

Good bugs gone bad: The microbiome in Heal - Pediatric Grand Rounds-20240906_082931-Meeting Recording

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1h 9m 27s

● **Kamat, Deepak M** started transcription



Kamat, Deepak M 0:06

I'll.

I'll take care of it.



Haslam, David 0:09

Great.

Thank you.



Kamat, Deepak M 0:37

Good morning.

It's 730 and is trying to start our grand rounds.

The CME code for this morning grand rounds is in the chat box and will keep repeating it every few minutes every 1015 minutes.

It's my great pleasure to introduce this morning's Gannon, speaker, doctor David Haslam, who is a professor of Pediatrics in the division of Infectious Diseases at Cincinnati Children's Hospital, where he is the medical director of the Antimicrobial Stewardship Program and director of the Microbial Genomics and Metagenomics Laboratory of the.

Haslam received his MD from the University of Calgary Medical School, and he completed his residency in Pediatrics at Children's Hospital of Eastern Ontario at the University of Ottawa.

Subsequently, he did his clinical fellowship in pediatric Infectious Diseases at Saint Louis Children's Hospital, Washington University School of Medicine and Research Fellowship in Molecular Microbiology, also at Washington University School of Medicine in St.

Louis, Missouri doctor Haslam's research focuses on childhood diseases caused by a

bacterial toxins and the role of microbium in health and disease.

His interest in microgram is closely related to his goal of improving appropriateness of antibiotic prescribing practices as antibiotic induced disruption of microgram appears to place children at greater risk of disease, including hospital based acquired bloodstream infections, not Haslam.

Thank you very much for accepting our invitation.

I know you are very, very busy here and clinical service right now and still decided to join us today.

So really appreciate it.

The floor is yours.

HD Haslam, David 2:22

Ohh, thank you very much.

It's my great pleasure to to be invoice invited.

Thank you very much and and discuss some of our our work both as in my role as director of the Antimicrobial Stewardship Program and role as Director of the Microbial Genomics and Metagenomics Laboratory, otherwise known as the Microbiome Laboratory.

So I'm actually going to talk about both of those roles and hopefully convince you how I think they overlap and how my interest in the microbiome is really driven by antibiotic prescribing practices and how they may influence the microbiome.

For better or worse, and I'll say mostly for the worse as well as we'll find out as we go along.

So I don't have any relevant financial disclosures or or conflicts of interest.

Umm.

And we'll start by saying uh, so I'm sure you're all aware that that the human skin and mucosal surfaces harbor a diverse community of microbes.

And this is now been well established over the past 15 plus years where.

P patherndon 3:32

Yeah.

HD Haslam, David 3:34

Uh, at that time, new technology allowed us to really document or investigate which organisms which microbes were present in different parts of the body.

So we know, for example, that the gut, the intestine harbors actually more than more than 2000 species of bacteria, fungi, and viruses.

The mouth is also not shown on this slide, but the mouth is also a very rich microbial environment, almost as many species of bacteria live in the mouth as in the interest.

Excuse me, intestine.

Surprisingly, and the skin as well, has a very diverse microbial and I would say as clinicians, we don't really think about the microbiome very often if at all, but probably as I'll as I go through the presentation, you'll see that I think there are clear cases where the microbiome does influence health and in particular from my perspective as an infectious disease doctor influences our risk for infection.

You're we do know now that these microbes usually serve beneficial functions.

They provide nutrients and metabolites that were still understanding how that influences health, but a very clear example, I think, for pediatricians that we've known about for a long time is that the intestinal microbes provide us with vitamin K so humans have no ability to make vitamin K on our own.

We rely on microbes in the intestine to metabolize uh, precursors to produce vitamin K, and we know that because one of the first things we do to babies after they're born is give them a shot of vitamin K, and that's to give them a boost until the babies are able to establish their own microbiome, which can produce vitamin K without supplementation.

We know sometimes in kids if we treat them with very broad spectrum antibiotics for prolonged periods, they're at risk of hemorrhage because of vitamin K deficiency.

And that's because we deplete microbes that provide that provide that vitamin.

But we're learning more and more about beneficial metabolites that are produced by the microbiome and and could influence health.

In that regard there, there have been many, many studies now that have looked at a link between the microbiome that is, which organisms occupy the intestine, the mouth or the skin of a person, and how that relates to disease.

Yeah, I like to show this graph looking at the number of publications every year that describe some sort of a link or putative link between the microbiome and disease states.

And some of those diseases that have been most implicated in as associated with the microbiome or allergies and topic diseases like asthma, heart disease, cancer, diabetes, many immune mediated disorders, especially inflammatory bowel disease,

but even neurologic inflammatory diseases like multiple sclerosis and even neurologic diseases, which seems a little bit counterintuitive if we think that there shouldn't be any microbes in the brain influencing risk for Alzheimer's or autism or Parkinson's disease.

But it's not so much the presence of the organisms in this central nervous system that that seems to contribute to these neurologic diseases, but rather it's the metabolites that are produced by organisms, for example, and that got or the the mouth or skin that may then go to the brain and influence risk for some of these other diseases.

I don't study these in any of these diseases directly, but but in my role as the director of the Microbiome Lab, we have worked with many investigators across the the university and the surrounding area to look at most of these disease States and and compare the microbiome, for example, and people with or without autism or or Parkinson's disease, areas that I have been directly involved with include C diff infections.

So this is probably the the disease where there's the very clearest link between abnormalities in the microbiome and risk for C diff infection.

We all know that antibiotics are the major risk factor for C diff infection and and we now know that replacing the microbiome, for example, with fecal transplant from a healthy person is a very effective preventative against C diff infection.

Now there are some some products on the market that are derived from mixtures of organisms that can colonize the Gut and protect against C diff colonization and infection.

So very clear associations between an abnormal microbiome and C diff risk.

But I'll show you some work that we've been doing that shows, at least in hospitalized high risk patients, there's a link between an abnormal microbiome and risk for invasive disease.

So we'll jump right in and and look at that data now.

So we did a study looking at patients at very high risk for blood stream infection, including bone marrow transplant patients, liver transplant, kids with intestinal failure or often called kids with shortcuts.

So many of these are babies or children who had small bowel resections due to necrotizing enterocolitis and were subsequently TPN dependent.

Umm.

And then children at our institution, who had undergone small bowel transplant.

So these are in our hospital or the children that are highest at highest risk of bloodstream infection.

And we did a prospective study where we collected stool samples from from these children and then monitored for children that subsequently developed bloodstream infection.

And you can see that the rates of infection were very high during this two year 2 plus year study period.

Umm.

13% of overall transplant patients develop a blood stream infection and you can see the rates and other disease states were even higher.

So that meant that we had stool samples in many patients before they developed the bloodstream infection, and then in some children pics use me.

We have stool samples after they developed bloodstream infection and so we could look at the microbiome and those kids and see if we saw any difference between children that developed bloodstream infection and those that didn't.

And our hypothesis was that we would see differences in the kids that developed BSI compared to those that that didn't.

And in fact, that's what we found.

So just to take a step back and and discuss how we measure the microbiome or how do we know which organisms and how many of those organisms are there, what we do is take a sample.

So in this case, we're looking at stool samples, which contain bacteria, different types of bacteria, fungi and viruses, and we extract all of the DNA from the stool sample, and then we go through a process of digesting the the DNA and then subjecting each fragment to a next generation sequencing.

And we use those sequences as a bar tag.

Basically, to align to a database of microbial genomes, and if there's a match between a DNA fragment sequence and the the genome database, then we assign that read to that microbe and then we can count up the number of reads that lying to different microbes and present the data in different ways, including this bubble graph, which is one way I like to look at the data.

So in this bubble graph, each circle is an Organism and then the size of the the circle represents the abundance or the amount of that bacteria in the sample.

And you can see for example in this sample there are many, many different bacteria. And in many cases it's a fairly diverse microbiome.

So there are lots of different organisms, one one or two or most prevalent.

But in general it's it's.

It has a good diversity, which is in the stool is a good thing.

And so when we looked in our patient cohort, we saw patients like this.

This was this is a bone marrow transplant patient who came into the hospital for conditioning for bone marrow transplant.

We had a stool sample shortly after the patient was admitted that looked like this.

So there were many different organisms similar to the previous slide, and most of these were normal commensal organisms.

So back to roadies.

Very common.

Uh, uh and other organisms that should be in that got.

And we would say are generally thought to be beneficial.

The child was undergoing chemotherapy and conditioning for leukemia on transplant, spiked a fever and you can see compared to this sample on December 6, after receiving antibiotics for a few days, the microbiome had totally changed.

And now was completely dominated by E coli.

Umm.

And the antibiotic treatment continued and the the patient continued to have low grade fevers and then became acutely ill, septic actually became hypotensive and had to go to the intensive care unit and was started on pressors and and I was very ill and the blood culture two days after the stool sample group, E coli from the blood and so we can actually not just look at the Organism, but we can look at relatedness and compare the the genetic sequence of this E coli compared to the Organism that was in the gut previously and were able to.

Show that these are essentially identical organisms, so to us this suggested that or indicated that antibiotic treatment had disrupted the normal microbiome allowed per proliferation of this E coli.

And then for your call, I went from the intestine into the bloodstream.

This patient's equali was resistant to antibiotics, and it took us a little bit of time to get the patient on the appropriate antibiotic, but fortunately the patient recovered and subsequently had the bone marrow transplant and and has done well.

This is kind of an extreme example of we we had many patients that didn't have as much disruption during antibiotic treatment.

But I would say there were.

There were several patients where we saw similar pattern and overall I'll show you how.

There are associations across all patients with between the microbiome and bloodstream infection, but I would say this experience and working with our BMT colleagues as well as oncology and critical care situations like this where we can say really antibiotic treatment in this case seem to predispose to infection has had a pretty big impact on the way we think about antibiotics even in our highest risk kids. So the previous slide was one individual patient experience.

When we look across all of the samples, we one way to look at the microbiome composition in children that have bloodstream infection compared to those that don't is to look at the microbiome composition in this 22D graphical ohh way method called principal component analysis and in this analysis each sample is a dot and then the closer together the dots are the more similar.

The samples are the more similar the microbiome composition is, and here we're looking at 55 control samples.

So healthy healthy patients and you can see in this analysis those 55 samples all clustered fairly closely together.

Then we looked at patients who develop blood stream infection 30 day within 30 days of having a stool sample and we when we looked at these kids that developed bloodstream infection and looked at their stool within the last 30 days before developing bloodstream infection, we could see that essentially none of them had a microbiome composition that would fall within the normal range.

And that was the Case No matter what type of Organism cost the bloodstream infection, we saw essentially all of the kids that developed bloodstream infection had an abnormal microbiome prior to developing the invasive infection.

So when we looked at which organisms were associated with risk of infection, we found a little bit surprisingly that it was mostly a decrease in the protective bacteria. That was correlated with risk of bloodstream infection and not so much the abundance of pathogenic bacteria.

Suetonius was correlated with risk for infection and lower on this list on things like E coli abundance and Klebsiella abundance were correlated with risk.

But it seemed in this analysis that it was in fact a decrease in these really commensal or beneficial bacteria that was a greater risk for subsequent infection.

So it seems like they really are protective against against developing invasive infection and so that leads to the model that gets may come into the hospital with a

diverse microbiome.

Yes, which we would say is protective against infection, receive antibiotics that in many kids really disrupts the microbiome and leaves.

Pathogenic organisms, often antibiotic resistant pathogenic organisms, and this is really a set up in high risk kids for developing and invasive infection.

Things like breakdown of the intestinal barrier through chemotherapy, alteration and feeds that that may impair beneficial metabolites may allow the gut to become leaky and let these organisms into the blood stream to cause sepsis.

Another area that we were interested in was whether there was any evidence that kids that harbored an abnormal microbiome might be the source of transmission of those resistant organisms to other children in the hospital.

And in fact, when we looked at the organisms in the stool that these high risk cohorts, we were able to see several examples where we thought there was evidence of transmission of an A pathogenic Organism from one patient to another. So this slide shows each point on this on this graph is an individual sample, a stool sample, and we're looking at in this case it E coli.

Within the stool of the of these patients, the samples are colored by patient identity. So this is one patient that gave us two stool samples with E coli and we when we compare the ecoli, these two are very close to each other, essentially identical in genetic sequence.

So this this child had the same equali at two different time points.

And when we look at different patients, we see that they're often colonized or carry the same equal Lian.

They've got over time, this was a patient, a liver transplant patient who carried the same equali in the stool, but also developed invasive bloodstream infection with the same equal eye.

When we looked at other patients, one of these was a liver transplant patient, but there was a bone marrow transplant patient who didn't seem to have any overlap in the hospital with these patients.

Nevertheless, these three kids seem to have exactly the same equal I in their in their gut.

This doesn't prove prove that there was direct transmission from one child to another.

In fact, it probably wasn't direct transmission, but we would argue this suggests that there's there was a a route through the hospital that E coli from one child was able to

colonize other children.

We looked at this expands this this section a little bit.

We looked at this hypothesis and a slightly different way, so last year or two years ago we were noticing an increase in infections with vancomycin resistant

Enterococcus among our bone marrow transplant and oncology patients.

So this is uh, number of VRE isolates invasive infections in our hospital, and you could see that we really had none for several years.

And then a real uptick.

And so we wondered, is this what's driving this?

And was this due to colonization?

Was it due to transmission or were these just random and just happened to to to be different organisms but but a random spike in the number of VRE infections and so one of our fellows, actually Catherine Murphy, collected these invasive isolates, the blood isolates, but also we had been screening for VRE colonization.

And then culturing that VRE?

Umm to collect those organisms and ultimately compare them between organisms. VRE organisms in the gut from patients compared to the invasive isolates, so in this case it's a little bit different.

We're not looking at the microbiome, but we're looking at individual organisms cultured from either the stool or from blood or respiratory tract and isolates.

And when we did that, we found that most of the isolates collected over this time period were exactly identical.

So that you know the genetic sequence was identical between these VRE isolates, even though some of them came from stool.

For example, two oncology patients in July of 2022 had an Organism in their intestine that was identical to subsequent isolates.

You can see from cology patients from the bloodstream 3 months later, so it did look like there were patients that were colonized with VRE.

These two kids didn't are one was an adult, didn't develop invasive infection, but subsequent patients did develop invasive infection with VRE.

And we would say likely either these patients or other patients that we weren't sampling could have been the source for these later infections.

So.

So that leads us to the to the point where we think that if we monitor high risk kids in the hospital by collecting stool samples or I would say even even skin or recipe

oral samples, we may be able to use methods like shotgun sequencing or metagenomic sequencing to look for the presence of an abnormal microbiome that would put a child at risk and subsequently hopefully be able to intervene potentially by, uh, yeah, ideally using fewer antibiotics in these high risk kids that might disrupt their microbiome less, but at least have a, A.

Clue as to which Organism?

The child's at risk of infection with and this extends not just to the high risk kids that I've discussed so far.

We've also been looking at the microbiome in preterm infants, and this is driven largely by Yanping, who's a neonatologist from Tsinghua University in China, as well as my colleague in neonatology, Enria Kimmel Kimbe here at University of Cincinnati. And we were interested in the known observation that the type of Organism that causes infection in preterm babies is different in the United States compared to China.

So in the US, now most bloodstream infections in preterm infants are caused by Gram, gram positive organisms that showing at the sort of.

A highest level here where we compare a baby's in the NICU from Cincinnati versus Tom, Joe Umm, where in Cincinnati, most bloodstream infections over a two year period are caused by gram positives, whereas in China, Hangzhou, China, most bloodstream infections are gram negatives and then particular Klebsiella accounts for most of the bloodstream.

Infections in Hangzhou, whereas in Cincinnati, most bloodstream infections in babies are from staff orias, and so our hypothesis was if that's the case, it probably is related to the underlying microbiome.

So probably the organisms that are present on the skin or in the gut of babies in Cincinnati is different than the baby's in Hangzhou.

And so we sought out to investigate that by collecting stool samples and skin swabs from babies at day seven of life and Day 21 in both uh centers and then sequencing the microbiome to look at for potential differences in the abundance of organisms.

And indeed, when we look overall at all of the samples obtained during the study and compare the organisms that are most abundant, we find that Staff orius is the most abundant bacteria in the microbiome of kids in Cincinnati, whereas in Hangzhou Klebsiella and ammonia was the most prevalent.

Similarly, for example, organisms like Acinetobacter were more and Enterobacter were much more prevalent in Hangzhou than uh Cincinnati, whereas other grand

positives like Staff Appy were somewhat more abundant in Cincinnati.

And that's showing graphically here.

When we look at either axilla, groin or stool at week one or Week 3 of life in either Cincinnati or Hangzhou, and we can see, for example, for Klebsiella at most time points and body sites, the abundance of Klebsiella was higher in control.

Then since Annotti, interestingly that was the case for axilla and groin, so skin samples had a lot of gram negative organisms and it was less notable in the stool, which was a little bit of a surprise to us because we would have assumed that if Klebsiella S causing bloodstream infections and these babies that it was probably coming from the stool.

But actually this information suggests that maybe Klebsiella infections in babies in Hangzhou may be coming from the skin rather than the gut.

We saw similar trends with Acinetobacter.

So recall that these organisms cause most of the bloodstream infections in Hangzhou and we saw increased abundance of Acinetobacter and Harjo, compared to Cincinnati.

And it was the inverse.

When we looked at staff like oxide, so staff warriors was by far the most abundant Organism on the skin of the axilla and groin, skin and babies, and Cincinnati and more abundant than compared to Hangzhou and more abundant than the stool.

And that was the case for staff apply as well.

So overall, we found that Staff Oreos and staff epidermis were more common cause of bloodstream infection and also much more abundant on the skin in Cincinnati and it compared to babies in Umm in Hangzhou.

So there is an overall correlate between differences in the microbiome and risk for infection and I guess in some ways that's intuitive, although I would say when I started out as an infectious disease fellow or junior faculty, we, I think we used to think that these organisms like a staff worries bloodstream infection just came out of nowhere.

It was because the line was put in incorrectly or or you know, there was transmission directly from one baby to another.

But now I think we're starting to see that.

It's what happens for weeks, days to weeks before a bloodstream infection that really put the baby at rest.

So what are the drivers that account for increased amounts of staff?

Or is on the scanning in babies and and Cincinnati and what are the drivers of increased *Klebsiella* and *Acinetobacter* in the microbiome and babies in China? And could we adjust those to influence the risk of bloodstream infection, ideally to decrease all of them with commensal organisms and hopefully prevent infection with with any of these pathogens.

So I'll revise an earlier slide to say that it may not just be the gut, but the skin microbiome is probably an important contributor to risk for bloodstream infection, both in the in the newborn nursery but also in our in our high risk patients.

So we were interested in not just identifying the organisms that were present on the microbiome that seem to correlate with risk for infection, but could we actually predict which antibiotic was likely to be the umm to be the most ohm effective against those organisms if the patient did develop bloodstream infection?

And so here we're using fairly advanced machine learning tools to try and predict antibiotic resistance based on microbiome data.

And I'll show you how that works.

So for example, this is that patient I highlighted earlier.

What if we could tell here that this *E coli* was resistant to multiple antibiotics?

It certainly would have influenced, I think what we did when the patient became septic, we probably would have been able to more quickly get the patient on the appropriate antibiotic.

So what we'd like to be able to do is not just identify abnormalities in the abundance of organisms, but be able to predict which antibiotic is most likely to be effective.

And so we started that by looking at individual isolates and developing a a neural network to go from the sequence of this Organism to predict which antibiotics were likely to be sensitive or resistant on active or inactive against this equali.

And so I had a summer student from Harvard who who had never done any programming.

He he didn't hadn't any experience with machine learning before him, nor did I.

But we decided we were gonna try and do this.

So what we took many, many different isolates of equal iclips, seela, summonings, and then have sequence data for them.

And we looked at the abundance of resistance genes and then the coverage, basically, how much of the gene was present in the Organism.

And then he developed this neural network to be able to predict resistance to various antibiotics, and by the end of the summer.

But here developed a neural network that was very accurate.

So you could feed in, we could sequence.

Uh, an equal I or a clepsydra?

Pseudomonas.

And then feed it into this neural network and it would.

It could predict meropenem or cephem or other antibiotic susceptibility based on known susceptible, you know, susceptibility of those organisms.

Umm, with what we predicted it very accurately and for example for staff orius and methicillin resistance, we were essentially 100% accurate in predicting resistance.

So.

So this was nice, at least from individual organisms that we already grew in the laboratory, had already tested in the clinical lab for resistance and then could predict their resistance based on their sequence.

We wanted to know if we could extend this to a sort of crude microbiome data.

So could we take a stool sample sequence?

Everything that was in that stool sample and then identify antibiotic resistant organisms in that stool sample.

And so I had another I was a Co mentor of PJ Van Camp, whose it was actually a Pediatrics resident from Holland who came to Cincinnati to do a bioinformatics PhD.

So I was a Co mentor of DJ and he took the work that Max had done previously and applied this to the microbiome and had a very productive couple of years and was able to develop a few tools that could take shotgun sequencing data.

So just all of the all of the sequence sequence information from a stool sample and then be able to predict the presence of an antibiotic resistance gene or Organism with very high accuracy.

And not only could he predict the presence of Ann resistant Organism, but he could say this.

It's likely to be equali that carries this resistant mechanism, or it's likely to be Klebsiella.

I don't.

And so we think now that from just taking a stool sample, putting it through the sequencing protocol, we can predict.

Ohh, the presence of a of a multidrug resistant Organism and if we could do that in real time cheaply, neither of which are possible now.

But if we could do that, we think it could be quite beneficial, especially for these kids

that are likely to develop bloodstream infection during their hospitalization. So umm, just to turn to one last project that we did, looking at antibiotic resistance and antibiotic effects, this follows up on the work that I mentioned from preterm infants and here we're looking at how antibiotic exposure affects the microbiome. It's developing in preterm infants and then I'll show you that we can look at not just that presence of resistant organisms, but actually expression of those resistant genes. So when we look at the microbiome at babies that at one week of life and these are preterm infants, they're microbiome.

This is again a principal component analysis where this looks at the whole abundance of organisms and that got where the skin and then looks how similar the samples are and then we can group them based on.

For example, if this is, these are samples from the axilla babies that week.

One this is at week 3 so you can see there's a shift in the overall microbiome composition based on where the samples fall on this PCA plot from Week 1 to Week 3.

So early in life, the samples are a little bit more closely clustered together and over a two week period there is maturation and differentiation of the microbiome and in babies that do not receive antibiotics.

So these are although these are preterm kits, actually our nick you now is and remarkably not treating most preterm babies and unless they have evidence of sepsis, that's a change from our recently when all preterm babies used to get a a sort of a rule out with antibiotics that's not the case then most preterm babies in our NICU anymore but many babies did get antibiotics during that first three weeks of life and compared to babies that didn't get antibiotics at week 3 here in the upper sort of circles we found that antibiotic exposure pushed or blunted.

Maturation of the microbiome.

So compared to babies without antibiotics, the microbiome was more blunted towards this less differentiated state compared to babies that didn't get antibiotics. So we we argued that antibiotic exposure blunts maturation of the microbiome and if that's the case, then organisms that are beneficial for gut development, for example maybe impaired in their in their maturation in babies that received antibiotics.

So we looked at genes for production of a.

Metabolites called short chain fatty acids, so butyrate and acetate are thought to be very beneficial for gut health and we found that in babies that did not receive

antibiotics, they acquired genes in bacteria able to produce butyrate from Week 1 to week 3 and this should be beneficial then forgot health and gut development. But if babies received antibiotics, we completely blunted their acquisition of these beneficial metabolic genes.

And that was the case for acetate as well.

We also thought we saw enrichment for antibiotic resistance genes in babies that received antibiotics versus babies that didn't.

This is perhaps not too surprising, but still worrisome.

I would say these are aminoglycoside resistance genes that were more abundant.

Babies that received sets of gentamicin compared to babies that didn't, and same with broad spectrum antibiotic resistance genes like CTX M and oxa beta lactamases. And this gets a little bit complicated as if I haven't given you lots of complicated data already, but here we're looking not just at presence of an antibiotic resistance gene, but how highly is it expressed.

So not only is the DNA present, but if it's being expressed at high level, the RNA will be expressed to an even greater degree than abundance of DNA.

And so we could look at expression of individual antibiotic resistance genes based on RNA versus DNA abundance and babies that had no or low antibiotic exposure compared to babies that had high antibiotic exposure.

And we saw really dramatic increases in expression of several antibiotic resistance genes.

We saw for example AMP C beta lactamases were markedly upregulated in the intestines of babies that received antibiotics, and this is actually a known thing. You may have heard of inducible beta lactamases, so those are the amp C beta lactamases where antibiotic exposure is known to turn on expression of these genes and can make organisms resistant to treatment on therapy.

So this was somewhat expected, but we saw increased expression of uh, for example, aminoglycoside resistance genes and other very extended beta lactamases.

And this hasn't been seen before, but what it suggests to us is that exposing these preterm babies during antibiotics not only enriches abundance of the genes, but turns on their expression to make it more likely that they'll be resistant if the baby develops an invasive infection.

So that's a lot of information, but at summarized the key points here, organisms present in that gut scanned or oral microbiome are the likely source of the most invasive infections in the hospital.

And I'd argue in that community as well.

Asymptomatically colonized individuals may be the source of transmission to other kids, at least in the hospital, and probably in the community, and that antibiotic exposure modifies the microbiome to decrease the presence of beneficial Organism and increase the abutments of pathogenic organisms.

Further antibiotics increases the presence and the expression of antibiotic resistance genes.

So antibiotic exposure increases the risk for later invasive infection with antibiotic resistant organisms.

So this is all well and good, but it's all research and right now not very applicable to real time actionable information, but nevertheless I will say that our work on the microbiome with our colleagues and bone marrow transplant, oncology and and other transplant services, I think has they've contributed all of these samples and and been involved in the analysis.

And I think it's really changed the way that that we think about what we're doing with antibiotics and kids that that are at risk of subsequent infection.

That's and and I would say for my first several years as a director of the Antimicrobial Stewardship Program, we were really focused on those high risk kids.

And we've had an impact there.

I'll show you some of that data in a minute, but in reality, most antibiotic prescribing happens in the outpatient setting and that's an area where we have been less focused.

But I think that's it's we've realized now that we've had some impact on our high risk hospitalized kits, but most antibiotic prescribing actually happens outside the hospital and we think much of what happens in hospitalized kits also happens in the community when we expose kids to antibiotics.

So I don't have to convince anybody that there have been increased rates of antibiotic resistant infections at least over the previous years and that antibiotic resistance results in worse outcomes for patients and also for the healthcare system accounts for lots of costs.

We know that young kids receive the highest rates of antibiotics, so this is number of antibiotic prescriptions in the outpatient setting at different age ranges.

So that gets less than 10 years receive the highest rates of antibiotic prescriptions per year compared to any other age range.

When we look at what antibiotics are being prescribed for, we know that in young

infants it's mostly otitis media.

And as kids get a little bit older, for example, in the early teenage and toddler years, there's a bit of a shift for otitis to pharyngitis as the reason for antibiotic prescriptions.

And yet we know that most cases of otitis media are viral.

It caused by viruses and similarly for pharyngitis around the same percentage of cases of streptococcal pharyngitis are caused by viruses, and yet those two together account for most prescribing in the outpatient setting.

Well, this is a huge problem because we know that babies get a lot of viral infections every year.

This was a study done by my colleague Mary Stat and one of our fellows, Tio Zahi, who's now in University of Seattle, so they Mary has been collecting samples from babies and moms during their first two years of life in a very intensive way.

So she collected nasal and stool samples every week from a large cohort of moms and babies.

And then also very intensively kept in touch with the family and the medical records to look at illnesses.

And so from this two year collection period, there were 13,000 nasal swabs tested for viruses and these are in babies that were symptomatic or asymptomatic.

And of those 13,000 samples, 2000 had a virus, a viral pathogen detected, and so they found that each baby in their study got an average of 9.4 viral infections per year.

And these were things like RSV, influenza parainfluenza this cohort was before COVID, although she's continued through COVID and umm and I would say the epidemiology changed a little bit, but still the rates of viral infection are are dramatically higher than I think any of us would have predicted.

So in fact, much of the time when a swab was obtained, there was a virus present whether or not the child was ill.

But I think this sort of gets to the point that babies are gonna get or young infants are going to get sick and they're gonna get lots of viruses.

And most times they're children are gonna recover without intervention.

Yet many of those kids are gonna receive antibiotics, so.

I mentioned already that up to 95% of antibiotics are prescribed in the outpatient setting.

30% of those prescriptions are probably unnecessary based on guidelines and about

half of them may be either inappropriate antibiotics or inappropriate durations.

We know from 2000 to 2010 worldwide there were increases in antibiotic prescriptions across all antibiotic classes.

This is globally looking at 2000 to 2010 and increases in antibiotic prescribing and there were increased rates of resistance for essentially all organisms that were looked at.

This is Klebsiella resistance in the US to different antibiotics over time and from early 2000s to 2010 there were increased rates of resistance.

Around that time, American Academy and the Infectious Disease Society came out with practice guidelines for most of the respiratory illnesses or other illnesses that (Dr.) antibiotic prescribing in Pediatrics.

Similarly around that time.

There was a there has been a requirement for each hospital to have an antibiotic stewardship program, and that was most recently implemented for Children's Hospital, where now it's required that all children's hospitals have a stewardship program.

And so I'm director of that program and and we've so there are a number of different ways to do stewardship.

We mostly have focused on black guideline development and education.

Although we know that that can be less effective than more restrictive practices like formulary restrictions where you just can't have an antibiotic to requiring prior approval.

So we focus mostly here on and and some of that shown on this next slide.

So we rely a lot on a on a computerized uh.

Third party system that monitors prescribing and and out and culture results, and we've used that information to help educate our colleagues and help develop guidelines.

I would say this has been a major activity as to collaborate with our colleagues to develop guidelines.

We do have some restrictions, although I would say the I'm I'm not a very aggressive person and so this is probably less effective for us than than some of our our guidelines.

And if we look at what's happened over time, we have seen decreases in overall antibiotic use that were not so much reflected in antifungal and antiviral use, although recently we have been starting to look at antifungal potential overuse as

well.

If we look at individual antibiotics that we were most targeting, we saw decreases over time and Meropenem, Vancomycin, Cipro and aminoglycosides in particular and that was I think has been very rewarding.

I'll just go back to this and say this is now all inpatient prescribing and we've had some really great advocates, including our director of the Bone Marrow Transplant unit, Stella Davies are oncology and intensive Care unit colleagues have all contributed to this decline.

But until recently, we haven't really focused much on outpatient antibiotic prescribing.

But we have now mostly through a collaborative run by my former fellow, actually run Alphaete, who's now a professor at at in Kansas City who has put together a collaborative where we share our outpatient antibiotic prescribing practices.

And here we're comparing Cincinnati and Orange over time to peer hospitals and looking at outpatient antibiotic prescribing practices.

And so we see, for example, on the emergency room, a child that comes in with a respiratory illness gets antibiotics about 15% of the time, and the urgent care, it's a little bit higher in primary care.

It varies and we see big differences in our individual practices in prescribing rate. So there's practice to practice variability.

One thing I'm not going to show here, but we've also been looking at outpatient equity equitable prescribing practices and we do see like many other centers that there are differences in antibiotic prescribing practices based on race.

For example, we also found that primary language spoken in the home.

This seems to be a driver of disparate prescribing practices.

So for us, spent that kids that speak predominantly Spanish at home, receiving antibiotics at a different rate than kids that speak English.

And I think Cincinnati, we're not a we don't have a large Spanish speaking population.

And so we're not a very well equipped with interpreters and we think that in addition to race and ethnicity that that language is a barrier to appropriate prescribing in in some of our in most of our our primary care practices.

Umm, if we look at, I'm sorry, this was all all visits prescribing rates.

This is respiratory illnesses.

So you can see that that if you if a child comes in with a cough or runny nose, they're

gonna get antibiotics about 40 to 50% of the time, although there is variability among again our sites and our providers.

We can look at which fraction of kids received amoxicillin, which is the first line recommended antibiotic for most respiratory illnesses, and this fraction in this case, the higher the better.

And Cincinnati, we are at the low end.

This is kids that are prescribed as if we're mycin as the first line antibiotic and here were on the right side of where we prefer to be.

So we have work to do.

If we look at the percentage of prescriptions that are greater than 10 days, which is almost always in a appropriate in the outpatient setting, we find that we're really outliers compared to most of our peers.

So we do have work to do and that's just goes to the, to the point that we haven't really focused on how patient prescribing Oriana and her group has shown that you actually can improve appropriate prescribing practices in the community.

And so they did this study where they looked at 4 interventions to see how that impacted impacted appropriate prescribing.

They did tracking of antibiotic prescribing and then reporting back to providers that provided education and handbooks.

They develop order sets.

So for example, if the diagnosis code was otitis media, they developed order sets that suggest that the most appropriate antibiotic and duration, and then they had providers voluntarily put up letters of commitment that, say, say things like I'm dedicated to appropriate antibiotic prescribing or and umm and looked at how that impacted prescribing practices and they saw over time and improvement in their first line or a guideline recommended prescribing.

So she, she would say, and her group would say that providing education and treatment guidelines to prescribers is helpful.

Information sheets for families.

Comparative antibiotic usage information to peers.

So I mentioned there are there are differences across our practices and across patient populations and we feel that just providing that information may in in some cases influence how our how our prescribers are using antibiotics and we've run into a little bit of we're trying to implement these in a study now that we're doing where we're looking at suitability or adaptability of some of these interventions.

And sometimes these commitment posters are not very popular, although I would say many of our uh primary care providers are are interested in having a poster in their office that that discusses this.

So I mentioned guidelines have been developed.

These actually most of these have not been updated since 2012, so that these guidelines are still the existing guidelines.

That's sad.

They're multi page documents and it can take some time to weigh through these and I think for a busy pediatrician to have to flip through a guideline each time a child comes in is not very convenient.

But there are some more succinct resources, and one that I really like is the American, the Redbook, the AAP Redbook that has in the back a systems based treatment table that, for example, acutis media lists the most common organisms.

What is the appropriate first line therapy?

What's the recommended duration based on age and what other things should you think about so this watchful waiting for example and in kids over 24 months of age is recommended for most kids with non severe otitis media and and so we're trying to develop resources just on one page resource with the most common respiratory and other like UTI illnesses and the the guideline appropriate therapy and I'll just finish by saying that although we often in stewardship are people that walk around spouting gloom and doom, there is actually good news since guidelines were developed so this figure.

Looks at antibiotic prescribing up until 2015 in different countries and looked at change in antibiotic prescribing practices and actually in North America and some other countries in the world, there have been decreases in antibiotic prescribing.

In the most recent period and that's been correlated actually with declines and antibiotic resistance, at least in this case, this is strep pneumo.

And here we're looking at penicillin and selfless foreign resistance.

And in our hospital and many places, rates of highly resistant strep pneumo have fallen over the last several years.

And I would say this is really because of the guideline.

Well, the guidelines almost certainly contributed to less antibiotic use for otitis media in the community and that's had a direct impact, alright, rates of resistance.

But if we look at other organisms like mersa abundance, that's actually falling across the United States over time, and we've seen that in our hospital where MRSA causes

fewer even scale and soft tissue infections than, say, 10 years ago, VRE rates are declining across the United States.

Multidrug resistant *S. aureus* infections, including CF patients actually are declining and if we look at clustering and *difficile* infection rates, which were rising steadily up until 2010, there we're seeing evidence nationwide that rates of *C. diff* are falling.

So it's not all gloom and doom.

And I think we can say that changes in prescribing practice largely or at least partly driven by.

Oh, oh, guidelines as well as antibiotic microbial stewardship and just increase the awareness by our prescribers in the hospital and in the community seem to be having an impact on resistance rates.

So in summary, antibiotic resistance accounts for large share of morbidity and mortality and rates were increasing until about 2010.

But umm ohh rates of prescribing practices among children have declined, corresponding to decreased rates of antibiotic resistance.

And so our word to our providers in the Community, I think having a succinct guideline or recommendation table available for for yourselves and also for families can be helpful.

Consider posting a commitment to appropriate antibiotic use and and support your local antimicrobial steward.

Some references and and some.

Thank you.

So I apologize, I haven't left a ton of time for questions, but would be happy to take any if you have them.



Kamat, Deepak M 59:23

That Haslam, thank you very much for that fascinating presentation.

I have a question in the chat box and then I'll go to Doctor Barton.

I'm curious, how much does it cost to do complete DNS tool evaluation?



Haslam, David 59:37

So.

So right now it costs about \$120.00 per sample, which is not terrible when we think about costs of other tests.

For example, the syndromic panels that we do for viruses and the nose or in the stool, those cost patients more than \$600.

In fact, sometimes up to 800, eight, \$100 per sample, we don't think about that.

But so the stool for us in on a research basis, it's about \$120.00 per sample.

I would say it's more the turnaround time.

That's a barrier.

We it's not cost effective to do them one at a time.

So we have to do them in batch and so I think that's a big barrier.

Another thing, and this is off a little bit off tangent, but I think there's still questions about how useful would it be clinically.

 **Patherndon** 1:00:21
You.

 **Haslam, David** 1:00:28

So let's say we screen every child that came into hospital and we found that 10% of them carried a multidrug resistant Organism.

Well, then I would say do we put all of those kits in contact precautions?

So we you know, what exactly how do we use that?

I think those are questions that we need to to answer, but one thing I I will say it may, it may influence our antibiotic prescribing.

So if we start to see a child develop an abnormal microbiome, then I think this antibiotic stewardship people have a strong case to say, let's hold off on antibiotics for that child.

But yes.

 **Kamat, Deepak M** 1:01:06

I don't know Barton, go ahead and ask you a question.

 **Barton, Theresa** 1:01:09

Aye.

So thank you so much for a great talk.

So my question I2 questions that are kind of just related to each other.

One is how impactful our probiotic products at at shifting the microbiome back to

something less pathogenic and and can that happen in the setting of actively giving antibiotics.

 **Haslam, David** 1:01:36

That's a great question.

I would say the short answer is they're not the the existing commercial probiotics are not very effective.

I wanna say not effective at all.

That might be a little bit of an overstatement, but we had plenty of kids.

Actually, our hospital does tend to prescribe probiotics.

We mostly use Lactobacillus Rudi and we saw lots of kids in our study that have, you know, a bubble of Lactobacillus.

But then they also had huge bubbles of Klebsiella or sudo monas, and so we didn't see any apparent benefit of of those probiotics.

Now we did see fecal transplant.

I didn't include this, but we're doing fecal transplant for C diff recurrent cdiff, and there you can really shift the microbiome.

The question is, you know, would we feel comfortable giving the bone marrow transplant patient fecal transplant?

Maybe, maybe not.

 **patherndon** 1:02:30

The.

 **Haslam, David** 1:02:31

But there are some.

There are some new products on the market for C diff that are mixtures of organisms that typically come from the God, and they seem to be bad.

They seem to be pretty effective at preventing C death, so maybe they would be good at shifting the microbiome to a less, umm, aggressive state.

 **Kamat, Deepak M** 1:02:57

Doctor Seidner, you have question.



Seidner, Steven R 1:03:03

Actually it was the same question as Doctor Barton.



Kamat, Deepak M 1:03:06

OK, good.

Uh, that the part done, you can go and ask your question.



patherndon 1:03:12

Doctor Herndon.



Haslam, David 1:03:14

Yes.



patherndon 1:03:15

OK ohh my question.

Well, I've been through a lot of experiences in this pandemic as well.

As you know, being a cancer doc where you end up being multiply recolonized and made sick by campylobacter or something like that with a providers come in and a sick patient Merrill transplant whatever.

But I'm wondering about in, in folding in the data.

Uh.

From prenatal exposures, known neuro as well as immunotec trigens like alcohol or barbiturate drugs, things like that might have have might affect the data that you're overall data constellation here about colonization in the sustainment of pathogenic colonization.



Haslam, David 1:04:05

That that's a great question.

There's there is a lot of interest in looking at other drugs and their impact on the microbiome.

I would say for sure chemotherapeutic agents do have an impact on the microbiome. In fact, some chemotherapeutics were initially being developed as antimicrobials and and I think they were found to be too toxic for for healthy individuals.

 **patherndon** 1:04:23

Exact.

 **Haslam, David** 1:04:29

But but but had chemotherapeutic activity so.

So chemotherapy?

Yes, for sure shifts the microbiome.

There are.

There certainly are other drugs that have been implicated and shifting the microbiome and mostly in a in a non healthy way.

I'm a diet is another, so we don't think about this very often, but we see kids that when they go on of what certainly parenteral nutrition, there's a huge shift in the microbiome in a more pathogenic direction.

 **patherndon** 1:05:01

1st.

 **Haslam, David** 1:05:02

But even say tube feedings is doesn't seem to to be beneficial, although I I don't think I shouldn't say that it it you definitely see differences in the microbiome.

I don't think we've looked to see if two feeding alone is associated with risk for infection, but yes, I completely agree with you.

I think there's a lot that we do to people that that affects their microbiome and potentially does increase risk of infection.

 **patherndon** 1:05:32

But I'm also worried about the things like alcohol.

 **Kamat, Deepak M** 1:05:33

You.

 **Haslam, David** 1:05:37

Yes.

Yeah, there's alcohol does shift the microbiome, and then there are actually interesting studies that show that microbes can produce alcohol themselves, ethanol.

P **patherndon** 1:05:49
Excited too.

HD **Haslam, David** 1:05:50
And there are circumstances where there is a there is a really fascinating publication that about a patient who had so much of an Organism that metabolized carbohydrates, that person would get drunk every time he ate pasta.
So that's kind of a, a, a little bit off off of your question.
But yes, ethanol does affect the microbiome, and in fact with with liver disease, both alcoholic and non alcoholic liver disease, there's a there seems to be a strong contribution in the microbiome to the inflammatory response and the liver like in Nash and natural and alcohol is part of that, but also microbial inflammatory factors and probably alcohols produced by the microbes contribute to that as well.

P **patherndon** 1:06:28
Yeah.
And I think about prenatal other or Natal other exposures like breastfeeding.
How does breastfeeding feed fit into all of this?

HD **Haslam, David** 1:06:47
Yes.
Yes.
So arditth Morrow here listed here, is really.
That's her.

P **patherndon** 1:06:54
Exactly.

HD **Haslam, David** 1:06:54
Her real area of interest is in breastfeeding and and in fact a lot of nick use now do supplement.
Commercial feeds with breast milk and there are actual there are proteins, breast

milk, derived proteins that are supplemented by some commercial of formula makers to try and make it look more like breast breast milk.

 **patherndon** 1:07:14
Click.

 **Haslam, David** 1:07:19
Think the jury still out about how well those work, and in correcting the microbiome, but it's an active area of interest and I think I think it definitely there's a, there's a lot to learn there.

 **Kamat, Deepak M** 1:07:32
The last time my last question I think you're partly answered this question what are your thoughts about using probiotics in the extreme premature population?

 **Haslam, David** 1:07:42
You know, I I there might be some neonatologists on the line and so I don't wanna say anything that's gonna get me in trouble.

 **patherndon** 1:07:47
Umm.

 **Haslam, David** 1:07:49
I think so.
Our neonatologist, we see real differences in opinion there.
I think there's data in both directions.
It's, it seems to me a little bit hard to tease out umm to tease out whether there are potential benefits and some people would argue one of my colleagues in Saint Louis feels pretty strongly that probiotics may be harmful and may risk increase the risk of infection due to probiotic organisms.

 **patherndon** 1:08:16
Right.

 **Haslam, David** 1:08:16

In fact, I think the FDA may be just I I don't, I don't wanna say this cause I don't know it for sure, but I think the FDA just suggested that probiotics shouldn't be included in in supplementation and then newborn, but that I don't take my word for that.

I what I will say is my understanding is that there's information on both sides of benefit versus non benefit and there may be some indication there in some babies there could be some risk.

So I think it's still a developing question.

I we'll probably learn more.



Kamat, Deepak M 1:08:56

That has them.

Thank you very very much for that.

Fascinating.

Wonderful presentation on antibiotic storage.

It really appreciate it.

I know you're very busy.

You are on service right now.

Truly appreciate it.

So I'm going to conclude this morning's grand rounds.

Thank you all for attending.

We'll see you next Friday at the same time and same place.

Thank you.



Haslam, David 1:09:14

Thank you.

● **Kamat, Deepak M** stopped transcription