National Antimicrobial Resistance Monitoring System (NARMS) Public Meeting Day 2 Transcript

Welcome - Presenter Dr. Patrick McDermott
Time- 00:01:00 – 00:04:02

All right. Very good. Thank you. So welcome again, everyone, to day 2 of the National Antimicrobial Resistance Monitoring System public meeting public meeting. Special welcome to those who are joining for the first time and weren't able to come for the first part of the meeting.

If you weren't, you missed some really good presentations and a technical workshop on Tuesday, where the different NARMS partners displayed some of the tools you can learn in order to access the data. We really dedicated to that, to transparency in the system and open access of the data that we can provide to all in making it -- in fostering easy methods to do that through interactive data dashboards. So that was a very good day 1 technical workshop.

Yesterday, we started into the meat, the agenda, let's say, the programmatic parts of the meeting, and I would say, and I think when we first published the agenda two years ago to the Web, we mentioned the major themes of the NARMS -- not the agenda, but strategic plan -- the major themes of the NARMS strategic plan are, One Health and the scope of testing that would comport with the pillars of One Health, namely including environmental components, animal health components and ultimately others such as plants and perhaps wildlife, so it's about the scope of the program.

And then also, the other theme of the current strategic plan is a commitment to fully exploit the technologies and next generation of DNA sequencing and all the analytical tools and machine learning tools that go with that to strengthen to the extent we can the scientific foundation for the program itself so that anyone who can and wishes to act on the data will have the scientifically robust data set. So those two themes of one health scope of testing and fully taking advantage of next generation DNA sequencing technologies have been the main theme of the strategic plan, and the main theme of the meeting. So we spent yesterday really focused on the scope of testing. Today we'll get into the goal 2 of the strategic plan, which is the genomics, the next generation DNA sequencing, thank you, Claudine, for the agenda. Employing advanced technologies to better understand the evolutions and spread of resistances is to take advantage of sequencing technologies, and then we'll have two brief moderated panel discussions looking at goal 3 and 4, improved data communication and collaboration and conducting research, sort of basically getting to the dynamics of resistance that we learned from the data itself.

And then in the afternoon after lunch, NARMS partners will be in listening mode and we'll hear from stakeholders who are interested in the work of NARMS and can use the data for advancing food safety priorities. So that's a sweep of the agenda today, and I want to welcome everyone to the meeting, and I'm looking forward to a fruitful discussion.

Goal 2: Employ Advanced Technologies to Better Understand the Evolution and Spread of Resistance among Foodborne Pathogens – Moderator Dr. Jean Whichard
Time- 00:04:02 – 00:04:56

So kicking off, then, today, our first moderator for leading the discussion presentations on goal 2 is Dr. Jean Whichard. And Dr. Whichard has been a part of the NARMS effort for many years. She had received her BA in chemistry from Mary Baldwin College, and her DVM and Ph.D. degrees from the Virginia Maryland College of Veterinary Medicine at Virginia Tech. As I mentioned, she's long been involved at NARMS at CDC where she leads the surveillance team and the enteric diseases laboratory branch, for the laboratory testing for the human surveillance component is done. Dr. Whichard's interests include transmission of resistance in humans, animals and the environment, electronic management presentation of the surveillance data for public health purposes, and antimicrobial stewardship. Dr. Whichard, thank you for moderating this morning's session. I'll turn it over to you.

Objective 2.3: Develop metagenomics approaches to characterize the resistome of animals, humans, and environmental samples and to link resistance genes to their microbial source – Moderator Dr. Jean Whichard
Time- 00:04:57 – 00:07:00

Thanks so much, Pat, and thanks so much, Claudine. This is a great meeting. Really appreciate the opportunity to participate in it. And this is a lot to put together, so a big thanks to the organizers and so glad to share with the public all the cool

stuff we're doing in NARMS. And so as Pat mentioned, we're in day 2, starting off with Goal 2, which is employing advanced technologies to better understand the evolution spread of antibiotic resistance among foodborne pathogens. So we're going to hit two of the objectives today. We've got four great speakers from our NARMS scientific crew, and we have a pretty generous Q&A at the end of the talk. So we might hold questions, depending on how long the presentations go. But I would encourage you all along the way to enter your questions that you have, any areas of interest into the Q&A so that we can pick those up during the Q&A session or the speakers can answer them as we go.

So the first objective we're going to hit is developing metagenomic approaches to characterize the resistome of animals, humans and environmental samples, and to link resistance genes to their microbial source. So our first speaker, Dr. Andrea Ottesen received her Ph.D. at the University of Maryland in 2008 in natural resource sciences. She was focused on agricultural metagenomics. She started a metagenomics program at FDA Center for Food Safety and Applied Nutrition or CFSAN in 2009 and worked for ten years there to provide metagenomic data to describe ecologies associated with high- risk crops and phytobiomes. Recently she's joined the team in the Division of Animal Food Microbiology (DAFM) at CVM to contribute to questions at the human, animal, environmental nexus of One Health, including our NARMS, National Antimicrobial Resistance surveillance efforts. So without further ado, I'll hand it over to you, Dr. Ottesen.

Using metagenomic and quasimetagenomic methods to look at AMR in surface water – Presenter Dr. Andrea Ottesen
Time- 00:07:52 – 00:30:35

All right. I'm really excited to be a contributor to this meeting. I've attended this meeting for years, and this is my first time getting to participate as a contributor, so this is incredibly exciting for me, and as Dr. Whichard mentioned, I am going to talk to you about our using metagenomics and quasi- metagenomics, which I'll explain what that means, to look at AMR and surface water.

So typically -- so I'm not going to spend a lot of time placing this in, you know, the One Health context because we've been doing that for the last couple of days. I will take a tiny snippet from one of Dr. McDermott's elegant descriptors about surface water and One Health in NARMS and just mention that from a one health's perspective, surface water serves as key environmental integrators and at the end of the day, almost everything is two- thirds water so the more that we understand about this important component of health from animals, humans and environment, the better positioned we will be to steward health.

And there's been a lot of talk about different water sources. And I think what we really want to see is all of these and the flow of AMR through surface water, through ground water, and certainly effluent is a well demonstrated tool for incredible epi achievements recently that we've heard from many of our colleagues in the past couple days.

So you've also heard a lot about this consortia of EPA, USDA, FDA and CDC and

how we're working together to try to coordinate methods.

I think that is really kind of the pinnacle, if we don't have methods that, you know, have some kind of apples to apples, we won't really be able to talk about point source, ecology, national or global AMR with very much resolution at all.
So I will talk briefly just about -- you've heard about ultra filtration. That's one of the methods that we're using -- or that is the primary method that we're using for water collection at this point. There's a lot of reasons for that. This is what that looks like. As people have mentioned, as we create these protocols, we post them publicly, so this is all available for anyone that wants to collaborate with us.
This is schematically what this collection looks like. You're pulling from a water source, using a pump. It goes through an ultra-filter, and then what was not collected by the filter is filtrated out.

This is what it looks like in real life. We've got a pretty low tech flowometer there, which is a bucket this time, but you can have very high- tech flowometers attached to this.

This is sort of the range that you're getting, so it's really exciting. These are used to clean kidneys. So you can imagine how much is going to be pulled through this. And we have a very nano scale to macroscale ability to track pretty much anything that we're interested in.

The reason we really want to use this one as a starting point for water collection is because of the great utility that can happen downstream. When you have

flushed clean water and tweened pulled organisms off of filter and you have this back flush, this starting material can be used for all of the traditionally validated assays, all the multiplex PCR assays you want to use, culture independent, EDNA and what we call quasi metagenomics, which we're using for the AMR initiative. So if something can do all of that, how valuable is that as a starting point. Quasi metagenomics is really just shotgun sequencing the enrichment. And the reason we have to make a special name for that is because metagenomics, the word means culture independent. So if you say culture independent shotgun sequencing of an enrichment, it's already an oxymoron. So we just started calling it quasimetagenomics and we've been using it since 2009 to look different dynamics in our methods. A really shocking thing that we noticed in 2009 when we attempted to culture *Salmonella* from tomatoes, we enriched the proteobacteria as we intend to. That's where *Salmonella* lives, phylogenetically. But we also co-enriched firmicutes and one of them has demonstrated efficiency as killing *Salmonella*. So that's really important to know if you're trying to recover these pathogens and respond to outbreaks.

We also used a lot, starting in 2016, sort of retrospectively looking at the ice cream outbreaks, and we were able to show, like, the purple in this graph is *Listeria*. Across the top is time, zero to 48 hours, the preliminary enrichment, and across the bottom was the USDA, FDA and FSIS methods to recover *Listeria*. We wanted to see that we're on the same page there, which we are.

And but you can see the dynamics, and like the starting microbiome and how that interferes with our ability to grow *Listeria*, and when we can just shotgun

sequence this at hour 28, for example, and have the exact same phylogenetic source tracking resolution that we would five days later. So this speeds it up. And this is really valuable for AMR because at hour 28, you would be able to talk about the AMR phenotype of that *Listeria* with very high resolution.

So you're seeing, like, in culture independent, which I will continue to abbreviate at CI and then QMGS for quasimetagenomics, really different taxa. You don't see your targets. You only see the *E. coli*, for example, once you've enriched so you're only going to see the AMR associated with that *E. coli* once it's enriched.
And this paper sums it up really nicely. This group took 200 million reads and applied those to pig ceca, effluent and upstream sediment and then they talked about the amount of AMR they can see using this. And so for pig ceca, it was 50,000 genes TET in this family. 22,000 in these 23S r RNA, those are going to be easier to find, and then when they looked at upstream settlement, the highest number of genes that they could find with the CI data is 22.

And this is really similar to what you find in water. And so this is why, when we're going to surveil water, we need a tool that can do it. And culture- independent metagenomics alone will not do it. So that's why we piloted this quasimetagenomics on two sites. One reasonably clean reservoir input to drinking water, Sligo, a creek with a really point source contamination from a hospital, and we just started, despite how different they are in terms of point source pollution, they were actually in pretty similar human impact zones. And I'll talk a little bit more about how we're organizing that in a minute.

But so that allows us to use this starting input, the backflush through the filter. We can get our metagenomic profiles on the top CI, and you see how different they are, so that's really valuable information that we do not want to lose. However, this area is where you'd see enterobacteriales, and there's nothing. You get very few hits. Certainly not hits that would talk about serovar, certainly not hits that are going to talk about AMR phenotype. So that's why we need the quasimetagenomics down here after 24 hours enrichment, and in just a universal pre enrichment broth, modified buffer peptone water, we now have a nice swath of enterobacteriales to look at, and this is what that looks like with the key. So both stories are very important, honestly. And then this slide basically, it's an entire workshop just the metagenomic annotation of these data. So we'll save that for next year. But in any case, we're using these different pipelines, trying to -- some of these are designed for short read data, short read metagenomic data like AMR Plus Plus, AMR Finder Plus. Not specifically designed for metagenomic data, of course. But also very useful. CARD, the comprehensive antibiotic resistant database, and then COSMOS ID is a proprietary group that uses kmers, so this isn't something that we'll be pushing forward, but it's nice to look at compared to all these databases.

And the thing that really comes across is that with the CI data, we do not have enough information to talk about the AMR in the water. So we need to complement these CI data with the quasimetagenomics data, and that's true across all different groups. In some cases, you will see some hits from the CI, but in general, it's never going to be from the list of critically important.
And because we get so much data in the quasimetagenomic data sets, we are

actually able to make biological inference after normalizing these data about, you know, that there may be more of these genes, or that there are very likely more of these genes in the Sligo, the one near the hospital compared to the reservoir. And so we use the list that all the people associated with this meeting are working to continually compile, like the list of critically important things for monitoring and surveillance, and we use this kind of as like a light in the fog because the data is so gigantic, and if we can at least sort of focus on reporting this, then we kind of have a goal.

And so if we look at the genes that are critically important, we see that we see none of those, again, in the CI and we see quite a few in the quasimetagenomics and we can begin to look at the differences between the water sources.
So we also sort of can pan for escape pathogens, the pathogens that are most commonly associated with mortality in AMR. We can look at European veterinary pathogen targets. We can just kind of comb through and create reports for all these important avenues.

And this one I put in here because it's very interesting that we do not see *Borrelia*, we do not see *Mycobacterium*. We do not see these sort of finicky, fastidious, slower growing organisms. We're going to not going to see them in the quasimetagenomic enrichment, because they're outcompeted. But we do see them in the CI. So that's pretty exciting. These need to be paired together and you need to be specific about what your goal is.

We have, I would say, maybe a low resolution ability to look at the plasmids just

because they're so scattered, or we just -- the long reads are going to help this. We're right now just looking at a data set that is comprised of short reads from the Illumina platform.

So that was kind of our pilot on this methodology, and that is almost completely accepted. You can still read it on the bio archive right now.

And now, we take this method that works and start trying to figure out how we're going to look at AMR across, for example, Maryland. And what we did was take the land use data that's available in the state through ESRI and this GIS mapping, USGS, lots of different people contribute these data. Maryland actually has very sophisticated data because of the Chesapeake Bay Foundation. So we take all the data. We compile anything that, like, sort of counts as human impact, impervious road, impervious surfaces, crop land as best as we can. I think ultimately we might pull crop land out of this to sort of differentiate that but trying to put all the human stuff together and call that high impact, and then contrast that against everything else, which is low.

And that's what this looks like for Maryland. The blue is high impact, and the white is low impact. These are all just math. We just add up the impact, you know, in these tessellations that are like ten square meters. And then we also sort of looked at HUC 8 level watersheds because that seemed like a practical one for our initiative. So those are outlined here, and then we selected points, the 15 points at the lowest point in the high impact watersheds and the 15 points in the lowest human impact watersheds. And then we also cross referenced those with

public access sites. You can do anything. There's hospitals. We can do proximity to hospital point source. There's so many things you can add to this. But just to start, we did high and low, cross referenced with public access sites so that we weren't hanging over bridges or going anywhere near people's private property. And this is just a broad look at low impact on the left versus high impact using AMR Plus Plus, this is normalized and so you can see there's a lot more AMR that we're seeing in the high impact with this annotation. That won't always be the case, for example.

What we've been trying to do to start is using the CARD database because they have an ontology that goes down to kind of, you know, species or serovar of these genes. I know that's probably not exactly how you say it, but usually you'll get the level that will tell you CMY- 4. You'll just see CMY in a lot of the ontologies for the databases, so we've been using CARD and we use it across 100 percent coverage across 100 percent identity so that we can be reasonably sure that what we're reporting is there. And then this is what that looks like with that and then there's less of a pattern here, honestly, and so that's an interesting take- away at this point. We were expecting to see, you know, the cleaner waters are not going to have as many of these resistance determinants as the higher impacted water, but we're seeing a really broad coverage of many of these genes across a lot of the sites.

So this is, again, just to sort of show you the difference between the CI and the QMGS, using that same data, the CARD annotation at 100 percent. You see how few you see in the low human impact, and you see a lot more in the high human

impact, in the CI, and then across the QMGS, the quasimetagenomic, you see a lot of stuff across both and kind of less of a signal.

So this is all pretty new. We just finished this summer sampling, sequenced it, and then I went on vacation for two weeks. So last night was actually one of the longest that I got to spend with these data and I'm so excited to make these stories more clear and more relevant.

So here is just a look at, you know, beta lactam resistant determinants across the state. This one, you know, sort of fits a little more with what we might have expected to see, twice the number of sites in the high- impact zones have genes, you know, from this list, the NARMS list, and then, you know, although many of the same genes, though, were in these supposedly kind of more pristine environments.

So it's really interesting. We're still just learning about it. Same thing for macrolide and colistin. It does look like maybe there's a pattern here and, you know, we have to learn to sort of apply more statistical models to how we're going to report this. But this is just really preliminary work, looking at what we saw from this first pilot on Maryland high impact and low impact human use. We do see separations by watershed. This is looking at bacteria now, and water body. So that's kind of reassuring.

Similarly in the enriched data and that’s where we see more of the important targets. Of course, we focus on the pathogens of interest to NARMS across the

top are the *Campy, Enterococcus, Salmonella* and *E. coli* and on the bottom, we have *Vibrio* and *Aeromonas*. These are all enriched data or you would not see these species in the same way.

Again, we can just sort of go through the different surveillance initiatives and pull out stories that are important.

One thing that we're excited to do, like another state that said they'd like to help New Mexico Department of Health reached out and said they would like to help so we applied the same human impact assessment using their land use data to identify the same number of sites there.

And because it's such a bigger state and there's so much desert and so much less human, the range of impact goes up to this 367,000, where that range was only at about 700 for Maryland. So we're hoping this might be a very interesting way to explore this approach, see how it's only a much smaller summed human impact. So this will be a very interesting data set to gather.

Again, these that were so different in our pilot, the point source hospital and the reservoir, are also pretty similar in their actual impact classification. So maybe this isn't the way to do it, maybe it is. Anyway, we're figuring it out.

But we're also working, of course, with Alison's group, and they're doing a bunch of sampling in Ohio so we've applied these analyses of human impact to the Ohio watersheds, and these are the sites they're sampling, and we can now sort of see

if this, you know, kind of holds up at all, or if this isn't, perhaps, the best way to approach this.

So those data will start arriving the end of this month, or those samples, and we'll start organizing those.

And another thing I just want to mention, though, like, it's been a real success, like all the methods that we've developed during this, and even if they're not identical, a lot of the work Manan did sort of showed this one does relatively the same as this one, does relatively the same as this one, make you don't see it with this one. If we even have an understanding of how to compare apples to oranges, to infer what it might mean then, you know, we'll be really poised to take advantage of the NRSA study, even if we can't use ultra filtration in 20 to 100 liters, we can understand what we can learn from the liter we will get, you know, and what we can infer from that.

So all of this has been incredibly valuable, even if we, you know, as a group, have not arrived on like the perfect set of methods that are going to, you know, really enable global surveillance. So anyway, it's been incredibly productive.
Again, yeah, this is just my point. Like different volumes we've looked at. Understanding what we can infer from different volumes. Understanding what we can infer from PCR panels compared to quasi. You know, collection methods, and then putting it all together. This is just a mess of a correlation network from some water sampling we did a long time ago trying to correlate what species we found with which enrichments. And we hope to be doing sort of a little bit more of this

with the data set that we just gathered. So that's all. I just want to really sincerely thank especially Errol and Pat, because scientists like to be disobedient sometimes because we think we know what we should be doing, but I've been very obedient and just started doing the water work because that was my job, and along the way I just felt so grateful to be part of such an important project and such an exciting project, so I just had this, like, moment of gratitude to my supervisors to have designed this, you know, that we can all participate in. So special shoutout to them and then of course the EPA folks and Manan and Shawn Behling has been sort of the GIS expert that pulls all this data together to ascribe the land use categories. This team down at the bottom, they are the architects of AMR finder, and they have been really helpful to us as we try to, you know, learn what their program can do for us. Anyway, so many, so many incredible people helping. It's just a beautiful team, and a special shoutout to this guy who pretty much helps me with everything. He is the best. Anyway, thank you so much. That's it. And I look forward to the question and answer period later.

Objective 2.4: Employ long-read DNA sequencing methods to establish a reference database of fully characterized strains and their plasmids– Moderator Dr. Jean Whichard
Time- 00:30:48 – 01:20:20

Thanks so much for giving us some data that was pressure off the presses -- fresh off the presses. Talk about timely sharing with the public participants here. That is awesome. We are going to move on now to objective 2.4, which is employing long- read DNA sequencing methods to establish a reference database of fully

characterized strains and their plasmids. And we've got three speakers for you today. First up is Dr. Lucas Harrison, who's going to talk about tracking resistance in plasmids. Dr. Harrison studied mechanisms of antimicrobial tolerance and acquired antimicrobial resistance in Dr. Nancy Hanson's lab at Creighton University. He joined FDA's center for veterinary medicine in 2019 as a staff fellow where he evaluated genomic markers for source attribution in *Campylobacter*. As Research Microbiologist at the Office of Research, he investigates the roles of genes and plasmid diversity in foodborne pathogens. Dr. Harrison, the floor is yours.

Tracking Resistance in Plasmids – Presenter Dr. Lucas Harrison
Time- 00:32:00 – 00:47:23

Excellent. All right. Well, good morning. Today, I will be talking about a new method we've been developing to characterize plasmids and how this method can be used to assist in tracking plasmid mediated antimicrobial resistance. Now, I like to make sure that everyone's on the same page and make a quick distinction between chromosomal and plasmid DNA bacteria. So the chromosome is usually a large, single- copy molecule that contains all the genetic material that defines an organism. So in other words, everything that makes an *E. coli*, an *E. coli,* can be traced back that chromosome. Plasmids, on the other hand, are smaller extrachromosomal DNA structures. They carry genes that can potentially provide a fitness advantage to the bacterium. Now, some plasmids may be transferred to the bacteria, allowing for introduction of the plasmid into new strains, new species, even entirely new genera. But most importantly, though,

plasmids can recombine with other DNA molecules, allowing them to gain or lose permissions their original sequence. This is especially concerning when this process allows them to acquire antibiotic resistance genes.

Now, regarding AMR, plasmids play a critical role in the transmission of resistance genes throughout the interactome. One aspect the interactome that inhibits the spreads of bacteria through all the sources is this concept of host restriction. Now, generally speaking, this refers to the trait that some strains of bacteria are better able to thrive in one source than in another.

Well, plasmids allow for the spread of AMR genes throughout the interactome, even when they're found in host restricted strains.

Now, an example of this is on the pictogram on the right. So as we can see we have our strains that are found in surface water. This particular strain is encoding a plasmid with an AMR gene represented by the red circle. Now, this particular strain can't colonize humans. However, it's possible that the surface water can pass this particular strain on to, say, your dog. And in your dog, the strain with the AMR gene can transfer the plasmid to another strain of bacteria. Now, if this strain is able to colonize humans, then your dog may then be able to colonize you, and at that point, the AMR gene has entered the human population. It goes with you where you go and allows you to contaminate the built environment.
Now, keep in mind this is more than just story of water, dogs and humans. You know, really any source can act as a site for plasmid transfer. So, you know, we've got the connection between food animals, humans and built environments, and

then maybe there's a transfer event where the plasmid goes to another strain and dogs and surface water, so plasmids really increase the connectivity of this AMR transfer network.

Now, as I mentioned earlier, plasmids undergo recombination events that result in them gaining or losing large portions of their DNA sequence, and this makes them fairly difficult to track. As an example, here are four of the major restructuring events that can affect the resistance phenotype associated with the plasmid.

First, up top, for the insertion event, we have the introduction of an AMR gene between plasmid elements 1 and 2. Next, a rearrangement event that swaps the positions of plasmid elements 1 and 2 may affect the promoter region of the AMR gene.

Third, a deletion event can entirely remove an AMR gene and finally recombination with another DNA molecule can replace large sections of plasmid DNA with the exogenous DNA molecule. So even though these events can affect the AMR status of a plasmid, current plasmid typing methods don't take into account these structural variations, and that's at least in part due to the limitations of assemblies from short read sequencing, and thus what I have an illustration of on the left. It's an assembly from short read sequences.
Now, ideally, what we would be seeing is one large circle that represents the chromosome and then several smaller circles that represent plasmids.
Instead, we have a jumble of about 50 different sequences and a lot of questions,

such as what sequences belong to the chromosome and what belong to the plasmid and if there are any plasmids, how many are there, and if multiple plasmids are present, what sequences belong to what plasmid.

Now, these are all questions that can be addressed with the addition of long read sequencing, and here we have a visualization of the assembly from the same organism, but this time, we're using long read sequences. And this addresses some of the questions that were by the short read assemblies. In other words, there's a clear distinction between the chromosome and the plasmid sequences. The exact number of plasmids is known. There are three at the bottom, and we can also know which sequence -- what sequences go with which plasmid. So there's no confusion if we wanted to attribute, say –these are closed sequences so we know what sequences go with what plasmids.

Now, one aspect of closed assemblies from long read sequences that's often overlooked is that closed assembly show both what is and is not present on the plasmid sequence. So in other words, if the sequence is missing from the long read assembly, the sequence isn't present in the plasmid, which I realize sounds obvious, but that's not a call that we can make with short read assemblies where we don't have a full accounting of the plasmid sequence.

So since we're now able to make a distinction between what is and isn't on a plasmid, we have the ability to generate a plasmid subtyping system based on the presence or absence of plasmid genes. And that's just what we did, easy as 1- 2- 3. So 1, we created a pangenome of plasmid sequences, which is

represented on the left. We identified the plasmid genes or the plasmid typing loci that were indicative of a plasmid type and three, we analyzed where each plasmid type was located on the plasmid sequences.

Now, I realize this is a very broad overview, but as we were analyzing positions of our typing loci among the plasmids, we noticed that certain sets of loci were often clustered together such as this set of three loci were often found together, this set of seven, and this set of eight.

Additionally, we noticed that the clusters of loci, so these clusters of 3, seven and eight, weren't always positioned in the same place on the plasmids. So, for example, this cluster of three in this particular example, it's near the 11:00 position, but sometimes we would see it closer to, say, the 5:00 or the 8:00 position.

And so knowing that there was a pattern to how the loci were grouped among the plasmids, we could begin to develop our plasmid subtyping system using both sequence and structural elements.

So for the sequence elements, we can take the sequence of the individual loci and develop a plasmid multi- locus sequencing type system so that allows us to compare the sequences across the plasmids to identified plasmids with similar genetic profiles. But next we could compare the order of the loci across the plasmid structures.

So if you'll notice in plasmid A, we have the locus that's represented with the barred pattern in position number 2, but in plasmid B, the locus with the bar pattern is positioned in number three. So this indicates a slight restructuring event in plasmids. But we can use this structural difference to differentiate between the plasmids.

We can also take a step back and look at the larger order of the clusters on the plasmid. So, for example, plasmid A, the order, if we're going clockwise, would be cluster 1, cluster 2, cluster 3. But in plasmid B, for example, maybe cluster 3 and 2 could swap positions, and so the order becomes 1, 3, 2. So again, another structural element we can use to distinguish between plasmids.

Now, finally, we're scientists, we like to leave no stone unturned. Why not look at the distance between the clusters? Because if we have an insertion or deletion event, that occurs in the space between the clusters, that's going to affect the distance between the clusters. And so we can use this element as part of our typing schema.

And what's great is that all of these patterns can be assigned a single unique numeric I.D. So, for example, in plasmid A, we might call this particular locus or the plasmid that contains this locus sequence type 5, and in plasmid B, sequence type 3, so that becomes plasmid subtype 5 and plasmid subtype 3, pretty straightforward. But we can then add on any or all of our structural developments to help subdivide our plasmid types. So for example this particular order of loci would be barred, locus in the second position might be pattern loci 8, and with

the barred pattern in the third position, we might call that loci pattern 6, so now this becomes plasmid A, sequence type 5, loci type 8 or plasmid subtype 5.8. And we can extend this to the cluster pattern, so a 5.8.2 and a 3.6.3 plasmid.
And then finally, we can enter in the intercluster distances to have a single line reporter that describes the subtype of the plasmid. So plasmid A would be a 5.8.2 (6000, 500) and plasmid B would be a 3.6.3 (1000,5500). So using this typing system, we can recreate the structure of the plasmid from these numbers alone. So if first three numbers correspond to a database of sequence types, a database of loci patterns and a database of cluster patterns while the numbers in parentheses detail the exact distance between the clusters and the plasmid. And this directly contributes to the NARMS objective 2.4 to employ long read DNA sequencing methods to establish a reference database of fully characterized trains and their plasmids. However, we can also view plasmid subtyping system to help differentiate between plasmids with identical AMR gene content, which I'll demonstrate with these three plasmids.

So if you'll notice, these plasmids not only have the same AMR gene content, so they all have the same four AMR genes, but the underlying structure of the plasmid is the same. So the order of the clusters is 1, 2, 3, as we're going clockwise, and the clusters are in the same position on the plasmids. So the only thing that's different is the position of the AMR genes. And we can use these positions to differentiate between the plasmids using our system.

So, for example, we can generate a table of the AMR genes from plasmid A that shows that AMR genes 1 and 2 are between clusters 3 and 1. And we can also say

that cluster or AMR gene 1 is 70 kilo bases from fragment 3, and AMR gene 2 is -- there we go, 8 kilo bases from fragment 3. Again, we can populate the table for positional information from AMR genes 3 and 4. Now, alone, this table isn't very informative, but we can layer in the AMR positional data for plasmid B and plasmid C, which allows us to quickly compare between the plasmids to determine where the AMR genes are in conserved position.

So, you know, between plasmid A and B, we can see that AMR gene 1 is in the same position from these two, but in plasmid C, over here, AMR gene 1 is 9 kilobases from fragment 3. So this helps us differentiate between plasmids.
So this subtyping method we have, all this detailed information about the layout of the AMR genes on plasmid while current plasmid type methods would only convey that the AMR genes were present. So in other words, we would know that all three plasmids would appear to be exactly the same using current typing system because they would say plasmid of a particular type with four antimicrobial genes.

Now, finally this new method that we're developing not only increases our ability to distinguish between plasmids with similar AMR gene content, it also enables us to focus our search on the region of the plasmid that carries the AMR gene. So in this way we can begin to establish a plasmid lineage of AMR genes in the data set. And because our plasmid data is already organized in a tabular format, if we have a plasmid of interest, we can quickly screen for plasmids whose AMR gene is in a similar plasmid environment. So this means we can track the AMR region of a plasmid, even if that region was acquired or transferred on to an entirely new

plasmid molecule.
And we can do this using the three steps in the middle column. So first, we can identify other plasmids whose AMR gene occurs between the same two plasmid clusters. So if this top plasmid is our plasmid of interest, we could quickly differentiate these plasmids where the AMR gene occurs between clusters 1 -- sorry, 2 and 3, from other plasmids where the AMR gene may occur between clusters 1 and 2.

Next, we can screen the intercluster region for markers of other plasmid types. So in our plasmid of interest, we can see that there are no markers of other plasmid types in this -- the region surrounding the AMR gene, but in other plasmids, we might find markers for other plasmid types surrounding the AMR gene, which would indicate that in these plasmids, the AMR gene originated from another source.

And finally, we can compare the position of the AMR gene relative to the neighboring clusters among the other plasmids. So in our plasmid of interest, again, this is about 75 -- or 750 base pairs from cluster 2, and 5 kilobases from fragment 3, whereas in other plasmids we might see these to be a larger distance from cluster 2, say 5.5 kilobases.

Now, this visual comparison works for pair wise comparisons, but we can actually expand this to look at the larger population or the larger data set of plasmids to identify trends and deviations in the AMR position. And so we have in this density plot, we can see that in our particular population, the AMR gene of interest is

most often found one kilobase element, but we do have some smaller subsets that this carry the same AMR gene roughly 5 kilobases from plasmid element 2. So the take- away message is, because I know there's a lot of theory involved in this presentation, plasmids enhance the spread of AMR genes in the interactome, long read sequencing allows us to close the plasmids and develop an enhanced plasmid typing method and our enhanced plasmid typing method augments our ability to track AMR genes as they're acquired by different plasmids and are transferred between hosts and with that I'd like to thank you for the opportunity to present some of the work that Shaohua, Cong, Greg, Pat and Errol and I have done on the new system for in tracking resistance to plasmids. Thank you.

Thank you so much for that, Dr. Harrison. Demystifying and finding a systematic approach to typing plasmids, which, if left to their own devices they will rearrange and do all sorts of restructuring events. So thank you very much for that.
We'll continue here in objective 2.4, employ long read DNA sequencing methods with two speakers from CDC who are going to talk about harnessing long read sequencing for plasmid and pangenome analysis. So I'll introduce these researchers. Dr. Hattie Web is a research scientist with our NARMS group here at CDC. Her work focusses on epidemiology of antibiotic resistance in foodborne pathogens such as *Salmonella* with a special interest in One Health. She completed her Ph.D. in 2016 at Texas Tech University and has been with NARMS since 2018 . Dr. Kaitlyn Tagg is a research scientist with us here at CDC at well. Her focus is on epidemiology of antibiotic resistance in foodborne pathogens such as *Salmonella*, and her special interest in plasmids and mobile genetic elements. She completed her Ph.D. at the University of Sydney in Australia and has been with

NARMS since 2017. So Doctors Tagg and Webb, you have the floor.

Harnessing long-read sequencing for plasmid and pangenome analysis – Presenters Dr. Kaitlin Tagg and Dr. Hattie Webb
Time- 00:48:55 – 01:03:46

Thank you, Jean. Just going to wait for Hattie to be able to share her screen. That was a great introduction to plasmids by Lucas, so thank you very much. Yes, we can see your screen. Thank you.

Perfect.

Okay. Good morning, everyone. We're really excited to be here as well and to be presenting our work. We did try and relate it to goal 2.4. We were a little disobedient. We kind of expanded a bit, but we're covering our bases. I'm Kaitlyn. Hattie and I will be co- presenting today on our work over the last couple of years, and again, like I said, hopefully how it relates to the NARMS strategic goals number 2 and beyond.

So firstly, some context on our work. All of the phenotypic and genotypic data that the NARMS program has collected and generated in the last 20 years plus has placed us really well to start investigating the ecology of antibiotic resistance. And our work predominantly focuses on plasmid ecology, because as Lucas mentioned, plasmids are one of the main vehicles that shuttle resistance genes

around. So knowledge of plasmid dynamics is really key to understanding the evolution and spread of resistance in the food production system. And of course feeds into our ability to develop mitigation and control strategies.

But before we can really understand plasmids in depth, we need to put adequate technology and tools in place, so the routine short read sequencing that we have available for all of NARMS isolates means we have a plethora of genomic data to work with but there are challenges and limitations with using short read data. Again, like Lucas mentioned. So what we've been doing is optimizing the long read sequencing process to get the highest quality closed plasmid sequences to study.

And optimization for us at CDC is occurred both on the wet lab and dry lab side, and importantly, currently we're coupling the long read data with the short read data because we have found through a number of different iterations that this is the best way to recover large and small plasmids.

So the long read sequencing has enabled us to generate reference data sets of plasmids that can be used for downstream investigations. So this is both internal and external to CDC. We're showing a few of those downstream investigations up on the screen.

And we've released this data -- well, these are the genome announcements. We've released this data as genome announcements, along with the metadata to make sure that it's publicly available. And we can use these reference data sets to

support other epidemiological investigations, other applied research projects and just more generally add to the body of plasmid knowledge in the public arena. So long read sequencing has been this great technological advancement for us to study plasmids but we are still relying on sort of outdated or older methods. In terms of plasmid classification our routine work is on replicon typing. Which uses just a single to gene to detect plasmids. It's definitely very useful, but because we have all of this data available to us now, we really need to be harnessing it to capture the true complexity of plasmids.

So as some of you know, we are already trying to do that. We've teamed up with Fernand De La Cruz at the University of Catabria in Spain. He's a leading plasmid biologist and he was already working on a more advanced tool to classify plasmids into robust biologically relevant categories.

The tool is based on average nucleotide identity of the entire plasmid sequence, not just a single gene. And they call it the taxonomy of plasmids, which where is called a plasmid taxology unit, or PTU for short. This tool is really for foundational classification of plasmids. And this table is akin to speciation. What it does is lay the groundwork for more specific detailed plasmid analyses like what Lucas just mentioned.

With this work we're able to map the pathways that plasmids travel, and thus the most likely transmission rates for the resistance genes that they carry with them. The tool itself is called COPLA. I think that's like a play on a Spanish word, actually, but the method was published last year, and what we're hoping to do is expand

the reach and impact of COPLA. So we're working with our collaborators to adapt the method for use in our routine surveillance, and ultimately, we hope to see the method adopted by our partners and by NCBI for global use and access.

For us, there are two short- term next phases for the work. So one is an expansion of the COPLA database to include more plasmids, which will improve plasmid detection rates. And secondly, application of this updated version to a large collection of *Salmonella*. We are focusing on *Salmonella* to highlight the PTUs of interest for us in NARMS, and also to detect the ones that have a higher propensity for resistance.

So these phases, both of these two phases are described in an upcoming manuscript that's still in preparation, but here's a glimpse of that work. This network represents different plasmid clusters, or different PTUs that are colored by their dominant association with different subplates of *Salmonella*. So some PTUs appear to be restricted to certain *Salmonella* clades or specifically to serotypes, like FS in the middle, while other plasmids are less restricted like the PTU I2. What we're doing here is mapping the landscape of plasmids more generally, which places us well to detect new emergences of resistance plasmids and perhaps calculate a risk metric for them arising in new serotypes.
And with the tools and technologies that Kaitlyn's been describing, we can perform more advanced genomic epidemiological analyses, and I'll take you a little bit through the pangenome work that we've been doing where we apply those methods.

So up until now, we try to understand resistance by visualizing our data like this, and this is a core genome multilocus sequence type phylogenetic tree, with the AR genes and the plasmids shown as a heat map, and in this way, we are, by default, giving more weight to chromosomal similarities or differences, and as a secondary thought, using this to attempt to make sense of acquired AR genes and plasmids.

Now, as you can see, there aren't always profound patterns that we can make sense of when we do it this way. By comparison, our pangenome approach is quite different. We use what's called Jaccard index analysis to measure relatedness using all core and accessory genetic material, and then instead of a tree, we represent it as a network, which is more reflective of bacterial evolutionary pathways.

Now, these two images contain the same genomes, but with the pangenome analysis, we can detect patterns that were not visible before, and we'll show you a glimpse of our proof of concept analysis and the types of data we can visualize. So this is our first large data set we applied the method to. It's *Salmonella* Typhi, and the network includes the complete CDC NARMS Typhi genome collection and a global reference collection, so in total, it's approximately 2,400 genomes, and they're represented by the gray and red dots.

And from this, you can see that they self-organize, the genomes do, in to 14 clusters, which are indicative of their relatedness to one another. Just to give you an idea, each cluster contains genomes that are over 99.99 percent identical to

one another, and that's across the entire genome.
Now, the value of this analysis comes when we overlay our molecular and/or epidemiological metadata.

So here we've highlighted resistance, where multidrug resistant strains are red and extensively drug resistant strains are green. And clearly, these profiles are not widespread across the network, meaning only some clusters are currently of concern for AR. And when we couple this with plasmid information, many of those resistance clusters also carry PTUs.

Now, this isn't surprising. In fact, it really makes perfect sense, but the distribution of AR is closely aligned with the distribution of plasmids. However, there's one thing that really stood out to us, and it's that the XDR and MDR genomes in JI Group A, that's this one here, the largest one in the middle, doesn't contain any PTUs.

And if we go back to the resistance that we showed you earlier, we were really curious about what's happening in the green genomes right here, and JI Group A. Now, using long read sequencing, we're able to confirm that the AR genes are integrated into the chromosome. So what we're capturing here is a snapshot of the evolution of extensive drug resistance from plasmid to chromosome, from mobile to stable, and the Typhi analysis has really been our proof of concept in what we consider a more well- described *Salmonella* serotype.

Beyond Typhi, we wanted to apply this method with a more One Health lens to

serotypes present along the farm food fort continuum, and where the pangenome is less understood.

Salmonella Hadar was the first serotype we chose to focus on, and our interest in Hadar stems from an increase in the serotype in 2020 and 2021 compared to previous years. And that's despite an overall decline in reported salmonellosis. Recent outbreaks of Hadar in humans have different epidemiological signals, namely exposure to backyard poultry flocks, for example chickens and ducks, and then consumption of ground turkey.

And strains from these two different exposures are often really hard to differentiate by current core genome methods. We decided to apply our pangenome approach to see if we could better understand the overall population diversity and to see if we could offer insight into these more recent outbreaks. So the first phase of that analysis only includes CDC data. But it's really clear from this that the contemporary strains form their own clusters, which means that they have distinct differences in their pangenomes. You can see this in Group A, in the middle, and Group D at 3:00.

And they almost exclusively are made up of contemporary genomes. If we zoom in on Group D, that difference is the presence of a new plasmid, but that plasmid doesn't occur in Group A, and so it's not the only defining feature of the contemporary strains. Using long read sequencing was really valuable at this stage. We were able to select genomes from each cluster and generate closed reference genomes or sequences, and then we have better representation of the

diversity across the serotype.
We aim to be able to pinpoint specific genetic regions that are unique to strains of interest like the contemporary strains I was just talking about, and to guide research to further understand their function.

So we know our inferences are limited right now because we don't have good representation from different sources. We're currently working with our partner agencies, and they're helping us expand the data set to include Hadar genomes from their surveillance systems and we hope this helps us better understand the interplay between molecular and environmental factors in the recent outbreak strains.

To close out, the purpose of investigating all of these tools and technologies over the last two years has really been to address the NARMS strategic plan. I think we fit really well within goal 2, but also goal 3 of collaboration, and so long read sequencing means we can generate complete genomes and contribute to reference databases. COPLA lays the foundation for more robust, biologically relevant plasmid analysis that helps us better understand movement of AR genes, and our Jaccard Index pangenome approach creates complex genetic maps to serve our epidemiological investigations.

And with these approaches, I think we're much more informed to thoughtfully ask and answer questions about the spread of resistance among foodborne pathogens. And we see a lot of opportunities to apply these methods, and we have really big plans for the next couple of years. So I can't wait to be able to

come back and update you at the next public meeting about what we've done. In the meantime, please reach out if you're interested in collaborating. And lastly, we wanted to end by thanking Fernando, his Ph.D. student, Arancha, who has done all of the Jaccard index work, as well as Santiago and Mattie. They've been incredible plasmid guides for us as we work to bringing plasmid ecology in to One Health. And that's all we have for you today.

Thanks so much, Doctors Tagg and Web for that great presentation and really understanding the ecology behind the ecology and how these plasmids move and their host strains, and just great stuff. Thank you so much.

Objectives 2.3 and 2.4 Q&A – Moderator Dr. Jean Whichard
Time- 01:04:05 – 01:20:20

Please, we definitely want you to put some questions in the Q&A for us to pick up for all of our speakers in Goal 2. And I see that we already have a couple that are in there. So we'll try to answer those. First we got a good question for Dr. Ottesen and compliment for the great work. Are you able to tie the antimicrobial resistance genes to the pathogen where they are housed with the quasimetagenomics approach?

Where they're housed. Let me read that. So we are able to, you know, based on the database, say that this came from *E. coli*. This is the exact same across 100 percent of 100 percent of the gene. With the quasi, when we do assemblies, we can get things that can go past the gene and we can sort of hand curate responses

that, I know it's just sort of visually in our mind, we want sort of reassurance that that gene, you know, also has flanking sequences that make -- you know, make it 100 percent that's nothing else it could be. But we don't typically do that. We have done a little bit of that on some of the data. But for a lot of it we are just relying on what the database actually has, which is very hand curated as well, you know, these were tests that were done on resistance in *E. coli*, resistant *Salmonella* and those sequences have been submitted and that's what we're matching 100 percent, so it's not exactly I think what you're hoping. Although we do have limited data sets where we've done that. We haven't explored that as much as we can and will. At this time.

Great. Thanks for that. So it sounds like sequencing out to see if it's associated with some *E. coli* determinants, for instance, and hope it's not on a plasmid that might be in different strains.

Exactly. Yeah.

Okay. And then we have a question for Dr. Harrison. Does the position of the AMR genes on the plasmids affect their expression?

Yeah, so I guess it depends on how you want to answer that. Does the specific position on the plasmid like base pair 50 or whatever, does that matter? No, not so much. But the sequence context of the gene does matter. So, you know, if we have an event -- so let's say an AMR gene is about 500 base pairs from one of our clusters. If that contains the whole promoter sequence, then let's call that our

baseline expression.

Now, if say we have an insertion sequence, that occurs -- well, that basically disrupts the promoter sequence of this AMR gene. That would displace the AMR gene and disrupt the gene expression. And so in that case, the position --the position of the AMR gene would be an indicator of disruption. So, how do I put this? Insofar as the position reflects a disruption of the surrounding sequence, then yes, the position will affect the AMR gene.

Now, moving forward, what I'm hoping to do is that this method will help standardize how we talk about the different locations on the plasmid, and so eventually we'll be able to say that when we see an AMR gene, you know, in say one of those 5 kilo bases away versus one kilo base away, we might start to make associations between that and the strength of expression. But for right now, we don't have those associations just yet.

Okay. Thank you very much, Dr. Harrison. And we have a question for Drs. Tagg and Webb, the analyses of global diversity in plasmids from long range sequencing shown today are very interesting. Do you have any concerns about the use of a similarity index like Jaccard to determine differences in population rather than using a weighted index such as Unifrac?

Thanks for the question. I will preface my answer by saying all of the complicated analysis has been done by our collaborators, so I'm probably not the best person to speak on it, but I will say -- well, firstly, my familiarity with Unifrac is minimal, but my understanding is that it relates a little bit more to phylogenetic trees, and

the Jaccard analysis is an assembly free kmer based method, so what it's really measuring is the relatedness is measured by the fraction of shared kmers between two genomes. So the JI is representing just a fraction. So I don't know that I can actually answer your question. I'm certainly happy to bring it back to our collaborators, but I think that the approach is quite different from the one that you mentioned. Please throw another question in the box if I need to clarify further.

Great. Thank you so much, Dr. Tagg, and I would encourage anyone who has any questions, we still have ten minutes or so left in this session, so you've got the experts here, metagenomics and plasmid analysis, so please ask anything that you would like to them.

I might just add a question in here of how you can frame the importance of studying these things, because we're trying to get it sort of the evolution of resistance in the spread of why it's so important to understand these things at the detail at which you are looking. Any of the panelists are welcome to respond.

I'm not sure if I can respond to -- well, so what I -- maybe not evolution, but certainly start to talk a little bit about the spread, and sort of Lucas prefaced that, you know, just talking about plasmids moving through water and so, you know, NARMS, as everyone knows, has looked at AMR and pathogens in humans with animals, and now, with this water, I found myself listening to Heather Tate's talk yesterday about resistome tracker and thinking how incredibly exciting it will be when we can put the data from these environmental reservoirs in some of that,

you know, kind of epi tracking capacity.
So I'm speaking more to the flow than any kind of evolution, but, you know, maybe that would be teased out on higher levels as well.

And I think to carry on with that, as Andrea mentioned, with the epi point of view, you know, one of the things I'm trying to do with these plasmids is, well, source attribution. We want to know where does the AMR gene come from. It kind of goes back to the question of what's more important? Is it the strain that we're concerned about or is it the AMR gene, and so what I'm hoping to do is to help some of the epidemiological investigations to identify what the actual source of the resistance gene is?

Wonderful. Do others want to come in on this question?

I think another interesting thing is, you know, we're finding all these genes, and I think it will sort of maybe sort of fuel a new era of emerging issues because, so we're seeing the gene, and we are seeing it in traditional pathogens, but there are a lot of things, you know, that are, you know, have the potential to become threats, and especially if they acquire a lot of these genes, so correlating what we're seeing in these environments with, you know, these kind of critically important resistance determinants I think is going to be incredibly valuable for, you know, just correlating predictive emerging resistance. So that's just another piece to add on to that.

And I would agree, understanding what is where and how it's arranged and its

host range of mobile elements and how and why things are moving is the key, then, maybe to mitigation and prevention, if you will.

Exactly. Yep.

Great.

I think we're going to learn a lot from this. It's been just really exciting to have this new matrix that may tie all of the others together in some way.

Wonderful. Yes, and please add any other questions that you have. We're not seeing any -- oh, here we go. We have one for Drs. Webb and Tagg. Great presentation. Do you foresee long-read sequencing approaches and plasmid characterization work being applied routinely in the future across public health labs across the US or internationally, for example, via PulseNet or other surveillance programs?

I think ideally -- well, it depends on what we're trying to look for, right? At this stage, we're using long read sequencing for a more applied research to complement the work that we're doing with our short read surveillance, but it can offer a lot more information. And so I'm wondering if it's sort of a dual part where alongside our routine short read, we're able to do long read to continuously refine what we're surveilling for.

I don't know the feasibility of using long read sequencing more routinely in

impulse surveillance system.

I think eventually that will happen but I don't know how far away we are. I don't know if other people have thoughts.

I'll just add one more point. It's about changing our methods as well, so we mentioned -- COPLA, for example, they're in the process of adapting it so it can be used on short read sequencing. So sometimes we're going to have to take the time not only to develop the method itself and implement it into surveillance, but the tools that we use to analyze the data as well have to be adopted.

Technology is a fast- moving train.

Okay. And we also have another question to the panel. Thanks for very interesting presentations. How quickly are antimicrobial resistance genes lost from plasmids in the absence of antimicrobials? Great question.

Yeah. And I think the answer, as it usually is in science, it depends, right? It depends which other genetic traits the genes are sitting with that are going to be co- selected for. It depends if they're -- if they have any kind of fitness cost on the plasmid or the strain, and whether it -- there's any drive for it to be lost. I think what we tend to see is – what we tend to see in Typhi and we've seen in the other serotypes as well is movement from plasmids into chromosomes and that really stabilizes that genotype, and the plasmid can be lost after that. I don't like to say lost because we don't know how much it actually happens. It's more that the

strain that does not contain the plasmid becomes more dominant in the population.

And yeah, so that is kind of -- that's my concern. It's kind of like we're not -- we're more concerned about the stabilization of these phenotypes and they're never going away.

Great insights. And I wonder about that movement to sort of stability in chromosome if the absence of antimicrobial selection, if maybe that is not seen so much.

Yeah. Again, great question. I think there's been a lot of sort of modeling work done with trying to understand how likely modeling in sort of the mathematical sets -- like theoretical modeling, how often that would happen, and under which selection pressures.

But I think you would imagine that it's driven by selection, but maybe it's another factor that we don't entirely understand yet.

I mean, we're sort of seeing this idea with Typhi is that these genes are being integrated into the chromosome and then the plasmid that originally carried those resistance genes the sort of not present in the population anymore. And you're getting new plasmids come in. So I've been playing with this idea of like it clears space within the bacterial population for novel plasmids to come in that may have been compatible -- or not necessarily compatible but may have been

not as well adapted to survive when other plasmids are there. So it kind of -- that stabilization in the chromosome might actually just open up the population for novel things to come back in as well. It's not necessarily stabilization of a particular phenotype of need.

Yeah. And Dr. Tagg really summarized that well because this is an incredibly complex subject. And just one thing to keep in mind with it is that, you know, we talk about these bacteria, these plasmids, as individuals, but in fact they're part of a colony and so when you talk about loss of an AMR between, sure, it may happen between one or two, or say within half of the population, but you have to keep in mind if the other half of the population retains the AMR gene, you know, I think they call that cheater phenotype, the phenotype that benefits from the production of the other one.

So, you know, it's -- when you're taking into account the dynamic and the modeling, you know, there's this whole layer of complexity involved with how the community evolved. So yeah, this is unfortunately not an easy question to answer.

Thank you so much for a great

Fun to speculate, isn't it?

Yes. What a great panel presentations, and also discussion afterward, and great questions from the audience. Really appreciate everybody participating so

actively in this Day 2 Goal 2 session. So thanks to our speakers and our -- all the great questions.

We are heading for a break right now, and we will have -- we will reconvene at 11:00, where we'll pick it up with Goal 3, Improving Data Sharing Communication Collaboration, a round table discussion. So thanks, and we'll see you back in ten minutes.

-Break-

Goal 3: Improved Data Sharing, Communication and Collaboration: A roundtable discussion– Moderator Dr. Mustafa Simmons
Time- 01:30:35 – 01:58:44

Okay. Welcome back, everyone, from the break. It looks like most people have returned. I'd like to introduce our next session for this morning's agenda, which is a panel discussion on goal 3, improving data sharing, communication and collaboration. And this will be moderated by Dr. Mustafa Simmons. Dr. Simmons is a public health specialist with the USDA Food Safety Inspection Service's Office of Public Health Science in Athens, Georgia. Dr. Simmons specializes in the use of bioinformatics to characterize and determine similarity of foodborne pathogens from the FSIS regulated products using whole genome sequencing data.
Dr. Simmons, I turn it over to you.

Good morning. And thanks for that great introduction.

As we've seen in the previous presentations, NARMS is now able to provide data in a much more timely manner. And this has largely been made possibly rapid advances in whole genome sequencing, as well as several advances in information technology, including web- based tools such as some of the tools we've seen for dashboards and data visualization. These advancements allow stakeholders to view data at nearly the same time as our NARMS partners and this advancement is the basis for goal 3 in the NARMS strategic plan.

Given these advancements, how can we best present, access and discuss these data for response for all stakeholders for the good of public health.
In this session, we'll discuss what NARMS agencies are currently doing in terms of data sharing, communication and collaboration, and what are their future plans. We'll also have a Q&A session to seek input from the meeting participants. Today our panel consists of several spokes persons from various NARMS agencies, from the USDA Animal Plant Health Inspection Service, we have Dr. Beth Harris who is an associate coordinator with USDA's National Health Laboratory Network (NAHLN). From USDA Agricultural Research Service, we have Dr. Kim Cook, who's a National Program Leader for Food Safety. From the USDA Food Safety and Inspection Service, we have Jay Gallons, who is a Senior Data Analyst with the Office of Planning, Analysis and Risk Management. From CDC, we have Jared Reynolds who is an Epidemiologist with NARMS in Division of Foodborne, Waterborne and Environmental Disease. From FDA's CVM, or Center for Veterinary Medicine, we have Amy Merrill, who's a Mathematical Statistician, and we have Olgica Ceric who is a Veterinary Officer Vet-LIRN at the Office of Research at FDA CVM.

And at this point, I would like each agency to first start by telling how their agency is handling NARMS data sharing and have there been any recent updates and what do they foresee in the future. And which challenges do they anticipate or have they had to overcome.

Let's start with Dr. Beth Harris from USDA APHIS.

Good morning, everyone, and thank you for the opportunity to participate in this round table discussion. As Dr. Simmons indicated, I'm Beth Harris, the Associate Coordinator with the NAHLN, where I coordinate the AMR project which monitors antimicrobial resistance in pathogens of veterinary importance. I also wanted to introduce two of my colleagues from APHIS, Dr. Chelsea Shively, who is our AMR, antimicrobial resistance coordinator in the Office of Interagency Coordination for USDA APHIS Veterinary Services and Dr. Christine Foxx, an ORISE post- doctoral fellow within NAHLN where she conducts bioinformatics analysis of whole genome sequence data and antimicrobial resistance data for the AMR project.
A few improvements in data sharing and collaborations that I wanted to highlight this morning are partnerships with our other NARMS federal agencies and veterinary diagnostic labs, our Web- based tableau dashboard and our AST proficiency panel.

So to start off with, our antimicrobial susceptibility testing panel is one of our longer term accomplishments and was produced through a collaboration with USDA national veterinary services laboratories. While this may not seem to be as

significant as, perhaps, some of the other information that has been presented so far during these meetings, this really has been a foundational gap for our veterinary diagnostic community that we've been able to address and really speaks to our ability to better improve standard testing methodology and improve our data quality.

More recent accomplishment is the development of our tableau dashboard, which is hosted on our NAHLN website and allows users to visualize our data from program nearly as soon as it's collected and reported to us. And so this is an interactive dashboard that allows people to focus on the information that's relevant to them, so we designed it to be able to look at information such as host animal, bacterial pathogen, date ranges and then for dogs and cats also looking at *E*. *coli* and *Staph* data either from urinary tract infections or non-urinary tract infections.

Again, this data is updated monthly, so information is being provided as quickly as we can.

And then finally, I wanted to touch on the collaborations with our NARMS partners on the combined data sharing, which was highlighted by Dr. Greg Tyson from FDA during the last couple days. And then also inherent to this collaboration is our partnership with our multiple veterinary diagnostic labs across the U.S. and Canada to help support these monitoring and surveillance programs by providing us with susceptibility test data and whole genome sequencing data and essentially near real time.

We really can't do these programs without our state and university diagnostic lab partners, and so I do want to highlight that collaboration.

And so that, I believe, is all the time I have for today, so I think I'll turn it back to you, Dr. Simmons. Thank you.

Let's move on to USDA agriculture research service, and that would be Dr. Kimberly Cook.

Good afternoon. Thanks for the invitation to be part of this panel. At ARS, we're a little different than other NARMS partners because we are non-regulatory research- oriented organization, so we don't have standardized data collection or surveillance. So a lot of our data is uploaded, as it would be by university as part of publications, but we do collaborate with NARMS stakeholders, NARMS partners quite often, and our data is uploaded along with publications that we have with them.

You, you know, as far as the future, ARS is really focusing in on creating databases, or we have, for example, Ag Data Commons where research data is uploaded and made available. The issue within ARS is that we -- our data -- our studies are very diverse. So I'm personally part of nutrition food safety and quality, but we also have animal production and protection, crop production and protection and natural resources. So standardization and harmonization are difficult for us, but we I think at ARS in the future, I do see that that data will be in

some ways standardized, and I'm talking especially about metadata, you know, weather data, environment data, sampling data, because, you know, you really need that data to be able to mine your studies properly.

And so as we look to higher level data analytics, I think that's going to happen more often. I did want to speak to the challenges. So I mentioned that, you know, we're not really in a position that we can standardize our data very well, but also, as I said, I think the right metadata, key metadata, or key to being able to properly interpret our studies and give out, you know, relevant recommendations for interventions, but we also have to be extremely cautious in that the data, there's proprietary and trusted relationships, and so for me, and in the future, it would be great if a little bit more focus was put on how do we protect confidentiality in a way that, you know, will still let us, you know, be this premier organization that NARMS is. Thank you.

Thank you, Dr. Cook. Moving on to USDA FSIS, we have Jay Gallons.

Hey. Can you hear me okay?

It's a little bit low.

Okay. I'll try to speak up a little bit. I'm Jay Gallons. Even though it was very impressive to see a Ph.D. next to my name on the screen, I'm not a Ph.D. I feel like I'm living a lie right now. But it was good to see. I'm a senior data analyst here at FSIS. I work in the Office of Planning Analysis and Risk Management, or what we

like to call OPARM. Here with me are some folks from our NARMS work group, Dr. Catherine Rockwell. She is in the office Of Public Health and Science, where she is a senior public health advisor working on the antimicrobial resistance. Also here is Dr. Glenn Tillman, who is also in the Office of Public health And Science, where he leads the laboratory work in antimicrobial resistance. And also he works with whole genome sequencing.

And then we have our moderator here, Dr. Mustafa Simmons, he's a public health specialist and also in the Office of Public Science in the Eastern Laboratory. Let me pass it off to Dr. Tillman to discuss a little bit about the NCBI.

Thank you. Thank you for that introduction, Jay. Appreciate that.

So since around 2016, in October beginning that fiscal year, we've committed in our agency to putting -- posting publicly our whole genome sequencing data. I think you saw some of that, our data sources yesterday in Dr. Tameru's talk, when he talked about FSIS NARMS sequel data sources, as well as the product verification sampling. So we're a very strong submitter of whole genome sequencing data from which AMR can also be obtained. That goes for *Salmonella*, *Campylobacter*, STEC *E. coli,* general *E. coli* and *Enterococcus*. That's our largest data repository. I know there's been some presentations on those kind of aspects. So that's one of our large data sources. Our other data source sharing, our public website, in which we post either aggregate level data or line by line data associated with antimicrobial resistance.

I'll turn it over to Catherine, Dr. Catherine Rockwell for any other comments as well.

Okay. Thank you, Dr. Tillman. So one of the uses of our NARMS data in collaboration with our public health partners is the use of NARMS data to help inform outbreak investigations by looking at similar resistance patterns in both clinical and non-clinical collected as part of NARMS testing. But we have to make sure that we recognize that it's necessary that we consider the context and the epidemiological evidence before considering whether isolates are connected to an outbreak.

And so it's important that we collaborate with our public health partners and we look at the focus on the importance of looking at the lab data, the epidemiology, as well as traceback information when we're considering the relationship between these AMR trends.

And with that, I will turn it over to my colleague Mustafa to talk further. Mustafa.

Thank you, Dr. Rockwell. So one thing I did want to add, just as a participant, is that one of the future advances that I do see coming that's kind of relevant to this discussion is our -- one of our GNFS work groups, which is a collaboration of the various NARMS agencies you see here, as well as NCBI, is that we are working on a new metadata package for NCBI, and it's called the One Health Enteric Package. Some of the agencies have already started using it and FSIS is in the early stages of implementing it, but what it will allow for is more standardized metadata, and

it will be a lot more useful for some of our stakeholders to get more standardized and more detailed metadata.

I think Kim Cook did a great job explaining that. And I do want to give credit to Dr. Ruth Timmy from FDA CFSAN. I don't know if she's on the call, but she's the lead of the GNFS metadata work group. But I just want to say that the one health Enteric Package is going to be more standardized and include more traditional data and make it easier for our stakeholders to mine our data. Thank you.

And with that, we'll move on to CDC, Jared Reynolds.

Great. Thanks, Mustafa. And good morning, everyone. Thank you for giving me the opportunity to speak to you all this morning.

So I'm Jared Reynolds, an epidemiologist with NARMS here at CDC and I'm joined by two colleagues from the CDC NARMS laboratory, Dr. Jean Whichard and Dr. Jason Folster. So CDC as an agency has made a recent push to make our data more transparent. We've heard many times of the phrase freeing the data, making the data available faster, so it's actionable, and NARMS has really been ahead of the curve here. I mean, we've had our public platform, NARMS now human data, since 2015, and in recent years, we've made efforts to release the data in a preliminary fashion. So before the data is perfect and we began pushing the latest data to our site each night. And so as we've talked about throughout this public meeting, one of the big advancements to our surveillance system, it has been the incorporation of whole genome sequencing.

And so these data are allowing us to look at antibiotic resistance much faster than what we had -- what we have with MIC data. MIC data from our phenotypic resistance monitoring relies on receiving physical isolates here at CDC and doing the testing. With whole genome sequencing, that process of sequencing is happening through the PulseNet network, public health labs, PulseNet as a seven- day turnaround time, we're able to import those records into our system, and analyze those for resistance genes much faster than we would have through our traditional surveillance sampling. And so having the ability to get our data out on the web faster and before, you know, before holding it until a whole year of data is complete has been really important given our access to these new data. And so that's really kind of shown its value, especially during the COVID pandemic where we actually had to hold back on our routine surveillance shipping of isolates for nearly a year, but during that year of 2020 and into early 2021, the sequencing that was happening at the states allowed us to continue to look for signals for whole -- through the whole genome sequencing and making a predicted resistance determination from the presence or absence of genes. And so, you know, we --in addition to putting our data out on to NARMS now, we also put our data out in to public outbreak web postings that our outbreak prevention branch manage, and so without whole genome sequencing, especially during the pandemic, we may have very little or no information on resistance that's occurring during outbreaks, but with the resistance that's happening, we're able to characterize these outbreaks from the resistance point of view. And one of the developments we're actually working on with the outbreak reporting is to have a NARMS- specific results page that links out to the traditional CDC outbreak

postings that you may be familiar with. We've heard calls from our stakeholders to provide more details about the resistance within these outbreaks and so we're working on a page that will -- for those that really want to dive in, to detail the actual resistance patterns by isolate, that we're seeing within these outbreaks. In terms of further developments as well, we heard about the BEAM dashboard from Dr. Tauxe in his opening remarks yesterday and that's a public facing tool for the PulseNet System. And we see that BEAM might be an opportunity for us to actually incorporate resistance among outbreaks in an aggregate level. So NARMS has been monitoring resistance among outbreaks for a long time, but we really haven't had a place to report those. It's very complex to link those isolate resistance patterns to actual information about the outbreak such as the outbreak size, how many people were ill, what were the implicated food sources. Having that aggregate level is something we've been working toward and having that publicly available, which we don't currently have, and so we think that BEAM, which is aimed to incorporate data from multiple surveillance sources here at CDC, could be a place that we put these resistance information in the context of the outbreak so that it can be analyzed on an aggregate level.

And then some of the challenges that we're -- you know, that are on the horizon, Jason talked about in our workshop, and we've heard a lot about metagenomics here earlier, but having the access to the isolate is something that we're worried about. Our current surveillance completely rely on having an isolate, either for our traditional phenotypic antimicrobial susceptibility testing, or an isolate for whole genome sequencing. And so, you know, one of the things that we're doing is encouraging reflex culture when the CIDTs are positive, we have an example of

a collaboration with one of our departments that actually lost our *Campylobacter* sequencing for a year and a half, but we were able to get an agreement established to get those reflex cultures done on CIDT positives. Of course, that has insurance reimbursement ramifications. It takes work, and it takes money. And so we are looking, as we've discussed, into metagenomics and one of the challenges will be how to incorporate that into the traditional way we've reported our NARMS surveillance and making those data publicly available and understandable.

So I think that's about my time, but I'll stop there and see if Jean or Jason have anything to add.

Yeah, for the sake of time, I'll defer for now, but if there's time at the end, I'll squeeze some other comments.

Great overview, Jared. The only thing I would add is to applaud all the partners that are here for freeing the data, or as Errol said the other day, getting the data in the hands of those who can use it. And I would plug the technical workshop that happened the day before yesterday. It is just a great primer for all of these systems. We got a public tool and display for just about every need. All the NARMS partners are making the data available. We've got NARMS human data, integrated data, tableau displays, resistome tracker, Vet-LIRN and NALHN, and all the visualization tools at NCBI and BEAM, so please check them out and that recorded session for the technical workshop would just be awesome.

Thanks Jean and Jason. And Mustafa, back to you.

Thank you, Jared and the CDC team. So moving on to FDA, we have Amy Merrill.

Thank you. So I'm Amy, and I am a mathematical statistician for the NARMS program at the FDA. I do statistical analyses and data visualizations.

Speak up, Amy. Your audio is low.

I've played a large part in the releases of our annual reports, as well as the development and maintenance of our interactive dashboards. Today I'm joined by Dr. Heather Tate and Dr. Errol Strain and we're going to discuss some of the more recent changes in data sharing and reporting at the FDA and where we see things going in the future. Our partner labs collect samples, isolate the bacteria and perform whole genome sequencing on an ongoing basis for the NARMS program. The sequencing data is submitted to NCBI's Pathogen Detection, allowing for public access to those results as they become available. Most of the recent developments for the FDA NARMS program involve taking advantage of the data available through the pathogen detection to report on our resistance findings and closer to real time.

So at the time of the 2018 NARMS integrated report summary publication, we began including predicted resistance data in NARMS now integrated data. We download data from pathogen detection on a weekly basis and provide the predicted resistance data for the years where the antimicrobial susceptibility

testing has yet to be completed.
Shortly after adding predicted resistance to NARMS now, we began publishing interim data updates. These updates are also based on WGS analysis and summarize recent, unusual or concerning antimicrobial resistance findings and bacteria found through the retail meat monitoring.

Building off of these interim data updates, we thought it would be useful to have a tool that allows users to explore emerging resistances of interest on their own. So we started developing an interactive tableau dashboard, the strain explorer that highlights snip clusters with NARMS isolates that contain genes that confirm resistance to the clinically important antimicrobial agents. This tool is used as new isolates are submitted Pathogen Detection. I presented the beta version of this tool during Tuesday's technical workshop, but if anyone missed that presentation and are interested in seeing it, please don't hesitate to reach out to me and I'd be happy to provide a demo. So moving forward, we will continue to develop new ways to present our data, report on key findings in a timely manner, relying heavily on access to the latest WGS data. We do see ourselves moving away from the current format of our annual integrated report summaries, which has led to delays in the releasing of the finalized resistance data. Instead, we will try putting together shorter summaries with the most important results which we hope we can publish a bit faster. As we continue making these transitions, we are looking forward to hearing from stakeholders about what you would like to see. The more information we have on your specific needs, the better we can present our data and beneficial and effective ways. Thanks. That's it for me.

All right. Thank you, Amy. And moving on to Dr. Olga Ceric as from FDA CVM.

Thank you. And good morning. I'm Olga Ceric and I'm the lead for Vet-LIRN AMR monitoring program which started in 2017. I'm joined by Dr. Greg Tyson, and I hope you were able to attend Dr. Tyson's presentation yesterday to learn more about Vet-LIRN AMR program.

From the beginning of our program, we were very focused on making sure that our data was shared with all of our stakeholders, and so our AMR data was included with 2016-20117 NARMS integrated summary, which was also the first time that AMR data from animal pathogens was included with NARMS integrated summary.

The next important development in sharing our data was forming the cross agency collaborating group with USDA's NAHLN to develop the centralized data collection and reporting process across participating laboratories from Vet-LIRN and NALHN AMR and monitoring programs. I presented the Vet-LIRN and NAHLN integrated data dashboards during the technical workshop on Tuesday, and the first integrated report for Vet-LIRN and NALHN data was released as a part of the NARMS integrated report summary for 2018.

Importantly, our dashboards also include resistance mechanisms from genomic data, which supports NARMS goal to useful genome sequencing data as a primary means of assessing the most recent AMR trends. Moving forward, we will soon be able to start looking at those trends, since we will have that three- year data

available in our dash boards by the end of this year. We currently have 2018 and 2019 data available, and we are working and finalizing our 2020 data.
Turning over to you, Dr. Simmons.

Thank you, Dr. Ceric, and thank you to all of our panelists for the great discussion and updates on how we're currently using our NARMS data.

At this time we wanted to have a Q&A. Unfortunately it's already 11:29, so that would really only leave one minute. Can we still just put the questions in the Q&A box and we can answer them throughout the day via chat, if possible? Because I think we only have -- we have less than a minute to go.

Hi, Mustafa, that fine, we can do that. We'll keep the chat open so we can monitor the questions and make sure they get a response.

Okay. Great. Thank you. And I guess I'll pass it back over to Dr. McDermott.

Objective 2.4: Conduct Research to Assess the Sources and Impacts of Resistance and the Effectiveness of Prevention Practices for Foodborne Pathogens: Panel Discussion – Moderator Dr. Kimberly Cook
Time- 01:58:53 – 02:32:04

Okay. Thank you very much. All right. Great. This brings us on to discussion of Goal 4 in the strategic plan, Conduct Research to assess the sources and impacts of resistance and the effectiveness of prevention practices for foodborne

pathogens. When I read the title of this goal, I think back to some of my opening comments where we talked about One Health and the need for broader partnerships, and this is certainly an area in which that's required to answer some of these questions. And so it's an interesting topic, and a vital one, and look forward to the discussion today, which is being moderated by Dr. Kimberly Cook, and Dr. Cook is National program leader for Food Safety with the USDA agricultural research service office of national programs in Beltsville, Maryland. Dr. Cook's been with ARS for 17 years as a research microbiologist, research leader and national program leader. She oversees ARM research associated with the national safety program and services as the ARS lead on many national, international interagency working group focused on AMR priorities of critical importance to Agriculture and Food Safety.

Kim, thanks for joining us. I'll turn it over to you.

Thank you. Welcome, everyone. Welcome to Goal 4. We will be -- welcome to the panel discussion on NARMS strategic plan Goal 4, focus on research to assess sources and impacts of resistance and the effectiveness of prevention practices for foodborne pathogens.

Goal 4 of the strategic plan emphasizes prevention, and in this session, you will hear from NARMS agency speakers about agency research and how the work contributes to the NARMS strategic goals.

We will then engage in a Q&A to seek input from all of you, so please add

questions to the Q&A box at the bottom of your screen.

Also, we will provide links to additional information discussed by the panel that you will see QR codes on your screen.

So to access those, open your smartphone camera, point the camera at the QR code and tap the banner to link to the information, and I think Claudine will probably put some information in the chat as well.

So it's my pleasure to introduce our panel today. Dr. Michael Neafsey is a - the veterinary services One Health coordinator for APHIS. Dr. Jonathan Frye is a research microbiologist at the U.S. National Agriculture Research Center with ARS. Dr. Louise Francois Watkins is a medical epidemiology in the division of foodborne, water borne and environmental diseases at CDC. Dr. Shaohua Zhao is a biomedical researcher at FDA center for veterinary medicine, and Dr. Sheryl Shaw is the applied epidemiology director with the Department of Public science at FSIS. So welcome to the panel. We appreciate you being here.
The first question that I have for you is what is your agency doing to address the research needs as laid out by goal 4, and its sub-objectives? And Dr. Neafsey, we will start with you.

Good morning. Thank you for the opportunity to be on this panel. In regards to your question, generally speaking, APHIS doesn't conduct research but rather supports monitoring and surveillance activities. Wildlife services and veterinary services, National Veterinary Services Laboratories, or NVSL, do have a couple of

projects that I'll cover, but ARS and NIFA support research more directly and APHIS just really collaborates with them on AMR questions. So APHIS has supported a couple of cooperative agreements. We have one specifically with NIAMRE, but the focus on that particular cooperative agreement is on data protections and information security, not specifically on AMR. This information will hopefully be used to support the future creation of an AMR dashboard for APHIS.

We do have a couple others, so our NALMs program studies do collect information about antimicrobial use as well as context about overall animal health that includes, you know, the diseases that lead producers to need to use antimicrobials.

NALMS also collects data on antimicrobial stewardship practices, and for many of their studies, NALMS routinely tests fecal samples for some combination of *Salmonella*, *Campylobacter*, *E. coli* and *Enterococcus*, and they typically test the *E. colis* and the *Enterococcus* solely to evaluate AMR.

Because the ongoing need for AMR data collection, NALMS has partnered with University of Minnesota and Pipe Stone Veterinary Services to collect data from U.S. Poultry and Swine Industries. I mentioned NVSL earlier. They do have a couple studies related to AMR, specifically AMR and *Salmonella* serotyping in which they're trying to analyze *Salmonella* isolates submitted to NVSL between 2014 to 2017 for AMR genes.

And I'll go back to our wildlife services partners. They do have a couple research projects as well, including developing alternative cost- effective and timely approaches for identification of AMR and *E. coli* and *Enterococcus*. They are also working on developing and optimizing methods for detection of AMR in wildlife fecal material and they are conducting a meta-analysis on the prevalence of AMR bacteria in wildlife species to help to identify target species for future studies. So really each of these programs and projects that I've highlighted show the cooperative and collaborative approach that APHIS embraces when working on AMR with our federal, state and industry partners. And I'll turn it back over to our other panelists. Thank you.

Great. Thank you. Next we'll go to ARS. Dr. Frye.

Yes. Thank you very much for the invitation to speak today. ARS is the in- house, non- regulatory research agency of USDA and is charged with researching, developing and transferring solutions to agricultural problems with high national priority, such as antimicrobial resistance.

ARS works with collaborators and stakeholders to approach the problem from both ends by combining basic research into the genetics of AMR and agricultural settings, with applied research in the interventions to solve the problem of AMR and pathogens of importance to human plant and animal health.

As an example, I'll talk a little bit about my own work. We learn from our NARMS partners that *Salmonella* Infantis was becoming an issue in poultry. We did

several studies to help elucidate that. We took a look at the pESI plasmid carried by Infantis and found that it had conserved genes that may give the *Salmonella* an advantage. We were also informed that this plasmid had made its way into other serotypes in turkey isolates, and so we did another research project looking at the chromosome of the *Salmonella* Infantis carrying this plasmid and found that it was clonal. This indicated that the plasmid was likely causing most of the issue. Because of this, we formed *Salmonella* Infantis working group at ARS and had a number of ARS scientists work together to try to determine what features on the plasmid were giving advantages to the various *Salmonella* that carried them. Along with that, we also developing interventions and alternatives to antimicrobials used to eliminate this problem. Similar problems are going on elsewhere in ARS to address other issues with animal and food products. ARS is also developing alternatives to antimicrobials as well as other interventions to help eliminate antimicrobial resistant bacteria. So throughout the agency, we are approaching this with our collaborators to get the research done that needs to be done so that we can understand what's going on.
Thank you.

Thanks. And next, Dr. Francois Watkins from CDC.

Thank you. Good morning, everyone. I realized, in trying to kind of organize some thoughts to respond to this question, that CDC has more activities in this area than I can possibly mention in just a couple of minutes, but I did want to try to highlight some of our activities that help to meet kind of each of the three major objectives of goal 4.

So in terms of burden and impact, our CDC NARMS routine and outbreak surveillance work really remains the cornerstone for examining trends and resistance in to tagging emerging resistance, but we've had a few notable changes in the last couple years.

After CDC's PulseNet platform transitioned to the use of whole genome sequencing in 2019, CDC has defined a number of what we're calling REP strains, REP strain is short for recurring, emerging or persisting strain, using case definitions based on an isolate genome.

So we can now identify isolates associated with important resistance strains and close to real time and better focus our efforts on the epidemiological side to collect additional information.

So some examples of REP strains with important resistance include multidrug resistant *Salmonella* Infantis linked to chicken and multidrug resistant *Salmonella* Newport linked to travel to Mexico.

In terms of assessing risk factors and source attribution, we're working to link our CDC NARMS data to information collected through other national surveillance systems. Currently we are analyzing the first two years of exposure data collected as part of extended case and other ascertainment through the FoodNet system and this will enable us to better describe the relationships between resistance and specific exposures, including travel.

We are also working to link resistance to data collected through the national outbreak reporting system, or NORS, so that we can see how outbreak strains linked to known vehicles align with resistance. And those who attended the technical workshop on Tuesday may have heard Jason Folster speak about our alert system for rare and concerning resistance, so we're also developing an enhanced surveillance protocol to collect corresponding epidemiological and clinical information for those cases.

We're working to make our CDC data more accessible to external stakeholders to enable more timely response to emerging trends. The publicly available BEAMS and NARMS now platforms were discussed in more detail during the technical workshop and also in the last Goal 3 session. And finally, we also have a new initiative to support industry partners through prevention office technical assistance. Thank you.

Great. Thanks. And next we have Dr. Shaohua Zhao from FDA.

Good morning, everyone. Thank you for inviting me to join this discussion, and to reduce prevent the resistance dissemination, one of our primary rule of our research team in CVM is to conduct research to understand the burden of resistance and to assess the resistance source attribution. This address the Goal 4 and the sub objective 4.1 and 4.2. We have a several project that use genomic and metagenomic data generated from a variety of sources to study to study the antimicrobial resistance source attribution. Here I'm going to just highlight a few

study and finding here. First one is ESBL ctxm *Salmonella* Infantis that Jonathan just mentioned. It was detecting in 2014 from a retail chicken through the clone expansion and widely spread in the United States. By 2019, every NARMS retail meat site had detected this strain, and it has the high prevalence in the cecal sample of the chicken and high incidence in the human as well.

So we shared all the information with the poultry industries through the Chicken Council and presented the data in some scientific media such as the IAFP. So we hope that, you know, the industry will take this information, follow the strategy to reduce and control this organism in the poultry farm.

The second study I like to point out is the *Salmonella* Kentucky led by Heather Tate. It's a collaboration study with CDC, I think Jason Folster may have mentioned this study yesterday. So we analyzed over 700 Kentucky genome isolated from different sources. The results showed that sequence type 198 and 152 are major ST type reported in human infection. ST 198 appear to count as most human infection in the United States further analysis included human exposure data which suggested that for quinolone resistant ST 198 infection may be linked with the consumption of contaminant imported of food or through the international travel. So this is quite an interesting study.

Another study, *Campylobacter* source distribution study. The study included both *Campylobacter jejuni* and *coli*. As you know, the retail beef and the pork has a low contamination of *Campylobacter*. So in this study, we collaborated with FSIS and used a *Campylobacter* isolated from cecal sample of the cattle and the swine and

the poultry, and the poultry MLST showed that in addition to the chicken both the beef cattle and dairy cattle played a very important reservoir for human *Campylobacter* infections. Our study showed that certain MLST are highly linked with a certain resistance gene. For example, we find with a variety of gentamicin resistant but if the *Campylobacter* carry aphIG and the afg the gene, they were mainly detecting the chicken, turkey and human sample. So that's indicating the chicken maybe the source to cause the human gentamicin resistance *Campylobacter*.

Another source attribution study is the metagenomic approach. We have 3 PIs conducting research in this area. Andrea, Daniel and Beilei. I think this morning, Andrea gave an excellent presentation of surface water, so they used a metagenomic approach to assess the AMR in different source. The findings are interesting. I'll give an example here. So Daniel's food animal cecal sample study shows that it identifies 194 amr genes that represent 17 different amr classes. The distribution and the relative abundance of amr genes observed vary by source but were consistent across the years. So I hope when you read their publications, it's quite interesting in this area.

Another very important research area to support the goal 4 is to analyze the genomic structure of the mobile elements. I think morning my colleague Lucas and our colleagues from CDC stressed how important the mobile elements. So they place an important role to spread the dissemination of AMR gene to different environment. So understand the MDR plasmids and genomic structure, AMR gene composition, along with heavy metal and the disinfectants genes will

allow us to understand the co- selected pressure for AMR development, origin, evolution and initiative. So we do have some publication on this. I just want to point out, you know, all study, plasmid subtype, you know, some subtypes are highly and associated with specific serotype, and the source of the isolates, and linked specifically gene. For example, the cmy gene is very important antimicrobial gene if linked with the incC plasmids mostly you can detect the *Salmonella* Newport isolated from cattle or beef. But if the incA is linked with the IncI plasmids, and you are mostly detecting the *Salmonella* Kentucky isolated in chicken. So that's quite interesting to look at the source attribution.
Also, we find out if the plasmid carries five or more antimicrobial resistance gene, normally it's highly linked with the disinfectants and heavy metal of genes. So this is a really great resource to look at is co- selective pressure.

Finally, I just want to mention about the resistant tracker, I think as Dr. Heather Tate has described in detail about this tool. It's epidemiological tool that allow us to track the resistant gene in bacteria isolated from a different source around the world. The program was established in 2019. Currently we cover the four organism of *Salmonella*, *Campylobacter*, *E. coli* and *Enterococcus*. The source of the WGS data is from NCBI. Isolates originated from over 180 country, and the isolates are from 75 different sources, the categories included food, companion animal, environment and various produce and crop type and a clinical samples. So far we have identified several hundreds of resistance new genes belonging to 32 unique antimicrobial classes among those isolates. I think the resource is there, updated weekly by Amy Merrill I think -- I hope you have a chance to look at this database, and if you have any questions, you can contact Amy, Heather Tate, or

Errol, so with that, I stop here. I normally have so many research here, and I just highlight a few. Thank you.

Thank you. Thanks so much. We'll move on quickly to Dr. Sherry Shaw with FSIS. All right. Good morning. And thank you for the question. So FSIS is a public health regulatory agency that develops and enforces the policies to ensure health, or the safety of meat, poultry and egg products. In support of our agency's mission, we publicize our research priorities and related studies that are needed to motivate the development of new scientific knowledge. We regularly present our information on our research needs and the accomplishments at scientific meetings such as this one. Our research priorities may be based on outbreaks, laboratory data, findings in the field, or other relevant information.
We currently have 20 priorities, one of which is to improve our understanding of antimicrobial resistance in pathogens in poultry and cattle.

To help accomplish our research priorities, we're supporting projects at ARS related to improve our understanding of *Salmonella* in a pre-harvest environment, including characterizing antimicrobial resistant genes in *Salmonella* Infantis. We also support the education of Ph.D. students as FSIS food safety fellows. Two of our students are working on projects related to antimicrobial resistance in foodborne pathogens. The projects aim to characterize antimicrobial resistance genes in foodborne pathogen with a view to improve detection methods and characterization of risk. With that I'll turn it back to you, Kim.

Thank you so much. Thanks, everyone. So we're very short on time if we want to

be able to have a Q&A session, but I do want to get to question 2, which is what are the challenges and opportunities of doing research on the AMR and enteric pathogens as related to the goals of NARMS? Dr. Neafsey.

Yes, thank you. So most of our work in this space relies on voluntary participation of producers, veterinarians, diagnostic labs, and participation in itself can be a challenge. So I had mentioned earlier our NALMS, national studies, and, you know, one of the things of those studies is the fact that they are voluntary, and they're designed in collaboration with industry. But because they're voluntary, we often struggle with, you know, reduced response rates, and especially in industries that are routinely surveyed.

And to counter this problem, we have tried our best to provide incentives for participation, and we do our best to reduce producer burden along the way. We're moving to an electronic data collection system to help provide multiple options for producers to respond so they can participate in ways that they're most comfortable with.

And meeting all the -- another issue that we have is meeting all of the needs for information around AMR data. So we ask questions in a way that producers are willing and able to answer. The type of information needed to examine AMR isn't always easy to get through a cross- sectional study, which our national studies through the NALMS program, that's what they are.

And these studies are points in time. And it happened over every so many years,

and the number of years between studies is increasing due to the addition of new industries or, you know, there's also reduced resources as well. So adding longitudinal data collection will address some of the AMR data needs along the way.

There are two issues that I can identify as being related to wildlife specifically, and those are funding and credibility. So we've submitted a number of proposals that are well received but are never funded. In terms of credibility, most people associate AMR with people and domestic animals, but don't really think of wildlife being as a potential problem.

I think that's being dispelled, but it's still a problem, especially when you're trying to acquire funding. So I will pass it back over to the panel. Thank you.

Thanks for that. And Dr. Frye.

Yes. I'll try to be brief. Agriculture is diverse and very complex with a myriad of stakeholders, including farmers, producers and ultimately the American consumer. Research solutions must be economical and practical to facilitate adoption so as not to contribute to rising food costs or food insecurity. Additionally, given the complexity of plant, animal and other agricultural systems, what may work in one system may not work in another. Thus, solutions must be tailored to address each production system individually. This requires the maintenance of scientists with expertise in multiple disciplines, cooperation, trust and engagement for multiple stakeholders, and if required, navigation of multiple

regulatory pathways. In comparison to public health, agriculture has received fewer resources to combat antimicrobial resistance.
As far as opportunities, ARS possesses that in depth knowledge about management practices and technologies associated with animal, environmental and public health and is uniquely positioned to contribute to the body of scientific knowledge around AMR. Mitigating AMR requires a science- based, comprehensive approach to minimizing risk of crucial important and create real- time detection technologies.

ARS will optimize its own efforts and harness the work of essential stakeholders and partners to understand, detect and mitigate AMR that can harm agriculture and public health. Thank you.

Thanks, Jonathan. Dr. Watkins.

Sure. On the human health side, I think we can also continue to contend with some of the ever- present challenges in public health work, including access to sufficient funding and staffing. I think it will suffice to kind of echo Dr. Neafsey to say that, you know, we don't always have the resources to pursue ideas, even when those ideas are good. I think it probably also bears mentioning that the last few years during the COVID- 19 pandemic have been challenging not only for our CDC team, but also for many of our state partners, and we haven't always been able to obtain all of the information about the cases of resistant enteric infections that we hope to receive. Many of our surveillance and response activities were delayed or had to take a back seat to more pressing pandemic work. But as we

look forward, I think that our major challenges will continue to be -- to develop and strengthen the types of partnerships that will enable us to accomplish some of our goals, especially those related to prevention and interventions.
Unlike some of our sister agencies, CDC has no regulatory authority, so in order to bring about change, we really have to rely on our partners to make changes and choices that are in line with our public health goals.

I think our surveillance work is as strong as it's ever been, but we now need to kind of take the next steps to ensure that our data are being used not only to describe resistance and monitor trends, but also to inform public health action. And we hope that some of our recent initiatives to improve transparency and timely access to CDC NARMS data will help our partners to work collaboratively to develop strategies to help address the problem of resistance in enteric pathogens. Thank you.

Thank you. Dr. Zhao.

Oh, sorry. Okay. I think I'm just look at as a researching challenging part, you know, the antimicrobial resistance is complicated, you know, it's continued growing, and the mechanism is complicated. So right now, we are completely, you know, relying on the antimicrobial resistance database, and that is to continue to update. Right now, it's not 100 percent correlated, but of course it gives us opportunity to look at, you know, the new mechanism. So I think that's, you know -- it's a challenge, but it's opportunity as well to say, you know, the new mechanism or new mutation all the mechanism, you know, we can continue to

study this area. So that's one area.

Another is metagenomic research, I think it will be great if we can link the resistance gene to the particular organism of the interest, whether this is long reads or improving bioinformatic, I think this is achievable, I think it will be great resource to study, you know, the AMR in a different commodity, and another challenge I feel is data sharing. A great example is the esi *Salmonella* Infantis, we have great data sharing and I think we also shared with the industry, I think it will be very nice that the industry share their strategy, how to use information to control the -- you know, this esi at the farm level. That would be very helpful for us to come up with a new idea for the research.

So I think the data sharing should go both way. That's all I have.

Great. Thank you so much. And Dr. Shaw.

All right. Thanks. So as you've heard, FSIS collects and analyzes many samples from many sources. So one of the challenges framing this data and information in a manner that tells the story that warrants further research. Back to you, Kim.

Excellent. Thank you. Okay. Well, we only have a couple minutes for questions, but there are some in the chat, and I thought I would go ahead and start with the first one. This has been an interesting conversation. Will the current scope of NARMS to monitor retail foods be maintained? How would the new focus affect the NARMS budget and what appropriations, changes are expected to reach the

goals outlined in the strategic plan? So I think maybe , Shaohua, will the current scope of NARMS to monitor retail foods be maintained? I'm not sure if that's a research question or if you want to -- oh, Pat may be able to answer that question.

Yeah. Sure. I'd be happy to answer that question. It's a good one, obviously, it's foremost in our minds for every budget cycle. You know, the work we're doing in this program we believe is leading to a more sound scientific basis for public health action or we wouldn't be doing it, and we think that the work we've done to date has been faithful in that mission and we continue to try to make the program more consequential and the data more robust, to maintain the things we put in place, including the retail piece, for sure. It's hard to know in this environment what budgets look like in the out years. It's a bit of a prognostication game. We certainly endeavor to pursue the things we think meet the criteria of sound public health service in terms of generating data for good decision making. I don't see a threat right now to maintaining the retail meat testing scheme right now. I hope you can hear me okay. I don't see a threat to maintaining the retail meat testing scheme right now. The work that's more aspirational in this strategic plan to move into some sort of sustained environmental monitoring, again, we think the data are starting to show that that would be a worthwhile plan, we have the resources I think to complete the work that we've put in the plan in the out years if we want, in its expansion, is to lead to an expansion of the scope of testing. It will be hard to do it under the current resources and we'll continue to strive to get what we need. So that's the best I can answer that, I'm afraid.

Thanks, Pat. And I will ask one other question. Does the CDC engage partners overseas in investigations to elucidate source persistent MDR infections such as *Salmonella* Newport? This would be a practical way to meet national action plan for AMR, and Dr. Watkins, do you want to answer that?

Sure. So we do. We do attempt to do this. I think we've heard earlier during the technical workshop that there are a number of platforms that bring together isolate information on the molecular side of things. So one of the advantages is that we're actually able to see when there's a strain, or, you know, a concerning resistant strain that we are following in the United States that's causing human illness here. We're able to see if other countries have uploaded isolates that match that strain to public platforms such as the NCBI pathogen detection pipeline.

And when we do see isolates appearing from other countries that match a phenomena that we are investigating here, we reach out to our colleagues in those countries.

Specifically here for the MDR *Salmonella* Newport, the countries that have shared isolates publicly are Mexico, which is one of the countries that we know to be involved, because a number of human cases in the United States that reported returning to travel from Mexico, and also Canada, so we're in the process of working with Canada right now, actually, to try to better understand the epidemiologic information that they have about the MDR Newport cases that they're seeing there. But yes, we do try to do this when we can to be able to pool

information is often very powerful and helps us understand better global transmission. Thank you.

Thank you so much. Well, thanks, everybody. Thanks to the panel and thanks to the questions. I think we'll have to leave it there because we've already stolen a couple of minutes of your lunch and I believe we're supposed to back at 1:00. Thank you, everyone.

-Lunch-

Dr. McDermott is speaking. Okay, welcome back from lunch, everyone. Let's start with our afternoon session. This afternoon we change gears and hear from NARMS stakeholders in different perspectives from people affected by the activities of NARMS. Moderating this session this afternoon is Dr. Katherine Huebner. Dr. Huebner is a Veterinary Medical Officer in FDA CVM's Office of Surveillance and Compliance. In this role, Dr. Huebner Coordinates a variety of CVM activities related to antimicrobial use data collection as well as antimicrobial resistance. Dr. Huebner obtained her degree from the University of Pennsylvania and then completed an internship in livestock medicine and surgery at Colorado State University. She also obtained her masters in Epidemiology at Colorado State University researching resistance in livestock production setting. Kate, thanks for moderating this afternoon. I'll turn it over to you.

Stakeholder Presentations – Moderator Dr. Katherine Huebner
Time- 03:30:59 – 05:07:47

Thank you, Pat. Good afternoon everyone, and thank you for joining our session today. We'll be holding a stakeholder presentations. Our first speaker today is Steven Roach. He is the Safe and health program director for food animals concerns trust and senior analyst for Keep Antibiotics Working, which is a coalition of advocacy organizations that have joined forces to combat the inappropriate use of antibiotics in food animals. He has worked on policy related to antibiotic use and animal agriculture for over 20 years. Without further ado, I'll turn it over to you, Steven.

Integrated Surveillance and Consumer Advocacy – Presenter Steve Roach
Time- 03:32:05 – 03:43:49

I'm going to get started. I've Steven Roach. I'm work for an organization called food animal concerns trust which is a Chicago based advocacy organization looks at issues around animal agriculture. We have two main programs, humane farming program which provides services to farmers, to help them raise their animals in healthy ways. We have a grant program aimed at improving access to pasture and improved marketing.

We also have mentoring and a series of webinars. So we have some other resources for farmers.

I actually lead our safe and healthy food program which looks at limiting the impacts of animal agriculture on human health. Primarily looking at pathogens that can be transferred from animals to humans. One of the issues we worked at is antibiotic resistance pathogens that moved from farm to people. We have taken a One Health approach to address this.

In my work on antibiotic resistance. I've always worked with the keep antibiotics working coalition. The coalition is made up by 19 advocacy organizations at this point. We've had our membership change based on the interest of members.

Our goal is we support the use of antibiotics in animals to treat sick animals. What we want to do is restrict the use of antibiotic in animals that are not sick. So that's looking at growth promotion and disease prevention.

We believe that you should prevent disease in animals by providing appropriate care to the animals and environments and not doing things that would lead to sick animals. We also consistently supported integrated surveillance antimicrobial resistance, and by that we mean surveillance of pathogens in the food system, resistant pathogens in the food system and surveillance and monitoring of antibiotic use in the food system as well.

It looks like we need to shift away from thinking from pathogens and more on resistance determinants and plasmid.

So I think the first thing I like to think about is why are we here? I could go to a meeting on antibiotic resistance every day of the week for months on end. Lately I've been doing that.

We're here because antibiotic resistance is causing lots if health problems in

humans and animals. You have increases in the severity of illness, hospitalization, and death. So 2019, antimicrobial was the leading cause of death worldwide. We have 2.6 million resistant infections in the U.S. And about 20% of those are easily directly tied to farms and food systems. So the *Salmonellas* and the *Campylobacters*. 40 to 160,000 deaths and an unknown number resistant infections deaths in animals. So again, why are we here? And by here, I mean having meetings have antibiotic resistance. My perspective is that the reason we're doing this is that we hope that in the future, through our activities through these meetings, we will have less resistance that fewer people will have resistant infections and that our antibiotics will continue to work longer.

What I say is, the reason we're here is we're trying to make resistance go down. That's the measure. I thought it was interesting, Dr. Beloeil from the European Union noted that they actually have an indicator that they're concerned about and that's susceptibility in indicator *E. coli* from that. I don't think we have something similar in the U.S. particularly in the animal side. At one point in the healthy people goals, there was a goal related to *Salmonella* from animals but it was removed.

So I think what we want to do is look at resistance going down. I've chosen one of the series from the CDC threat report from 2019 resistant *Campylobacter* these are resistant- either decrease susceptibility to fluoroquinolones or reduce susceptibility to macrolides both of which are used to treating serious *Campylobacter* infections. If you look at the data there, you don't see it going down. There is a reduction in the poultry side. Which I was kind of surprised, but it is down.

I would say the other thing is, you know, 20% is not great. But we can look at other countries where there's higher use of fluoroquinolones all of *the Campylobacter* in those countries would be resistant. The other thing is that with this slide, it was challenging for me to create a slide that looks at or had to do it on my own that takes the threat report and I had to create it myself. And important thing for NARMS suggestion for NARMS we actually on the NARMS now slides we actually do reporting consistent with the threat report. So you can go and look and say, ok CDC says this is a threat in *Salmonella*, this is a threat in *E. coli* and actually look for those on the NARMS Now without having to do the calculations yourself.

And how do we get reduced use? From our perspective, the primary was to reduce resistance is reduce use as a primary driver for resistance. The keynote speaker was clear that in the European Union, they accept that fact that reducing use is important and it needs to go down.

What I show on the right of the slide is a comparison of reductions in the U.S. versus reductions in several of the European countries. We see steeper reductions in several of the European countries where they've taken efforts to do it.

So I think one of the challenges we have in the U.S. is that we don't like to talk about reducing use. There is recent report of from the FDA or the Reagan-Udall foundation said it's a problem if the public thinks how use is bad and low use is good. And I think this is a problem. In the U.S. we like to talk about promoting stewardship, reducing inappropriate use, reducing need for use, but are really careful, and I think this is definitely FDA and USDA staff that we may never talk about reducing use.

And I think we need to be clear about that. The goal of these things is to reduce use so that it limits the drivers of resistance.

And so, I think the way we can reduce use is create ways that we raise animals where we don't cause animals to become sick. What we are calling on here, is for the U.S. and the FDA to acknowledge the need to reduce use in agriculture. There is no reason to believe that this sector is somehow a shining light of perfect use. We know there's ways to reduce use.

We should set national targets for reductions and report on progress towards use reductions. We've to avoid metrics in this country because it keeps us from avoiding accountability.

We believe that there's enough data. The data we have on use right now is the sales data. And I think that is an effective proxy for it. We would like to see better data on antibiotic use. You know, the FDA and reports on the FDA have called for collection use data for 20 years now. But we never seem to have a system that collects use. S o we're forces to use the sales data which is consistent with the world animal health organization recommends anyway. Use of sales data as a proxy for use.

We recommend begin collecting antibiotic use data starting with feed distribution data from feed manufacturers. We say feed the feed distribution because FDA already has regulatory authority to collect it and it doesn't create the representativeness problem you have from voluntary systems which FDA is currently considering.

In terms of the NARMS strategic plan, we support it. We think that we really like the surface watering monitoring. We need the one hole One Health approach.

The water connects the different pieces of it. I think again there was disturbing yesterday we learned that the water monitoring program is unlikely to capture where animal agriculture as an applying manure to the environment.

So we support the feed sampling. There are new studies out just recently and older studies showing that feed can an important way to spread around *Salmonella* and resistant *Salmonella*. And the meta genomics approaches being explored are really exciting and interesting and give us new information that we need.

A couple of gaps in the strategic plan, I think we really need to think about how does NARMS tied into our efforts to avoid the next pandemic? So how do we tie this better with other zoonotic monitoring systems for viral pathogen. How can we look at them both?

I think the wastewater project is looking at that on the human side. How do we find something similar on the animal-side? And clear glaring gap in this the crops and antifungal co-resistance where we're looking at what we're doing on farm, applying antifungal is creating human health problems. And then there is some room for improving reporting , as I've said before, it would be really good if we can go onto NARMS Now and pull up information on the threats that CDC has identified and see how prevalent they are in the food.

We would like USDA data by state. Right now we only have the retail meat data by state, the CDC's data by state. The problem is gives us few isolates. This chart shows lots of states with data usually it's one or two or three isolates. Ang that also leads me to the next one, be clear on the limitations on the data because you can't tell much from having three isolates from Iowa whether you have a problem

or not.

And we have a support training integrated reports that include use and resistance.

And then the last set of recommendations are attempting to address equity, racial equity and marginalized groups within NARMS. We need to seek input from historically marginalized groups on how can NARMS better address it their needs. One of the areas we really think is important that has been recognized but I don't think it's been given thought. Look at food chain workers which are from marginalized groups or here socio-economic groups but they are also on the frontlines of getting impacted resistance that come from farms, either handling animals or large quantities of raw animal products.

And with that, I'd like to end these are some of the farms that my, animals from the farms my organizations work with. On these farms, there is very low antibiotic use if any in general. And what this really illustrates to me is the reason we use the antibiotics that we use is not because it takes antibiotics to raise these animals. It's how you raise the animals lead to high levels of use. So thank you.

Thank you, Steve. Thank you for sharing consumer advocacy perspective through your work.

I'd like to remind the audience to please feel free to submit questions as we be having a Q&A session at the end of this talk, and if there is time allotted, at the ends, individual talks we can potentially take questions as well.

Okay. So, our next speaker today will be Lola Olabode. Lola works a Research

Program Manager at the Water Research Foundation where she has worked since 2000. Prior to the foundation, Lola worked as an epidemiologist investigating food and water borne outbreaks at the Maryland Statement Department of Health and Mental Hygiene. Lola is on scientific advisory boards for the global stewards national science foundation, national research traineeship program at the University of Maryland and the emerging contaminants summit.

Lola directs a comprehensive and dynamic research portfolio on compounds of emerging concern, micro plastics in water and receiving water linkages in water quality. She is an active member of the water environment federation's disinfection and public health committee, as well . Welcome, Lola. And I will turn it over to you.

WFR's Research on Antibiotic Resistant Pathogens and Genes – Presenter Lola Olabode
Time- 03:46:04 – 03:57:18

Thank you very much. I'm Lola Olabode research programmer with the water research foundation. I would like to highlight a snapshot of our research on antibiotic resistance pathogens and genes. This is just a snapshot because the three projects that I'm going to highlight are in the same stage, the draft final ,and they have exciting preliminary information.

So first before I get into that. A little bit about the water research foundation. I realize maybe not everyone is familiar. We're a global organization at the forefront of public health and environmental protection leading the water sector. So I'm bringing a water perspective with water, dripping water, surface water,

storm water. All those water. So we're a merged organization and it's to really meet the evolving needs of the water sector.

So a little bit about our mission basically about advancing the science of water to improve the quality of life.

We have-- we want to be the one stop shop in all things water. And we certainly have quite a number of values. So we have an access to expanded collection of water research. We have the opportunity to leverage funding and communication with government partners. And we have strong relationships with the water partners organizations that you would also be hearing from today.

And we have a dynamic model for collaboration across the water community. I heard earlier and so this is the all the water matrices that waters community that was about to lose my breath describing.

But so our research benefits all of the areas of the water sector. And there is agriculture, energy, which we're not necessarily strong at. We lean heavily on our partners for that.

So a little bit of snapshot of like our research program areas. So we have several in your research priority where 60% of the research funding comes from. And then we have this unsolicited every other year, and-- it's ideas we're not thinking about that others are using to advance. They remain like an incubator for trends in the water sector or a nexus into food energy. The tailored collaboration is subscriber driven, subscribers are basically mostly a majority of the water utilities, drinking water plants, wastewater plants and storm water plants. And then there's emerging opportunity. Things like outbreaks or what we like to say what keeps our subscriber and water sector partners up at night. That comes through

that. And facilitated research is 100% funded by the partners and utilities. And so we give somewhat of a peer review totally funded by them.

And here I won't go too much into details but it's basically giving like an idea of the schedule and so some are on rolling basis like emerging opportunities and some are really structured and batches of RFPs will come out which we're about to release in the fall.

So this year, we restructured our research portfolio into five themes. And to this group, it will mostly be in the healthy communities and the environment. And suddenly the efficient resource use and recovery for ideas because we want to partner with the ag sector. And we have optimization and intensification, resilient infrastructure, utility operations and management. I mention healthy communities and environment first because that's where a lot of my research for my portfolio. And the intercross link with all of them will be climate reassessment and adaptation, communication, and environmental justice as our commitment to D&I.

So now moving onto why we're here, like Steve said. The WRF's research on antibiotics resistant pathogens and genes. Yes we take the one water, One Health approach, involving the water system health, the environmental health, human health, animal health. And here in the bottom left section, is like a global database that our global partners.

Right now, last week we were in the international water association meeting where two of the projects I'll be talking about was featured with our global partners.

So the first one which is very exciting, because it's in that draft final stage, critical

evaluation and assessment of health and environmental risk from antibiotic resistance in reuse and wastewater. And so the goal of this one is to develop and disseminate a risk assessment framework of water based sources of antimicrobial resistance. Just a step back, I think that they water sector was really worried about the contribution of current state monitoring because the wastewater plant are seen as a point source. Well They are. That's what a lot of people see versus the discussion versus the other norm point sources.

Our global partners are from around the world-- we have the French and their similar organizations to ours. And what started as a discussion in 2017 where our global partners especially the Dutch described as a puzzle we have some really exciting findings. Their puzzle now fits into a database that Kerry Hamilton that Arizona State University just recently-- so when I say Q2 to 23 for the deliverable, that's a report itself.

So one of the motivational for reassessment that Kerry listed, you know, I have some of this on the screen. But we need to understand and prioritize AMR determinants, characterize context and predict and prevent disease and many others. And again you see that, our framework which I've seen in a lot of research and presentations, I won't repeat a lot of what Steven had said. So I had that over 35,000 deaths. But we know the mathematical can be used to estimate risk to a given population.

One of the highlights of our global meeting was it has to be site specific. The model may not work for countries like India where there are no septic system compared to the U.S. But we know that .8 million annual infections in U.S. and over 85,000 annual deaths due to AR infections. So we're really excited to move

forward with doing research in this space.

So this second one here is this was one of the ones that came through other people's ideas.

So it was understanding the fate of antibiotic resistance genes ARGs and antibiotic resistance pathogens and full scale activated sludge processes. This is really water treatment process. And the goal was to further the knowledge of the antibiotic resistance bacteria and the genes through secondary treatment process.

This has really exciting results as well. It's showing so far that of the five AR associated in anaerobic digestion there's seen reduction in decline and both long-term and short-term SRT. That is retention time processes and also in the anaerobic digestion. This the exciting at least for wastewater treatments and there were six that were sampled. This last one is a partner one. So the research partner here is the California State Water Resources Control Board. We attained a $4 million grant. And part of the grant was to look at standardizing methods with QA/QC standards investigating the occurrence, and removal of antibiotic resistance bacteria and antibiotic resistance genes in surface water, wastewater and recycled water. This is completed but the deliverable will be out shortly.

So, this particular project is going to host the database from the first project that I mentioned. The co-PI Amy Pruden, on the first one is the principle on this one. And so she serves as a reviewer. She's really in the depths of things. And again the goal was to identify and develop validate standard methods for monitoring ARBs and ARGs in whatever environment including water metrices that I mentioned.

And so I mentioned we have a lot of use-- you saw the five theme areas. So in the Fall we are going to be having some batches of RFPs released not anything

specific on ARG or ARB. The one might be interest to this group is integrating wastewater based epidemiology and clinical surveillance for public health decision making and utility operations.

And I'll be managing that. And I just highlighted this IWA World Water Congress and Exhibition where we are and our global partners really showcase on highlighted in those two projects that I mentioned. It had almost 4,000 people in attendance.

And so that's all I have. And if you want to stay in touch, please send me messages especially on opportunities to collaborate maybe through that emerging opportunities program or what not.

I'm happy to stop right here.

Thank you, Lola. Some very exciting updates about the water research foundation's program. Next in our session today I will be introducing Anna Mehrotra. Anna has recorded her talk, for the Q&A session, her colleague Claudio -- can answer any questions that may arise.

So Anna is a licensed professional engineer with a master’s in environmental engineering and Science from Stanford University, as well as Ph.D. in civil and environmental engineering from UC Berkeley. She's the Director the water federations wastewater program. She oversees entertaining, collaboration, pilot testing and other activities focused on strengthening relationship between wastewater utilities and public health entities, advancing the practice of wastewater surveillance and expanding participation in CDC's national wastewater surveillance system.

So I will turn it over to her recording. Just take a moment to get that up and running.

National Antimicrobial Resistance Monitoring System 2022 Public Meeting
Water Environmental Federation (WEF) – Presenter Dr. Anna Mehrota
Time- 04:00:32 – 04:09:49

Hello. Thank you so much for the opportunity to present to you today. My name is Anna Mehrotra. I am the Director the wastewater surveillance program at the water environment federation or WAC. And I want to answer two questions for you today. The first is what is WEF's role in the water sector and the second is why is NARMS important to WEF? So let's talk about the first. What exactly is WEF? What do we do and what we do for the water sector? We are established not-for-profit organization founded in 1928 as a federation of sewage works associations.

We rebranded ourselves in 1950 as the federation of sewage and industrial waste association. Then again in 1960 was the water pollution control federation and finally in 1991 was the water environment federation. In this evolution, our branding reflects our evolution on focusing solely on sewage treatment to focusing more on the entire water cycle. Although I will say we have enough spots for wastewater and wastewater treatment.

WEF is global. We are a membership organization representing a little overs 30,000 individual members plus 75 affiliated member associations. So these member associations come from around the world. They are clustered in North America, Canada and United States. But we also have member associations in

Latin America, in Europe, in Africa, and the Middle East and in Asia and the pacific.

And these member associations provide activities and services to our members around the world, things like conferences, operator trainee and certification, local and regional legislative and regulatory activities, educational programs, affiliations with other professional organizations and so on.

So when you become a member of WEF you also become a member of your local member association. WEF is also very committed to the water sector. As you see we're a federation organization with all our member associations, and we're focused on connecting and enriching expertise of water professionals to improve water quality and protect environmental and public health around the nobody. Who have these water professionals? They are engineers, plant managers, operators, regulators, researchers from academia, consulting, industry, government and, of course, utilities.

So that's who WEF is. But what exactly do we do? A big part of what we do is run events. As I mentioned our member associations run their own events and training activities. But WEF runs conferences, summits, webcasts and workshops to provide education and networking opportunities for water professionals.

So here's a peek at what we're planning for 2023 in terms of conferences and summits. We're running a forum of intensification of resource recovery. This is through the wastewater treatment process. We run conference on utility management in collaboration with AWWA. There's also residuals and biosolids conference and an odors and air pollutants conference. An innovations in process engineering conference. My personal favorite, the wastewater disease

surveillance summit that’s at the end of June of to 2023. Storm water summit. Collection systems and WEF TeC. It's the biggest event. So WEF TeC is the Water Environmental Federation’s the technical exhibition and conference. And it really is the world's most comprehensive gathering of water quality professionals and has a whole wide range of programming. Lots of different technical sessions, workshops, learning exchanges, technologies, spotlights. Of course there is the exhibit with hundreds of vendors exhibiting and other really fantastic events like the operations challenge.

In addition to events, WEF puts out a lot of resources. So these include things like publications. Our water environment and technology magazine is a monthly magazine that provides information on new technologies, innovative solutions, operations and maintenance, regulatory and legislative considerations, professional development. You know, things that are of interest to water professionals.

Water environment research is our international, multidisciplinary water resource management journal and we also publish books. These are technical publications things like manuals of practice, study guides for professional operators and so on. We offer continuing education credits for successful completion of our training programs, workshops, seminars. And these resources are available on our online platform called access water. It's free to get a log in to access water. There are over 20,000 items up there now, things like conference proceedings, FAQ sheets, some of the standards, manuals of practice.

Not all of these things are free to access on access water but many of them are.

And finally another important thing that we do relates to advocacy. So this

includes legislative affairs. In other words we track, review, and actively comment on legislation impacting clean water issues.

And we do this in collaboration with other organizations working in clean water. And we also equip our membership to be able to educate Congress on clean water issues in their own districts and states.

We issue clean water position statements to guide the work that we provide in clean water policy. So here is a QR code that will take you to our position statement webpage and actually the position statement that will pop-up when you get there is the wastewater surveillance statement. We also call water advocates program which provides information to our members to help them inform their government decision makers about the importance of water.

So that what's what WEF does which brings me to my second question is, why is NARMS important to WEF? And a lot of this relates to the work that I am fortunate enough to be doing at WEF which is on the subject of the wastewater disease surveillance.

The answer to this question has two parts. The first part is that we want to help leverage our wastewater based disease surveillance work and the work that's being done nationally by CDC for NARMS.

So WEF represents the wastewater utility stakeholders participating in the national wastewater surveillance system or NWSS. And you heard yesterday from Amy Kirby how NWSS has expanded rapidly in the last two years and encompasses 1100 sampling sites across the U.S. Most of these sites are wastewater treatment plants and many of those are members of the NWSS community of practice which is something that WEF hosts to enable exchange of

information among utilities interested in participating in wastewater surveillance. We view wastewater surveillance data as being complimentary to NARMS.

So WEF can help forge partnerships and enable communication between the water sector and the agencies participating in NARMS.

Which now brings me to the second part to the answer to my question, why is WEF interested in NARMS? And this relates to the data. We want to help maximize the use of NARMS data. I think I demonstrated how's WEF knows how to collect, verify and distill and share water quality information with the members and the sector through our events, resources and our advocacy.

So we want to use that infrastructure to help share NARMS data with the water sector and work with NARMS stakeholders to maximize the use of NARMS data for promoting better stewardship of the water environment.

With that I want to thank you for your attention today and encourage you to reach out to me. Send me an email with any questions about WEF, about our wastewater surveillance work. You can also find out more about WEF generally at WEF.org or about our wastewater surveillance work at NWBE.org. Thank you.

Okay. Thank you to Anna and I think we're a couple of minutes ahead of schedule. But I believe that's a good thing. So I would like to introduce our next speaker who is Dr. Michael Costin. Dr. Costin is a 2003 graduate of Kansas State University college of veterinary medicine. After graduation he worked as a veterinarian both in mixed animal and dairy practices for several years in the state of Wisconsin.

In 2012 Dr. Costin took a position as a technical services veterinarian with an

animal health distributor and also completed his MBA at the university of Wisconsin's executive MBA program.

In 2015 Dr. Costin's current program became the Associate Director with AVMA where he currently serves as the staff liaison to AVMA's animal agricultural liaison committee, AALC. And the committee on antimicrobial as well as the AVMA's liaison to the United States animal health association.

Welcome Dr. Costin. I will turn it over to you whenever you are ready.

Update on the AVMA Committee on Antimicrobials – Presenter Dr. Michael Costin
Time- 04:13:39 – 04:27:02

Good afternoon, my name is Michael Costin Associate Director in the division of animal and public health with the American Veterinary Medical Association. I'd like to express to Dr. McDermott and the NARMS program for their invitation to speak with you all today. They've asked me to share with you the actions, AVMA has taken to promote antimicrobial stewardship within the veterinary profession.

AVMA has a long history stretching back 20 years of working in this space. Back in 1998, the AVMA created a steering committee to address antimicrobial use in veterinary profession. The steering committee created the first stand-alone policy addressing antimicrobial use in veterinary practice and created the policy judicious therapeutic us of antimicrobial. This policy was then expanded upon by our allied veterinary groups as they used it as a foundation document to create judicious use policies specific to the species under their care.

In 2009 the AVMA created the antimicrobial task force to clarify the role of the veterinarian and the level of veterinarian involvement in all uses of antimicrobials. In 2011 AVMA created the veterinary oversight steering committee to work with the FDA on the development and implementation of the veterinary feed directive. The steering committee's influence led through the inclusion of a requirement that a proper veterinary client/patient relationship or VCPR exist with when a veterinary feed directive is issued. In 2013 the task force for antimicrobial stewardship in companion practice was created. This task force developed a report with recommendations for implementing antimicrobial stewardship in companion animal practice and numerous resources for practitioners, all of which are available on AVMA website.

Additionally during this stretch of time, numerous standing AVMA committees and councils were involved in helping the AVMA develop positions and comments with regard to proposed legislation and regulation.

Now before I continue, I want to explain what the veterinarian client patient relationship or VCPR is for those who are unaware. The VCPR is the basis action among veterinarians and their clients and their patients and critical to the health of animals. VCPR is established when requirements listed on the slide are met. These are the AVMAs, AVMAs definition of VCPR which is similar to the FDA's, these do vary slightly depending on the state of veterinarian practices in.

The VCPR is a foundational principle, it's included in the AVMA's principles of veterinarian medical ethics and model veterinarian practice act.

So as mentioned previously for nearly 20 years to AVMA addressed antimicrobial issues through a variety of existing AVMA entities or through the creation of topic

specific steering committee or task forces. Over time it became apparent we needed a standing committee to specifically handle antimicrobial issues. In 2016, AVMA created the committee on antimicrobial or the COA. The COA has nine seats, eight are held by allied veterinary associations, one seat is held for members at large.

Each seat is represented by both primary and alternate representative. And in the case of the COA, the alternate representatives are active participants and committees activities. We put them to work. We have to. There's too much to do for nine people.

So everyone who is involved is actively participating.

We also have four advisors one each from FDA Center for Veterinary Medicine, one from USDA APHIS and CDC and Animal Health Institute. The COA serves as AVMA's lead entity on antimicrobial issues. We are tasked with the oversight and development of AVMA's antimicrobial policies, crafting AVMA's responses and positions to legislative and regulatory proposals, creation of tools and resources for AVMA members and others and interacting with stakeholder involved in antimicrobial issues. Back in 2016 when COA was created, conversation around antimicrobial was shifting. People were talking about antimicrobial stewardship and one health. I can remember the COA's very first meeting, we had everyone in at AVMA headquarters in Schaumburg, Illinois. We were sitting around the conference table and we went around the table and asked everyone to define antimicrobial stewardship and explain how that was different than judicious use of antimicrobial. We got a different answer from everyone involved.

That moment, COA knew what their first task was how are we going to promote

antimicrobial stewardship in veterinary medicine if we couldn't agree what it meant. So the COA worked. The created a definition of antimicrobial stewardship in veterinary medicine. We developed a set of core principles of stewardship in veterinary medicine. This document was adopted as AVMA policy in 2018 when it was unanimously approved by the AVMA's house of delegates.

For those if you who are not aware, AVMA's house of delegates is the veterinary version of Congress. Every state veterinary medical association as well as every allied veterinary medical association is represented. So a unanimous vote meant that the veterinary profession now had a unifying definition of stewardship regardless of the practices or the species veterinarians work with.

Here's the core principles were developed to practices implement stewardship plans. But if you take a closer look at them, you'll see that there are no specific how to instructions.

That wouldn't work as veterinary practices vary widely depending on the species that we work with. The core principles are created more to serve as a foundational document which the allied associations can build upon, creating more specific recommendation for implementing a stewardship plan within their specific species types.

Other things that COA has undertaking, we developed numerous resources for practicing veterinarians to help them have discussions with their clients about judicious antimicrobial use and if an antimicrobial is needed. Some examples are on the slide here. We call these our dos and don'ts posters available to members on the website veterinarians can download and hang them in exam rooms and help initiate conversations with clients and determine if antimicrobials are

needed for specific diseases or not.

In developing these resources we realized that we needed a place we can make team readily available to our members and other interested parties. So working with AVMA's implementation division as well as digital service division, the COA developed a landing page on the AVMA website for antimicrobial. This is antimicrobial.avma.org and from that landing page, one can connect to numerous informational page as resources.

Shown here are excerpts from our resources page. On this page we have links to both AVMA developed resources as well as other groups materials which can help veterinarians practice and implement stewardship in their practice settings.

So in 2018 COA embarked on an ambitious plan. The CDC had been putting out a yearly report titles antibiotic resistant threats the United States. The CDC document focused on human medicine. The COA wanted to develop a similar report focused on veterinary medicine. We began working with microbiologists, epidemiologists and species experts to identify bacteria affecting multiple animal species for which there is evidence of resistance available to antimicrobials. This report includes actions that veterinarians, and our teams, our clients, producers, breeders, and others seeking medical care for animals can take to collaboratively to combat antimicrobial resistance.

The full report which you can get to from our main landing page includes the overview impact of antimicrobial resistance has on animal health in the United States. We have report cards that summaries pathogens of concern for different host species as well as the technical appendix. This report was published in 2020. We have a review plan that begins next year with hopes that an updated of this

report will be published in 2025.

We've also come out recently with some additional resources for veterinary medicine similar to some CDC resources. We created flyers to assist veterinarians in their discussion with clients to determine whether antibiotics are needed or not. We have client-focused resources to help veterinarians have that discussion with clients and also have a veterinarian focused resource which helps veterinarians work through their decision tree to determine if antibiotic is needed, and if so how to determine which antibiotic might be best in that specific case. These resources are also available on our resource page.

In addition to the resources and the reports, COA is also developed a new policies for AVMA. In 2018 AVMA approved a policy titled AVMA definitions of antimicrobial use for treatment, control and prevention.

COA developed this policy because at that time there was state and legislative actions and some recommendations from the WHO which seemed to suggest when it comes to antimicrobial stewardship, use if antimicrobials for prevention control or treatment could be ranked in an order of appropriateness which in turn led to some instances attempts to limit or specifically oppose the use of medically important antimicrobials for prevention of disease. In contrast the AVMA's committee on antimicrobials believe that attempts to evaluate the degree of antimicrobial stewardship on the basis of therapeutic intent were misguided and use of antimicrobial for prevention control or treatment of disease may comply with the principles of antimicrobial stewardship. It was important to the COA that veterinarians and animal caretakers were clear about the reason they may be administering antimicrobials to animals in their care. Therefore, they developed

some definitions of prevention, control and treatment of both individual animals as well as populations of animals to avoid confusion and to help veterinarians clearly communicate their intentions when prescribing or recommending antimicrobial use.

In addition to this policy, the COA also developed and published within the AVMA's journal an accompanying white paper which further explained their thought process behind the development of this policy. This white paper was used by the U.S. delegation to the CODEX task force in antimicrobial resistance in their work when reviewing the codex code of veterinary practice.

Earlier this year, AVMA house of delegates approved another COA developed policy-- titled support for the collection of antimicrobial use data for antimicrobial stewardship. This policy established a clear position for the AVMA to guide our efforts as we participate in some ongoing discussions regarding the collection of antimicrobial use data.

The policy recognizes that the collection and the evaluation of antimicrobial use data does adhere to the core principles of stewardship but makes clear that the methods of collection must preserve veterinary client confidentiality and as well as include acceptable data anonymization.

Accompanying this policy once again the COA developed and published in our journal a white paper which further explains their thought process behind the development of this policy.

That concludes my report. Thank you to the NARMS program for the invitation to share this with you. I'd also like to acknowledge the efforts of the members of our committee on antimicrobials. Over the last six year, nonpaid all volunteer

workforce has put forward a tremendous amount of work on behalf of the AVMA and the veterinary profession. And I would like to give my thanks to them for all that they have done.

Thank you, Dr. Costin. That's an impressive portfolio of tools and work. Thank you for sharing.

So our next speaker today in our panel is Dr. Paul Plummer. Dr. Plummer is an Executive Director of NIAMRE, as well as a professor and Anderson Chair in veterinary science in the department of veterinary diagnostic and production medicine for Iowa State University. He also serves as the Associate Dean for research and graduate studies at the Iowa State University college of veterinary medicine.

He is both a board certified food animal internal medicine and infectious disease specialist, and a Ph.D. in veterinary microbiology and leads an active research laboratory which places him in the intersection of translational research that is focused on antimicrobial resistance. In addition he serves a as a voting member on the presidential advisory council for combating antimicrobial resistance bacteria or PACCARB and also serves on the AVMA committee on antimicrobials which we just heard about in the previous talk.

So I will turn it over to you, Dr. Plummer. Thank you for joining.

We also have a question about whether we'll have time to for questions. And the answer is yes after following this talk to I continue to encourage participants to submit questions at this time. All right. Go ahead, Dr. Plummer.

NIAMRRE Coordinating action to combat the global threat of antimicrobial resistance – Presenter Dr. Paul Plummer
Time- 04:29:03 – 04:45:02

Dr. Plummer. All right. I appreciate and echo the appreciation of others for the opportunity to speak on this NARMS public meeting. Appreciate all of the organizers and the other speakers and it's been an interesting meeting.

On behalf of the National Institute for Antimicrobial Resistance, Research and Education (NIAMRRE), I also appreciate the opportunity to discuss some things that we're doing as well as some comments more broadly. So a little bit for those who of that aren't familiar with NIAMRRE. We're an organization that's been around for about the last three years. That emerged out of an effort of the American association of veterinary medical colleges and the association of public and land grant universities, to promote a One Health approach and industry public private partnership interaction around the issue of antimicrobial resistance, antimicrobial use and antimicrobial stewardship.

And core to that component is very near will dear to us is really truly taking that One Health approach that balances and optimize health outcomes for humans, animals plants, and the environment.

The vision of NIAMRRE is to be a trusted leader in coordinating one health efforts

that preserve the ability to prevent and treat infectious diseases for generations to come and our mission is to drive cross sector engagement and coordinate action to combat the global threat of antimicrobial resistance in humans, animals, the environment and plants.

To do that we are largely membership based organization with members from coast to coast. This is representation of a number of our current members at this time. We have academic industry as well as affiliate members and represent a large number of different sectors of human, animal, plant, and environmental health.

Collectively we work together to approach antimicrobial resistance use and stewardship from what we define as a broad approach. By that I mean, that we're not purely focused on antimicrobial resistance for instance at the research bench, or in diagnostic samples but more broadly the broad approach to technically address the need to use antimicrobials for the health, welfare, and One Health approaches across humans, plants, and the environment.

So within this approach we would consider development of new vaccines, development of new technologies precision medicine on the human side, precision agricultural approaches on the animal side that allow us to detect disease earlier to identify individual animals that need to be treated as early as possible as well as on the crop side to evaluate how those precision technologies can be applied to assure our continued development and continuous improvement and stewardship around the use of antimicrobial and antifungals.

We also appreciate the approach that focuses on stewardship in all sectors and really promoting inter-professional education across the sectors to learn from

each other and to further our ability to address this issue from that One Health approach.

I'm sorry to interrupt you. I want to let you know your slides are not advancing. I'm not sure if you're aware.

Dr. Plummer is speaking. Let me go back. I'll stop sharing. Can you see it now?

I can see it now, yes.

Dr. Plummer is speaking. Okay. So I'll just quickly show, our current NIAMRRE members and then this broad approach.

So we've seen several slights on One Health already presented here. And I want to commend NARMS for the desire and the approach to take a One Health approach. As I mentioned we truly believe that's important.

And the reason I put it here a define mission here, collaborative, multisectoral and transdisciplinary approach with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants and environment. We view One Health as that true intersection in the middle.

So it's really a, if you will, a translational science that in many ways pulls those other sectors together and creates a new convergence science that focus on the One Health aspects of that. So you start to blend altogether lose the distinguishing silos and areas of human, animal, environmental or plant health.

So within NIAMRRE and our work in this One Health sector we have four priority

focus areas those are research, collaboration, advocacy and education.

And very briefly I will talk through several of these and how we see that they potentially fit in with the great work that's being done in the NARMS program and moving forward.

Before I do that, a quick overview on the research side. We don't have research laboratories. Everyone involved in NIAMRRE do. But really on the research side we're focused on research gaps, building teams to address those gaps from finding new funding opportunities and promoting new funding opportunities that are strategically positioned to address those gaps that have been identified.

We work to build collaboration across the sectors, collaboration between academic institutions, collaboration between academic and industry, and collaboration between public institutions. And then also certainly with the stakeholder groups and other important sectors in this very complex issue of antimicrobial resistance. We work in advocacy related to strong policy, respond to a number of public comments that might be issued by a variety of Federal Agencies related to policy, but also advocating for new opportunities to address those research needs that we've identified in gap analysis as well as mechanisms to promote the data protections that allow us to aggregate that data and continue to use that data to advance in a continuous process improvement.

And finally the fourth of those is education. We are very interested in education and educational opportunities that both focus on prescribers across the health sectors and broadly on policymakers and on the lay public and consumers as it related from childhood up through adults on the importance of antimicrobials in maintaining the health and how we can optimize our use of those to assure their

continued utility.

So with those kind of brief introduction, I'd like to make specific comments about that relate to the One Health approach here for NARMS and continue to help and assist in moving this process forward from that One Health approach.

We believe that research interpretation is critical and One Health research space really requires a One Health team of researchers to interpret that data. Within that context we caution against having sampling and all of the different environments.

But then not considering and utilizing the collective One Health expertise that you have on your team and that you have in demonstrated in the last couple of days to interpret that research outcome.

So we support the work that you're doing and continue to encourage you to pull the researchers together as you look at interpretation of this data. If our organization can be of help over our membership, we have identified and resource map over a thousand researchers working in different areas of AMR represented by the NIAMRRE members most of those in academic institution, but many of them, at other places that have deep experience in this One Health approach to AMR data interpretation. We would lend our expertise if there's a need. Examples of current relevant extramurally funded projects through NIAMRRE are one that is looking at promoting interprofessional One Health education as a means of mitigating antimicrobial resistance across the food chain. I'll speak briefly more about that here at the end of the slides.

We're working on developing educational training for a variety of industry folks, recently started a cooperative agreement with FDA looking at multi-pronged

standardized methodology to identify key diseases and prioritize antimicrobial alternatives in production. And have been working as well through cooperative agreements on white papers related to the evaluation of AMR data privacy and security necessary to allow us to make these comparisons across One Health sectors and do such in a research and evidence-based manner.

Through that process, we continue to recognize as does the NARMS team that trust and collaboration are essential. And so we've had some experience in doing this through a prototype AMR dashboard that we are developing. This is still a prototype. I t's not public available or anything like that. But we're really working through many of these data security privacy issues as well as how do we represent data may truly equal playing field across the One Health sectors which is not quite as easy as often is kind of thought to begin with.

Certainly NARMS has been active in this area and well aware of these challenges and so we appreciate that. The dashboard just a bit of background, it's really the prototype is design to think about how we can strengthen national One Health surveillance for antimicrobial resistance. And right now we have a significant lack of coordination across veterinary diagnostic labs, antimicrobial susceptibility testing across institutions and significantly differences across those platforms across the One Health spectrum.

So even though we might be doing microbroth antimicrobial susceptibility testing in human, animal, and other diagnostic labs, those results and the platforms that we use are slightly different and making direct comparisons quite difficult. NARMS is well aware of this issue and working to address that.

But the goals of this prototype project are to improve harmonization and

collection and integration of data across the VDLs with the eventual goal of a One Health data integration on the human and environmental side.

So just as an example, this is just prototype. But in our current prototype we have upwards of 283 different bacterial organisms, a broader scope than the current focus in NARMS.

And across 17 host species including companion animals as well as livestock and then other pocket pets and fish and variety of different ones. And then right now over 22,000 isolates which really represent a very small component of the potential data that could be pulled together in this type of prototype dashboard if we can fully gain the trust and confidence and collaborative approaches through data security and privacy controls.

So currently in our prototype, we can look at this data through MIC charts, and those mic charts are developed in real-time. We're not using predetermined interpretations from the different diagnostic labs. We use Realtime interpretations that allow us to look at both human, animal breakpoints, ECOFFS, across variety of different breakpoints by toggling which breakpoints we want to turn on, in that real-time calling. We also have charts around multidrug resistance. Charts that allow us to compare genotypic and phenotypic results when we have whole genome sequencing. And geospatial charts that were really prototyping and developing mechanisms that assure those data protections and security while still allowing us to potentially look for areas of difference in how resistance patterns are occurring.

And then the final point here before I finish up my time, would just be through our education efforts we really believe that education and clarity in dissemination

are critically important.

So towards that, I mentioned earlier the interprofessional task force workshops. I'll show a slide of that here in a minute, but we're doing AMR learning outcomes committee that's developing one health AMR outcomes applicable across all the health sectors. We work hard in the science of science education and how we disseminate and communicate science to nonscientists in a manner that's evidence-based and results in improved understanding. So finally, I mentioned a couple of times this One Health Inter-Profession Education (IPE) AMR workshop. This came out directly out of an effort or paccarb report issued on the importance of interprofessional education and AMR. And so we have 33 inaugural cohort members across the one health disciplines, human, animal, plant, and environmental health, diverse of careers, practitioners, consultants staff, faculty and students, geographic diversity across the country and we have been working as a group over six months here working through One Health AMR cases having those discussions, learning from each other's section various health sectors and expertise and really at the end of the day most excited to hear one of our participants say at the end of the day, we're all working together for the same thing.

So we'll be doing a Capstone virtual presentation of the six teams in our NIAMRRE October research symposium. If you're interested in watching that or considering in being part of a future One Health IPE cohort that we will be launching, feel free to go to our website, niamrre.org, that listed there for more information on both of those activities.

With that, I appreciate the opportunity to talk and will turn it back over to you,

Kate.

Thank you, Dr. Plummer. That concludes our speaker presentation for this session and we'll be moving into the Q&A session.

Stakeholder Presentations Q&A– Moderator Katherine Huebner
Time- 04:45:11 – 05:00:47

The first question that we received was for Dr. Olabode regarding there were thoughts from the Water Research Foundation perspective on the NARMS strategic plan and activities. Dr. Olabode I don't know if you're ready to take that question.

Yes, I just put-- responded to Jay in the CHAT. So goal two and three especially on the technologies and information sharing will be really important. I know that during our global partner meeting they all had to prioritize their next immediate need. And one of the concepts we are probably coming out with this year is some white paper bringing it altogether.

And Dr. Amy Pruden will probably be helping that global group with that. Because she's helping some other countries as well.

But anyway, it's definitely on our radar. And it's really important except the utilities have not said that is what is keeping them up at night. I think it's topics

like PFAS right now. But they see AMR as almost like the next micro plastics because of the pandemic.

And so I think our driver is just leaning on our global partners and we really like the simplicity of WHO's recent framework. I don't know if that helps answer your question, Jay.

Okay. I'm not sure who would have the ability to speak on-- he can respond in the Q&A.

So moving onto our next question. There was a question, I think what could potentially be directed to both our speakers on water research. And follow-up question whether it would facilitate surveillance if implemented by raising awareness about the benefits of monitoring drug resistant microbes. So Claudio or Dr. Olabode please feel free to take that question.

I'll take it first, Lola. I answered in writing that it is becoming a key point. It is actually the target of CDC has funded WEF to take on the analysis, evaluation of what it might take to do a wastewater surveillance to set up a wastewater surveillance network worldwide. And the first target in that would be AR.

So there is in the works a thought process to do that. WEF is giving that a lot of thought and we'll set up a group of experts to help advise us, Lola that's one of the things we'll be reaching out to you to help us with.

We know that there's been a lot of monitoring happening on the animal side. So we want to learn from that as well. But I think that it's very exciting time from a

perspective of wastewater monitoring even worldwide. I don't know if you want to add anything to that, Lola.

I'm not sure I really understand the question per se. So Kate, could you help me. Is it saying what is the research? I'll not sure I understood it.

I can repeat it. And then maybe we can follow-up if need ed. So it's whether, would surveillance be facilitated through raising awareness about the benefits, about the benefits and monitoring drug resistant microbes in like it speaks to about education and outreach to facilitate surveillance.

I have an RFP coming out shortly in the fall. And it would -- we want that clinical data. But we're leaving it to the creativity of the proposer and researcher. So some might come with-- I think we've outlined the need for that communication in there. But it's a very nonprescriptive RFP and it will get to bridging the gap. We want to bridge the gap between wastewater epi and clinical data of significance. So that would help.

And I know example we got excited about is pathogen loading when there's a norovirus outbreak. So wastewater epi could help during that time in the pre-treatment with the pathogen loading. That was an example but we're not saying that is the example. We just want to articulate some of the ways that we could bridge the gap.

And like Claudio said, we're already doing that. We got a lot of feedback from

WEF hosted a public health summit with the CDC earlier this year. And recently got, the two sectors come together. So the public health folks and wastewater folks. You know it was very interesting the communication back and forth, but it's at first time the two groups had come together to articulate how they can help each other. So I think that is our first step. It's certainly my first time in the 20 years in the water sector that I saw a meeting like that.

Exactly. I agree, Lola. That was the exciting part about that meeting. I know for-- you folks are mostly health public health related. So you wouldn't appreciate this so much. We for the first time in my entire career, somebody got up in the meeting and asks what is a CSO was, and that was exciting. Combined sewer overflow. If you are on the wastewater, you know was a combined sewer overflow is, but to get a question like that, it showed that we actually had different people in the room. And that was exciting.

And that goes back to Anna's presentation of why, you know, why being here and talking to you all is exciting to us. It's because we think that there was a lot of wastewater surveillance can bring to the table and research are and important work that Lola is doing, the WRF is doing will fill the gaps we have. But WEF is moving forward with some of the logistics and some of the infrastructure that needs to be there for us to be able to do that kind of work and set up the network that I think will be needed.

Also benchmarking many we're looking seriously of what it will take to get a benchmark that will have value in a global setting. And we want to learn from what has already been done on the animal side.

Thank you both. Excellent answers. I think that dovetails into a question I thought of in listening to these presentations. Kind of getting back to the One Health approaches and framework many.

I know we heard a lot in this NARMS meeting about how a One Health framework is needed to adjust the topic of antimicrobial resistance. And many of you touched on it. And I think, Claudio, your story about having different people in the room and is it really relevant as well. And I'm impressed by the diverse backgrounds in this panel.

And it highlights to the importance of taking that multidisciplinary approach to this topic. I guess my general broad question, if anybody wants to take it would be, you know, if you could elaborate further on what One Health means to the respective organizations and maybe touch on some examples of One Health in practice.

I know there were examples that were brought up. But I want to throw that out there in case there were additional that could be added.

So Kate, I'll comment on that. I commented quite a bit, I think in presentation on our perspective from the NIAMRRE perspective.

But I think really as I said, you know there's this concept and this interest of NSF and others around convergent science. The way I described this, a friend of mine that's a science communicator talked about this that as we start doing team research which One Health is collaborative research, you know that goes through

iterations.

And they described those iterations using food example. So they said, where many of these team collaborations start is kind of like your dinner plate. And so your potatoes and your broccoli and your entrée maybe all on the same plate, all in the same area, the same room if you will. The same virtual room, the same place.

But each have their own characteristics and they're not mixing together but they are in close proximity in talking. As you continue to breakdown those kind of walls or build bridges between those different groups, if you will, then they start to take on the appearance of a fruit salad where they're all chopped up and mixed together. And so they're in closer proximity. They're working together to make one meal, if you will. But you could still pick out individually a strawberry or a cherry or whatever much like you might be able to pick up a veterinarian or a physician or an epidemiologist or whatever.

Our goal in convergent team science is to get to the fruits smoothly if you will. To where the language, the concepts and understanding of each other's science is so tied together that it becomes one new science. And you're able to communicate without those barriers without the jargon. And then build products that represent, you know, the collective output of a completely unified approach.

And so for us that's what we strive for. I won't say that we're there yet. But that's what we strive for, continuing to breakdown and integrate those more and for NARMS that's not just sampling in water and sampling in, you know, retail meats and sampling in humans. But it's really having to pull those together and have that data interpretation where the scientists completely understand each of those

sectors and look at the data in each sector using the One Health approach, not just collecting data in all of the sectors of One Health. If that makes sense.

Thank you Dr. Plummer. I know we have a break coming up but we have a couple of minutes to respond to that. I don't know about others but that analogy made me hungry. I want to respect people's time to take that break. Steven Roach would you like to go ahead and respond?

I actually think that interdisciplinary resource doesn't mean you make a smoothie that people have different perspectives. And you come-- actually agree, disagree at times. And so kind of my example of the One Health issue would be something I learned from working with Swedish colleagues on antimicrobial resistance where you have to look how to raise animals and what is the health systems there.

For me a very clear example of that in the U.S. in our feed lot systems most of the cattle are given diets unhealthy for them. We use antibiotic to treat the liver abscess they get, it's a critically important antibiotic and then that creates problems with resistance like in those *Campylobacter* isolates I shared with you, from cattle.

So I think one of the problems we have in the discussion is that there's an assumption that everyone actually is on the same page. I don't think that we are. I think when we all believe that we're all working for the same goal, it's not effective.

You know, different people have different interests. And we need to acknowledge

that and look at those. I would also say different, you know, there are different perspectives that actually lead to different answers to problems.

And part of the challenge is how do we resolve those differences and how do we do it in a fair way that doesn't bias against certain people.

So I would just leave it there that I think we really don't want a blender. We want to have people that have different perspectives on issues. And then try to think about how do we work through those different perspectives and do it in a fair way.

I think that's really interesting perspective. That's, important to mention as well. So with that, I see that we're at 2:30. And it's time to take a break. Ten-minute break. So I think we'll be returning at 2:40 for further stakeholder updates. Thank you. Thank you.

Kate, I answered the questions.

Oh, thank you.

Yes, I wrote the answer to that last questions.

Take care, thank you.

Thank you everyone, I appreciate your participation today.

-Break-

Dr. McDermott is speaking. Welcome back, everyone. I hope you enjoyed your break and got some more coffee. Now we enter into the final phase of the agenda. It's my pleasure to welcome speakers for the next section on stakeholder updates and to introduce the moderator for this session, Dr. Chelsey Shively. Dr. Shively is currently the Antimicrobial Coordinator in the Office of Interagency Coordination for Veterinary Services at USDA's Animal Plant and Health Inspection Service. Prior to this, Dr. Shively spent 3 years working on National Animal Health Monitoring System on the NAHMS team, focusing on collecting the data on antimicrobial use and resistance in animal agriculture at USDA. Dr. Shively serve as a AVMA AAAS Congressional Science Fellow in 2016 and 2017 working on the U.S. Senate Committee on agricultural nutrition and forestry. Originally from Michigan, Dr. Shively completed her undergraduate and Veterinary Degrees at Michigan State University and then completed her Ph.D. in animal behavior and welfare at Colorado state put with Dr. Temple Brandon. Dr. Shively is also board certified in American college of animal welfare. Dr. Shively over to you.

Stakeholder AMR Updates – Moderator Dr. Chelsey Shively
Time- 05:11:54 – 06:02:07

Thank you, Pat. And it's my pleasure to moderator the session today. I think one of the key themes we've heard throughout the meeting over the last several days together is the need for collaboration. So I think during this session, we'll hear some updates from our industry partners because they are integral components of how we address antimicrobial resistance. So first up we have Dr. Ashley Peterson Senior Vice-President of Scientific and Regulatory Affairs of the National Chicken Council. Her responsibilities include scientific and technical expertise on a variety of topics including food safety, poultry inspection and broiler health and welfare. She oversees regulatory policy development and scientific initiatives within the regulatory agencies in Washington D.C. and represents industry views on policies impacting the broiler industry. She earned her Ph.D. in animal science from the University of Maryland, her Master of Science from Michigan State University and her Bachelor of Science from the University of Kentucky. Dr. Peterson, over to you.

NARMS Public Meeting: National Chicken Council Update – Presenter Dr. Ashley Peterson
Time- 05:13:30 – 05:24:02

Good afternoon and thank you for the invitation to speak at this public meeting addressing the NARMS program. My name is ash lie Peterson and I'm the Senior Vice-President for Scientific and Regulatory Affairs at the National Chicken Council based in Washington D.C.

The national chicken council is a trade association that represents approximately 95% of the broiler chickens raised in the United States. Broiler chickens are meat chickens not egg laying chickens. And we also represent companies that have goods and services that they supply the industry.

The national chicken council focuses on forming areas including federal legislation. Regulatory affairs, communication and trade.

And before we hop into talking about NARMS, I want to provide you with a few industry statistics. In the U.S. there are approximately 30 companies that are involved in raising, processing, and marketing chickens are there are about 25,000 family farms that have contracts with companies and about 95% of the broiler chickens are produced on these family farms.

To put a little context in the size of the U.S. industry, in 2021 over 9.2 billion, that is with a B, broiler chickens were raised weighing almost 60 billion pounds live weight. That's almost 42 billion pounds of ready to cook chicken.

Clearly everyone eats chicken. Americans consume chicken more than anyone in the world, consuming almost 100 pounds per year. And it is the number protein consumed in the United States. Again for a little context that's about 160 million servings of chicken each and every day.

The top five broiler producing states in the U.S. are North Carolina, Georgia, Arkansas, Alabama, and Texas. And we export about 17% of our U.S. production.

The U.S. is the second leading exporter of broiler meat in the world just behind Brazil. Our top five export destinations from a value perspective are China, Mexico, Cuba, Canada and Angola. That's a 2021 statistic stat as well as our top

five export destination from a volume standpoint in 2021, were Mexico, China, Angola and Taiwan.

Antibiotic stewardship is a top priority for the broiler chicken industry. It is of at most importance that antibiotic be used judiciously to maintain their effectiveness to treat diseases both within human and animal populations.

It's also critically important that our poultry veterinarians have effective antibiotics to protect our birds' health and well-being. The broiler industry has diversified its product offerings to meet consumer needs and preferences.

For example, in 2015, not quite 10% of the industry was raising birds without antibiotic. However the numbers have changed over the years. By 2017 almost 45% of the industry was raising birds without antibiotics. And as of last year many almost 70% of birds in the U.S. were raised without antibiotics and all that data comes from USDA.

However, we continue to see resistance in certain pathogens to antibiotics that have not been available for use in the broiler industry for decades. This leaves the industry, scientists and our public health partners all scratching our heads.

Reducing *Salmonella* from farm to fork is a criminal strategy in combating antimicrobial resistance. Keeping birds healthy is important in our antibiotic stewardship efforts. It's important to take note that farm interventions such as against organisms like *Salmonella* can take many months to years to have an effect specially if that mitigation strategy involves vaccination in our breeder flocks. The industry has done an exceptional job in reducing the prevalence of *Salmonella* moving through the production continuum, but there is still room for improvement. And the industry has many widely adapted practices such as

vaccination of boiler breeder for *Salmonella*, robust sanitation and biosecurity practices at the breeder farm, the hatchery, the feed mill, and grow out houses, treatment of feed to reduce the presence of *Salmonella* and potential pathogens, inclusion of feed additives such, as pre and probiotics, the treatment of bedding and water lines in grow outhouses, feed withdraw strategies prior to moving birds to a processing facility. Minimizing the stress of our birds to minimize *Salmonella* shedding and at the processing plant there are numerous steps taken to reduce *Salmonella* including cleaning chickens to reduce potential foodborne pathogens keeping meat at a proper cold temperature to prevent bacterial outgrowth and conduction very robust microbiological testing to guarantee that products are meeting FSIS performance standards.

Speaking of which, on performance standards, if you look at the recent data at APHIS regarding *Salmonella*. The industry is doing an excellent job. The performance standard for whole birds is 9.8%. Right now there is only about 3% positive across the industry. That's for all production sizes.

Also with regard to *Salmonella* if you look at chicken parts, the performance standard for chicken parts is 15.4% and the industry is less than half of that or half of that 7.2%.

And if you look at *Campylobacter*, the most current data out of APHIS indicates that as an industry we're around 16% for both broiler carcasses and parts. There is not a current performance standard for *Salmonella*. However the old performance standard for whole birds was 15.7%.

So I think while it's important to talk where the industry has come over the last several years, we're really here to talk about NARMS. As discussed the NARMS

program was established in 1996 to monitor antimicrobial resistance in bacteria that are found in retail meat, human and food producing animals. NARMS' charge distribute information in a timely fashion to promote interventions that reduce resistance among foodborne bacteria. However, based on previous experiences within the broiler industry specifically, some information collected and monitored by NARMS has not reached stakeholders in a timely fashion to allow for those stakeholder to react to potential emergence of antibiotic resistance bacteria that could cause public health issues. We appreciate the presentation yesterday by Dr. Errol Strain and his team on NARMS-Now and how the agency is working to make information available in a much more timely fashion.

I would also encourage the agency to engage with the broiler industry on an ongoing basis to review current information and discuss trends that the agency is seeing.

Of course, as mentioned in previous presentations as with any real-time distribution of information, it is important that NARMS be ready to engage should this information be misinterpreted or misused as we have seen this the past. We certainly would appreciate our public health partners standing with the industry to help correct any misinformation should it be used in an improper and unscientific fashion.

As I mentioned at the previous public meeting in 2020, industry was surprised by not only the contents of the strategic plan published in August of 2020 but the wide net that was cast. As far as we're aware there has been little stakeholder engagement as the strategic plan was developed and that lack of engagement continues today as we're halfway through the implementation of the strategic

plan and we've had very few discussions with NARMS over the last two years.

And hopefully this is an opportunity for us to have further engagement and heightened engagement moving forward. Given the focus of this as a One Health approach, one in which NCC experts it's difficult to see this is truly a One Health approach when the main focal points of the strategic plan including livestock and poultry were largely missing from some of these discussions. Moreover the agency's response to a few questions raised in the 2020 meeting were published only 20 days ago. And NCC for one would appreciate ongoing or continue discussion with NARMS as we have further questions and would like details to those questions.

We are hopeful that the agency will reconsider this approach and begin routine engagement with the industry next prior to the next public meeting.

We believe that stakeholder involvement timely distribution of information and a true One Health approach is important for the ongoing success of the NARMS program.

While the industry has been supportive of this program since its inception, we are hopeful the agency will improve on this approach moving forward. Information collected should be collected and distributed in a timely fashion. NARMS and other public health partners should be ready to assist should information be misinterpreted and misused. And finally we're all here today committed to public health. It's imperative that NARMS and all public health agencies evaluate the effectiveness of their policies and programs and answer this very specific question. Does this impact public health? Sound science, robust data, risk assessment and alike will aid NARMS and all public agencies in answering this

question.

I want to thank the organizers for inviting NCC to participate in this very important public meeting and we stand committed to work together to advance our common goals of improving public health.

With that I will turn it back to you Dr. Shively.

Thank you, Dr. Peterson. Next we have Dr. Heather Fowler, Dr. Fowler completed her veterinary medical degree at the University of Pennsylvania school of veterinary medicine in 2010. A masters in public health and applied biostatistics and epidemiology at the Yale School of Public Health in 2011 and a Ph.D. in environmental and occupational hygiene from the University of Washington School of Public Health in 2017. She is board certified and veterinary preventive medicine and has expertise in the area zoonotic disease, public health, worker safety and health and of One Health application.

In the summer of 2017, Dr. Fowler began work as Director of Producer and Public Health at the National Pork Board, where she oversees public health as well as occupational safety and health issues as they relate to swine production if the United States. Dr. Fowler, the floor is yours.

NARMS Public Meeting: Swine Industry Update – Presenter Dr. Heather Fowler
Time- 05:25:25 – 05:34:45

Thank you for that introduction, I'm excited to be here today. It's been a really

exciting past couple days, a lot of data, a lot of information to take in and excited to share this update.

As mentioned in any bio, I am a public health veterinarian by training. And really a One Health champion. So excited to hear all the references to One Health throughout. Thought, I think there are opportunity for continuous improvement here.

As I speak on behalf of America's pig farmer, I'm excited to give you the update from the past couple of years since our last update in 2020.

As a reminder, the national pork board has a very long history in guarding and protecting the use of antibiotics and making sure that our producers are using antibiotics appropriately and that is really represented in our pork quality insurance plus or PQA plus program which almost predates me started in the 1980s and has continued to grow and have new content added to it every three years with a program update.

The program itself covers the six we care ethical principles which describes ethical responsible pork production in the United States.

You can see there are six principles here and they span human, animal, and environmental health.

And that's because within the swine industry and really in animal Ag in general, as we think about producing products and animal protein we can't help but think of protecting and sustaining the environment in which these animals are raised, optimizing their health and well-being and from a food safety and public health perspective, thinking about the people we serve and feed and are caring for the

animals in our farms.

We've often times referred to that as a not just a One Health approach but making sure we're doing right what's right for people, pigs and the planet. We have a very long history in doing this and before we even used the term or coined the term One Health our farmers were doing this every single day because it's ingrained within in the process. Since the last time we chatted about our different work here and the stewardship space, the industry has shifted towards the concepts of sustainability. As One Health champions, One Health sustainability what's the difference? That is that sustainability is an ultimate goal in the way we reach that is through One Health approach.

All that we've heard today as we focus on a specific topic of antimicrobial use and resistance and food safety we're really still taking that One Health approach to address that goal and ultimately it's one of the many components that we'll move towards sustainability objective at the end.

With the national pork board sustainability efforts, again we take that One Health approach. We are grounded in those six we care ethical principles meaning that we apply that One Health approach through those principles to reach that end. Within those activities themselves we have a component that maps back to antibiotic stewardship, food safety, et cetera. I'll show you more in a second.

As I mentioned before, as we think about those six we care ethical principles, our people, environment, animal well-being, public health, food safety and community, we're thinking about our production system and our different stakeholders, consumers, the supply chain stakeholders, et cetera, and making sure that not only are we looking at each individual component within those we

care ethical principles, as we move to a more sustainable pork production, but that we are making sure that we are continuously improving in those spaces.

Some examples that are relevant to this meeting today include food safety, where we've made the commitment to produce the safest food in the world. So speaking from the NARMS perspective, making sure that we're following the withdrawal periods, as driven home in the PQA + program, making sure we're using antibiotics and other animal health products responsibly, safely, that take into food safety concerns and food safety compliance.

For public health, we're committed to producing the highest quality food possible while increasing the enjoyment of pork and the well- being of people around the world. So beyond the food safety piece, beyond responsible antibiotics, we're thinking about the people themselves, our consumers, making sure that the food they eat is delicious, nutritious and contributes to their well- being.

Again, these areas still may seem slightly out of scope for what we're talking about today. All come together from a One Health approach, and it's what our producers are doing every single day. We have to balance all of these different pieces to make sure that we're producing a sustainable product.
Beyond the sustainability efforts, as a reminder, as a check- off organization, we have a three- fold mission, that's to conduct research, that's to promote pork, and to participate in education and outreach opportunities like we're doing here today.

As it relates to specific stewardship research and outreach, we've continued to participate in various activities and initiatives that allow us to fund the research to answer questions, key questions within the industry, as well as to assist the barn yard in that space as well.

Most notably is our involvement in the FR, foundation for food and agriculture research's international consortium for antibiotic stewardship and agriculture. I believe a speaker in a previous panel mentioned kind of losing their breath going through they acronyms. So the FR ICASA initiative brings together key stakeholders across the barn yard to address the topic of antibiotic use, stewardship and resistance.

Of note, the National Pork Board has partnered with the USDA, Pipe Stone Veterinary Services and others to fund what is called the Imagine Project, which was referenced earlier. This project is the first of its kind to track resistance not just of NARMS pathogens, but of pathogens specific to swine. As we think about the NARMS project, or excuse me, the NARMS activities, one of the questions posed to us in the industry is how are we using that data? How is the NARMS activities contributing to our work? And it's through research like this that can continue to explore ways that the -- these pathogens are impacting our production that we are able to not only utilize the data but augment it and make sure that it is meaningful to our industries and allow us to continue to identify research questions and answers.

As we move towards a One Health Approach, I want to just reiterate, again, thank

you for the opportunity for allowing me to present today to give you this update from the swine industry. As you see, we're focusing on sustainability. We're taking a one- health approach to get to that sustainability, and we're collaborating with our partners across the barn yard, leveraging our research dollars through different initiatives to continue to address not only issues with antibiotic use, stewardship and resistance, but other areas that span our six we care ethical principles which map back to that One Health framework.
Again, the One Health approach is ingrained within the swine industry and across the barn yard. To reiterate the statement my colleague, Dr. Peterson made earlier, involve us.

Involve us early and often. Yesterday, we talked about the impact of the pandemic on resources and what that meant for the NARMS program and other related programs tracking overall resistance. I totally understand that those resources needed to be addressed, or to be, excuse me, redistributed to those efforts.

Now, as things start to calm down, we start to meet in person from time to time, I'd ask that we spread out these meetings, that we have updates more regularly, that we engage industry and other key stakeholders and to reference something Mr. Roache said earlier, we may not always agree. And that is fine, but involve us in this conversation, whether -- wherever we fall on the RACI chart, or responsible, accountable, consulted, informed, involve us early and often. We're committed to the One Health approach. We're doing everything we can within our span of control, within our industry from a One Health approach and are

leaning into the larger global conversation in One Health. Involve us early. Often. Thank you.

Thank you, Dr. Fowler. Following that, we have Dr. Mandy Carr- Johnson.
Dr. Johnson is the senior executive director for the science, culinary and outreach team at the National Cattlemen's Beef Association. Dr. Johnson also leads the Beef Safety Research Program and facilitates the functions of the beef Industry Food Safety council, a group of industry food safety professionals working together to provide industry- driven research, guidance, materials and educational programs.

Dr. Johnson was raised in a small rural community of Sudan, Texas, where her family still lives and raises beef cattle. She is a graduate of Texas Tech University with an undergraduate degree in food science and technology, a master's degree in food science, and a Ph.D. in animal and food science, specializing in food safety. In 1999, Dr. Johnson joined the faculty at Angelo State University. During her almost eight- year tenure, she developed a meat and food science, undergraduate and graduate program and designed and managed the federally ASU laboratory . Dr. Johnson joined NCBA in 2007. She and her husband live in Highlands Ranch, Colorado. Dr. Johnson, the floor is yours.

WFR’s Research on Antibiotic Resistant Pathogens and Genes – Presenter Lola Olabode
Time- 05:37:51 – 05:47:38

Well, it doesn't seem to want to cooperate. My apologies. So we'll try to make this as large as possible. Okay. Hopefully that's acceptable on that end, for whatever reason. That's the best we could do. So thank you for the opportunity to share with you today and really provide a perspective from the National Cattlemen's Beef Association. As mentioned, my name is Mandy Carr- Johnson, and I'm the senior executive director for our scientific affairs, which encompasses all of our research programming. One thing I'd like to really overview today is we know that as mentioned earlier, NARMS is noted as the U.S. public health surveillance system that tracks antimicrobial resistance and enteric bacteria. NARMS is to work closely with several government and industry partners who play complementary roles in addressing the threat of developing antimicrobial resistance.

NCBA, National Cattlemen's Beef Association is one of those industry partners and has supported the work of NARMS to protect the public against resistant bacteria through the beef industry's commitment in several ways, including food safety, the beef quality assurance programs, educational practices to advance standards of cattle care, the industry's environmental sustainability goals, as well as the industry's research efforts that promote optimal animal health, safety, wholesome beef and our environmental conservation.

Like NARMS, the beef cattle industry operates under this One Health perspective. First off, just to reiterate some things that my colleagues have also mentioned, is that the safety of our product, beef, and the beef supply is critically important to all segments of the beef cattle industry and supply chain. What we note there is

that this goes from producers to packer processors to retail and food service. Today, the industry is challenged to recognize and address an expanding and complex food delivery system. Meeting these new and complex needs really requires a collaboration across all sectors of the industry. An example of this is our Beef Industry Food Safety Council which brings together representatives from all segments in industry- wide science- based strategies. That really address our challenges of beef safety. From its inception in 1997, BIFSCO, as we call it for short, has facilitated input across all members of the supply chain to coordinate a frame of reference for action.

BIFSCO also enjoys a collaborative effort with the beef check- off program. The beef check- off program not only funds beef safety research, but it partners with BIFSCO for an annual beef safety summit. Each year, research is presented, and at the most recent event it included things like *Salmonella* transmission networks, shedding of pathogens for cattle, antimicrobial resistance, as well as longitudinal evaluation of *Salmonella* in the environment.

Additionally, BIFSCO works together to provide a unified best practices document that really serve as a blueprint for making a safer beef product based on the scientific information. These documents are available to the public and on the BIFSCO Web site.

The industry's beef quality assurance program, or BQA, instructs cattlemen and women who use best practices for raising beef cattle at all stages of production. Over 85 percent of the U.S. beef comes from BQA certified producers, and for

over 30 years, this program has worked to ensure that cattle farmers and ranchers are continuously improving the ways that they raise beef, including the ways that they use antimicrobial drugs.

In a significant part of this BQA program involves the antimicrobial stewardship training about appropriate use and administration of these products, the honoring of withholding or withdrawal times to avoid antimicrobial drug residue violations, the prevention of environmental contamination, and the need for accurate record keeping, as well as the importance of a valid veterinary client- patient relationship.

The Beef Producers Guide for the Judicious Use of Antimicrobials in Cattle addresses 14 major considerations for using antimicrobial drugs, as necessary, in beef cattle production. NCBA believes that its responsible use of antimicrobial drugs will aid in the preservation and future effectiveness of antimicrobial agents across common pathogens in both human and animal species.

According to the U.N. FAO, the U.S. beef supply has the lowest greenhouse gas emissions footprint of all the beef- producing countries in the world and has been a global leader since 1996. U.S. cattle producers have a personal stake in protecting the environment. For generations, ranchers have raised cattle on native grasslands, in steep mountain sides, on coastal Plains, working in harmony with nature to produce this nutrient- dense food product we call beef. Ranchers protect that habitat for wildlife, maintain the health of native ecosystems and employ grazing management practices to sequester carbon and

reduce the threat of wildfires. Cattle producers work hard to protect the air, as well as water quality, not only to preserve the health of their animals, but the health of their families and their communities. Cattle producers in the U.S. play a vital role in mitigating climate- related risks, and in the face of growing concerns related to this topic, the cattle industry is committed to showing that we are part of finding a solution and have established four sustainability goals, which you see here on the screen, demonstrate climate neutrality by 2040, create and enhance opportunities that result in quantifiable increases in producer profitability and economic stability and sustainability by 2025, enhance trust in cattle producers, as the responsible stewards of their animals and their resources, as well as continually improve the workforce safety and well-being.

These goals are a major benchmark for the industry as we continue to work towards the balance between live animal production and a sustainable planet. When we look here at the information previously mentioned by my colleague on the ICASA, I want to share just a couple of things. One is that improving the antimicrobial stewardship and modern agriculture settings requires partnerships across all aspects of the supply chain. With the participations from livestock producers to meat packers, all the way through retail and food service, we also have participated in the International Consortium for Antimicrobial Stewardship and Agriculture, or ICASA. As mentioned, it was a program started by FBAR as a non-- profit created to fund bipartisan support by congress. FBAR's initial investment of 7.5 million into the program is matched by private sector participation for a total investment of $15 million in animal health and antimicrobial stewardship projects.

Importantly, this framework of diverse organizations to work collaboratively on issues span across the value chain and share the resources, knowledge and results. NCBA is a founding participant in ICASA and an ex- officio member of their executive committee with over 25 percent of fed U.S. beef cattle directly represented by producers in this consortium to illustrate that power of a program to advance with research this topic.

Lastly, in conclusion, the opportunity to really talk about NARMS and its monitoring for antimicrobial resistance trends, among foodborne pathogens in humans, animals, retail meats and then disseminate timely information on antimicrobial resistance to promote interventions and to conduct research in decision making regarding the program. The beef industry is engaged, as mentioned earlier, by colleagues in a similar meeting two years ago. We encourage the NARMS program engage with animal agriculture industries on a more regular basis and timeline for true collaboration on targeted issues, as we all work collaboratively towards a vision of a world where antimicrobial drugs are effective to treat both human and animal infections and the burden of antimicrobial resistance is minimized.

So with that, thank you for the opportunity and I will turn it back to you.

Thank you for that. We have our final presenter today, Beth Johnson. She brings over 25 years of food policy experience, serving inside and outside the government, including the Food and Drug Administration, U.S. Department of Agriculture, the Senate Committee on Agriculture, Nutrition and Forestry, the

National Cattlemen's Association and the National Restaurant Association where she builds strong relationships with a broad group of stakeholders and a unique and in-depth understanding of the issues. Over the years, she has been involved in policy negotiations and development at the local, state, federal and international level. She also helped lead USDA's work on CODEX and food safety. She lives near Annapolis with her husband and two daughters. I'll turn it over to you, Beth.

National Turkey Federation – Presenter Elizabeth Johnson
Time- 05:48:35 – 06:01:38

Thank you so much, and I do not have slides at all, so you just get to see me providing some remarks here. I want to absolutely thank you all for the invitation to speak today. I am speaking on behalf of the National Turkey Federation, and we are pleased to be part of this discussion. My background personally is as a dietitian, but in the public health area, so I'm certainly speaking on behalf of the animal health sector today, but I think it helps to show that, you know, that there is a very close connection between animal health and public health.
NTF represents nearly 100 percent of all turkey producers. Or processors, I'm sorry, as well as processors, breeders, hatchery owners and allied companies. It's the only national trade association representing the turkey industry exclusively.
In addition to proper animal effective and timely treatment and prevention of disease is an important aspect of our food safety, animal health and sustainability efforts to protect the health and welfare of turkeys.

As many but not all of you, understand, food animal veterinarians at times need antibiotics to protect the health of livestock and poultry, just as doctors need antibiotics to treat people and veterinarians use them to protect our pets. Although I cannot speak for physicians and other veterinarians, I can say that our turkey veterinarians are committed to judicious, responsible use and have worked long and hard to better understand and enhance microbial practices.
We are committed to a robust food safety program, and to a strong public health program.

To begin with, I want to share how the turkey industry's use of antibiotics has decreased over the years. To determine this change, NTF has been pleased to participate with the U.S. Poultry and Egg Association in their FDA grant to collect antibiotic use in poultry products.

Through that effort, Dr. Randy Singer analyzed the use of antibiotics across almost 70 percent of the turkey production over the past several years. Dr. Singer's work has been effective in showing that antibiotic sales to livestock and poultry do not equate to antibiotic use.

Additionally, while our industry has long known that our use has gone down, Dr. Singer's work was able to document it, specifically between 2013 and 2017, U.S. turkey companies that participated in the study dramatically reduced their use of antimicrobials, especially those deemed important for treating people. Some examples, in feed, tetracycline use decreased approximately 67 percent. Water soluble penicillin use decreased approximately 42 percent. Water soluble

tetracycline use decreased approximately 28 percent, and water soluble Lincomycin use decreased approximately 46 percent.

And I said earlier as far as the fact that sales, antibiotic sales don't equate to use, I would also say that antibiotic resistance doesn't always equate to use. As Dr. Peterson noted earlier, we do see antibiotic resistance in some antibiotics that aren't used anymore.
Of course, as many of you here today know, the interpretation of this data can and has been completely construed. Opponents of our industry and those who do not support the care and well- being of turkeys and other food- producing animals choose to misuse this data, which, just like the NARMS program, remains a top concern for our industry.

NTF and its member companies are staunch supporters of the NARMS Program. As a collaboration between the government and industry. We've partnered with USDA and FDA on the NARMS Program, including advocating for robust funding. NARMS can provide and does provide valuable information as we seek to ensure the health of animals and the safety of our food supply.

Along with our colleagues in the pork, chicken and beef industries, we have appreciated the collaboration that was -- that has been in place prior to the '21 -- 2021 through 2025 strategic plan announcement. Since that announcement of the draft plan in 2020, we've been disappointed about the lack of communication and engagement with NTF and other animal agriculture stakeholders, and I think you've heard that consistently now from all of us.

In 2020, we, along with other providers -- we, along with others, provided comments on the draft's strategic plan, and we then had a follow- up meeting with leaders at the Center for Veterinary Medicine to further outline our thoughts, concerns and ask questions.

However, since that time, there has been little communication, and no update to the draft strategic plan from our perspective. We have no idea if our comments were heard or taken into consideration. The only document that was shared was a list of questions and answers, which, at least according to our read, provided information that had already been put into the draft strategic plan.
So to that end, we're largely repeating our comments from 2020, and we're hopeful that even though the plan is in the implementation phase, you will consider and even think about amending the strategic plan to address our concerns raised today.

And I'll also repeat what Dr. Fowler said is, include us, include us. Please, early and often. We want to be a part of this. We want to be working with you. NTF supports the longstanding objectives of NARMS as outlined by the NARMS review subcommittee of the FDA Science Board, or the NARMS review committee, NRC. These objectives are monitor trends in antimicrobial resistance among enteric bacteria from humans, retail meats and animals in the time of slaughter -- at the time of slaughter. Disseminate timely information on antimicrobial resistance in pathogenic and commensal microorganisms to stakeholders in the U.S. and abroad to promote interventions that reduce resistance among foodborne bacteria. Conduct research to better understand the emergence and persistence

and spread of antimicrobial resistance, provide timely antimicrobial resistance data for outbreak investigation, and provide data that assists the FDA in making decisions related to the approval of safe and effective antimicrobial drugs for animals.

The expansion of the program, as was proposed, and now being implemented, in the strategic plan dilutes these objectives, and this loss in focus unfortunately diminishes the value of the program. NTF believes the value of the program is in foodborne AMR, and while we understand that this strategic plan is based on expanding the One Health approach to capture as much as information as possible, we strongly suggest the government agencies identify the public health challenges and specific questions to be addressed before expanding data collection. Again, I repeat that these were from 2020, so that a lot of that work has already started.

Research begins with a hypothesis, and that appears to be missing from the NARMS 2021 to 2025 strategic plan. Given the fact that the strategic plan is already underway, it is frustrating that as your partners in this program, this was not done in 2020 when this concern was first raised.

To be more specific, the strategic plan has four goals, and multiple objectives. Yet none of the goals or objectives identify the specific questions that will be answered with the significant increase in data. Both the goals and objectives use words like enhance, initiate, conduct, develop, et cetera. Instead of identify, determine, ascertain. These are not subtle differences. As we noted in 2020, NTF

would be pleased to work with the government agencies and other stakeholders, and all stakeholders, to identify appropriate questions and directions for NARMS to address.

Without such focus, NTF continues to question if what is -- what is often referred to as a fishing expedition. And I noted yesterday in one of the presentations there was a question about looking at water systems from closer to animal production sites, and one of the panelists noted that they actually see a difference in the data when there's been more rainfall, or other instances in the environment as opposed to being close or farther away from an animal production site. Those are the kinds of things that we want to talk about. We want to be a part of that. Another significant issue we must address is the communication of the data. We continue to see irresponsible use of information obtained through NARMS and other sources. NTF and its members have strong concerns about whether the various government agencies involved in the expansion of NARMS truly understand the extent to which the information gathered could be misinterpreted and miscommunicated. Thus, diminishing the findings in this effort as it is related to the public.

This is a significant expansion of the program, and without an understanding of how you are seeking to analyze and communicate all the disparate pieces of information, we frankly are beginning to question the value of the strategic plan. A safe food supply and consumer confidence in that safety is critical. Something turkey producers take very seriously, and we understand and appreciate how data collection can help demonstrate actions taken to ensure safety. However, in

the wrong hands, that same data, if not reported or communicated correctly, can cause unnecessary fear or concern because those hearing it have no context to analyze the information. For example, reporting the finding that there is no antibiotic resistance -- resistant bacteria in animal feed or surface water means what? Will that be reported as there's no problem? Or will that data collection methods be changed until a problem is found?

Likewise, if antibiotic resistant bacteria is found, what does that mean? And how will it be communicated in the appropriate context? How is the water contaminated? Is there a certainty of the pathogen source? Past data collected from surface waters in Minnesota showed significant issues downstream from municipalities. How will that be factored into the data collection and reporting? There's no system that can completely assure data will not be misused but if we don't at least discuss the issues up front, we have guaranteed the misuse will not only occur, but it could be rampant.

Additionally, past experiences have shown us that in the wrong hands, information can be shared inappropriately, even by those within the government. The National turkey association -- or the National Turkey Federation supports NARMS, but we feel strongly that the government must report its findings in a manner that puts what is happening in a clear, easily understood context. Specifically, we ask that you work with us and others in the livestock and poultry sector to ensure that data is not collected so broadly that multiple conclusions could be reached when looking at the same data set. Data is collected in a fashion that makes it possible, and maybe even easy to produce a balanced assessment of

risks and benefits. Data sharing and communication is responsible and productive for all stakeholders.

As we all experience each and every day, the lens through which information about food production is viewed has changed significantly over the last 50 years. In some way, the changes are helping, and in some ways, they are making the producers and veterinarians' jobs and passions to care for animals even more complicated.

Our ask is that as we approach today's challenges, including the need to communicate expansive and complicated sets of data to a wide variety of interested audiences, including veterinarians, academicians, government agencies, lawyers and the general public, we seek to minimize the unintended consequences which could make caring for animals -- for animals a practice of the past. NTF generally supports partnering with the federal government to understand the issues we all face. The partnerships allow for an ongoing dialogue and seek to work for all parties involved.

As FDA seeks to form another partnership within the livestock and poultry sector on antibiotic use data, we are hoping to look at NARMS as an example of how we can work effectively together.

In summary, the national Turkey Federation looks forward to working with you to ensure NARMS remains a helpful tool in our joint goal to minimize antibiotic resistance and protect human and animal health. Thank you.

Thank you again to all of our panelists today. We really appreciate the feedback from our stakeholders and we look forward to working with you all. Again, that was reiterated through all of the presentations that you all want to hear from us and work with us, so we appreciate that, and again, we will continue to work together to address AMR.

And with that, I will turn it back to Pat. Thank you.

Public Commentary – Moderator Dr. Patrick McDermott
Time- 06:02:07 – 06:04:04

Thank you. Thank you, again, to all of our speakers in this latest session. That brings us in the agenda to the public commentary period. My understanding is we had two individuals who requested to make comments during this time period. It's not clear to me from the participant list whether they are on the call or not. So I'm going to ask them to identify themselves. The first person who did register for this section is Rory Faulkenburg, and Rory, I don't know, I can't tell if you're on the call. There is one person phoning in that is perhaps one of our speakers in this session.

So I would ask either Rory Faulkenburg, or Lia Biondo is the other person who signed up for both of you or either of you to please identify yourself and introduce yourself if you are on. I'm going to give it just a minute to make sure that I make room for technical connection challenges here. All right.

Okay. Well, I'm -- if anyone else is looking at the attendee list and can help me not miss if anybody is identifying themselves, I don't see anybody. These are our two registered speakers from the session. So I'm going to assume they're not present to give their five- minute speeches.

All right. Well, maybe we'll move on, then, to a few closing comments, if I may. Let me see if I can share my screen. And while I'm trying to do this, if anyone sees that I've overlooked our registered speakers, just interrupt me.

Closing Remarks – Presenter Dr. Patrick McDermott
Time- 06:04:04 – 06:30:00

Okay. I'm going to assume that my slides are available, and I think I'll proceed. So like I said, interrupt me if I have overlooked our speakers for the public commentary section. Well, again, thank you, everybody, for joining the meeting the last several days. I think starting with the technical workshop and moving through the discussions yesterday and today, to provide updates on where we stand with different elements in the strategic plan, I hope it's been helpful to show where we stand. I think it has been incomplete in answering questions, which maybe is partly inevitable, but it is our goal to endeavor to answer people's questions about what we're doing, where we're going, seek your input, and use that to plan to look ahead.

So I wanted to just finish by just giving a sort of brief overview of where we've

come from, where we stand today and the vision for the future that's articulated in the strategic plan, and if you look back when NARMS began in 1996, it began with the CDC testing non-typhoidal *Salmonella* and Shiga toxin *E. coli* in 14 laboratories -- laboratories in 14 states in 1996, and grew over the years to -- over the next seven years to have laboratories in all 50 states for *Salmonella*, and they also added other organisms that you can see listed here. Including some that are tested throughout the program. And so some of the data are linked to CDC in terms of the pathogens under surveillance.

And the year before reaching all 50 states for human testing, the retail meat program began with five partner states, and we still have all five, except Colorado won't be testing next year, but the other four, who were foundational to the retail meat testing program since 2002 have been with us since then, and we've expanded the scope of retail meat testing now to just about half of the states, including Puerto Rico, and next year, including Massachusetts.

And so that part of the program has become more comprehensive over the years, and starting in early 2017, we added the animal pathogen surveillance networks, through the Vet-LIRN and NAHLN network of veterinary diagnostic labs and this map shows an overlap of the retail meat to human testing and the animal pathogen testing.

And for those of you who have followed the program over the years, you know on the food animal side, we began in 1997 with testing *Salmonella* from the HACCP program for product verification testing, and this was, you know, in the early

days, as most programs like this grow or begin anyway, it started with pre-existing infrastructure that was in place, and that was the HACCP testing was being built at that time as well. And so those product verification samples became the animal component of NARMS. And while *Salmonella* was available from all the most terrestrial food animal sources, the *Campy*, *E. coli*, and *Enterococcus* were only from a subset of chicken carcasses at the Eastern FSIS Laboratory. We carried on with that program for some years until 2013 when FSIS led an effort to get individual animal samples from intestinal contents, and this was something of a compromise with the injunction that we've been offered by many stakeholders to have an on-farm component to NARMS and we reasoned that the product testing was closer to retail meat and that cecal testing could be as close as we could get to the farm, and so at the very least we could do it in a nationally representative way, we could distinguish more production classes and have more complete microbiology for animal species and reasoned the cecal sample better reflected animal samples than samples compounded by the microbial status of a given processing plant.

So this began in late 2013. And I think where that brings us today, if we think of the themes of the strategic plan being the attempt to help define best practices or One Health AMR surveillance through the adoption of an environmental component, probably that's our biggest challenge, and also the affordability of how to through put DNA sequencing technologies, those are sort of the big themes of the strategic plan.

In terms of scope of testing, I think, and someone can correct me if I'm wrong on

this, I believe NARMS represents most comprehensive national system of its type at this point. We track -- if we take *Salmonella* as an example, and human isolates of *Salmonella* are now compared to *Salmonella* from 17 different food and animal sources that are listed here.

We also have regular testing of seafood, although *Salmonella* is not a target pathogen there. We have a growing database of animal pathogen, AMR, which is one of the pillars of the one health model, and throughout all this sort of let's call it organic growth, we've continued to conduct different pilot studies to look at other animal and commodities raised with antimicrobials. And those pilot studies have different effects. I mean, sometimes they are translated into permanent features of NARMS, and other times, not.

And this slide lists a few of those that have happened over the years. Retail veal study done in 2018 to 2021, which we published. We anticipate that this is worthwhile if done on a periodic basis, but we haven't determined what that would be yet. It will depend on competing priorities in resources, as is the case for many of these. A seafood pilot has led to seafood being done for a couple years now in NARMS. If you saw Dr. Tate's presentation, I think the data there warrant a close examination, and maybe a determination on if and how to continue that sort of testing and what sampling interval might be appropriate, given the return on investment and the limited resources.

You heard presentations on looking at sheep, Lamb, goat and Catfish from FSIS. Maybe you might call minor food animal species that have antibiotics approved

for their production. We've looked at also the study early on lymph nodes as an alternative sample type for cattle at slaughter.

There's a chicken Giblet study that we conducted, operating both on data showing Giblets as a form of foodborne illness, but we also started to get a higher recovery of *Salmonella* from them so we looked at them as maybe an alternative sampling type for broilers.

You heard from Dr. Ge about animal food testing and establishing base lines for resistance in animal food. As I mentioned sort of a symmetry to the -- in One Health to the approach of looking at human food. We're trying to expand our scope beyond the tunnel vision that we have looking at just the limited number of target organisms, and as I mentioned, CDC is -- long ago has added to their list of human pathogens they put under the NARMS umbrella. Seafood comes along *with Vibrio* and *Aeromonas* as new bacteria we hadn't looked at. We've looked beyond *E. coli* to other lactose positive enteric. I haven't reported those data yet but done some selection for carbapenem resistant enterobacterae.

So, you know, this work we sort of looked at in comparison and contrast with what can be gleaned from metagenomics, which liberates us pretty much from having to cultivate and isolate pure culture. So those two sort of activities to enhance, say, broaden the scope of the AMR data are sort of working in parallel.

We do epidemiological and statistical research across the program to try to understand different aspects of the dynamics of resistance, and of course you've heard a lot about microbial genetics and genomics, which is still a research project, but it's also now an activity that's become a permanent -- or a regular

feature of data generation and surveillance. Not just in NARMS, but FoodNet and other places.

The other thing I think is salient about the NARMS evolution to date is I think you could identify 2019 perhaps as when the whole genome sequencing capacity was built out in all 50 states through the efforts of the CDC, and really shows really a great advantage to us in FDA in terms of the value of that partnership. We wouldn't have been able to do that.

And so when CDC built that capacity, our retail meat sites, for example, now had instrumentation in their core labs, if not in their NARMS labs, too, to take on responsibility for doing the primary data generation from WGS and submitting that to NCBI, and I think at that point you can say not across every, you know, COVID being an exception, not -- say month- to- month, but on a fairly regular basis, some component of NARMS is updating their data within a fairly short time from collecting the sample. So real time is a soft term, but I think we're the first and still the only real time of the AMR monitoring program.

That means that annual reports cannot continue to be the primary means of communicating the findings, and so you heard a lot about that, I know, throughout this meeting that, you know, NARMS Now has become more now than ever, and the NARMS reports are such a difficult thing to put out in the timely fashion that they don't really present data that's actionable in terms of just timeliness, and that's been the hardest thing.

I think we kicked off the last public meeting by admitting that that's the one goal we didn't achieve on the last strategic plan. And I think we're farther along that path than ever, and in some sense can say that we're -- real- time would help AMR program.

So WGS data published sometimes weekly, up to public committed NIH, and for global access, and I want to emphasize global access. And one of the things that this program can be proud of is that we're one of the few countries that does this, even today, even after 26 years. Well, let's be fair. Maybe after eight years of affordable DNA sequencing, which was really the enabling technology. We're one of the few countries that does this. I think the U.K. has come quite a ways on this as well, and maybe there's some other countries, but, you know, I look at things that are spread around the globe in terms of multidrug resistant *Salmonella* like D T104, and there was a nice study from Denmark on genomics to try to recapitulate the history of the spread of that strain, and it arrived in countries sometimes with five resistances packed on its chromosome, and it gained ascendancy and spread widely, and we didn't have the systems in place at that time to see it coming, let's say.

And Infantis is another example of this. The data suggests the strain evolved and spread internationally and arrived on our shores and had preexisting resistances, as Dr. Peterson noted, on antibiotics we don't use in poultry, although it gained a strong foothold in poultry. Had we had more information from other regions of the country on these ascendant strains that were causing local problems, we would have been in better position to anticipate them. And I think we're there

now with the Kentucky situation. We know a lot about it and how it spread in Europe and we see signals of it popping up here and there in the U.S. We ought to be thinking about and anticipating ways in which we can try to keep that wolf at the door, if you will, to keep that from becoming a local problem that evolved elsewhere.

And it brings up an interesting question about what are the -- until there's true global in health, what are the limits that an individual country or industry or agency can do to combat some of these multidrug resistant pathogens that spread internationally.

But along with the let's say real- time data, we've switched more to these NARMS interim updates, that is to say here's a signal we see in our information. We think it's worth pointing it out to you.

And that should be part of the dialogue that's being called for, too, in this meeting, is sharing information like that. I'd like to see more of a sharing of data between different data collectors in a true partnership like that. We're working to continuously develop data dashboards for data accessibility. I think it's been mentioned that everyone sees the data now at the same time we do. There's not much delay there. And we want that. That's a value of the program, and I think we've made good progress there.

And not only does the data allow us and enable us to say announce what we think are important signals, but it allows anyone to do that and to take down -- to

download the isolate level data and to look at it for their own purposes, and for other public health and food safety priorities, including developing methods for rapid detection and response.

The bioinformatics side, I said that the status of the program is the great effort that's been put into genomic information gathering has given us the largest set of foodborne strains with both MIC testing and whole genome sequencing data and probably around 70,000 isolates now.

This is a great resource for developing models to fully exploit these data. And the first cut of that was to say, hey, if we see known resistance genes in our genomic data, does that correlate with MICs at or above the clinical break point. And we showed quite some years ago now, yes, it's very high reliability for predicting resistance, and so now we'd like to take that farther -- further and look at other features related to AMR and to the spread of pathogens, including MICs in the susceptible range, or features that allow organisms to out- compete and gain ascendancy in the production industry or in public health.

NARMS has developed and deployed work flows in galaxy tracker. CFSAN Genome Trakr scientists have helped us a lot in all of these bioinformatics enterprises. This is the software that allowed the labs generating sequence data to identify AMR genes of concern without having to do it in a centralized fashion, depending on others. And so I invite you to learn more about that. Dr. Strain can help with that if anyone's interested in those tools, they're free.

You heard from Lucas Harrison and others about what that long read DNA sequencing is enabling, and it's enabling us to really get a deeper analysis and enhance our reporting of AMR dynamics over time, and the example is one of the interim updates where we pointed out that this plasmid, in Infantis, this pESI plasmid has moved to another serotype, and this has iron acquisition features, it has cell binding features that seem to give it an evolutionary advantage, so if it makes it to other serotypes, that's important.
And this technology, this element in our strategic plan, and the work that's been done by our scientists has now made this level of sensitivity possible, and we can see genes and their plasmids moving and we can compare them at individual nucleotide level.

And I can't skip this slide without mentioning the work done at NIH, National Center for Biotechnology Information and the development of AMR Finder Plus. All of the NARMS partners worked in the early stages of this to help build the catalog of AMR genes and point mutations, and they're curating that database and they're performing the annotation for submitted genomes and making it available to everyone and adding other important food safety factors such as virulence and biocide acid, heat maps and heavy metal resistance. So these are important things. They're studies showing how metal resistance tracks with AMR quite strongly.

So I think that it's no small achievement that the program's come to a point where we have the possibility of early detection like never before, and transparent data sharing with few restrictions, and where do we go from here.

And you heard some talk about our progress in exploring the future of the program in terms of let's say helping to -- helping to identify sound practices in the one health paradigm for AMR monitoring.

The genomic and metagenomic sciences, although they already brought us a long way, they're going to continue to be an important part of this work as we move forward, working to understand how the environment mediates resistance in bacteria from human, ag and wildlife sources, and in plants, we need to include in that.

We want to do that to develop a relevant and reliable One Health data system. It's not a hobby. We're not here just to be curious. We're here to understand, according to the One Health paradigm, we're here to try to understand what a healthy environment looks like in terms of AMR. We know that the soil teams with antibiotic resistance producing organisms. That's been the source of these medicines for a hundred years. We know there's genes there that confer resistance that don't make it to the mesophilic pathogens. We know there are evolutionary bottlenecks, and so finding a gene is not finding a hazard and we need to sort through that to understand what are the activities that might be mitigated that help protect human and animal health. That's the ultimate goal. So at the very least, this work will help to find the minimum data objectives needed to make comparisons between watershed studies. A know a lot of people on the stakeholder presentations today work for organizations supporting this same type of work. We're on the same team, and so we want to understand how to keep resistance and use environments, whether municipal or agricultural, from

reaching the environment where, when they might be mitigated and doing our job to help ensure safety.

It also provides a template for consistent data collection and reporting. So again, even if the data at the end of the day from the environmental works suggests that this isn't the right way to go about, say, the environmental piece of One Health, let's put that out there, we will certainly put together valid methods and metadata standards and other things that can help people compare their studies, whether they're done through -- well, regardless of who's supporting the work. By 2025, if you heard on day one, we have to have a national estimate of resistance in surface waters and validation of these minimal requirements for data comparability, and at that point we'll evaluate the effective -- effect of all this work and make some decisions about whether our priorities need to be reviewed.

So what does this all mean? Well, you know, as I mentioned at the start, the themes are One Health in genomics, and we've seen tremendous advances in the sequencing instrumentation and chemistry. We're seeing advances in artificial intelligence. We're trying to expand the scope to get an ecological perspective of NARMS, its intersectoral, multidisciplinary, recruits people with expertise from the far reaches of the scientific community -- and public health communities who are all going to participate -- who, in the One Health paradigm will be participating for different purposes perhaps, and I think that needs to be emphasized. I think one of the questions was what will NARMS do with environmental data in reviewing new animal antimicrobials. And I think what that

shows is perhaps a reluctance to realize that one health doesn't make NARMS only about that, right? So now it might be that EPA's interested in -- they close down beaches sometimes because of MRSA, so EPA has an interest and an obligation to look at AMR mitigation steps in environmental waters. And so it's one health, but it's become much more diverse in terms of its purpose and its participation.

And I think maybe that's worth repeating.

So I think with the expected and continued advances in sequencing chemistry and instrumentation, artificial intelligence, software tools, it's possible to envision monitoring using integration of large continuous data sets across the ecosystems, and superimposing resistome profiles with other information relevant to microbial communities. Flood waters was mentioned, right? Rain events. These do affect watersheds, and that's part of the data that would be looked at in seeking to understand these data. But the shared microbiome across the One Health domains, I think it's a useful analogy to look at and conceptualize it as something like a weather map with resistant microbiota that are mixing across niches affected by different natural stressors and also by those who have built human and ag environments.

And the data, they become more complete, I think, as our visual systems like NARMS become more expansive, but they also become more complex and they become more complex when you this that we now have four million nucleotides of data on every isolate, let's say.

And I appreciate that the elaboration of complex systems, you know, can be intimidating, with concerns about false attribution, you know, falsely attributing a threat to sources. It's serious concern. Somebody you know who have been with us for years know that the FDA has spoken up when data were misconstrued in serious ways, and it leads to the wrong response and the wrong tools and it does damage at the very least, shows confusion.
And as I mentioned at the outset of this meeting, the new returning FDA commissioner, Robert Califf, has made that one of his priorities, that complex information moving in a rapid rate is often misconstrued, and it lends itself to being misconstrued perhaps more readily than before, and the context, it's an ongoing challenge, you know, we're a vigilance program and vigilance over resistance is the main purpose, but vigilance over how the data are interpreted and shared is not something we take lightly.

At the same time, I think complexity must be embraced. I feel confident there's every reason to think we can get it right in this age of genomics, and we can get it right without being afraid that it's complexly right, because it is a complex issue, and we have data to reach down into the lacunae, if you will, of those complexities, and it does increase the challenge of communication, but I'm confident that we're up to that challenge, and I think I could speak for all the NARMS partners and say we're all committed to that as well.

So I hope the meeting gave everyone a clearer view of NARMS work, as well as the work of our stakeholders, and I hope it will rekindle collaboration and

communication and information sharing. Certainly we heard you loud and clear that we need to sort of, you know, get back out of our COVID caves and start reaching out more and talking more and sharing information more. I think that I've shared with some of you that, you know, we -- it's certainly an important thing that we need to do a better job of.

If I could, you know, summarize it all, I would say the one- word mission of NARMS is vigilance. We're here for, you know, alert and purposeful watchfulness, I think that's how the dictionary defines vigilance, and NARMS is a vigilant system, and more than ever before, we have a live view of resistance that will I think enable a much greater understanding and a much better informed response to AMR threats to protect human and animal health and we promise to continue to work tirelessly to always try to make the program better.

And with that, I will thank you, and I will stop sharing because I believe Claudine has -- oh, one last slide here to put up. And I would be remiss if I didn't offer some special thanks to the people who made this meeting possible, especially Claudine Kabera who you all know now, I think, as she was your contact even to register, but just did so much of the work to get this meeting to pull it off, and she's got all the skills from computer and dealing with computers to understanding epidemiology and AMR. I don't know how we could have done it without Claudine.

But also Ryan Holofcener and David Cameron, who were our IT support, and Siobhan Delancey on communication pieces, and Denise Benton helping with Web

pages and Heather and Errol and Jason from FDA who all helped in preparing for this meeting, gathering information and organizing us. Thank you to all the speakers and moderators for your contributions, and to all the -- my friends and colleagues in NARMS that are trying to steer this program and bring it to a place where the next generation of scientists can take it even further. That's what we're always trying to do, and thanks to all of them as well for helping to get this meeting together, and I hope you agree that it was helpful to everyone in terms of understanding and sharing information.

I think, with that, unless I'm looking, there's no chats to suggest that the public commentary speakers who had registered have shown -- raised their hand yet. I think without further ado, we can adjourn, and thank you again, everyone, for this week's meeting.

[Meeting adjourned at 4:02 p.m.]