albeit on dust or on anything, you could easily amplify up because they are gonna principally be using molecular methodology to probably characterize this.

Elio: Good point, absolutely. Anyhow, so let's assume that these people did it right, which they seem like they did. And the paper deals with the fecal fossils, the coprolites, of extinct birds of New Zealand. The Moas. I remind you that moas were huge birds. They were many species, they lorded over the landscape mainly in the south island.

Vincent: They were flightless, right?

Elio: They did not fly, they were flightless and they were, some of them were huge.

Michele: Weighing up to 500 pounds.

Elio: 500 pounds. 3.6 meters tall. As tall as an African elephant. And they, yeah, they were huge. And they were, there were several kinds, and they became extinct about the year 1300 which is when the first humans came. They were easy to kill and they were apparently very good to eat. So moas are no longer. There has been an occasional statement that they found moas in some remote place but that has not been confirmed at all.

Vincent: So you would say there's no moa moa?

Michael: Augh.

Elio: Oh boy, I had it coming.

Michele: (laughs)

Michael: You had it coming. But Vincent doesn't get that one, you get one, Vincent.

Elio: May I proceed, please? (laughs) Anyhow. The coprolites of moas which turn out to be 7,000 years old are both abundant and well preserved. So they studied these coprolites from five kinds of moas in different habitats in New Zealand, and they used high throughput screening as far as I can tell, they did it very well. And they found that the DNA was significantly different. You can tell moa DNA from mammal DNA and from modern birds. Here is the surprise. One of the big things they ate were mushrooms!

Vincent: (laughs)

Michael: Of course!

Elio: You know me, I have a thing about mushrooms, so this is really what caught me, this combines two interests of mine, copros and mushrooms. (laughter) Okay. Anyhow, most of the fungi that they found in the coprolites are ascomycetes, mainly molds and yeasts, probably derived from the

environment. They didn't study those further. But for the rest, they found that the DNA of these mushrooms, mainly Basidiomycetes, are from such species as, let me rattle off a few names, Cortinarius, Inocybe, Armillaria are honey mushrooms, in case you wondered about that, as well as some puffballs. All of these are mycorhizomes, that is they grow in association with tree roots. So plant roots.

And this is surprising. I mean, why, we didn't know this was what Moas ate, and they combined not only the eating but also they helped in the dispersion. Now here is a slight mycological digression. Allow me one. Most mushrooms are like the mushroom you buy in the store, white button mushrooms or portobellos. If you look underneath you find that they have gills that have lamella in, partitions. That is where spores are made. And the spores in most mushrooms are spread by spores falling off the surface of the gills and being wafted along the currents that exist in the forest and the fields.

However, some mushrooms don't do that. Notably, truffles. Truffles don't have gills and they don't open up and what do they do? How are their spores spread? Well, we know they are spread by from the fact that they made nice odors. This is why truffles are so delectable. And they call it the smell, the odor, is noticed by animals and animals like voles, field mice, go in there and eat them and they spread the spores by the digestive tract. They don't digest the spores. Now there are some mushrooms, they don't look like regular mushrooms, especially in the genus Cotinarius and others. They are common in New Zealand, which don't open up, they look like essentially like a truffle, essentially enclosed in a membrane and therefore they do not spread the spores by this mechanism, by the usual mechanism.

So it turns out that according to the authors, they make this proposal, and it is a serious proposal, that the moas ate these particular mushrooms, and that by doing that they spread their spores around. So that's kinda neat. Now the problem is that other extant birds apparently do not do that. They don't eat mushrooms. Some do but not in New Zealand, apparently, and so the survival of this mushroom is threatened because they have no good way to spread the spores. That's a guess for it. So. Anyhow. A lot of this is guesswork at present. So we need more sequencing data, especially down to the species level, to see what really it is, what species of mushrooms that they in fact sequestered, what kind of mushrooms is unknown.

And what else did they eat? Turns out that they ate a lot of fern, mosses, and local shrubs and herbs, and material from trees. So they eat all of this, can be told by looking at the coprolites in different kinds of moas preferred different food, which is not surprising because they live in different habitats. In addition to this, you can look for parasites. And sure enough, the DNA of coprolites of moas reveals the presence of parasites like protists and worms like nematodes and flukes. And so this is a lot of information.

Think about it. We know much more about Moas than we thought we did. So this tells you about habitat predilection, this tells you about parasites, it tells you what they ate, what they preferred to eat, so this is pretty good. I think, you know, it is not surprising that this study of coprolites is gonna be really, it's gonna extend it, it's gonna be extended, we're gonna hear more about it. It's remarkable how much information can be extracted from this, let's call them down to Earth remains.

(laughter)

Michele: I agree, I was really impressed with how bold this approach is, to be able to make inferences based on the DNA sequences that are available from these fossilized dung. It's very cool.

Vincent: Now Elio, they say that some of these parasites are no longer around which suggests that when the moa went extinct so did the parasites, right?

Elio: It's not surprising, some parasites are very species specific, host specific, when the host goes the parasite goes.

Vincent: Now as for the fungi that they find, are all of them still around today or are any of them extinct?

Elio: We don't know, the sequencing they did is down to genus, essentially. So we don't really know what species they were dealing with and they, it's probably gonna continue. I imagine we are gonna see more papers and the papers are gonna tell us more about just that point.

Michael: They need additional samples in order to get more rich, in order to enrich their data set.

Elio: That's right, that's right. They also have to, there is a sequence down to species, which may not be easy to do, I don't really know. Right now it is all 16s RNA so maybe more information is needed. You know, the way sequencing is going on there is so much richness, so many possibilities to do more studies that it would be surprising if we don't hear more about it.

Michael: It's getting automated and that is actually the analysis pipelines are becoming automated, and as long as you set the boundary fields properly, you can then phrase the question so you can answer things like is the parasite unique to the bush moa or the giant moa or the upland moa, and you can begin to by setting the threshold tolerances and doing the principle component analysis, I think anybody who is in microbiology today that doesn't at least take one or two classes in how to do some of this genome analysis is going to be really short in their ability to follow the literature. Because you have to understand what is actually going on in order to make those informed questions like they are doing here.

Michele: And as the databases get larger it'll be easier and easier to make inferences when you identify a new sample. You can place it in the phylogenetic pattern.

Michael: The one figure that I was most impressed with in this paper was the figure 3 where they give you the distribution of the select taxa amongst the species and you see, they were able to pull out fungi, plants, and parasites, and across the, if you will, the X axis they show what species of moa they were actually pulling these out and it is beautifully color coded. They give you the size of the animal. So again, the moa was the principal herbivore of New Zealand. It was the elephant, if you will, of Africa, but this was a bird that was effectively responsible for consuming most of the plant material in this island nation. And they quickly went extinct just like the poor elephants are going extinct in Africa as habitat is being destroyed and they are being hunted.

Vincent: They couldn't swim to another island.

Michael: Yeah.

Michele: And the birds probably got that big because they didn't have competition, right, from large mammals?

Michael: No predators.

Vincent: And that's why the humans could walk up to them and kill them. They had no fear. That often happens with species where there is no predator and they live on islands. They're not used to it, they're not weary.

Michele: Fat, dumb, and happy.

Vincent: Yeah. And they get extinct. Now, there's an interesting note, tortoises have an interesting story where they were present on some islands off of eastern Africa and those islands got settled and people would eat all the tortoises because they were good, but tortoises can actually float in the ocean and they colonized other islands just by floating in the waves because they don't have to eat for weeks, they're reptiles, very low metabolisms. So the poor moa I guess couldn't swim and get somewhere else.

Michael: The other thing that folks may not know is that moas don't have any vestigial wings. I mean, they literally only have the two legs they're standing on. They don't have--

Elio: Oops. (laughter)

Michael: Looking at this, and that's why their necks were so long, so they could get at the plant material and eat different things, I mean it's an absolutely fascinating bird and that is what was curious. I went out and learned all about moas on the internet as I was going through the paper.

Vincent: Elio, you could do this with any coprolites and study other--

Elio: Well, they do. It's being done. So human coprolites are extraordinarily well studied.

Vincent: I'm thinking of other extinct animals, right?

Elio: Extinct animals, sure. Of course. You have to know how to tell, how do you tell the coprolite is from a given animal, but there are ways of doing that. There has to be auxiliary information.

Vincent: Is there dinosaur coprolites?

Elio: Yes indeed, and how.

Vincent: Could you get DNA from it, you think?

Elio: Gosh, I think so.

Vincent: Wow.

Elio: I think so but I'm sorry to say I don't, I haven't gotten to the subject deep enough (laughter)

Michael: Deep enough.

Vincent: I know dung.

Michael: I know dung.

Michele: The authors also close by making a smart point that by getting more information about the past, and these past extinctions and ecological relationships, we can better identify risks to our ecosystems poised by future extinctions. So that's one of their motivations for doing this work.

Elio: Yeah.

Michele: And aren't they lucky that New Zealand is the perfect place for it? What a lovely country, I'm told. I haven't been there yet but it is on my wish list.

Vincent: Has anyone been? Have you been, Elio, to New Zealand?

Elio: I don't know.

Vincent: (laughs)

Michael: I was there for four hours transiting to Australia.

Elio: I'm sorry, I haven't been there.

Michael: I got to see the Ausland airport, which looks like all other airports.

Vincent: You know, Michele, you say this information is good for future management but depending on who is in charge, they may ignore it, you know.

Michele: That is the problem.

Vincent: That's the problem. Thank you, Elio! That was great.

Elio: My pleasure. Boy, I enjoyed this one.

Vincent: Good. It's kind of weird that you would enjoy ancient dung, but, you know, I can understand it. Alright, before we go to Michael, let's give away this antibiotics book which we mentioned a couple of weeks ago. I said just send an email with antibiotics in the subject line. I received 19 entries from

James, Peter, Theresa, Kendall, Richard, Sonia, Michael, Kendra, Santino, Jian, Rajesh, Anthony, Garret, Kim, Trudy, Scott, Rebecca, Kelsey, and Brad. 19 entries!

Michele: Wow.

Vincent: Some of them wrote little notes which we will read in the next couple of weeks, but let me pick a winner randomly out of 19, so we will pick a random number between one and nineteen, that would be number 2! That's Peter. Peter is the winner, so there was just one Peter, you know who you are, send me your address, twim@microbe.tv. We'll send you Antibiotics by ASM Press.

Michele: Congratulations, Peter. Thanks for listenin'.

Vincent: Alright, now our snippet comes at the suggestion of Trudy, who wrote:

Dear TWIMmers, can you please help me make sense of this article? From what I know as a laboratory scientist, 70 is the magic percentage of alcohol needed to sanitize a surface according to Michael Schmidt on the True or False video at ASM Microbe this summer, that number is actually 67%. So why do hand sanitizers contain a concentration of 60% alcohol and is it really possible for bacteria to become more tolerant or even resistant to alcohol? My impression has always been that this is very unlikely. I would appreciate any insight.

Michael, enlighten Trudy and us.

Michael: Alright, so I have it built in to the episode because I saw Trudy's question ahead of time, and the title of the paper that we are going to work through is called "Increasing tolerance of hospital Enterococcus faecium to hand wash alcohol." This paper is from a group in Australia, there is a large number of authors, Sacha Pidot, Wei Gao, Andrew Buultjens, Ian Monk--

Elio: You aren't gonna read em all, come on.

Michael: Alright. But they're down in Australia, and so we go from the New Zealand to Australia today, and before we get into the details of this paper, I'd like to compliment the authors and the editors of Science Translational Medicine, because there is so much more to this paper than the headline or the flashing Read Me light because many of you probably have heard of this paper, it's been popularised in the press, the press was very much concerned about it.

But the authors and the editors who published this paper go to great lengths to take us through the observation and then show us how these traits can move easily through a bacterial community and then through your hospital. The headline is the title, and it is effectively giving us that flashing check engine light, but the authors really have done this a tremendous service with the level of rigor that they bring to this important finding that the Enterococcus is becoming increasingly tolerant to alcohol hand sanitizers. So this paper was in Science Translational Medicine, it came out about the tenth of August, and--

Elio: There are 22 authors, by the way, I counted them.

Michael: There are 22 authors. I even learned how to pronounce most of their names, so. I'm gonna start the snippet with a question to help frame the topic for the uninitiated. So if a wide bodied jet crashed killing all on board, and if this occurred every day for the foreseeable future, would any of us ever fly? Well, we all know the answer to that question is a profound no. Yet this is precisely the number of lives lost attributed to healthcare associated infections or HAIs just in the United States on an annualized basis. One planeload of people die each day in the US from HAIs.

The paper is sending us this check engine signal in that one of our best methods and approaches for combating health care associated infections, namely good ol hand hygiene, through the use of these hand wash or hand rub alcohols, is being called into question as the microbes are becoming resistant.

Elio: Let me ask a question. They claim that this is better than soap and water.

Michael: It is indeed better than soap and water for the average microbes that you are encountering in a healthcare environment. But alcohol of course will not touch the spores of C. diff. And C. diff is becoming of increased concern in the United States and other areas of the developed world because of the widespread use of antibiotics. So for the most part, hand hygiene through the use of alcohols principally at that sweet spot of about 70%, I said 67% because that happens to be the concentration of ethanol that is in our hand hygiene material, and I'll get into why the concentrations vary here in a minute.

Michele: Before we go from the soap versus the alcohol washing, isn't part of the benefit of these alcohol based hand rubs is that you don't need a sink? You can take it with you and it's quicker so in a hospital setting it is more convenient.

Michael: Absolutely, and that is one of the most essential pieces in that most of these alcohol rubs work by dehydration and evaporation and you just need to apply and rub them and within 15 to 30 seconds, all of the alcohol has evaporated and your hands have been debulked of the microbes that were associated with them. So these alcohols that are in hand gels like ethanol and isopropanol have been effective in limiting the spread of multidrug resistant pathogens such as Staph aureus which is a normal resident of our nasal cavity and most folks have heard of the scourge called MRSA or methicillin resistant variants of Staph aureus, and then of course Enterococcus faecium principally the ones that are resistant to vancomycin.

And Enterococci, the organism that they are modeling in this particular study, are normal residents of our digestive system. They are, if you will, what we were talking about in the last episode or the last paper. This is what DNA they are gonna find in our stool, the Enterococci are big in our stool. But they generally have very low virulence. We all have them in our guts and we are all walking around perfectly healthy. But when Enterococci get into the wrong spot, they can wreak havoc. And Enterococci now account for about 10% of hospital acquired bacteremia, that's where the bugs in your gut leave your gut and begin to move through your bloodstream. And they globally, so they are 10% of hospital acquired bacteremia cases globally, and in North America and Europe, they are the fourth and fifth leading cause of sepsis in those two particular locations, North America and Europe.

Again, sepsis is something that we haven't talked about much on TWIM, but in brief, it is your body's extreme response to an infection. Your immune system really goes crazy and should you develop

sepsis, it is an unfortunate life threatening emergency resulting in rapid tissue damage, organ failure, and it often ends up in death. So sepsis is maybe something we wanna explore on a future TWIM. The other issue is that hospital acquired Enterococci infections are difficult to treat because this microbe has intrinsic resistances. It is resistant against bile and all of our other things that are normally taking out bacteria in our gut. It's happy because it's living down there.

The other thing is it has acquired resistance traits against many classes of antibiotics such as the beta lactams, the amino glycosides, and the quinolones. The authors also point out that in a hospital situation there is a particular rapidly evolving clade that has been associated with the hospitals in Australia. It's referred to as the A1 clade or the clonal complex CC17. And this particular microbe is of concern because it has a high number of mobile genetic elements and is enriched for genes altering carbohydrate utilization in transporter proteins that distinguish the hospital variant of this microbe from the community acquired in the non pathogenic variants of Enterococcus faecium. Now think about it. Alcohol catabolism is a carbohydrate and if it is enriched for genes that have altered carbohydrate utilization you can begin to see the crux of the problem that they are going to begin to investigate.

Elio: Can I make a little point here?

Michael: Yes, you may.

Elio: Normally when you think about a bug becoming drug resistant it is because it has been exposed to the agent. When it is resistant the bug becomes resistant by being exposed to penicillin. This is not the case here. If you expose bugs to alcohol, unless they are already resistant like spores or maybe bacilli they are gonna die. They don't have a chance to develop resistance. So the resistance has to come from something else.

Michael: And that's the last figure of their paper.

Elio: Okay.

Michael: That's the last figure of their paper, why they are doing us a great service explaining how this conundrum happened. So as Michele pointed out, we love alcohol hand rubs because you only need about 30 seconds. You allow your hands to naturally dry. And if you follow this guide and routinely drop the concentration of bacteria by about 3.5 to 4 logs, or you take about 10,000 bacteria off of the population that is on your hands. And for most of us, unless we have been working in an extremely rich environment where there is lots of microbes cleaning toilets or something like that, that routinely will drop the critical concentration where it doesn't matter and we're not gonna transfer microbes from ourselves to a vulnerable patient or to the environment.

Now all of this is regulated by a set of standards. The effectiveness of alcohol based hand rubs are typically evaluated by using the standard method developed by the American Society for Testing and Materials, or ASTM, which is the international standards organization that many regulatory agencies defer to in making a decision about hwether a product is effective. ASTM has been around for a hundred plus years and the United States FDA specifies that if you are evaluating the effectiveness of a product that you use a particular ASTM protocol specifically designed to evaluate the product in a

health care personal handwash situation. And the FDA requirement is the products are only required to achieve a minimum of a two log reduction from baseline after one application.

So you squirt the juice on your hand, you rub it, and it only need drop it two logs from whatever concentration is on the particular material. And that is why you see the variation in the concentrations of what is actually being used in hospital settings. And it is also in the compounding of what surfactants you are putting in, what emollients you are putting in, and you can change the concentration of the active ingredient which is the alcohol if you are controlling the evaporation rate or making so it is not so harsh on your skin but at the same time, you still have to be sensitive that you must hit this FDA mark of two logs reduction from baseline or a three log reduction if you are applying it ten consecutive times. So it gets into the weeds of how the FDA regulates these problems.

Michele: So the suspense is killing me, Michael, let's look at their data.

Michael: Okay, we're gonna go to the data (laughter) So the data begins, their fundamental question that they ask was whether or not tolerance to isopropanol was being selected for to get back to what Elio just alluded to. You expose somebody to penicillin, you'll select a mutant. So they went to their freezers and they took 139 different isolates and they separated them into three groups. The two hospitals where they got the isolates from didn't start heavily using alcohol hand based rubs until 2002. So they start in 1997 and they move forward and the first group is 97 through 2003. The second group is 2004 to 2009, and then the third group is 2010 through 2015. To address the issue of selection pressure, prior to 2002 they were only using 100 liters of alcohol hand based rub a month. After 2002, that number climbed to over 1,000 liters a month. So they increased their consumption tenfold, or they increased the selection pressure or--

Elio: Wait a minute, why did you call it selective pressure?

Michele: Exposure.

Michael: Exposure pressure.

Elio: The test they did is to 25% alcohol, isopropanol, it's not considered 67%.

Michael: We'll get to that in a second. So what they found with, they took these isolates and they exposed them for 5 minutes to 23% isopropanol. Why did they use 23% and not 70%? And the reason is that 70% killed too quickly. And they weren't able to assess whether or not from the 139 isolates they would have had to screen many more isolates to find a trend, and what they did is they found a Goldilocks concentration of isopropanol that they worked out was 23%. And what you can see from their data is that, and they only exposed these organisms for 5 minutes and they started with an initial load of 10^8 CFUs.

And so they measured the reduction that they were seeing when only exposing them to 23% isopropanol. And what you see is that as the isolates move forward in time, so does the number of isolates tolerance to isopropanol. You just look at the dot plot and what you see is a quickly rising as time move forwards, you see them clustering more and more to the 0 mark in their particular figure, which is at the top of the graphs because they are effectively plotting the negative log. Yes, Elio?

Elio: Let me ask you a question.

Michele: Figure 1A, for those of you following at home.

Elio: The question I have is what does resistance to 23% alcohol tell you about the resistance to 67% alcohol? Does it say anything?

Michael: No, it was just enabling them to effectively ask the question whether or not they were seeing an increased tolerance to ethanol, or excuse me, isopropanol.

Elio: But the concentration is important here. It could be that they become resistant to 23% and not resistant to 67%.

Michael: They address that in the second figure, but before I get there, let me finish with their, they refine their question of looking at this 23% by asking, does this behavior manifest in the predominant clinical isolates that they were collecting out of their freezers, and that it wasn't just a freak 1 mutant, 2 mutant that they were seeing. They were asking the question, was this a predominant phenotype that was expressing itself in the hospital? And that is the remaining pieces of figure 1, that's panels B, C, and D, and what you again see is the curve shifts upward, illustrating that even among the predominant clones recovered from the hospital patients, that they are becoming more tolerant to isopropanol.

Elio: At 23% isopropanol!

Michael: The second question that they ask is whether or not this observation had any clinical relevance. And so here they developed a contaminated surface transmission model using Enterococcus faecium in mice in order to assess if the alcohol tolerance uncovered when using 23% isopropanol was relevant to when using a 70% impregnated surface wipe to clean the surface, and 70% is the types of wipes used in hospitals. The model is interesting in that they first ask whether the isolates could move this trait, this alcohol tolerance from 1 environment or animal to another, and whether or not 70% isopropanol could stop this horizontal transmission.

To get back to Elio's point, they measured this with 23% isopropanol. So the first step is they wanna know how many Enterococci does it take to infect a mouse, or colonize the GI tract of the mouse? They had 2 isolates. The first by the 23% sensitivity assay was sensitive, and the second isolate was alcohol tolerant. They learned that the colonizing dose, or the CD50, was about 14 bacteria for the sensitive strain, and about 3 bacteria for the tolerant strain.

They point out that that wasn't statistically significant, it just happened to be what they saw for that particular suite of experiments. And when they coated the floor of a cage with 3 million bacteria and subjected it to a defined disinfection regime with either wiping it with water or wiping it with isopropanol, and this density of 3 million bacteria on their cage surface gives them a density of Enterococci of between 0.4 and 30 CFU per cm^2, which is relevant to the concentration of enterococcus routinely seen on environmental surfaces. So you have water and you have isopropanol and you have the control where they did nothing.

Michele: It's 70%.

Michael: It's 70% isopropanol. What they learned is the alcohol tolerant enterococcus was better able to withstand the 70% isopropanol disinfection and was able to colonize the mouse gut, than was the more alcohol susceptible isolate. And it was significantly, a p value of 0.01, so it is not like it's a fluke. But remember, they picked the isolates because it was either sensitive or tolerant at this 23% concentration. They next challenged the alcohol tolerance in transmission hypothesis by selecting a pair of vancomycin sensitive enterococci with a different tolerance to alcohol.

So again, it's a paired system where they have one sensitive vancomycin sensitive isolate, one tolerant genetic isolate, and these two microbes were much closer in their genetic identity to each other than the VRE isolates that they used in the first part of the experiment where one was isolated in 98 and the other in 2012 and so there was some more genetic divergence. And they point out that the vancomycin sensitive isolates while differing in alcohol tolerance by about 4.5 fold, they shared only 29 core genome single nucleotide polymorphisms or SNP differences from each other.

So they thought that they were equivalent with the only exception being their ability to tolerate more alcohol. And again, the sensitive isolate for vancomycin took 19 CFUs to colonize a mouse and the tolerant to isopropanol only took 12, so again there is nothing magic about alcohol tolerance and the ability to effectively attack it. So we have this beautiful set, these first two experiments, and they could have literally called it quits. And what they show is that the microbes are increasing their tolerance to alcohol. But they have not addressed Elio's specific criticism of what is going on with the microbe, whether or not we are truly isolating a unique creature.

Elio: I don't understand this, I really don't get it. The whole point is to show that bugs have become resistant to the alcohol you use in practice. They didn't test that.

Michael: They did, they did in the second experiment.

Elio: In the mouse they did, but not just directly.

Michael: No, and the mouse is the only thing that matters, because that is what they're concerned about.

Elio: No, I'm sorry, my fingers matter, that's not true. The reason for using alcohol is to avoid the spread of the bug. If the alcohol doesn't work, then the bug will spread. If it works, then it wont spread. Nothing to do with mice.

Michael: That's what they're effectively showing with the mice, that the mouse, go ahead Michele, you may have an easier…

Michele: I think that when you get to Figure 4D, we'll talk about that, and maybe we can return to Elio's rigorous experiment?

Michael: Okay. Alright. So they next looked for the bacterial genomic signatures of adaptation. If you will, the equivalent of the antibiotic resistance marker. Here they learned using the tools you might expect, sequencing, principal component analysis, that the alcohol tolerant Enterococcus faecium accumulated mutations involved in carbohydrate uptake and it's subsequent metabolism. This third figure in their series represents a whole lot of work but the cool thin is the alcohol tolerant and alcohol susceptible, and when you look at their third figure and you just look at, they first went and they looked at the extra chromosomal element.

And they looked to see where the differences between the alcohol tolerant and the alcohol susceptible changes were, and what they noticed is that you would anticipate a plasmid, that would be the first place, you would acquire an adaptation to a particular condition. And that's where they first found it. The conclusion that they reach from their very exhaustive phylogenetic conversion and discriminate analysis of principal components, or DAP analyses across these distinct Enterococci lineage, identified changes within several genetic loci that were contributory to the alcohol tolerance.

Michele: And they said as a criteria that they needed to see the same genetic mutation in multiple strains in their collection, so they did set up a threshold for that.

Michael: They did, indeed. So after that, they could have called it quits at the genetics, but they take it up one more notch and they validate that bacterial genetic factors that are linked to this alcohol tolerance threshold that they set. They used allelic exchange to make targeted mutants in an isopropanol tolerant isolate. So this is a tolerant isolate as defined by their 23% test, and this is then all rolled up in that fourth figure. And it's really quite humbling to look at what they were able to accomplish, and that's where you get into figure 4D.

Originally this was only gonna be a snippet, so I wasn't gonna get into the weeds of 4D and I'd simply leave it for the audience to take a look at this figure, but what inspired and humbled me was the amount of work they did in this allelic exchange were we saw RPOB and again, they are comparing the wild type phenotype. They're looking specifically at doubling time change and this is the fold difference relative to wild type. What you see is in the mutations that they identified from the genetic analysis that they are indeed tracking to this alcohol tolerance.

Michele: And the experiment in figure 4D that I found most convincing is they are looking at the growth rate of wild type versus these particular clean mutants or their complement. Now they are challenging them with 3% isopropanol, but they do find that mutations in a particular set of genes do increase the, do equip the strain to grow faster in 3% isopropanol. So consistent with the idea that accumulation of these mutations does provide a fitness advantage.

Michael: And this reminds me of the old days of enrichment plates or gradient plates that we would use when we were trying to isolate various mutants. We would put the compound at its most maximum concentration in the bottom of the petri plate, allowing the agar to solidify on a pipet at an angle, so you would get a 45 degree slope. And then we would plate agar without the selective agent on the top to level out the 45 degree angle.

You mutagenize the microbes and then spread them on the plate, and what you would see is there would be a particular gradient or location in which you could isolate the ones that were more resistant

or sensitive to the agent based on where it was in that gradient, and then you would ask the question, did that mutation read true? And that is effectively what they are doing with their elegant growth rate experiment. If they were to have used 70% isopropanol, they would have killed everyone. Because they are not resistant.

Elio: That's the point!

Michael: They're not resistant, they're tolerant!

Elio: They're not resistant to 70% alcohol!

Michele: There's good news, there's good news and there's bad news. The good news is, as Elio points out, there is no evidence that these strains can tolerate 70% hand washing practices that are still being used clinically.

Michael: That is correct.

Michele: The bad news is if you increase the sensitivity of your assay, I do think they have compelling data that there are mutations accumulating in the background that are equipping these bacteria to tolerate it. So it is maybe the tip of the iceberg.

Vincent: And they have increased over time, as alcohol use has gone up, right.

Michael: That is the take home message, something that we have always known, that bacterial adaptation is complicating infection control recommendations, necessitating additional procedures to prevent the spread of these horrible pathogens in our hospital settings. They are not resistant. They are becoming tolerant.

Michele: So in another 5 or 10 years or something the problem could be huge.

Michael: Worse.

Michele: But with this data that we have now, perhaps we need to start thinking about modifying our practices so that we don't select for superbugs.

Vincent: What would you do in terms of hand washing, Michele? What are you thinking?

Michele: I don't know, I don't have a lot of experience in a clinical setting to know what the options are, but I guess if we think about antibiotic resistance often use a multi drug appraoch to decrease the odds of getting a resistance. So I don't know if we're gonna have to wash our hands with soap and do a drying with alcohol, something like that. I don't know.

Michael: It's a concentration effect. It's simply debulking. I think if you routinely at least one hour wash your hands well, and then use these alcohols, you are effectively debulking it. If you can keep the concentration at a threshold, then it is less likely to transfer to the patient. This is not only happening with isopropanol, it's also happening with hexaquorifine. And hexaquorifine, if you've ever

had knee surgery, they probably sent you home with a bottle of the stuff instructing you to bathe in it the night before your surgery. Because what the hexaquorifine does is it debulks the normal flora on your skin so they are not introduced during the surgery into the wound and hexaquorifine has this unique attribute of sticking to our skin, but more and more of our skin flora are becoming tolerant to this particular antimicrobial agent, and it's causing concern because it is resulting in failures of the joints simply due to nosocomial introduction of the microbes from the patient, even if they go to great lengths to decon the wound, decon the area before they do the surgery, and so--

Elio: Let me ask a question. Based on this paper is there any reason not to use alcohol hand wash?

Michael: No.

Elio: In the hospital. The answer is no. The answer is clear.

Michele: The authors are careful to say that, the authors are very careful to say that we should ocntinue to use these products. But it is a fair question whether infection control units at some hospitals should start monitoring this. I don't know, what do you think?

Michael: That's their recommendation, is that they should monitor it in order to switch up or change because this was just to isopropanol. They didn't do the next experiment and ask the question, is it to ethanol, as well? Or to some of these other antimicrobials that you can put in hand sanitizers, because there are others that you might be able to use.

Vincent: So Michael, the concentration is 60% in these sanitizers?

Michael: 70, in Australia it is 70% in their isopropanol, and they're using isopropanol.

Vincent: Trudy's letter, she said 60%, so what's going on there, do you know?

Michael: It depends on the manufacturer and what the manufacturer got from the FDA for their label tolerances. I have one sitting on my desk that is 62%, the ones in our hospital are 67%, and they are made by different vendors. It has to do with the evaporation rates and all sorts of other complicated things adding to it.

Vincent: I heard a talk this summer on hand hygiene at the hospital by a nurse who is head of infection control, and she said all the surgeons use alcohol sanitizers now, they don't wash their hands with soap anymore.

Michele: Hmm.

Vincent: Did you know that?

Michael: That's scary.

Vincent: They said it's better (laughs) so. I don't know if that's universally true or what, but it's here.

Michael: Yeah.

Vincent: Now Elio, you're not worried at all?

Elio: Not yet. Not yet. I think it's appropriate to call a warning shot, but that's all it is. It's a warning shot, it doesn't show that this is a problem yet.

Vincent: It's not a problem but they're clearly here, there is a molecular mechanism that can increase tolerance to lower concentrations.

Elio: That's good to know.

Vincent: Yeah, I think it's good to know.

Michele: It's good that it has to, it requires multiple mutations that are happening stepwise, so it's not like a giant plasmid has come in and is instantly switching from sensitive to resistant. So that's more good news.

Vincent: Michael, when you add-

Michael: Multi-gene effect.

Vincent: When you add 70% alcohol to bacteria, what happens to them?

Michael: They principally dehydrate and it disrupts their membranes.

Vincent: So these genes--

Elio: Isn't it dissolving the lipids in the membrane?

Michael: Yes.

Elio: But they pop instantly, it doesn't take any time, so there's no way to develop tolerance that way.

Vincent: Yeah, it seems to me that if you're delipidating and dehydrating that will be tough. Maybe lower concentrations would involve these transporters, right, but I don't know if you would ever get it to the higher. I guess someone is doing that experiment, right? Just keep growing, I mean you could start with low passage and higher and higher concentrations. I would think that at 70% you're just gonna break them open. Sort of like copper, Michael.

Michael: It is indeed, it's the same mechanism, you're attacking the membrane and you're causing them to effectively, thermodynamics is your friend. They are effectively lysing.

Vincent: This made a big media splash, didn't it, Michael?

Michael: I'll commend the authors because it got a big media splash that only focused on the first figure showing alcohol tolerance.

Vincent: Sure, sure.

Michael: But figures 2, 3, and 4 is where the bulk of the work was, even though Elio took us to task on it, they do indeed show that they are becoming tolerant. As Michele points out, it is a multi gene event which is the good news.

Elio: But, but, but, but! So what! I mean, I hear what you're saying. It's nice microbiology. But does it deal with the fact that alcohol is being used at a concentration of 60 to 70% in hospitals very effectively? Is there any reason to doubt that, is there anything exchanged?

Michael: No. No.

Michele: I do have one question, how safe is it to assume that if you just give your hands a little squirt and rub quickly that you get 70% concentration across all your hand, the entire surface of your hand?

Elio: Why not?

Michael: That's the fundamental problem, it's a distribution of concentration and coverage.

Michele: And the volume that you squirt into the palm of your hand.

Michael: The volume that you squirt and whether or not you apply it properly.

Michele: How quickly you spread it.

Michael: There's a specific--

Elio: The volume is determined by a machine. The machine is set up to deliver the same volume all the time. So it's not like you have a choice. If you put your finger on the button that's what you get.

Michele: I was also thinking about people that use these alcohol based washes in that they carry around with them in their backpack or whatever.

Elio: Most of it is used by machines in the hospital. The doctor doesn't walk around with a bottle of the stuff. Most of them do a dispenser, the dispenser does it the same way all the time.

Michael: And they have been doing a lot of work looking at foams because foams will increase the surface area to volume ratio which will then get to the issue of distributing it across more area on your hand so that the effect of concentration reaching the hand is apparent. In order to, rather than requiring that the human rub it all over themselves, the foams will effectively spread it more easily.

Elio: So that's a problem, that's the point, that's not the problem.

Michael: No.

Elio: So I don’t know where the problem is.

Vincent: Elio, before you were saying something about the publicity, could you repeat that?

Elio: I don’t know who is responsible for the fact that this has been hyped up all over the place. I'll show you the newspaper here. It seems to me that this is not right. It implies that the effectiveness of alcohol in hospitals is waning. We have no evidence for that.

Michael: That is indeed the case. But what we do see is tolerant organisms emerging. That’s the only conclusion.

Elio: That’s right.

Vincent: I don’t think that the press or the general public could really make a distinction between that the way microbiologists can.

Elio: Exactly.

Vincent: So Elio, what happens is the universities where the work goes on, they typically send out press releases, then news organizations will look at them and say ah, this seems interesting.

Elio: They should have been careful. This does not deserve a press release, in my opinion. I’m sorry.

Vincent: The problem will arise if people start to get rid of alcohol sanitizers and try something else, right?

Michael: Yes. If they just say they’re no damn good and they stop it all together. This is why--

Elio: That’s my point!

Michael: Before I uttered a word I commended the authors and the editors for including the other figures in the paper. Because they could have just, it could have been a throwaway, and a quick report. But they actually, there’s a fair amount of work that the authors need to be commended in their thoroughness in which they attack this to address--

Elio: Look, come on. This is true for most of microbiology. This is not a sloppy science. Why are you commending him for, for doing the work? I don’t understand that. It’s a nice paper.

Michele: I agree with Michael. I agree with Michael on that point. They were using a large cohort of strains over a 15 year period, 18 year period, they did a lot of genomic analysis, classical genetic analysis, phenotypic analysis, so I think they were very careful in their approach.

Vincent: Bottom line, everybody, hospitals, people, keep using hand sanitizer. It works.

Michael: Yes.

Vincent: And Trudy, I hope that answered your question (laughter)

Michael: We had a good time doing it!

Vincent: It was fine, I think it is good to point out our suspicions or criticisms with any work. It's good, it's important. Thank you, Michael. Alright, since we went a little longer, let's wrap it up. We will save our email till next time. That's TWIM 185. You can find it at asm.org/twim. If you listen on a tablet or a telephone, you know there is an app that you use to listen, please just subscribe, search for TWIM, subscribe to it, it helps us if all of you subscribe and get every episode automatically twice a month. If you like what we do consider being a supporter financially of our work. You can go to microbe.tv/contribute for a number of different ways that you can do that. Of course, we always like getting your questions and comments. Next time, we will read some more of them. twim@microbe.tv. Michele Swanson is at the University of Michigan, thank you, Michele.

Michele: Thank you.

Vincent: Elio Schaechter is at Small Things Considered, thank you, Elio.

Elio: My pleasure. I hope I wasn't too cantankerous today. (laughter)

Vincent: Hey, no, it's great, I love it!

Michael: Makes it lively!

Vincent: Makes it lively. Michael Schmidt is at the Medical University of South Carolina, thank you, Michael.

Michael: Thanks, everyone.

Vincent: I'm Vincent Racaniello, you can find me at virology.ws. Thanks to ASM for their support and Ray Ortega for his technical help. The music on TWIM is by Ronald Jenkees. Thanks for listening, everyone, see you next time on This Week in Microbiology.

(music)

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