



Fiona Wood Public Lecture Series

Transcript for 'Gut health – are we still groping in the dark?' Presented by Dr Oliver Waters

That was a big introduction. I am not sure I can live up to that but, so I am a gastroenterologist. That is often met with sort of a quizzical look. You can see people thinking, why? Or you can get the reaction my 12-year-old daughter gave me when she asked what I did which was "that's disgusting" followed by a devastating eye roll. So, the short answer for those that want to nip out the back quickly is "are we still groping in the dark"? A bit, but there is some really interesting data coming out, and as Kim said, there is an enormous amount of data out there. I did a quick Medline search and there are 22,000 citations that are new scientific papers, in the last two years, just on the gut microbiome. So there is no way I am across all of that. I have an interest and we are still finding out where we are going.

So, I do not have any conflicts of interest to declare. I have yet to invent the Dr Waters special fermentation for lush hair, but I did go and have a look at what do we mean by healthy and there is a problem here. The world health organisation defines it state of complete physical, mental and social wellbeing and not merely the absence of disease or infirmity. So, the problem about that is that we do not actually spend that much money investigating healthy people, we spend a lot of money investigating sick people and so we do not have that much data about the healthy gut, and on top of that what would you define as a healthy gut if you had an absence of disease at that particular moment in time but you were about to develop bowel cancer five years down the line. Would you still define that as a health gut? I am not entirely sure what a social wellbeing is when it comes to your gut; maybe that is just a control of wind.

So, we are going to spend some time on the science, and I think it is really important that you understand these scientific terms because if you do not then the rest of what you read just will not make any sense. So the microbiome literally means all the genetic material in a particular environment. So, you could have the microbiome of your garden, or your microbiome of your skin or your eye or in this case your gut. The microbiota are the actual bugs, they are the bacteria, the fungi and the viruses that live in that environment. We tend to study these microbiomes using metagenomics. Now what metagenomics are is that you take these very fast sequencing machines and before when we sequence genes it would take ages and was really expensive, but now they can do it incredibly quickly and incredibly cheaply, and you assess all the DNA and the RNA material in that particular environment. Now it is important to understand what the RNA is. So, your DNA carries a code, mostly for proteins that go off and do stuff. There are lots of non-coding DNA as well, but how you make a protein is the DNA unzips itself, it then makes a copy, an antisense copy of that with messenger RNA and then the messenger RNA is then fed through a ribosome, and the ribosomes; these are in bacteria, are also made of RNA. The ribosome reads the code and then adds a amino acid and each three base pairs encodes one amino acid and there are 20 essential amino acids. Then off that you make this big polypeptide chain of amino acids which then goes off and gets further processed, chopped up and made into proteins which then go off and do stuff.

So the important things about understanding the ribosomes is that ribosomal RNA is highly conserved amongst species, and so you can analyse the RNA within the bacteria and it tells you what bacteria are there, and it tells you how many of those bacteria are there. The big problem is, that it does not tell you what they are doing, and it does not tell you whether they are dead or alive. So how this system works if I analysed everyone's DNA is here would tell me who is here and how many people, but if you are all dead it would tell me the same thing. At dysbiosis we define as an abnormal balance between your particular microbiota within a system, so a classic dysbiosis would be an algal bloom in the river.

So, proteomics. Proteomics is the study of the proteins that are in a particular environment, and we can start to do this when we look at all the proteins. Now the benefit of that is that we start to look at what these bugs are actually doing. Because they are making proteins and those proteins go off and do stuff. The problem about proteomics is that it is in its infancy and it is quite expensive, and the other problem is that you are only looking at proteins, and there are also a whole load of other molecules inside your gut that are doing really interesting stuff. Things like butyrate and short chain fatty acids and other small molecules produced by bacteria. So we do not get a picture of that.

So the next thing is metabolomics. So, metabolomics is looking at all of that stuff, so the short chain fatty acids and all those other things, including the proteins and the RNA. Now we are very lucky here at Fiona Stanley Hospital, we have just had the Australian Phenome Centre set up here for Harry Perkins and this is the sort of work that they do. So it is a very exciting time for us in research in this place. The bioinformatics, and I think that what we are seeing in the revolutionising gut health is all driven by computer power. As you can imagine, you generate these enormous data sets of millions and millions of bits of data which you then have to crunch through. They have had to invent new forms of statistics just to deal with this, and this is also part of the problem. Is that we have very little data that really shows causality and it is really important to understand the difference between associative data and causative data. I think the best example of this would be with smoking, and when they were first looking at smoking and its link to lung cancer they noticed that people who worked in pubs were more likely to get lung cancer. Of course the reason for that is that they are passive smoking, but you could conclude that handling beer causes lung cancer. I am very pleased to hear it does not, but just because there is a relationship does not mean that relationship is causative. That is something that is really important to understand when reading these papers or hearing this information. Because you hear all of this stuff, you know this bacteria, that bacteria, oh go and buy that, but actually it may have nothing to do with it at all.

Then the final thing I want to say is about culture, so this is where you actually take the bugs you put them in the lab and you grow them and then you study them in that environment. Now the problem is a lot of bugs in your gut are really hard to grow and in fact they can only grow about 80 to 100 bugs in the actual lab. So, the difference in all of this information is really important, and this is just a picture of these sort of data sets you see. I have no idea what that actually means, it is very complicated though.

So, the anatomy of the gut microbiome basically changes according to where you are in the gut, and so you have got quite a lot of bacteria in your mouth and we know that we can manipulate your bacteria in your mouth and that changes the way your mouth health changes by not eating too many lollies and brushing your teeth, chewing gum also helps because it raises the PH. In your stomach there is not very much at all because it is very acidic there and then you go through to the small bowel. The small bowel has some bacteria there but the small bowel is a bit like a fast flowing mountain stream. Things are flowing through there really quickly and that constantly keeps it washed out. It is when you hit the colon, that is where you hit most of the microbiome. That is where a lot of fermentation occurs and there is lots of bacteria there and fungi and viruses that are doing really important jobs for us.

It is also related to this interaction about where you are in the gut. So, if you are in the middle of the stream of the gut it is like being in the sea, in the deep ocean. There are some fish there, there is a bit of life but it is not an awful lot, but the real action is what occurs on the fringes of the ocean. Like in a coral reef. It is where you have these interactions of multiple complex systems and that is where the gut microbiome interacts with our mucosa and interacts with our immune system, and that is really the key. One of the problems is, is to sample that environment is quite difficult. Because if you look at a poo sample or a swab from the mouth, you are not actually getting that environment. You are actually getting a lump of poo which may have got nothing to do with that. So again it has little problems.

So, to give you an idea of the complexity, in everyone's gut in this room there is somewhere between 10 to 100 trillion bacteria. We are not entirely sure about the virome; that is the viruses and the fungi, there is far less study there. There is probably more bacteriophages there and to give you an idea of the genetic amount, there are 20 times more genetic material from microbiota on you and in you than there is actually of yourself. So it kind of redefines what you is. The gut microbiota is predominantly gram negative; gram stain is a way of looking at cell walls of bacteria, and it is predominantly anaerobic. So anaerobic is where you reproduce without oxygen. Most of those bacteria obligate anaerobes so they have to have no oxygen, whereas some are facultative where they can have some oxygen. Again this is an issue because the more you expose poo to air so these obligate anaerobes die off. There is a massive variation amongst people. So if you look at the hunter gatherers of Southern Africa compared to the Western suburbs, there is an enormous difference. However, what is really interesting is it does remain remarkably stable throughout your life, assuming it does not take massive insults. Your gut microbiome is actually more similar to your parents or your siblings than it is to someone you cohabit with, even if you have been cohabiting with that person for 30 years.

There is a very small overlap of core species. There is only about 50 to 100 core bacterial species that all humans have and the rest is all about variation. So, how it develops is obviously it develops actually in utero. The uterus is not sterile, there is some bacteria translocated in utero. Some interesting data about obesity where having antibiotics given to the mother whilst she is in the final trimester seems to infer a risk of obesity in the child, but interestingly if the mother is obese then antibiotics actually reduces that risk, which is interesting. The mode of delivery is often very important because if you have a caesarean section you do not pick up any of the gut microbiota from your mother as you pass through the birth canal. They are now moving to the stage where they take sort of a wipe from the birth canal and they smear it onto the baby's mouth after they have done it by select caesarean section. There is obviously a big difference between whether you are bottle fed or breast fed, and that is not only with you picking up the bacteria but it is also with the type of sugars that you find in particular breast milks and how that alters the gut microbiome which you can pick up.

There is also very interesting interaction with your genetics. So your genetics do dictate what bugs can actually live there, and once you get to about the age of between 2 to 5, it depends on many factors, but then your microbiome is basically the same as it is in adult life. Once you have passed the age of 60 it then declines like so many things unfortunately. But all of this points to the fact that environment is absolutely key to all of this. So, this is one of those complex pictures that are looking at your immune system and how it interacts with the gut microbiome. I am glad to see some of you did bring pens and papers, there will be a test. So, what you have got at the top is the lumen of the gut and that is where all the bacteria, viruses and fungi hang out, and then you have got this band of enterocytes underneath it. So, the barrier function is really important to keep the bugs away from our immune system, and their barrier function comes up with several things. First of all there is a mucus layer that lines the whole gut and in this mucus layer is embedded proteins called defensins, and these are natural, they are like barbed wire to bacteria and viruses and they

just prevent them getting to your immune system. Very interestingly you can look at patients and their bacterial profiles and it shows that bacteria that eats mucin as opposed to promotes secretion of mucin seem to be associated with more autoimmune diseases analogy. Then the actual enterocytes themselves, they get a lot of their energy from the short chain fatty acids and butyrates, which are not produced by humans. Only bacteria have the enzymes to produce these. So the bacteria that are there are really fundamental for the health of the gut lining itself, and there is good data that shows that the tight junctions between these cells are influenced by how well-nourished they are and how much short chain fatty acids are there.

Then the immune system itself is clearly very, very complicated and it is basically broken down into the adaptable immune system and the innate immune system. Your innate immune system is very, very highly conserved. So we have similar features to a fruit fly, for example, in our innate immune system, and this innate immune system is sort of permanently loaded ready to go off and it recognises particular [TIME: 23:35] on cell walls. So things like flagella that you find on bacteria that will trigger your innate immune system and other sort of bacterial cell wall features as well. Then you have this whole population of regulatory T-cells which are basically calming the immune system down and your immune system is in this permanent state of being switched on and switched off. Switched on and switched off. There is again a load of data that has come out to show that things like caesarean section rates, things like early introduction of solids, this influences the gut microbiome and there seems to be some form of association with how this immune system is regulated.

So we are just going to talk a little bit about allergy and the gut microbiomes. So, when you are born your immune system is immature and it learns how to behave, and I think this is really fascinating. Anyone who has got kids here, I will anonymise this child, although you would not recognise Fiona Wood from the photo. Anyone who has taken their kid to the beach, you know they have loaded the car up with the tent and the surf board and god knows what else, its been a major mission to get them there. You sit them down and the first thing they do is they start to eat the sand, and then they put sand in their eye and everyone has to go home early. So, this is really fascinating, and I think; we are hypothesising here, but is it that the child is actually, its immune system is sampling its environment and going this is okay, this is not so bad. There is a whole load of data that shows that if you are born in a rural environment and you have multiple pets very early on in life and you have got lots of siblings, then you have much lower rates of immunity, much lower rates of asthma, much lower rates of atopy which is things like eczema. Whereas if you are a single child, you live in an urbanised area, you don't have pets etcetera, etcetera, then the rate of allergy, asthma, atopy and all these other things seem to be increased. There are a whole load of other associations that show this as well including regulation of toll-like receptors. So toll-like receptors are these part of the innate immune system and actually we share those with the fruit flies.

So, how do we have some evidence, direct evidence about this? Well unfortunately we only have a lot of sort of data from observational studies, looking at these associations and I would emphasise they are associations, but we do have mouse models. So we use a lot of - I don't, but researchers do - use a lot of germ free mice. And what germ free mice are, are these are mice which are born via caesarean section and they are raised in completely sterile conditions. So they have no bacteria inside their gut, and they are very good models for studying those interaction of the microbiota and what it does to your immune system. They are sort of sickly, you know they are not the most healthy looking of mice. The other good thing about mice to study is that they are coprophagic, and that means that they eat their own and each others poo. So they are very easy to do poo transplants on. And what they find with these mice is that if you expose them to, if you introduce the microbiota to them and then kill the microbiota with antibiotics that does exactly the same thing as generating the allergies within the standard mice. They also show that if you give them an infection with a particularly adherent type of E. coli, this predisposes them to get further

gut problems later in life. And so what we are coming up with is this idea that you have this multi-hit theory that causes these problems that we are seeing.

Now, to give you an idea of the problem with allergies, is that allergies now effect 6% of all under 5 year olds and effects 3% of adults and teens. So, that is a massive ten-fold increase over the last 20 years and on top of that peanut allergy has gone up three-fold. So it is a massive problem. So these are all the factors that seem to be key in developing this. One of the things I think we have sort of spoken about most of these already, before you go out and buy the probiotics though; we will talk more about the probiotics, but the interesting thing is the junk foods and the baby foods. So, there was a very good paper in nature which looked at the effect of emulsifiers on this mucus layer around the gut. Now emulsifiers are food additives, they are very, very common. The reason you add them; they are basically detergents, they dissolve fat in water. So, peanut butter, mayonnaise they will all have high levels of emulsifiers in them and if you ever make your own mayonnaise it separates out, so that is the thing they put in the mayonnaise that on the shelf it never separates, ever. So this showed that by eating emulsifiers in high doses, this completely removed the mucus layer and removed the defences and essentially exposed the immune system to the gut microbiome. So, I think that is interesting. I think you cannot say "we should not eat emulsifiers". We all eat emulsifiers all the time, I love a bit of mayonnaise, but it is interesting.

So the probiotics, and the reason I think we want to talk about probiotics is that they are massive, there is a massive market for probiotics. Everyone I see always asks me about "should I take a probiotic"? The problem about probiotics is that first of all they are not regulated by the drug industry. They are not regulated by the drug industry, they are regulated by the food industry, and so there is lots of data out there that shows when you buy a probiotic you might not actually be buying what it says on the tin. It may also be dead as well and it can also be contaminated with things like milk proteins and all sorts of other stuff. The second thing to say about probiotics, when you look at the studies, these probiotics are very carefully selected for these studies and they are very carefully tested. So when you see a study that says this probiotic it is not necessarily what you get. What seems to be the case with probiotics is they do not engraft in a lot of people. So this is a very good study where they looked at exposure to a particular probiotic and they found that it only engrafted in 30% of patients. In the other 70% of patients whatever factors were at play, be it the immune system, be it the other bacteria, whatever it was in the system, the probiotic just disappeared with time. So you have got to be very careful when you read these studies that say oh this probiotic did this, this probiotic did that, and actually there is really not very much evidence that probiotics are really that helpful.

Again the problem is there are a lot of these studies looking at people with irritable bowel syndrome is that it is such a diffuse population and what I find in my experience is, probiotics actually do help some people. I definitely have some patients going, "this has made a major difference to me", but I just don't know which patient that is and I don't know which probiotic to give them and I don't know in what dose. Is 8 billion colony forming units enough? Do we need 16 billion? Do we need 24 billion? I have no idea, and that is the problem. But the interesting thing is the developments in probiotics we are seeing. So, what they are now looking for is they are looking for these small molecules and proteins using the proteomics and the metabolomics and then isolating probiotics that produce these or seem to promote production of them.

This is a great study, I think this is very clever. So in this study they took three forms of mice, they took a wild [TIME: 30:54] mice, which is the RF mouse, they took two germ free mice and one of the groups they gave human poo too, they loved it and they ate it. They gave a different form of human poo with a very different microbiome to the other mouse, again they loved that and ate that and so they engrafted these sort of three separate microbiomes. And then they gave them this probiotic MB001. Now what is interesting about this probiotic is that they have put a gene into this probiotic that gave them an enzyme that could break down something called perforin. Now perforin

is a carbohydrate that you have in seaweed and as humans and mammals most of us do not have the enzyme to break this down, as do most bacteria don't have it either. So what they then did was they then measure - is there a laser on this - let's try this yeah - so they measured the baseline and there was a baseline in the three groups of how much was there at the level of 7 and then they gave inulin, so inulin is a type of carbohydrate and that promoted some bacterial growth of this probiotic in some patients, in some of the mice rather but didn't make any difference in this one. Because this is obviously being suppressed by some microbiota. But then they added perforin to the diet of the mice. So you've then given this particular bacteria an advantage. You've created a niche for the bacteria and as you can see, foomp, it all goes up. So I think this is going to be where the future of probiotics sits, is where we look at first of all designing proper probiotics that have a targeted action and secondly creating niches for them where they can actually engraft and do stuff as opposed to just taking random probiotics.

So, I just want to talk a little bit about inflammatory bowel disease. I have a interest in inflammatory bowel disease, so that's predominantly Crohn's disease and ulcerative colitis. I would be really interested to know how many people in this room know someone who has Crohn's disease or ulcerative colitis? Yeah. So, it is a horrible disease. It effects young people, it makes them feel terribly unwell, just at the time when they are defining who they are. It is increasing, and we will talk about that later. There is certainly a genetic component but we know that really it is driven by something inside the poo, and we know this because you can do something called a faecal diversion procedure which is where if you give someone a stoma so the poo is diverted away from the diseased bowel distally to that, then the diseased bowel gets better. And if you revert that back the diseased bowel gets worse again. So there is clearly something in the poo that drives it.

There are numerous studies that show that IBD patients have a dysbiosis. The big problem is, have they got a dysbiosis is because the environment is different or have they got a dysbiosis that is driving the disease. And there is only study recently published that looked [TIME: 33:45], it looked at patients who had a high genetic risk of IBD and they found quite a marked dysbiosis in these people even though they didn't have IBD. Which is interesting. There is some great data that came out of Denmark that looked at antibiotic exposure. The great thing about Scandinavian countries is they have these fabulous health records. You will get given a number at birth and then the government records every time you get given a script of anything, every time you have an operation and if anything happens to you, and you get this wonderful data. This is a good thing, My Health Record is a good thing. And so you get these great studies that come out of there, and they showed that if children under the age of 10 are exposed to antibiotics, their risk of IBD goes up substantially. So if you're exposed under the age of 5 your risk goes up by threefold, and it depends on what antibiotic you get. So the more broad spectrum, the more gram negative, the bigger the risk of getting inflammatory bowel disease. Now of course this is an associative study. So you could argue well these children have got abnormal immune systems and that's why they are getting infections and that's why they are getting antibiotics. So we don't know the causal relationship, but it is interesting. And it was repeated in the UK, there is another big database in the UK which showed the same thing. The more broad spectrum the antibiotic, the younger the age of exposure, the bigger the risk. They have also shown that these adherent E. coli events seem to be important and we know that rates of inflammatory bowel disease in immigrants are much lower and there are a few other things that we seem to see this association between this dysbiosis and this disease.

So, this is what IBD looks like; apparently there's a podcast of this so I will have to describe this for those listening, it is pink and healthy and normal, you can see blood vessels. This is what IBD looks like which is a horror show, deep ulcers, red, angry and there is an exudate, it is a really unpleasant disease.

So there are lots of studies that looked at the immune dysregulation that occurs in inflammatory bowel disease and how that relates to the gut microbiome, and what we see is this significant barrier problem that these patients have. So we see decreased mucus production, we see abnormal antigen presentation, so that is where the immune system packages up these proteins that come, or molecules that come from the bacteria and present them to your immune system and if they don't present them right they produce a big reaction. And we see lots of abnormal secretory function as well. We also see the problems with cell immunity and that you get this massive dysregulation so the T-reg cells are not doing what they should do and your immune system is out of control, and that is what causes the ulceration. It is collateral damage to this overwhelming inflammatory response in the gut. We also see overexpression of this thing called madCAM. madCAM is an adhesion molecule in the lining of blood cells and all different tissues have different types of madCAM in them and that is the way white blood cells localise into tissues. And for those who are taking notes there is that diagram again, there will be a test. And then you have immune-mediated dysregulation. We know that all of these pro-inflammatory mediators are right up in the bowel wall of people that have inflammatory bowel disease.

So, as I said you have multiple studies showing that you have problems with the microbiome, you have these exaggerated immune response, particularly to certain proteins and we can also see in these mouse models that we can ameliorate this response using some probiotics. Again, what does that mean, I don't know but it is interesting. And again the short chain fatty acid production is down. So the genetic susceptibility of this disease there are more than 200 genes that they have identified which are associated with inflammatory bowel disease and 70% of them are shared between ulcerative colitis and Crohn's disease. The most important one seems to be NOD2, it is also called CARD15 and this codes for a protein which recognises a particular bacterial cell wall, and in people with inflammatory bowel disease there is dysregulation in this and this is permanently switching on the innate immune system in these people. But essentially all of these genes point to the same areas, and those same areas are killing bacteria once they get through the defences, the cytokine pathway which is controlling the immune function, the barrier function and the adaptive immunity. And that is where all of those genes are focused around.

So interestingly the incidence of Crohn's disease and ulcerative colitis is increasing. So this is a study from Victoria looking at the incidence of Crohn's disease in dark blue and ulcerative colitis from the 1970s through to 2005 and you can clearly see that it is going up. And what is interesting about that is that our genetic material is not changing nearly fast enough to explain that. There are many other things that we see. So, we are constantly seeing more and more patients with this problem. And again this is a study from the states and they have seen exactly the same thing. In a 10 year period they have seen a 34% increase in ulcerative colitis and they have seen a 41% increase in Crohn's disease, which is concerning. I was hoping that the CEO would be here because then I could say we needed more resources in inflammatory bowel disease.

This is a phenomenon that we see globally. So the first case of ulcerative colitis was first described in 1859 in the UK and then we have seen a slow and steady increase throughout industrialisation and then we saw the first case described in China I think it was in 1956 and again we are seeing an increase. And if you look at countries like Indonesia, Crohn's disease was unheard of in the 1980s, we are now seeing an explosion as we see an explosion of the middle-class and there are interesting reasons why that could be. So you can certainly have IBD and there is certainly evidence that if we manipulate the microbiome we can alter the course of the disease. So exclusive enteral nutrition is what we give kids a lot for inflammatory bowel disease, so it is just where you feed them polymeric feeds which are like sort of milkshakes, they are a bit sweet. It works really well in kids and it works really well in kids because we control what they eat. We give it to some adults, it does not work so well in adults because we don't control what they eat. What is interesting about this is if you look at the microbiome in people who are on exclusive enteral nutrition and what you see is a narrowing of diversity. You see a reduction in the bacterial volume that

seems to be driving it. So although all the other studies have said diversity seems to be a good thing, in certain diseases sometimes actually reducing the diversity is important.

There is data showing that probiotics can be effective in certain types of inflammatory bowel disease. Again it is difficult to extrapolate from them about what you get and so on and so forth, and there is some interesting data about Crohn's disease. After a patient has had an operation for Crohn's disease and then we put them onto a drug called metronidazole, which is an antibiotic, that reduces the recurrence of Crohn's disease but it only works for as long as we give them the antibiotic, and the problem is that after three months a lot of patients develop side effects to the antibiotics, but it is an interesting signal all the same. And then of course we have got faecal microbiota transplantation which of course is the ultimate probiotic.

So before I talk about FMT, I really want to talk about Clostridium difficile infection. How many people in here have heard Clostridium difficile infection? A fair few. So, Clostridium difficile is a really important hospital acquired infection. It is not only hospital acquired though. To give you an idea of the problem, it kills more people in the United States than AIDS kills. So it kills a lot of people, and it was first characterised in 1935. The reason it is called Clostridium difficile, it is a Clostridium which is a type of bacteria, but it was very hard to grow in the lab and that is why it is called difficile. But if you get an infection in hospital with C. diff you have a 5% death rate which is significant. What we are seeing is an emergence of this infection in the community, and it also appears to be moving into animals and this may be related to our use of antibiotics and the production of meat, which is an issue. Just saying that all your [TIME: 42:23 ?grass] that you buy from the shops, that is grown on pig manure which is interesting. The problem about C. diff is that it forms spores and these spores are really hard to kill. They are very resistant to degradation and we find spores on toothbrushes and all over the place. So, once it is there it is hard to get rid of. So the risk factors are exposure to antibiotics. If you are exposed to an antibiotic your risk goes up significantly and that risk lasts for about three months. If you have gastric acid suppression, that also increases your risk because the acid in your stomach is a really good barrier to kill it. If you are immunosuppressed, obviously that is a risk and unfortunately if you are aged over 65, we are not sure why, but that seems to be the problem.

So to tell you how I ended up getting into faecal transplants is really I have to tell you about the case that we saw. So this is back in 2011 when I was working at Fremantle Hospital, I came across this gentleman who was in his 60s, he had a stroke a couple of years earlier and was in a wheelchair, but an independent member of the community. He fell over and broke his leg and ended up needing a total knee replacement and so had that and then shipped off to rehab. Unfortunately he developed a wound infection whilst he was in rehab and then got given some antibiotics for the wound infection and then subsequently developed diarrhea. He was diagnosed with C. diff. So he was given some antibiotics, he got better and went home. But unfortunately for him the diarrhea returned within a few days of stopping the antibiotics and he was C. diff positive. Then in the next eight months this poor chap, he had five admissions to hospital. He spent 100 days in hospital. He had a couple of visits to ICU, he became within a hair's breadth of having his bowel removed and he gave every antibiotic we gave him but we just could not get him off the drugs. This is what C. diff looks like. It is really horrible. It is called Pseudomembranous colitis and you can see these horrible pussy bits and it is really very difficult to get rid of.

So, what do we do next? So we took a blender and a gastroenterologist and we found a donor, which is actually harder to do than you think, and then we found a willing registrar. Yeah it is pretty high tech isn't it? So, I described - this was an interesting day - I described this as a low and a high in the same day. So, it was, I mean I have got a pretty high threshold for disgust and I thought it was disgusting. We invited the photographer, the medical photographer in - lovely lady - and she was sort of sitting in the corner having a quiet vomit into the back of her mouth, and I think she has

got mild PTSD because she can no longer look at me when we pass in the corridor. That was the low point. What the high point was though, so we did it in the morning on the end of the list and then we went to see him at about 3 o'clock, so about four hours after the procedure. This is a guy who had been really, really sick for 100 days, and he is sitting up in bed and he is sitting there and he just goes, "I'm better, I want to go home". It was one of the most phenomenal things I have ever seen, and all his inflammatory markers just went "boof" back to normal. Then 24 months later he was diarrhea free, he has had no further admissions, he has got no C. diff. Now, yeah so that is how I ended up doing it.

So, this is the sort of history of FMT in the western world. There are actually stories of FMT in China a thousand years ago, it is called yellow soup over there. So if you are ever in China and offered yellow soup - say no. So, interestingly we are one of the few mammals that don't eat our own poo. Just something interesting to put it out there. I am not suggesting we do but ... So, the first case was in 1958 where a physician was looking after four patients who had intractable diarrhea related to *Clostridium difficile* and he did four poo transplants and they all got better. Then what happened was that someone invented some good antibiotics and everyone lost interest and FMT was the realm of the nut jobs and then as we saw a sort of increased risk with this *Clostridium difficile* infection we start to see more and more of these done by this case series. People have gone through literature and found these case reports. So, okay well we've got this patient we can't cure, let's do an FMT. So then there was a case review in 317 patients in 2011 and then there was a seminal paper in 2013, and we will have a quick look at those. So the case review found that 317 patients, there was a lot of differences. You know, how do you give the poo, do you give it via a nasogastric tube, do you give it via a colonoscopy, how much do you use, 10 g, 20 g, 100 g and do you freeze it? So there were lots of problems. But anyway what the message was, it seems to be really effective.

This is the key study that came out of the Netherlands. So this was a double blinded placebo controlled trial where they took patients who had recurrent and relapsed *Clostridium difficile* infection and they randomised them to either poo transplant or they got vancomycin which is standard of care, or they get vancomycin plus they get colonic lavage which you have to do colonic lavage if you're going to do a colonoscopy. What they found was that with the poo transplant, had an 80% cure rate. With the vancomycin at best 30 per cent. On the second poo transplant they had a 93% cure rate. So they stopped the trial early because they thought it was unethical to continue and continue to offer patients ineffective treatment with vancomycin, and that's a seminal trial. What they also looked at was the diversity of the bacteria there and no surprise to see this is the donors, this is the recipients before their FMT and this is after they had their FMT, and the diversity goes straight back up again.

So, this is what's called a systematic review which looks at lots of different studies which have looked at the same problem and it puts them all into one, does some special statistics and you come up with what's called a forest plot. So the forest plot if you're on this side of the forest plot this says the FMT is good and if you're on this side of the forest plot this means FMT is bad, and once you add up all of these things you see that FMT seems to be good if you've got relapsing *Clostridium difficile* infections. So there is really overwhelming evidence that in this setting it is a good thing to do. One of the problems is, is poo a medicine? This is actually a very tricky problem. So, in the states the Federal Drug Administration has classified it as an investigational new drug. Now you have to bear in mind that the FDA is 50% funded by big pharma and they're not going to make any money from doing poo transplants. So that meant if you wanted to do a poo transplant you had to fill out enormous amounts of paperwork and it was very expensive. So there was this big up in arms from the medical community and they said okay we will call it a biologically active agent, which means that there is only one sheet of paper you need to fill in, and it was left at that. So, Europe has taken a more pragmatic approach, they have regulated it in the UK and in Europe and currently here the Therapeutic Goods Administration initially didn't want to know. I've

got some interesting e-mail correspondence with them about what it is they did or didn't want to know. The big problem is that if we over-regulate it, unlike every other that they regulate, there is a limitless supply.

So, the TGA is currently undergoing a consultation process where they have received submissions from lots of people about how to regulate this and they should come back to us within the next 12 months or so with some sort of regulatory requirements. I mean I say when you can do DIY. You can get on the internet and someone will, there are lots of YouTubes that show you how to do a DIY poo transplant and this is the ACME enema kit, that's what it's called. They've kept the red colour. For those listening to the podcast it's got some strange looking rubber and plastic attachments. So, yep you can go and do your own poo transplant. I certainly do not recommend that you do that. I did hear a case the other day of - patients do some strange things - I had a case of someone who gave himself a coffee enema, piping hot coffee and presented three days later with abdominal pain and bloody diarrhea. I have a colleague in the UK who had a patient who wanted a fecal transplant that couldn't get one so they gave themselves Dettol enemas for three months. Ended up having their bowel chopped out. So, you know, yeah amazing.

Anyway, so following all of this, we set up this study to look at the efficacy and safety of FMT and the treatment of recurrent and relapsing *C. diff* in WA and really the purpose that we set this up for was to get round the legislation. We know that FMT is effective but to do a clinical trial was the only way we could actually deliver it. But the secondary endpoint is trying to work out a way of actually recruiting donors and doing those things and we are working hard to try and set up a poo bank basically here in WA. There's a registrar at the back who's scarred from that experience. So the results to date, so we've done 38 FMTs, a whole age range. I think the interesting thing is they've all had lots of antibiotics. They, on average, had 20 days in hospital. On average they waited 321 days before they got their FMT, but we have a 94% success rate which is great. So by the time they get to us they're begging for it.

The sad thing is, is that we're clearly not doing enough. So looking at the data of how many *C. diff* infections there are, actually we should be doing roughly 250 FMTs a year but we're doing about 20, and that's limited by supply. That's the problem. That's not that we don't have enough people donating, it's we don't have the source of processing. So just in the last couple of minutes because we're running out of time, sorry I've run over time. This is looking at FMT and active ulcerative colitis, and I think this is really interesting. So, there's a couple of studies here where they looked and they gave fecal enemas and they have one a week over seven weeks and they had a significant response, which I think is interesting. This is a photograph, so this is pre-FMT, you can see it's horrible, inflamed and this is post FMT which is much, I mean it's still scarred, it's not quite normal but it's still much better than it was. Now that is interesting. This is one by, this is from a Dutch cohort, and these guys they gave it via a nasogastric tube. They got one, quite a high dose but [TIME: 53:41] in three weeks, and this made no difference. So that's interesting. How you give it and how much you give seems to be important. This one came out of Sydney, and I would term this as 'intensive'. So these people got one via a colonoscopy and then they got 40 self-administered enemas. You've got to be committed to want to do that. But interestingly it was statistically significant, 27% of the patients in the enema group improved, only 8% in the placebo group. Again some really impressive photos about what these patients looked like before and after the FMT. So clearly there's something going on.

We took part in this study, we were one of the centres, and again we had 73 people. These people got an FMT via a colonoscopy and then two enemas, so not quite as intense, and we found that there was a statistical improvement. So, orange lines going down are good, these are the FMT group so this is an improvement, this was at the start, and this was at the end. This is the placebo, no different placebo, much more orange lines over here. So it works. Again if we look at our forest plot here we see probably FMT in UC is possibly a good thing. But, before you go off and do your

own FMT or ask your friend with UC to do it, there are some risks. This is one of the risks that we definitely see a flare of IBD. We see that in 10% of patients, roughly, and there have been case reports of patients flaring their IBD and requiring their bowel removed. So, we still don't know what it is and I certainly don't think FMT should be offered in any other setting other than in a trial setting.

We have got FMT in capsules which is affectionately known as crapsules. Unfortunately not my gag. So, this is just an interesting study that was recently done. So this is again UC patients, active patients, they were taking 25 capsules a day, roughly 12 g of stool, and what we see ... It's open label though, so these are patients what they report, how they feel and high numbers of bad, low numbers of good and you can see that within two weeks they're all feeling better. It's interesting, open label though. This is a measure of an actual inflammatory marker in these patients, and this is less convincing, but you still see a drop in the first two weeks. So, I think it's an interesting idea. But these patients all have active disease. I think this is more interesting. So, this is a study out of India where they looked at patients who are in remission. So they've got their disease under control and were trying to prevent them flaring again, because ulcerative colitis it flares in a very unpredictable way. What they found was that these guys got an FMT via a colonoscopy every eight weeks, is there's no difference in the clinical outcomes when you measure the clinical levels but, if you look at them endoscopically, those that got the FMT are significantly more likely to be in endoscopic remission, it looks normal. If you take biopsies they're significantly likely for the biopsies will be plum normal compared to the other people. So are we going to move to the place where FMT becomes capsules that you take to keep your bowel well?

So I think in summary, hopefully I've got across to you that it's really complicated. We still don't know what's going on, we're still documenting what's there but there's some interesting signals coming out. Don't take antibiotics unless you absolutely have to, I think that's a really important message. They are amazing, they've revolutionised health care but, used wrongly they are very bad. A bit of dirt probably won't hurt you. Eat a healthy balanced diet, I think that's important, we should all probably be eating more fruit and fibre and no one size fits all. I'll just leave you with two quotes, one from Hippocrates to say "let food be thy medicine and medicine be thy food". That's very esoteric and very Greek. My personal favourite though is the Romans. The Romans were a much more interesting civilisation I thought because they were less bound by ideas and more by practicality, and in their forum they invented waterproof concrete so they were the first to invent proper drains. They had the main drain running under the forum and there was a little alter to the goddess Cloacina, and that's where we get the word cloaca from for birds, and this is a poem to Cloacina. "Oh Cloacina goddess of this place, look on they suppliants with a smiling face. Soft yet cohesive, let thy offerings flow. Not rashly swift nor insolently slow".

Question:

What is the difference with irritable bowel syndrome and inflammatory bowel disease?

Response from Dr Oliver Waters:

Yes, although they do coexist. So, irritable bowel syndrome, you have to understand the history of irritable bowel syndrome. As gastroenterologists we used to see patients that come to us, they have all these symptoms, diarrhea, constipation, bloating, abdominal pain and we could never find anything wrong with them. So we chucked them all in the same bucket and we called it irritable bowel syndrome. Then we went through a phase of thinking they were all crazy and it turned out they were not all crazy, and then we sort of elucidated all of these mechanisms that actually drive this. Unfortunately none of those test were available so these were all experimental tests but they've identified at least 40 mechanisms that would explain irritable bowel syndrome. But it's really common, sort of up to 30% of the Australian population has irritability in one form or another and it can be transient.

Question:

What's the difference between prebiotics as opposed to pro? Some doctors say prebiotics are better. What's the difference?

Response from Dr Oliver Waters:

So, what a prebiotic is a substance and they're typically complex carbohydrates, fibres, which we can't digest and the only thing that can digest them are the bacteria, fungi in your gut. So it's really a bit like throwing compost on your garden, it promotes bacterial growth and it promotes other bacterial growth in your gut. As opposed to a probiotic which is actually a bacteria or group of bacteria's which are cultured and live and you eat those and hoping they germinate there. So that's the big difference. You can have what is called a symbiotic which is where you have a prebiotic and a probiotic together.

Question:

You mentioned about acid in the stomach, that you need a certain level of healthy acid I suppose. The PH level in your body, to keep that in balance, is there a way of measuring what your PH level is in yourself?

Response from Dr Oliver Waters:

You can, we do PH studies in people that have reflux but we don't do it routinely. As with all of this stuff, we talk about risk, not everyone that has acid suppression has problems with gut infections and people get gut infections with adequate acid production, so it's all multifactorial. So, what is interesting is we now have these fantastic medications called proton pump inhibitors which are very effective at suppressing gastric acid, and why that's amazing is because 20, 30 years ago what gastroenterologists used to spend their time doing is dilating oesophageal strictures which is where these people would get recurrent ulceration of the bottom of their oesophagus. So although again antacids generally should be avoided if you can, they are very good medications given in the right setting.

Question:

Have any tests been done on diverticulitis?

Response from Dr Oliver Waters:

So, there is some data on diverticulitis but again not really much in the way of data to say you should do this. What we can say about diverticulitis is that it is related to not consuming enough fibre throughout your life. There are other factors though which are how strong your connective tissue is and whether you're likely to develop diverticulum. So, I mean what I say to my patients that have diverticular disease is go and eat more fibre, it won't get rid of the diverticular disease but it will prevent it getting worse. There is some evidence to say that prebiotics, which are in the fibre, also produce "a less inflammatory", but there's no hard evidence to say if I eat this I'm going to get less attacks of diverticulitis.

This document can be made available in alternative formats on request.

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