National Antimicrobial Resistance Monitoring System (NARMS) Public Meeting Day 1 Transcript

Welcome - Presenter Dr. Patrick McDermott
Time- 00:02:00 – 00:05:40

All right, I think we can go ahead and get started everyone. I will start my camera. Thank you, everyone for joining. Welcome to the public meeting of the US National Antimicrobial Resistance Monitoring System. We have a two-day conference planned for you today. I think we have a record number of attendees. We are happy to be sharing the latest developments in our five-year strategic plan.

Before we jump right in let me see I would like to raise a couple of housekeeping issues. First of all, if you have a question and answer, there will be a Q&A box and you can enter Q&As in the Q&A box and the Moderators will be able to handle questions through that forum. You can select a microphone to do that efficiently. If we have questions and answers throughout the course of the conference, they can be answered on time to keep us on schedule, you can certainly email the speaker for their information. Also, the meeting is being recorded and will be posted on the NARMS website along with the transcript. Also, if there are other questions about NARMS or the program in general, you can send those and email to NARMS@FDA.HHS.gov.

So that is just a few issues. We will get into the outline of the agenda a little later, but before we do that, I'd like to welcome Dr. Robert Tauxe who's going to kick off the NARMS meeting this year. As you know, NARMS is an inter- agency enterprise we work closely across the U.S. government. We are trying to rotate the meeting around the NARMS partner agencies to host year to year. This is the

year for CDC to fulfill that role.

So, it's my pleasure to introduce Dr. Robert Tauxe who will be giving our welcoming address. Dr. Tauxe is Director of the Division of Foodborne, Waterborne and Environmental Disease at the National Center for Emerging and Zoonotic Infectious Diseases at the CDC in Atlanta. This is the Division that is charged with prevention and control of foodborne, waterborne, and fungal infections. The Division monitors the frequency of these infections in the United States, investigates outbreaks, and develops strategies to reduce the disease, disability, and deaths that they cause. Dr. Tauxe graduated from Yale University in 1975 and received his medical degree from Vanderbilt Medical School in Nashville, Tennessee. He holds a Master's degree in Public Health from Yale and completed an internal medicine residency at the University of Washington. He then trained at CDC in the Epidemic Intelligence Service for two years and joined CDC staff in 1985.

His interests include bacterial enteric diseases, epidemiology and pathogenesis of infectious diseases, epidemiologic and clinical consequences of bacterial genetic exchange, antimicrobial use and resistance to antimicrobial agents, and teaching epidemiologic methods. His faculty appointments include the School of Public Health and the Department of Biology at Emory University, Atlanta. Dr. Tauxe has written or co-authored 308 journal articles, letters, and book chapters. Dr. Tauxe, thank you for kicking off that meeting this year at the microphone is yours.

Welcome Address – Presenter Dr. Robert Tauxe
Time- 0:05:40 – 0:14:34

Thank you very much Pat, and welcome all to this public meeting. Thanks to the organizers for the effort that went in to putting this event together. I'm delighted to see so many people joining - 149 is the tally I have on my screen.

We have been a long-term partner with FDA-CVM in this effort and I want to thank them particularly for their support over all these years along with our partners at FSIS.

Today is day 990 of our national response to the COVID-19 pandemic. Fortunately, case numbers are declining now, but it is by no means over. Unfortunately, the monkeypox epidemic continues to expand across the country and the world. Response to both events is still absorbing a lot of our staff time here at CDC and, also for our local and state health partners.

At the same time, we do continue to move forward on five key areas to address antimicrobial resistance in the organisms we are responsible for. Promoting prevention by encouraging the appropriate use of antimicrobials, with many partners, is one example.

Our food safety Centers of Excellence have been developing and evaluating training materials and addressing stewardship and infection control with food animal veterinarians, small animal veterinarians, veterinary staff and pet and feed store employees.

We are also improving diagnostics and building metagenomic laboratory capacity to prepare for the time when we will no longer need to isolate the organism first in order to fully characterize it. We will be exploring that in this meeting.

We are conducting research to better understand the burden, outcomes, and risk factors for infections with these pathogens, how they emerge and why they persist. We will strengthen international collaboration to improve detection and prevention of extensively resistant typhoid fever and other highly resistant infections. We are collaborating on expanding our surveillance activities.

I want to describe some of this progress in our collaborative programs, NARMS itself and related surveillance activities. With great effort, our partners in state public health laboratories have continued sequencing *Salmonella* and Shiga toxin-producing *E. coli* and *Listeria* in PULSENET, our national network for molecular surveillance. They are now operating very close to pre-pandemic numbers.

Our partners in the states are now also again submitting a subset of isolates to be characterized by our NARMS group here at CDC. After a pause in 2020, we are making progress although we are not caught up yet and we are still coping with staff deployments and supply chain challenges.

Back in 2019, PULSENET made the major transition from pulsed-field gel electrophoresis to whole genome sequencing as the subtyping methods, bringing this capacity to public health laboratories in all 50 states. This new capacity and the precision of the method meant we have greater ability to detect outbreaks of specific strains than ever before even as the number of isolates dipped under the impact of COVID. And it has had other benefits as well. One unforeseen benefit of having sequencing in each state was that it was possible to start monitoring coronavirus variants with sequencing using some of the staff and equipment that was already in place. There have been other benefits.

Within PULSENET, sequence can now be used to protect serotype for *Salmonella* and antimicrobial resistance patterns providing a helpful supplement to the

information gathered in NARMS. Sequencing has opened a new window on source attribution as we compare strains from sick people from those with known sources like cattle, poultry, and produce. This is clarifying the central importance of poultry as a source of domestically acquired salmonellosis, and the public health benefit of actions that would reduce poultry contamination.

As we apply the greater precision of whole genome sequencing to surveillance, a more complex spectrum of epidemiological events has emerged, events that lie somewhere in between the single point-source outbreak at one end of the spectrum and the truly sporadic and unrelated cases at the other end. For example, it became clear that some specific strains cause outbreaks that reoccur each year at the same time and are often related to the same location or source like the *E. coli* O157 appearing in Romaine lettuce fields out west.

If systematic contamination events are repeating each year, this means we can figure them out. If we can figure them out, we can better prevent them.

There are other strains that are emerging. For example, changing from being extremely rare to becoming common causes of illness. A decade ago, the multiple drug resistant strain of *Salmonella* Infantis was only seen in a few people returning from travel to Peru. It then shifted to become established in our poultry flocks and is now an increasing cause of infection in people. There are still other strains which simply persist causing illnesses every year, year after year. Investigating these reoccurring, emerging, and persisting strains which we refer to as the shorthand of “REP”, to better understand their sources, may accelerate efforts to prevent them.

We are also making more surveillance information publicly available in closer to real time. For example, our new BEAM dashboard is provided preliminary

summary data on *Salmonella* isolates and updates and will be updated monthly. We plan to add other pathogens and predicted antibiotic resistance results coming from sequencing soon. You can search for this using the search term “CDC BEAM dashboard”. And we anticipate in the future the uses of sequencing will benefit other issues. Currently, sequencing focuses on the chromosomal DNA of the pathogen. But there is also progress in exploring the genomics of plasmids, the loops of extrachromosomal DNA that bacteria may carry and exchange. These plasmids often carry resistance genes we aspire now to describe the genomes of plasmids, for example, the ones that *Salmonella* carrier including the resistance genes.

While the COVID pandemic response has been stressful, it is also brought new attention and tools to bear on the long-standing challenge of antimicrobial resistance. I'm looking forward to the discussions over the next two days. Thanks to all involved in the efforts to maintain surveillance efforts throughout the pandemic. Thanks to all of you attending for your participation and attention.

Over to you Pat.

Thank you very much, Dr. Tauxe, for those welcoming comments. And thank you for setting the stage nicely for what has been done so far. I think we will be able to hear more about the groundwork that CDC laid and has benefited all of us at NARMS and all of us in food safety and public health response. Thank you for joining us today. I hope you can stay for the whole meeting.

I will do my best.

Introduction and Meeting Agenda – Presenter Dr. Patrick McDermott
Time- 00:14:34 – 00:45:56

Okay. Very good. I would move onto the next part of the agenda if we can.

Thank you again Dr. Tauxe. I'd like to just join my sentiments with his and welcoming everyone again to the public meeting of the National Antimicrobial Resistance Monitoring System and progress on the strategic plan.

That is the main goal today. In very simple terms, what we would like to achieve is to give a sort of progress report, to answer questions that people have about the priorities we have placed in our strategic plan and to seek input on the direction we were taking and perhaps opportunities that we are missing - things of that nature. We like to hear it from a broad base of stakeholders as we try to do something really that hasn't been done before in broad terms which is to develop a One Health AMR monitoring program. There's a lot of talk about it, but we are at that point where we are actually trying to build a solid framework that comports with the priorities of One Health and address as the broader public health needs of human, animal, and environmental, health.

So, answer questions, seek input and look ahead. “What does success look like?” We can't say right now what that is as we continue to gather data, but, again, that's the purpose of this meeting to see if the direction is getting us to that objective of successful and fully mature One Health AMR monitoring program.

This slide is a slide I showed in our last public meeting in October of 2020. We have taken a lot of direction - not all of the direction in the program - but significant portions of it we sought guidance from an external science board review of the program back in 2020 just ahead of our last public meeting where we outlined the major elements of the new strategic plan at the time.

The main themes we took from that review was that to fully meet the One Health paradigm, we need to include animal health and look at animal pathogens and develop an environmental surveillance piece. There was not much specificity in that recommendation, but it is an important compartment of One Health.

We need to look at food commodities and animal types to get a broader picture of resistance in different environments in which antibiotics are used. Also test more bacterial species. If you think about it, we are looking through a very narrow window at the microbiome of animals and food and looking at it through six different bacterial species. It gives us a foreshortened view of the true resistance profile present in these complex biological samples. We are trying to explore that also with genomic and metagenomic approaches. A recommendation for appropriate on-farm testing which continues to be a high hurdle. I will say a bit more about that later. Develop methods of microbiome surveillance and whole genome sequencing.

Another more general recommendation was to collect more metadata to make NARMS valuable beyond its primary purpose of tracking AMR. I will say a few words about that hopefully again at this meeting. A recommendation to better exploit WGS and, again, this gets to data being made available for other purposes. And then lastly, to broaden collaboration with other programs nationally and internationally.

This is still sort of our polestar, if you will, this report. We combined the recommendations of that report with some of our own internal deliberations, our understanding of the status of the science from the literature, and from talking to other experts as well. That's what led to the last strategic plan, and we are about halfway through it so it's an appropriate time to have a meeting.

I think a subtitle to this meeting could be, as I said before, what does AMR monitoring look like? There are several One Health definitions out there. Some are more of an essay than a definition; some of them are quite long. It's important to try to describe the scope of One Health and what it seeks to achieve. I found this description developed in 2021 by the One Health High-Level Expert Panel to the tripartite, which is now the quadripartite, a partnership made up of the of FAO, WHO, WOAH, and now the quadripartite piece is the addition of the United Nations Environmental Programme (UNEP). What you will see in the discussion today following the agenda is that NARMS is also now a quadripartite program, at least in the early stages of that, with the addition of the environmental piece. Our goal in that is to at the least help to contribute to the conversation about what's valuable, necessary, affordable and provides information that can lead to improvements in One Health, let's say.

So, their definition or description is: *One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, and ecosystems. It recognizes the health of humans, domestic and wild animals, plants, and the wider environment (including ecosystems) are closely linked and inter-dependent. The approach mobilizes multiple sectors, disciplines, and communities at varying levels of society to work together to foster well-being and tackle threats to health and ecosystems, while addressing the collective need for clean water, energy and air, safe and nutritious food, taking action on climate changes and contributing to sustainable development.*

You may or may not like every element of that definition and some of them may be more aspirational than is practical to implement in the structural of an AMR monitoring program. But it does paint a very nice picture of how broad in scope One Health is, and what it means to strive for that sort of model for what we are

trying to do in US AMR monitoring systems.

Another piece of information that follows on of that definition is to describe the scope of One Health. This comes from the human health commission. It touches on some but not all of the different elements of One Health that should be considered in formulating a national strategy.

It occurred to me in reading this you can see there's a lot of different human activities in here and a lot of different health-related fields of expertise involved, etc. and so on. It occurred to me when I was reading this that the antimicrobial resistance mitigation is an element of One Health. You could change the title of the slide to show how resistance is such a good example of One Health in action. You could change the title of the slide to “The Scope of Antimicrobial Resistance Mitigation” and almost nothing would fall off of this table. Resistance is related to agricultural production and land use. Clinical medicine needs for interrelationships between the health professions. Obviously, that's part of it. Disaster preparedness and response is related to AMR and is now being discussed in that context by different government initiatives. Obviously, AMR is related to disease surveillance, economics, environmental health, food safety and security. Two concepts are sort of inseparable and almost trade definitions in term of the scope of One Health.

I emphasize that because it changes the sort of historical silos in which we have operated. Where we used to think should we do this or not, it's outside the scope of NARMS. Well, in a sense, if we are serious about our commitment to a One Health solution, the scope of NARMS is much broader than it has been before. I showed the slide also at the 2020 meeting. As mentioned in that definition, it mandates that we recruit more expertise and partner with new agencies because AMR, from a One Health perspective, touches on all the different government

agencies, public health officials, food producers, pharmaceutical companies, the environmental aspects of all of that as shown on that slide.

Historically it has been FDA, CDC, and USDA as the primary partners in the NARMS program, including both USDA-ARS and USDA-FSIS, along with State and regional labs and universities. And them being mainly involved in helping collect samples for analysis. And now, especially with the addition of genomics, NIH is part of the NARMS franchise, let's say. And EPA, as I mentioned, as we move toward a quadripartite approach in examining the environmental component to try to come up with best practices for proper environmental monitoring. CDC is working in different areas of waterborne surveillance and monitoring including sewage for COVID and other things including AMR. Looking at the animal health side of it, the Vet-LIRN network and the National Animal Health Laboratory Network at FDA and USDA, respectively, are now helping to address animal health aspects of the NARMS One Health approach. And we are working with FDA-CFSAN as there are overlapping responsibilities in terms of water testing and food safety.

The point of this so far has been to remind people that we have been sort of pulled out of our traditional swim lanes, and our identity has evolved, as it has to, if we are going to take this new approach. With that in mind, I thought it would be worth to remind people to familiarize people with the last strategic plan because it also is basically the framework for the agenda for the meeting.

The main change was the scope of sampling as you might guess for the change in scope that comes with the One Health paradigm. The main ones were to address animal health and the environmental piece. That was not an explicit part of NARMS in the past. If you look at the first goal in the strategic plan, the first goal is to enhance sampling for foodborne pathogens within a One Health framework. That means looking at disease-causing pathogens in animals. We will hear talks on

that today.

The environmental piece is a very complicated piece. The environment is complicated by nature. There is no consensus on what that should be in terms of AMR monitoring, and we are trying to contribute a solution to that. You will hear a lot about that, especially today. The rest of today is really devoted to Goal 1 in the strategic plan.

Just as we look at human food in NARMS, we think a symmetrical approach to One Health model is to also look at what animals are eating in terms of animal food. We will hear from Dr. Beilei Ge on that later today. It may be that it's not an important contributor, or maybe it's something that should be looked at periodically and not on an ongoing basis. We think there's a lot still to be learned about that pathway, as well as other places where antibiotics are used like, for example, seafood products under Objective 1.4 and other commodities such as minor food-producing animal species. You will hear from speakers on what we've done so far in that arena. We will also look at other microorganisms. We are combining that with metagenomics to include uncultivatable microorganisms.

We've gone now since our last public meeting – or right before our last meeting - from this traditional NARMS interagency partnership of CDC, FDA and USDA looking at the major terrestrial species for the main foodborne pathogens, as well as indicator organism to know what is more of a One Health model. So, for animal pathogens, as I mentioned, data are now being harvested from diagnostic labs, so you get an idea of the resistance that's happening outside of the food and animal domain and looking at pets, but also pathogens affecting food producing animals. For this work, I listed "other" animal pathogens for bacteria because they add them so fast, I can't always keep up with them. It's basically getting information that's available in a passive approach by partnering with the diagnostic labs.

I mentioned EPA's work on the environmental piece, and we will hear a lot about that today. You'll hear about the three-phased approach to the development methods and applying them in the field to take them to a national survey. That's going to include a metagenomics analysis. And the NIH NCBI is a partner of NARMS included here because they are the ultimate repository for all the genomic sequences that we are doing now, and that's pretty well all that the *Salmonella Campylobacter,* and *E. coli* and many *Enterococcus,* as well as other pathogens at CDC, so we are doing a lot of sequencing as Dr. Tauxe said.

When we brought this strategic plan up at the last meeting, we got feedback that we didn't answer in as timely a manner as maybe we might have. I think a lot of us, as Dr. Tauxe mentioned, were trying to just keep the program running during COVID. It really did shift our center of gravity in terms of resources that could be dedicated to the COVID response and how that impacted NARMS. It did have an impact. What we did try to do was put online if you notice a couple of weeks ago to bring up some of the major questions about the NARMS strategic plan. The goal is for these to be answered more fully at this meeting. I wanted to point you to some of the general responses we gave to the largest number of comments we got on the strategic plan originally.

One is why are you looking at surface waters that are beyond the scope of NARMS. That was along with another recommendation that we weren't ambitious enough and that we should be adding drinking water and wastewater and other matrices such as biosolids. The rationale for surface waters is that they act as key integrators. If you think about it, they are the receivers of human, livestock, ag, and wildlife inputs and, also, the source of water for the same. It seems like the natural point of confluence of these built and natural environments would be the right place to look at in what constitutes a healthy ecosystem in terms of AMR.

We are working to understand the role of the environment in mediating resistance and coming up with recommendations of what ongoing work would look like in that domain.

There are questions about what methods will be used and what metadata will be collected. I think you will hear answers to those questions. Also, what types of water, what locations, how will the data be used to inform strategies to track AMR. As I alluded to, the data will basically give us at least the minimum necessary information and data quality standards so that comparisons could be made between different ecosystems regardless of the structure on which we continue into the future with this as a component of NARMS. So, it's to seek methods to make data comparable and able to be harmonized.

A point that can't be overlooked or minimized at all is this concern about false attribution. If you get a sample here, are people going to falsely conclude that the cause came from there. Dr. Calif just rejoined the FDA as Commissioner and has made it a top priority of his to deal with misinformation and provide accurate information and context surrounding information. It's a never-ending struggle and we appreciate that and are sensitive to that and aren't interested in that. We understand that that's certainly a possibility especially as society gets more complex and general data sets get much larger, much faster than sometimes it seems you can keep up with. I put that in there to reinforce the notion that we are very sensitive about that possibility.

Questions about pet food and animal feed. Again, some of them were, we treat animal food so why are you looking there. And this isn't a significant source of AMR and what will you do with the data again, and, also, is this issue of false attribution. As I said already, what we are looking for is to determine whether the data justify long-term testing. We will have to make that determination at some

point whether it does or not, but it is a formative part of the research as is the environmental piece.

I couldn't get through them all today, but we had other questions like is cecal sampling representative? Can CDC do more? We got a lot of specific individual feedback like this. Can we look at more bacteria? I can assure you that I can speak for everyone in the program in saying that we always wish we had more samples. One question was, could you please start doing seafood testing and another recommendation was, could you please stop. We will talk about seafood testing and some of the data there. I think it's interesting. It's a good time to pause and consider what sort of sampling interval would be appropriate for these newer commodities we've added.

Question about missing breakpoints for some species, some of the technical microbiology questions. We should get these questions answered during the meeting. Better connectivity of the data for local action. Hopefully that can come up in some of the Q&A. There was a comment that with a One Health focus, that we've shifted to an "ecosystem health for humans," it's not truly one health. We would like to hear more if anyone wants to add to the thinking behind that. I wasn't quite sure what that meant. We want to talk about all these concerns.

Goal two is focused on the next generation DNA sequencing technology. This has really revolutionized our surveillance. It is astonishing what can be gleaned from the genomic sequence. One way I have described it is that it's the highest practical resolution you can get to distinguish two organisms. I mean, we are down to the sequence of nucleotides. Where you go from there, counting electrons? It's that basis of granularity that gives us comprehensive, exhaustive information on what strains carry in terms of known genes and how they compare evolutionary. It's really changed everything. It's made the data more available to

people who need it for reasons other than just AMR, such as looking at outbreaks, or evolutionary relationships, or understanding microbiomes, etc. You'll hear presentations on metagenomics approaches and their value in NARMS surveillance. This is a tremendously powerful technology as most of you are aware. We will take advantage of all the technologies and to make best use of the data in terms of understanding the concomitant adaptive features that might contribute to the persistence and spread of resistance. It also gives us information about virulence and heavy metal resistance, all sorts of things. We can now do different types of analysis and look at the relationships.

In Goal 2, there was also a comment on whether whole genome sequencing is able to identify emerging resistant trends. I hope you were able to join the technical workshop yesterday led by Dr. Errol Strain. It was just astonishing good work by all the agencies and taking advantage of genomic data and how it can do an extraordinarily good job at identifying emerging resistance trends but identifying the signature sequences associated with the evolution of these resistances as pathogens and their genes move from one ecosystem to another. The technology is really tremendous and useful and powerful.

The last two goals we will hear tomorrow. They are meant to be addressed through the Q&A sessions and panel discussions. Improving data sharing, communication, collaboration. Yesterday was focused on data sharing. Dr. Tauxe mentioned that we've gone to a nearly real-time system here. I think it's extraordinary and it's one of the great achievements of the NARMS partnership. All the agencies have contributed tremendously to this. I think maybe Dr. Beloeil will tell me I'm wrong later, but I think NARMS can say that it's the first real time One Health AMR monitor system. We get signals to examine at least on a weekly basis and not just us, but everybody with Internet access can see those data. That

is quite extraordinary, and the data scientists should be given a lot of credit for that.

For Goal 3 there were questions about making data more available for other purposes beyond NARMS. I've alluded to that. Where trying to get more metadata and more isolates with MICs to NCBI. NCBI is also looking at other food safety traits like virulence factors to add to the genomic data and allow people to search by that.

The improved data sharing, I think as I mentioned as one of the great accomplishments. We've gone from these static and really cumbersome fixed data tables that used to be present in reports with hundreds of pages to now making near real-time data online in data dashboards. The important point I want to make about this is that the genomic sequencing is faster than the MIC testing. And so, we have predictive resistance out here now. If you look at the NARMS Now Integrated data, you will see it in the form of this colored box at the right hand of this line graph. That is resistance predicted from the genomic sequence. As we close out the data and confirm it with susceptibility testing, we will move that blue box along to the right each year we go. What this does is it liberates the data from being held hostage from the annual reports. It's out there, it's for everyone, it's real-time, it's loaded with metadata, and can be explored with very sophisticated tools. It allows you to ask questions that maybe no one has thought about of the data. You can download the isolate level data that goes with each sequence. It is an extraordinary data set. Our intention is to not make the annual reports be the only means of communicating the findings. Please go to the webpages for NARMS Now if you want to see the latest and understand what is emergent and what signals we are seeing.

Maybe I will skip over this slide because Amy Merrill did a good job of describing

this yesterday – some of the features of the program. This happens to show the Infantis phenomenon that been the chief problem in salmonellosis resistance for the last few years. This is a strain as far as I can tell, it seems to have evolved in other countries where it appeared before reaching here, and now it is showing multidrug resistance including those we don't use in the U.S. So, it is a tough problem. Having the interactive live data will enable anyone to see what might be an emergent problem.

Another way to liberate ourselves from the annual reports, which are always too late to be useful. It just takes so long to summarize the data and do all the repeat testing to get cleared language for the report. One thing we've been trying are these NARMS Interim Updates which summarize recent unusual or concerning resistance findings from retail meats. The two that you may be familiar with is the finding of *Salmonella* I 4,5 12i- with extreme drug resistance. And also, the movement of the Infantis plasmid into *S*. Senftenberg. It's always a concern as an MDR plasmid gets into a region through a strain and then moves from that strain to other strains or serotypes. That is something we were watching very closely because it's concerning even when it's not widespread because, as we've learned from other things like Infantis and DT104, they always start out as a single event. And you never know if and when they might become widespread. Given the nature of that resistance we are watching it closely.

Goal 4 will be discussed through a panel discussion to look at some of the issues related to measuring the burden of resistant infections, risk factors for AMR infections, and attribution to their source. And then trying to understand the impact of prevention. It gets back to the injunction in One Health to partner with more experts than ever before and we need to do that to answer some of these questions as well.

So hopefully that is a helpful high-level flyover. I hope that I set the stage to address questions that people may have about where we are going and how we are doing - the status of the program. And whether the goals are clear and make sense to help us address different elements of one health. We don't have the plant piece in there, plants and the environment as part of One Health. We are working on that with our partners in CFSAN to see if we can contribute in that arena. We want to hear from people that are participating in the meeting, some feedback on the status and perhaps forming new partnerships because that is the best way to tackle these complex problems is through networks of experts that are dedicated to the same mission.

That being said. If I have time, if I have a couple of minutes, it looks like there are a couple of Q&A.

Question. It appears animal pathogens are still exclusive to pathogens that can be transmitted from animals. Will there be any work on pathogens that can cause illness in animals? Excellent question. I'm going to let our speakers who are speaking this morning, Greg Tyson and Christine Fox, will be talking about the animal monitoring. I will defer to them on answering that.

Question. The resistance graphs are based on DNA not MIC? They are based on both, but they are based on primarily in time we get the sequence data first and so this blue box on the right of the slide shows resistance predicted from the DNA sequence and on the left of that, in the gray box, starting in 2014 based on sequencing and confirmed by MIC testing so we continue to do the MIC testing. It's just a lagging indicator, but we continue to do that for several reasons. Hopefully that answers your question.

Claudine has posted some things in the chat box that link to some of the things I

brought up in my presentation.

One more question. Have you considered expanding animal related AMR case data to veterinary university-based teaching hospitals? Thank you for that and the answer to that is I should really defer to our experts there. They are going to address that, but it does include veterinary-based teaching hospitals.

Hi Pat, it looks like Rob has a question.

Yes, very quickly one thing that we have not focused on very much with NARMS, but it's on the outer reaches of the definitions of One Health and its scope is its issues around plants. Just to share with the group that we are confronting one rather curious new development of a highly resistant fungal pathogen which is an innocent bystander in many fields. It is not a pathogen of plants, but it is becoming resistant to agents that are used, antifungal agents that are used in plants. These are occasionally showing up in humans. It raises a whole new arena for us of what could be the impact of the antifungal agents that we deploy onto plants and whether that has impacted public health. The agent in question are the azoles and the pathogen is Aspergillus, but that is going to be one for the future. The plants are out there, and the fungi are out there, and we will be thinking about them more in the future, I think. Thanks so much.

Thank you Dr. Tauxe, that is an excellent point. That has been an emergent problem that will take a lot of attention to address.

Let's move on to our keynote speaker for the NARMS public meeting. It is my pleasure to introduce Dr. Pierre Alexander Beloeil who is a veterinary epidemiologist and has dedicated his professional career to preventative veterinary medicine and food safety. His interests include *Salmonella* and *Listeria* in French swine production. He’s helped lead implementation and coordination of

the French national control program for *Salmonella* in poultry production. Dr. Beloeil has been responsible for developing and implementing harmonized surveillance of zoonotic agents in food-producing animals and food through routine monitoring and specific baseline surveys at both national and EU levels.

Dr. Beloeil currently works at the European Food Safety Authority, where he leads work on surveillance, analysis, and annual reporting of antimicrobial resistance in food-producing animals and food among EU member states. In conjunction with colleagues from ECDC and EMA, he has led work for EFSA comparing data on antimicrobial resistance with data on antimicrobial use - something we have not focused on as much in NARMS and to investigate those associations. We will hear about that topic today. Dr. Beloeil, thank you so much for joining us and I will turn the microphone over to you. Thank you.

Shall I share my presentation.

Yes, please. I'm going to stop sharing so that I can share yours.

Can you see the presentation?

Yes, thank you.

Keynote Speaker: Summary of EU. Efforts in One Health Antimicrobial Resistance Monitoring – Presenter Dr. Pierre-Alexandre Beloeil
Time- 00:45:10 – 01:18:34

Thank you so much. So first I would like to thank you for giving me the opportunity to give this presentation in order to present the efforts made in the EU and the EU member states to tackle antimicrobial resistance. This has been achieved by announcing AMR monitoring over a number of years and the AMC exemption monitoring in food producing animals.

I would like to start by setting up the scene a bit and recalling the EU actions to fight against antimicrobial resistance as it has been implemented. This started a number of years ago. In 2004, the member states started to implement monitoring of resistant zoonotic bacteria in food producing animals and food. Another important milestone was reached in 2006 when an EU wide ban of the antibiotics in feed as growth promoters went into effect. More recently in 2017, the EU adopted One Health action plan to tackle antimicrobial resistance. This action plan notably aims at promoting prudent use of antimicrobials and cross-sectional action as well as improving infection prevention and consolidating monitoring of antimicrobial consumption and antimicrobial resistance.

As well, the Council of the European Union issued a recommendation for a common approach for the EU member states for implementing actions against antimicrobial resistance. This common approach could touch on possible targets for AMR, ways to strengthen patient safety, and how to design impactful one health national action plans against AMR. Member states should implement such action plan and as well, stewardship of antimicrobials and surveillance of antimicrobial resistance.

Considering more specifically the harmonized monitoring of AMR in food producing animals and food, member states should implement detailed proficiency regarding monitoring of antimicrobial resistance.

A first EC implementing decision was implemented over the period 2014-2020. A second decision has been adopted recently and covers the period 2021-2027. The European Food Safety Agency has advised the European Commission and the EU member states for antimicrobial resistance monitoring legislation while drafting the first decision and as well the more recent decision.

The announcement of the harmonized monitoring of antimicrobial resistance in food producing animals and foods as set up by the 2020 decision not only rely on scientific and technical guidance provided by EFSA, as well as on the field experience gained by the implementation of the first decision. This field experience has been gathered through audits of implementation performed either by direct regional authorities of the health of the EC and as well by the European court of auditors.

So, summarizing quickly the main features of the harmonized monitoring of antimicrobial resistance in food producing animals in food, this monitoring is performed in zoonotic *Salmonella* and *Campylobacter jejuni* since 2021 in C. coli. Also, AMR is monitored in indicator *E. coli.* The resistance is a monitored in healthy food producing animals. As well, there is a specific monitoring of ESBL producers. The monitoring is performed in the main food producing animal populations in Europe. Mainly broilers, turkeys, slaughter pigs and bovine animals less than one year of age. Monitoring is performed on a rotating basis. Poultry is monitored in even years and bovine animals and pigs are monitored in odd years.

Because of the legislation, the monitoring is rather nicely harmonized and the

monitoring of the antimicrobial susceptibility relies on the micro-dilution. It's phenotypic monitoring. The set of antimicrobial substances tested and the dilution range are fully harmonized. The resistance is interpreted using the epidemiological cutoff values as defined by EUCAST. As well, the sampling designs for collecting samples in the slaughterhouses are fully representative and harmonized. An external quality assurance system is there to support member states that's been implemented by the EU reference laboratory which is the Danish Technical University in Copenhagen Denmark. The EURL provides regular trainings to the national reference laboratory for antimicrobial resistance in food producing animals and food, as well as yearly PT trials and yearly complimentary testing exercises.

In 2021 onwards, based on the new features of the harmonized monitoring, the scope of the monitoring has been enlarged and there is now a new part related to the monitoring of resistance in bacteria from imported fresh meat from third countries, as well as the use, the progressive use of a whole gene sequencing, in particular for the specific monitoring of ESBL producers. This is still on a voluntary basis. The intention is to progressively install genomic monitoring over the period 2021-2027 and around 2028, to switch for routine monitoring based or whole-genome sequencing still accompanied by some phenotypic monitoring.

Member states are in charge of implementing harmonized monitoring of resistance in bacteria from food producing animals and food. They should on a yearly basis report data to the European Food Safety Authority. In parallel, member states should report data on resistance in zoonotic Salmonella and Campylobacter from humans to the ECDC. Both agencies – the ECDC and EFSA - analyze together the data and issue on a yearly basis, the EU summary report on antimicrobial resistance. The most recent data covering the last two years are

summarized every year in this report. This is done in order to account for the monitoring performed on a rotating basis.

The objectives of this monitoring. This monitoring is clearly performed from the public health perspective. It is not performed in pathogenic bacteria in diseased animal. That's on the zoonotic or indicator bacteria from healthy animals. It is performed from the one health perspective.

What is monitored? The occurrence of resistance, the proportion of resistant bacteria to the harmonized set of substances as well as the combined resistance to the critically important antimicrobials as defined by WHO. The key outcome indicators of resistance have been defined and there are two of them in animals. The rate of complete susceptibility in indicator *E. coli* and the prevalence of ESBL producers and temporal trends are set up and carefully followed up.

So, the intention here for this presentation is to exemplify the results of the monitoring focusing on data on resistance and indicator *E. coli* from healthy food producing animals, domestically produced. Those data are intended to be compared with data on antimicrobial consumption data in food producing animals. So, the data of antimicrobial consumption are collected by another EU agency, the European Medicines Agency. We will see this later on in this presentation.

So, a very broad overview about the situation of AMR in indicator *E. coli* from food producing animals. Here we can see the situation across the EU member states. Each data point is a member state, and we have here summarized levels of resistance to ampicillin, sulfamethoxazole, tetracycline, ciprofloxacin, cefotaxime, and the level of combined resistance to critically important ciprofloxacin and cefotaxime.

Clearly, resistance to the commonly used substances in veterinary medicine are rather high to very high in the four main food producing animals: pigs, cows, broilers, and turkeys. Regarding resistance to fluoroquinolones – ciprofloxacin – the level of resistance is much lower in pigs and cows compared to the levels of resistance observed in the indicator *E. coli* from poultry, broilers, and turkeys.

Regarding level of resistance to third-generation cephalosporins, cefotaxime, the level of resistance is much lower as well as the level of resistance to the combined resistance to critically important antimicrobials.

The marked difference is observed across countries. Highlights the potential benefits of policy actions to tackle antimicrobial resistance. Considering the combined resistance to critically important antimicrobials, we can see that this is extremely low in certain member states. No combined resistance can be observed with the monitoring system in place. Still there are some discrepancies between member states.

Considering temporal trends, we can see that we observe in an important number of member states statistically significant decreasing trends. We can see, as well, that the starting point for different member states is not the same, in particular considering the level in 2014 where the fully harmonized monitoring has started. Some member states are at the forefront while others are a little bit behind. What we like to do in the report is to really highlight and promote statistically significant decreasing trends. Whatever is the starting level of resistance and what is important is that the resistance decrease.

Similar results, considering resistance in indicator *E. coli* from fattening turkeys. There are different levels of resistance and an important number of decreasing trends. Of course, more detailed information can be found in the national reports

issued by the member states.

Now considering the indicator, the overall indicator of resistance. In order to address the phenomenon of co-resistance and of co-selection, the indicator of complete susceptibility in indicator *E. coli* has been set up and is used in follow-up. What is the rate of complete susceptibility? It is the proportion of indicator *E. coli* at which do not exhibit any resistance to any classes of antimicrobials included in the harmonized set of subsets monitored.

Here again we can observe disparities between the situation observed in the different EU member states. We have marked variations. We can observe typically a north to south gradient. We have much higher levels of complete susceptibility in northern Europe compared to southern Europe and in a lesser extent, a similar east to west gradient having a little bit higher levels of complete susceptibility in the western countries than in eastern Europe.

Considering trends over time, and as well, considering what we have defined as the key outcome indicator of complete susceptibility. This key outcome indicator is a combined indicator. It's the weighted mean considering the rate of complete susceptibility in for different animal populations. We can set up and calculate a unique indicator in food producing animals. What we can observe beyond marked variations among reporting countries is a statistically significant increasing trend registered in 44 percent of the member states as well as in other reporting countries.

Considering the other key outcome indicator which is the prevalence of ESBL producers. So, the proportion of samples, either animal samples or meat samples having at least one ESBL producer, we are able to follow at least to assess this prevalence and to follow up over time. In food producing animals, the key

outcome indicator, which is a weighted mean, the weight is proportionate to the animal population exposed to antimicrobial treatment. We can construct a key outcome indicator for broilers, turkeys, calves, and slaughter pigs. Here is what we can observe - interesting statistically significant decreasing trends in the prevalence of ESBL producers in 55 percent of the EU member states.

We believe that the outcome indicators show that some encouraging progress has been recorded in reducing antimicrobial resistance in food producing member states over the last few years. Still, we have some member states which do not register a trend going in the right direction. There is still work to be done.

As well, we believe that it is an interesting achievement to integrate data, monitoring data on antimicrobial resistance and antimicrobial consumption. Again, it is a One Health activity and a one health project to compare data on antimicrobial resistance and data on antimicrobial consumption in animals and humans. This exercise relies on existing monitoring systems by ECDC for data on antimicrobial resistance in humans and by the European Medicines Agency for the monitoring of consumption of antimicrobials in animals and EFSA for the resistance data in animals.

In 2021, we were able to issue the third report for the Joint Interagency Consumption and Resistance Analysis., JIACRA.

So, comparing first the situation on consumption of antimicrobials, we can see that now in the EU, the consumption of antimicrobials has been lower in food producing animals than in humans and it is believed that this decrease in animals derives from the major stake to reduce the use. We believe that those measures are effective.

Comparing data on antimicrobial consumption and antimicrobial resistance and

considering a number of combinations of the bacteria and the antimicrobial substance, we very often observe associations between consumption and resistance within each sector considering separately the animal and human sector. Here we can see a nice graph where we see a clear association between the consumption of quinolones in food producing animals with the rate of resistance to fluoroquinolones in *Campylobacter jejuni* from poultry.

We can as well and this again, it's a One Health analysis. We can analyze together in a multivariate model data on consumption and resistance in animals and in humans. For certain combinations, again, considering the combination of *Campylobacter jejuni* and resistance to fluoroquinolones, we can observe a clear association between consumption in animals and resistance in animals, as well as an association between resistance and animals and resistance in humans. Here is another example for *C. jejuni* and this association is believed to derive from the zoonotic aspect of *Campylobacter*.

As well, we use the key outcome indicators. Our primary key outcome indicator of resistance in food producing animals is this rate of complete susceptibility in indicator *E. coli.* And regarding data on antimicrobial consumption, the key indicator is overall consumption of antimicrobials. We were able to compare both indicators and to clearly observe a statistically significant negative association between both indicators, meaning the higher the overconsumption of antimicrobials, the lower is the chance to be kept completely susceptible indicator E. coli from food producing animals.

It is believed or it is interpreted that the action to combat antimicrobial resistance should rely on the reduction of antimicrobial consumption and that this reduction should concern all classes of antimicrobials used.

So how to achieve the prudent use of antimicrobials so that consumption can be reduced? So, the agencies issued a number of years ago, a report where the recommendation was really to reduce the use of antimicrobials, to replace where it is possible using vaccination in particular, and as well, to rethink the way how to raise food producing animals by implementing good farming practices and good hygienic practices. As well, we use typically what we call the regulatory toolbox in order to really encourage the industry to reduce the use of veterinary medicinal products and as well, promote biosecurity good hygiene practices and good farming practices.

About the monitoring of antimicrobial resistance and the ongoing enhancements. So, in parallel of routine monitoring described previously over the period 2021-2027, the intention is to perform three one shot cross-sectional epidemiological surveys we call baseline surveys. They should be run in parallel with the routine monitoring. The first one should assess prevalence, genetic diversity, and antimicrobial resistant traits in MRSA from pigs. The second one should be in antimicrobial resistance and enterococci. The third one is in monitoring AMR in bacteria from seafood and most likely we will focus on shellfish.

I said as well over the intention is to promote the use of whole genome sequencing. There is a generalization of whole genome sequencing for monitoring antimicrobial resistance. It will be a stepwise approach.

As well, further to the third JIACRA report we are performing the fourth analysis and we should issue the fourth JIACRA report in December 2023. And the objective is not only to compare antimicrobial resistance and consumption year by year, but to try to account for what we may call the time effect and to compare trends in consumption in trends and resistance.

So, the take-home message regarding the monitoring of AMR in food-producing animals, as well, the monitoring of antimicrobial consumption in food-producing animals and the integrated analysis of both of them, we believe that the positive association between antimicrobial consumption and antimicrobial resistance in both humans and food-producing animals is confirmed. I have illustrated mainly the results concerning food-producing animals.

The need to have prudent use so as to reduce the consumption of antimicrobial for both food producing animals and humans is underlined. Further interventions to reduce antimicrobial consumption will have a beneficial impact on resistance. We believe that this reduction should concern all classes of antimicrobial classes. There is a clear need to promote in both humans and food-producing animals, prudent use of antimicrobial agents, infection control, and prevention of infection.

Still, we now observe high levels of antimicrobial consumption and antimicrobial resistance in a number of member states. So, all those interventions should be reinforced. It's time to act and there is still a lot of work in front of us.

So, thank you so much for your attention. Here I would like to acknowledge the immense number of persons involved in this integrated monitoring of antimicrobial resistance and antimicrobial resistance global consumption across Europe as well as the EU. Thank you so much, any questions now I welcome.

Thank you so much. We have a few questions in the chat box. I think we are 1-minute past time. One from Steve Roach. Are the outcome indicators in other organisms beyond *E. coli*? If not, why choose *E. coli*?

Go ahead.

Up to now, we were focusing on indicator *E. coli* as an indicator bacterium. Before

2014, there was as well monitoring performed on enterococci. But this has been appended I would say and that is why we perform soon in the coming years a cross-sectional baseline on resistance in enterococci.

Another question. The north to south gradient on complete susceptibility in indicator *E. coli* could be related to climate. What variables could account for the east to west gradient? Does this track with wealth?

It's an excellent question. Likely, it is multi-factorial for sure. There may be a climate of effect in explaining why in northern countries, the rate of complete susceptibility is higher than in southern countries. Still, there is likely all the factors which is that northern countries in Europe have started to fight against antimicrobial resistance a number of years before and I would say a number of decades before other EU member states and that's why they are at the forefront. As well, the size of the production sectors may be a little bit smaller in northern countries than other EU member states, but clearly it is multifactor.

Very good. There are a couple other questions in the chat box. Maybe you can perhaps address them by email. I wish we had more time. It's a fascinating conversation. We are grappling with consumption and use. There are so many questions that would be good to talk about. We will make opportunities to due date later on.

Thank you.

Thank you very much.

Goal 1: Enhance Sampling for Food Pathogens within A One Health Framework – Introductions of Moderators- Presenter Dr. Patrick McDermott
Time- 01:18:34 – 01:20:05

And in interest to keep on time. We are going to entertain presentations on Goal one of the strategic plan. Quickly, I will introduce our moderators and turn over the microphone to them to work with our speakers. Our moderator today for this session on Goal One, enhancing sampling for food pathogens within a one health framework, is Dr. Allison Franklin, a biologist with the EPA's Office of Research and Development in Cincinnati. She received her Ph.D. in soil science and biogeochemistry at Pennsylvania State University in 2019. Her work focuses on emerging contaminants of concern in the environment, and her Ph.D. work looked at the presence of antibiotics and antibiotic resistance in soil and groundwater when wastewater is reused for irrigation purposes. In her role at EPA, Dr. Franklin is currently helping to lead the pilot effort to analyze antimicrobial resistance in surface waters nationwide and you will hear from her later in today's presentations.

Along with Dr. Franklin is Commander Catherine Rockwell. Commander Rockwell is a veterinarian and Senior Public Health Advisor at the USDA Food Safety and Inspection Service's Office of Public Health Science. She has served in various food safety roles in FSIS over the past 17 years, including field operations, training, and policy, and has led projects for various food safety programs including the National Antimicrobial Resistance and Monitoring System, the U.S. National Residue Program, poultry slaughter modernization, and industry guidance development.

Thank you both for moderating today and I will turn the agenda over to you.

Objective 1.1: Enhance and maintain routine resistance in select pathogens causing illness in food-producing and companion animals – Moderator Dr. Alison Franklin
Time- 01:20:05 – 01:20:49

Thank you. I'm going to be moderating Objective 1.1 which is focused on the enhancement and maintenance of routine resistance monitoring in select pathogen causing illness in food producing and companion animals. Our first speaker today is Dr. Gregory Tyson. He is the director of Vet-LIRN, that's the Veterinary Laboratory Investigation and Response Network in the FDA Center for Veterinary Medicine. He was previously a research microbiologist supporting FDA's NARMS program. He received his Ph.D. in microbiology from Northwestern university. With that, I will turn it over to Dr. Tyson.

The Veterinary Laboratory Investigation and Response Network (Vet-LIRN) AMR Monitoring Program– Presenter Dr. Gregory Tyson
Time- 01:21:09 – 01:32:20

Great, thank you. I will go ahead and share my presentation here.

Okay. Hopefully you all can see that. Thank you for that introduction. Yes, I'm here representing the Veterinary Laboratory Investigation and Response Network or Vet-LIRN and our AMR monitoring program which focuses on animal pathogens. The Vet-LIRN mission is to advance the CVM mission of protecting human and animal health by coordinating a network of veterinary diagnostic laboratories. We help advance the CVM mission in many ways including responding to food complaints, working on COVID believe it or not and obviously

our work on AMR.

And so, AMR monitoring as has been described with NARMS really has been very strong in NARMS for a couple of decades now. In particular, we do work with food animals, retail needs, and humans. But really in trying to address the whole One Health framework, some things that were missing are the environmental component which will be discussed later. Also looking at pathogens in animals as well as opposed to just say foodborne pathogens from animals at slaughter, but also looking at the things that are actually making animals sick.

In the CARB report that was released about seven years ago now, the action plan specifically named NARMS, but then also Vet-LIRN and NAHLN which at the time didn't have AMR monitoring programs. The development of one in Vet-LIRN supports FDA's CVM strategy to identify and slow the emergence of resistance arising from antibiotic use in animals. It's filling a gap in the One Health framework and helps us understand both the pathogens that are specific to animals, as well as those that may be foodborne pathogens or simply one health pathogens that impact the health of both animals and humans.

As part of our network of laboratories, we actually have 46 laboratories in our network, and a subset participate in the AMR monitoring program. We have 30 source labs which provide isolates all the time. Then we have six WGS labs that sequence a subset of isolates from the source labs. The overall framework basically has 30 source laboratories that continually get bacterial isolates all the time. This happens independent of other Vet-LIRN's efforts. They are veterinary teaching hospitals, departments of agriculture that are getting pathogens all the time. What we say is you are already getting the isolates from the list of pathogens of interest, please do susceptibility testing on those isolates. And so, we get that data from the laboratories, and we test them on the plates that are

relevant for the treatment of the animal that might be sick. Then we receive the various data from all the different laboratories, and we use it as part of the reports that are integrated with the NARMS reports. I will show you later as well. Then importantly, we have sequencing as part of that. It's been mentioned a lot already how important it is - sequencing - to understand the genetic determinants of resistance and how it's changing over time, comparing resistance from an animal to that in humans or in foods. A subset of isolates is sequenced. That data and other data is immediately made publicly available. People can see what's going on with the animal pathogens in Vet-LIRN. You can find it pretty easily in NCBI in the isolate browser.

In terms of which pathogens we're looking at, we started out with the top three that are listed here which includes *Salmonella E. coli* and *Staphylococcus pseudintermedius*. That addresses some of these same pathogens looked at in NARMS such as *Salmonella* and *E. coli*. It includes a Gram-positive pathogen that is commonly found in companion animals, *Staphylococcus pseudintermedius.* The *E. coli* and *Staph* are from dogs, the *Salmonella* could be from any animal host. It could be from a cow, it could be from a dog, or could be from a sick bird or a pet of any other kind. And so, all these isolates undergo susceptibility testing as I mentioned and a subset of them are sequenced as well.

We also have somewhat less or fewer isolates that are collected and have susceptibility testing and sequencing but are also part of the program including *Klebsiella, Pseudomonas, Enterococcus, Enterobacter, Campylobacter,* and also aquaculture pathogens. These help fill in a variety of different pathogens that are relevant to those of you that are aware of illnesses in humans, but also because plenty of illnesses in animals. These are not surveillance samples collected just as part of a sampling strategy. These are animals that are actually sick with the

particular pathogens.

Just to kind of show the context of what is collected across the different programs, NARMS focuses on the food animals and humans. In terms of the animal component, they have *Salmonella, Campylobacter* and *Enterococcus* from food animals at slaughter that are from FSIS. And then, Vet-LIRN collects a lot of other pathogens from companion animals as well as from food animals and then NAHLN gets some of the same pathogens as us and we actually combine our data and report them together, by including E. coli and Staph. They collect some other pathogens. I think one question that was in the chat that I can answer now is we are looking, as you can see, many of these pathogens are the same in humans, but some of them are more vet-specific pathogens such as *Staph pseudintermedius* that I mentioned, and the aquatic pathogens, then NAHLN also with the *Mannheimia* and some of the other ones.

And so, just in terms of the scope of the program, as I said there are 30 labs. We are getting quite a few isolates. We started in 2017 when we had a certain level of susceptibility testing of isolates in the program. By 2019, we ramped up fully and are getting over 3,000 isolates per year, and overall, we have over 15,000 isolates that have undergone susceptibility testing as part of the Vet-LIRN monitoring program. As far as sequencing, we have over 4,000 isolates that have been sequenced. All this data is publicly available. The focus is on the main three pathogens, but we are seeking to sequence a subset of the other pathogens to understand the AMR determinant are there, how do they differ from those in humans. Are they related to human isolates? Those are things that haven't been answered.

In terms of our reporting component, we have our integrated AMR data. NARMS has its dashboards. Vet-LIRN and NAHLN have our own AMR dashboards. We

have 2017-2019 data available. The WGS data is available as it happens. In terms of what the dashboard looks like, you can see it shows resistance prevalence and the focus is mostly on the dog isolates because that's where we are getting a lot of isolates from. It's easier to look at the *E. coli* and *Staph* and *Salmonella* from dogs. We report prevalence across many drugs, many specific to animals but some with similar comparators to those used in humans. If you click on one, you can see number of isolates different MIC levels and the presence of resistance genes in the isolates.

There are some results I can share. We published a paper on early genomics findings early in the program. We found carbapenem-resistant E. coli as part of an outbreak at one of the Vet-LIRN labs. That was really important for us to be able to identify and help understand the genetic mechanism and to see if it was spreading to other places. We've helped identify resistance mechanisms in *Staph pseudintermedius*. It's a major animal pathogen and actually, to some extent, in humans as well. It's like the veterinary Staph aureus. We helped uncover the resistant mechanisms that haven't been previously described to a great extent. We started monitoring AMR in fish pathogens which is a gap that also exist.

A few other findings are just the One Health nature of animal illnesses. These things don't happen in a vacuum. So, 63 percent of the *Salmonella* isolates were within 27 SNPs of human clinical isolates. If you're not too familiar with SNPs, it just means they are pretty related to human isolates. It is good that we were sequencing these isolates so that we can identify zoonotic outbreaks.

What's interesting as when we looked at the data was that we weren't seeing many unusual or new resistance genes, for example. We are seeing a lot of the same stuff you might see in humans or that are just kind of sampled from animals at slaughter compared to something that might be causing an overt illness in an

animal. These things, again, it's not in a vacuum, it's part of the One Health framework. You can see that resistance genes are probably flowing across these different sources. It really highlights the importance of antimicrobial stewardship across humans and animals. Again, we are all connected. Antimicrobial use and resistance can really impact other places as well.

I can finish up with some resources. Here is our Vet-LIRN web page and then the dashboard for the monitoring program. You can search these things easily, but if you want to scan a QR code, I believe that is it for me.

I'm happy to take questions if we have time for it. I saw some things may be coming in.

Dr. Tyson, we have a Q&A session after Objective 1.2 that will cover both of these.

Okay.

Objective 1.1: Enhance and maintain routine resistance in select pathogens causing illness in food-producing and companion animals – Moderator Dr. Alison Franklin
Time- 01:32:23 – 01:33:36

Feel free to put your questions in the Q&A box and then we will get to those questions after the end of Objective 1.2. So, with that we will move on to our next speaker which is Dr. Christine Foxx. She is an ORISE postdoctoral fellow for the USDA APHIS National Animal Health Laboratory Network (NAHLN). She received her BS in biology at Seattle University, MS in biological sciences at the University of Northern Colorado, and Ph.D. in integrative physiology from the University of Colorado Boulder where she studied Mycobacterium’s effect on stress coping

behavior, the gut microbiome and the gut and plasma metabolomes in mice. At the NAHLN, Dr. Foxx conducts bioinformatics analysis of whole genome sequence data and antimicrobial resistance data from the NAHLN AMR pilot project and with that I will turn it over to Dr. Foxx.

Antimicrobial resistance monitoring in select pathogens causing illness in food-producing and companion. The NAHLN AMR Pilot Project – Presenter Dr. Christine Foxx
Time- 01:33:48 – 01:42:07

Thank you for that kind introduction. Can everyone see my screen and hear me?

Yes, we can see your screen. Could you increase your volume a little bit.

Yes, I can try.

Thank you.

Yes. Okay. Hi, everyone. Thank you for allowing me to participate in the NARMS public meeting today. I would like to discuss how the National Animal Health Laboratory Network conducts our AMR monitoring on select pathogens that cause illness in food producing and companion animals through the AMR pilot project which I'm very happy to report is a USDA/APHIS priority goal slated to become a permanent program. My mentor is Dr. Beth Harris, one of two associate coordinators of the National Animal Health Laboratory Network who you will hopefully be hearing from tomorrow in some of the round table discussions around the NARMS goals.

The NAHLN and participating veterinary diagnostic labs have been monitoring AMR in bacterial pathogens of veterinary interest over the last 5 years in several

livestock and companion animals. These include swine, poultry, cattle, horses, dogs, and cats. With the inclusion of *Campylobacter* sequencing, we can now include small animal ruminants like sheep and goats. The main objectives of this pilot project are to develop standards to track antimicrobial resistance at a national level and to identify trends of interest to the veterinary diagnostic community. These include laying out methods for determining AMR whether that's by laboratory techniques such as antimicrobial sensitivity testing on broth microdilution platforms, or by whole genome sequencing which is where I come in. Identifying standardization guidelines or areas of needs for standardization in the interpretation of these results and then establishing reporting mechanisms to share the data with other agencies and stakeholders.

So ultimately, we hope that these data will facilitate antimicrobial stewardship and the judicious use of antimicrobials by clinicians as we get a better sense of what's going on in sick livestock and companion animals across the country.

It's important to talk briefly about how the data is collected from participating VDLs. Isolates must meet three criteria which is the identification of pure cultures to the genus and species level. Even to the serotype level for *Salmonella*. Association with the clinical disease or diagnostic finding, and the date of isolation by the veterinary diagnostic lab which we then further validate by information on the anatomical source of that isolate and representativeness on a national survey by isolating only once per year from a unique animal source, whether that's from a single herd or farm, flock, household, or owner. We require that laboratories do validate this information by including host animal species and state of origin. And then assigning a unique identifier for each isolate for tracking longitudinally.

Once isolates have met those requirements for inclusion in the pilot, participating laboratories do susceptibility testing to determine AMR phenotypes for that

isolate using broth microdilution test. We ask that labs isolate to a standardized number of CFUs per mill in double passage subcultures and then instruct them to use commercially available Sensititre plates for feasibility of use on the Biomic or Swinn platforms as indicated by the host animal and bacterial pathogen information to the right.

NVSL staff and our bacterial reference laboratories give orientations to new labs on how to read the MIC results from the platforms and have specific protocols in place for reading trailing endpoints for specific antibiotics in Gram positive coccis such as those highlighted here.

But it's also important to note that lab staff and external laboratories maintain ISO accreditation for susceptibility testing by participating in annual proficiency tests, so standardization and data accountability go both ways here in USDA APHIS.

I just wanted to point out that we do share our annual reports through the link that Claudine shared with everyone. That includes information on how many isolates we collect each year. The annual reports are published one year in arrears. We collect a total of 3,000 to 5,500 samples each year from those participating VD laboratories. That information includes companion animals such as dogs, cats, and horses which are not covered by livestock surveillance programs. We do publish longitudinal reports on antibiotic resistance across those Sensititre microdilution tests and also report out on where the data came from in terms of the sample source within each isolate. An example is *E. coli* from dogs that specifically did not have any urinary tract infections.

We rely on those laboratories to provide us that minimum inhibitory concentration data and aggregate that into those reports but also report them

out to these public Tableau dashboards displayed here. This information can be messaged directly by laboratories through messaging systems or emailed to using standardized Excel macro templates and then uploaded to a secure database where we perform data aggregation and any necessary transforms that are automatically processed through a coded pipeline.

I do want to note that that covers our phenotypic testing, but we do also conduct whole genome sequencing and we train laboratories on how to utilize bioinformatics tools to conduct their own sequencing and their own analysis. Isolates are sent directly to the NVSL or sequenced by participating laboratories using one of the four sequencing platforms indicated here, which include the ones listed here. We have specific library preparation kits that have been approved for use as well as other external kits listed here for any lab which has no Illumina iSeq or MiSeq. And then the raw sequence provided by labs is uploaded to a secure drive which our computational biologists, or myself, can then use to transfer data to where it needs to go.

The raw sequence files submitted by laboratories are usually accompanied by assembled data in fasta alignment format. But since we've incorporated sequences from long read platforms, we found the need to incorporate multiple assembly tools such as the UniCycler or SPAdes which is standard across the NARMS groups that are presenting today.

All of these submitted sequences are screened for quality and mean coverage to a reference genome that matches the isolated bacteria listed. Generally speaking, these isolates are also screened for sequence identity using Kraken which maps those to reference genomes in the curated databases such as NCBI to verify the cultures are pure, which in this example here is not strictly true.

That's actually it for me, but I would like to briefly say a special thank you to my supportive colleagues and mentors in the NAHLN program office, the NVSL scientists who screen and isolate and sequence isolates from around the country. And especially the 31 NAHLN labs that participated in the pilot program over the last few years. Before I take any questions, I just want to invite you to scan the QR code to learn more about the AMR pilot program and the strides that we've made toward antimicrobial stewardship as a result. To learn more about the laboratories that curated the data that I've shown you today and read up on some annual reports if you care to do that and other bacterial isolates in the study. Thank you very much for your time. I will be happy to move on from here. Thank you.

Thank you, Dr. Foxx. We are actually three minutes ahead of time for starting Objective 1.2. If there were any quick questions that people want to ask for Dr. Tyson or Dr. Foxx, please go ahead and put them in the QA box. If not, then we will just move on to Objective 1.2. My fellow moderator Catherine will be taking over.

Objective 1.2: Implement geographically representative monitoring including surface waters to establish baseline AMR data in aquatic ecosystems – Moderator Dr. Catherine Rockwell
Time- 01:42:41 – 01:44:02

Thank you, Dr. Franklin. Good afternoon, everyone. I will be the moderator for the next series of presentations under Objective 1.2 which is to implement geographically representative monitoring including surface waters to establish baseline AMR data and aquatic ecosystems.

Our first presenter today is Dr. Jay Garland who will be presenting on the surface waters pilot study. Dr. Garland joined the EPA's office of research and development in 2011. Dr. Garland received a Ph.D. in environmental science from the university of Virginia and spent over 20 years working on NASA's efforts to develop closed bioregenerative life-support systems for extended human space flight. He has worked on a range of topics including effort for microbial community analysis, factors affecting survival of human associated pathogens, and various biological approaches to recycling waste. His current efforts are focused on advancing innovative approaches to water infrastructure including decentralized water reuse and mitigating risks associated with antimicrobial resistance in the water cycle. Dr. Garland, I will turn over the speaker to you.

Thank you.

The NARMS Surface Water Pilot Study – Presenter Dr. Jay Garland
Time- 01:44:25 – 02:02:49

Okay, thank you for the introduction. I'm glad to be here today to talk about this surface water pilot. So, what I'm going to do in this introductory talk is emphasize the points that Pat had covered earlier and then also give you a broad overview of this pilot and set up the other speakers to talk in more detail about the design and the analytic methods for the study.

To reiterate, this has been an interagency collaboration between the EPA, FDA, USDA, and CDC. We began meeting as an environmental working group near the beginning of the pandemic and met throughout the pandemic and then to go back into the lab as we implemented the work. It's been a pleasure working with this group. I would say that the expertise and engagement has been exemplary

and I want to specifically call out the FDA, Pat and folks from CVM, both for their foresight in making this happen and also for their tenacity in keeping it going and really kind of maturing it to the point now where we are in the middle of it and moving forward.

As I mentioned, I will give you some background on the context and relevance of AMR in the environment. Talk about an overview of the surface water pilot then I will give an outline of the other talks in this session.

I think AMR, as most of us would agree, is a complex one health challenge. Not because of the interplay between humans and animals and the environment, but also because the contaminant here is very distinctive in the sense that we are not looking at the attenuation of the apparent molecule or the parent contaminant as we often are when we are looking at contaminants in the environment. We are looking at something that is not only the genes and the resistance themselves, but the mobile elements that they travel on and their potential amplification in nontarget animals and in this case microorganisms. It's a very complex situation and this slide reflects that.

In terms of thinking about antibiotic resistance as an environmental contaminant, it's important to understand the relationship to baseline levels. We know that this is a naturally occurring phenomenon. It's not something that is very unique to anthropogenic activities such as say perfluorinated compounds or pfas. This is something that naturally exists and trying to understand its importance and the dynamics in the environment requires you to have a reference to the baseline levels. We know that our activities increase it. This talks about one study in particular from the Dutch studies from long-term Rothmanstead soil studies where we know we have soils from before the antibiotic era and we know there is an increase over time because of the anthropogenic use. We also know that there

are a number of studies that show increasing levels due to discharges of wastewater and other kinds of contaminants in the environment. That can range from, as this picture shows, both the antibiotic production, humans in our waste, as well as the waste from agriculture.

When we look at our environment, there's been a number of white papers and summaries over the past decade or so. This is one from one of the leaders in the field. I like this because it breaks it out in these four major aspects that we need to focus on. One is understanding with the relative contributions of different sources are in the environment. This puts a real premium on doing robust statistical design so you really can understand what those different sources are in a meaningful way. Then also looking at what the eventual drivers for amplification. What is the role of evolution on this resistance including the amplification in nontarget organisms that I mentioned.

Also, that is important, but what are the impacts on human and animal health? They are really trying to build the data that you generate into risk models to understand in a quantitative sense what the role of the environment is in AMR risks. And then finally, really develop monitoring programs where you can understand the efficacy and feasibility of different interventions. You can both assess what might work and then also see how well it is working once they are implemented.

Pat talked about the NARMS strategic plan. The bottom panel there describes the recommendations and the response to the Science Advisory Board, focusing on surface water as kind of a confluence point as he mentioned for a variety of different impacts from the built environment on the watershed.

At the top box represents another relatively recent review article on the role of

antimicrobial resistance in the environment. In that document, it specifically calls out environmental waters as one of the areas to emphasize. And it brought up those three key points, one geospatial distributions of resistance to inform risk which touches on some points I made in the original slide that Larson brought up, also understanding the sources and selective pressures for implication and transmission.

Also, looking at and this is critical to our work and what we really focused on was defining and standardizing sampling and analysis methods so that you can compare across a lot of data that is being generated.

This graphically kind of illustrates what we were talking about that the watersheds -- there are multiple impacts on watersheds. The upper left-hand graph there, those dots represent the percentage of treated wastewater that's in the intake of different water sources which can be considerable at different parts of the country. The upper right one, the black dots represent where there are combined sewer overflow systems in the US which is untreated sewage that flow into the environment including antimicrobials and antimicrobial resistance from the human use. The bottom left one is a map from EPA's envirorales that kind of maps out the level of manure in the different watersheds. No watersheds or integrators of the different impacts. That is why we began with water. The point to make is that we are not saying this is the only environmental pool to monitor, but it's when we wanted to start with as an effective kind of integrator of impacts.

The environmental working group within NARMS, as we met our first goal was really to define what our objectives were. The overarching goal is to develop a pilot environmental effort within a newly focused One Health program. We want to develop a national scale, quantitative assessment of AMR within surface water. The quantitative is important because we want to be able to use the data for risk

models. The four sub-objectives are all important as we develop this program. One is to develop a standardized measure and a library of samples so we can monitor trends as part of NARMS. Then also develop data, quantitative data, that we can use for AMR risk models as we were developing them for different sources and different uses of water; recreational use, which is a big aspect of EPA's work, as well as drinking water, agriculture, and now increasingly water reuse.

Then we want to try to develop a monitoring program that can look at the drivers of occurrence and the selective pressures for potential amplification.

Then again, as emphasized earlier, really use this to understand where the critical control points are and to understand where we can assess current and new mitigation strategies.

We realize that these are pretty ambitious goals and pretty broad goals. It's hard to think of a program that can meet them all simultaneously. One of the key questions is when you go about designing this kind of pilot study and monitoring program is, do you go big and slow or small and fast? I guess big relates to the scale of the study, national scale as the top panel; or maybe a single watershed which is the data from the Chattahoochee which is in the bottom group which the colleagues at CDC developed. Slow and fast relates to the frequency of sampling. If you to go at a national level study, it's hard to imagine with the available resources doing that at a very high frequency, but therefore you might miss some of these very important temporal impacts of things like rainfall which is what the bottom graph illustrates.

So, you have these divergent kind of strategies and so our solution to this was to do both in phased approach.

Pat mentioned the phased design of our efforts. We broke it out into three phases

in the pilot. Phase four is beyond the pilot and the eventual ongoing program would look like this. But we focus on first three. Step number 1 was the initial testing of methods so that we could standardize the methodology we will use across all these studies. The second phase is to look intensively at a watershed use that initial study both as a way to evaluate the methodologies that we want to apply, but also serve as a basis for how we want to conduct the overall watershed study.

Oftentimes we generate a lot of data in watersheds. That can be very valuable, but we wanted to develop a kind of coherent statistically valid and robust way to sample a watershed and use this initial phase is a way to define what that is that could then be multiplied across many watersheds eventually, in phase four.

Phase three is to leverage an existing program which is the national rivers and streams assessment to really look at the national picture. I will show a couple figures on that NRSA program. The first shows a blow up of one of the panels from the earlier slides which is all those red dots or all of the sample sites in the EPA's National River and streams assessments. It's about 1800 sites. The sampled over a two-year period at low flow conditions, were talking about each sight one sample a year and technically one sample every five years because we do this over a two-year period and that we move on to a different type of water body or water resource or coastal waters, lakes, and reservoirs.

That graph there, you can see where the different colors or eco-regions of the U.S. This study is stratified according to eco-regions because this program is put in place to look at the integrity of the water. We are looking at all of these impaired waters or not.

There's a range of testing that's done on the waters from fish to invertebrates to

algae to other water quality parameters.

That's an existing study that's been going on. We've added in analysis of AMR to that.

Recently over the last couple of cycles of NRSA, we've done some piloted efforts looking at antimicrobial resistance genes. These are maps from a previous cycle where we've looked-- can see some of the gene markers for *E. coli*, Enterococci and 16-S at the bottom, but then at the top for *intl1*, *sul1*, *tetW*, and *blaTEM*. We are able to map these out by the eco-regions that I mentioned because of the stratification. Each one of the data points, and Mark Bagley is going to talk a little bit later about this, is a probabilistic design where each one of the data points represents a certain amount of river miles in the U.S. so you can translate that into the national maps. This data was recently published in the environmental science and technology.

So, that is the big picture which allows you to look at some of the questions. You do miss some of the mechanistic understanding of what's happening at a high frequency within watersheds. What we've done is leveraged a different EPA program, the East Fork Little Miami watershed which is very close to our labs in Cincinnati which is where most of the EPA people that are talking today are from. So, it's an existing water quality monitoring program that focuses our nutrients that are in place. In the upper left you can see a gradient of suburban to kind of traditional to rural areas. There's a lot of agriculture. There are wastewater treatment plan discharges. There are septic tanks in that area. The middle graph there shows the density mapped out of septic tank influent into the waters. It could potentially get into the waters. In the bottom on the far-right graphic shows where the sampling points are in the system that have been implemented over the last decade or so to do the water quality monitoring. But also shows who we

are the point sources and wastewater treatment. You notice at the bottom of that if you can see my cursor there is a reservoir there that is a drinking water and recreation reservoir that we can sample and look at some of the data to link to some of the other risks of water use.

That's what we are doing with the phase two study. That is ongoing now. It will last the next year. Again, I'm going to turn to -- and Mark will talk more about the details of the two different studies and how the different studies mapped out to different objectives that I mentioned earlier of the overall program.

In terms of the targets, we're going to use across those sites both the watershed study and the NRSA study, we are looking at three-tiered approach, looking at culture-based approaches, targeted gene analysis like I showed from the NRSA results and then metagenomics. This standard three-tiered approach is one that we adopted, but then we work closely with the concurrent effort that's ongoing by Amy Pruden from Virginia Tech that's funded by the water research foundation to develop a standard method for wastewater and water. And they've also adopted this kind of three-tiered approach that was done independently. I think this makes a lot of sense for the different values.

Culture gives you an understanding of potential pathogens themselves, but it is more limited. You can't look at all the pathogens. We decided to look at *Enterococci*, *E. coli* and *Salmonella* and, Allison, is going to talk in detail about those methodologies. We'll also look at targeted gene analysis which allows us to look at a greater range of resistant genes and a very quantitative way. And then, also more broadly, we can look at metagenomics to look at the entire resistome maybe not in a quantifiable way as the target gene analysis, but definitely a deeper understanding than the microbiome. So, the outline of talks that you're going to hear next is that Manan Sharma is going to talk about the method

development that happened on the culture side, that needed to proceed the watershed study and he's going to talk about that. USDA-ARS did a lot of that work. Mark is going to really lay out the two different study types in phase 2 and 3, the NRSA and watershed, and really crosswalk those through the different objectives on how those studies meet that from a statistical perspective. Alison will talk about the standard sampling and analysis procedures that we already started to employ in the watershed study, and we will do throughout the work.

But that is to develop a robust statistical pilot study. Then Amy Kirby will talk about the national wastewater surveillance system which speaks to the explosion of work that is happening and wastewater monitoring, this is untreated wastewater in response to the COVID pandemic.

I will come back and wrap things up and talk about next steps and we can have some Q&A.

I'm going to turn it over to you Manan.

Thank you, Dr. Garland. And so, our next speaker is Dr. Manan Sharma. He will be speaking on methods for water testing. Dr. Sharma is a research microbiologist in the environmental, microbial and food safety laboratory with the USDA agricultural research service in Beltsville, Maryland. His research focuses on preharvest produce safety issues and their intersection with environmental sustainability including the persistence of pathogens in biological soil amendments and in irrigation water sources.

He investigates methods for the detection and recovery of antibiotic resistant pathogens in water as part of the NARMS EWG pilot program. He received his BS degree in microbiology and cell science in the university of South Florida and his MS and Ph.D. degrees in food science and technology from the University of

Georgia.

Dr. Sharma.

Dr. Sharma, I don't know if you are muted or not.

(no audio)

Dr. Sharma, we can see your video, but we've lost your presentation.

We cannot hear you Manan. We can see your presentation, but we can't hear you speak.

Perhaps you are double muted because it looks like your speaker within Zoom is not muted.

(No audio)

Okay. Since Dr. Sharma seems to be having some technical difficulties, I'd like to move on to Dr. Mark Bagley on his presentation. So, Dr. Bagley will be presenting to us on statistical design of water surveillance and Dr. Bagley is a senior science advisor in EPA's watershed and ecosystem characterization division. In this role, he helps to oversee a broad environmental research portfolio aimed at understanding the dynamics of chemical and biological pollutants in watersheds, the risks they pose to human health, and our supporting ecosystems and methods to medicate those risks. Dr. Bagley's science training is in molecular ecology and population genetics which he has applied to study population responses of aquatic organized into anthropogenic modified habitats, as well as to understand mechanisms of pesticide resistance development in agricultural pests. Dr. Bagley, are you available to present?

Yes, I will try to start sharing.

Thank you very much.

Let’s see. Is that coming up?

Yes, we can see.

We could see it, but if you could put it in presentation mode.

How is that?

That is perfect. Thank you.

Sampling Design Considerations for the NARMS Surface Water Pilot – Presenter Dr. Mark Bagley
Time- 02:08:30 – 02:31:09

Great. Okay. So, this is a follow-up basically of what Dr. Garland. It doesn't get a whole lot deeper. I was going to say this is about sampling design more than it is about statistical design. We do address statistical considerations but work I’m going to describe is really about how do we implement the sampling program. Again, like in the case that Dr. Garland mentioned. This was a highly collaborative effort. Involving a number of members from the EPA, the USDA, FDA and the CDC. I co-led this effort with Dr. Jim Wells from the USDA ARS. What I'm going to be describing is really the results of about two years of discussions from subgroup of the larger the environmental committee focusing on the sampling design. This really isn't even everybody. This was the core group and invited others as discussions delved into different areas where we needed more expertise. I wanted to mention that I thought this was a great group. It was highly productive and I'm proud of what we accomplished.

This is a slide that Dr. Jim Wells developed actually. It is really to get at the

complexity of what we are looking at. I need to turn off some things on my screen so that I can actually see it here.

Okay. It shows how complex AMR is in the environment. Also shows why surface water was chosen for monitoring because it is a key integrator. But we really want to understand the flows of resistance and resistant bacterial through the system from the different sources the production as well animals, humans, and pets. And how they go through different environmental media and then get into the surface water, and that's kind of on the left-hand side of the slide. And then once it's in surface water, there are interactions that happen within the biome, within the surface water, there is transport within the surface water, then there is uptake directly by the same source animals contributed in the first place, and then sometimes the water itself is applied to different media and which provides another medium where these sources can uptake the AMR again.

So conceptually what we are looking at, in this next slide, is something of a summary of that actually. So, we want to consider the various types of sources and activities that contribute to AMR. Both from an urban and a rural landscape. We want to look at the different animal sources and activities, different productions systems that are used to distribute AMR in the system.

And then we need to be aware of the potential of actually putting AMR and how that might interact with what we're trying to look at. And as I mentioned on the previous slide, we need to look at the different environments that are important here and how the different soils and chemistries affect how water moves through the system, and how the AMR moves through the system. And the hydrology within the watersheds affects how things are moving around.

So, this slide again is -- I'm sorry, but I can't see the screen, but this is, again, the

slide that Jay showed which is the goals that were given to us as the committee designing the sampling side for the study. These are the objectives that we need it when tried to design the sampling study. We highlighted in bold the things that we thought were most important. As Jay mentioned on the previous slide, it is very difficult to envision a single monitoring program that can really address all of these different goals.

What we did was go back to the committee and say, can you prioritize these and tell us what would be most important. Really what we got back was that they really were all important.

Which is why we came back with a hybrid study design. Again, this is the slide that Jay provided. The hybrid study design was to look at both the national study to get the sense of how prevalent the AMR is across the nation in different types of ecosystems, but also to get what's happening in the watershed, the sub watershed to try to understand how the AMR is moving through the system and what is affecting the dynamics of AMR within the watershed and how potentially we might be able to control that. And that is what we decided we can accomplish with feasibly with the pilot efforts we were to propose. We know that this was still not going to really address everything that needs to be done. So, it’s more of something that we could build off of. Particularly by putting together standard methods that perhaps other groups could use to build off of what we are building and provide more of a national study.

Once we decided that we wanted to have the national scale study and the watershed study, we went to look at what different programs were out there that could be of use to us. The programs we looked at in particular were an SF NEON program, the National ecological observatory network. I think it's 25 sites that are water oriented and they are trying to create a long-term study to look at the

ecology of these system so that was a fairly interesting one for us to look at and then we talk to them a little bit. The other one is the USGS National Water Quality Assessment program which is basin-scale assessments of water quality over a period of time. They rotate these assessments on different days over multiples years.

The other one is Army Core water resource program mainly looking at reservoirs, but they are also looking at other large water bodies. They resource management responsibility and due some water sampling.

And then the last one is EPA's Aquatic Resource Surveys and as Jay mentioned, these are surveys that happen on a 5-year cycle. One of the is the National Rivers and Streams Assessment survey, others are the National Coastal Conditions Assessments and the National Lakes assessment, as well as a wetlands assessment.

We focused in on the National Rivers and Streams Assessment because we thought that it would be most valuable for AMR monitoring for this pilot.

Moving to the watershed scale, we had more trouble actually identifying program that could be used. The problem with the watershed scale is that studies don't tend to have lasting power, they tend to be short-term studies. So, we had problems finding something that we could actually leverage.

We did look at USDA's Conservation Effects Assessment program which is designed to look at landscape studies to look at conservation practices and how impactful they are.

And then NSF has long-term ecological research network (LTER) sites do have a long term, which usually last about 5 years or so, and they do have both urban and rural ecosystems projects.

Getting near the end here. I want to highlight where we'd like to build this out, which is that the National Probabilistic Survey is very good at answering this question and I should say that the rows of these are the original goals that were described broken out more so we could analyze them for the pilot. The national probabilistic survey does a good job determining the extent of AMR across the nation, but not so good at trying to get at things like the drivers and attenuators of AMR within surface waters.

The single watershed study compliments it quite well, but again, the single watershed study is not something we can extrapolate to other watersheds. To get the full understanding of AMR we'd like to have, and you can see the full complement in the last water is the multiple watersheds that capture the variation across the country both regionally and in terms of types of sources and water chemistries you might see across the country to really understand what's happening in watersheds across the country in a way we think it's useful.

To end what I wanted to talk about today, again, what we need are these additional watershed studies. We're not doing this as part of the pilot, but we'd be interested in hearing from other people about what watersheds they might be able to look at or have an interest in building out this kind of watershed study across the country. Particularly interested in getting because we don't have it in system, watersheds that have different types of high livestock inputs high hog farm watersheds, high cattle for example.

We're missing the highly urbanized systems might contribute a lot to AMR and a regional variation.

A good description is a midwestern system that is suburbanizing but we're missing regional variation across the country.

With that appeal for people that might want to partner and this by those things moving into the future, I'll end this talk.

Catherine Rockwell is speaking. Thank you, Dr. Bagley. At this time, we're going to take a break and we will start up again at 2:40 p.m. Eastern Time and I would just like to invite our attendees to again, submit their questions to the Q&A chat box in the bottom of your screen, and we will be following our next presenters at the end of our presentations with a Q&A session. So, thank you all and enjoy your break.

-Break-

Catherine Rockwell is speaking. Okay, it is 2:40 p.m. Eastern Time. Welcome back to our next series of speakers and we're going to return to Dr. Manan Sharma and his presentation on methods for water testing. Dr. Sharma is presenting on behalf of the USDA Agricultural Research Service. I'll turn the mic over out, Dr. Sharma.

Methods for Water Testing – Presenter Dr. Manan Sharma
Time- 02:40:22 – 02:56:20

Manan Sharma is speaking. Thank you, Dr. Rockwell. I hope everybody can hear me now. I appreciate the opportunity to present this work. On behalf of my ARS colleagues and EPA and FDA colleagues who advised us all throughout this process. So, Jay and Mark did a really good job of introducing the concepts of phase one and what was the overview. We wanted to talk more specifically how to collect some of the data, specifically on the pathogens and indicators, fecal indicators of interest that can tell us about antibiotic resistant isolates and prevalence in water samples. We'll focus on *Salmonella* and *E. coli*. Our focus was to recover these isolates. NARMS has good procedures and protocols developed already to identify and characterize antibiotic resistant isolate status. Our focus was on how to efficiently capture the *Salmonella* and antibiotic resistant *E. coli* present there. And in the case of *E. coli*, to give us the opportunity to collect quantitative data on specific AMR phenotypes that could be present there. As Jay mentioned, we wanted to identify these methods and evaluate them, then deploy them in a pre-pilot watershed study which is the East Fork study that's underway. We want to do this in a practical manner and practical in this case meant consulting a lot with the EPA team and the NRSA coordinators that Jay mentioned on how exactly we could do the sampling. What were the field and laboratory capabilities and limitations? We found out that the field crews really don't spend a lot of time at a specific site because they are in a rush to hit a lot of different sites to cover as much ground as they can, so it is better to try and ship the water to a laboratory for the analysis as opposed to filtering or concentrating in the field, and then shipping that out. All that was really helpful on how we went about evaluating what we wanted to include and which methods we wanted to include. As I mentioned, we are focused on *Salmonella* and *E. coli.* For the

Salmonella methods, is the determination of the presence or absence of *Salmonella*. For *E coli*, it's the presence and absence but also quantitative recovery different phenotypes. And as we talked about in Phase one, we wanted to identify and compare these methodologies for *Salmonella* and *E. coli* using the standard inoculum to evaluate that pathogen and specifically in the case of *Salmonella*, pathogens at low levels and take different surface water inoculated with the standard inoculum and identify what scheme we could recover that low level from.

There are four different methods we use for *Salmonella*. The first was called a bulk water enrichment. A rationale to evaluate this was the EPA uses a lot of contract laboratories to execute the laboratory tasks. We wanted to make this more broad so you don't have to have a lot of microbiology experience to do these methods. Basically, you get a one-liter sample of water and put some nonselective enrichment broth and mix it with the water and go and try and analyze your *Salmonella*. It's pretty easy and quick, not too complicated.

Next method we wanted to evaluate was modified Moore Swab. We added the vertical part to it. Modified Moore swabs are a commonly used way to recover and evaluate pathogens and surface water for a long period of time. We've used it in our lab at ARS here. The FDA researchers used this in the past.

And the benefit of this is, you can filter a large volume of water using this method. It's relatively cost-effective because cheese cloth is pretty cheap and simple to cut it, roll it up, sterilize it. Making the PVC canister is more complicated, we put steps

together there and gives you versatility. You can use this in the lab or the field. But it does require more training.

The next method is the modified standard method 9260.B2. I'll call this the standard method from here on out. Our rationale to evaluate this was a proven method used by EPA and ARS researchers to recover pathogens from water. It involves a glass wool filter where you filter your water through and diatomaceous earth, pool filter fiber, enter the water sample and it binds the bacteria, collects it on the filter and you analyze the filter.

When you have multiple water samples, it's easy to create a high through-put scenario. You can set up the ability to analyze multiple samples simultaneously with not too much complicated equipment, basically vacuum filtration, flask, tubing and filters. We wanted to compare the three methods against each other to see what our *Salmonella* recovery would be and then we wanted to take those three methods, see which one was the best and evaluate it against this method, the dead-end ultrafiltration method. The dead-end ultrafiltration method is used by the FDA, described in chapter 19C of the FDA bacterial analytical manual for the recovery of the parasite *Cyclospora cayetanensis,* which has been a parasitic foodborne pathogen involved in several recent outbreaks. It involves using a Rexeed 25S dialyzer, which is a really nice filtration mechanism. It has a high surface area inside the filter, it can capture a lot of different, can run a lot of volume through it, can capture a lot of different types of pathogens. This is a really good method to compare it to. There's interest and the FDA is using this method for several different types of studies so we wanted to compare whatever

was the best out of the three previous methods I described.

As Jay mentioned, we have a lot of people, a lot of hard- working people who worked on this project. In this case, all ARS labs participating in this. This is done during the pandemic when we had limited opportunities to visit our labs, so really hats off to all of my ARS colleagues who did the work and got their lives organized to do this. We had laboratories in California, Nebraska, Georgia and Maryland that were involved in this and what we did was we went to a site in the Mid-Atlantic U.S. we had used before in a research study, where that site was likely to have *Salmonella* in the surface water, and we knew that from previous sampling.

We collected four liters of water from that site and we would ship four liters of water to each of the participating laboratories. We did these five separate times so five dates or five replicates.

Each time we'd do that, we'd ship them four liters of water but we were interested in comparing the three methods I mentioned, and before each lab analyzed the water samples for those method, they'd inoculate the one liter with the *Salmonella* Typhimurium bioball. A bioball is a *Salmonella* strain that has a green fluorescent protein and the bioballs have basically about 30 CFUs in each ball. Adding one of the bioballs to the volume, it's about 30 CFU per liter. This is pretty low for *Salmonella* detection, I think. What this led to is about 60 water samples being analyzed over five replicate trials of 15 samples at each location and 20 individual assays of each of those three methods. You can see in blue here,

we did also refrigerator storage here. We'd store the samples. We had inoculated the bioball into the one-liter samples, store them for one week and then try to recover the *Salmonella* again. We had 12 water samples because of a Round Robin and so to Pat's earlier point at the beginning of the discussion, we wanted to be transparent and upload methods. All the methods we used have been uploaded to a platform called protocols.io. All the methods have a doi associator with them. If you want to know what we did, you can download the methods or go here. We have uploaded the methods so people can see what we did to evaluate this.

A note about our *Salmonella* recovery, this is the same *Salmonella* scheme that NARMS uses for sample currently. It's a nonselective enrichment, followed into a selective enrichment and get a presumptive *Salmonella* on XLT4 or brilliant green sulfa agar. This is a picture of an XLT4 and *Salmonella*. And then we had the added benefit of confirming this using the green fluorescent protein expressing *Salmonella* typhimurium of strain. So, this is a pretty efficient scheme for us to use.

So, getting to our results now, we had four ARS locations. We had three methods evaluating at each location. 15 samples at each location, so 15 samples that show location here and 20 water samples evaluated by each method.
If you look at the percent positive by location, you see that there's a pretty big spread there, that some locations were really good at recovering *Salmonella*. Some locations were not, and that's really to be expected. There's a lot of lab variation here.

We're interested in, too, is the percent positive each method recovered. So, we can see that the bulk water and modified Moore swab recovering *Salmonella* 60% of the time but with the standard method we recovered *Salmonella* 75% of the time. So overall, a 65% recovery rate, 39 out of our 60 samples were positive for the *Salmonella*, for the fluorescent *Salmonella*.

Concurrently we're also evaluating the environmental *Salmonella* present in the water sample from the one site and you can see that environmental *Salmonella* recovery is a little bit lower, the standard method is 45%, we're raising it between 40% and 50% for each three methods and a spread in the location effect here and 60 samples, and there's a lot going on trying to simultaneously recover environmental *Salmonella* and inoculated *Salmonella* strain. There could be enrichment bias and competition between the *Salmonella* strains, et cetera, but we did attempt to do that as well.

Just our refrigerated strains quickly. We had 12 samples, but we got again a spread of methods here, and overall 75%, so I think that shows that you could store the water for a week and still recover the fluorescent *Salmonella*. So, I think that this is beneficial to this effort as well.

What do we do with the data? Applied a logistic regression to the data and used location, date and method in our model, location was the significant term in that model. As you can see, the laboratory location was the most significant factor there, but it also showed that the standard method had the highest recovery of

Salmonella more frequently. That led us to identify that standard method and compare it to the dead-end ultrafiltration method for the next set of experiments to see which one recovered *Salmonella*. We only did this at two ARS sites comparing the dead-end ultrafiltration and the standard method. We did 20 water samples at each site, one liter inoculated with the *Salmonella* bioball. We had a total of 40 samples in the site with good recovery, 39 out of 40 times we recovered *Salmonella* and you can see that the dead-end ultrafiltration and standard method were equivalent how they recovered *Salmonella*, there was no significant difference here when we put this into our model. This identifies the standard method as something we want to go forward with because it fits well into the logistic and laboratory considerations we have to do with the study and pre-pilot program.

I wanted to mention the *E. coli* work quickly. My colleague Lisa Durso came up with an exquisite set of protocols to analyze the *E*. *coli* data to attempt to quantify extended-spectrum beta-lactam producing *E*. *coli* and tetracycline resistant *E*. *coli*. We used a standard inoculum that was a pan-sensitive strain and a strain resistant to both cefotaxime and tetracycline with an evaluated membrane filtration methods and most probable member assay, a colilert some of you might be familiar with. What we found was that the TBX media was supplemented with antibiotics provided a more consistent quantitative recovery than MI media. We did this with surface water from each of the four ARS locations. The local surface water was shipped in a round robin way to each lab and these *E*. *coli* strains were inoculated into those surface water samples as well for this evaluation.

And Lisa contacted a commercial company that is going to make a commercial preparation of these *E. coli* strains so they could be used as a standard inoculum in future studies.

As our conclusion, we have a method that can recover low levels of *Salmonella*, the modernized standard method 9260.B2 and incorporates well into the pilot studies and some of what we learned about the NRSA's study and I think it can be practically integrated there.

We did identify quantitative methods for the recovery of *E. coli*, and we showed that they were equivalent to the NPN methods. I think the NARMS group has decided to use a little bit different methodology based on membrane filtration, but I think that these are good data to have. Finally in the effort to upload data and be transparent, we are putting our methods on protocols.io and plan on uploading the data sets to ag data commons where USDA can house data for large experiments and we're trying to be transparent and standardize some of the inoculum used in the studies so people can repeat them and see the data and make their own conclusions about that.

Finally, just want to thank all of our ARS colleagues throughout the country. They've been outstanding helping us do this, a lot of investment. Our FDA and EPA colleagues are essential to this as well and specifically, I want to call out our ORISE fellows, Autumn Kraft and Betty McConn who have been helping lead, along with Autumn Kraft a systematic review on the recovery of antibiotic resistant pathogens from water. And also acknowledge our funding and inner

agency agreements between the FDA and USDA for this project. Thank you all very much.

Catherine Rockwell is speaking. Thank you, Dr. Sharma. Our next presenter will be Dr. Alison Franklin, who will be presenting on the status of sampling and analysis in surface water monitoring and for those who may not have been in the meeting earlier today, Dr. Alison is one of our moderators but I'll briefly re-introduce her. She is a biologist with the EPA's office of research and development in Cincinnati, Ohio and her research focuses on emerging contaminants of concern in the environment. In her role at EPA, Dr. Franklin is currently leading the pilot effort to analyze antimicrobial resistance in surface water nationwide for the NARMS program in collaboration with FDA, CDC, and USDA. Dr. Franklin, the floor is yours.

Developing a Pilot Environmental Efforts for NARMS – Presenter Dr. Alison Franklin
Time- 02:57:14 – 03:11:14

Thank you, Dr. Rockwell. Appreciate the introduction. Good afternoon, everyone. I'm Dr. Alison Franklin. I work for the EPA and I’m heavily involved with this pilot environmental effort for NARMS. I'm going to talk about the status of sampling and analysis in surface water monitoring. It wasn't great Dr. Sharma had issues but I think it kind of works best that he was right before me because I’m going to continue a little bit with what he was talking about. I want to recognize all the people involved in the interagency collaboration. It was a group effort. Everyone from EPA, FDA, USDA, and CDC have been involved, created a very unique project that I think is going to be very successful.

So first, is a brief outline of what I'll be talking. I'm talking about the methods that are selected for culture and molecular work after we did the method developments and reiterate for culture, we're looking at *E. coli*, and *Enterococcus*, *Salmonella*, with molecular, targeted gene analysis, metagenomics, and whole genome sequencing. I'll briefly talk about the protocols selected for field sampling, I'll provide an update on where the project is right now, specific to the watershed study that just started, and I'll talk about upcoming work with finishing the watershed study and starting the national study. First, I want to talk about the final decisions for culture work.

Dr. Sharma did a wonderful job of really explaining especially for *Salmonella* all of the work that they did that was a very thorough and we thoroughly appreciate having USDA collaborate with us. First with *E. coli*, we selected the modified mTEC method a modification of EPA standard method 1603. We're looking at cefotaxime and tetracycline resistance. For *Enterococcus*, modified mEI method, and that's a modification of EPA standard method 1600, and with that, we'll be looking at vancomycin and tetracycline resistance. And then *Salmonella,* it's the modified standard method and has the presence or absence of *Salmonella* being in the sample.

Some reasoning for the selection we made specifically I wanted to point out for *E. coli* and *Enterococcus* and as well for Salmonella. We wanted standard methods that would be utilized by similar efforts. So even though during the method developments, the USDA collaborators weren't able to work with modified mTec

and modified mEI due to issues of the pandemic and getting supplies, it was noted that there has method development already been done looking at the methods, specifically with the WRF effort that Dr. Garland pointed out during his talk that Amy Pruden is leading. It was noted while our method development wasn't able to use these specific methods, they would be equivalent and reasonable to use. So, because other efforts similar to ours, specifically the WRF, which is looking at standardizing culture methods and molecular methods looking at AMR in surface waters and other waters, we decided to go with those methods for *E coli* and *Enterococcus*. In addition, we also have an EPA beaches study going on right now, that is also using the modified mTec and modified mEI methods. We also need isolates for susceptibility testing and whole genome sequencing. One of the things that was used during method development for our project was the IDEXX. But to get isolates from that is very tedious and it can be difficult to do. We also wanted to define media, this goes back to IDEXX II, which is easy to use but has high priority media.

I wanted to talk about the final decisions for molecular methods since we didn't get into a whole lot of detail about that. First, we're doing metagenomics on whole water samples and FDA will be doing that with this project. Some targeted genome analysis using a fluidigm system, high throughput PCR looking at relative abundance, presence and absence and droplet digital PCR to quantify select genes of interest.

We'll also be doing whole genome sequencing courtesy of FDA with all *Salmonella* isolates as well as a subset of resistant *E. coli* and *Enterococcus*. The fourth item

which hasn't been mentioned is a quasimetagenomics, which is taking the culture enrichment from the *Salmonella* work then extracting and doing metagenomics work on that. Andrea from FDA will be talking about that tomorrow.

Reasoning for having this variety of methods for our molecular work, we really wanted to fully characterize the presence of AMR in surface waters and understand the microbial populations that's in the surface waters. No single molecular method fully characterizes AMR in environmental samples so using these compliments will give us a lot of information that we need.

Targeted gene analysis will help us with determining relative and absolute numbers of known genes of interest, and with fluidigm, you can do that quickly.

With metagenomics, that will helps us determine the resistome of environmental microbial population and with the whole genome sequencing of the select isolates, we can create a database of resistant organisms in surface waters.

Going more in-depth with what we did with the method development work specifically here to aid with metagenomics work, we wanted adequate sample volume. We went with 500 milliliters both because of logistic reasons and also, that seems to be the amounts of water that could easily be filtered through and provide us with the amount of DNA we felt was adequate for all of the work we were going to be doing with the samples. We did some method development with DNA extraction procedures and went with the power water DNA kit comparing it with other popular kits. We got broad recovery and the highest yield in quantity.

Those wholesale cell standards for quality assurance and quality control. Both with metagenomics work and with the targeted gene analysis. And those wholesale standards came from Zymo and ATCC. Both were equivalent whenever we worked with them. Whichever one we use will be likely due to cost and availability. Sometimes ATCC has lead times up to a month or so, whereas with Zymo, they're typically readily available. The major advantage that metagenomics provides to the pilot study is that characterization of the full complement of the environmental microbiome and resistome. And also, can identify early signal of emerging resistant genes.

And in the figure, here is a great example of looking at the environmental resistome and the food animal resistome, and how you can find what the shared resistome is. The figure work and credit work for this goes to Daniel Tadesse from the FDA. The next part of the molecular work is the targeted gene analysis. And I just want to touch base on these two methods because they are going to provide us with a lot of information at the gene level. First is the fluidigm, it is a 96 by 96 IFC can nanoliter reactions. We have the ability to screen 96 samples for 96 targets at the same time if you're not doing duplication or triplicates but typically, we do do duplicates and triplicates within one IFC. We have a suite of antimicrobial resistant genes that we will look at, fecal indicators and mobile genetic elements and you're looking at presence and absence and gain a general idea of relative abundance with this system.

Then we have the droplet digital PCR where we can quantify select genes without the need for standard curves and we'll continue looking at the antimicrobial resistant genes previously monitored during NRSA and the link for article for that

work is at the bottom of the slide for anyone who is interested. And we're going to look at genes detected via fluidigm and our metagenomics center of interests. Now, moving on to field methods because we had to make some decisions what we were going to be doing with regard to sample collection, where we're going to be collecting samples, and so on.

First with sample type, we ended going with the whole water grab sample. We had looked at or considered doing some concentrations as Dr. Sharma mentioned, dead-end ultrafiltration, however due to the number of samples that we would be collecting in the watershed scale study and the logistical considerations for the national scale study, most time with the national scale study they're on a boat, or they're backpacking into locations and they have X number of other things they need to be doing and setting up a dead-end filtration system, either taking it in or setting it up would be very difficult. We decided we'd stick with the whole water grab sample and then sample, and location is the surface water confluence if possible and that's in order to understand the main flow of water that is going through that system at that specific location.

With the watershed study, sampling is either going to occur by walking or wading in and there's a picture there that shows one of the walk-in locations, or bridge sampling, because some of the locations it's not safe to get down to it, or it's just not possible, and so you can see a picture of an example of a bridge site location that's part of the watershed scale study. For the national study, sampling will occur at the end of the sampling day so the whole time is shortened at transect K, which you can see there as the end of the transect. They have various different

transects, where they collect a variety of different samples, and these samples will be taken either by wading in or by boat when the sampling location is non-wadeable.

Where are we right now? We completed the method development. We're in this transition phase, where we're just at the point where we're beginning to start the watershed scale study but it's not full scale yet. I'll go into details about that. But essentially, what we're doing is testing out the methods that were decided upon during the method development and defining the workflow for the watershed scale study and getting that down. We also are getting the field and laboratory personnel working with the sampling procedures and methods and becoming proficient and comfortable with them.

So, the status of the watershed scale study, we, one, started work in early July, so we have nine weeks of sampling so far and to reiterate what was stated before, there's 35 sites that have been sampled three times essentially. There are four locations that are being sampled weekly within the 35 sites. An analysis we performed to date, all the samples have been filtered for molecular work and DNA has extracted and we're getting ready to do fluidigm analysis on those. And we're also doing *E*. *coli* and ESBL *E*. *coli* for culture work. The upcoming work, we have *Enterococcus* and *Salmonella* work begins late September/early October time frame and we'll start working with vancomycin *Enterococcus*. And then we'll add in the tetracycline resistance for both *E. coli* and *Enterococcus*.

With regarding to the upcoming work, first thing, we're going to continue with

the watershed study analysis and that’s going to run until June of 2023, again with sampling 35 sites 17 times total for most of the locations and for the locations the four sampled weekly that would be 52 times they've been sampled.

The national study will start in April/May time frame of 2023 and as stated previously, that runs for two years over the summer months. So, in 2023 that will be from April/May until September time frame, and then again in 2024. In total, that's 2,000 samples but about a thousand samples per year. As for 2025 and beyond mentioned previously, it would be great if we could do additional watershed scale studies and other works that could occur would the national scale waterway assessments include coastal, lakes and wetlands.
With that, I want to thank you for your time and I'll be happy to answer any questions during the Q&A session.

Catherine Rockwell is speaking. Thank you, Dr. Franklin. Our next speaker I'd like introduce is Dr. Amy Kirby, who will be providing us an update on the national wastewater surveillance system. Dr. Kirby is an environmental microbiologist in the waterborne disease prevention branch and program lead for the national wastewater surveillance system at the Centers for Disease Control and Prevention. She has a Bachelor of Science in agriculture from the University of Georgia, a Ph.D. in microbiology from the University of Buffalo, SUNY and a Master of Public Health in epidemiology from Emory university. At CDC, Dr. Kirby is interested in leveraging environmental microbiology methods to measure pathogens, antibiotic resistance genes, and other health indicators in natural and manmade water systems. Dr. Kirby, the floor is yours.

Implementing Community-Wide Disease Surveillance Through the National Wastewater Surveillance System (NWSS) – Presenter Dr. Amy Kirby
Time- 03:12:15 – 03:28:18

Dr. Amy Kirby is speaking. Thank you. We're going to change gears, still talking about environmental testing but instead of talking about environmental testing to understand exposure and risk, in this case, we're going to be using environmental testing to get information on what is, what infections are circulating in the population that's contributing. I think Claudine has my slides. I don't see them yet. Maybe others do.

No, sorry, I guess you can share your screen but I'm happy to pull your slides up.

Dr. Amy Kirby is speaking. If you have them, that would be better. I don't have them pulled up right now.

Just give we one minute.

Dr. Amy Kirby is speaking. I'll dive in. I'll do a little on the fly to get us back on schedule. Basically, what I'm going to be providing today is an overview of the wastewater system established at CDC in September of 2020 as part of the COVID response. And the idea is basically using wastewater coming into wastewater treatment plants as essentially a pooled community stool sample. So, all of the things that we can test for in an individual stool sample and understand, learn about the health of that person, we can do the same thing at the community level

by taking wastewater coming into a treatment plant before it is treated. That gives us information on the population or health overall.

That was the idea behind the national wastewater surveillance system but there was no infrastructure in the U.S. to support that surveillance in 2020. You can go to the next slide. A lot of our early efforts were around building the infrastructure for this system and making sure that our public health practitioners know how to use this type of data, because it really hadn't been used for a domestic health issue prior to 2020.

So, there's four big advantages to wastewater surveillance, and these are really COVID- specific, but that can apply to other infectious diseases as well. So, the first is that we know that people infected with SARS COVID 2 will shed detectable viral RNA in their stool, symptomatic or not and it happens in adults and children. And so that means that by looking at wastewater, we can get information on the full spectrum of disease that's in the population, as opposed to clinical surveillance, which tends to over-represent symptomatic cases.

Second and I think most importantly, it's independent of health care- seeking behavior and access. So, it requires no action from the individual other than using a toilet connected to a wastewater system. They don't have to go to the doctor, don't have to get tested. If they're using home tests that aren’t reported to the Health Department, wastewater surveillance will work in their communities, so it gave us a reliable source of information on community infection levels as behaviors changed over the course of the pandemic. In between communities.

As I mentioned, it's very efficient too so that one sample coming into the wastewater treatment plant can give us information on hundreds, thousands, even millions of people in our largest wastewater systems and finally, it's fast. From the time the toilet is flushed to data in hand is about five to seven days. We've seen consistently over the course of the pandemic, we can detect trends in wastewater data four to six days before we see the same trends in COVID case data, so it is serving as the earliest warning of changing infection trends.

Next slide. Next slide. We spent a lot of time building up the infrastructure to support wastewater maintenance. And this is where we are right now. On the left is our funding map. We currently have 46 states, five major cities and two territories that are using CDC funds to support wastewater surveillance activities in their communities. This year, we were able to establish two NWSS centers of excellence in Colorado and Houston, which will be critical for driving forward the practice of wastewater surveillance for public health and on the right is a map showing all of our sites that are submitting data to the national system, starting with September of 2020 to I think this map was updated last in August. So, we have over 1,200 sites routinely submitting data to CDC, that's in all 50 states, again those two territories and eight tribal communities. This is actually a bit outdated. We have over 96,000 unique wastewater samples represented in our data system and collectively, that represents over 133, again, slightly outdated from August, 133 million people so we're already covering 40% of the U.S. population with these sites.

Next slide. The heart of our system is really our data analysis. So, this is a screen shot of our DCIPHER data platform. So, all of the raw data comes in here along with a host of quality control and quality assurance variables and the data is analyzed and reported back to health departments for public health action and the real work horse metric we rely on most heavily is percent change. So that tells us how much wastewater levels are changing in a community, are they going up or down and how quickly are they changing. The second question, if it's going up, is it going up were from a low level or up from a high level. We use a relative measure of percentile to get at that. So, we look at the current measure compared to all of the historic data from that site and ask is this most recent measure in the top 20% of concentration ever measured at that site or the bottom 20% or somewhere in between. So, between those two you can see where we are now and where we're going in our community.

And then finally we have detection proportions. So, of all of the samples collected in the last two weeks, what percent of them have any detection at all? Unfortunately for COVID, we're almost always at 100% positivity for all of our communities but for more rare pathogens, this is going to be a very important metric that's going to be our first indication that these pathogens are present in at community. Right now, this is applied specifically to COVID but we can apply them to COVID variants and other pathogens as well. Next slide.

This is also an exploratory system so we can look at other things, we can bring in other data sources like data from COVID data tracker about vaccination coverage, you can explore all of the data down to individual data points, as you see in

number three, and then number four is the one I particularly want to draw your attention to. Sewer systems have boundaries of their service areas that tend to not align with other jurisdictional boundaries like counties or zip codes or cities, and so it's really important that we have these sewer shed boundaries available in our data system, so that we know what the population is that's being represented by that data.

Next slide. Just a quick note to say we can also do this, so what I was showing you a minute ago was PCR-based data. We can also do this with sequencing data. This is a tiled amplicon approach to detect SARS-CoV2 variants. So we can receive that data directly into our DCIPHER system and can analyze it using a bioinformatics pipeline developed by FDA and report that back to our Health Department partners. So, you can see on the left is a map of the predominant variant in communities and as we're seeing in clinical, it's almost all ba.5 and you can see a time line of different sequencing results over time for a site and you can see that bar along the bottom is showing how the predominant variant has changed in this example location over time and the stars there represent samples where we have sequence data.

Next slide. Next slide. I'm actually going to skip this.

I want to show you a case study instead. One of the questions we get, how do you use this data? I think this case study out of North Carolina is a really great example. They had wastewater surveillance in one of their coastal communities and in June of last year, they detected a really strong increase in wastewater

coming out of the community. At the state level, it didn't make sense. They didn't understand it was the only community seeing the increases, there wasn't anything that could obviously explain it, and so they weren't seeing it reflected in clinical cases and in testing. None of our other indicators were aligning with that.

So, they reached out to the local Health Department and started asking questions and it turns out there was a big fishing event that weekend, and they had a lot of people coming in from out of town so a lot of tourism and the evidence indicates the tourists brought COVID with them and went home to get tested. We saw it in wastewater but didn't see it in cases and they used that information to follow up with messaging to the community about we have detected an increase in COVID in our community, it's important that you take protective measures if you're feeling sick, get tested, stay home, all of those things. This is a great way to that wastewater can show us things we wouldn't otherwise see.

Next slide. Okay, so changing gears, that is what we have built for COVID but we recognize from the beginning that wastewater surveillance can be used to get information on a lot of diseases in our communities, and so it was developed for pandemic, clearly having can in existence prepares for the next pandemic. I want to think about core surveillance. How do we supplement our ongoing core surveillance? Next slide.

So, this is how we were think being building out the system. Built for COVID. The next thing we wanted to add was this core surveillance for endemic or common diseases, like influenza, food-borne infections, antimicrobial resistance, and the

think about emergency needs, sporadic but expected, things like *Shigella*. So, if for example there's a flood, we get worried about shigellosis outbreaks in the wake of that. Wastewater surveillance in the local area for the short term could be useful but it's not a good investment to do *Shigella* surveillance nationwide all the time, so it would only be that emergency deployment.

Finally, thinking more far ahead of pandemic preparedness, what other diseases are out there in the world that might show up in the U.S. that we need to be prepared for. Some of you are probably noticing that last word, Monkeypox, this is one that wasn't in our pandemic preparedness bucket and came to our shores before we expected it to so we've been able to rapidly adapt this system and we already have data coming in on Monkeypox and that will be scaling to national surveillance in the next couple of weeks. The system has already shown that it does have that responsiveness, which is great.

Next slide. But we are still thinking about those core targets, and at the top of our list is antimicrobial resistance. Because we know that the first issue of a lot of asymptomatic cases that never get tested, that's huge for antimicrobial resistance. We know we're missing a lot with our clinical surveillance. Wastewater surveillance might help us address that. As we think about the first core surveillance deployment, we wanted to target AR genes that are clinically relevant, emerging, high-priority targets so I listed the ones that we're considering here, right by their priorities. So carbapenemases, followed by colistin resistance, ESBLs, Fluoroquinolone resistance, particular those plasmid mediated resistances, and macrolide resistance. We don't know which will end up in our panel. It

depends how well the assay optimization and multiplexing goes so we know how many assays we can run in a reasonable panel.

The timeline for this expansion did get pushed a little bit because of Monkeypox so we have those assays in development now. Again, this is PCR-based for right now, and our hope is that we will start pilot testing what those two centers of excellence in early 2023 for AR. Assuming no or limited technical issues plan to roll that out to the full system by August of 2023 and we'll of course be updating our internal DCIPHER dashboard to receive and analyze AR data and as well as developing a public-facing dashboard. Right now, our COVID data is available on COVID data tracker but we can't put non-COVID data on there so we'll be developing a new specific dashboard that can handle all of our non-COVID data, including AR, so that will hopefully be launching around the time that we roll out to the full system.

Next slide. And then really quickly, you can click through the next one. Quickly, I want to touch on challenges we still have for developing the science of wastewater surveillance, and particularly for AR. I want to focus on these four, so we really need to improve our metrics, so trends, percentiles and detection proportion are the baseline, but we would really like to get at is how many cases does this represent in the community? What's the disease prevalence? That's hard to do even with virus and much, much harder with AR so I think we need to put some effort into that question of how can we understand what this means for case numbers in the community.

Second, we need to think about the appropriate sampling frame. For COVID, we want to sample as many communities as we can we recommend twice a week sampling. That is probably not needed for AR. We certainly want to get to a lot of communities but twice a week is too frequent, and not a good return on investment for this funding. What is the appropriate frequency for AR sampling?

We're working on method development that will continue, that's always needed, but certainly for AR, we really want to be able to link those genes to what their host organism is. It's not something we'll be able to do in the first round of testing, but hopefully in the future, that is something that we'll be able to do.

And finally, I really need to say that we really need to have a good and think clearly about the ethical framework for wastewater surveillance. This is a new type of surveillance. There is, it is not clinical surveillance the way we typically think of it, but it is getting information about a population so we need to be clear with how we're using this data, what we're doing the testing for and especially as we start thinking about sample archives, how do we put guardrails on future use of these samples so that they will continue to be used for the good of public health.

Next slide. With that, if you have questions about NWSS, find more information on our website cdc.gov/nwss and you can reach out to our team at NWSS@cdc.gov. Catherine, back to you.

Catherine Rockwell is speaking. Thank you so much, Dr. Kirby. We have one final

presenter for this segment of our meeting today, we'll close out our objective 1.2 session, going to be Jay. Jay Garland from EPA to discuss next steps, and before I turn it over to Dr. Garland, I'd like to encourage our attendees to submit any questions you have to our panelists and to our speakers, because following Dr. Garland's presentation, we're going to have our Q&A session. Dr. Garland, the floor is yours.

Next Steps – Presenter Dr. Jay Garland
Time- 03:28:57 – 03:33:39

Dr. Garland is speaking. I'll make this quick so we have time for questions. I'll pick up the end of my introductory talk about next steps for the work. I think the primary thing is, as Alison indicated, we've got to get the implementation right. She mentioned what we're doing right now and kind of reflected the large sample load we have really over the next two years. We're ongoing in the watershed study, there's going to be an overlap early next summer with the watershed study and the first Summer of NRSA followed by the second Summer of NRSA and with any gas we have staying on top of the data to compile it, analyze it and assess it. Because this whole pilot is about to determine if and what a surveillance system should look like. We really need to stay on top of the data. So that’s the primary step over the next couple of years.

The second thing is to continue to work on developing risk assessment models. This isn't really part of the NARMS effort directly but we're working on that with colleagues, primary working with Carrie Hamilton, Arizona State University, who developed a framework looking for quantitative microbial risk assessment specifically applied to resistant bacteria and we've worked with her on that framework and applied it to a couple water-related issues, one specifically on reuse for showering and the risk for MRSA as one example that's already published and we're working right now on ESBL *E*. *coli* and recreational exposure risks and finally think about planning for phase four, beyond the pilot what the ongoing effort would look like. Mark mentioned it, talking about other kinds of watersheds but we also need to think broadly about that, is maybe links to other monitoring that's going on. Amy described NWSS. I'll mention briefly next about wastewater affluent monitoring that may be developing and really try to align those different efforts, both from the terms of what's coming from the human population, what's going through treatment, what's going in and out of the environment. Something we need to pay attention to, and then also think about other environmental components. We mentioned water, but we know that soils can be important especially as a transfer into the aquatic systems, also biofilms within the systems. We do do some sampling of the epilithon or rocks and the biofilms during NRSA, so we can think about doing some pilot efforts comparing AMR within that the environmental component and surface water itself.

I put in wildlife because I heard an interesting talk from USDA colleague from their wildlife group, who looked at whether it's birds or even mammals who maybe using riparian areas and potential transfer between as vectors between

the human and even agricultural entities. That's another potential connection point to look at.

So, this is just a diagram of the AMR model is the framework. I don't want to go into specific there is.

Briefly, there's a 2021 last year NAS came out with a report, broad-reaching report on debating antimicrobial resistance. Surveillance was part of that. And they point out that the challenge for environmental monitoring is what factors amplify the resistance in the environment as we've already discussed. And they point out this issue of the treatment plants, and in particular the wastewater treatment plants not equipped to remove the resistant traits or drug residues. There may be some reduction but not complete removal so they become this important bridge between human-made contamination, which NWSS is looking at and kind of mirroring back to the population, also look the wastewater treatment plants as this connection point and mirroring out into the risk in the environment too. They've recommended that EPA develop guidance and resources to really provide the states to do this testing at a national scale so we're really actively looking at that and how we would build that kind of pilot effort there through some mechanism as early as next fiscal year and again, this gets back to thinking about how we would align that kind of monitoring of the point sources with what we're doing in the surface waters. I'll leave it there and kind of stop sharing and open up for questions.

Objectives 1.1 and 1.2 Q&A – Moderator Dr. Catherine Rockwell
Time- 03:33:44 – 03:46:33

Catherine Rockwell is speaking. Thank you so much. So, we are now at our Q&A portion for our first two objectives. Looking at our Q&A chat box, it looks like the questions we received earlier had been responded to during the presentations or following the presenters' presentations and so I'm not seeing any new questions at the moment. However, I'd like to get the ball rolling with a question, and I'm going to open it up to our panelists.

It's my understanding that much of the current research into water and antimicrobial resistance is focus on surface water rather than ground water. Can our panelists speak to any of the research going on with regards to the prevalence of antimicrobial resistance genes and the presence of antimicrobial resistance residue in ground water environments, particularly those ground water areas where agricultural fields exist and the fields are irrigated by treated wastewater. Is there any data on ground water and any correlation of the findings to the surface water testing and can you also speak to the role that aquifers may play in differences seen green ground water and surface water? I'll open it up to our speakers.

Dr. Franklin is speaking. During my Ph.D. work I studied a system where treated wastewater was being used to irrigate agricultural and forested lands. I looked at the presence of antibiotics and antimicrobial resistance in that system, and first speaking of the antibiotic compounds there was antibiotics present in the wastewater that went out and was being spray irrigated and first, we saw the

antibiotics in the soil profile, sometimes rather high levels, especially compared to what was actually going in as an input, and we did see very low levels of antibiotics in the groundwater, typically anywhere from 100 to 1,000 times lower than what was in the wastewater water, and another part of it, we did see uptake of antibiotics into a wheat crop. I know in a previous talk it was mentioned the concerns about food and so I wanted to mention that. With regard to antimicrobial resistance, definitely saw some correlation with the presence of antibiotics in the soil and antimicrobial resistance. It was less so in the groundwater. So typically, it was more so seen in the soil itself versus in the groundwater system, but there was some elevated antimicrobial resistance gene presence in the groundwater, just not as strongly correlated as with the soil. With regard to aquifers, I do not have any knowledge about that right now.

Catherine Rockwell is speaking. Great, thank you very much. We do have a question that's been submitted through the Q&A, and it's from Steven Roach. It is disappointing that the watershed study is looking at agriculture impacts in a watershed without animal agriculture. Nobody anticipates much resistance from agricultural systems without animals. There is no lack of watersheds in the Midwest that are impacted by animal agriculture. For the NRSA study, the sample is during the summer when crops preclude manure spreading. What is the plan to look at impacts of animal agriculture on water? I'll just open that up to our speakers.

Dr. Garland is speaking. I can start and Mark, if you want to add anything about the selection of watersheds but I think Mark went into the rationale for why we

selected the pilot effort to be in East Fork but emphasizing we believe that's a starting point. We want to look at watersheds that have more of an animal footprint, animal ag footprint in them and that would be part of the next step so it's not because we don't think that that's important to look at, but you know, for the initial pilot effort, we had to focus on one watershed. We had this long-term study with a lot of infrastructure in place that facilitated an effective evaluation of how to best do the watershed study. In this case, it's more of probably a human footprint, largely from septics, and some wastewater but not that animal footprint and that really is a priority, as Mark emphasized about selecting the next types of watersheds to look at. And with NRSA, it is a limitation. There's only a single sample done. It's the baseload conditions in the summer period, so that is why we need to kind of look at both the national scale and more focused watershed studies and in the future look at a greater diversity of watersheds.

Mark Bagley is speaking. I was just going to say, we recognize these deficiencies and tried really hard to find a watershed that had a large livestock input. We couldn't identify one, but this is why we really think that we need to get additional watershed studies somehow in the queue, particularly livestock but also highly urbanized system, which this also does not address very well. That's a follow-up question we need more discussion on.

Manan Sharma is speaking. I would also argue that the environmental drivers are equally as important as the landscape. In our studies, we see there's sometimes tenuous, sometimes stronger correlations between say rainfall or turbidity of the water and elevated *Salmonella* levels and certainly water temperature and

Listeria monocytogenes , which isn't a concern with AMR to this point but some other environmental factors may drive this than the actual source of the water.

Catherine Rockwell is speaking. Great, thank you very much. I'm not seeing any additional questions at the moment, but I would like to ask a question of Dr. Franklin regarding her talk. During your presentation, you mentioned that the national watershed study would start in spring of 2023 and be conducted over the next two years over the summer months. Can you elaborate on why sampling will be specific to the supplements rather than year-round and is there a concern about seasonality and its impact on what patterns and prevalence we're seeing with regards to antimicrobial resistance and maybe even the presence of antimicrobial drug residues in?

Dr. Franklin is speaking. I'm not specifically involved in the development of NRSA. Maybe Dr. Garland or Dr. Bagley can add anything because I don't know why they selected only summer months. My gut feeling is that feasibility, because during the summer months the weather will be optimal to be doing field work. There's a lot of, there's locations throughout the U.S. and some of them they have to backpack in, and I would assume during the colder months it would be more dangerous to do so.

What was the other part? Yeah, we do believe seasonality would most likely play a role, where the watershed study comes into play because we're sampling all year long and hope with the watershed scale study we'll get an idea of what seasonality may play with regard to the presence of AMR and with the NRSA

study it's really trying to get a snapshot of what AMR looks like at a national level, and as Dr. Bagley pointed out, it doesn't get into the more nitty gritty details of what the actual drivers may be, and how seasons may affect the rainfall events. With NRSA they're doing it at base flow, so if there's a rainfall event, they wouldn't be collecting samples.

Dr. Garland is speaking. I guess your question is a good one, but it speaks to and emphasizes the comment Mark made. No single study will address all the questions, both providing that national scale picture as well as kind of looking at some of these drivers for occurrence that might be higher frequency, so that's why we adopted this dual approach, and as the first question addressed, the dual approach eventually would have to involve more than a single watershed in our mind, but watersheds are different types, but I think I would urge people to, if they're interested, look at that ESNT paper that we referenced. There are some interesting trends that you can see, even with the single point measurements at the watershed. Given the amount of data that we collect on those samples, and we know about the watersheds, and the probabilistic design, there's some pretty good insight that you can generate. That was doing limited ARG targets. The factors that are driving the occurrence and a lot of that is about the overall health of that watershed, not necessarily specific driver, specific inputs of ag versus human but the overall state of the system.

Catherine Rockwell is speaking. Thank you very much. There are again still no new questions in the Q&A chat but I did have another question that's changing gears a little bit, and I'm not sure if our panelists can speak to this, but it has to do with

chlorination, proven effective in reducing waterborne diseases but concerns raised about the impact of chlorine disinfection on the development of antibiotic resistant genes and bacteria. It's my understanding the factors that trigger this resistance are not well understood. Can any of our panelists comment and whether this is a focus of current studies?

Dr. Garland is speaking. I am aware of people looking at that question within wastewater treatment systems, looking at co-selections or selection for resistance. From the point of the view of the design of this study, the wastewater treatment plants, say in Ohio and a lot of places do seasonal disinfection. So, there is some chlorination that occurs during the summer months, when there is supposed recreation of the waters but during the winter months, there isn't chlorination. So, the chlorination itself is not a uniform driver or uniform selected pressure within the wastewater systems but trying to understand what, you know, that may influence what's discharged but not consistent across the entire year.

Catherine Rockwell is speaking. Thank you very much. Well, again, I don't see any additional questions in our Q&A chat box, and we are approaching the end of our Q&A session, and so I'm going to turn the moderator duties over to Dr. Franklin, who will take us into our next objective.

Objective 1.3: Initiate an AMR testing program for animal feed and pet food, including their ingredients, and share the data in an integrated database and in NARMS reports – Moderator Dr. Alison Franklin
Time- 03:46:33 – 03:47:57

Dr. Franklin is speaking. Thank you, Dr. Rockwell. If anyone has any other questions from previous talks, please feel free to put them in the Q&A box, and whoever can ask them will get back to you through the Q&A box.

Our next objective is 1.3, and that is to initiative an AMR testing programmer animal feed and pet feed including their ingredients and share data in an integrated database and in NARMS reports. Our first speaker is Dr. Beilei Ge, research biologist at the FDA CVM at the Office of Research's Division of Animal and Food Microbiology where she's been leading the microbial food and feed safety research program for over ten years. The research for polio in support of the regulatory mission includes pathogen detection, mitigation strategy and antimicrobial resistance. Dr. Ge's research employs traditional microbiological and molecular methods, and newer genomic and metagenomic tools. Dr. Ge received her Ph.D. in Food Science and Food Microbiology from the University of Maryland. With that I will turn it over to Dr. Ge.

Thank you, Dr. Franklin. I'll share my screen. Am I doing okay with the screen?

It looks great.

Antimicrobial Resistance (AMR) in Animal Food – Presenter Dr. Beilei Ge
Time- 03:48:21 – 04:02:25

Thank you. So, I'm going to continue the same with this NARMS public meeting 2020, progress on the strategic plan. My talk is going to focus on a goal objective 1.3, so in this objective, we are trying to initiate our AMR testing program for animal food. Before I start my talk, I want to mention that Dr. Jenny Murphy who is the Deputy Director with the Office of Surveillance and Compliance is also here, so if during the Q&A session she will be joining us for questions. Using the term animal food, we are referring to pet food, animal feed, raw materials, and ingredients. In terms of progress on this objective, I'm going to share with some of the recent findings we had. Like Pat mentioned in the introductory remarks, why testing animal food? We have a data gap in terms of AMR contribution of animal food to the overall one health AMR burden and they're literature showing bacteria in animal food can be linked to human illnesses and, also, there's some antimicrobial resistance organisms in this commodity.

We are trying to conduct some research studies to determine whether long-term testing of any animal food is necessary or beneficial for the overall NARMS program. So here I have some literature showing the concern of animal contributing to human illness. This dates back to 2002. CDC has a review of outlining bacterial contamination of animal feed and its relationship to human foodborne illness. Following that, there are also some studies showing that different kind of animal feed or pet food or more recently raw pet food has been linked to some human illnesses, some studies were supported by the whole genome sequencing data. The first study over here is actually conducted by Vet-LIRN. This slide shows some of the prevalence in the AMR study published specifically by FDA researchers. This is again dating back to a little bit earlier,

1995, FDA survey determined *Salmonella* contamination in animal feed. In that study, the contamination rate was above 50%. More recently published, Xunde publish this study on the *Salmonella* surveillance program conducted by CVM at FDA, that shows some of the antimicrobial susceptibility data and then we also have some retrospective analysis of pathogens in the animal feed and also pet food. Today, I want to spend a little bit of time talking about this 2020 publication on prevalence of antimicrobial resistance ability of indicator organisms *E. coli* and *Enterococcus* isolated to the U.S. animal food. This was also a more retrospective study.

So, in this study, we collected over 1,000 samples. A majority of them are pet food samples and about one-third of animal feed samples. You can see overall the *E. coli* prevalence of 12.5% and 45.2% was positive for *Enterococcus*. And the prevalence differs between these two commodities so animal feed on the higher end and pet food was lower.

So, we specifically took an animal feed data and compared the antimicrobial resistance with NARMS retail and NARMS animal cecal sample, around the same time period. So, this bar chart is showing the *E. coli* data. As you can see, the animal feed was blue and NARMS retail was orange and gray is NARMS animal. The asterisk is showing not a significant difference, so, as you can see, majority of the antimicrobial drugs tested, animal feed do carry significantly lower resistance risk compared to NARMS retail or NARMS animal. So only for this drug, these three drugs there was not a significant difference, so they are a similar rate.
So, another slide for this indicator organism study is that the takeaway from this

study is that we found generic *E. coli*, not pathogenic *E. coli*, these are the indicator organism we're looking at, and *Enterococcus* were commonly found in animal food commodities, and the AMR in animal food was significantly lower compared to NARMS retail and NARMS animal samples. Nonetheless, among the samples we tested, we found the multidrug resistance was observed in about 3.3% of isolates from *E. coli* and *Enterococcus*.

This study was a little bit outdated, 2005 to 2011. It is a historic funding to have the baseline for AMR of these indicator organisms in animal food. In the past decade, we had many regulations related to animal food including preventive control for animal food safety that's part of the FESMA regulation and also we have judiciary use of antimicrobials in animal feed so those regulations may have affected the scenario of AMR in animal food so maybe this is worthwhile to conduct a periodical look into this commodity.

So, now I'm going to talk about *Salmonella* AMR in animal food. *Salmonella* is considered a major microbial pathogen in animal food, among the hazards in animal food. So, in 2021, we presented an oral presentation at an IAFP meeting, this presentation was on phenotyping and genotyping characterization of *Salmonella* resistance within the US FDA's foods program. For the remainder of my presentation, I'll talk about findings from this study.

Background and objective. So, some of you know that FDA's foods program collects and tests regulatory samples including a lot of human samples and some of them are animal food samples, and there are also environmental samples

including swab samples collected from food facilities during inspections and during surveillance compliance program, during outbreak investigations, or when they need to follow up with consumer complaints. Many matrixes covered in this program are not routinely captured by NARMS. The objective is to characterize *Salmonella* isolated from those sample collections. We summarized over 20 years of data from AMR including by *Salmonella* collected from this program.

Sample processing workflow. So, sampling was conducted by FDA's regulatory laboratories at the Office of Regulatory Affairs. Their processing was following standard methodology, including AOAC and some methodology in bacteria analytical manual published by FDA's BAM. After isolation, there is genotyping performed by whole genome sequencing, as some of you were able to hear, the very wonderful technical presentations on WGS yesterday. In the meantime, phenotypic AST was performed by ORA laboratories, and for WGS, all the data uploaded to Genome Trakr and part of NCBI's workflow are AMRFinderPlus, the reference gene catalog was used to find the AMR genes among those isolates. This slide gives you a snapshot of isolates collected in this program. We have about over 8,000 *Salmonella* isolated from over 5,000 samples spanning from 1999 to 2021. Among the sample composition, you can see about 15% are from animal food and then majority of them was from human food. In terms of distribution by country, 37% are domestic and followed by other countries, Mexico, India, Indonesia and other Asian countries and Canada is somewhere here. For major *Salmonella* serovars detected in the sample collection, we have Weltevreden as the leading serovar, Senftenberg is the second one, followed by Newport, Enteritidis, Typhimurium, and others. So, for the AMR testing, here is

the presentation on the phenotypic testing, basically the MIC testing, looking at over at over 8,000 isolates like I mentioned earlier. We break the data into a human isolate and animal isolates. You can see the animal food isolates. Human food isolates was in blue and animal food isolates was in red. Keeping in mind that we do have a large majority of the samples were from the human side. So, while the general trend that you can see the resistant trends together, you can see when there's no resistance to meropenem, neither isolates from animal or human food had resistance, and when there's resistance with trimethoprim, both had similar levels around 2%. The highest resistance we can see in this data sets is less than 14% for tetracycline from animal food isolates. The general idea from this slide is that we do see that Salmonella from animal food and human food tended to have similar trends for resistance, but in general, the resistance level is low so most of them are under 10%.

Nonetheless, we do find multidrug resistance in animal food isolates, so here are some examples. You can see a lot of them are from pet food and pet treats, in terms of the resistant genes identified, some are not only multidrug resistant. It looks like extensive drug resistance and those are the only examples we were observing from the over 8,000 samples isolates.

So, the takeaway from this food program data is that we see a diverse *Salmonella* serovars found in both human food and animal food collected in the last 20 years of data, and AMR occurrence was not common. Over 80% of isolates lacked any AMR determinants by WGS and multidrug resistance was detecting some in animal food isolates.

So, we think more research is needed to determine if we do want to implement AMR monitoring program for NARMS. So, whether this is necessary or beneficial way to spend NARMS resources, but we do think continued the research is needed to shed more light and to fill more knowledge gaps in this field.

So finally, I want to acknowledge Office of Research, many of the microbiologists who are involved in this work and from the Office of Surveillance and Compliance, including Jenny, who is able to be here with us and ORA analysts who did a lot of the Food Program testing and the NARMS leadership team for the opportunity to speak here. Thank you. I'm going to turn this over to you, Dr. Franklin.

Objective 1.4: Add routine testing of seafood products and imported foods and conduct pilots to explore other possible sources of resistant bacteria affective health such as minor food-producing animal species, produce and wildlife – Moderator Dr. Alison Franklin
Time- 04:02:34 – 04:03:14

Thank you, Dr. Ge. With that, we'll move on to our next objective which is objective 1.4 which is to add routine testing of seafood products and imported foods and conduct pilots to explore other possible sources of resistant bacterial affecting health such as minor food producing animal species, produce action and wildlife. Our first speaker is Dr. Heather Tate.

Dr. Tate is an Epidemiologist in the Division of Animal and Food Microbiology and has been working with NARMS since 2008. And with that, I will turn it over to Dr. Tate.

An Update on Seafood Testing in NARMS– Presenter Dr. Heather Tate
Time- 04:03:20 – 04:20:33

Thank you, Dr. Franklin. All right. Let me see.
Hopefully you can see the presentation mode for my presentation. All right.
Thank you.

All right. So, I'm going to give an update on what we've been doing with regard to seafood testing in NARMS over the last couple of years. In the 2021 NARMS strategic plan, we decided to incorporate seafood testing as we shifted to a one health paradigm, and that's because, as you can see by the diagram, seafood is an exemplarily intersection of all of the spokes of one health.

And because we had never tested seafood before, we had to develop a pilot study to decide which bacteria and commodities were best to establish our existing long term monitoring program. Our pilot study, in collaboration with the eight states and university lab, was published in a June issue of the Journal Frontiers in Microbiology and for the remainder of the talk, I'll walk us through the pilot work and then at the end, I'll show a bit of our more recent monitoring data.

The samples that we collect reflect seafood consumption patterns in the U.S. population. According to NFI, the four most highly consumed products have consistently been shrimp, salmon, canned tuna and Tilapia. Because we test raw product, we excluded canned tilapia and then shrimp, salmon excuse me, we excluded canned tuna and then shrimp, Salmon, and Tilapia comprised almost

half all seafood consumed in the U.S.

To select the target bacteria, we relied heavily on published text showing the prevalence of various bacterial species in fish, as well as preliminary results from metagenomics testing that was conducted by Dr. Daniel Tadesse.
We also wanted to look at carbapenem resistant organisms. Other studies had shown that seafood are carriers of these organisms which is important, especially in countries like the U.S. where carbapenem are not used in food animals. For the pilot, we worked with 8 retail meat sites and they collected a total of 710 salmon and 710 shrimp and later in that year, we added Tilapia. Samples were collected from supermarkets on a monthly or twice per month basis.
From each seafood package, we recorded the country of origin and other information to analyze as possible risk factors for isolation of our target bacteria and for association with resistance to at least one antimicrobial. And here's some other sample descriptions that we recorded.

Every purchased sample was tested for *Vibrio*, *Pseudomonas aeruginosa*, *Enterococcus*, *Staphylococcus*, and *Aeromonas*. However, less samples were tested for *Salmonella*. We actually stopped testing for *Salmonella* midway through our pilot study due to the extremely low recovery we had.
All of the salmon and shrimp samples were tested for carbapenem resistance organisms as you can see in the red, but carbapenem resistance organisms or CROs, but that testing stopped before we began our Tilapia sampling.
We asked the participating sites to take a 25-gram sample of the seafood and enrich it in broth or different peptone waters and then plate to the different agars

that you see here. For the CRO, we used a M super carba chrom agar that had color markers for different Enterobacteriaceae.

Once the states recovered the isolates, they were shipped to the FDA where we used Vitek confirm the organisms and then we performed susceptibility testing on the confirmed target organisms. We didn't confirm susceptibility testing on the carbapenem resistant organisms and I'll discuss what we did with those in a bit. So, here you see the prevalence of the target bacteria and the carbapenem resistant organisms. The orange line represents our preference for an organism to be included routine monitoring. Ideally, we wanted an organism that had at least 15% recovery. Of the target bacteria, so not looking at the CRO bars, the predominant genus in all was *Enterococcus* and then *Salmonella* had the lowest recovery with only one of the 506 salmon samples yielding and only 2 of the almost 500 shrimp samples yielding *Salmonella* and then we didn't get any *Salmonella* from the tilapia we tested.

We also found low levels of *Pseudomonas aeruginosa* in seafood. *Aeromonas* and *Staphylococcus* were present in approximately 20 to 30% of all seafood samples and *Vibrio* had the most variable rate of recovery from the different sources from about 7% in Tilapia to about 41% in shrimp.

Over 75% of the seafood samples harbored at least one presumptive CRO and I'm calling them presumptive because we did not confirm carbapenem resistance with antimicrobial susceptibility testing.

Here you can see the 28 different genera that we identified among the presumptive CRO isolates tested with the most predominant being *Pseudomonas*, followed by *Stenotrophomonas*, *Acinetobacter*, *Serratia*, and *Aeromonas*, which rounds out the top five.

And here's what the susceptibility testing revealed for the gram-negative isolates.

Overall, there was very low AMR. All three *Salmonella* were susceptible to all of the antibiotics tested. Less than 8% of Aeromonas isolates exhibited resistance to any drug tested.

Twenty-Seven to 40% of the *Vibrio* isolates ampicillin depending on the source and that really wasn't that surprising to us, given *Vibrio* are known to have some intrinsic beta-lactam resistance mechanisms.

Less than 6% of the Vibrio had resistance to all the other antibiotics that had interpretive criteria.

For *Pseudomonas aeruginosa,* there are only four drugs that have interpretive criteria under CLSI standards which is what we were following for susceptibility testing interpretation and all 19 *Pseudomonas aeruginosa* isolates were susceptible to these four drugs.

For the gram-positive organisms, we found out that *Enterococcus* was the most resistant organism with the highest levels of resistance, with the highest

resistance to tetracycline followed erythromycin. And *Enterococcus* isolates from tilapia did appear to have levels of resistance that were two to four times higher than isolates from shrimp or salmon for about five of the antibiotics that we tested.

We did find two salmon isolates that were resistant to Avilamycin, which is orthosomycin intended, at least in the U.S., only for use in broiler chickens and wiener pigs. And for *Staphylococcus* isolates, we saw that there were Tilapia isolates that had levels of daptomycin resistance that were approximately three to three and a half times higher than what we found in shrimp and salmon but less than 8% of Staphylococcus from all of the other all sources were resistant to all of the other antimicrobials.

If we want to compare what we're seeing in seafood to what we've seen in retail meats, *Enterococcus* is really the only organism, other than *Salmonella* which, again, we had very low isolation of that, it is common between the retail meat and the retail seafood testing. And *Enterococcus faecalis* were the overwhelming majority of the species that we identified in all of our seafood commodities. So, here we're comparing *Enterococcus faecalis* from seafood to *Enterococcus faecalis* from retail meats and we see that the resistance frequencies are similar so that when resistance is high in retails meats, it is also high, comparatively, in the seafood and when it is low in retail meats or nonexistence in retail meats, it is also low or nonexistence in seafoods.

The biggest difference observed is in resistance to tetracycline and resistance to

at least one antibiotic which is driven by tetracycline. But here you can see the proportion of land meats with tetracycline resistance was much higher than seafood.

Okay. So here are the results of the demographic analysis that we did to determine risk factors for growth. We found that shrimp had the highest odds of recovery of our target bacteria, so we determined that that's a top priority commodity to include in our long=term monitoring.

We also found that samples collected from Asia, Latin America, and the Caribbean were more likely to produce the bacteria. So again, that's something we want to consider as we continue with our routine monitoring. And then we also found that fresh or previously frozen seafood, which we call seafood sold at the counter, was also more likely to yield our target bacteria than seafood that was purchased frozen.

And then here are the results of the demographic analysis that we did to determine risk factors for resistance.

We found that shrimp are less resistant than salmon and, also, interestingly, farm-raised salmon and shrimp were less likely to yield a resistant organism than wild caught seafood, which is not something we would have expected but it did come out of the data. And what that means is that we definitely want to continue monitoring wild caught product for long term monitoring.

After performing susceptibility testing on our target bacteria, we select the those that were resistant to at least one drug and then we sequenced them along with isolates that were grown on the carbapenem containing medium. So, all together approximately 300 and so isolates.

Most of the CRO organisms did not undergo susceptibility testing as I said earlier, but we would do susceptibility testing to confirm an interesting genetic finding. With regard to the AMR genes we found, we saw that 33% of the target bacteria that were sequenced carried no known resistance mechanisms despite having phenotypic resistance to at least one antimicrobial and we found that 34% of presumptive CRO did not harbor any known antibiotic resistance genes even though they all exhibited decreased susceptibility to carbapenems because they grew on the carbapenem containing agar.

We just determined that it was probably intrinsic or other as yet to be discovered acquired mechanisms that were likely responsible for this phenotypic resistance in isolates that didn't have any known AMR genes.

We found 156 unique AMR genes among the 340 strains that were sequenced. Only 3 isolates had known transmissible carbapenemase genes and all of these isolates were isolated from the chrom agar. So, we found an *Acinetobacter* and *Aeromonas* both containing blaNDM-1 and Enterobacter containing blaIMI-2.
I do want to note that many of the organisms had resistance genes common to those bacteria such as qnrVC in Vibrio and various blaOXA genes and blaADC genes that is common to *Acinetobacter*.

Also, we did find that not all genes were indicative of a resistance phenotype and that's not surprising. There really has not been much done on genotype phenotype comparisons in the organisms that we isolated from seafood, particularly the organisms that were isolated from the chrom agar.

So, these are the final decisions we made for the routine monitoring program. Due to low isolation, Pseudomonas aeruginosa and also *Salmonella*, we decided not to include those in the routine monitoring program. We decided to keep Vibrio and also to keep Aeromonas, but expect that these organisms would yield low levels of resistance. We also decided to keep *Enterococcus* because it had the highest level of isolation among all the organisms tested and it could be compared to the terrestrial animal sampling that we're currently doing for NARMS. We decided to discontinue *Staphylococcus* because it had less CLSI breakpoints than *Enterococcus*. And we had our gram-positive organisms with *Enterococcus*. We also decided to discontinue the chrom monitoring because looking for carbapenems in seafood was like looking in for a needle in a haystack. So, that might be something we want might want to do less frequently.

So, since initiation in 2020, now seafood sampling has occurred in all NARMS sites. They collect two samples each of salmon, shrimp, and Tilapia each month and they test them for Vibrio, Aeromonas, and Enterococcus. We also ask them to send us MacConkey broths or colonies grown on MacConkey agar. This is actually part of our original attempt to isolate *E. coli* from the organism, but we could not figure out why we were not getting *E. coli*. So, we figured we would use these

lactose positive organisms for another project that we're currently working out.

Here is the 2021 data compared to 2019 and as you can see, there really is still very low overall antibiotic resistance among the organisms that we're monitoring. There has not been much of a change compared to 2019 except in the red where you can see we have increased resistance to tetracycline among *Aeromonas* from tilapia. And it looks like also now in 2021 compared to 2019, there's increased resistance to quinupristin-dalfopristin in *Enterococcus* from tilapia. And just quickly, in the future we're going to be comparing the genotypes and phenotypes of seafood organisms and we're going to be using machine learning potentially to do MIC prediction. We're also going to interrogate the contigs with important resistance genes for associated with mobile genetic elements and plasmid content. We are, again, evaluating lactose positive bacteria for resistance and other attributes.

And as Pat discussed earlier in his beginning, in the beginning of this conference, we are considering what the future of seafood monitoring will look like and how frequently we will collect seafood. And I want to thank all of the folks. This was a Herculean effort to get it off the ground and running and these folks had a huge hand in it. Here's the QR code for the paper which I discussed. Thank you.

Objective 1.4: Add routine testing of seafood products and imported foods and conduct pilots to explore other possible sources of resistant bacteria affective health such as minor food-producing animal species, produce and wildlife – Moderator Dr. Alison Franklin
Time- 04:20:40 – 04:21:27

Thank you, Dr. Tate. And with that, we'll move on to our next speaker who is Dr. Tameru Berhanu. He's a Senior Risk Analyst with the Risk Assessment Analytics Staff and the Office of Public Health Science in the U.S. Department of Agriculture's Food Safety and Inspection Service.

He is a member of FSIS's National Antimicrobial Resistance Monitoring System's workgroup team. He has vast experience in risk analysis, biostatistics, and mathematical epidemiology. Prior to joining USDA FSIS, he was in academia as a tenured professor.

And with that, I will turn it over to Dr. Berhanu.

NARMS Expansion Projects: FSIS Update – Presenter Dr. Berhanu Tameru
Time- 04:22:08 – 04:36:17

Thank you, Dr. Franklin for the introduction. Good afternoon. My name is Berhanu Tameru from FSIS. Today I'm presenting on behalf of the FSIS NARMS Team, the NARMS Expansion Project Update. The key person, Dr. Glenn Tillman, the Branch Chief, from our laboratory is also here. So, this is the outline of my presentation. I'll start with rationale and goals and summarize findings with concluding remarks.

I believe this group is all familiar about FSIS, the Food Safety and Inspection Service. FSIS is responsible for ensuring the meat, poultry and egg products are safe and that they are properly labeled and packaged. Inspections conducted in fiscal year 2021 include 165 million heads of livestock, 9.6 billion poultry carcass, 2.8 billion pounds of liquid, frozen and dried egg products. Samples test and results for fiscal years 2021 include 130,000 samples, 795,000 tests, 2.9 million results.

About FSIS NARMS, given FSIS mandates, the Agency has the opportunity to participate in NARMS to sample food producing animals and products for pathogen and indicator. FSIS's NARMS program focuses on antimicrobial surveillance in pathogen, *Salmonella* and *Campylobacter*, and indicators, *E. coli* and *Enterococcus*. FSIS operates the cecal part to traditional NARMS and the NARMS expansion project through interagency agreement, or IAA with the FDA. And 2017, the NARMS review subcommittee of the science board recommended that NARMS surveillance adapt a strategic one health model antimicrobial resistance and AMR monitoring.

In 2020, NARMS expanded testing of animal species, food products, and bacterial type and included environmental testing for antimicrobial resistance.
As Dr. Patrick McDermott described in the introduction and other presenters, one health is defined as a collaborative multisectoral and trans-disciplinary approach working at the local, regional, national, and global levels with the goal of achieving optimal health outcomes, recognizing the inter connection between people, animals, plants, and their shared environment.

The NARMS program adopted the one health model, human, animals, and environments in 2018 and was used in developing the new 2021-2025 strategy plan.

The goals for NARMS expansion project was to explore antimicrobial resistance which may exist in food producing animals are not routinely sampled under NARMS.

New sampling. The NARMS expansion project included new sampling, adding analysis to existing sampling and some lab related investigation.
New sampling and analysis included *Salmonella* in cattle mesenteric lymph nodes (MLN) and *Salmonella*, *Campylobacter*, *E. coli*, and *Enterococcus* in veal, goat, sheep, and lamb.

Existing sampling new analysis. Additional *E.coli* and *Enterococcus* analysis were added to the existing explanatory Siluriformes *Salmonella* sampling.

Lab projects. One part of the lab investigation included examining microbial diversity to ensure that by looking for antimicrobial resistance in *E. coli* only we do not miss out on additional resistance which may be present in other bacteria closely related to *E. coli* such as *Citrobacter*, *Klebsiella*, *Pseudomonas*, etc. The other part of the lab investigation included looking directly for group bacteria that are resistant to critically important antimicrobial called carbapenem and this group is called that resistance carbapenem resistant Enterobacteriaceae.

The finding for NARMS expansion as a take home is summarized below. New sampling analysis, especially veal, will conclude in fiscal year 2022. 40% of E. coli isolates and 25% of *Enterococcus* isolates were multidrug resistance, MDR. 33% of *Campylobacter* isolates were resistance to ciprofloxacin, nalidixic-acid and 93% of multidrug resistant *Enterococcus* isolates were resistant to erythromycin.
And goat sheep, and lamb analysis will conclude in fiscal year 2022.

32% of *Campylobacter* isolates resistant to ciprofloxacin and nalidixic acid, plus at least one other class of antimicrobial. Cattle MLN analysis, *Salmonella* concluded in fiscal year 2021 and MLN *Salmonella* isolates were mostly pan-susceptible. *Salmonella* serotype among MLN isolates compared to cecal and ground beef were not the same. And Siluriformes analysis concluded in July 2022.
28% of *E. coli* is resistant ciprofloxacin to plus at least one other class of antimicrobial. Lab analysis, AMR diversity analysis concluded in fiscal year 2021. Genus *Escherichia* capture represents AMR diversity in enteric microbes. Carbapenem resistance (CRE) concluded in fiscal year 2021. Very few findings of concern. The NARMS program will not include the NARMS expansion surveillance items in fiscal year 2023. However, we will revisit these categories for inclusion in fiscal year 2024. We note here that *Salmonella* in cattle MLN are predominantly pan-susceptible, meaning we do not need to be concerned about MLN as a potential source of AMR to other limp nodes.

The sampling design similar to traditional NARMS. Establishments selected for cecal sampling are part of a stratified sampling schema that stratifies establishments based on size and slaughter volume, 12-month head count as you

see on the right side of the table, with each size group having an establishment, number of monthly samples are assigned to it, based on the percent type. Establishments are then selected at random from each size group.

The objective of this study was to see *Salmonella* in cattle mesenteric lymph nodes of an antimicrobial resistance and to see if these are similar to those seen in cattle, ceca or gut, as the ceca gut pathogens are known to transfer/translate to MLN and then to other lymph nodes via the lymphatic system. Note here, even though two of them are the same, the third serotype in MLN was Anatum and for cecal it was Newport, so they are different. *Salmonella* isolates in MLN was recovered from 63 samples, almost 15%. Note this recovery rate is similar to what's recovered from cattle ceca or gut.

Here we see the veal finding reveals that 95% of *Salmonella* isolated from veal is pan-susceptible. 40% of *E. coli* isolates and 25% of *Enterococcus* isolates are multidrug resistant. Very small percent *Campylobacter* recovered,33% of *Campylobacter* isolates are resistant to ciprofloxacin and nalidixic acid. Differences in *Salmonella* serotypes and their ranking was observed. Also difference in serotype diversity and AMR were observed among the different veal categories.

Currently, lamb and goat sheep products are not sampled for antimicrobial analysis and regulatory verification program. The ceca NARMS sample is the only source of information for any AMR information goat, sheep, and lamb. So, it is very good information.

So, while a direct comparison between fecal and ceca gut samples cannot be made, in USDA FSIS's 2011 national sheep study, resistance to ciprofloxacin or nalidixic acid was about 6% in fecal *Campylobacter* from slaughter. Our ceca gut *Campylobacter* resistance of 33 % seems to be several fold highs. We also observed that MDR in goat and lamb and sheep is low and *Salmonella* serotype distribution in goat is different compared to lamb and sheep. Siluriformes analysis concluded in July 2022. More than 90% of *Salmonella* isolates were pan-susceptible, 28% of the *E. coli* isolates were resistant ciprofloxacin plus at least one other class of antimicrobial. About 28% of *E. coli* from Siluriformes being resistant to ciprofloxacin and nalidixic acid is a notable observation. We note here that a comparison between Siluriformes aquatic and other food producing animals which come from land-based animal should be made with caution as the rearing environment, production practice such as feed and drug usage, and so on are different, and that Siluriformes are fillet and not cecal samples. The diversity and CRE lab studies focus was to ensure that by focusing on *E. coli* as the only gram-negative indicator, we do not miss out on additional antimicrobial resistance that may be present in other closely related bacteria, despite the variety of gram-negative bacteria recovered, example *Citrobacter*, *Klebsiella*, *Pseudomonas*, etc. The genus *Escherichia* is a good indicator of antimicrobial resistance for this group. The NARMS program will not include the NARMS expansion items in its surveillance in fiscal year 2023. However, we will revisit these categories for inclusion in fiscal year 2024.

Here, we note, *Salmonella* in cattle MLN are predominantly pan-susceptible, and

we do not need to be concerned about MLN as a potential source of AMR to other lymph nodes. And thank you. That's all.

Objectives 1.3 and 1.4 Q&A – Moderator Dr. Alison Franklin
Time- 04:36:26 – 04:48:45

Thank you and now we'll move into Q&A session. So, this is for both objective 1.3 and 1.4. As of right now, I don't see any questions in the Q&A box, so please feel free to put your questions in there. And while we're waiting for the audience to come up with some questions, I had a question for Dr. Ge. Did you see, I noticed you listed different types of animal foods. So did you see any difference with regards to rate of resistance by animal food type?

The thing that made me think about it was the fact that you listed raw food so I was thinking maybe with raw food you might see increased resistance in that versus dry food.

Thanks for the question. We are, for this dataset, are just at the beginning, we still haven't come down to the final checking of the data. We have the overall picture. But I do think like your comments, it’s very important. It’s likely that the raw pet food will trend similar to the meat and poultry, but that will depend on further analysis.

Thank you very much.

So, I have a question from Dr. Tate.

Do you have any idea on what could have resulted in those increases in resistant organisms in Tilapia from 2019 to 2021? I wasn't fast enough to write down what the resistance was to, but it was, I know one was with *Enterococcus* and it went from 6.9% up to a hundred seemed like a huge difference, like a huge leap to go from that fairly low to high. I didn't know if you had looked into any possible reasons why and especially because it was the same with Tilapia with both instances.

So, the QDA, that actually, a lot of it has to do with the number. So even though these were statistically significant increases, there were very few isolates that were tested because QDA is really only applicable to *Enterococcus* faecium which is the proportion of faecium in seafood all together is very low. So, there were I think less than 10 isolates in 2019 and also less than 10 isolates in 2021 that showed resistance to quinupristin/dalfopristin. And still with that and the tetracycline, there were more isolates, and that was *Aeromonas*, there were more *Aeromonas* isolates. You know, I don't know, we can postulate that it might have something to do with increased tetracycline use. I haven't looked at the 2021 data. I haven't interrogated it enough to see if these particular Tilapia samples came from a specific country or region. And that gives use some clues about what might be used in that region. And then we could look at whether there was a trend in an increase of use in that region or something like that. So, I really don't know at this point.

Thank you very much. Yes, I can understand. It is complicated to tease out all

these little details. No, we do have a question in the chat and once I read this and it is answered, Dr. Garland had a question for Dr. Tate. This is a question for Dr. Berhanu. As far as I know, fluoroquinolones are not used for sheep and goats. Do you have an explanation for the observed high ciprofloxacin resistant rate in *Campylobacter*?

Thank for the questions and thanks for the presentation, Dr. Tameru. That's a great question and that's something we want to look in to more. We are going to most likely not do expansion project here, but this gives us a little time to delve a little further into what we're seeing. We've collected data over the last 2 to 3 years and we want to spend a little bit more time actually evaluating the data in a really super meaningful way. But at this point, we don't have any great kind of thoughts on why that resistance might be noted in the *Campylobacter*. Really appreciate the question. I'll take note of that. Thank you.

Dr. Garland if you wanted to ask your question.

Heather, I was wondering about your result that you had higher resistance in the wild type versus the aquaculture. And I wondered, I didn't see the actual data itself. Do you think that this is a strong consistent effect? And if so, any thoughts on what would lead to what you might think is a surprising result?

Yeah. It was surprising and we mentioned it in our paper as well, we didn't really have any rhyme or reason behind it. It would be interesting to see if we continue to see that if that observation is sustained in the 2020 and 2021 data as we go

through it but I really don't know. Like you said, it's you would expect that farm raised are receiving the antibiotic so wild caught are not. It could have to do, I think in the paper we suggested it may have to do with run off, going into oceans or estuaries or wherever these wild-caught fish are being caught from agricultural areas or sewage. But again, we're postulating at this point.

Thanks.

Dr. Tate. So, this question is for Dr. Ge. Considering the antimicrobials that are mixed into in animal feed and the fact we know that there are bacterial genera in animal feed, there may be selection pressure for AMR. What do you think about probiotics strains being added to animal feed it with respect to interacting with the bacteria in the animal feed?

Thank you for the question, Dr. Harbottle. I think that can go both ways. There could be a gene transfer between those two genera of organisms but there's not study in terms of the literature on that so that's definitely a research question we could look in to further in the future. Thank you.

And the next question is for Dr. Tate. Do the differences in fresh versus frozen seafood for AMR bacteria indicate that the establishment or food chain is playing a role in transmission, cross-contamination during transport, or at the sales establishments?

Yeah, that is a possibility. As we said, previously frozen or fresh, we labeled as

sold at the counter, and so when these fish are at counter, they are in an ice box with other fish, so there is possibility for cross contamination there. But it could also have to do with just the matrix and perhaps the bacteria are easier to isolate when the matrices are not frozen as opposed to when it is frozen. In the retail meat sampling, we are sure to collect meat that are not frozen, that are fresh as a result of some pilot work that had been done decades ago, and I imagine that was also part of the rationale there was that the bacteria were easier to isolate from foods that were fresh or thawed and not frozen but cross contamination is a possibility.

Just to follow up on that, just to clarify, so when you were isolating from what was concert for the seafood, so you were doing it when it was still frozen, you did not allow it to thaw?

Yes. Packages were purchased from the store and brought back and stored in the manner they had been purchased and then if they had to be stored, if they were not processed right away and then they were processed.

Thank you very much. Okay. This is a question for Dr. Tate again. Why do you think the yield of bacteria was higher in seafood sold at the counter?

Again, it could have something to do with the cross contamination, but it could also have to do with just the matrix allowing us to better isolate bacteria from things that are thawed or fresh.

So that covers the questions we've received so far in the Q&A box. if anyone has any other questions, and if you feel more comfortable saying just speaking it instead of typing it, by all meansm raise your hand.
That could be only for people who are panelists, I apologize. But for anyone else, please put your questions in the Q&A box.

It doesn't look like we have any more. I went through my prepared questions so Claudine and Pat, if you wanted to move to the adjournments.

Day 1 Adjournment – Presenter Dr. Patrick McDermott
Time- 04:49:23 – 04:50:31

Thank you, Alison. Thank you, everyone. Thank you for staying on. These meetings make for long days and there's no escaping that. But I want to thank all of the speakers especially who put in time to prepare for today's presentation. I hope you found it informative. I hope you were given a good sense of where the program has progressed. It is our last public hearing over the last 24 months so since we announced a new strategic plan. Tomorrow we'll carry on, it is an even longer day but mostly half the day will be the NARMS partner agencies listening in and hearing from stakeholders which has always been sort of a traditional form of this meeting to end with presentations from stakeholders. So, looking forward to that very much.

I won't keep anyone any longer than necessary. Thank you again for joining, and we'll reconvene tomorrow at 9:30 a.m. Eastern time. I don't know, Claudine, if you have anything you wanted to adhere at the end.

Nope. Thank you.

All right. Very good. We'll see everyone tomorrow.