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DEPARTMENT OF HEALTH AND HUMAN SERVICES

FOOD AND DRUG ADMINISTRATION

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OFFICE OF FOODS AND VETERINARY MEDICINE

CENTER FOR VETERINARY MEDICINE

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2014 SCIENTIFIC MEETING OF THE NATIONAL ANTIMICROBIAL RESISTANCE MONITORING SYSTEM (NARMS)

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August 13, 2014
8:00 a.m.

FDA White Oak Campus
10903 New Hampshire Avenue
Building 31 - Great Room
Silver Spring, MD 20993

INTRODUCTORY SPEAKER:

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SESSION V: RESEARCH

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SESSION VI: LOOKING INTO THE FUTURE

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SESSION VII: PUBLIC COMMENT PERIOD

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Food Animal Concerns Trust

SUSAN VAUGHN GROOTERS
Keep Antibiotics Working

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American Association of Swine Veterinarians

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M E E T I N G

(8:00 a.m.)

DR. McDERMOTT: Can we please take our seats and we'll get started?

Welcome back to the second day of the 2014 NARMS Scientific Meeting.

Well, just to briefly recap yesterday, so yesterday we spent some time sharing perspectives on how NARMS data are used by each of the agencies that rely on it and contribute to generating NARMS data. We touched on efforts in database development to improve data sharing and reporting, and we heard some very good presentations on efforts in the international arena. And today we're going to change and look at more of the research angle of things from the microbiologists and epidemiologists in NARMS.

But before doing that, to hear from representatives of the three agencies on how NARMS research aligns with the Agency goal. Yesterday we heard about AMR activities within the government, but as part of the broader scientific portfolio, this type of surveillance and research has a place in each of the agencies. And so our first three speakers will touch on that.

Then we'll hear from an on-farm pilot update from Eileen. Historical research -- or research on historical isolates from Daniel Tadesse.

Feed survey from Beilei. All at FDA. Those two are at FDA. And then some of the research into the molecular work that's ongoing.

We have one, two, three, four, five presentations from the epidemiologists on different aspects of NARMS-related work.

And then some time looking to the future. And we looked at this meeting as partly to take stock of where we've been since we started the current five-year strategic plan, but it's also to get ideas about how we might formulate the next strategic plan, and there are certainly issues of drug use that play into that. How that type of data are incorporated into NARMS and whole genome sequencing are two of the most salient examples.

Then we'll have a public comment period at the end, and I think we should be able to adjourn on time.

So, with that, I will introduce the moderator for the first session today, Dr. Maureen Davidson. And she will introduce and welcome our first set of speakers.

Dr. Davidson joined the FDA in 2007 at CDER as part of the Special Pathogens Group, working on Medical Countermeasure Initiatives in CDER. We were fortunate to get her -- steal her away to CVM in 2010, and she's been the Director of the Division of Animal and Food Microbiology there for several years, and that's the division within FDA in which retail meat testing is conducted.

And so I'll turn it over to you, Maureen, to get us started this

morning.

DR. DAVIDSON: Good morning.

Our first speaker this morning is Dr. David White. And many of you already know him. He is currently the Chief Science Officer and Research Director for the Office of Foods and Veterinary Medicine at the FDA, and his previous positions at FDA, he's also been the Director of the Office of Research in the Center for Veterinary Medicine; the Division Director for the Division of Animal and Food Microbiology; and the past Program Director of NARMS.

Dave.

DR. WHITE: Thanks, Maureen. Thanks, Pat. A pleasure to be back. I've been gone from CVM for two years, and it's great to see so many people that I have not seen since then. So I look forward to catching up at the break with many of you.

What Pat asked me to do this morning was to kind of give a brief story of what we're trying to do and how NARMS fits in with the bigger strategy of research and science across the Office of Foods and Vet Med. So if I can figure this out, I'll try to show you.

This is the FDA structure right now; it's very complicated. And most, you know, federal bureaucracies are very complicated and very large. And if you look in blue, here, this is what was created a few years ago, was the Office and Foods and Veterinary Medicine. This is run by Mike Taylor,

who is our Deputy Commissioner. Under the Office of Foods is the Center for Food Safety and Applied Nutrition, as well as the Center for Veterinary Medicine.

So my position was created about two years ago. I detailed for close to a year and then accepted the position. And function and responsibility was to coordinate the research activities across CFSAN and CVM, as well as the food and feed activities of the Office of Regulatory Affairs, where we have 13 of those laboratories across the country. So, overall, we have about 18 laboratories conducting some type of food or feed safety research and there were some pockets that talked well and others where there was no communication, whatsoever. So what I've been doing the past year is building infrastructure to kind of coordinate across these 18 labs.

And a little bit of what we do. So we created a team. My team is growing. I have now five people: three scientists and two project managers. They're a fantastic team. What we did at the beginning is we evaluated our inventory and what worked and what didn't work, and we realized that we needed some type of steering committee. So we crafted something called the SRSC -- as you know, the government is full of acronyms. This stands for the Science and Research Steering Committee. It's an interagency committee made up of senior science leaders from CFSAN, CVM, ORA.

We also added the National Center for Toxicological Research, because they do have a microbiology program. This past year, we've added someone from the Office of International Programs, which is our group that oversees our international collaborations with our federal partners globally. And then the Office of Chief Scientist, which is Steve Ostroff at this point.

Our primary goals are to develop strategic planning, figure out a way how we prioritize research across these groups, how we coordinate, in particular, methods development process, because we're very driven by methods development and validation, you know, both for microbial pathogens and for chemical hazards. We have a lot of laboratories doing this, and I would say three years ago we had a lot of laboratories potentially duplicating the same thing. And you would go to meetings and you would see the same titles of posters, but the authors were completely different. So the idea was to get these groups together so we could kind of focus on working together and also develop those priorities and figure out what those are.

So one of the things we're trying to do is align FVM's strategic science with plans that are already in existence, and we have the Foods and Veterinary Medicine Strategic Plan, which is online if anybody has an opportunity to look at that. We are crafting a new one that will be a 10-year strategic plan pretty soon. Again, if you have trouble sleeping at night, this is a great document to pull out. It's very long.

(Laughter.)

DR. WHITE: We also have the Advancing Regulatory Science at FDA. This was back in -- this was in response to the 2007 mission at-risk report that the FDA Science Board put out there saying, you know, we've got to invest in science at FDA. You know, we're a regulatory agency, but we're also a public health agency, and we have a significant science enterprise and a laboratory enterprise, and let's recognize that and get the funds necessary for us to do our jobs. And that's happened, which is nice.

So on the right, this is kind of the language from both. We tried to make sure it was the same. And, really, our goal is to strengthen scientific leadership, capacity, and partnership to support public health and animal health decision making. And you can see -- some of these bullets I won't go over, but it was established and implemented a centralized planning and performance measurement process, which I'll show you kind of what we've done.

So one of the key things I'm tagged with is how to establish priorities and manage crosscutting laboratory activities. And as you can imagine, it is an interesting responsibility and one where we've made a lot of good effort, but I think we still have a ways to go.

So, again, here's the steering committee that we created. We had a charter that was just signed. We had an initial charter from two years ago, but we had to revamp it. And here's our -- one, two, three, five -- our five top items that we're pursuing right now. One is to develop a single FVM

science and research strategic plan. We're working on that right now.

Develop a process for prioritizing research. That's a tough goal, if you think about it. Anybody that is working with a program, how do you prioritize what you do and what you don't do? And especially when we have hundreds of priorities across chemical hazards, microbial hazards, allergens, cosmetics, you know, veterinary drugs -- you name it. We don't have a money tree in the back. You know, we only have so many scientists, so we have to develop a process where we direct our people to engage in these highest priorities for public and animal health. So that's taken a lot of our time.

Again, develop and implement a unified analytical methods program. That's key. And we are just about ready to come out with a brand new document on validation that we'll be putting out on the internet at some point. That shows that any laboratory, be it CVM, CFSAN, or ORA, they're going to follow these guidelines. In the past, we've had three sets of guidelines. Unfortunately, that's not a good thing. So we need to have one set of guidelines that everybody agrees to.

Again, improving tech transfer to the program offices and field labs. So we have a lot of scientists in our center laboratories, and one of their main goals sometimes is to transfer methods to the field labs. We need those field labs to be engaged in the conversation at the beginning rather than at the end. Does that make sense? Right, we don't want to send them a

method and they go, I don't even have that instrument. Why did you spend two years doing that when I can't use it?

So the membership is quite large, it keeps growing. We've added many people. I don't expect you guys to read this. And I can provide these slides to anybody that would like them after the meeting, if you just send me an e-mail. But the key thing is we have the senior science leaders from CVM, CFSAN, ORA, NCTR, OIP, Office of Chief Scientist, and FVM trying to coordinate all this.

So one of the things we're trying to do is, again, how do we prioritize these crosscutting research projects? And, again, you first see all these damn acronyms, but it's the best thing to capture this rather than spelling it all out. Ideally, in red here in the box is what I'm trying to do, is the research is directed at the important strategic and regulatory goals and optimize the research capability of FVM and ORA.

So even though we're scientist-driven, the priorities have to come from the organization and have to be done together in a conversation. There just can't be a scientist running with something and then deliver something to us later on and we go, that was really cool science; tell me how I'm going to use that at FDA? And then the scientist goes, well, okay, I'll have to get back to you on that. You know, I mean, I found a new gene in *Listeria.* That's fantastic. How is it going to help me do my job at FDA? And if they can't answer that, that's something we have to have a conversation on.

So we established these Research Coordination Groups, or RCGs, which are based on discipline. We have four of them. We have a nanotechnology RCG, since nanotechnology is coming at us fast and furious in both foods and veterinary medicine. We have a microbiology RCG that Maureen Davidson chairs and Chris Elkins co-chairs. So what we try to do is have a chair and a co-chair from each of the operating units. So we'll have someone from CFSAN, someone from CVM, someone from ORA, and then they serve a one-year term and then we rotate again. So the idea is to kind of train people across the discipline so that we're coming up with a cadre of science leaders that understand that we're trying to do crosscutting research.

We have chemistry, which is Phil Kijak at CVM, John Callahan at CFSAN, and a brand new one that started this year was toxicology, which is Susie Fitzpatrick from CFSAN and Kevin Greenlees from CVM. So that's a brand new group, and we felt there are a lot of talks going on, you know, are we doing gut on a chip, lung on a chip, where are we moving with our toxicology characterizations? Are we moving from in vivo to in vitro mechanisms? This is going to be a big area for us to invest in and coordinate across the centers.

So when the RCGs got together -- they're not huge, they only may have, like, 8 to 10 people and they're made of division directors, branch chiefs, and so forth. We needed to get the bench scientists involved, so we created something called TAGs, which stands for Technical Advisory Groups,

and these are our bench scientists, our subject matter experts. And, again, they're made up of the bench scientists and the experts across CFSAN, CVM, and ORA. And we have quite a few of them.

So the MRCG stands for Micro, M for Micro. The CRCG is Chem for Chemistry. And you can see we're very -- these are all the disciplines we're working with. So this is what we're trying to manage, this really incredibly diverse research portfolio, in both. Now, we don't have any toxicology TAGs yet, but I can envision that happening in the next couple years, once it gets more specific and we get the experts together.

But for NARMS, to bring it back, we do have a drug resistance one, and Pat McDermott chairs that, that TAG. And you can see where it would have a lot of interaction with some of the other TAGs. We have a molecular epidemiology working group; we have an OMCS working group, which you're going to hear more about later today. I think Ruth is going to talk about -- from CFSAN -- how we're doing the whole genome sequencing. And you'll hear, I think, from Barbara and Jean about non-culturable diagnostics and how that's making an impact and how it will in food safety. And then we have a *Salmonella* group. So you can see where these TAGs are working together to kind of align our priorities.

So one of the ways we do this is, how do we get a handle on all the research we do at FDA? Well, we were lucky enough that years ago, CFSAN had a program that was called CARTS, which stood for CFSAN

Automated Research Tracking System, and it was a software-based system that captured every single research project, the resources involved, the title. It had a plain-language abstract, a number of FTs devoted to it. So when I came to FVM, I said well, we need CVM to participate, and we need ORA to participate. So we could no longer call it the CFSAN Automated Research Tracking System, so we tried to figure out -- we wanted to keep the C. What do we do? So we figured Components; I don't know why. At least it didn't change the acronym that everybody was familiar with.

But this is a tracking system that captures all research activity so you can query it. So if I wanted to look up *Listeria*, I could find all the *Listeria* research ongoing within FDA. This is, unfortunately, on the intranet, not the Internet. But one of our goals for next year is to put all of our information somehow on the Web so that people can see. Because one of the things we want to do is establish public-private partnerships, where if we get out the information in the gaps that we feel need to be looked at, let's go out there with the brain trust that's outside of FDA, as well as our federal partners, with universities, with industry, let's figure out where we can invest our brains and solve these questions that have plagued us for so long. So just kind of a glimpse of what we do in there.

And what we adopted this year, you'll see on the bottom, very simple. Quickly, we've adopted kind of a traffic light system for status of the project. So I can quickly go in and look. If it's green, I know the project is

progressing. If it's yellow, something has slowed it down. If it's red, that means something has stopped and I need to really take a look at it and see what happened. You know, has someone left? Do we need to get a new instrument or something's gone down? So it's a matter of us trying to track this research. Again, we have 300 active studies ongoing right now across CFSAN, CVM, and ORA, and that's a lot to manage.

So one of the things we found out with CARTS, unfortunately, it's not the answer to all of our questions. It's a great input-in tool, but the business objects part of it, excuse me, sucks. So we're trying to figure out how to do that. The information I want, unfortunately, like the federal government, most of these are being run by contracts to IT organizations. If anybody deals with them, they're black holes. You know, for anything you want to do, it's another $100,000 and four months later, they'll get you something. So to me, again, I hate to say, it sucks. So what we have to do is take this data out and put a new -- simple Excel spreadsheets or Access spreadsheets and figure out the best way to look at this.

So this is one of the examples we've done. It's a lot of work, it's a lot of manual effort. That's what we were trying to get away from. We want something that's automated that we can quickly run a business objects report and I can figure out where our scientists are working.

So, from left to right, we call this strategy-to-tactic. Strategy is the big end at the left, what we're trying to accomplish. And for this one, I

don't know if you can read it, but it says: Reduced incidents of illness outbreaks involving fresh produce. That's a pretty good public health call. Here's our regulatory goal: Agricultural and process practices that achieve no detectable human pathogens. And then we have knowledge gaps. So what's preventing us from getting here? So we have a series of knowledge gaps. And then to the right is: What research can be done to satisfy those knowledge gaps?

And then we have actually rankings here, so we have this whole process that I don't have time to go over that looks at priorities and we get people together. It's a long, drawn-out process, but by the end of the day, we have agreed-upon crosscutting activities, what our scientists are going to work on next year. Now, that doesn't bar, of course, emergencies or other things that rise up because something may happen like a melamine, that we have to change our -- you know, what we're doing and focus on that. And then, again, we have the status update on here so we can see what's happening. With the greens, are going good. Blue, something happened here. I forget all the color acronyms, but -- Maureen, do you remember what blue is?

DR. DAVIDSON: It's finished.

DR. WHITE: It's finished, fantastic. So that's a good one.

(Laughter.)

DR. WHITE: So thank you.

So here's the operational plan, if you look at it from a flowchart. We have the TAGs at the bottom, the Technical Advisory Groups. They feed into the Research Coordination Groups that then feed up to the CFSAN, ORA, and CVM line management.

(Cell phone rings.)

DR. WHITE: Unbelievable, sorry. That's the Commissioner calling. He says you better sit up. Don't say "sucks" anymore.

(Laughter.)

DR. WHITE: This goes up to the SRSC, and then this goes up to something called the FVMGB, which stands for the Food and Veterinary Medicine Governance Board, and that's made up by Mike Taylor and Howard Sklamberg, who are the Deputy Commissioners for Food and Vet Med and then Global Ops.

So this is kind of -- you can see it's very convoluted. It is vertical, but the idea is to do top-down, bottom-up, side-side, so that we're taking all this information and at the end of the day we have institutional support for what we're doing in that, you know, when someone goes out there, they have the backing of the organization behind them rather than, you know, this was an idea, I took it, I ran with it, I don't know where it's going. I mean, I don't want to stifle innovation; we need to have obviously some of that. But the majority of our research portfolio should be applied and not basic. Basic is for other organizations besides FDA.

Again, looking at it from on a calendar basis, from left to right is January to December. This is our schedule of where we're engaged in this process, and at the top is the planning prioritization phase. And at the bottom is the operational phase, and the bottom is the reporting. So we have these Research Coordination Groups report to the SRSC three times a year on the status of the crosscutting project. So it's a great discussion, and it's very open, and we have, again, branch chiefs and division directors and team leaders talking about the status of the projects, and the idea is to have each other talk to each -- you know, so we can figure out where there are problems, where we need to coordinate, and maybe areas where we need to invest in.

So right now, as you can see in this, we're in the midst of our timeline up to August, which is identifying our needs for next year for FY15. And each of the centers are doing this. CFSAN just completed theirs. I believe CVM is working on theirs, and ORA is working on theirs. And then what happens, in September, we have an annual two-day conference where all of the organizations bring their priorities. We break into these discipline groups, and we, again, push those ones up that we feel are crosscutting, meaning that there's interest between CFSAN and CVM or CVM and ORA. It has to be two of the three. We also have center-specific projects that are tracked by the centers because they're not crosscutting. But the idea is to kind of have the senior management of CFSAN, CVM, and ORA monitor where

the research is going, who the stakeholders are, right, and make sure that the research gets done and has the resources necessary to get done.

And then we come up with our EROs, which I don't know if I've mentioned yet, but those are Expected Research Outcomes. So, ideally, what we're trying to do in FY15 is push those out to the public so people know what we're trying to work on. That way we can start more developing public-private partnerships.

So, again, this is a nice segue in, that's one of the things I'm trying to do, is establish partnerships outside of FDA. I think NARMS is a great example of that, with CDC and USDA, with academics, with the states. I mean, you name it. NARMS is a great example, I think, of collaboration in science.

One of the things we host, this was our fourth year, we have a fourth -- I'm sorry, the FVM program science and research conference, it's internal. But in the past, we've had outside people. I think we've had Scott Hood from General Mills talk. We've had -- Rob Tauxe has talked at our conference. Caroline Smith DeWaal has talked at our conference. The idea is to bring in scientists, leaders from our federal partner agencies, as well as academia, industry, to talk about food safety and get our scientists to start thinking outside of FDA.

So these are just a few people who gave a talk. We had Stan Bailey this year from bioMerieux. For anybody that was at the food

protection meeting, there was a big session at bioMerieux about whole genome sequencing and the future of it in food safety. And it was interesting because there was some dichotomy in presentations. We had Eric Brown from CFSAN, who talked about whole genome sequencing being the next best thing since sliced toast, and then you had Paula Cray talking about well, we're not there yet. I'm going to say it's the next best thing as sliced toast, frankly. We're that close to implementing whole genome sequencing in everything we do.

But to give you a description of what we had at the program, we had 161 abstracts presented; it was really well attended. We had over 300 people from CFSAN, CVM, and ORA attend. And you can see, in terms of NARMS, we had 11 posters on antibiotics and antimicrobial resistance. Right now, one of our posters are online intranet, so we're trying to figure out if we can put them on the Internet so people can see them. So would people be interested in seeing these? Yes, great. It's going to get the IT people to work with you, it's a little complicated at times.

So, again, to segue into whole genome sequencing, you're going to hear more later today about the impact of whole genome sequencing in outbreak detection and attribution and so forth. It is a huge initiative across not only FDA, but CDC, USDA, and NIH. It's a great collaboration that's going on.

One of the things that FDA is looking at is something called the

Genome Trakr, and this may be something where NARMS would want to partner, consider partnering with Genome Trakr in the future. This is a state and federal lab network collecting and sharing genomic data from foodborne pathogens. The big thing about the Genome Trakr, it's an open-access genomic reference database. It's hosted by NCBI. And they are incredible partners in this. The talent goes beyond anything I could even comprehend. We're also involved with something called the Global Microbial Identifier, and I think Ruth will talk more about this, this afternoon.

Still many unknowns, obviously, with the use of whole genome sequencing, but we are moving there as the earth -- one of the things that we can show you that we -- how important it is to FDA, it was recently used to shut down or suspend registration of Roos Foods because they were able to identify the same *Listeria monocytogenes* in the cheese in addition to recovered from sick people. That was the first time whole genome sequencing was used by FDA to suspend registration. This is huge. I mean, to me, this is where we're going, and this is going to be in more and more of our activities day to day, is using whole genome sequencing.

So the non-culturable diagnostics and everything moving in, you can see we're rapidly looking at a revolution in microbiology that is actually already here. So we have to start talking now to plan for the future.

Again, I'll throw it out there. If anybody ever saw this quote. I try to make this applicable to whole genome sequencing. When

Steve Ballmer was asked, in 2007, about the iPhone, he said there is no chance that the iPhone is going to get any significant market share. How many of you have iPhones? I would say he was wrong.

So, again, if you put this into whole genome sequencing, I do feel that there is an incredible chance that whole genome sequencing is going to become a daily part of our activities for both attribution, for outbreak detection, for research. There are obviously questions with that, and like we were just talking with Cathy Logue about closing, closing the genomes is going to be a lot of information and a lot of work needs to be done on that. The bioinformatics part is going to be where we really need to invest.

The technology in doing the sequencing is already here. I mean, we can get it down to between $20 and $30 per run on a HiSeq from Illumina. And you can do 96 strains at the same time. That's just amazing compared to years ago where it was what, $5,000 an isolate? That's crazy. I mean, we're getting down to a point where this is really going to become economically feasible to do it on every single strand. That's the future.

So just to let you know, this also was a Secretary pick, the HHS Secretary. We have something called HHS Innovates. And this was an idea thrown out by the CDC, and it was called "Whole Genome Sequencing: The Future of Food Safety." And it was recognized by the Secretary, just a few weeks ago, as an innovation idea. And this was fantastic. It's a CDC/NIH/CFSAN partnership, and again, we're talking about the potential

biggest transformation of public health microbiology probably since the adoption of PulseNet. I think, if you look at that, this is kind of the next step forward. And it really does have the capacity to revolutionize foodborne disease tracking.

So just to let you know, this is really something we need to embrace as a food safety and both -- I would say veterinary medicine as well. I mean, I don't think vet med diagnostic labs have embraced this yet, but sequencers are getting so cheap that it should be something that AAVLD and other organizations should consider in terms of veterinary diagnostics getting into sequencing.

So, summing up, we're trying to look at a unified approach between foods and veterinary medicine program leadership, among the researchers and between the researchers and the policymakers, ideally geared to strengthening our core science and research capabilities in AMR. Antibiotic resistance is one of those core science areas we need to be expert at. We're trying to implement a unified analytical methods development validation program and improving our tech transfer to our program offices and field laboratories.

Again, the research is integrated across FDA and aligned with FVM strategic goals with a focus on public health, including the health of food-producing and companion animals. That's obviously critical for CVM. It's linked back to agency responsibility of why we're doing it, and we're

strengthening the linkages between the regulatory science researchers and the program office. That's key, is really making sure our scientists talk with the programs and that they're identifying their gaps, and then we go back and forth and it's a two-way conversation and not just the one.

So, lastly, the research outcomes are identified, documented, and communicated throughout FVM, ORA, the federal food safety research enterprise, and stakeholders. And I have "and stakeholders" there at the end because that's our goal for the next year and a half, is to take this information and get it out to the Web so people can see what we're trying to do.

So, with that, I thank you very much. I'm not sure what my time is because I don't have a watch, but I don't know if there's time for questions?

(No response.)

DR. WHITE: Thank you very much.

(Applause.)

DR. DAVIDSON: Okay, our next speaker is Dr. Barbara Mahon from the CDC, and she spoke with us yesterday already. Her master's of public health is from the University of California at Berkeley, and her M.D. is from the University of California. She is Deputy Chief of the Enteric Diseases, Epidemiology, and Surveillance Branch at CDC, which is responsible for the national surveillance of bacterial enteric diseases. She is also the acting lead for the NARMS team at CDC.

DR. MAHON: Thanks. Good morning. That looks like me. So that was a fantastic overview. Thanks, Dave.

I'm going to be taking a little bit -- I'm coming at this with a slightly different approach, to offer you a CDC perspective on NARMS research. And I wanted to start by going back to the slide that I showed you yesterday about the four core actions that CDC has identified to prevent the development and further spread of antibiotic resistance.

We focused yesterday on NARMS tracking, how NARMS is showing us where the problems lie, whether they're getting better or getting worse. NARMS research at CDC takes us out of tracking and into the other three corners of this box. NARMS research gives us information that's important for preventing infections, preventing the spread of disease, improving antibiotic prescribing and use, and developing new technologies, drugs, diagnostic tests, and so forth.

So a lot of NARMS research is based at the core on NARMS surveillance. So this slide shows a hypothetical distribution of MICs, minimum inhibitory concentrations, for a pathogen, for a drug. And what you see is that over here, at the left, the great majority of these isolates are in the lowest MIC and in the susceptible zone of the antibiotic, so 95.9% in this example. But you also see that looking across the higher MICs, going into the intermediate zone and into the resistance zone, we are seeing a few isolates there. And that's where NARMS research tends to be focusing.

So for those isolates that aren't just that plain old wild-type susceptible bug, we're looking at what genes are present that account for their different behavior. Are these infections worse when people get sick with them? Where are these infections coming from? What are the sources? If we know the sources, then maybe we can prevent people from being exposed to them. And how are patients responding to treatment?

So CDC NARMS investigates new and emerging resistance threats however they come to light. So this is an example of something that came to light not through routine NARMS surveillance, but it's an outbreak. So there's an outbreak -- this is a Morbidity and Mortality Weekly Report, an MMWR report, from last year on an outbreak of *Shigella* infections with decreased susceptibility to azithromycin that happened in Los Angeles in 2012. Now, azithromycin is a very important drug for treating *Shigella* infections, and so this is really concerning that this outbreak had happened. We hadn't really seen anything like this before. And so NARMS investigators working with state partners in California looked into this outbreak and wrote it up, got the word out to the public, and documented this recent emergence of *Shigella* with decreased susceptibility to azithromycin.

Now, what was also important was that they pointed out that clinical laboratories had no method available for testing *Shigella* for susceptibility to azithromycin. So this is a problem that our clinical labs have no microscope for, no tool to see. And so in subsequent work, NARMS

researchers have developed and validated methods that clinical laboratories can use for testing *Shigella* for decreased susceptibility to azithromycin and have shared those with CLSI and are working to make them available for clinical labs to actually be able to track this problem. This is very practical work.

Looking at another emerging threat, this is a paper that was published a year or so ago reporting on ciprofloxacin-resistant *Salmonella* Typhi. This is typhoid fever, a really bad disease with a high mortality rate when it's untreated. And in this case, NARMS researchers used linked surveillance datasets to look into what are the risk factors, where are people getting exposed to this resistant typhoid fever.

So they linked the data from NARMS, the resistance data from NARMS, to travel information from our National Typhoid and Paratyphoid Fever Surveillance System and were able to show that most, if not all, of these infections had actually been acquired when people traveled to India. And so this is important, not only for travelers and not only for the physicians who are advising travelers before they go to India, but also taking care of them when they get back and they're sick, but also for India, which doesn't have NARMS. So this information has practical value beyond the United States.

A lot of our research, both in NARMS and more broadly, in the enteric diseases groups at CDC, are focused on food source attribution using

NARMS data as one of multiple angles that we're using to try to get a better understanding of the sources of enteric infections.

Here's an example of a paper led by Maria Karlsson, who spoke to us yesterday afternoon, looking at the genes that were responsible for ceftriaxone, ceftiofur resistance in humans and in retail meats and in food animals, from NARMS. And what they found was a remarkable overlap in the specific genes that were responsible for this resistance and concluded that this information supports -- it doesn't absolutely prove, but it's one more line of evidence supporting meat and food animal sources as reservoirs for these human infections. Again, obvious practical implications to this work.

We spent a lot of time, a lot of effort, looking at genetic mechanisms of resistance, and this work is increasingly including whole genome sequencing, as well as other methods for characterization of mechanisms of resistance. This gives us insight into how resistance is spreading from one bacterium to another, and that, of course, informs control measures.

So, in this paper, led by Jason Folster, who will be speaking to you later in the morning, he and his colleagues looked at the plasmids, those little rings of DNA that were carrying the genes responsible for ceftriaxone and ceftiofur resistance in *Salmonella* Heidelberg during the large increase in resistance that we saw both in humans and in retail meats in 2009. And they showed that likely this increase was caused mainly by acquisition of plasmids,

in other words, horizontal transfer of those genes between bacteria in poultry, rather than by clonal expansion or just division of those bacteria.

NARMS research, as Dave White was saying, the research group in the enteric diseases laboratory branch is actually called applied research. All of our research is applied research. It's intended to provide information for action, and if it doesn't, then we don't want to be doing it. So NARMS data, NARMS research, looking at the consequences of fluoroquinolone, *Campylobacter* infections in humans, and the sources of those infections were essential to FDA policy decisions that led to the withdrawal of poultry fluoroquinolones that we talked about yesterday.

So I wanted to kind of connect this to what you're going to be hearing later this morning. NARMS research focuses largely on the topics that are listed here; we also go beyond these, but some of the major questions that we need to know to be able to control resistant infections. Trends in resistance: Are specific resistance patterns going up, going down, and how can we measure that most accurately and most usefully? You'll be hearing Cita Medalla give a talk on the work that she's leading on this.

Risk factors for resistant infections: Where are they coming from? What are the travel sources, food sources? What exposures do these people getting these infections have in common? Allison O'Donnell will be talking to you about her work on *Salmonella* Enteritidis, which is just one small piece of information that adds to our overall knowledge on the risk

factors for resistant infections.

The clinical outcomes of resistant infections: Do people have longer illnesses, worse illnesses? I talked to you in a very general sense about some work that's been done showing that yes, these infections do tend to be more severe. Beth Karp is going to review some work done in NARMS that adds to this body of literature.

Food source attributions: What are the foods that are associated with resistant infections? Allison Brown will be talking about that.

Genetic mechanisms of resistance: Jason Folster will be giving us a talk on genetic mechanisms of resistance that casts a very important groundbreaking way of looking at food source attribution. So that will be coming up later this morning.

And then, of course, we also, as I've mentioned, developed laboratory methods for researchers and for patient care to be able to track these problems.

The methods that we use obviously involve both epidemiology and microbiology and usually the two together, because when we have both epidemiologic and microbiologic data linked, we can learn a lot more than we can with either one alone.

On the epidemiologic side of the house, a lot of our recent research has involved using linked surveillance datasets. And, again, by linking information from more than one surveillance system, we can get

insight that goes beyond the insight that we can get from one of them alone.

And then, on the laboratory side of the house, most of our work involves advanced molecular characterization of resistant isolates and increasingly whole genome sequencing. I don't think we're talking specifically about our work on whole genome sequencing today, but I'm sure that Jason would be happy to talk with any of you who have questions.

So I'll be happy to answer any questions if you have any. I think it's going to be a really interesting morning. I know I'm really looking forward to hearing all the talks.

(Applause.)

DR. DAVIDSON: Our next speaker is Dr. Eileen Thacker from the USDA. She received her bachelor's of veterinary science and doctor of veterinary medicine degree from the University of Minnesota. She moved to Michigan State University and she also got a Ph.D. there. She was a member of the faculty of Microbiology and Preventive Medicine at Iowa State University, which is where I first met her when she was working on mycoplasma diseases. And she eventually moved to the USDA as the head of ARS, National Program Leader in Animal Health, where her responsibilities include overseeing the bacterial and parasitic research projects for the agency. She is currently the interim National Program Leader for Food Safety, and she is overseeing the ARS research on pre-harvest food safety for animals and leads the research and direction on antimicrobial resistance research and

policy at ARS.

DR. THACKER: Good morning. Now, my talks on research with USDA are going to be a little bit different than the last ones because USDA is such a very large department with multiple agencies and the multiple agencies have a very diverse background. But we all recognize NARMS as being extremely important.

USDA, like I said, is multiple agencies, and while in the past year antimicrobial resistance has drawn us together, as we've recognized how important this is, we have even been mandated by the White House to really start working on antimicrobial resistance. So, in the past year, all of our agencies have worked together. However, as we look at the USDA and our missions and we look at NARMS, each of the agencies has a little bit different relationship, often minimal relationship, but I think all of us are very aware of NARMS.

Of the multiple agencies, ARS and, of course, NIFA are the predominant research agencies. That is the sole goal of ARS and the National Institute for Food and Agriculture, and ARS is the Agricultural Research Service. APHIS has non-regulatory surveillance and through their National Animal Health Monitoring System, over the years they've also been involved with food safety and antimicrobial resistance. We have the Economic Research Service and the National Agricultural Statistical Service, and they are also involved. And this is only part, of course, of the USDA because this

doesn't even begin to discuss things like the food stamps and things like that.

So all of these agencies that are listed have recognized AMR as being very important and have recently worked together to develop an action plan that will hopefully someday be released to the general public. But at the base of all of this, all of these are very aware of NARMS and the important work that NARMS does.

Now, I'm going to start talking about ARS, and yesterday, as you listened to FSIS and different groups talking, they often discussed the Agricultural Research Service. The Agricultural Research Service is the intramural, the internal research program within the USDA. We have a $1.1 billion budget with multiple emphasis on all sorts of things. But, in addition, what's really important is that we have a strong research program in food safety, and this covers everything from animal health, food safety, crops, and antibiotics, which people don't talk about very much but is a very real situation, as well as, for example, last week I was in Tucson at a meeting about antimicrobial resistance and the environment, and we have a strong environmental program also looking at antibiotic resistance even within the environment. Now, all of these are working on antimicrobial resistance independently of NARMS, but most of them are aware of it.

The group that works the most with NARMS, of course, is our food safety program, and now I'm not interim, I am now the permanent National Program Leader, so I'm actually National Program Leader fulfilling

two jobs, both animal health as well as food safety. The vision of our food safety program is to enhance and protect public health and agriculture through the development of technology, strategies, and data that safeguard food from pathogens, toxins, and chemical contaminants during production, processing, preparation, and thus increasing the safety of the food supply. And as you can see, that is a very strong vision, and it crosses both pre-harvest, which I predominantly do, as well as post-harvest with Dr. Jim Lindsay, and we cover the entire gamut of food safety. However, NARMS has always been acknowledged as a very important part of our national program.

In this program, we have a $98 million budget in 2013 with an additional $10 million in external funding raised from our scientists. We have 180 scientists plus post-docs, visitors, and students. So, as you can see, we have a very large food safety program within ARS with 64 appropriated projects.

ARS has been involved with NARMS since the inception. We used to do all of the culturing. Paula Fedorka Cray, as you saw her name repeatedly, and has recently unfortunately left our agency to become the department chair down at North Carolina State -- their gain, our loss -- has actively been involved with NARMS, and we're hoping to replace her. But, currently, we still work very closely with FSIS as they have taken over the culturing for the animals. And we also work with all of the different agencies

to provide research background and support. We consider the federal government agencies as our stakeholders, and that's very important to us.

So NARMS helps ARS refine some of our research goals on antimicrobial resistance because if NARMS has certain findings, we will explore further. Sort of, in a similar manner that Barb just talked about, we will take individual isolates and work that up and find out more that we can about them and what we need to do and, of course, this is with the animal isolates. But we work closely with the FDA and FSIS for outbreaks and providing support as needed. In addition, we also provide additional research to develop new technologies to improve food safety protocols, as well as some of the protocols used in NARMS.

Now, I'm going to change to the National Institute of Food and Agriculture, NIFA. Now, NARMS itself does not directly impact the requests for proposals that come from NIFA. NIFA's proposal process is internal and they -- while they recognize food safety and they have a very large food safety research program, NARMS would not play an important part of that. However, NARMS is critical for the researchers that are applying for NIFA grants because they will take the data from NARMS, identify the gaps, and therefore NIFA indirectly supports NARMS by supporting the research of the university or governmental scientists that are performing research. So this just shows that NARMS is also very important to NIFA.

Of course, FSIS, I'm not going to spend much time on that

because we know how important NARMS and what an inter-important partner in NARMS that FSIS is currently doing, and they are doing the culturing of the animal samples and working it out. However, whenever I contacted FSIS and asked them, they said that NARMS helps them to meet their strategic goals, and that is to strengthen collaboration among internal and external stakeholders, to use science to understand foodborne illness and emerging trends, and to implement effective policies to respond to emerging risks. But, in addition, it helps them to determine the relatedness.

And similar to what Barb was talking about, the animals component side of NARMS is also important in trying to trace back outbreaks in a real-time method, and so FSIS is working closely, of course, with NARMS to do that. They are active with whenever there's an animal outbreak -- or a food outbreak that's traced to animal sources and the molecular characterization of those isolates. And similar to what Dr. Hill talked about yesterday, I think that we're looking at more and more doing that in real time, and I think that's going to be critical as we piece together this antimicrobial resistance problem.

APHIS was historically engaged with testing for NARMS, although they're not directly engaged. You heard yesterday that they have signed an MOU to work with FSIS on outbreaks in a voluntary fashion, and they're becoming more and more, as all of our agencies are, engaged in addressing antimicrobial resistance through the action plan, through doing

surveillance, increasing surveillance. APHIS has always done surveillance through their national health monitoring system, and I think this is only going to increase. While that's not directly connected with NARMS, it's also very important for animal foodborne pathogens.

So bottom line, you've heard several times yesterday about how important that stakeholder meeting was in 2012. USDA is very interested in what stakeholders say, we meet with stakeholders, all the different agencies meet with stakeholders on a regular basis. And they all say that NARMS is important, and we all recognize that. We also recognize the importance of antimicrobial resistance and maintaining the information to the stakeholders is critical. The stakeholders recognize NARMS as being important and want USDA to remain active and of course, we will do so. NARMS is recognized as a very important tool to help measure antimicrobial resistance in production animals.

So, to conclude, it's difficult to say, unlike what Barbara was able to do with CDC and say they followed this outbreak and that outbreak, NARMS has been very important. Over the years, ARS has worked with the NARMS isolates to prove -- and if you saw, Paula's name was on that when she was representing ARS. However, we also recognize, by FSIS stepping up to the plate and now increasing the culturing, doing cecal samples, whether it's APHIS that's helping doing on-farm studies or surveys from NAS and ERS, we all recognize that NARMS is a critical component and a unique system to

monitor foodborne pathogens in food-producing animals. And there are not a lot of other tools that actually collect this type of data and collate it and make it into something that can be used for the greater good of public health. So we certainly recognize the need to maintain and even to expand surveillance for antimicrobial resistance in production animals.

Thank you.

(Applause.)

DR. THACKER: Are there any questions?

(No response.)

DR. THACKER: Will I just stay up here?

DR. HILL: You can stay there.

I'm Joe Hill with FSIS, and Jean Whichard with CDC, and we'll be the moderators for the microbiology section. And without further ado, we'll turn it back over to Eileen to continue the talks.

Thank you.

DR. THACKER: Let's see. I think I have to do something different here. Okay, so this is going to change a little bit of directions and this is going to provide an overview of our on-farm studies that we have been recently doing in conjunction with NARMS with multiple agencies, multiple researchers, and also funded by FDA.

So these on-farm studies were begun actually in about 2011. And it was thought up by Dr. Mary Torrence, who was the National Program

Leader for Food Safety at that time with the FDA. I wasn't involved at all at this time and so -- and Mary left about a year and a half ago, so I stepped into this in the middle of these projects. But I think that it's been a very exciting and informative process, and I think that, we're hoping that this is something that we can really continue on.

Within each of the commodity groups, there were two to three projects over the three-year period, and the important thing to know is that they're not quite completed. I don't think any of the groups are completely done with all their data analysis. It was a tremendous amount of effort and an amazing amount of collection of isolates that had to be characterized, and so we're hoping within the next few months that this will be completed.

The goal of this project was to evaluate the relationship of food safety bacteria on farm as well as in slaughter plants. Also, it was conducted as a feasibility study for foodborne pathogens and AMR to be looked at as potentially a long-term process that could be added as a pre-harvest component of NARMS. So it's actually a feasibility study that needs to be determined whether this is something that can be done long term and what it will contribute.

Although not an original part of the study, since this time they've also begun to explore obtaining antimicrobial use information on the farms. This is something that some of the groups have been more successful in getting started than others, and it is providing a lot of information that

Dr. Craig Lewis will talk about later, as there is a lot of interest in collecting antimicrobial use on the farm. And also, to evaluate the logistical challenges and the potential value in adding a pre-harvest component to NARMS. Is this something that we need to be looking at of animals? What is the pattern of AMR on the farms compared to slaughter and then on down from farm to fork?

I'm not going to present any data today. The data is not really mine to present anyway. It belongs to the researchers. The researchers will be publishing, and this will all be available in the public forum in the future as the researchers reach their conclusions, just like with any other study that's done. So these were studies that were done independently, contracts were drawn up with the universities or with ARS, depending on the source of the researchers, just like any other study. And it is expected that they will be publishing their research in peer-reviewed journals.

One of the first challenges of this was to ensure the confidentiality of the producers and the people that were participating in the trials. It's critical, as we look at going out to a farmer and saying we want to come on to your farm, we want to collect your antimicrobial resistance pattern and take it back and do something with it, that they feel that they can do that without reading their name in the front page of the *Washington Post* the next day. Most producers can't afford that, and that would be an impact on their livelihood. So if we're to find out this information, confidentiality is a

critical component. And it did take a while to get that set up, so that this was guaranteed. So we're extremely grateful to the producers that were willing to allow our researchers onto their farms and their production systems.

Then we also had a number of challenges. This could be anything from the government shutdown to getting buy-in from the different industries, things like that. It's amazing how much time it took to just get this project going. The species that we evaluated were dairy and beef cattle and broilers and turkeys, as well as swine.

I will provide a brief overview of each of the studies by species, saying who did it, what they looked at, and just a very brief overview. Like I said, no data will be presented today.

With dairy cattle, dairy cattle was especially challenging because while we were trying to look at on the farm and in the slaughter, dairy cattle don't move out like swine and poultry and beef cattle in groups to slaughter. So it was a very big challenge for our researchers to be able to follow cows, sample them on a farm, and then to go to slaughter. So this is something that we've really found was a challenge for dairy cattle. The studies were conducted in Pennsylvania. They looked for *Salmonella* and *E. coli,* and the research was conducted by Drs. Van Kessel, Karns, and Harhay from ARS, as well as Drs. Wolfgang and Hovingh from Penn State.

They conducted three studies. The first one just started to figure out optimal sample collection, culture methods, how they were going

to do it. The second study they collected from two commercial dairy farms in Pennsylvania. Interestingly, one was positive for *Salmonella*, the other one was negative, and the results of this are really quite interesting as they followed those animals to slaughter. And then the third study, which the data is not yet complete, is a sampling of a cross-section of the dairy herds, pre- and post-weaned calves, dry cows, and lactating cows.

With our beef cattle, we had quite a group that -- three different groups that were sampling different populations with a little bit different goals. We had Dr. Loneragan that was testing cattle in Texas. We had Dr. Schmidt from ARS doing Nebraska. And we had Morgan Scott, who is here, looking at some of the antibiotic resistance.

And so this has actually been a very good study and provides a lot of prevalence, statistical variation, and antimicrobial resistance data across feedlots in both the major cattle producing states of Texas and Nebraska. They sampled for *E. coli* and *Salmonella* and then also looked at developing protocols for the detection of carbapenemase and ESBL genes. And this is important; if we're going to start looking for emerging problems and changes in antimicrobial resistance, this is going to be important.

Now, this was a group that was really impacted by the government shutdown because John had seven groups of cattle that he had been following for months. The government shutdown occurred right when they were going to slaughter, and all that data was lost. So this shows you

the kind of problems that can happen with research and when people say, well, why is this taking so long. I want to give you some explanations, that it wasn't that the people weren't trying.

With poultry, we're looking at broilers and turkeys. Dr. Hofacre from Georgia and Dr. Singer, who is here, from the University of Minnesota, have done a wonderful job in getting buy-in from the poultry industry. There have been three studies from 2011 until present. And they currently have enrolled over 60% of the broiler and turkey production. It took a while for them to convince the poultry industry that it was in their best interest to participate, that this knowledge needs to be collected, and so they have done a great job in gaining the trust and the participation of the poultry industry, because as you know, poultry is a very important commodity in association with food safety.

They also are beginning to collect some drug use information, and it's been interesting, as we've worked with the different commodities, the differences and willingness to participate in even providing on-farm drug information voluntarily. It's been interesting over the course that I've been involved with this. Some are very open and some less so.

But with the poultry industry, it's been great because Drs. Singer and Hofacre have provided a close relationship, they provide value-added information, and at this point, the isolates are not submitted to the FDA. This, again, goes back to that problem that we have that many

producers do not trust government employees, and I'll be the first one to say that that's a real problem that we have. They don't think that the government will maintain confidentiality and that they'll turn around and use the information in a regulatory fashion. So it's something that we have to be able to convince them that their information is safe with us.

If you look at swine, there we had a little bit of a different challenge. This research was conducted by Drs. Jim McKean, Frana, Catherine Logue, who is also here, Annette O'Connor at Iowa State. Annette kindly stepped in because unfortunately Dr. McKean passed away during the course of the study. He's greatly missed by all of us. In addition, we had the second complication of Paula leaving ARS to go to the North Carolina State in the middle of this trial also. So it was kind of challenging, as you look at a three-year study and what all happened.

The objectives of the swine group was to look at normal lairage practices on *Salmonella* and *Campylobacter*, as well as to look at the uptake of antimicrobial bacteria in lairage and looking at that in the cecal samples because, as you know, FSIS is starting to look at cecal samples and potentially use those as an on-farm measurement, and we were interested in looking to see how that stacked up. And so they've got some very good data; they're finishing up some of their culture.

Now, what they did was because, as you know, biosecurity is a major concern for the swine industry, and this was all happening at the same

time that porcine epidemic diarrhea virus was breaking out across the country. Needless to say, they did not welcome people onto their farms to wander around and collect isolates. So what Jim arranged was for dedicated, clean trucks to take the pigs directly to the slaughterhouse. They met them at the slaughterhouse prior to them unloading, did the testing then, as well as throughout the time that the pigs were at lairage. So, therefore, they did not go on to the farms, and they were able to prevent compromising biosecurity at the farms.

So, in conclusion, in 15 minutes it's very difficult to give a very definitive overview of a very large, complicated trial, multiple species, multiple institutions. I will have to say I really admire our researchers, and I really appreciate all that they have done and the participation of the producers.

We have gotten some really important information from the start: (1) Our sampling protocols and what we need to do has to be developed individually for each species and each commodity. We cannot just have one thing for swine, poultry, and cattle, beef or dairy. So we're going to have to be -- if we want on-farm testing, we're going to have to work with the subject matter experts for each commodity group to find out what works best for their system.

We have to be able to show the trust of the producers as well as educating the industry and the producers why we're doing this, that we're

not doing this because we're trying to shut them down or stop animal production, but this information that we need for public health and is critical going forward in controlling antimicrobial resistance. And I think that's something that we've really learned and we worked through pretty well for most of our groups.

Next steps. Big question. We're currently waiting to get the final results. We're working with the researchers and FDA to work out what the next steps will be. So I can't tell you exactly -- maybe Craig and Pat will be able to provide a little more, but I think that we have "the jury is still out" until we finally get the results and figure out where we're going to go.

One other small issue will be money. As you know, budgets in the government can be fickle, and with it being election years in the next two years, who knows what the U.S. government's budgets for even doing this kind of research will be.

So we have a lot of questions that will have to be resolved before we know what for sure the next steps will be. However, I do know that the information that we have gotten here is valuable, it will be able to be used to go forward, and I think we will be using that to go forward to determine if we're going to continue on-farm research. Dr. Lewis is going to discuss also the potential for gathering antimicrobial use in the animals, so this is going to be an ongoing topic.

Thank you.

(Applause.)

DR. THACKER: I don't know. Do we have time for questions?

DR. WHICHARD: Yeah. Do we have any questions?

(No response.)

DR. THACKER: Well, I guess I answered them all. Thank you very much.

DR. WHICHARD: I just have one. Will we collect up the papers, the peer review papers that come out of the on-farm pilots anywhere and post them on a website?

DR. THACKER: We'll have to see if we want to try and set up a webpage for everything that comes out. We haven't gotten to that stage yet, so we kind of have to wait and see. Because some of the studies may have actually been incorporated in the papers already, it's something that would be really good to put all together. But the studies are not completed yet, so we're still waiting. I think a lot of the researchers have already been telling me that they're going to be starting to present at meetings and things over the next year, so I think a lot of this will come out.

DR. WHICHARD: Thank you.

So our next paper in the Micro section is from Dr. Daniel Tadesse. Dr. Daniel Tadesse received his doctor of veterinary medicine degree from Addis Ababa University in Ethiopia in 2000 and a Ph.D. degree from the Ohio State University in 2009. Dr. Tadesse is a research

microbiologist working in the Division of Animal and Food Microbiology at the Office of Research at FDA. His research focuses on the effect of antimicrobial use on antimicrobial ecology in selection of antimicrobial resistant foodborne pathogens.

DR. TADESSE: Thank you.

For the next 15 minutes, I'll be talking about -- I will give you a historical perspective of antimicrobial resistance and how we can use historical data to explain the resistance that we are seeing in the current or modern isolates. Just to begin with, I will give a brief background.

The discovery of antimicrobial agents was a turning point in human history and drastically changed medicine in many respects and saved millions of lives. For example, from 1900 to 1980, mortality rate associated with infectious disease in the U.S. dropped from 800 per 100,000 population to less than 40 per 100,000 population. And since the discovery of the effect of antimicrobials in the late 1920s, antimicrobials have been in use for therapeutic purposes in humans and animals, for metaphylactic, prophylactic, and growth-promotion for animals.

And resistance is -- I know it's kind of natural selection, and generally the drug use has been complemented by the development of resistance. Whether we are using antimicrobial for prophylactic, therapeutic, metaphylactic, or growth-promotion, what's common in all cases is the antibiotics are released to the environment and thereby providing selective

pressure for the development of resistance.

Currently, there are more than 100 antimicrobials approved for clinical use, and here is the timeline for antimicrobials that we commonly use in human and vet medicine. And the first antimicrobial to be introduced for clinical use was sulfonamides. And sulfonamides was introduced in 1936 and have been in continuous use for more than 70 years now. And in 1940s, four antimicrobials from four different classes were introduced, and these are streptomycin, penicillin, chloramphenicol, and tetracycline. And in the 1960s, the first cephalosporin, which is cephaloridine, was introduced followed by cefoxitin in 1970s and ceftiofur and Cetraxal in 1980s.

If you see, most of these antimicrobial classes were introduced between 1940s and 1960s where we consider it as a golden era in the age of antibiotics. But what's important to note here is resistance has been developed for every major class of antimicrobials following their introduction to clinical use. For example, resistance to cefpiramide and penicillin were reported in the 1940s; tetracycline, streptomycin, and chloramphenicol resistance in 1950s; and cephalosporin resistance in 1960s.

Particularly over the last two decades, between 1990s and 2000s, bacterial pathogens are becoming increasingly resistant to most frontline antimicrobials, including extended-spectrum cephalosporin and fluoroquinolone. I'll say a word of caution here. The appearance of clinical resistance doesn't necessarily mean that the antibiotic lost its clinical utility

and that's why we are still using some of these antimicrobials.

While we had such success pre-1990s, there was no monitoring or surveillance systems that monitors the development of antimicrobial resistance. And, in fact, in many countries, a resistance monitoring system was established in the mid-1990s, and for example, one such program is enormous, the National Antimicrobial Resistance Monitoring System in the U.S. It was established in 1996 to prospectively monitor antimicrobial resistance among foodborne pathogens including *Salmonella* and *E. coli*.

To fill the information gap pre-NARMS, especially in terms of the antimicrobial resistance trend, we assayed historical *E. coli* and *Salmonella* isolates collected before the beginning of NARMS with a goal of (1) to better understand the historical emergence and trend of resistance since the beginning of antibiotic age, and (2) to provide a broader picture of evolution of resistance and help explain the range of resistance seen in modern isolates.

For this part of our study, we have looked at more than 2,000 *Salmonella* isolates obtained from human clinical cases between 1948 and 2003. Most of these isolates were recovered from stool samples and a few from blood and urine samples. And this table shows the distribution, the temporal distribution, of the isolates. And in terms of the serotype composition, Enteritidis was the most common followed by Typhimurium and Naples.

For the *E. coli* part of the study, 8 out of 1,729 historical isolates were included, and 57% were from human and 43, the remaining 43%, were from animals. The animal isolates were from cattle, chicken, and pigs. Again, this table shows the historical temporal distribution of the isolates.

I just want to say, this is a retrospective study, which means it relies on preexisting culture collection, and there is also limited or no information in terms of the rationality behind preserving these isolates, so there is a caveat on the dataset.

In any case, we did the antimicrobial susceptibility testing using microbroth dilution for a panel 50 antimicrobials representing these eight classes. And these are the same antimicrobials that I presented at the beginning in terms of their approval timeline.

And, generally, antimicrobial resistance was observed for almost all the drugs tested with different frequency and not expected. Most common resistance phenotypes were for older drugs. For example, 41% of *E. coli* and 10% of *Salmonella* were resistant to tetracycline, a drug approved in 1948. And similarly, 36% of *E. coli* and 11% of *Salmonella* were resistant to sulfonamide, a drug approved in 1936. And on the other end, a much lower proportion of isolates were resistant to a drug approved in the 1980s. For example, ceftriaxone, approved in 1984, and less than 3% of E. *coli* and less than 1% of *Salmonella* were resistant to this drug.

In the next few slides, I will first talk about the *E. coli* data, the most important part of the *E. coli* data, followed by the *Salmonella*. And this graph shows the change in antimicrobial resistance among *E. coli* isolates over the past six decades. Here the x-axis shows the year, and the y-axis shows the percent of proportion and the blue bar shows the proportion of *E. coli* isolates that were pan-susceptible. When I say pan-susceptible, it is resistant, these isolates were resistant to all the tested antimicrobials, the 50 antimicrobials that we tested. And the red bar on the other end shows multi-drug resistance, and again multi-drug resistance here refers to resistant to three or more antimicrobial classes.

The take-home message from this graph is the proportion of pan-susceptible isolates reduced or declined over time, while the proportion of multi-drug resistance increased. And bear in mind, during this time, more antimicrobials were approved and were being used for treatment and other purposes.

In terms of trend analysis, resistance trend analysis, human *E. coli* isolates showed increasing trend in resistance to ampicillin, sulfonamide, and tetracycline, while animal *E. coli* isolates showed an increased trend in resistance to 11 of the 50 antimicrobials tested.

Just to take two examples, ampicillin and tetracycline resistant trend in *E. coli* between 1950s and 2002. Here the x-axis shows the timeline for drug approval and the bar, this bar, is for ampicillin resistance. And

ampicillin was approved in early 1960s, 1961. And as you see, between 1960s and 1970s, there is a huge spike in terms of the isolates being resistant to ampicillin, and that increasing trend continues until recent.

Similarly, the tetracycline drug was approved in 1940s, as you may see on this figure. We have observed tetracycline resistance in 1950s, and that trend increased over time. And the blue bar here shows the animal isolates, and the red bar shows the human isolates, as you may appreciate on the graph. The animal isolates are more resistant to ampicillin than the human.

This is a graph to show you the distribution of multi-drug resistance among different sources. Again, the x-axis here shows the source: human, cattle, chicken, and pigs. The y-axis shows the proportion. Again, the blue bar shows the pan-susceptible, the proportion pan-susceptible isolate, and the red bar shows the proportion of multi-drug resistant. As you may see on the figure, a significant proportion of human *E. coli* isolates were pan-susceptible while on the contrary, a significantly higher proportion of *E. coli* isolates from animal sources were multi-drug resistant.

This graph is to show you the evolution of antimicrobial resistance in *Salmonella* between 1940s and 1990s. We just focused between this period because then in 1996 NARMS started. And here the x-axis shows that period or the year with the approval timeline for different antimicrobials, and the y-axis shows percent resistance. I only selected eight antimicrobials

here from eight different classes. And if we only focus on those antimicrobials that showed increase in trend -- this is an example, streptomycin, is approved in 1940s and we have been seeing resistance to streptomycin since then with a continuous steady increase in terms of resistance. Similarly, we have seen the same trend in tetracycline, as well as ampicillin and chloramphenicol. And sulfonamides.

Again, this is looking at the same data differently, and this is a trend, an antimicrobial resistant trend, between 1948 to 1996. If you remember, one of the objectives that we wanted to see this isolate was, can we learn something from the historical isolate that can help us explain the resistance that we are seeing in current NARMS isolates?

And if we compare the historical *Salmonella* isolates, both are human *Salmonella* isolates. If you compare the one historical human *Salmonella* isolate with a NARMS human *Salmonella* isolate, it really explains in terms of the resistance that we are seeing in the current isolates. And if you see, we have been seeing an increasing resistance trend in historical isolates for older drugs, like tetracycline, streptomycin, ampicillin. And, similarly, here we can see a larger proportion of recent isolates, NARMS isolates, also are resistant to the older drugs.

And I think later today there will be a presentation on the trends, on the *Salmonella* resistant trend in human isolates, so I won't go into detail. But what unique things that we are seeing here is, in terms of

ceftriaxone resistance in amoxicillin/clavulanic acid combination resistance is relatively higher in recent isolates than what we were seeing in historical isolates. And these two drugs were approved in 1980s.

And this table shows the multi-drug resistance by serotype, and as I've already indicated, the proportion of the isolate in terms of their serotype composition where Enteritidis was the most common, followed by Typhimurium and Newport, as expected and as previously indicated on yesterday's talk, resistance in *Salmonella* -- by serotype and that's what we are seeing in historical isolates also, and *Salmonella* Enteritidis tends to be susceptible, while *Salmonella* Typhimurium tends to be resistant. And we have seen, also, the ACSSuT pattern, which is a common pattern that we usually see in recent *Salmonella* Typhimurium isolates also were common in historical *Salmonella* Typhimurium isolates.

In summary, multi-drug resistance is not an innate feature, and generally, the use and resistance are closely related. And we have observed a significant upper trend in resistance to all the drugs. Particularly in animal *E. coli* isolates, we have seen increasing trend in resistance to 11 of the 15 antimicrobials tested.

And our results provide foundational information on resistance development over time, laying the groundwork for understanding the evolution of multi-drug resistance and help explain the range of resistance that we are seeing in modern *Salmonella* and *E. coli* isolates.

Finally, the molecular work is ongoing to elucidate the details of underlying genetic mechanism and also to compare with the recent isolates.

And thank you so much.

(Applause.)

DR. WHICHARD: Thank you.

Do we have any questions for Dr. Tadesse?

DR. FELDGARDEN: Mike Feldgarden, NCBI.

Two quick questions. First, how are the historical isolates stored? Were they stored in stab cultures, that kind of thing? And, second of all, does molecular characterization include whole genome sequencing?

DR. TADESSE: To begin from your second question, yes. It does include -- we are currently doing whole genome sequencing on the historical isolates, and we hope to put it to the public very soon. In terms of their storage, they were stored in stab for -- particularly the older isolates were on -- especially pre-1980s, they were kept in slants, and that's how they were. But the recent isolates were frozen.

DR. FELDGARDEN: Thanks.

DR. SCOTT: Morgan Scott, Texas A&M University.

I realize the more ways you chop the data, you end up with sparse cells. But can you confirm for me that the Category 3 to 8 for the *E. coli* in the '50s and '60s and '70s were more likely 3 than 8? So your

Category is 1 and then -- or 0, pan-susceptible, 1, 2, and then 3 to 8. And there's a big difference between 3, which would be tet-strep-sul and 8, which is ACSSuT plus who knows what. Do you have that in your head? Were those in the '50s and '60s more likely to be 3 than 5, 6, 7?

DR. TADESSE: Generally, that's correct. But I don't have the specific figure, but what we were seeing in all the isolates were resistance to the drugs that were approved before the date that we have the isolates. So most of the older isolates were resistant to streptomycin, tetracycline, and sulfonamide.

DR. SCOTT: Right.

DR. TADESSE: But the recent isolates, they showed resistance to all the 15 -- there are a few isolates that showed resistance to all the 15 antimicrobials tested. And, generally, MDR pattern was increasing over time. And, again, when I say 3, it's not antimicrobial agent. It is antimicrobial classes.

DR. SCOTT: Thank you.

DR. TADESSE: But I can provide more if you need the composition.

DR. HILL: Okay, our next presenter is Dr. Beilei Ge. Dr. Ge is a research microbiologist with FDA/CVM. Dr. Ge's research focuses on feed and food safety microbiology including rapid method development feed surveys and antimicrobial resistance issues in feed. Dr. Ge received her Ph.D.

degree in food science and food microbiology from the University of Maryland.

DR. GE: Good morning.

Animal feed surveys has the very beginning of the farm-to-table continuum. There has been continued interest in looking at feed as a format for introduction and transmission of foodborne pathogens into the animal production environment and also for feed as a vector for transmission and dissemination of antimicrobial resistant genes.

At FDA we have a continued program monitoring the bacterial contamination/antimicrobial resistance in feed commodities. It is my good pleasure to present some of the feed survey tests here with you which are conducted at FDA/CVM and FDA/ORA laboratories.

First, some background information about feed production. According to a recent survey by Alltech, the global feed industry is estimated worth greater than $500 billion. Among the 130 countries surveyed, there are 963 million tons of animal feed produced each year from over 28,000 feed mills. China and the USA rank the top two feed producers, and in the United States, there are 169 million tons of animal feed produced each year from over 5,000 feed mills.

So this pie chart shows the total feed by animal species. Among a total of 963 million tons, poultry feed takes the largest slice at 444 million tons, followed by pig feed ruminant, aqua, pet, and equine.

So at FDA, FDA/CVM is a primary agency regulating animal feed production in the United States. There are two anchor programs in terms of feed surveillance and compliance activities by FDA/CVM. The first program is called Feed Contaminants Program. This was started in 2002, which is umbrella program not including just microbials, also chemicals such as mycotoxin, elements, and other contaminants, chemical contaminants in feed. In terms of microbial contaminants, primarily *Salmonella* is a pathogen under surveillance, and we also look at *E. coli* O157:H7 that now has been identified to date.

Another program is FDA nationwide *Salmonella* assignment. This was implemented since 2007. The type of samples surveyed each year varies differently. In recent years, we have pet food, poultry feed, and also milk producers being sampled. Those programs are in collaborative effort between CVM Office of Surveillance and Compliance, FDA/ORA laboratories, and Office of Research at CVM.

So over the years we have done multiple surveys to look at bacterial contaminants and antimicrobial resistance in feed commodities. So here are some examples. Back in 2002 to 2003, we looked at feed ingredients survey, look at it for bacteria. Those are the same bacteria examined by NARMS program. And starting in 2002, the Feed Contaminants Program start to take place. Primary *Salmonella* is surveyed there. And a few years later at CVM Office of Research, we started to look at the feed samples collected by

OSC and ORA laboratories to look at *E. coli* and *Enterococcus* primary resistance monitoring. And recently, in 2013, we had a feed pilot survey, look at *Campylobacter* and *Listeria* in feed. So we want to see, besides *Salmonella*, what other contaminants are found in feed. For today's talk, our primarily focus on the Number 2 and Number 3.

The survey objectives are threefold. First, to determine the occurrence of bacteria contaminants in animal feed products. And we also want to further elucidate *Salmonella* serotypes commonly associated with feedstuff. And this hasn't been really clearly understood. The third one is to characterize antimicrobial susceptibility profiles of microorganisms recovered from feed. We use *E. coli* as gram-negative indicator organisms and *Enterococcus* as gram-positive indicator.

In terms of sample collection and processing, FDA/ORA field investigators collect samples for CVM assignment following routine sampling procedures. Each sample collected as 10 sub-samples and shipped to ORA laboratories for *Salmonella* culturing and to CVM laboratories for *E. coli* and *Enterococcus* analysis. Primary BAM procedures were followed. That is Bacteriological Analytical Manual of FDA. And from the 10 sub-samples, we made two composite samples, and from there 25 g of each composite were cultured.

So overview of the feed sample collected. Approximately, we have 680 samples each year, which are from the two programs. In the Feed

Contaminants Program, we have about 280 samples each year. Some of those samples are imported from other countries. For CVM-directed *Salmonella* assignments, we have, in recent years, pet food assignments collect about 300 samples each year. Milk replacer or poultry feed or sometimes other types has about 100 samples each year.

From here, I'll be presenting some of the prevalence and susceptibility data of *Salmonella*, *E. coli*, and *Enterococcus* for all the y-axis bars, which are percentage of either prevalence or resistant rate, and x-axis could be years or either the feed commodities.

So, in terms of prevalence of *Salmonella* in feed. Keep in mind that the feed assignments program was started in 2007, and we have some data sources from the two programs. We can see, overall, the range of the *Salmonella* in feed ranges from 5% in 2012 to over 25% in 2003. And the samples from feed assignments program tends to have lower *Salmonella* prevalence.

In this slide, I'm placing the NARMS retail *Salmonella* data into the chart you just saw earlier. So we can see the NARMS retail. In NARMS retail the *Salmonella* prevalence fluctuated over the years, which range about 5% to below 10%. In comparison, the early years of feed surveillance, the prevalence was higher than NARMS number, and in recent years, the number starts going down. So we clearly see the *Salmonella* prevalence in animal feed is decreasing, has a decrease in trend.

So this chart looks at *Salmonella* prevalence by feed type. So we have poultry feed, cattle feed, and other type of feed. We can clearly see the feed ingredients seem to have the majority of problem. The data was divided up by different years. So 2002 and 2006 were grouped together. That's before the feed assignments was implemented. And 2007-2009 data were grouped together. And the ingredients tends to have the highest prevalence.

The leading *Salmonella* serotypes present in feed. So we have three major columns here. The first column are those serotypes identified in animal feed. The second columns are comparing NARMS 2012 retail top ten serovars. The third column is CDC 2011 human top 20 serotypes identified by *Salmonella* surveillance. And in the first column under animal feed, those highlighted in red have been identified in the second and third columns. So we have Montevideo, Infantis, Typhimurium, Anatum, Agona, Enteritidis. So those have been found in top serotypes, both NARMS and CDC human.

So this slide shows the *Salmonella* resistance profiles in feed. The percentage is topped at about 15%. So the highest resistance is to tetracycline followed by streptomycin, ampicillin, and others. So there's a clear trend. It looks like isolates from pet food has higher resistance rates compared to those from animal feed. So keep in mind that those are all below 15% and some are below 5%.

So now I put NARMS data, NARMS retail data there. So this is

August 2012 data, which is preliminary data that Emily presented yesterday. So, in comparison with NARMS retail data, antimicrobial resistance we see in *Salmonella*, feed isolates are relatively lower, is much lower compared to the NARMS retail.

So, in terms of multi-drug resistant *Salmonella* in feed, on the left panel, which shows resistant profiles, the first resistant profile was resistant to seven drugs, seven drug classes. The second one was six drug classes. We found one isolate from pet food that has the first resistant profile. And we also found one isolate from animal feed had the second resistant profile, which are resistant to greater than six antimicrobial classes. So I took a closer look at the data. Both isolates were recovered from feed that were imported from other countries. And if you move down the chart, they are resistant to four classes and three classes. Those, we classify them as multi-drug resistance.

I want you to pay attention to the last row of data which show the pan-susceptible isolates. So we can see animal feed, 88.8% of isolates are pan-susceptible, which means that they are susceptible to all the drugs we tested. And in pet food that was 73%, but in NARMS 2012 retail data, there was 37.7%.

So now I want to move on to the CVM feed survey on *E. coli* and the *Enterococcus* data. So this pie chart shows the sample source, primarily composed of pet food samples and also ingredients and

supplements for pets and others. In total, we have over 1,000 samples. The prevalence of *E. coli* and *Enterococcus*, we had 12.5% prevalence of *E. coli*, 45.2% *Enterococcus*. Over 90% of *Enterococcus* isolates recovered were *E. faecium*. So when you compare animal feed and pet food, you can clearly see that pet food has a lower prevalence in terms of *E. coli* and *Enterococcus*. So, again, comparing NARMS retail 2012 data, both *E. coli* and *Enterococcus* were isolated at a higher rate from NARMS retail.

E. coli resistant profiles. Those data were separated by animal feed and pet food. Again, tetracycline had the highest resistant rate, followed by streptomycin. Interestingly, we found a small percentage of isolates that were resistant to ciprofloxacin and nalidixic acid. So compared with NARMS data, so that is shown in the green bars; again, NARMS appears to have higher resistance for most of the drugs examined except for ciprofloxacin and nalidixic acid.

For *Enterococcus* resistant profile, mycomycin has the highest resistance, followed by tetracycline, QD, and we also found ciprofloxacin resistance in *Enterococcus*. Again, comparing with NARMS data, again, NARMS has a higher prevalence in animal feed isolates except for -- there are a few exceptions there. Ciprofloxacin is one of them.

So, in conclusion, we think our worst populations of *Salmonella*, *E. coli*, *Enterococcus* commonly present in animal feed commodities. Antimicrobial resistance is not as common among bacteria in

animal feed as those in retail NARMS surveillance. Continue to monitor prevalence and antimicrobial resistance in feed is necessary.

I want to acknowledge this group of researchers at FDA for their partnership with the feed survey studies.

Thank you.

(Applause.)

DR. HILL: Any questions for Dr. Ge?

MR. ROACH: This is Steve Roach, Food Animal Concerns Trust. And I appreciated the presentation. I just had a question.

Where you showed that the amount of *Salmonella* going down over time, it seemed to me if you break out the animal feed ones as opposed to the pet food ones, you actually don't see that decrease; is that correct? And the other -- just a question I have is, have you looked at some of -- I couldn't see in the data any types of imported feed.

You know, one of the things my organization is concerned about is, there are certain strains of *Salmonella* in other parts of the world that we would rather not have be introduced into the U.S. food population. One of them would be Kentucky with ciprofloxacin and ceftriaxone resistance, and there are also, again, some other regions where you have pretty scary other versions of *Salmonella.* So I'm just curious about that.

And I guess I'll ask the final one. Do you have any idea why we're having that higher level of ciprofloxacin resistance in some of the feed

samples as opposed to NARMS?

DR. GE: Okay. So three questions.

(Laughter.)

DR. GE: The first one, in terms of the prevalence in pet food, where you divide them into pet food and feed commodities, you may see a different trend? No, that's not the case. Even if you divided by that, you still see a clear decrease.

The second question is on the imported for Kentucky, right?

MR. ROACH: Or any of the imports, were there are differences --

DR. GE: Yeah.

MR. ROACH: -- for imported feeds or feed ingredients?

DR. GE: Right, yeah. We haven't really looked at the data closely in terms -- to separate them into imported and domestic. We would be looking at that closely. In terms of the specific serovar, you talked about Kentucky. It's rarely seen actually in our dataset. So ciprofloxacin are those shown in the chart. It looks big, but only a few isolates. You keep in mind the ratio, the percentage is quite low. It's only two or three isolates has resistance. But we want to take a closer look at resistance mechanisms and maybe use the whole genome sequencing data to look at their evolution.

MR. ROACH: Have you also looked at the origin of those few with cipro? I'm just curious.

DR. GE: Yeah. That's a good question. We will be looking at that, yeah, for the cipro.

MS. GROOTERS: Hi. Susan Vaughn Grooters with Keep Antibiotics Working.

So I'm just thinking about point of contamination in this animal feed and pet food. And did you look at grain versus animal byproduct in both commodities, and how does that break out? So to sort of think about that point of contamination.

DR. GE: Yeah. As I mentioned at the beginning, we did do a feed ingredient survey by dividing them from animal byproducts and plant-based byproducts. That study has been recently published. And we can clearly see that animal byproducts has higher *Salmonella* prevalence compared to plant-derived material. That's a clear trend from there.

MS. GROOTERS: Okay, thank you.

DR. GE: Thank you.

DR. WHICHARD: Please join me in thanking these three speakers.

(Applause.)

DR. WHICHARD: And we're scheduled for a 10-minute break now. And don't go far, come back soon, because we're going to be talking resistance genes and plasmids.

(Off the record.)

(On the record.)

DR. WHICHARD: All righty. Well, welcome back to the second part of the Micro section. We've learned about on-farm sources of resistance and the studies going on there, and also historical resistance and something about feed sources of resistance. And now we're going to get down to some genes and plasmids.

It's my pleasure to introduce Dr. Shaohua Zhao. She is a research microbiologist at the Division of Animal and Food Microbiology at CVM Office of Research. Her responsibility is to conduct and coordinate research to support the NARMS program. Her research fields include the mechanisms of antimicrobial resistance and molecular subtyping of foodborne pathogens. She's really involved in the molecular interagency working groups, and she is a great collaborator in understanding the correlation between resistance genes and phenotypic resistance.

DR. ZHAO: Good morning. And I think this slide has been presented by my colleague from CDC a few times yesterday.

And, currently, more than 100 antimicrobial drugs have been approved for use in clinical medicine with a tremendous benefit to both human and animal health, and however, resistance has developed for each antimicrobial in different time and with different frequency.

So Interagency Task Force on Antimicrobial Resistance was created in 1999. So the Public Health Action Plan to Combat Antimicrobial

Resistance was created by the task force. There are four principal components: surveillance, and prevention and control, research, and new product development. So today I'm going to present some data on surveillance and the research on gentamicin-resistant *Campylobacter* from the NARMS program.

Okay, I'll give a little bit of background about the aminoglycosides and their resistance. Aminoglycosides, they are highly potent broad-spectrum bactericidal antibiotics commonly used in the treatment of infection caused by aerobic gram-negative bacteria and also selected as some gram-positive bacteria. The most common aminoglycosides antibiotics include gentamicin, tobramycin, amikacin, kanamycin, neomycin, and the streptomycin. The top three, gentamicin, tobramycin, amikacin, is mostly commonly used in the clinical medicine. The drug kills bacteria by binding the 30S ribosomal subunits -- you know, inhibit the protein synthesis.

The resistance to aminoglycosides is mediated by four different mechanisms: efflux pump, changes in target sites, impermeability, and there's inactivation or modification. The most clinically relevant resistance mechanism is in the modification. Currently, three major aminoglycosides are modified and have been identified, include aminoglycoside acetyltransferases, aminoglycoside nucleotidyltransferases, and aminoglycoside phosphotransferases. APH is mostly highly related to the high level of the resistance. Currently, more than 100 aminoglycoside-

resistant genes have been identified.

Based on the NARMS data, the gentamicin-resistant *Campylobacter* in the U.S. is very rare. They first detected the gentamicin-resistant *C. coli* in humans in 2000, and in the retail meat, it was 2007. Since then, you can see that the resistance has gradually increased, and for the retail meat, chicken isolates reached 80% and the human reaches at 12%. And the chicken slaughterhouse reached to 6% in the 2011. And since 2012 resistance dropped again.

To understand the epidemiology of gentamicin resistance and the mechanism of resistance, we have collaborated with CDC, our colleagues, and they get all the human isolates isolated from 2000 to 2011 and compare the retail meat isolates. So we get those isolates, we characterize them by pulsed-field gel electrophoresis with -- and also use a count panel to do the AST, look at the AST profile and use PCR to screen the gentamicin resistance gene. The subset of the isolates, we did the whole genome sequence.

So this slide looks very busy, but in general, you can get a sense of that, you know, that this is a PFGE profile and this is the ETA black square, representing the resistance of two different antimicrobials. And you can see that those, you know, they have more resistance, those isolates, human isolates. However, you can look at this cluster here, we put Cluster E here. The shared -- immuno PFGE profile, both isolates are from retail chicken as well as human isolates. And we did a PCR and the whole genome sequence.

They all carry the APH-IgG. I would, you know, describe it in a little more detail later.

Based on the literature, you know, they would have -- you know, there are three gentamicin-resistant genes that have been identified. That's including the APH-If and the bifunctional, the APH-Ia. So another one is aacA(4'). But when we designed the PCR to detect those seeds of the isolates, we were not -- you know, we have very difficult time. You know, some of them cannot detect it, and some were very weak, you know, they're bad by PCR.

So we selected the subsets of isolates, then we sequence -- use the whole genome sequence and we identified, you know, nine gentamicin-resistant genes, and this red one here is first time identified in the *Campylobacter*. And you know, the APH-Ig, If.1, If.3, and the Ih, and there's a bifunctional If, as a novel gene, has been never reported before. So you really can say that whole genome sequence is so powerful in terms of, you know, detecting gentamicin-resistant gene.

This slide shows that, you know, the immunoassay phylogenetic tree. So now you say then why use PCR? We cannot detect those genes. The immunoassay identity among the APH family is very low, so is a very diverse family. It can be low as 25%. So that's really -- you know, you can say that PCR has a difficulty detecting them.

This slide shows the gentamicin-resistant gene distribution in

C. jejuni and the *coli* from a different source. Here is a human isolate, so we get a total 79 from a CDC human isolates. Forty-one of them are *coli* or *jejuni.* I cannot see here. And 38 *jejuni* and 41 *coli.* And here is, you know, different types of resistance genes. And the process of this one we did the whole genome sequence. So you can see that the *jejuni* contain 36 of APH-If, lh. Sixteen of them are confirmed by whole genome sequence. We identified one of them contained APH-Ib and one contains bifunctional APH-If or Ia. That's *jejuni*. For *coli*, we also identified 10 of them contain APH-If and 27 of them APH-Ig and four of them contains bifunctional in that.

Now, look at the retail chicken isolates here. Chicken isolates -- we also identified both *jejuni* and *coli* has APH-If, but the majority of them are APH-Ig gene. And you can see on the previous -- you know, their PFGE profile. They are, you know, very closely related to the PFGE profile. We also sequenced the additional -- you know, the isolates from FSIS. This is cecal isolates. So it also again contains APH-If and APH-Ig gene.

Now, look at the timeline of the gentamicin-resistant *Campylobacter* by phenotype and they also look at the risk-specific gene. As I mentioned it before, the first -- you know, the gentamicin-resistant *coli* was identified from a human in 2000 and the *C. jejuni* in 2004. And we didn't see any retail chicken isolates until 2007. Of course, the retail meat started in 2002, but again we do not see it until 2007. And then, for the 2012, we were forced to say that gentamicin resistance in *jejuni*.

I mentioned it before, you know, there's two genes shared between human and the retail chicken. That is APH-If and the APH-Ig. Now, let's look at the APH-Ig gene. The first detected is from retail chicken. That's in 2008. I said it was 2007 first, and that's one isolates we didn't identify the gene which caused the resistance to gentamicin, even we did the whole genome sequence. So the first detected the APH-Ig gene is from the chicken isolates in 2008. Following year, 2009, we saw the human isolates. And between 2000 -- you know, or 2011, as the previous PFGE showed, that's -- expansion. That's lots of retail chicken and the human isolates share this gene and they shared similar PFGE profile.

And the 2012, that is the first time that we saw gentamicin and this is the *jejuni* from retail chicken, which it carries the APH-Ig gene. Unfortunately, we didn't have the human isolates from 2012 to 2014. It would be interesting to see human isolates and whether or not the *C. jejuni* carry this APH-Ig gene.

Okay, now look at the APH-If. APH-If is mainly identified from human isolates. So the first that we see this gene, *C. jejuni* in 2003 and the *C. jejuni* in 2004. They're all from human. And we do not see any of the APH-If, the *Campylobacter*, from the retail chicken until 2012 in *C. coli a*nd in 2013 in *C. jejuni*.

So, you know, animal and human were leaving the global -- and the resistant gene can transfer from animal to human and the animal can --

you know, human can transfer the gene to animal as well. Of course, there's so many -- you know, a researcher can go here. How this APH-Ig gene, you know, happening in the human much earlier and so prevalent, they eventually go to the animal whether it's directly transferred or through the intermediate, like a *Staphylococcus* or *Enterococcus*. So there's lots of research needs to be done to answer that question.

Okay, this slide shows the PFGE ST profile of gentamicin and *coli*, you know, just breaking down *coli* and *jejuni*. Although many people agree that PFGE may not be the best subtyping methods for *Campylobacter*, but in this case, I can say that, you know, the PFGE has -- you know, give us a very good correlation, not only just to say that, you know, the PFGE profile and also the resistant gene profile, as well, too.

So, in this case, you know, Cluster E, they're all co-resistant to tetracycline except one. And all those clusters carry the APH-Ig gene. And in this here, they have a different, you know, resistance profile. So, for example, the resistant Cluster C, you know, by PFGE and by PCR and the whole genome sequence all identified that they carry the APH-If. Under D, they also carry the bifunctional, you know, aminoglycoside resistant gene. So I think the PFGE really give us a certain correlation, you know, not just a resistance profile, but the genes they carry.

The last two here, there's two retail chicken isolates. They have a unique PFGE profile. They carry the APH-Ic gene. This gene has been

identified as *Enterococci* because of the high level of the gentamicin resistance.

Okay, this slide shows PFGE and AST of the gentamicin-resistant *jejuni*. So, again, you can see this cluster. So you can see the human isolates, there's quite, you know, resistance here. And if you look at it, that most isolates are from the west coastal state. And, again, here, we know by PCR, we identified that it carries APH-If, but when we did sequence, seven of them by whole genome sequence identified as APH-Ih. Between these two groups, their immuno identities, 88%. That's why we use PCR. It can pick up the APH-Ih.

And then Cluster B, here, none of them are covered by second -- but, again, in this Cluster B they're all carried by APH-If and the whole genome sequence of two of them, they contain APH-Ih gene. Again, you know, we only see three chicken isolates until 2013 and two of them that carry the APH-If, and that's only when we saw the *jejuni* carry the APH-Ig gene.

We have sequenced a lot of gentamicin-resistant isolates, but we have closed one of the gentamicin-resistant *C. coli* genome. Both chromosome DNA and the plasmid DNA. So this plasmid DNA, we call it pN29710-1. We identified it as, you know, resistant genetic -- and they carry besides the APH-Ig, they also carry the tetracycline -- so that's why you see why the whole gentamicin resistance is co-resistant to tetracycline. Also, we

identify additional, the aminoglycosides, genes such as AAD3, APH(4'), et cetera .

We compared this, you know, the plasmids with the literature, and we also identified, you know, the one has been sequenced before, pCG8245. This is a gentamicin-resistant *jejuni* isolated in 1999, but another from the NARMS program is published, you know, by other scientists there. It's actually from a U.S. soldier deployed in Thailand. But the gene identified in this cluster is APH-If. But, you know, even though the isolate is in 1999, but the gene was identified in 2005 because of the sequence technology.

And also the last one here, we also look at and compare with the resistant genetic -- this isolate is the *C. jejuni* isolate from Chekiang in China; also share the same resistant gene, but except the gentamicin-resistant gene in this case is a bifunctional APH-Ia.

So when we get this APH-IgG because of low, very low, you know -- their identity was published. So we want to confirm that they are truly resistant to the gentamicin. Also, we want to make sure whether or not they can transfer. So we did two experiments. One is do the conjugation. So we selected two, you know, gentamicin-resistant *coli* as donors here and one recipiency by conjugation, by conjugation. So you can see that is the kanamycin, gentamicin. The phenotype expressed was transconjugant as well as the tetracycline resistant phenotype because that's a whole plasmid, they transfer their recipients here.

And also we did the cloning. So we cloned this APH-Ig gene in the plasmid pack 57. And we transferred this, and we cloned the plasmid to the

E. coli DH5 alpha. Again, you can see after the cloning, this phenotype is expressed there. Of course, the tetracycline will not because we didn't clone the -- gene there. That really confirmed that APH-Ig gene is indeed the cause of resistance to gentamicin.

And, you know, in the NCBI database, I think they have lots of -- genome of *Campylobacter*, *C. coli*, but we're the ones, the first one, to clone this genome and it has been submitted to NCBI. I think it's good for the reference in the future of whole genome project.

So I'd like to summarize our gentamicin resistance study. Gentamicin resistance has increased rapidly in *Campylobacter* in the U.S. We have identified the nine variants of the gentamicin-resistant genes. Seven of them are the first time in *Campylobacter*, five of them are novel aminoglycosides, the resistant gene. Human isolates contain more diverse gentamicin-resistant genes than retail chicken isolates. And the PFGE and gentamicin resistance and the genotype indicated that as contaminated retail chicken could serve as a source of gentamicin-resistant *coli* effect in the human. And the whole genome sequence is a powerful tool to detect the resistant genotype.

And now I'd like to just give you a few slides about these FSIS

isolates. Many people -- actually, Emily talked about it yesterday as well, too. Since 2008, in the NARMS retail meat program, we no longer culture *Campylobacter* from ground beef and pork due to the low prevalence. So, in 2013, we collaborated with FSIS. And, again, this is a cecal sample from, you know, a slaughterhouse. We look at, you know, the *Campy* prevalence. And, surprisingly, you can see that cattle has, you know, over 40% *Campylobacter* and the swine has more than 30%. Both the young chicken and the turkey is between 10% to 20%. I mean, we were so shocked with how much *Campylobacter* in the cattle and the swine, which we would never be able to detect it from retail meat before.

If you look at the gentamicin -- and again most, you know, young chicken has about a 16% or 18% and the young turkey has more than 20%. We identified two cattle isolates, their gentamicin resistance. They both carry the APH-Ic gene. I mentioned it before. But we do not see any gentamicin-resistant isolates from swine.

And the more interesting is that, you know, when we get so many -- you know, first time we get so many cattle and the swine isolates, and we want to see what's the PFGE look like, you know? So surprising to us, you know, so many isolates. We compared the CDC PulseNet. It has an indistinguishable PFGE panel with human clinical isolates.

I was talking to Jean, you know, we had a collaboration project looking at the entire 2000 retail chicken and the human isolates. We have

less than 5% of isolates that has the same -- you know, share the same PFGE profile. I know many people think the PFGE may not be the best -- you know, the method to subtyping *Campylobacter*, but here, I think it -- so right now, I know CDC has launched a big project for source attribution using whole genome sequence. I think that we have several hundred isolates from cattle, and this is why we like to, you know, participate in support of their projects. I think of this as a kind of significant contribution for those isolates.

Finally, I'd like to just update a little bit the whole genome sequence in CVM. You know, we only had one -- in the last two years. So far, we have finished -- completed more than 500 *Salmonella*, more than 100 *Campy,* and more than 90 MDR *E. coli* isolates from retail meat and food animal and some of *Salmonella* human isolates associated with outbreak, and we supported CDC's foodborne outbreak, you know, investigation using this whole genome sequence.

We're also working with the CFSAN, and all the states send those old -- so-called older, you know, the retail meat isolates to state. So far we have been sent 800 isolates. I think, you know, this whole genome sequence give us lots of -- you know, great opportunity to do the research. Later this afternoon you will hear my colleague Yuansha to talk about the phenotypic, genotypic, the comparison, you know.

Finally, I'd like to thank all the people who work on NARMS and particularly who worked with the *Campy* project there in CVM, the *Campy*

team of Melissa and Shenia, and the PFGE team, and Jason and Sharon, Thu, and Jonathan, and the epi group, Heather, Claudine, and Emily. Of course, the whole genome sequencing group, Yuansha, Thu, Sampa, and Claudia. And of course, CDC, you know, Jean and Maria and Jason for great cooperation, you know. Also, thanks to USDA, ARS, and the FSIS. And also FoodNet and the state and public laboratory participating in the retail meat program.

Thank you.

(Applause.)

DR. HILL: If it's okay, we will hold questions until the end of the Microbiology session. And we have one more presenter, and that's Jason Folster. And Dr. Folster is a research microbiologist with the National Antimicrobial Resistance Surveillance Team at CDC, and his research focus is identification of antimicrobial resistance and characterization of mechanisms of resistance.

DR. FOLSTER: Good morning. So today I'm going to tell you a little bit about what we do in the applied research unit of NARMS CDC.

The applied research team is made up of myself, Maria Karlsson, and Davina Campbell. And I really feel like the priority for us is to identify the mechanisms of resistance and how they're spread. However, we also think a lot about how else can we use that data to help us protect people from resistant infections. So today I'm going to tell you about three different studies, all that we're using to help understand source, attribution of sporadic

illness, and also to assist in outbreak investigations.

So we've already heard a lot about resistance of third-generation cephalosporins, specifically ceftriaxone and how important it is. These are commonly used for the treatment of invasive *Salmonella*, especially important for treatment of children. And because of this, there's a great deal of research that's been performed on the mechanisms of resistance.

We know that in the U.S. resistance to *Salmonella* is mainly due to the production of a beta-lactamase called CMY, encoded by the *bla*-CMY gene, and that these beta-lactamases are usually encoded on plasmids. And these plasmids can be characterized and typed by different size, additional resistance genes, incompatibility, and also plasmid MLST when available.

So this project initially started approximately seven years ago when I first joined the lab. We knew that we had ceftriaxone-resistant *Salmonella,* but we didn't really understand a lot about their mechanism. So we set out to take a snapshot of a single year. So, in 2007, we looked at all of our clinical isolates of ceftriaxone-resistant *Salmonella.* I'm just going to briefly mention methods for all three studies, and then I'm going to talk about some more methods that were done.

PCR was done to identify the bla-CMY gene, plasmids were purified and transformed. The reason for that is there is commonly more than one plasmid there, so we need to purify the plasmid that we're studying. Plasmid incompatibility and replicon typing was performed. Plasmid pulsed-

field gel electrophoresis was done. This is done to size our plasmids. When available, plasmid multi-locus sequence typing was done and also antimicrobial susceptibility testing was performed in all the transformants for us to know what resistance genes were transferring along with the CMY gene.

And I should note that all this work that I'm going to talk about today was done back in the dark ages of molecular biology, when there was no whole genome sequencing being performed. But most of the data that we're looking at can be mined from whole genome sequencing. And the future looks bright for that.

So, in 2007, this is what we saw. And on the left-hand side you'll see the different serotypes that we saw and then the number of isolates, the plasmid type, size, any sort of additional resistance genes, and ST type, where available. So the first thing that we noticed is that we saw mostly just two different CMY plasmid types, and that was an Inc I1 plasmid, which carried only the CMY gene, no other resistance genes. And we saw a multi-drug resistant Inc A/C plasmid.

And the second thing that we noticed is when we looked specifically at the serotypes, we saw that some serotypes that are normally associated with cattle -- so Newport, Dublin -- we only saw Inc A/C plasmids, while serotypes associated with poultry, we only saw Inc I1 plasmids. And for Typhimurium that we know is coming from probably both sources, we saw both plasmid types. So this got us thinking, can we use the molecular data

that we collect to help identify the source of infections?

So we decided to use serotype Typhimurium as our model. It's the second most common disease causing *Salmonella* in the U.S. We also know that it's found in diverse agricultural niches, making its identification for the source of infection difficult.

And this is where I think NARMS really shows its strengths in that -- well, we don't just have access to clinical isolates, but we can also look at isolates from food animals and also from retail meat. So this was a collaboration. We decided to look at all of the ceftriaxone-resistant Typhimurium that we had in a single year, 2008, in retail meat, food animals, and from humans. So all these were confirmed. These were all bla-CMY positive. And then PFGE analysis was done, AST was performed, plasmid typing, and pMLST, where appropriate.

And this is what we saw. I don't expect you to be able to read this. So this is a dendrogram showing the genetic relatedness of these isolates. I will say for plasmid type, which is all the way on the far right, we again only saw two plasmid types, Inc I1 plasmids and Inc A/C plasmids. And when we looked at the dendrogram, we found two main groups of isolates. So NC, we saw 92% of all of our poultry isolates were in that group, and 92% of all of our Inc I1 plasmids were in that group. While in Group A we saw 93% of all of our cattle isolates, 70% of all of our Inc A/C plasmids, and somewhat surprisingly, 70% of all of our human clinical isolates were in that group.

And this is just another simpler way of looking at that data. And so this is just looking at *bla*-CMY plasmid type versus the source of the isolate. And so in blue are the Inc I1 CMY plasmids, in red are the Inc A/C CMY plasmids, and green is where we didn't find a plasmid associated with the CMY gene.

So, in chicken and chicken retail meat, you can see the vast majority of the isolates have an Inc I1 CMY plasmid. In cattle, we only saw Inc A/C CMY plasmids. And somewhat surprisingly, in humans, more than two-thirds of the isolates had a plasmid that looked more like a cattle plasmid. This is somewhat surprising in that we don't see a lot of *Salmonella* coming from ground meat or ground beef. So, to me, this is just that we're somehow missing those isolates. It may be that they're coming from a different source that we're not sampling. It could be that these are coming from a secondary source, so I mean, this could be vegetables.

So then our very simplified hypothesis is that we have ceftriaxone resistance coming from *Salmonella* isolates, and this is primarily due to *bla*-CMY plasmids. We see two main plasmid types. So we have Inc A/C CMY plasmids and Inc I1 CMY plasmids, and Inc A/C plasmids are coming from a cattle source, and the Inc I1 plasmids are coming from a poultry source.

So then the last study is: Can we use this molecular data to help us identify the source of infection during outbreaks? So, from 2011

through 2012, we identified nine ceftriaxone-resistant *Salmonella* outbreaks. These were caused by serotypes Typhimurium, Newport, Heidelberg, Enteritidis, and Thompson. Whenever possible, we identify the source of these outbreaks. We then chose one representative outbreak isolate for additional characterization. And I should say at least one of these outbreaks was a mixed outbreak where we had both resistant and pan-susceptible isolates within the outbreak. And so in those cases we chose the resistant isolates since that's what we're studying. We then characterized the sub-tracks and resistant genes by PCR and plasmids by PCR-based incompatibility and sequence typing.

These are the different outbreaks that we studied. I'm sure people here recognize some of these: Heidelberg/chicken livers and Typhimurium/ground beef.

And this is the data that we saw, and we'll return back to this in one moment. I just want to give an overall -- so we found that all of the outbreaks were caused by -- or all had a plasmid encoded bla-CMY gene. In plasmid typing, we identified five Inc I1 plasmids, three Inc A/C plasmids, and a single Inc F/B plasmid. All of the bla-CMY Inc I1 plasmids and the *bla*-CMY Inc F/P plasmid conferred only the CMY resistance phenotype. So, again, they had no additional resistance genes on those plasmids, while all of the Inc A/C plasmids were multi-drug resistant.

Poultry was implicated in two of the five Inc I1 positive

outbreaks, and the Inc I1 plasmids were ST12, and this is a common sequence type plasmid that we find in isolates from poultry. The remaining Inc I1 outbreaks were associated with ground beef, tomatoes, and an unknown source. We'll come back to that. In contrast, beef was implicated in two out of three of the bla-CMY Inc A/C positive outbreaks. And the third outbreak was unknown. But, really, I think the devil's in the detail here when we look at these outbreaks. So let's just go through them.

So we had some very good examples of where it matched our hypothesis, so here are two different poultry outbreaks, both of them were positive for Inc I1 CMY plasmids. And, again, these had the common ST12 sequence type plasmid.

And here are two examples that also match our hypothesis: Two beef outbreaks; both of these had Inc A/C plasmids and were multi-drug resistant.

We had two examples that really don't fit our hypothesis because they're not in our hypothesis. So the Enteritidis. This was an unknown source, and it had an Inc K/B plasmid. This is the first time that we've actually seen this plasmid carrying the CMY gene. We also had an outbreak caused by *Salmonella* Newport, that the source was identified as tomatoes. You know, I would say by looking at the plasmid, the fact that it's an Inc I1 plasmid and it's this common ST12, if I had to predict where this outbreak was coming from, I mean, I would say it probably points to an avian

source.

We also had two outbreaks where we had an unknown source. But, you know, for the first one where we had *Salmonella* Newport, this had an Inc A/C plasmid; it was multi-drug resistant, and it looks like the common MDR Inc A/C plasmid that we see coming from a beef source. So my prediction would be that this was probably a beef source. And, secondly, we had *Salmonella* Thompson that was an unknown source, and this had the Inc I1 CMY plasmid that was also ST12. So, for me, this also points to a poultry source.

We really only have one example that did not match our hypothesis, and so this was a Typhimurium outbreak that came from ground beef, and this was a grocery store. In this case, we identified an Inc I1 CMY plasmid. But let me point out that the sequence type ST20 is actually not a common sequence type that we've seen. We've actually only seen this sequence type once before, and that was in an *E. coli* O157, and that the outbreak itself was associated with grinding of beef and there was not a very good record of what was actually ground at the time. So my guess is that this may have been an example of a plasmid transfer from something else to Typhimurium.

So we identified a likely association between the source of ceftriaxone-resistant *Salmonella* outbreaks in the U.S. and the type of resistance genes/plasmid that it carries. Outbreaks linked to poultry

exclusively contained Inc I1 CMY plasmids, while the majority of the beef associated outbreaks contained Inc A/C CMY plasmids. But it's important to remember that outbreaks are complex situations, and we certainly don't mean to suggest that by simply identifying a plasmid and the resistance genes that we'll be able to say exactly what the sources of these outbreaks are. And, therefore, it's important to take into context all the epidemiological data, serotype, PFGE analysis, AST data, along with these resistance genes and plasmids that we're identifying.

And then just lastly with recent whole genome sequencing advances, I mean, this really should allow us to identify additional resistance genes/plasmid and source associations in the future.

Thank you.

(Applause.)

DR. WHICHARD: Do we have questions for these last two speakers?

DR. TAUXE: Rob Tauxe at CDC.

I have a question for Dr. Zhao, and that is, I was not completely sure I followed the story of the clonal -- it appeared to be a clonal expansion of *Campylobacter coli* of a particular PFGE type which had this particular gene. And that gene was on a plasmid?

DR. ZHAO: Yes.

DR. TAUXE: Did I understand right?

DR. ZHAO: Yes.

DR. TAUXE: It was? Yes. So, yes, it was on a plasmid. And the plasmid could transfer?

DR. ZHAO: Right.

DR. TAUXE: It was a transferable -- a mobile plasmid?

DR. ZHAO: That's right.

DR. TAUXE: Yeah. We need to have your answer here on the mike, as well, I think.

DR. ZHAO: Yeah. Yes, the APH-Ig gene is carried in the plasmid, and we did the conjugation. It can be transferred to susceptible isolates. That's correct.

DR. TAUXE: And it could transfer to susceptible *coli*, also to susceptible *jejuni*?

DR. ZHAO: We only used the *coli* as the recipiency in that case, you know. So we only transfer to *Campylobacter coli* as a recipiency, you know. But we used *jejuni* as a recipiency, but it could be transferred because the APH-Ig gene, if you look at -- by the 2013, the first time so that APH-Ig gene is in *jejuni*, that's the first time. So between the species can be transferred as well.

DR. TAUXE: And the last question I had is, do you have a sense of gentamicin use in poultry that would propel this?

DR. ZHAO: Yeah.

DR. TAUXE: That would select for this?

DR. ZHAO: Actually, I wasn't looking for -- you know, some in a later -- unfortunately, I didn't see that. I know that gentamicin steers this, quite a bit used in the companion animal. So, for food animal, then -- you know, does anybody know? I didn't find that information, how often you use a food animal.

(Off microphone comment.)

DR. WHICHARD: Could you please come to the mike?

UNIDENTIFIED SPEAKER 1: Probably one of the advocacy groups here requested a freedom of information request of a 2004 survey of hatchery use of gentamicin in ovo when gentamicin was used.

(Off microphone response.)

UNIDENTIFIED SPEAKER 2: Post-hatch, too. It has approval for day of hatch as well. So it can either be used in ovo or at day of hatch as well.

DR. TAUXE: And when was the approval? Just wondering --

UNIDENTIFIED SPEAKER 2: It was a long time ago.

DR. TAUXE: Okay. A long time ago, right. Okay, thanks very much.

DR. ZHAO: Yeah. I want to just point out, you know, during this study, look at a later -- how this APH-Ig gene, you know, is so dominated in the *Enterococci* and the *Staphylococcus. S*o it's possible they're originating from those gram-positive bacteria, maybe in the chicken gut, you know. So

that's based on the literature, yeah.

DR. WHICHARD: All right, we'll take one more question before we change it over to the Epi group.

DR. WHITE: Thanks, Jean. Dave White, FDA.

I had two quick questions for our speakers. One is, it sounds like NARMS needs to get together and develop a whole genome sequencing strategy among the partners, how it's going to be used, including the states and, you know, there are academic partners as well. What priorities, what are we going to do, what technologies are we going to invest in, how the data is going to be put out there.

The second is great presentations, they've all been published in great journals. Is anybody reading these articles in the journals? So it goes to my question again. How do we get this data to our stakeholders that are not reading these scientific journals? We have to do a better job of communicating this data out there in some type of consumer update, a constituent update, some way that we tell the research part of NARMS and this is why we're doing it, it's linked with public health priorities, and here's the data. I just throw it out there for consideration. Thanks.

MR. HALLBERG: John Hallberg from Zoetis Animal Health.

A question on your *Campylobacter* paper. You saw the resistance in people, but it was not in young chickens. So what's the possibility that the people are contaminating the chicken as they process it

versus the chickens contaminating the people? I'll throw that one out.

DR. ZHAO: That's very good question. I was wondering as well, too. You know, based on the literature -- yes, I mentioned it before -- lots of gentamicin resistance genes is originated from gram-positive bacteria such as *Staphylococcus* and *Enterococcus*. So for APH-If gene, it has dominated for many years in the human, but eventually you saw that in the chicken. Whether this is directly from a human to chicken or slaughter in the intermediate organisms such as *Enterococci* or *Staph aureus.* So, you know, that is to more investigation. I think the whole genome sequencing will help us to look at those molecular epidemiologies, the gene transfer for how the environment between human to animal, animal to human, or through the intermediate organism. I think, you know, the whole genome sequencing probably will provide a great tool for us to -- for the study, investigation, in this field.

DR. TAUXE: Perhaps I could add one comment. Whole genome sequence won't tell us one thing, though, and that is whether the people who were infected with that particular strain several years before it appeared in chickens traveled to other parts of the world. We saw fluoroquinolone resistance in *Campylobacter* infections in people several years before fluoroquinolones came into use in the poultry industry, and they had traveled to mostly Latin America, to other countries where, in fact, there was a lot of use of fluoroquinolone in poultry. So the fact that we see it in a person does

not mean that that person then subsequently transmitted it to chicken. That person may have acquired it somewhere else in the world. And thinking about the information we need about the person who is sick is -- we can't leave that out.

DR. ZHAO: Rob, I totally agree. I think I mentioned one of the comparison, the -- plasmids. That's the first human isolates are from a U.S. soldier in 1999 and who deployed in Thailand. That carries APH-If gene. That's actually forced to report and the soldier in Thailand. So, certainly, I think the international travel is associated because if you look at it, most of the human *jejuni* -- gentamicin-resistant *jejuni* is in the East Coast. You know, I mean, I talking to, you know, Maria on the CDC side, so it would be interesting if we have some epidemiology information about those cases, but unfortunately, this is kind of the older isolates, and those information are not available. But I totally agree with that, you know, the resistance we're not just looking at the domestic picture associated with the international travel, and the import of food, I think will play important role in terms of the transfer of resistant gene from this country to another country.

DR. WHICHARD: Well, thank you very much. That was great discussion after those two papers. I really appreciate it. We're going to turn it over to the Epi section now.

DR. McDERMOTT: Our moderators for this section are Dr. Barbara Mahon and Dr. Heather Tate, who have both been introduced,

and so I won't introduce them again. So we'll turn it over to you for the next session.

DR. TATE: So the first speaker for the Epi section is Dr. Allison Brown. Dr. Brown is an epidemiologist with NARMS at CDC. Her work focuses on surveillance in epidemiology of antimicrobial resistant infections. She holds a Ph.D. in molecular microbiology and immunology from the Johns Hopkins Bloomberg School of Public Health and an M.P.H. in epidemiology from Yale.

DR. BROWN: Thank you. Good morning.

CDC NARMS is using data from multiple surveillance systems in new ways, and these new ways of using surveillance data now allow us to answer questions we previously weren't able to explore. I will be discussing how we're linking NARMS data to other surveillance systems of human enteric disease and how this linking is helping us use antibiotic resistance data to better understand the food sources of enteric infections.

The U.S. has a comprehensive system for foodborne disease surveillance in humans, and this overall system is composed of many interrelated surveillance systems, each of which has a different intended purpose, meaning each system provides us with some unique information about the sources and impact of enteric disease.

First, I'd like to give you a brief overview of the surveillance systems I'll be discussing other than NARMS, and these include the National

Outbreak Reporting System, also called NORS, which supports national reporting of enteric disease outbreaks transmitted by food and captures data on the sources of outbreaks, including the agents, foods, and settings responsible for illness.

PulseNet is a national network that is currently using PFGE to subtype isolates from humans, animals, and foods based on genetic relatedness, and it is most often associated with outbreak detection but also helps us identify possible linkages between pathogens and sources.

The Foodborne Disease Active Surveillance Network, also called FoodNet, is a sentinel system of active surveillance in 10 states. FoodNet provides foodborne illness estimates and patient data on exposures, clinical outcomes, and travel history.

We linked data from NARMS, PulseNet, and NORS to describe antibiotic resistance among outbreaks of foodborne non-typhoidal *Salmonella*, which I'll refer to as *Salmonella*. And we focused our attention on outbreaks occurring in the United States from 2003 through 2012 and the foods that caused these outbreaks. First, some brief background information.

Outbreak-associated illnesses comprise only a small fraction of all *Salmonella* illnesses. Nevertheless, as you've heard from a few other folks, investigations of foodborne disease outbreaks provide us with unique information about foods causing outbreaks. Although the foods that cause sporadic cases of foodborne illnesses are not known nor are they knowable,

the information provided by foodborne outbreak investigations can be used for overall food source attribution. Moreover, antibiotic resistance testing of human isolates from foodborne *Salmonella* outbreaks can help determine foods associated with infections caused by resistant and non-resistant strains. Determining which foods are associated with infections is important for hypothesis generation during outbreak investigations and for general food source attribution.

CDC NARMS asks state public health laboratories to submit *Salmonella* isolates from foodborne disease outbreaks for testing. All states are asked to submit isolates from outbreaks caused by *Salmonella* serotypes Enteritidis, Newport, and Typhimurium, which are among the most commonly occurring serotypes that exhibit clinically important antibiotic resistance patterns. In addition, FoodNet sites are asked to submit isolates from all foodborne outbreaks of *Salmonella* regardless of serotype. CDC NARMS then tests these outbreaks, outbreak isolates, for antibiotic resistance. So our study analyzed isolates submitted for outbreaks occurring from 2003 through 2012.

We linked isolate resistance data in NARMS to data on *Salmonella* outbreaks reported to NORS using a combination of variables. It's pretty complex. It includes the laboratory identification number, specimen collection date, first and last illness onset date, state, and serotype. We also linked NARMS to PulseNet so that we could use the PFGE information to

validate these NORS NARMS links.

Once our data was linked, we categorized outbreaks into two groups. Outbreaks are considered to be caused by strains with resistance detected if one or more isolates was resistant to at least one antibiotic agent tested on the NARMS panel. Outbreaks were considered to be caused by strains with no resistance detected if all isolates were either susceptible or intermediate to all antibiotic agents tested. So, for brevity's sake, I will refer to these outbreak categories as resistant or non-resistant for the remainder of my talk.

Across 10 years of reporting, 1,492 NARMS isolates from foodborne outbreak associated *Salmonella* infections were submitted. Across these same 10 years, 1,266 foodborne outbreaks of *Salmonella* infections were reported. We linked 695 or 47% of NARMS foodborne *Salmonella* outbreaks isolated to NORS. The fact that we were only able to link 47% of NARMS outbreak isolates to NORS did not come as a surprise. NARMS requests isolates from clusters of infection that are still being investigated, yet many of these clusters do not eventually result in a solid outbreak that would quality for NORS reporting.

I will now turn your attention to some of the results of our linked data analysis. This figure shows the proportion of *Salmonella* outbreaks by resistance. We see that 21% of 76 linked outbreaks were caused by resistant strains, and 79% were caused by non-resistant strains.

This table shows us some basic demographics of the outbreaks by resistance. When we compared resistant outbreaks to non-resistant outbreaks, we found that a significantly higher median percentage of patients under the age of five years were among the resistant outbreaks. We also saw a significantly higher median proportion of males among patients of resistant outbreaks.

Looking at the geography of outbreaks, we see a higher proportion of single state outbreaks reported than multi-state outbreaks. In reality, approximately 99% of outbreaks that occur are single-state outbreaks. Nevertheless, both resistant and non-resistant outbreaks seem reflective of overall outbreak reporting to NORS. The only difference in geography between resistant and non-resistant outbreaks is a slight, though not significant, increase in the percent of resistant outbreaks reported in the Northeast census region of the United States.

Looking now at the proportion of outbreaks caused by resistant and non-resistant strains by serotype, the graph on the left presents 37 outbreaks caused by resistant strains. The top five serotypes were Typhimurium, Newport, Heidelberg, 4,[5],12:i:-, and Braenderup.

The graph on the right presents the 139 outbreaks caused by non-resistant strains. Whereas Typhimurium, Newport, and Heidelberg were again present among the top five serotypes, the most common serotype associated with non-resistant infections was Enteritidis, which was notably

absent among outbreaks caused by resistant strains. For reference, it was expected that Enteritidis, Newport, and Typhimurium be among the most common serotypes detected overall, since the majority of NARMS sites were only submitting isolates to NARMS from outbreaks of these serotypes during the majority of our study period with the exception, of course, of the 10 FoodNet sites. We also know, from our nationwide surveillance of sporadic infections, that these serotypes are indeed among the most common.

Foods implicated in outbreaks are categorized according to a detailed categorization scheme. The most basic division is into four food groups, including aquatic animals, land animals, plants, and other. These food groups contain increasingly specific food categories, notably dairy, eggs, and poultry are included in the land animal food group along with meat, which is further subdivided into specific categories to include beef and pork. Similarly, the plant group is further subdivided into more specific categories like sprouts, seeded and row crop vegetables, fruits, and nuts.

These graphs describe antibiotic resistance among *Salmonella* outbreaks attributed to a single food group. Of 83 such outbreaks, 20 were caused by resistant strains and 63 by non-resistant strains. Among outbreaks caused by resistant strains, presented on the left, 80% were attributed to foods from land animals, whereas for non-resistant outbreaks, shown on the right, only 44% were traced to foods from land animals. Thus, among *Salmonella* outbreaks attributed to a single food group, foods from land

animals were the primary source of resistant outbreaks.

Looking at these same numbers in a different way, among all outbreaks attributed to foods from land animals, seen in the leftmost pie graph, more than one-third were resistant, as indicated by the yellow piece of pie, compared to about one-tenth of the outbreaks attributed to foods from plants, as seen in the middle graph.

Taking a closer look at the resistant outbreaks attributed to a single food group, we see that foods from land animals were more commonly associated with multi-drug resistant infections defined as resistance to at least three classes of antibiotics and resistance to at least one clinically important drug, defined here as ampicillin, ceftriaxone, ciprofloxacin, or trimethoprim/sulfamethoxazole. We also see that, as we would have predicted, resistance to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline, otherwise known as ACSSuT, was found only among outbreaks caused by foods from land animals, and this resistance pattern is indeed most commonly associated with cattle.

So which specific food categories were implicated in these resistant outbreaks caused by foods from land animals? Eighty-eight percent were traced back to either poultry or beef, and the remaining 12% were split between dairy and pork. In contrast, foods causing non-resistant outbreaks were more equally distributed.

Whereas poultry and beef cause 88% of resistant outbreaks,

these same foods cause only 32% of non-resistant outbreaks. And unlike the resistant outbreaks, a sizable proportion, another 32% of non-resistant outbreaks, were traced to eggs.

There are limitations to this particular study. First, approximately half of all outbreaks reported to NORS were not attributed to a single food group and thus could not be included in specific food group or food category analyses. In addition, isolates were submitted for resistance testing for only a proportion of outbreaks, and it's unknown whether these are representative of all outbreaks or all sporadic cases. In 2011 NARMS began efforts to increase the submission and testing of outbreak isolates of *Salmonella*. Now all 54 sites are asked to submit isolates from every single state outbreak, and CDC submits specific isolate requests for each multi-state outbreak investigated.

To give you a sense of outbreak isolate submissions and reporting, this figure shows year-by-year tallies of the total number of foodborne outbreaks of *Salmonella* reported to NORS, which is shown in the blue bars, and the percent of outbreaks for which we were able to link NARMS outbreak isolates, which is indicated by the red line. Although the percent of outbreaks with linked NARMS isolates generally hovered around 15% through 2011, initiation of enhanced outbreak isolate submissions in 2012, the final year of data included in this analysis, improve this measure by twofold. And we anticipate this upward trajectory to continue as we further

enhance both isolate submissions and linking capabilities.

Another limitation to our analysis is the fact that a plasmid can be gained or lost over the course of an outbreak, and as a result, outbreaks can be caused by strains where resistant isolates had differing resistant patterns or where some of the isolates tested from a particular outbreak may be resistant and some may be non-resistant. These mixed outbreaks demonstrate the complexity of defining an outbreak as resistant or non-resistant based on a select number of isolates from each outbreak. Nevertheless, no matter which definition we use, we do know that non-resistant outbreaks remain non-resistant outbreaks, and with all isolates testing negative for resistance to all antibiotics on the panel.

So, in conclusion, this linking project illustrates how antibiotic resistance data on isolates from *Salmonella* outbreaks can help identify sources of antibiotic resistant infections. These data can also assist with food source attribution analyses and inform hypothesis generation for outbreak investigations. In this analysis, we found that foodborne outbreaks of *Salmonella* infection were more likely to be caused by resistant strains when attributed to foods from land animals. And relatedly, outbreaks caused by food from land animals were more likely to involve resistant strains.

By helping us to better understand the biology, epidemiology, and ecology of food source contamination, linked data can provide useful information that can help inform research priorities as well as policy decisions

and prevention efforts.

One way we're attempting to enhance the use of NARMS resistance data to understand sources of infection is by making data more easily available to partners, including state and federal surveillance partners and the Foodborne Outbreak Response group at CDC. CDC is now reporting resistance data, including those generated from outbreak isolates to our state partners through the web-based database that both Jean and Regan had discussed earlier.

NARMS is also working to add linked resistance data into a web-based platform that currently provides users with easy visualization of PulseNet and NORS data. We're also working to collect more information on clinical risk factors and outcomes of resistant and non-resistant infections by deploying standardized questionnaires for both sporadic illnesses with worrisome resistance and outbreak-associated illnesses. Two versions of these questionnaires are now in development.

Two ways we're working to improve human isolate data collection and analysis are to enhance isolate testing by strengthening local capacity for collection and testing, and these local enhancements will increase the percent of isolates tested and decrease the time to results, as well as the timed reporting, all of which will help us to detect and determine the food sources of more resistant outbreaks. We are also working to improve our ability to link NARMS data to other surveillance systems through

IT upgrades and enhanced isolate ID formatting. Improved linking will further expand our abilities to answer questions we previously could not explore analytically.

Thank you.

(Applause.)

DR. TATE: We'll take questions.

MS. GROOTERS: Susan Vaughn Grooters from Keep Antibiotics Working. Thank you for that presentation.

One thing I didn't see but I noticed in your conclusion, things that you're looking for in the future, clinical risk factors of invasive infections and things. Did you see an increased hospitalization rate in the antibiotic resistant infections versus non, and if so, by what -- was it twofold, was it onefold? What did you see?

DR. BROWN: So looking at hospitalization is a little difficult when you link to NORS because you're looking at summary data. We did look at it. It's not what you would expect. In this data, it was approximately the same. We also did look at median proportion hospitalized, which was slightly increased. I mean, there are a lot of data that we're still delving into, and there are a lot of ways to parse it out, so that's definitely something that we're looking to answer. But whether the NORS database is the correct database to use for that, we're still unsure. We're also looking at the FoodNet database, which is much more clearly associated with clinical

outcomes.

MS. GROOTERS: Great, thank you.

DR. BROWN: Sure.

DR. TATE: I actually have one question. So were any of the outbreaks attributed to food vehicles which aren't traditionally consumed in the U.S., like lamb? I don't know how traditional that is, but lamb or ox or anything like that?

DR. BROWN: So there is an "other" category, which I didn't describe. There's game. There are very few outbreaks associated with game. I think there was maybe one. But that is something that you can look at. Actually, there were no resistant outbreaks associated with game. Yeah, none. None for game among the resistance. And then among the non-resistance -- I don't have that data. But a lot of these outbreaks are unclassifiable. They're in mixed categories. So there is a lot of information that we just don't have, but we have looked at these sort of non-traditional foods, if they can be classified, and didn't really see anything very notable.

MR. ROACH: Steve Roach, Food Animal Concerns Trust. And that just reminded me there was a question I wanted to ask.

We generally are talking about foodborne outbreaks, but I'm also concerned -- you know, there are other outbreaks, and it would be interesting to know, are you all looking at those, as well? Particularly, we've seen in this meeting, you sometimes find -- you know, we don't find very

much *Salmonella* in beef. We do get some beef outbreaks, but there may be more -- and people may be more likely to get sick from *Salmonella* from contact with animals, you know, from beef cattle or dairy cattle. And I'm just curious about the non-foodborne outbreaks, and have you all looked at those, as well?

DR. BROWN: We certainly intend to. When we did the initial linking, we did include animal contact outbreaks so that we wouldn't have to go back and do the linking all over again. So for this analysis, we just did foodborne, but that's something that we're very interested in looking at, and we are now able to pick up animal contact outbreaks a little bit more, you know, now that people are so heightened, as far as looking for that association.

Any others?

(No response.)

DR. BROWN: No? Okay.

DR. MAHON: Thank you.

DR. TATE: Thank you.

DR. MAHON: Thank you very much, Allison.

We'll move on to our next speaker.

(Applause.)

DR. MAHON: Our next speaker is Dr. Beth Karp. Dr. Karp has a doctor of veterinary medicine degree from Cornell University and a master of

public health degree from Johns Hopkins. She is also board-certified in veterinary preventive medicine and epidemiology. Before joining the NARMS team at CDC four years ago, Dr. Karp worked at the Maryland Department of Health for five years and at CVM for nine years, where she was the coordinator of the NARMS program during her last three years.

DR. KARP: Thanks, Barbara.

Good morning. I'll discuss the results of a study that was conducted by Amy Krueger and colleagues at CDC, as well as FoodNet sites. And it was published in *Foodborne Pathogens and Disease* in May. None of the authors were able to be here today, so I will do my best to speak on their behalf and highlight some of their key findings.

The objective of the study was to examine clinical outcomes of antimicrobial resistant non-typhoidal *Salmonella* infections and determine whether or not they differ from those of susceptible infections. During the remainder of the talk, I will refer to non-typhoidal *Salmonella* simply as *Salmonella*.

Before I discuss the paper, I will provide some background information about the public health impact of *Salmonella* and antimicrobial resistance in *Salmonella*.

Each year, *Salmonella* causes an estimated 93.8 million cases of gastroenteritis and 155,000 deaths annually worldwide. In the United States, *Salmonella* causes an estimated 1.2 million illnesses, 23,000 hospitalizations,

and 452 deaths annually.

Salmonella is the leading bacterial cause of foodborne illness and the most common foodborne pathogen causing hospitalizations and deaths in the United States. Each year, in the United States, there are an estimated 100,000 antimicrobial resistant *Salmonella* infections.

When a *Salmonella* infection is resistant, early and para treatment may fail and treatment choices will be limited. Studies have found there are more illnesses and more severe illnesses with resistant *Salmonella* infections than susceptible infections. When strains are resistant, they have a selective advantage in people who are taking antibiotics for some other reason, and this results in more illnesses.

A number of studies have reported more severe illnesses for patients with resistant *Salmonella* infections than for patients with susceptible infections. Resistant infections have been reported to have higher risk of death and to cause more bloodstream infections, more hospitalizations, longer hospitalizations, and longer duration of illness. The studies assess different serotypes and antimicrobial agents, some adjusted for potential confounders while others did not.

To further explore the relationship between resistance in clinical outcomes, a prospective multi-center cohort study was conducted. The two-year study was a collaboration between NARMS and FoodNet, the Foodborne Diseases Active Surveillance Network. FoodNet conducts active

surveillance for laboratory confirmed infections with nine pathogens transmitted commonly through food, including *Salmonella*. And surveillance is conducted in seven states and counties in three additional states, shown on the map.

When the study was conducted, the FoodNet surveillance area represented about 15% of the U.S. population. Some FoodNet sites enhanced sampling and sent more isolates to NARMS to achieve the power needed to detect key differences in clinical outcomes. Isolates were tested for susceptibility to 15 antimicrobial agents in 8 antimicrobial classes using the NORS panel.

Patient interviews were conducted using standard questionnaire within 85 days of specimen collection. Demographic information about symptoms and antimicrobial use was collected from patients. Information about hospitalization and death were obtained using standardized chart extraction forms. 1,057 patients were eligible for the study; 875 or 83% were enrolled, and 182 or 17% were excluded. Most of these patients, the patients who were excluded, refused to participate in the study.

Isolates from 705 or 81% of patients were susceptible to all antimicrobials tested; 165 or 19% were resistant to one or more antimicrobial agents; and 5 or 0.6% isolates were intermediate. Patients with susceptible isolates were compared with patients with resistant isolates.

Let's now look more closely at the resistant isolates. These isolates were categorized into nine overlapping groups based on the number of antimicrobials in the resistance pattern. Isolates resistant to only one agent were classified as R1, those resistant to only two agents were classified as R2, and so on.

You can see here a quarter of the isolates were resistant to one agent and about a quarter were resistant to five agents. The rest of the isolates were distributed among the other categories. Two of the isolates were resistant to all eight classes tested, and they were resistant to both cephalosporin/ceftriaxone, as well as quinolone/nalidixic acid. As we've heard, cephalosporins like ceftriaxone and fluoroquinolones like ciprofloxacin are commonly used to treat salmonellosis. Resistance to the quinolone/nalidixic acid is correlated with reduced susceptibility to ciprofloxacin and may be associated with treatment failure.

In addition to categorizing resistant isolates by the number of antimicrobial classes in the resistance pattern, isolates were also placed into additional categories based on specific resistance patterns. We looked at resistance through a pattern known as ACSSuT. As was mentioned before, that is resistance to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline.

We looked at resistance to "at least ACSSuT" as well as "ACSSuT only." We also looked at resistance to "at least ceftriaxone." All the

ceftriaxone-resistant isolates were also resistant to other agents. And resistance to "at least nalidixic acid" and to "nalidixic acid resistance only."

There were no significant differences in age, antimicrobial use, or diarrhea for patients in most resistance categories, as shown here. A higher proportion of patients with R7 resistant infections had taken cephalosporin in the month before illness onset. All R7 resistant isolates were resistant to ceftriaxone. A lower proportion of patients with nalidixic acid resistant infections were female. In addition, both patients with R8 resistant isolates were 5 to 17 years of age.

Now, let's turn to the main question of the study: Are clinical outcomes worse for resistant infections?

We looked at the following outcomes: bloodstream infection, hospitalization, duration of hospitalization greater than three days, and death. We conducted analyses to estimate the relative risk for each outcome, comparing patients with resistant and susceptible infections. Logistic regression was used to adjust for serotype and age. We adjusted for serotype because resistance and invasiveness varied by serotype. Serotype may therefore confound the association between resistance and outcome, as illustrated in this diagram. We also adjusted for age because it may also be a confounder. We used four categories for serotype and age. We included the three most common serotypes: Enteritidis, Typhimurium, and Newport, and a fourth category of "other and unknown serotypes." For age, we used less

than 5, 5-17, 18-64, and 65 years or older.

This slide shows the percentage of patients with the four main outcomes. As you can see, the percentage was higher for patients with resistant infections for each of the four outcomes. Further analyses were performed for the first three outcomes. We were not able to do further analyses for death because there was only a single death among patients enrolled in the study.

First, we looked at bloodstream infection. Adjusted for age and serotype, the risk of having a bloodstream infection was higher for patients with isolates in most resistance categories, including resistance to the quinolone/nalidixic acid. The risk of having a bloodstream infection was 3.1 times higher among patients with isolates resistant to at least nalidixic acid than among patients with susceptible infection.

Next, we looked at the risk of hospitalization. Adjusted for age and serotype, the risk of hospitalization for patients in several resistance categories -- R3, R8, and ceftriaxone resistance -- hospitalization was higher for these patients in these categories than among those with susceptible infections. Patients with ceftriaxone-resistant infections were 1.7 times more likely to be hospitalized than patients with susceptible infections.

Finally, we looked at the risk of hospitalization greater than three days. Adjusted for age and serotype, the risk of hospitalization greater than three days was higher for patients with isolates in several resistance

categories -- R5, at least ACSSuT, and ACSSuT only -- than among patients with susceptible infections.

This study had some limitations. The role of resistance versus serotype can be difficult to sort out. Resistance categories had small numbers, which prevented an examination of outcomes by serotype. However, we did control for serotype in the analysis.

Another limitation is that some patient characteristics or conditions, for which we do not have information, may have increased chances of both acquiring resistant infection and developing a bloodstream infection, for example, frequent contact with hospitals or nursing homes.

In summary, the study found that patients with resistant infections had more severe outcomes. After adjusting for age and serotype, bloodstream infection was more common in patients in most resistance categories studied, including resistance to the quinolone/nalidixic acid than among patients with susceptible infections. Hospitalization was more common among patients with isolates resistant to ceftriaxone or resistant to at least three antimicrobial classes -- to only three, excuse me -- only three antimicrobial classes than among patients with susceptible infection. And hospitalization at least three days was more common among patients with isolates resistant to only five antimicrobial classes, ACSSuT and ACSSuT only.

By analyzing isolate data by both number of resistance classes and by major resistance patterns, we confirmed and extended findings for

previous studies showing that more patients with resistant infections have more severe illness. And we demonstrated that adverse outcomes are associated with several different resistance categories, including resistance to quinolone/nalidixic acid and cephalosporin/ceftriaxone.

In conclusion, antimicrobial resistance in *Salmonella* is associated with more severe clinical outcomes, so it is critical that we use antimicrobial agents prudently in both humans and animals. This will help minimize the emergence and spread of resistance genes and resistant *Salmonella* infections. And it will help keep antimicrobial agents effective for treating disease in both humans and animals.

Thank you.

(Applause.)

DR. KARP: I'll try to answer any questions.

DR. MAHON: We're running a little bit late, but I think we have time for a question or two.

DR. ROBERTSON: Hi. This is Kis Robertson, USDA/FSIS.

So you looked at infections, *Salmonella* infections, in general, but with outbreaks, have you noticed a correlation between resistance and the size of outbreaks, which would suggest maybe infectiousness increases with AMR?

DR. KARP: With outbreaks, the study looked at the sporadic cases of illness, so I'm not sure. I can't answer that question.

DR. ROBERTSON: Okay.

DR. KARP: I'm not sure.

DR. MAHON: You might be able to talk with Dr. Brown afterwards.

DR. ROBERTSON: Okay, thank you.

DR. MAHON: Okay, I think we can move on to the next talk.

Thank you very much, Beth.

(Applause.)

DR. TATE: So our next two speakers will be giving a collaborative presentation. The first speaker is Ms. Allison O'Donnell.

Allison O'Donnell is a surveillance epidemiologist with the Atlanta Research and Education Foundation working at CDC with the NARMS program. She received her Master of Public Health from Boston University in 2010 and her research interests include the surveillance of antimicrobial resistance in enteric pathogens.

MS. O'DONNELL: Thank you, Heather.

Good morning, everyone. Today Claudine and I will be presenting on nalidixic acid-resistant *Salmonella* Enteritidis linked to international travel and imported foods. We will begin with some background on *Salmonella* Enteritidis.

SE is the most common non-typhoidal *Salmonella* serotype isolated from humans in the United States. In 2013, 19% of all non-typhoidal

Salmonella cases reported to the Foodborne Diseases Active Surveillance Network, or FoodNet, were serotyped as Enteritidis. Common *Salmonella* Enteritidis sources in the United States include chicken and eggs.

Resistance among SE isolates is generally low. Eighty-eight percent of SE isolates tested by CDC NARMS in 2012 displayed no resistance to any antimicrobial agent tested. Among SE isolates with resistance to an antimicrobial agent, resistance to the quinolone/nalidixic acid was most common.

This graph shows the percentage and number of SE isolates resistant to nalidixic acid from 2003 to 2012. The percent of SE isolates with nalidixic acid resistance has risen in recent years. After dropping to 3.7% in 2009, percent resistance rose to 7.7% in 2012. Among all non-typhoidal *Salmonella* isolates tested by CDC NARMS in 2012, 50% of nalidixic acid resistant isolates were serotyped as Enteritidis. Nalidixic acid is not used for treatment of *Salmonella* infections in the United States. However, resistance to nalidixic acid is correlated with decreased susceptibility to ciprofloxacin, a fluoroquinolone used to treat severe *Salmonella* infections in adults. So identifying sources of nalidixic acid resistant infections is important.

Among the meat products tested by NARMS, SE is most commonly found in chicken. In the FDA retail meat program, 188 non-typhoidal *Salmonella* isolates have been serotyped as Enteritidis from 2002 through 2012. However, nalidixic acid resistance in SE from retail chicken is

rare with only one nalidixic acid resistant SE isolate since testing began.

In the USDA animal testing program, 1,286 SE isolates have been isolated from chickens at slaughter since the beginning of testing in 1997. Nalidixic acid resistance is also rare in SE isolated from chickens at slaughter. Only one nalidixic acid resistant isolate has been found since testing began in 1997. Domestic chicken does not appear to be a source of nalidixic acid resistant SE, so we must consider other possible exposure sources. Eggs could be a potential source of nalidixic acid resistant SE, but we are not aware of any recent nalidixic acid susceptibility data from SE isolated from domestic eggs.

For today's presentation, we will be focusing on international travel and imported food as possible exposure sources. To begin, I will be presenting the results of a study examining the association between international travel and nalidixic acid resistant SE infections.

For this analysis, we linked *Salmonella* Enteritidis data from CDC NARMS and FoodNet from 2004 through 2010. The NARMS program at CDC systematically collects isolates of non-typhoidal *Salmonella* from humans and tests them for susceptibility to a panel of antimicrobial agents. Currently, NARMS applies interpretative criteria to the minimum inhibitory concentrations for 15 antimicrobial agents from the 10 antimicrobial classes listed. For the period of the study from 2004 through 2010, all classes listed had drugs tested other than the macrolide class, which was added in 2011.

Two quinolones were tested: ciprofloxacin and nalidixic acid.

CDC NARMS isolate level data was linked to case data from FoodNet. FoodNet is a collaboration among CDC, 10 state health departments, USDA's Food Safety and Inspection Service, and FDA. FoodNet conducts active surveillance for laboratory confirmed infections with nine pathogens transmitted commonly through food.

Data collected through FoodNet includes patients' history of international travel in the seven days before illness onset, along with information on travel destination. If a patient traveled abroad in the seven days before illness onset, the infection is considered travel associated. This information has been systematically collected from all FoodNet sites since 2004.

We linked NARMS and FoodNet data for SE isolates with specimen collection dates from 2004 through 2010. Eighty-eight percent of NARMS SE isolates from FoodNet sites were linked to FoodNet data.

Among the 445 CDC NARMS isolates linked to FoodNet, 368 were from patients with travel history information. Seventy-seven isolates were excluded due to lack of travel history information. We first broke down the 368 linked isolates by reported history of international travel. A history of international travel before illness began was reported by 75 or 20% of the 368 SE patients with travel history data. Among patients with a reported history of international travel, 24% had nalidixic acid resistant SE infections

compared with 3% of patients who reported no travel.

Next, we looked at the 368 linked isolates by nalidixic acid resistance. Among the 368 patients, 28 had isolates that exhibited resistance to nalidixic acid, of which 18 or 64% came from patients with international travel before illness began. Seventeen percent of the nalidixic acid susceptible infections were from patients reporting international travel. The proportion of patients with recent international travel was significantly higher among those with nalidixic acid resistant SE infections than those with nalidixic acid susceptible infections.

We next took a closer look at the 28 nalidixic acid resistant SE isolates. Eighty-six percent of the nalidixic acid resistant isolates exhibited reduced susceptibility to ciprofloxacin, and all 18 nalidixic acid resistant isolates from patients with travel had intermediate ciprofloxacin MICs. Five of the 28 nalidixic acid resistant isolates had co-resistance to other antimicrobial agents. Three of the five co-resistant isolates came from patients with a history of international travel. When the five co-resistant isolates were excluded, there was no appreciable change in the proportion of patients with a history of international travel.

Information on countries visited was available for 14 of the 18 patients with nalidixic acid resistant SE infections. Seven patients reported travel to Latin America with four patients reporting travel to the Dominican Republic and three reporting travel to Mexico. Four patients reported travel

to Europe with one patient each traveling to Poland, Russia, and Spain, and one patient reporting travel to England, France, Poland, Greece, and Germany prior to illness onset. Three patients reported travel to Asia with one patient each reporting travel to China, India, and the Philippines. Overall, patients reported travel to several regions of the world, so we were not able to link to any specific region.

We next took a closer look at the 340 patients with nalidixic acid susceptible SE infections. Among the 340 patients, 19 or 6% had infections with resistance to at least one of the 14 antimicrobial agents tested in NARMS other than nalidixic acid; 321 of the nalidixic acid susceptible infections displayed no resistance to another agent. For this analysis, no resistance includes isolates that were susceptible to all agents tested and those with intermediate MICs.

In order to investigate whether international travel was associated specifically with nalidixic acid resistance or resistance to any agent, we compared the proportion of travel between patients with infections resistant to an agent other than nalidixic acid to patients with infections with no resistance. There was no significant difference in the proportion of patients with a history of international travel between these two subgroups.

In conclusion, we found that a high proportion of nalidixic acid resistant SE infections were associated with travel outside the United States.

However, no specific region of the world was linked to travelers with nalidixic acid resistant SE. Not all nalidixic acid resistant SE infections in the U.S. are travel associated, so other exposure sources, such as imported food, should be considered.

Claudine will be presenting next on imported food as a possible source. Thank you.

(Applause.)

DR. TATE: Claudine was introduced yesterday, but just briefly again, she is an epidemiologist with the FDA NARMS program.

MS. KABERA: Thank you. As Allison said, I'll be briefly talking about our findings with the imported foods from the RA partners.

To begin, I want to provide a brief background on the data that I will be presenting. So one of the reasons we gathered this data is that NARMS is interested in looking at potential resistance that may be coming from imported foods, and we were looking for different resistance-based data that we can explore this information from, one of which was the ORA field laboratories. So beginning in 2000, NARMS began conducting antimicrobial susceptibility testing on *Salmonella* isolates from imports that were collected from the field laboratories, and these isolates were then tested against a NARMS panel, which Allison has already mentioned. The drugs are tested on a NARMS panel. And these were tested at our Denver laboratory. And then the serology and antimicrobial susceptibility data were submitted to our lab

at CVM.

So between 2001 and 2013, we received nearly 4,000 *Salmonella* isolates from imported samples and I've broken them down. So they included some water samples, environmental samples, food, feed, and pet treats. And I also wanted to give a brief note regarding the limitations of this data. The isolates, the information that we received, some isolates that were received from the imported foods and feed and other samples were risk based. They were collected as a risk-based type of analysis where certain countries are more likely to be targeted by ORA as they came in through the imports, so the data is not completely representative of all the imports that are received through the U.S.

And now going back to the data. So nearly 4,000 *Salmonella* isolates that were received between 2001 and 2013, 92% or 3,586 were covered from food samples.

And then here's a brief breakdown of what the *Salmonella* serotypes from these food samples were. And specifically looking at -- as you'll see, the top serotype was Weltevreden followed by Newport, Senftenberg, Bredeney, so on. And Enteritidis, we found, was the ninth most common *Salmonella* serotype that was recovered from these imported foods, totaling about 67 isolates in all, or 2% of the total isolates that were part of this dataset. And then in looking at these *Salmonella* Enteritidis sources, we found that of the 67 isolates, 34 were from seafood and then 13 were from

herbs and spices.

So when we looked at general resistance among the *Salmonella* isolates from these imported foods, we found that 91% of the isolates had no resistance to any of the antimicrobials tested, and that of these imported food samples, only 2.8% showed any resistance to nalidixic acid. And so when we broke down those 2.8% of the isolates, we found that 15% were from *Salmonella* Enteritidis; 13 from Kentucky; and 6 were from Albany and Virchow.

And then when we looked closely at *Salmonella* Enteritidis data, we found that 29% of *Salmonella* Enteritidis isolates -- so this is from the 67 that I mentioned earlier. They were resistant to at least one antimicrobial tested. And then when we looked at nalidixic acid resistance, we found that 22% of them showed resistance to nalidixic acid.

And this was the most common resistance to the *Salmonella* Enteritidis isolates, and then we also saw other resistance to these particular isolates. 19% of them showed resistance to ampicillin. Streptomycin, 14% were resistant to streptomycin, and 11% were resistant to tetracycline. And those are the other common resistances that we saw in these 67 isolates.

So, again, the total number was 14 nalidixic acid resistant isolates, and then when we broke them down by sources, we found that six of them came from seafood. And I have broken down the countries where these seafood were linked to. And then the other -- three of them were linked to

herbs and spices, so I also included the countries here. And as you can see, predominantly, these food sources came from Asia and then predominantly China, but there were only four isolates that came from China.

So, from our data, we concluded the nalidixic acid resistance seems to be higher in imported foods when we compared it to what we have from retail meat and the meat at slaughter; and that seafood and herbs and spices seem to be the most common source of *Salmonella* Enteritidis and they also were more commonly associated with nalidixic acid resistance.

So, in looking at both the travel-related data and the imported food data, we found that there may be -- there appears to be a link to nalidixic acid resistance from *Salmonella* Enteritidis that came from import and travel. But we also -- given the limitations of the ORA sampling, we need to do additional analysis on imported foods to see if this link can be provisional, so further research needs to be done on other sources that could be potential sources of *Salmonella* Enteritidis, sources such as eggs, which have been previously linked to outbreaks.

And then let us know if you have any questions.

(Applause.)

DR. TATE: I think we have time for one or two.

DR. SHRYOCK: Tom Shryock, Elanco Animal Health. Probably a question for Allison.

I'm just curious. Many travelers, internationally, particularly

business travelers, have medical kits that they carry with them which do include fluoroquinolones, which they may self-administer if they're feeling a little bit upset, perhaps for travelers' diarrhea, but anything goes. So I'm just wondering, do you have data on that sort of thing, perhaps, in a wider sphere of research?

MS. O'DONNELL: So we don't have data on when the isolate was taken, whether or not it was taken before or after they took an antimicrobial. And we do have a questionnaire that we administer in NARMS that does ask that question, so that is something we could look into, but we don't have that data for this study.

DR. TATE: Okay, thank you both.

DR. MAHON: Our next speaker is Cita Medalla. Dr. Medalla is an epidemiologist with the National Antimicrobial Resistance Monitoring System at CDC. She earned her medical degree and M.S. in epidemiology from the University of the Philippines and completed residency training in family medicine at the UP-Philippine General Hospital.

DR. MEDALLA: Good morning. Or should I say good afternoon? I will be describing trends in antimicrobial resistance among *Salmonella* from 1996 to 2012. And at the end of my talk, I will also be describing some of our future directions.

As described by previous speakers, NARMS began in 1996 and is the only source of nationwide information on resistance in *Salmonella* and

other bacteria that are commonly transmitted by food. Since 2003, all 50 states have been participating in *Salmonella* surveillance, and states have been submitting every 20th *Salmonella* isolate to CDC for testing.

NARMS tracks resistance, including resistance to individual antibiotics and multi-drug systems. Most important, resistance patterns include resistance to antibiotics used with -- infections including ceftriaxone, a third-generation cephalosporin, and ciprofloxacin with quinolone.

A few speakers have articulated the importance of tracking resistance, and it is one of the core actions to prevent resistance. NARMS tracks resistance, and a lot of the ways it does this is by reporting national data on resistance percentages annually. NARMS has detected both increases and declines in resistance, and both increases and declines are important, and they have important public health implications. Increasing resistance, however, is of great concern as it may reflect important emerging trends. Some of the emerging trends in NARMS have contributed to detection of new highly resistant infections leading to investigation of their sources and discovery of new and ongoing ways -- acquire resistance and spreadability to other bacteria.

Here is a summary of our methods and how we assess changes in our systems. We compare resistance proportion in the current surveillance year versus the baseline, and thus comparisons are between two points in time. We include citing the analyses to address potential state-to-state

variations in the resistance, and we use the nine U.S. census regions to categorize these. We test for statistically significant changes. And I will not go into the details, but I will be describing these changes as either an increase or a decline where we found significant differences between the years that are compared.

Baseline source is selected to address specific questions about changes in the resistance. In NARMS, we have used two baselines: historical 1996 baseline to describe changes in resistance compared to what we found in the first year of surveillance. We have used this baseline in previous publications in earlier and other parts. We are currently using our 2003-2007 baseline to describe changes in more recent years. These years represent the first five years of nationwide surveillance for *Salmonella*. And we use the five-year out range of resistance to have more data in our baseline and to smooth out yearly fluctuations. This baseline we are using in our analytic part since 2008.

The next six slides, I will be presenting some highlights on the paper that we published last year describing changes in resistance among non-typhoidal *Salmonella* and four major serotypes from 1996 through 2009. These results refer to non-typhoidal *Salmonella*, which I will simply refer to as *Salmonella* for the remainder of the talk. We focused on the four major serotypes, as these major serotypes are among our five serotypes which have accounted for nearly half of isolates for *Salmonella*. We compared resistance

in 2009 with the 1996 baseline.

This slide shows resistance to two of our important antibiotics, ceftriaxone and ciprofloxacin. From 1996 to 2009, resistance to the two important antibiotics increased, although remained at low levels.

Resistance to ceftriaxone, shown by the blue line, increased from 0.2% to 3%. And reduced susceptibility to ciprofloxacin increased from 0.4% to 2%. Although our title says non-susceptibility to ciprofloxacin, for the remainder of this talk, I will refer to this pattern as reduced susceptibility. And let me remind you about our definition of reduced susceptibility. It includes full resistance as well as some degree of resistance to ciprofloxacin.

When we look at resistance in overall *Salmonella*, our typical next step is to look at what serotypes are driving this increase, for example, this trend. And for ciprofloxacin resistant isolates, the majority are actually three serotypes: Typhimurium, Newport, and Heidelberg. Each accounted for about 80% of our isolates. And, further, these accounted for over half of isolates with reduced susceptibility to ciprofloxacin.

This slide shows multi-drug resistance in overall *Salmonella*. Multi-drug resistance is defined as resistance to at least three classes and has declined from 17% to 10%, as shown by the dark blue line. And the serotype that's really driving this multi-drug resistance factoring was serotype Typhimurium, which accounted for about 60%. And I'll be showing the data for Typhimurium after this slide.

We found that this decline in multi-drug resistance was actually mainly driven by decline in MVR pattern, ACSSuT, shown by the red line. And other speakers have defined ACSSuT as resistant to the five drugs: ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline. ACSSuT declined from 8% to 4% in overall *Salmonella*.

You will also note that there are two other MVR patterns shown on this graph. And unlike ACSSuT, these patterns increased and these patterns include ceftriaxone resistance. And I included the light blue line to show that these patterns are actually showing the same direction as ceftriaxone resistance, which is the blue line. So these two other patterns, the brown line and the orange line, have actually increased.

This slide shows resistance in serotype Typhimurium. Again, the dark blue line shows multi-drug resistance, and the red line shows ACSSuT. Multi-drug resistance declined from 50% to 28%. And as I noted earlier, the multi-drug resistance in overall *Salmonella* was mainly driven by this serotype, specifically by decline in multi-drug resistance in serotype Typhimurium. And you will see that the parallel decline in multi-drug resistance in ACSSuT is similar to the results that I've shown from the previous slide.

You will also see that resistance to ceftriaxone increase, shown with the light blue line, from 0% to 7%. Typhimurium, along with Newport and Heidelberg, as I mentioned earlier, accounted for the majority of isolates

that are resistant to ceftriaxone. But the increase here, in Typhimurium, is not as dramatic as the increase that we observed for Newport and Heidelberg. And I'll be showing the results for those two serotypes later.

This slide shows resistance in serotype Enteritidis. From 1996 to 2009, reduced susceptibility, shown by this purple line, increased from 0.4% to 4%. And this is a very remarkable finding, mainly because Enteritidis is typically not resistant to other antibiotics. Multi-drug resistance remains at low levels. Our previous speakers have actually shown the association between nalidixic acid resistance, which is our marker for reduced susceptibility in international travel.

This slide shows resistance in serotype Newport. Again, we define multi-drug resistance as resistance to three or more antibiotic classes. So the blue line shows multi-drug resistance, and from 6%, you're seeing a peak here in 2001 at 31%. So this is the line for Newport, 6% peaking to 31% and then declining to 8% in 2009.

There are two patterns in the next two lines, although you can hardly see them because they are overlapping and they're almost identical. These are ceftriaxone resistance, represented by the light blue line, and an MDR pattern that includes ACSSuT plus ceftriaxone, represented by the brown line. I will simply refer to this pattern in Newport as MDR Newport.

MDR Newport emerged in 1998. It was the first time we detected this pattern in Newport, and it peaked in 2001 at 25%. And the

emergence of this pattern in Newport was found to be the main driver in an increase in ceftriaxone resistance among overall *Salmonella*. Note that in the early years of NARMS, particularly those years from 1998 to 2001, investigations of cases in New England states identified cattle as a reservoir of infection for this MDR Newport strain.

This slide shows resistance in serotype Heidelberg. Multi-drug resistance increased from 12% to 26%. And resistance to ceftriaxone increased from 3% to 8% in 2008 and then it jumped to 20% in 2009. And this serotype has been implicated with outbreaks related to poultry, and Dr. Tauxe has kind of described that in the past two years we've seen these outbreaks of Heidelberg. And this is kind of big news, the same way that Newport was big news back in the early 2000 -- in 2001. So this is currently what we're really focusing on lately, because we've had quite a few outbreaks of Heidelberg.

Before I give a summary of the 2012 results, where we compared to 2003-2007, I would like to kind of just summarize what we found in the first 15 years of surveillance. So we found declines in multi-drug resistance. We found increases in important antibiotic ciprofloxacin and ceftriaxone, although the levels have remained low.

So what are the trends that continued after 2009 through 2012? Multi-drug resistance continued its declining trend since what we observed in 1996. It was 12% in our baseline, and it declined to 9% in 2012.

Two important MDR patterns in Typhimurium and Newport declined. ACSSuT and Typhimurium declined from 23% to 17%. The MDR pattern in Newport declined from 13% to 4%. So we are seeing that continued decline for these two MDR patterns. For Newport, after the peak, it continues to decline.

Other resistance to ceftriaxone and susceptibility to ciprofloxacin remained low. Resistance was higher in some serotypes, which is a cause for concern. I described an increase in Heidelberg that we found beginning in 2009, and then through 2012 resistance has remained high at 22%. Nalidixic acid resistance, which is a marker for the susceptibility to ciprofloxacin in Enteritidis is still of concern even if it's still considered at low levels at 8%.

There are issues and limitations in our analysis. First, identifying the appropriate baseline to address important questions is really key. I have also described that the comparisons are between two points in time, so meaning what we're testing is mainly a comparison between the current year versus the baseline. So our comparisons do not really capture what happened between these years. There are multiple findings between these years. We do describe them, like the peaks that we noted for serotype Typhimurium.

Site categories are limited to the nine U.S. census regions, so it's very limited. We're not able to adjust for -- at least for the 50 states. We're only using the nine U.S. census regions for -- including site in the

analyses. There are issues with sparse data, particularly, the resistance-based serotype because of lower numbers. We recognize that the limitation at NARMS is that we cannot calculate the incidence of resistant infections directly from NARMS data.

NARMS sampling includes every 20th isolate, and thus, there's a fraction of all infections reported in the United States. Resistance proportions are what we can calculate directly from NARMS. Incidence is the rate, and like resistance proportions, it is a rate and it represents a number of infections per person per unit time. So we can define annual incidence, for example, as the rate of resistant infections per 100,000 persons per year.

So why do we need to estimate incidence of resistant infections? Antimicrobial resistant infections represent a distinct public health problem. We need to understand the size of that problem and how it might change over time. Because annual resistance is a rate, it is a measure of the occurrence of resistant infections per 100,000 persons per year and thus can provide information on the magnitude of the problem and changes over time. NARMS does not report the national incidence estimates of resistant infections, and yet changes in resistance first suggest that we detect in NARMS -- I mean, not necessarily reflect changes in the incidence of resistant infections in the United States.

And here's an example. Again, this is Heidelberg. And I've described the increase that we have observed since 2009, and it's too high in

2012. But we've observed that the number of isolates that we've been testing in NARMS have continued to decline over the years, and when we looked at reports of the total number of infections, we also found, shown in the green bars, that the number of infections in the United States has gone down. And this actually will translate into decreasing incidence of Heidelberg infections.

So we're concerned about this increasing resistance, but we know if the incidence of resistant Heidelberg is going down, the same way that incidence is going down -- or is it higher in the more recent years, even if the number of infections have gone down, so this is clearly a question, and this illustrates the need to measure the incidence of resistant infections. Clearly, there is a need for estimates of resistant incidence, and we have started working on this. We have developed methods for estimating resistance incidence for these four major serotypes: Heidelberg, Typhimurium, Newport, and Enteritidis, and overall *Salmonella*. And we're focusing on the important antibiotic ceftriaxone and ciprofloxacin.

So what are our next steps and future directions? We are enhancing our approach for assessing changes. We would like to expand our knowledge issues in other baselines to address specific questions of public health importance. For certain analyses, we need to explore other methods and approaches to address issues that I have described. We will continue efforts and develop methods for estimating resistance incidents. And major

issues include combining data from 50 states and dealing with sparse data for certain serotypes and resistance patterns. This approach will enhance reporting of NARMS data.

Resistance incidence estimates are of great value in public health. It can be used for a target setting and can be used to measure impacts of intervention and new policies. And this is why we will continue our efforts in estimating incidence estimates because they can be used to increase our understanding of the burden of resistant infections and how it may change over time.

We would like to thank our NARMS sites for their contributions to NARMS and thank you for your interest in NARMS.

Thank you.

(Applause.)

DR. MAHON: I think we have time for one question.

DR. SCOTT: Morgan Scott at Texas A&M University.

It's perhaps a comment and it relates to Jason Folster's presentation earlier. What I would like to point out is that there has been discussion about the classification of multi-drug resistance on the basis of three-plus categories. And what your data show, and what Jason's analysis of the CMY being on an Inc I in poultry versus an Inc A/C with a large multi-drug resistant cassette in cattle, points out the problems of relying simply on those in terms of regulation and policy.

My suspicions are -- I am not a physician, but there are those in the audience that, faced with a Heidelberg that had ceftriaxone resistance on an Inc A/C but not meeting the threshold of MDR versus a tet-strep-sul Heidelberg, that one would probably suggest that the ceftriaxone is a worst scenario. But it also has an impact in terms of how one would approach management in the pre-harvest sector. And the example was given yesterday about Quebec and the Heidelberg in the broilers there; that also was an Inc I. You remove the selection pressure, resistance went down almost in a waterfall effect. But it isn't clear that that would happen if it had been Inc A/C with ACSSuT.

So I think, taken together, these three presentations and the discussions yesterday point to the importance of not using simple categorizations of three or more as being somehow more evil or dangerous than the particular beta-lactam that we're really, really concerned about.

DR. MEDALLA: Thank you for comment. We're using that definition more or less like a generic definition for multi-drug resistance, but we agree that it is definitely not a standardized definition for multi-drug resistance, and perhaps the more important way of looking at it is looking at the clinical important drugs like ceftriaxone. And we agree that there are limitations with the use of our definition of multi-drug resistance.

DR. MAHON: Thank you, Cita.

(Applause.)

DR. TATE: Our final speaker for the Epidemiology portion of the research talks is Dr. David Dargatz. He is an epidemiologist at the USDA/APHIS Center for Epidemiology and Animal Health in Fort Collins, Colorado. He received his D.V.M. from Washington State University, a master's and Ph.D. in the Animal Science Department at Colorado State University, and as an epidemiologist at APHIS Center for Epidemiology and Animal Health, he has served as a beef cattle specialist since 1988. And in recent years his focus has been on infectious disease control, food safety, and antimicrobial resistance.

DR. DARGATZ: Thank you, Dr. Tate.

It's a pleasure to be here and to share some information about the National Animal Health Monitoring System studies. You've heard the NAHMS program alluded to in several cases, and I will talk a little bit about -- give an overview of the NAHMS program just to refresh some of the tenets of the NAHMS program; talk a little bit about the history, where we've come from over the years; some of the current and future studies that are under way and how they relate to the objectives of NARMS and this issue of antimicrobial resistance; some changes in the types of data and the samples that the NAHMS program has been collecting over the years; give a few examples from a recent feedlot study that we conducted in 2011; and then finish up with some conclusions.

So the NAHMS overview. Indeed, NAHMS, or the National

Animal Health Monitoring System, is a USDA program. It is a non-regulatory program, and it started in the '80s as a series of state-level pilot projects and transitioned into a national program in the late '80s and the early '90s. So since 1990, the NAHMS program has been a national, centrally coordinated program. Our mission is to estimate health and management parameters for livestock and poultry operations across the U.S. So some key points there. We use a stratified random sample of producers within subsequent weighted analyses to represent the source population, so indeed we're not just describing the participants in the study, but we're making estimates for that population.

And, typically, when we do sampling, we strive to represent at least 70% of the operations with the commodity of interest and at least 70% of the animal population in those production streams. And we will generally eclipse those threshold numbers by a large amount by focusing on key states and perhaps some thresholds in terms of inclusion with animal numbers into the operations that are eligible to participate.

It does rely on the voluntary participation of producers. Typically, we administer structured questionnaires through a personal interview or a telephone interview with producers. Sample collections often occur in association with these studies, and over the years we've collected quite a variety of different samples including feces, blood, air, water, dust, milk, insects, feed, environmental samples, nasal swabs, ear notches, all

driven based on what the questions posed by the stakeholders are that we're trying to address. So, as part of the design phase of these studies, we reach out to the stakeholder community, identify what are those key questions, and then see if there's a biologic sampling component that will help us answer those.

Another important point, confidential record level data. So, as has been mentioned multiple times, confidentiality is a key concern. And so the individual record level data that are collected are indeed confidential. Summary data are published, though summary data typically at the national level certainly, but also at the sub-national level where the sample size will allow that. So oftentimes in the reports you'll see references, estimates based on operation size, region of the country, operation type or production class of animal on those operations. So those would be typical sub-national estimates or subpopulation estimates that we would provide in the reports.

Again, over the years since 1990, in the transition to a national program, we focused on a variety of different production systems. We're now getting to the point where we've visited some of these production systems on many occasions. We're finishing up analysis on our fifth round of swine studies. Currently, we have data collection ongoing and analysis ongoing on the sixth round of dairy studies, and you can see that many of the other commodities have been represented multiple times in the NAHMS portfolio. And we've also covered quite a broad range of commodity

production streams over those years in trying to develop information about health, production, management practices on those operations.

Just a brief commercial to point you to the website where all the reports are posted, invite you to go there and explore what might be available across all of those studies. I won't begin to scratch the surface of what's available in those studies even for the AMR issue, but -- and I'll revisit this slide at the end of the presentation as well. Hard copies are available for those that might not want to dig through the electronic version. And if you want to join the mailing list which provides updates and alerts when we post new information to the website, you can contact Anne Berry. And also, there's a phone contact for her as well.

So some recent studies, just to sort of illustrate the link and the interest area to the NARMS program:

- The feedlot study conducted in 2011.
- A dairy heifer rearing study, so those heifers that generally go offsite from a dairy operation for rearing purposes.
- A cow/calf study in 2007-8.
- 2007: A lactating dairy cow study.
- And a swine study previous to the one that we're currently analyzing, occurred in 2008.

You can see the number of states that are represented, so we try to reach broadly across the U.S. again, to represent a variety of different

production types in a large majority of the animal production class under study. Usually, typically a number of questionnaires administered in sequence to the various participants in the studies. And I've highlighted some of the sampling that's gone on, particularly with an emphasis in commensal organisms and potential foodborne pathogens as a component of these various studies. And I'll talk a little bit more about the characterization of those isolates as we get further into it. But you can see a variety of different organisms that we've collected samples and evaluated.

Studies that are either currently under way or in some form or being planned:

- 2012: Another swine study, again focusing on operations with at least 100 pigs. Three questionnaires, a variety of enteric organisms identified. We're analyzing those data now.
- 2013: The dairy study focused on dairy/milk production operations, and again, a variety of samples collected for evaluation of the organisms.
- And planning right now for an equine study in 2015.

So some changes over the years in terms of the collection of data and the collection of samples. Historically, almost since the very beginning of the NAHMS program, we have collected some antimicrobial drug use information as a component of these studies, but typically, early on, a lot

of that was qualitative collection, so qualitative in the sense of are antimicrobials used in this or delivered in this way?

We've transitioned more into the use of named products or product classes, and we've developed some more quantitative use where we actually look at the numbers of animals that might receive these products and duration of use. And I'll show you some examples of that. We've also collected information on indications or reasons for use of those products.

So here's an example from the recent feedlot study. So you see that here are some of the sub-national breakouts, subpopulation breakouts. But if we focus over on the right-hand column of all feedlots, these would be feedlots with at least a thousand-head capacity and across 12 major cattle feeding states in the U.S. We've broken down the antimicrobials delivered in feed and talked about the percent of cattle that might receive those various products. So some look, then, at the amount of use. We also have estimates for the percent of feedlots that are using these various products. So, typically, we would look at both: operation prevalence of that management practice as well as the animals.

In addition, as I mentioned, we collect some information about indications or reasons for use, and these are why the producers would attribute the use for those particular products when requested.

In terms of antimicrobial use by injection, we've expanded that section of the data collection, as well, to include -- instead of primary product

used for a particular disease scenario, we'll say, we've gone to collecting the use of name products and classes, and some quantitative in terms of numbers of animals that are receiving those products and the disease condition for which they would receive those. We've also begun to incorporate some information about outcomes of treatment and subsequent products used or additional products that might be used in conjunction with antimicrobials for treatment, as well as subsequent rounds of treatment when animals don't respond to those products, what's typically used.

So here would be an example, again, from the feedlot study where we've estimated the percent of animals that experience bovine respiratory disease in these feedlots. So about 16% of animals placed in feedlots would experience a course of bovine respiratory disease commonly called shipping fever, as well as a variety of other disease conditions.

When those animals experience bovine respiratory disease, oftentimes they'll receive an injectable antibiotic; a certain percentage may receive an oral antibiotic, and then a variety of other ancillary treatments might be delivered for those animals as well. So, again, this is one of the charts showing the percent of placed cattle that might receive those products.

We've got some further breakout of that information as we look at placement weights, so animals that are placed at a lair weight might have a different animal health experience as they're coming into those

operations, and so we've collected data on different, sort of, classes of animals as they come in to that production system, again with an eye towards looking at the occurrence of some of these disease conditions and then subsequent treatment of those disease conditions.

So just in terms of conclusions, not to belabor the data, nearly all the cattle with respiratory disease or shipping fever typically associated with a bacterial bronchial pneumonia were treated with injectable antibiotics. The most common antibiotic treatments for initial therapy would be something in the macrolide class with about 46% of the cases being treated that way; phenicols, 25%; and the quinolones, about 16% of cases treated as a first-round treatment with those products.

As I mentioned, we've been collecting some data recently about success of those initial treatments or patient outcomes, if you will. So on those initial treatments, about 82% of those animals responded and were not relapses. And we also collected this information based on those animals' placement weight, so how large the animals were when they were coming in. Again, that's related to the levels of disease occurrence. So we can see, then, what's happening in terms of the health history of these animals and then retreatment and success rates for retreatment rates. We would also have some information about the selected products used in subsequent treatments for animals.

In terms of additional data, we would also collect some

information about decision making in terms of producers for product selection and ancillary therapies used. So who influences those decisions, how are those decisions made, what are the key factors that are considered when a producer makes a decision to use an antimicrobial product, all with an eye towards understanding how those decisions are made in the production environment.

In terms of sample collection and evaluation, we are in the process of moving from, I'd say, largely individual animal sampling to increased use of composite sampling on operations. And many of you will appreciate the fact that that's in, at least part, a financial efficiency approach to be able to look more broadly either at more operations or at more housing units within an operation to try to handle lab capacity, as well as cost. We've also moved from some individual animal sampling to environmental sampling to try to do that as well.

Initially, much of our focus was on *Salmonella* in the early days of NAHMS. Since then, we've expanded the focus to additional organisms to encompass the commensal organisms. And you'll recognize that these organisms are the same organisms that the NARMS program has focused on for recent history. In addition, we've had, on occasion, as there's been a need or a desire, a gap identified, we have looked at some other organisms such as MRSA and *Clostridium difficile*. And in the most recent dairy study, we'll be looking at *Listeria* as well.

We've moved from simply culture in order to estimate prevalence. We've incorporated susceptibility testing and driven toward more in the way of serotyping or speciation, and we're continuing to move into more molecular typing, as is everyone else, to further delineate these organisms. In addition, we're also looking at follow-up studies for subgroups of isolates. So exploratory evaluation or deeper evaluation of some of the isolates that might be of special interest.

So just an example to show the continuing series of estimating prevalence. So, again, from the recent feedlot study, prevalence of *Salmonella* isolates at the sample level, about 9% of samples were positive for *Salmonella* of some serotype; about 36% of the pens had one or more positive samples when we collected 25 samples per pen. The most common serotypes, three serotypes representing about half of the isolates that we saw, those being Anatum, Montevideo, and Kentucky; a variety of other serotypes represented in there, usually at very low individual prevalence levels.

In terms of the susceptibility profile for these *Salmonella* isolates, 75% of the isolates that we recovered were pan-susceptible, everything on the panel, a variety of other resistance attributes that you see, but far and away, the largest percentage of these were resistant to tetracycline and uniquely resistant to tetracycline. For all the other antimicrobial drugs, less than 10% of the isolates were resistant to any of

those.

There are some limits to the NAHMS data and to the sample collections. In terms of while we've been moving towards more quantitative measures of antimicrobial drug use, we have no information currently on regimen, which would include dosing level interval, duration of the injectable products. And so we're not truly able to calculate use in a quantitative sense. We have no information on the inclusion rates for the feed delivery specifically, and we have no information on combinations of products. So when products are used in particular combinations, we've not necessarily captured them.

However, we have used some other approaches to augment the NAHMS data, and many of you will be familiar, some of you very familiar, with this paper that really took another approach and augmented the data that we had from a swine study to apply contemporary use practices to those base data that we had within the swine study to extrapolate and to try to develop more quantitative measures. And so that is certainly an area that we're interested in and one that we've used in the past and plan to continue to use.

Again, returning to the commercial interruption, the reports and the data are available on the website. We'd be happy to help you find what you're looking for, if you want to contact us, or to supply hard copies of those or to enroll you in the mailing list.

A few summary comments then. The NAHMS data provide a historical perspective on antimicrobial drug use and resistance. We've been increasing the specificity of the data, that is the amount of data and how specific we've gotten in terms of those management strategies and the health experience of the animals. We've been increasing the diversity of the organism and doing more characterization of those organisms that we've been recovering from those production units as well. NAHMS data cover many of the major animal production streams and estimate for the national population. So, again, we're using fairly strict sampling design to be able to infer back to the source population for those participant groups.

NAHMS data do have some limits. The studies are periodic and cross-sectional, so they occur at intervals, varying intervals, depending on the commodity that we look at, so that's certainly a challenge when there is a demand for information that's timely, so these intervals don't necessarily always lend themselves to delivering important information quickly. It's also difficult to assign causality in these cross-sectional studies, as many of you will appreciate.

The laboratory capacity often limits the scope of our AMR evaluations and hence our move toward more composite sampling and environmental sampling to evaluate AMR and to evaluate prevalence of the organisms. The specificity of the data collection has to be balanced against burden, so after all we're there, we're enrolling these producers, we're

relying on their voluntary cooperation, and so recognizing that that exerts a burden on those people and takes them away from the other business that they're doing, we've tried to balance between more specificity in the data collection and trying not to be too much of a pest in the collection process for fear of jeopardizing participation.

However, there are other approaches to supplementing that. I mentioned the augmentation studies to where we can marry together data from other sources, and there is certainly the possibility of alternate study designs such as prospective studies, sentinel operations, similar to what you saw described earlier with the ARS pilot studies, in terms of ongoing work with a select group of operations, to be able to develop more information in terms of the temporal relationships between things that we may see happening on those operations and the possibility of getting much more specific with the data that we collect.

And with that, I'd be happy to try to respond to your questions.

(Applause.)

DR. MAHON: Thank you very much. I think maybe we'll have you take any questions outside because I definitely heard stomachs grumbling.

(Laughter.)

DR. MAHON: So we'll break just now. We'll be back at 1:30 for the afternoon session. Thank you very much to all the speakers and to the

folks who asked questions.

(Whereupon, at 12:42 p.m., a lunch recess was taken.)

A F T E R N O O N S E S S I O N

(1:33 p.m.)

DR. TAUXE: Okay, come on in and have a seat. Our next speaker, first speaker, for this afternoon session, is Dr. Craig Lewis, a Veterinary Medical Officer at the FDA Center for Veterinary Medicine. Dr. Lewis received a D.V.M. in 2006 from Cornell University and then worked in mixed animal private practice after completing an M.P.H. and a residency in food animal production medicine. Dr. Lewis is certified in veterinary preventive medicine. He's currently working in the Office of the Center Director at CVM, assisting the Deputy Director for Science on issues related to antimicrobial resistance.

Dr. Lewis.

DR. LEWIS: Hi, I'm Craig. I am currently using antibiotics. I feel --

(Laughter.)

DR. LEWIS: -- obligated to disclose that. It's for sinus infection. It's a second-generation cephalosporin. The diagnostic workup included the physician inquiring if I had discolored mucus and me encouraging him that if there was any reason not to prescribe antibiotics that I would be okay with that. But, anyway, I feel I did my part. It's probably a relatively minor use, but I feel obligated to disclose that.

Today I'm going to talk about antimicrobial use data in animals,

which I know is a topic of great interest here. In general, I'm going to go through highlighting the need, why we want antimicrobial use data in animals, and as an example of context, talk a little bit about how that relates to our current strategy. We'll cover, sort of reemphasize the existing data that we have; Dave and Eileen and others have highlighted some of these sources of data, the focus in particular on the work that some of the NARMS partners, through an interagency group, have done over the last few years; highlight another collaborative effort which we have under way; and wrap it up with a few conclusions.

So, first, starting with the why? Why do we want on-farm use data? And I guess before we go into what's actually on the slide, I wanted to acknowledge and maybe emphasize another need, which, I think, is confidence. You know, apart from the scientific inquiry or identifying appropriate interventions or identifying targeted interventions to address the public health concern, another, I think, primary driver of getting additional information is just confidence. And I think it relates to the fear of the unknown.

And as it's been stated, 2-3% of the population is currently directly involved in animal agriculture, and that means that animal agriculture, in general -- and therefore, the vast majority of us have no idea, frankly, of -- you know, first hand what's actually going on in NAHMS. So I think that fear of the unknown is a primary driver of the benefit of having

more information, and I just want to acknowledge that as we go forward. But most of the rest of this talk is going to focus on the scientific motivation, and I sort of see those as two distinct but concurrent motivations.

Talking about the scientific motivation, it's a huge priority getting on-farm use and resistance data, in particular, the two of those things. And I think the case has been made throughout the rest of this meeting that there's a gap between what we have right now and what we would like to have. And the reason for this is, is for meaningful metrics in order to assess both stewardship and policy initiatives or anything where we're attempting to mitigate the risk of resistance, the public health concern, by way of intervening on the way that we use antimicrobials.

So what it gets to, again, on the science side of things, that what we want to do is appropriately refine our stewardship principles and our regulatory policies. In order to do that, we need to improve our understanding of the associations between different uses and resistance, and in order to do that, we need long-term studies capturing a range of different use patterns and the associated resistance patterns in those different circumstances across all the variety of different production settings. So this sort of three-step logic here kind of reframed ending off with what we're going for: appropriate action, appropriate targeted, specific action. And in order to get there, we need long-term studies, and I think that's been emphasized and a lot of the work that the other groups have shown here as

well. And they came as two things together as improved understanding.

So I'm trying to frame this as the overall need or the overall why is for appropriate stewardship and policy, and FDA's Judicious Use Strategy is one example of such a policy. This is an important step forward in terms of addressing concerns related to the way these products are used in animal agriculture. It certainly isn't the only thing that FDA and others have done, and I think Bill Flynn showed a timeline of some of the things we've done over the last few decades, and those, in and of themselves, are just one example of the work that's ongoing in the past. There's work going on concurrently today in the human medical side, and even within the affected industries in vet medicine and animal production, and there will certainly be more work to be done in the future.

The overall policy objectives for FDA's current strategies, though, are to eliminate the use of medically imported antimicrobials for production indications and to move the use of these products into a situation that requires veterinary oversight. And these are two fairly substantial changes that have been demonstrated; these products have been used for a variety of purposes for decades and decades, so these may not be sufficient changes to us of all the concerns related to antimicrobials used in food-producing animals. These are big changes in the way the products have historically been used.

And that's over a three-year time frame, which kind of

culminates at the end of 2016. So it has sort of a definite period of time that this intervention is occurring, and there's been great interest in getting baseline information to assess the impacts. So I wanted to put that context, the assessing of the impacts of the strategy, in the context of this overall strategy, what the various components of that strategy are from implementing the changes. What we're actually trying to get done is to change the way these products are approved.

And as part of that, again, public notification, transparency to the public in terms of tracking progress, assessing the impacts related to making those change, we're going to change the way the products are approved. The intention is we're going to change the way the products are used, and ultimately, over time, we're going to change the impact on resistance.

And highlighted in red is the focus of this talk here, which is additional data, but also under Assessing Impacts is: Continue to collect and report the data that we already have. And I would like to emphasize that there is a tremendous amount of data, actually, that are going to be very valuable in terms of assessing those impacts over time that provide great baseline information, and it will be very useful. So I want to emphasize that existing data, the value that it has, as well as the need to get some additional data to make it even more easier to assess the impacts.

The last component is reinforcing stewardship, so making

changes on the labels up front and the way the products are approved, assessing the impacts of those changes by monitoring sales, use, resistance, but also reinforcing behavior, enforcing the principles of stewardship to the parties that are affected.

Dave Dargatz and Eileen Thacker have covered some of this information here, and actually, I somehow omitted an important slide between these two. I think I deleted it in the process of editing, but it was the on-farm pilot work is another source of information that has helped build this background information. But walking through here, we had NARMS at the top; this is why we're all here today, providing great information on antimicrobial resistance through the foodborne pathway.

Dave mentioned the NAHMS studies, which have actually a tremendous, although periodic, source of information regarding animal management practices, livestock situations, including antimicrobial resistance or antimicrobial use indicators. And in addition to that, some biological sampling already.

The ARS dataset, the ARMS surveys, themselves, are two very, very interesting -- in that they cover a lot of different technologies that the animal production groups may be using. And a great way to track the change as this intervention unfolds, how these different production systems are going to adapt, so that's another dataset that's going to be extremely useful in terms of tracking the impacts of this strategy and any others frankly.

The National Residue Program, very interesting one. It gives an indication of perhaps the misuse of these drugs, whether it's from using too much of them or from an inappropriate withdrawal time or a variety of other things that may impact the defining of the violative residue. There is some confusion, I think, in the public that the relationship between residues or antibiotics in the food and antibiotic resistance, the presence of resistant bacteria in the food, so hopefully this on this slide doesn't blur that line even further. But this is another interesting mis-source of data which is, again, we have historically as a baseline, and this will continue in the future.

Lastly, here, we have our antimicrobial sales and distribution data, and FDA has collected data on the sales of products from the pharmaceutical companies, and we put this in an aggregated manner. But we also have access to this internally on a product-by-product basis. So for internal purposes, in particular, this would be a very useful way to track the individual changes to specific products.

We do take assessing the impacts of this strategy, we have taken it very, very seriously, and I believe Eileen perhaps mentioned that there was a meeting in May of 2012, a public meeting, a stakeholder meeting that focused on this topic, and actually, fairly soon after that, in a follow-up, we had a July *Federal Register*, an opportunity for public comment on issues related to data collection, essentially three different questions that we posed to the public to give us input.

One is enhancements on the way that we presented the sales data that we already collect: How can we make the data that we collect and that we have access to more available and more useful to the public?

So this is an interesting one in that it doesn't necessarily change the information that FDA has access to and therefore may not necessarily impact our ability to directly assess the impact of our efforts, but it was in an effort to provide increased transparency and increase utility to the public in general.

I'm sorry, I might have mixed up the order of things.

The second thing that we asked for comment on were additional, potential additional, reporting requirements; additional things that the animal pharmaceutical industry could report to make the data that we do collect a little bit more useful. And this data would potentially be an enhancement to the data that FDA receives and therefore could make use of in terms of assessing impacts, as well as potentially for reporting to the public.

And the third question that we asked in that July 2012 notice was for suggested methods, additional mechanisms, under existing authorities that we could obtain additional information on the use of antimicrobials in the food-producing animals. And we made that a clear statement there, and I just want to reemphasize here that we think that having additional information that allows us to better understand the extent

of use and, even further than that, the relationship between use and resistance will support the implementation of our current strategy and beyond.

So, in response to that 2012 opportunity for public input, essentially all three questions we initiated, a response to each one of them.

The first one, the reformatting question, the question of making the data more useful for public representation, based on the comments that we received, we came up with additional ways to break the data down and to re-summarize it and report it. In September of last year, we presented, granted without any detail and then without releasing any information, but we provided another opportunity for public feedback on these proposed additional tables. And we're currently in the process of building that extra round of public input into our 2012 annual summary report. And we're also looking at going back historically at 2009 through 2011 to enhance those reports similarly.

The second question: What possible additional reporting requirements related to the sales and distribution of antimicrobials might be useful for FDA to receive and to require as part of our current requirements?

The current ADUFA 105 requirements are part of a legislative effort, and we currently don't have any regulations that implement that legislation; not that that stops us from receiving bad data, but in the effort to codify that legislative requirement, we're considering possible additional

requirements, as indicated by the public comment that we received.

And the third question: What can we do to get more information on the way the products are actually used in food-producing animals?

Frankly, there weren't any clear, in terms of mechanisms -- there was wide support for obtaining additional information, and frankly, it came from a number of different stakeholders on all sides of the equation. I think there's a common desire for appropriate metrics and for appropriate information. And I think that there is a common belief that the information that we have right now is maybe useful, but it would be desirable to get even more appropriate information.

So, in response to that, and as a way to move forward into concrete action, we reached out to our partners, CDC and USDA, formed a group to work on this in a dedicated way to develop approaches for obtaining this information in order to get that implemented on the ground before the end of our Judicious Use Strategy implementation, which, you know, in other words means before December of 2016.

And this is actually the place where I had omitted that slide, but the origins of this interagency group, the first thing that we did or the context that this group's action arose was actually in the context of discussions related to the on-farm pilot work, and that being ongoing work, as Eileen has mentioned, the utility of the information that that effort has

already given in terms of context for a path forward has been invaluable and so was some of the context of the initial work of this group. The additional work proceeding from that included a rigorous mapping of the distribution channels for antimicrobials, including medicated feeds, as well as other antimicrobial products, such as the water products that are affected by our current strategies but also the injectables and so forth.

We also reviewed literature for analytic approaches to developing this surveillance system effectively, this monitoring system we want to be sure that had analytic methods established to help guide the development of that surveillance system, so review of the literature as well as other international programs for methods, analytic methods in particular, that have been utilized to link these two things together.

And hence, sort of subsequent to Steps 2 and 3, coming up with a relative absence of a clear analytic method to design this monitoring system around, we submitted a proposal for a working group to develop an analytic method that could be used to build the surveillance system around and ultimately to analyze the outputs. And not stopping there and waiting for that effort to complete itself, we're continuing to develop the approaches for collecting this data.

So, in brief review of some of the findings of those -- a variety of different activities of this interagency group. This is a little bit of a brief slide, perhaps, but -- and perhaps a bit of an obvious statement, but the

distribution channels are extremely complex. The fact that we receive our antimicrobial sales and distribution data as a bulk annual total sort of belies the underlying complexity of what happens to it once it leaves the pharmaceutical company, or the manufacturer, before and in some cases if it is ever ultimately used in an animal or exposed to animals in any meaningful way.

So part of that initial -- the motivation behind that assessment of the distribution channels was the understanding, then, that capturing on-farm use patterns is a complex thing given that the state of the United States agriculture -- but this effort really drove home to us how complex it was or -- and frankly how challenging it would be to -- you know, in any sort of targeted way gather information in midstream in a distribution channel between the distribution from the pharmaceutical companies and the ultimate end use almost to the point that the real value of what we're looking for was the way they're used in the products. The complexity of that system made it appear unreasonable or unhelpful to look for points in between there that we could monitor that would give us added value with a reasonable amount of effort.

So what we were really looking for was on-farm use information. It didn't seem like there were any shortcuts to getting that by looking sort of midstream in the distribution channels, for instance, the Veterinary Feed Directive forms or even the amount that was distributed

from a given feed mill with the possibility for further distribution to other feed mills and wastage and whatnot.

So, moving on from the distribution channels, we looked at the analytic approaches both in the literature and in other countries, and the overall sense that we got, or our overall conclusion there, at least for the question that we were targeting, which is really a national population level intervention, how to analyze the impacts of a national level change in use patterns and national level changes in resistance. There does not currently appear to be a clear analytic tool available.

There were some analytic methods available using in vitro or individual or even a local scale phenomena; some of them were purely descriptive, some of them use complex modeling techniques. None of them were quite appropriate, we felt, to the question that we are ultimately trying to tackle. Similarly, our work that's been done in other countries and with the expectation that there is -- everybody is struggling with this issue across the world and that they may be developing additional analytic methods that aren't -- you know, that we're not publicly aware of right now. But as of this time, the time represented by the work of this group, the work that had been done in other countries, from an analytic standpoint, to link these two things together, we didn't find anything that we felt quite appropriate.

So that led us to this proposal to the National Institute of Biological and Mathematical Synthesis. Keeping with the spirit of

collaboration and federal agencies working together, I think that this is a great example of different federal groups: NSF, Homeland Security, and USDA being the main collaborators in this NIMBioS -- in NIMBioS, the institute. And the specific charge of this group and the mission of NIMBioS, to integrate modeling and mathematics with biological studies, seemed incredibly appropriate for this issue and for NARMS partners in general.

This is, perhaps, a little bit of an obscure group or a program, so just a little overview of what a NIMBioS working group, in particular, is. NIMBioS actually has two main sort of activities that they fund. One is workshops, which are a bit larger and a bit more general. And the other is working groups, which are smaller, focused on a specific question. And so the proposal that we submitted was for a working group for a well-defined scientific question, which is a question of analyzing the relationship on a population level between use and resistance.

Typically, these working groups meet two to four times over a two-year period. Participation is closed or that -- it's sort of inherent in the approval process of the proposal is approval of the participant list, so it's a closed invitation, but summaries of the meetings are publicly posted. So we submitted this proposal, and it was accepted. Here's the title of the proposal, "Evaluating the Association Between the Shifts in Use Practices and Resistance Resulting from FDA's Risk Mitigation Strategy." That focuses and kind of provides some of the urgency that we would like to get this in place or

get this analytic method and ultimately the on-farm additional data collection in place in advance of the completion of the changes that we're looking for under our current strategy. But that's not to say that the utility of such an analytic effort couldn't be much, much more broader than our current strategy.

The objectives. The specific objective is really to develop analytic methods and to prioritize data sources or particular types of data that are appropriate for monitoring and associating these population level changes in use patterns and resistance.

So the first meeting is scheduled next month, and within a month or so after that, we should have a public posting of the minutes and the outcomes from that meeting. And there's a link, there's a webpage that gives you a little overview of that work. And in the future, as updates are posted, that will be available so you can find it on the NIMBioS site by searching for Antimicrobial Resistance Working Group, or if available, you can follow this link here.

So that was, in some respects, a bit of an aside; I think a very important one in that we recognize relatively early on in the work that we were doing last year that it was somewhat of a deficit if we didn't have an analytic method in place to prioritize the variables that we wanted to go after based on the analysis that we ultimately wanted to do. We sort of recognized that as a deficit. But instead of blowing past that and not doing anything, we

put forth this proposal to kind of spin something off to get some work done on that front. But on the other hand, we didn't stop there and wait for it to happen, so we're proceeding to develop some approaches for getting this additional data even in the absence of a clear analytic method, even in the absence of a clear method for prioritizing variables. We're still moving forward with our effort to obtain additional information.

And that's where we are right now. We are developing these approaches, and within the next calendar year, next fiscal year, our intent is to seek public input on our plans to move forward, possibly through an additional public meeting, so this forum right here may be perhaps a little bit unsatisfying in terms of the amount of information that we can present here today, but our expectation within the next year is to provide another public forum specifically targeted at providing input on this issue and our plans to move forward with the goal that we begin collecting data in the subsequent year, during 2016, the end of our Judicious Use Strategy. The end of the three-year implementation of that strategy is in December of 2016, so we want to be collecting data prior to that time.

So, to sum it all up where we are right now, useful baseline information is currently available from a number of different sources, including the drug sales in antimicrobial resistance. And there is data available on antimicrobial uses, as Dave Dargatz highlighted, on the work that's going on with the on-farm pilots, is another example of mechanisms for

obtaining such information, what level of information might be obtained through those ways. But there is a gap in terms of linking use and resistance on farm -- or getting access to both those pieces of information at the same time, which is another sort of downside of looking at the distribution channels, is the missed opportunity to get resistance data as well.

This is a big priority for us, both in terms of our current strategy -- as you can see, one of the main components of our current strategy is to assess the impacts, and in order to do so, we would like to get additional information, but also, in the broader sense, to appropriate stewardship policies and interventions that may not even be directly related to what FDA is doing. This additional information, understanding the relationship to these two so that we can target the most risky uses, is a very high priority.

And the third thing in terms of an analytic method, what are we going to do with this information once we get it all? We have an effort in motion to attempt to attain or develop an analytic method that will allow us to target appropriate variables and to develop a monitoring system that is useful for doing just that, for understanding how these two things relate to each other.

Here are a few our websites, the FDA websites, in particular, that relate to this; our Judicious Use site, as well as our sale data and another shameless pitch for the NARMS program. So thank you very much, everyone, and open up for questions.

(Applause.)

DR. TAUXE: If there are questions, please come to the mike and identify yourself.

MS. STULBERG: Hi. My name is Elizabeth Stulberg. I'm from Congresswoman Louise Slaughter's office. I have a question about -- I really love the idea of this integrated way of looking at how use and resistance are connected, and I was wondering how a report from that kind of a group would be different from the reports that Bill Flynn was talking about yesterday that come from FDA where every six months they're going to give a report on how Guidance 13 is going.

DR. LEWIS: Thank you, great question. Bear with me. I'm going to scroll back so we can kind of see it in context of our overall strategy, if at all possible. And this sort of breaks down the three major or four major components of our strategy as we currently see it. And the third piece, the assessing impacts, is related to sort of internally how we make sense of this, how it increases our understanding of the effect that we're having, you know, on use practices, the changes that were happening in the first component, are having on use practices and ultimately on resistance.

The second piece, the reporting to the public, is related to that but a little bit different. The second piece, reporting progress, is transparency, keeping folks in the public up to date with what's happening, how the changes under the first component are progressing, as well as how

any assessments that are ongoing are really happening as well. So I think that second piece related to -- you know, one of those kind of primary motivations to just have some awareness of what's going on.

Specifically, you mentioned our periodic or our six-month reports, and those are indicated here under "Reporting Progress." Mainly, those are focused, at least right now, on the label changes, so are mainly going to be indicators of how many products have changed, aligned themselves -- how many drug companies have aligned their products with the recommendations and any summary of pending submission. So, in the short term, the public reporting and particularly the six-month reporting, is going to focus on actually the label changes for the most part.

Moving forward, in the years and decades beyond, I think that there is going to be a public reporting component of assessing the impacts, which is going to be a little bit different. Thanks.

Must be after lunch.

DR. NELSON: Harry Nelson, veterinarian with the American Association of Swine Veterinarians. Craig, I appreciate your presentation and more a comment than a question, I think. And it goes, actually, to how you began your presentation, which, I think, leads to one of the key issues that we're dealing with when we talk about antimicrobial resistance in the human population, and that is the ability for us to call up our family physician, describe our clinical signs and get a prescription for something, whereas had

you been a pig in today's swine world, a veterinarian actually would have come to your house, examined you, taken samples, unfortunately, might have submitted you or parts of you to the diagnostic lab along with samples from your closest neighbors who were exhibiting similar clinical signs. And all of that would have been for the purpose of determining: (1) What is the diagnosis? (2) Are antimicrobials appropriate to administer in this case, and if so, which antimicrobials? So I guess my point would be that -- or comment would be that yesterday and today we talked a lot about getting on the farm and finding ways to get to assess antimicrobial use on the farms, but I've not heard a whole lot about how we get into the human population and determine appropriate antimicrobial use within the human population. So I just make that comment.

DR. LEWIS: Thank you. I'll avoid the temptation to say much more than that other than, you know, I thought that that was where you were going, your comment on me being a user of antibiotics.

(Laughter.)

DR. LEWIS: I'm trying to be part of the solution, and right now, I'm probably a little part of the problem.

Any other questions? Are we on target?

DR. TAUXE: We're on target, I think. Thank you very much.

DR. LEWIS: Thank you.

(Applause.)

DR. TAUXE: I will say that the focus of this meeting is veterinary use. There are extensive and extraordinarily dense conferences throughout the medical community trying to communicate the need to reduce unnecessary use in human populations. That is not an ignored area at all. And I appreciate the comment. I think we have lots of work to do in that arena as well as in this. And that work is being pushed forward.

Our second speaker is Dr. Ruth Timme, a research microbiologist at FDA's Office of Regulatory Science. Dr. Timme received her Ph.D. in plant biology and has a research background using comparative genomics and phylogenetic methods to answer revolutionary questions that go way beyond plants. At the FDA, she is implementing phylogenomic methods for tracking foodborne pathogens throughout the U.S. food supply.

Dr. Timme.

DR. TIMME: I'm going to talk a little bit about the whole genome sequencing program we have over at the Center for Food Safety and Applied Nutrition, and then a little bit about how that relates to the NARMS database.

Okay, so a little perspective on the food supply and what the Center for Food Safety -- their role in trying to make the food supply safe. So CFSAN monitors over 200,000 registered food facilities; 80,000 of them are domestic, a lot more are foreign. We watch over 300 ports of entry; 130,000 importers; 11 million import lines per year; and over 2 million U.S. farms. So

it's quite a lot in trying to protect the food supply.

So most of the recalls that you hear about in the news, I think most people think about meats, sometimes cheeses, but actually the contamination can creep up into all different kinds of food, processed and fresh, a lot of produce. And so here's just a picture of kind of the recent recalls we've had over the past two or three years.

Okay. So I'm going to introduce the whole genome sequencing program over at FDA/CFSAN and then talk about the solution that we came up with, with trying to track the sources of these contamination events back to their food source a lot quicker.

All right, so this is a slide from 2008, just as our whole genome sequencing program was getting started. And at that time, we thought that we would collect the whole genome from each pathogen that was a contaminant of a food and we would use the whole genome to look for, like, little micro-changes of the genome that then could be used for industry to make assays to screen for a certain pathogen. There is a lot of emphasis on microarray; you could screen for antimicrobial resistance, look for several target genes. But what really stood out and took off is the outbreak response using the entire genome in the context of its evolutionary history to trace back to the source food.

And so I'm just going to tell you a little story about one of the early applications of this technology. So about two years ago there was a

Salmonella outbreak linked to spicy tuna. And there are a lot of ingredients in spicy tuna; it was in a wrapper, the rice, spice, the dish. So this slide right here, we got in a whole bunch of clinical isolates. Clinical isolates here. And they were all typed. This is like a banding pattern, kind of like a crude fingerprint for the genome. And we could tell that they were all the same here, and there were isolates coming in from Maryland and New York from clinical labs. Then we matched, looked in our database, and found some other PFGE patterns that were the same but not part of the outbreak. Then we had all these other of the same Bareilly type in the database.

And so what we did is, we got the isolates from all of these strains, and we sequenced whole genomes for all of them. So it was over 100 whole genomes that we sequenced. We were like all hands on deck for two weeks, we all kind of really committed to trying to solve this.

And so the isolates that had the same PFGE pattern, you know, those would hopefully give us a lead, like where this was coming from, but really, they were distributed all over the world, so our FDA investigators really were wide open for input. And after we sequenced all the whole genomes, we could narrow it down. The genome that was closest to the clinical isolates came from this location in India. And after we analyzed all the genomes and put them in their evolutionary context, here's -- called a phylogenetic tree.

What you can see here is here's the outbreak, right here, and

these are all extremely closely related, almost indistinguishable, and here's the same PFGE pattern, same banding pattern, but not part of the outbreak. And here you can see huge resolution here. And when we added in the eventual isolates from the contaminated food, they fell right into the outbreak. We identified them from a Moon Fishery in India, and those isolates actually came from a fishery only six miles away from here.

And so this really was a huge eye opener for us, and we thought wouldn't it be great if we already had those background isolates already sequenced in a database so that when we got new isolates in, they could just drop in, match their nearest neighbor, and provide an investigative lead right away? So that's where we went out to -- went about building this whole genome sequence database.

Okay, so we call this database a Genome Trakr. With this network, we are relying heavily on the fact that public health labs all across the U.S. already have these incredible storage databases of strains that they've collected over the past 20 years, and they're in freezers, they have complete metadata, and they're just sitting there. And so our group thought let's buy each of these public health labs a genome sequencer and a person to run it, and in exchange, they turn through those freezer stocks and start sequencing the genomes for all those strains they collected over the past couple decades.

So what we have right now are six state health labs, in blue,

and we also have 12 FDA labs participating as well. It's about 18 labs that are contributing to this network. We also have partners with sequencers that we aren't supporting financially. We have USDA as a partner, of course, and then partners all over the world, the UK, Denmark, Italy. Actually, Argentina is our new actual Genome Trakr partner. They're going to come online later this summer. And then we also have partners with isolates from all over the world. So they're sending us isolates and we're sequencing them.

And I emphasize this new Argentina lab. This has been huge. We partnered with the WHO and allowed them to pick a country anywhere in the world where they thought it was most appropriate to put a sequencer and fund a person to run it. And so we're excited to have the Argentina lab come aboard and get a lot of South American isolates in the database this way.

All right, so what do we want this database to look like? We want it to be as high-resolution genomic database as possible. We want it to be completely open access for the whole world to view the data. We want it to function in real time. So you'd submit a genome and expect some kind of an answer within a day or so. And we also require that the genomes going in have a minimum set of metadata so it's not just a genome sequence with no context. And then the sensitive metadata about firm names or specific locations like -- that would be all hidden behind a firewall of the FDA, CDC, wherever the data resides. And then in the future, right now, the only

submitters are our group at the FDA, but we envision that this would be open access for public health lab submitters from all over the world.

Okay, so the FDA minimum metadata. This has become extremely political and the most contentious part of this. So our minimum metadata for entry into the database is when, where, and who, basically. And pretty crude information or rough, you know, coarse information.

So the source information, like, what is it? We want more than "it was isolated from food." If we can get pepper or ground beef, that really provides a lot more investigative leads for tracking down the source.

When it was collected. We'll take the year, if that's all you have. But the requirement is at least at the year level.

Where it was collected. In the U.S., we require state level and no deeper than that.

And then who collected. Who can you contact if you have further questions about that genome.

This is a little bit of a dataflow on the data pipeline. As I said, we have 18 labs submitting data. They actually submit all the strain metadata to me first, and I register it over at NIH. We're partnering very closely with NCBI over at NIH, and they have a 25-year history of housing DNA sequence, and so this is just a further outgrowth of that. So the strains -- everything is all preregistered, then the data kind of comes to us after we already have -- kind of expecting the data. And then so all 18 labs transfer the data in

different ways to us. Then we do a quick batch QC, we convert it to the format that's required to upload it, and then we upload it over at NCBI. And then they build a phylogenetic tree of complete evolutionary history of that pathogen, and you can see -- you can kind of browse the tree and see your two new samples, where they landed in the tree. Okay.

And we envision -- we're trying to set up triggers now to figure out like when should we be alerted to something, like, is there a growing outbreak, is there a new strain that was added, is there a new food that was added that matched to a clinical? These are all kind of triggers that we want to set up that are automated. So we're in the process of doing that now.

So this is how the system's working now. In the future, we hope that it becomes a little bit more real time and that the field labs submit their own data and get their own results back, cutting the FDA out of this. Not out of it completely. I think we'll still stay in the curation business, but out of the data processing. And so we're developing tools right now to allow all those field labs to convert their own data, little software tools to convert their genomes and upload it themselves.

Okay. So where does the data go? I said it's going over to NIH. You can actually look up the database, just a simple URL; it's all public. And this is the database for *Salmonella*. Currently, we've registered over 11,000 bio samples, so complete strains with full metadata. And we have 5,700 genomes submitted so far. And this is about -- we've been very active in this

for little over a year. So that's -- we're pretty excited about -- I mean, not in my wildest dreams did I ever think we'd collect 5,000 sequences for this in the first year.

And then all the metadata I mentioned is public, too. And so you can click through these databases and browse the metadata yourself. Right here you can see "Collected by FDA." There's a strain identifier. It was collected from avocados. We have this huge sampling effort right now in avocados. You know, we have an exact date. It was collected from Florida. It's pretty high-level resolution there.

So how are we doing as far as the database diversity? Like, if we had 5,000 clones of the same thing, we wouldn't be doing very well. And so the kind of metrics we're looking for are spatial diversity, genetic diversity, temporal diversity.

As far as spatial diversity, we actually have samples from all over the world, just given our first year. Obviously, most of them are centered in North America, but we actually have some from almost every continent. Across the U.S., the samples, we have representatives from 43 states. You can kind of see where the labs are that are contributing the isolates, so we're looking at a couple more labs this year. We're hoping California, some of those states with -- border states and high agriculture states. Fill in some of these gaps.

Genetic diversity for the *Salmonella* database. We have mostly

subspecies enterica, which is the one that causes the most human illness, but we actually have representatives from all of the subspecies, in case there are other outbreaks. Within *Salmonella*, there are 2600 serovars, and we have over 400 represented in the database. So we're on our way there.

Temporal diversity. This largely matches what the public health labs have in their freezer, so over the past 15 years or so we have pretty good representation.

And then I just want to talk a little bit about how this is related to the NARMS database. So we've had this distributed sequencing network up and running for, as I said, two years, maybe a year and a half of really collecting data. And so we talked to the CVM folks and how we can include the NARMS sequencing in this. And so these are some of the health labs, public health labs that are contributing data, and when they run out of strains in their freezers, we know -- we have a contract with them. They have to sequence 400 strains a year. And so when they run out of their own, then we said, oh, you can sequence some NARMS, and so we coordinate really carefully with the NARMS database to make sure that the IDs all get transferred over and then those strains get sent out to those states and they're churning through them. So we have over 588. I think I just did a quick query on this genome sequence through Genome Trakr. So it's really exciting.

And here's a little bit about what bio samples look like for

NARMS. The only thing really different is that we have this NARMS ID that will be an easy way to query them. And I think in the future, I'm not sure that I'll be directly involved with this, but I envision that we'll have the phenotypic data, all of the phenotypic data that you have and antimicrobial resistance will be added to this in a more regular way.

So I've just been describing a bit about our *Salmonella* database. We actually have now these Genome Trakr databases started for four other organisms. Three of them have data. We have a real-time *Listeria* effort in close partnership with the CDC. And so since last August actually -- yeah, a full year now -- we have sequenced every single clinical *Listeria* isolate that's come up in the U.S. in real time, and we have 852 clinicals and FDA has sequenced 757 food and environmental isolates. These are all public. We have just a little smaller *E. coli* database, and also we just started sequencing *Vibrio* as well.

So I mentioned that NCBI is building these huge evolutionary trees. So this is what they look like. They're enormous, and you can zoom in on them. You can query them pretty easily. Here's the one for *Salmonella*. Here's the one for *Listeria*. And I mentioned all the data is public. All these, all the trees are public, too, so they get updated once or twice a week, and they just get put out to a public FTP site that anyone can download and look at the tree of the week.

And I'll just touch a little bit on a real use kit scenario, a real

use case for this data. You know, it's great that we have 5,000 genomes, but have we done anything with them? We have. And so I'll just tell you a little story about a recent *Listeria* outbreak. And the little outbreak is here, just give you a little context. So this past year, there were four clinical cases -- these are in blue -- of listeriosis. And three of them were in the Maryland-Delaware area, and one of them was in California. And they came in and they got fingerprinted, you know, with the PFGE patterns, and they matched, but there wasn't enough epi data to link them, and they just kind of sat there and nothing was followed up. And then all of the genomes were sequenced as part of this real time effort. And they clustered in the tree really closely, so that sent off a little curiosity.

So we started following up to see if they were really linked, and we learned that soft cheese might be the link, and so we partnered with our public health labs in Virginia and Maryland and D.C. They all got samples from this cheese, unopened cheese product. And so we sampled all those isolates. They all clustered in together, and then our FDA investigators went out to this company and took environmental swabs from the plant, and those all came back enclustered exactly the same. So here is a big outbreak cluster right here.

And so we were able to say conclusively that these were all linked, that this cheese, the contamination from this plant, the *Listeria* that we collected from this plant made it into the cheese, made it into the people,

caused them to be sick, and then we were able to -- the first time ever, we think, from a federal regulatory standpoint, used whole genome sequence data in this phylogenetic context for regulatory action. And we shut the plant down.

We were also able to, with this, exclude this California case. It's very close, but it's not within this outbreak swarm. So there's probably a common link way back here somewhere of a common source, but as far as this Roos cheese plant, we couldn't say -- we were able to definitively say it appears that they weren't the cause of this California case. And so I think this exclusion is going to be just as important as inclusion, especially as far as -- for industry.

We have a public communications site that anyone can join. It's fairly new. We post only public types of communication tools on here. So we have documents with all the wet lab SOPs and dry lab SOPs for processing the data. We also have a discussion forum where people can ask questions, and we respond in fairly quick order. And then the whole network can see the answer instead of all the e-mail going through me, which is what was happening in the last year. I also post progress reports for all the labs here, as well, and so everyone can monitor their strains and get accession numbers and stuff like that.

And I guess I just want to -- you know, as the NARMS -- as whole genome sequencing starts -- as we start collecting whole genome

sequence of all the NARMS strains, I really think that putting them in their evolutionary context will help answer a lot of questions about how antimicrobial resistance is evolving. We'll be able to track lineages over time. Each of the lineages in this tree that's associated with an outbreak is getting an accession number that we'll be able to track over time. So even though you get new samples in that lineage, that lineage will stay fixed, and so we'll be able to see, like, we'll be able to look at microbial evolution in real time, like does it happen -- you know, once you have an outbreak, does that outbreak continue to evolve and cause other outbreaks or just stay there and pop up from the stem. So I think this is what really helped us do trace-back, and I think will help NARMS as well.

And then I want to put a plug in for just the power of open data. I like the analogy of collecting of these satellites circling the world, collecting weather data, and they all get logged into a public database, and then people from all over the world can use those data to come up with their own models to predict hurricane paths or weather patterns. I think we're doing the same thing here. We have distributed data collection sites all over the U.S. right now, and then hopefully all over the world, all those data will get uploaded to the respective genome database, they all share data, and then scientists from all over the world can look at the evolution of the pathogens and look at really pathogen evolution in a global context.

And then in 10 years they'll probably predict stuff I haven't

even thought of. But I think that this only can happen if the data is open, the genome data and at least a minimum set of metadata. And so this is a huge effort with a lot of people involved, and so I just want to acknowledge that it's a multi-agency/industry partnership that has made this possible so far.

So thank you.

(Applause.)

DR. TAUXE: Any questions for Dr. Timme?

MS. GROOTERS: Susan Vaughn Grooters with Keep Antibiotics Working.

What's interesting here is we're going to be shortening the timeframe for outbreak. Can you give an estimate about how quickly you think it will shorten that from where we're at now?

DR. TIMME: Yeah, that's a really interesting question. So we can get the genome sequenced in the same amount of time as we're getting the PFGE patterns done. The difference is -- and I think that that will only shorten as technology improves. The difference, I think, is that the data are going to be public, and so the public, the press, industry can all be looking at the data in real time, just as CDC and FDA are looking at the data.

And so I think outbreaks, and especially sporadic illnesses, we'll be able to link those much quicker because the evolutionary history provides really good investigative clues. It's not going to be the only evidence we use. We need the epi data as well. But I think just knowing that genomes are

matching in real time and having that be public is going to be the real -- it's really going to speed things up.

DR. ZHAO: Shaohua Zhao from CVM.

Ruth, I have one question. You use some *Salmonella* Bareilly as the example, you know, to tier out the outbreak strain from the non-outbreak strain, even there was the same PFGE profile. So you say that there are two to three, five-SNP difference. Did you look at this as a core gene or entire gene, you know, include mobile elements?

DR. TIMME: For that Bareilly data -- so it was early. That was a bit more of research. We have really standardized our pipelines. I'm trying to think of the one that we used there. But it would just use the core genome.

DR. ZHAO: Core genome.

DR. TIMME: So all the SNPs that were found would have to be found in all the genomes, and so if there is a mobile element, then it's not moving. It's not moving within that dataset, I guess, to say.

DR. ZHAO: Okay, follow up with this question. Is any work going on between CFSAN and did Cita say PulseNet had come out with kind of a standard, you know, enter pre-table criteria, okay, use -- came out or use as a core gene into --

DR. TIMME: Yeah, yeah.

DR. ZHAO: Is any --

DR. TIMME: So that's going to be really important in

standardizing our analyses. We're publishing right now our SNP pipeline, in that it's public and so anybody can download it and use it and replicate it. And we're having regular meetings with the CDC to -- even if we use slightly different analyses, that our results are exactly the same, that we can replicate each other's results. So that's what we're working on right now.

DR. ZHAO: Thank you.

DR. TIMME: Yeah.

DR. TAUXE: Thank you. Thank you very much.

DR. TIMME: Thank you.

(Applause.)

DR. ZHAO: Okay. Our next speaker is Dr. Yuansha Chen. Dr. Chen received her Ph.D. from the University of Maryland in microbiogenomic. Currently, she is a researcher/bioinformatic analyst in the FDA/CVM with expertise in microbial genetic analysis. She has developed the genomic analysis of pipeline investigation of antimicrobial resistance and track of spread of resistant gene in pathogens, using whole genome sequencing. So the title of her talk is Comparing resistance genotypes with phenotypes - building a resistance gene library.

DR. CHEN: Thank you, Shaohua.

I will be talking phenotype/genotype associations study, using the whole genome sequencing. So, from the previous talk -- and then also whole genome sequencing has been mentioned in several talks before, that

it's a very powerful technique and that it has a potential to replace many of the traditional molecular and microbiology methods we used before.

For example, we can trace the phylogeny of the pathogens and to look at the source attribution, and we can look at the typing. And then the information that would traditionally derive from PFGE and serotyping, and we can identify the versions factors from the whole genome sequencing, and we can potentially also identify the antibody resistant genes and then predict a resistant profile from the whole genome sequencing.

So my talk will be focusing on the antibody resistance. So, in the last year or so, we have launched a couple of phenotype/genotype associations body. We want to test the possibility of using whole genome sequencing to predict antibody resistance. So we want to ask the question, do we have the knowledge and tools to do that and how well are the known genes' mutations correlated to resistance? And what genes and mutations should be included to predict resistance?

So it's obvious that in order to use the whole genome sequencing to predict resistance, we need a good collection, a good library, of resistant genes and mutations that leads to those resistance. And thanks to the research community worldwide, we actually know quite a lot about antibody resistant mechanisms. So the resistance can come from acquired genes, and it can also come from over-expression of target proteins or over-expression of efflux pumps to pump the drugs out, or it can also come from

permutation of some of the target genes.

So this is just an example of one of the acquired genes, the CTX-M genes. The CTX-M gene causes the so-called resistance to extended-spectrum beta-lactam. So by the time we launch our study, there has been 155 genes of variance, you would call it, reported and then the sequence are very diverse.

And this is another example. The CMY genes that Jason talked about this morning. And we can see there are two major groups, if we look at the sequence diversity.

And this is a different example of another mechanism of resistance. In this case, there is a permutation in the housekeeping gene 23S rRNA. So, in *Campylobacter*, if there is a permutation, permutation at this position, specifically positioned in 2075, and if it's a mutation from A into G, then the bacteria will be resistant to macrolides, like azithromycin. So, in order for the whole genome sequencing to be able to predict all the resistant phenotype, we should be able to capture the acquired genes and the mutations in the housekeeping genes.

So there are a couple databases out there in the public that we can use, and they include the CARL database, which is put out by the Canadian group, and it has a web interface. And also there is the ResFinder that is maintained in Denmark, and you can also use the web interface. And the new one would be the ARG-ANNOT. It's by the French group. And then I

think the ARG-ANNOT also includes some of the permutation information. So there is a little bit older one, it's put out by the University of Maryland, Antibody Resistant Gene database, and then this database it's comprehensive by a tree; it also has a log of the sequence from the genome database that has not been validated yet.

So all of these databases can be downloaded and then used with a program of your choice, such as the CLC Genomic Workbench or BioEdit or use the standalone blast or even your own script. So before some of those databases are published in FDA/CVM, we have compiled our own database. So we build the database based on, at that time, the available database publicly, including the University of Maryland antibiotic resistance database, and the beta-lactamase collection maintained by the Lahey Clinic, and the tetracycline and antibiotic resistant genes maintained in the Washington University.

And we also go through the literatures and pool our genes and then pool other sequence from NCBI and then collect all of these sequence together and then compare them one to each other, and build a non-redundant database. And since those public database published, we have cross-checked our database against those, that include CARL, ResFinder, and ARG-ANNOT.

So, currently, our database has 2,738 genes, and then if we group them by the 90% identity cutoff, there are 669 groups. And this will

give us an idea how diverse these resistant genes are, and they fall into different resistance with different drug classes. So this is the workflow we use to generate our resistant phenotype/genotype data. So we sequence our isolate from our pure culture, a single colony. The DNA was sequenced in the MiSeq instrument and then assembled by CLC Genomic Workbench. And then from there, we have one dataset to go through two rounds.

So for the acquired genes, we compare it to our database using the program BLAST. And then we retrieve out the acquired genes. And then for the permutations, we pool these target genes, including 23S rRNA and the gyrase A genes and then align them and then look at the permutations and the -- data and combine the two together. We have the resistant genotypes.

So we use our antibody resistant database to pool out genes of the resistant genes, but we also use the whole genome sequencing in turn to validate our database because for all these strains we sequence, we have the antimicrobial susceptibility test data, the AST data, for every single isolate so we can compare the phenotype versus the genotype and see they agree to each other.

So I am going to talk about two studies, one in *Campylobacter* and one in *Salmonella*. For the *Campylobacter* study, in the study we included 74 isolates, all from retail meats, and they include all of the representative MDR patterns, the multi-drug resistant patterns, from 2004 to 2010 and also include the gentamicin-resistant isolates from 2012 to 2013.

So just a quick reminder. The NAHMS AST panel for *Campylobacter* only include -- and then four for macrolides and two from quinolone and one each from the other classes. So this table is a small portion of the results for the *Campylobacter* phenotype/genotype association study. So the first column is isolate number, and the second column here is the AST data, which we consider as the phenotype. And the third column is the genes we found from those whole genome sequencing data, which we consider as part of the genotype. And the last three columns are the permutations from those genes that people have reported to us to associate with the resistance.

So without going through the detail, we found that the phenotype and genotype correlate very well for the *Campylobacter*. In all 74 isolates, we only had found that one isolate that has resistance to gentamicin, but we couldn't find a gene or mutation to associate with this phenotype; but for all the rest of them, they all correlate very well. And *Campylobacter* has many isolate that are resistant to quinolone and macrolide. And for quinolone resistance, we found that the gyrase A position 86 is a very good indicator and almost -- actually, all of the isolate resistant quinolone have a mutation at this position from threonine to isoleucine, and all the susceptible strains do not have this mutation.

And for the macrolide resistance, and people have reported the 23S rRNA mutation are very important for the macrolide resistance, and also some other ribosomal proteins will contribute to that, too. But in our data, it

looks like the 23S rRNA alone will give us very good prediction, so almost all of the strain, except one, if they're resistant to macrolide, we have a mutation in 23S, the position 2075. And the one of them does not have a mutation in this position has a mutation in 2074. So it's a 100% correlation in this case.

So if we break the results down into different drug classes, the correlation, the concordance for most of the drugs of tetracycline, quinolone, macrolides are 100% for *Campylobacter* in our datasets. And for the gentamicin, we have one resistant strain that we couldn't identify a gene for that. So when I talk about concordance -- on the resist side that, for example, like these isolates, we have found resistant genes for aminoglycoside resistance, but since those genes have not been reported for gentamicin resistance, but gentamicin is the only -- and aminoglycoside, we test it, so we would call these not correlated.

So then we launch a larger and well-designed study on *Salmonella* phenotype and genotype, and this is a collaborative effort between FDA NAHMS and CDC NAHMS. And the selection criteria for the study is we selected all the samples from the 2011 and 2012 isolates, and they represent all the unique combinations of different resistant patterns, source, and serotype. And that included 181 retail meat isolates and 104 clinical isolates. So the NAHMS AST panel for *Salmonella* has more drugs than the *Campylobacter,* and it has three aminoglycosides and five beta-lactams,

and also other drug classes.

So for the retail meat, again, this is only a small proportion of the results, not to make the slide too crowded. So for the retail meat *Salmonella*, again, that's the AST is the phenotype and the gene are the genotype, and then we don't have any quinolone resistant or quinolone or macrolide resistance for the retail meat samples in those three yet. And, overall, they correlate very well, and there are only a couple cases that the genes -- we have the phenotype and we don't have the genotype, or we have a gene but we don't see the correlated phenotype. And most of them are related to aminoglycoside resistance.

So if I break down the results into drug classes, again, the correlation are very good for the strains, the pan-susceptible strains, 100%, and 100% for tetracycline, chloramphenicol, sulfonamide and -- and even for the beta-lactam, yeah, it's very good concordance. But for aminoglycoside, the correlation is a little bit lower; it's 96%.

And this is the results of the clinical *Salmonella* isolates. The resistant profile of clinical *Salmonella* looks somewhat different to the retail meats, and the clinical isolates have a few more. The quinolone resistance has 15 of them out of 104; they are resistant to quinolone. So we include the permutation data here. But, overall, the phenotype and genotype correlate very well. It's very similar to the retail meat samples that if we -- and most of the correlation comes from the aminoglycoside resistance. In most cases, it's

AAD genes that we couldn't detect that is streptomycin resistant here. And then for the quinolone resistance, there are -- people have reported gyrase A and ParC genes, important for the quinolone resistance in *Salmonella,* and that if we look at the mutations in those positions, and then we found that there are three positions are critical for the quinolone resistance, and that was B gyrase 83, gyrase A 87, and ParC 80. So if one of these positions has a mutation or two mutations, the strain looks -- it's resistant to nalidixic acid. But if all three of them have permutations, then it is resistant to ciprofloxacin.

But in the quinolone resistant isolates, we also found one strain that do not have any mutations in these three positions, but it is resistant to ciprofloxacin, and in this case, we found a gene, a plasmid-mediated quinolone-resistant gene, the qnrB genes. So normally we don't expect qnrB genes to cause resistance. We normally increase the MIC but won't make it to the break point. But in this strain, it also has the other two genes, the oqxAB genes, which is the efflux pumps; they're related to quinolone resistance. So we think there is additive effects here for this strain, but the qnrB genes and several other strains, as well, but the strains are not resistant to quinolone.

So, again, to break the results into different classes, the correlation are very good for the pan-susceptible strains. For beta-lactam, 100%; tetracycline and sulfonamide and quinolone. And the quinolone,

again, I consider the qnrB gene here to predict resistance, but it would consider only increase MIC then actually can -- the correlation can be 100%. So the only -- classes that do not show 100% or a high percent correlation is aminoglycoside, and we think there are maybe -- the reasons for this disconnect may be due to the AAD genes, the expression level that varies. And also for the streptomycin resistance, we only have two dilutions in the AST panel for those two years. So it's either resistance or susceptible, so if there is one well wobble, the data may look different.

So to summarize these studies, so we think, based on the current technology and knowledge, whole genome sequencing can predict resistance very well. And a comprehensive and accurate database of antibody-resistant genes is critical for this implementation. The concordance is 98% to 100% for most of the drug classes. And then the correlation is a little bit lower for aminoglycoside, but still it's between 92% to 96% from the *Salmonella* data and *Campylobacter* data.

So we think the disconnect can come from the interpretation from AST data, for example, the streptomycin case, and also it can come from esperimentos and medical errors. You know, it could be like mixed cultures or could be misassembling of the data. Anything happen from once we get a strain until we get the results. And it also could be due to the variable gene expression level, the aadA genes seem to fall into this category, and we see many cases, the AAD genes is there, but we cannot call the strain resistance

to streptomycin. And there may be also unknown resistance mechanisms that we need to do more study. The gentamicin resistance seems to fall into this category. There seems to be something I know that exists there for the gentamicin resistance.

And I also would like to discuss -- put some discussion here, questions here. And from our data, it looks like, from the whole genome sequencing we can predict the resistance profile very well, at least for the surveillance data. However, in order to make this into a routine report, a routine testing, there are a lot of things we need to discuss. The most critical thing would be the interpretation criteria needs to be standardized. And also, the nomenclatures of all of these genes need to be standardized.

And like when we encounter a gene that is not 100% identical to the known genes, what are we going to do? So, like, what is the cutoff we are confident in saying that this gene is resistant to these drugs? And so question to think about and for people to discuss. And I would like to thank the NAHMS FDA team and the NAHMS CDC team for putting all this together.

Thank you very much.

(Applause.)

DR. CHEN: And I can take some questions.

DR. SCOTT: Thank you. I'm Morgan Scott of Texas A&M.

It's obviously a wonderful technology. I would just caution you a bit about, for example, concordance often implies that it's both ways and

you give an example of tetracycline; you have some isolates that are tet(A)(B)(C) and (E). Or (D), I can't recall. It seems to me that if you know those genes are there, you can predict, perhaps, resistance susceptibility, but if you have simply the susceptibility data, you can't predict the genes that are there. So concordance/discordance, you have concordance from the genome data to the susceptibility data in one sense, but it doesn't go the other way.

Secondly, I would also challenge you to go further. Our data suggests that tet(C) has a very broad range of MIC values, many of which fall below the resistance cut point. And you actually may have the opportunity to explore some of those different MICs, the ones that don't simply shift to the right of the CLSI breakpoint, for example. I know it's early; it's new. I think it's tremendous.

But my question is, can you take it further and determine location, plasmid versus chromosome and other transposons, integrons? Is that present in here? Is that also too much to ask, say, for example, with those four tet gene isolates that you have in your tables?

DR. CHEN: Well, first, thanks for the comments.

Yes, we'll follow up and then look at the MIC values for some of these genes, especially, for example, the AAD genes and also the tet genes, as you suggested. But yeah, for the plasmid data, we could dig it out from the whole genome sequencing, and it's not difficult to locate in the plasmid or whole genome sequencing. Or from the chromosome. And what is

challenging from our data now is to close the plasmid. But to locate into a plasmid or chromosome, we can get that data most of the time. I don't have the data here with me now.

DR. ZHAO: I can tell you that, you know, for the whole genome sequencing, we can identify the gene within the plasmid or the chromosome, and we also, we can tell you the plasmid type, incompatibility type where -- we just didn't present it here, but yes, we do have that data through the whole genome sequencing. And I think this brought out lots of research opportunity for us, like as a single gene and what's the MIC range, because, like, streptomycin do not correlate well. Always has an A, D, and G.

We do not assess the phenotype. Like Yuansha indicated, that -- we only have two dilutions, 32 to 64. So say -- do not have the breaking points, but we determine the 64. So maybe, I think, in the future we can present data to say -- you know, to reveal, to say what's the breaking point? Should it be based on the larger studies, every single genotype that correlated with MIC? Maybe could it be lower, you know? We don't know. I think we need more data to see this correlation.

DR. CHEN: All right, thank you.

(Applause.)

DR. ZHAO: Okay, our last speaker by CDC. Both of them have been, you know, introduced before. So Jean will start first? Okay.

Jean is the team leader for NAHMS program at the CDC. And

Dr. Barbara Mahon also is Deputy Director, Chief of Enteric Disease and Epidemiology Branch. She also is the acting team leader for the NAHMS program. So I will not -- because it has been introduced before, so I'll not repeat it again.

Thank you.

Jean.

DR. WHICHARD: You're watching me struggle with technology here. Now we're going to look into the future. Everybody ready? We're going to jump in that souped-up DeLorean with Dr. Mahon and me and -- no?

This is a great session. Pat and I were just looking at each other the whole time, going this is so cool. And I think it's just great to hear what's being done with whole genome sequencing and these concordant studies, because I think it's going to prepare us for the inevitable changes on our horizon when it comes to the paradigm shift that's happening in clinical laboratory confirmation.

So today I'm going to go over just a few questions: Why do we measure antibiotic resistance the way we do? What are some of the culture-independent diagnostic tests on the horizon? What would loss of bacterial cultures mean for antibiotic resistant surveillance? And how can we adapt and take advantage of those changes?

So Dr. Mahon and I are going to do a little tag team here and carry it through. So a lot of what we do is based on bacterial isolates. And

when I say isolate, I mean the thing that is isolated from the patient's clinical specimen, the thing that shows that person had that particular disease. If you suspect it's *Salmonella*, you're going to take a stool specimen and you're going to go and cultivate it and do some enrichment techniques and some identification techniques, and you're going to get that live thing out of there.

It helps you prove the cause of the infection or a contamination event, and it also gives you access to the whole package of things that we're used to studying for subtyping and determining virulence and all those great things. Think of a bacteria as a big bag of stuff, including DNA/RNA proteins. If you preserve that bacterial isolate well, such as we tend to do by freezing, you'll have an infinite supply of future material to study, so for doing things like conjugation studies, transformation studies, to see how these things behave. It's really necessary to have that live organism that you've isolated from a patient's infection that enables you to measure biological behavior.

And we're measuring behavior when we're measuring resistance and all of our current susceptibility testing methods. Everything we've done with NARMS heretofore is based on that phenotypic susceptibility testing. We've gone a pretty great length to get really objective and consistent data. It depends on very well-established, validated test methods using that standard concentration of bacteria and exposing it to the standard concentrations of antibiotics under standard conditions to get that number, to get that all-important minimum inhibitory concentration.

Use of appropriate internal control bacteria: We have an internal control to make sure that all of our conditions are working. Again, you're measuring biological behavior, granted in an in vivo assay, but that's the proxy we're using to establish whether that drug is likely to be useful clinically.

Here's how it might work: Person gets sick and a person doesn't just assume it was some bad potato salad and ignore it. They go to their healthcare provider. Ideally, their healthcare provider takes a specimen and submits that for culture. Bacteria are isolated, and if there happens to be *Salmonella* there, they're going to be forwarded to the state public health lab. And then that 1 in 20 isolate is going to come to NARMS for antimicrobial susceptibility testing. Talk about exposing it to those doubling concentration of drugs.

I realize some of this might be a repeat, but there's somebody who may have missed some of those sessions.

You incubate that test, and then you get that all-important minimum inhibitory concentration. And that's really the number that all the data is based on. From there, we get the Clinical and Laboratory Standards Institute guidelines out, and we determine whether that number means it's susceptible, intermediate, or resistant. And that's the type of stuff that you see on all of our resistance graphs, that's what you see on our resistance tables, it all comes back to that MIC data.

We also look at the MICs themselves, because we're looking at that part that doesn't look well typed. Those isolates down here, they're kind of wimpy. You know, little bit of drug, hey, I'm done; whereas up here, all these isolates that require a lot more drug to prevent their growth, those are things that we're looking at. We're looking at what genes are present there. Are those infections worse? Where are they coming from? And how are the patients responding to treatment? I mean, that's ultimately the question: Is this affecting the efficacy of the drugs?

We have a real-world example where we've looked at the so-called MIC creep over here because we know that physicians are using tetramycin to treat *Shigella* infections, but there is little data available about the susceptibility of this organism to the drug. No interpretative criteria for the susceptibility testing. But, hey, we've got a lot of *Shigella* in the freezer, why don't we see what we've got? And do we see any evidence that we've got resistance lurking out there? And our testing indeed showed these outliers; they have macrolide phosphorous genes. And those are an issue. Those are the ones that we're really going after and seeing is there some clinical outcomes effect?

Everything we do in NARMS is based ultimately on that isolate and the MIC data that we get to monitor those trends, to get that information out there in our reports, to conduct the research to better understand persistence, emergent spread, and all the clinical stuff associated with it. So,

by now, you've gathered that it's very important to have an isolate and an MIC. But what if that all went away? And that might well happen with the advent of culture independent tests. These are tests that are used by clinical labs to determine what the cause of a disease is, but they don't necessarily require doing the traditional culture identification steps.

In many cases, these tests provide information more quickly than traditional culture and all those techniques. And the kits and procedures don't necessarily yield an isolate, which is what most of what we do and all the subtyping systems like PulseNet that relies on an isolate. You can see the attraction. And I don't want to get into the alphabet soup of what all this is, but this is basically what you need to do to work up a stool specimen, determine the viruses, parasites, and bacteria that might be contained in that sick person's stool specimen.

This is from Kim Chapin -- she's the Director of Micro and Infectious Disease Molecular Diagnostics at Lifespan's Rhode Island Hospital -- by way of John Besser, who is kind enough to provide this for us.

A lot of steps, a lot of moving parts, a lot of people involved, a lot of transfer of things that are in process to determine what the cause is. It's very attractive to have a much simpler approach such as multi-agent panel that could detect 12 or 15 things simultaneously. Specimen arrives at the lab, orders are entered. Instead of having all those different steps and culture disks and looking for different pathogens by different techniques, but

have a single platform.

What would that mean for NARMS? Well, no isolate basically means no MIC. We need to find other ways to measure antimicrobial resistance. And you've already heard a lot about the shift to sequence-based methods, possibility of detection of looking for certain resistance genes and seeing the concordance between that and the phenotypic results, ultimately detection out of primary specimens. And we might be needing to look at that sooner rather than later because even our traditional whole genome sequencing methods, they're all based on having that isolate. So think in the mindset that the isolate goes away, might need to fast-track some things. And the clinical labs are already embracing these, some of the tests that are coming out on the market.

This just shows you some that have come up over the last few years. Some of them are immunoassays, some of them are multi-panel PCR assays. You've got the Luminex xTAG, the ProGastro SSCS, a few names I can't quite rattle off. But this just gives you a feel for how many of them are coming on the market.

And this graph shows you the shift from culture confirmed *Campylobacter* infections over time. The blue line represents the culture confirmed, and then the yellow line is a culture confirmed plus culture independent method. So changes are afoot. We haven't seen a drastic reduction in the number of isolates coming in yet, but I think it's something

that we are anticipating.

So how can NARMS adapt? Well, first we can try to fight it, deny it, try like heck to keep those cultures as long as possible because we've still got a lot to do in terms of concordant studies. The news is very promising, certainly with the work Dr. Chen and others have done. It's great to see how well the concordance is coming out. But we do want to keep those isolates coming as long as possible. But we need to determine how the DNA sequences correlate with the phenotypic resistance. And I feel really good about knowing or looking for what we know about today.

You know, we know that CMY genes are responsible for cephalosporin resistance. So we can look for those, and we can be really confident about the concordance there. For things that we don't yet know about, looking at the emerging resistance mechanisms, you know, that's something that we're still going to need to rely on, some phenotypic surveillance to find those things that we're not looking for.

Who would have known aminoglycoside-acetylating enzyme would go across the hall and try to go after some fluoroquinolones? Is that something that we would have predicted based on sequence alone? No, we would have needed some phenotypic data to see that this was a mechanism that was also affecting fluoroquinolones.

How does presence/absence of genes correlate within MIC? Because we have grown used to looking for those early signals as opposed to

the plus/minus result. And I don't want to pooh-pooh what's happening. I think it's great that we're getting good concordance data, but we just need to consider some of these things. We need to consider about DNA that has unknown function, how do we know how it affects resistance? There are a whole lot of genomes that are not defined that we don't have good predictive data to say what those genes actually do.

Do they contribute to resistance? How many do we need to look at to be comfortable with the fact that these pieces of DNA don't contribute to resistance? And I think, given the changes that have happened over the years, it's going to continue to change. We're going to need to explore detection and resistance out of primary specimens. And that comes with a whole new set of challenges because then you have to tie that resistance gene to some DNA sequence that you've gotten out of a primary specimen. You didn't have the luxury of knowing that that DNA sequence came from *Salmonella* or *Shigella* or *E. coli*. If you're getting it out of a primary clinical specimen, is it coming from *Salmonella* or one of the other many bacteria in that soup? There's also food DNA in a stool specimen, there's human DNA mixed in there, so lots of challenges.

But we're excited about the opportunities, too. I mean, it's just such an interesting time to be in science. I would never have envisioned the rate of change in our field with regards to sequencing and technologies that are eventually going to be available for, in essence, patient-side detection of

virulence, subtyping the organism that's causing the disease and probably antibiotic resistance, integration of DNA sequencing and surveillance at the state or regional lab; our routines will be quite neat. I mean, Genome Trakrs are already building some of those right now. Possibility of single workflow for identification and subtyping. It's a truly exciting time to be involved with it. It has scientists even levitating.

(Laughter.)

DR. WHICHARD: So we're going to tag up now, hand it over to Dr. Mahon and take us home.

DR. MAHON: So hello again, everyone. This session has been talking mostly about changes on the horizon and the future from the laboratory point of view. And Jean and I wanted to spend just five minutes on some changes that we are anticipating and hoping will come on the epidemiologic side of human surveillance in NARMS.

So I mentioned yesterday that CDC has an initiative for 2015 called the Detect and Protect Initiative. And this includes several activities to get started right now, starting in 2015, to combat antibiotic resistance.

So detecting and tracking patterns of resistance: We want to do more of that in NARMS. Responding to outbreaks involving antibiotic resistance bacteria: There are changes on the horizon for NARMS surveillance that will let us do that better and faster. Preventing infections from occurring, resistant bacteria from spreading: There, too, we think that

the activities that we're proposing can really make a difference. And No. 4, discovering new antibiotics and new diagnostic tests, also comes in to our sites.

So, essentially, what the initiative proposes is that there would be a new five-region antibiotic resistance laboratory network. Steve Solomon talked about this briefly yesterday, and I think I did, too, actually, but this is for all resistant pathogens, not just NARMS pathogens, but all of those respiratory pathogens and those hospital-acquired pathogens as well. But this would allow NARMS to expand its surveillance also. And, specifically, we would like to increase resistance testing for NARMS pathogens starting with non-typhoidal *Salmonella*.

We've talked about how we currently do every 20th *Salmonella,* and that gives us some pretty good information about what's going on, on the national level. But there are time lags with that. The states have to batch their specimens and actually ship them to CDC for them to be tested, and even though we request outbreak isolates in real time and put those to the top of the list for susceptibility testing, there are still some delays.

With the use of these regional laboratory networks and testing of every *Salmonella* at the time it's diagnosed, those delays would be hugely reduced. And even for the outbreaks, where we currently request isolates as soon as we find out that a cluster is occurring, we would already have the

resistance information before the cluster was even detected. So that would make a big difference in terms of detecting outbreaks faster and being able to solve them more quickly.

The initiative also calls for a new bank of resistant bacteria that would be available to partners, pharma, biotech, academic researchers for the kinds of research that many of you have talked about over these last two days to develop new drugs, new tests, new -- perhaps -- non-antibiotic interventions, but a whole range of research to try to reduce the problem of resistance.

And then on the epidemiologic side, this would also include getting more and better information about resistant illnesses. We really need to have detailed standard information that's collected from people as soon as possible after they're identified with these illnesses so that we can find out where they went, what they ate, which animals they patted and so forth. That's going to be the best way to find out what the sources of these infections are.

And it's incredibly powerful to have that information linked to the laboratory results, linked to the whole genome sequencing results so that you can see that yes, these match, and there's the history of the contact that you would think would be there.

Finally, a commitment to continuing to make the resistance data as broadly available as possible, as quickly as possible. So we think that

a goal of reducing MDR *Salmonella* by 25% by 2020 is achievable. This method, I think -- we're really all pretty excited about this and optimistic, although I probably should say cautiously optimistic. There are a lot of changes afoot that CIDTs -- you know, CIDTs get -- they're big before we have the work-arounds in place that -- it's a moving target. But we do think that this is achievable and would really be a great success for public health.

So since others have been advertising, I'm going to advertise as well. Here are some of our websites on resistance on the threats report, the Detect and Protect Initiative, NARMS, outbreaks, and so forth. And I guess Jean and I can take any questions if you have them. Thanks.

(Applause.)

DR. TAUXE: Don't all race to the microphone at once. But if there are any questions, now is a good time. I know that these same CIDT issues are playing through in food microbiology or playing through in veterinary diagnostics, and I think they are going to be a general challenge and a general opportunity. And I really think, if I could just editorialize for a moment, that the answer to it is -- whole genome sequence is only a very partial answer to it because the newest diagnostic platforms that are being developed for clinical use in human medicine start with a step that sterilizes the specimen and then something is done to extract the DNA, and so there is no moment to recover the organism possible with these new diagnostic strategies.

See, that lets you turn over the whole process to somebody that has no particular technological skill and won't infect themselves as they handle the specimen. Because you cannot -- there's nothing alive in it. You can't get an organism from it. And that means, really, we need to accelerate the process of getting to something that's on the far side of whole genome sequence. That's meta-genomic recovery of information about what is present in a very complicated specimen like food or like stool. And that's ahead of all of us, that's in our future, and that's going to make life incredibly interesting, I think.

DR. SCOTT: Morgan Scott, Texas A&M. I feel like I've been monopolizing the microphone today. But my question to you is, do you foresee these reductions, optimistic reductions, as being -- and I know the answer is going to be both, but you can reduce MRSA infections, and it sounds like the approach is to tackle resistance, but by reducing *Staph aureus* infections, you're going to bring both down.

Are you aiming for -- or which proportion of your success are you aiming to reduce the proportion of *Staph aureus*, for example, that are met with some resistance versus reducing the force of MRSA infections? And that could apply to the CREs, *C. difficile*, maybe not the *Staph* -- or the *Salmonella* non-Typhi.

What's the amount of effort that goes into achieving these reductions and goes into reducing resistance amongst the pathogens versus

reducing the force of infections through those pathogens?

I hope I'm not being too obtuse. Or I am?

DR. MAHON: No, I --

DR. SCOTT: No, both.

DR. MAHON: No.

DR. SCOTT: But really I mean --

DR. MAHON: Of course. I'm just being glib.

No, it's an excellent question and I think that -- so there are things that this group here is doing to decrease the proportion of *Salmonella* that are resistant, and those are great. It's also true that preventing *Salmonella* infections themselves decreases both susceptible and resistant *Salmonella* infections.

You might have noticed in Allison Brown's talk about outbreaks earlier in the day that the proportion of the outbreaks she looked at that included at least one resistant isolate was way, way higher than the proportion of *Salmonella* that we were report in NARMS that have any resistance. And that's not entirely a fair comparison because this is a very selected sample, and there are lot of caveats to that.

But it seems quite possible that by targeting outbreaks quickly and doing the things that we need to do to end outbreaks and to prevent future outbreaks that have been caused by the same sort of chain of events going wrong, that we could have an outsized impact on resistant *Salmonella,*

while at the same time decreasing all *Salmonella*.

DR. SCOTT: Thank you.

DR. TAUXE: Okay. Well, thank you very much, everyone.

I think we have a 10-minute break now, and we should plan on coming back at 3:45.

And let's applaud all of our speakers.

(Applause.)

(Off the record.)

(On the record.)

DR. McDERMOTT: This brings us to our public comment period for the NARMS public meeting. We have four individuals who have registered to speak during this public comment period. I think we have an hour for this set aside on the agenda, and if there are those who have not registered, I believe you are free also to get up as long as there is time, if you wish to speak during the public comment period.

I think what I will do is, in no particular order, introduce in turn the list of names that we received ahead of the meeting, and we'll begin with Steven Roach, who is representing Food Animal Concerns Trust.

Steve, are you prepared?

(Off microphone response.)

DR. McDERMOTT: So I think maybe just to the microphone right here in the middle. And I'm not sure if it's on. Well, actually, let me --

why don't you come up here, because I think the camera is only focused on the podium for those on the web, so that might be -- it might be better.

(Off microphone response.)

DR. McDERMOTT: Okay. Yeah, please come up. Thanks.

MR. ROACH: Hello. As Dr. McDermott said, I'm Steve Roach, and I am the Food Safety Program Director of Food Animal Concerns Trust. And Food Animal Concerns Trust is a not-for-profit. We're based in Chicago, and we focus on two areas. One of them is humane farming practices. And with that program, we primarily provide small grants to livestock producers to take steps to improve the welfare of animals on their farms. And then the other program, which I mainly work with, is our food safety program. And we try to find steps that livestock producers can do to reduce the public health risk from the animals that they raise. And one of the risks that we're concerned about is antimicrobial resistance.

Because antimicrobial resistance is a very challenging problem, we mainly work on this issue in coalition with other organizations. So we're a member of the Keep Antibiotics Working coalition, which is a coalition of about 13 advocacy organizations from public health to environmental to animal protection organizations. But, again, because antimicrobial resistance is a worldwide problem, we also work with it through Consumers International, which is an umbrella organization of over 200 organizations around the globe. We have also worked with another newer coalition that

started before the World Health Assembly this year. It's called the Antimicrobial Resistance Coalition, and it basically had the first meeting in Geneva earlier this year. So we try to work on a lot of levels on this, but my organization is primarily concerned with how food animals may impact antimicrobial resistance affecting human health.

Many of you have seen me many times on these NARMS meetings, and I've spoken at them before. So I think one of the important things is Dr. Ostroff yesterday talked about a tipping point. And he was talking primarily about public awareness of the problem, and I think that does seem to be true. And I hope that this tipping point will lead to more action on this problem. But I also think we should think in terms of biological tipping points. At some point, are we going to reach a point where routine treatments are not going to be available? So then we're going to routinely run into untreatable diseases, and that's what we're concerned about. And that's another type of tipping point. And I hope that we are able to keep from actually going over there, that the rate of drug innovation and drug stewardship and infection control can kind of outpace the rapid development of antimicrobial resistance.

But getting back to what we're talking about here today is NARMS. And the first thing I would like to say is, you know, since 2007 with the Science Advisory Board, I provided some comments to that one. There has been some, definitely some progress, and I think probably what we are --

my organization, Food Animal Concerns Trust, is probably most excited about are the improvements in sampling, because that is something that we think really needed to be done, particularly on the animal side.

And I heard several comments today about people were surprised to see the *Campylobacter* in the cattle isolates, but that was something we really expected and we felt that the program failed to pick up in previous incarnations. So we're really happy to see that.

Another thing is in terms of the improvement of the reports. Again, we would like to see them come out a little bit more quickly, but I believe Dr. McDermott's promises that we'll speed them up. But I think having more interpretation in reports is really helpful. I mean, it used to be just dump the data on us and figure out what it means, and I think right now we're more in terms of there is interpretation, what are the important findings on them.

Another area that we think there has been progress is with the CDC reporting resistance information on outbreaks. I think, again, that's a very important step, and that has happened in the last 10 years, at least since I've been working on it. If nothing else, it helps to raise public awareness that this is really a problem that affects people, particularly on the enteric disease side.

And I think the new methods that we're seeing in the whole genome sequencing, that sounds like it has some promise. But then we also

have the problem where we're losing isolates, so how to balance out the technological change? But I think there is progress in that area.

But there is a need for more work. Dr. McDermott described the U.S. system as the most extensive, but I would say it is definitely not the most comprehensive. And the area where we really feel there is a problem is with this idea of collecting data on how antimicrobials are used. And sadly to say -- and I feel sorry for Dr. Lewis, who is feeling sick today, but his presentation did not really make me feel much better about this. It has been a priority -- it was a priority on the Public Health Action Plan in 2000, but we still have not really made much progress on that. And seeing some of the work, again, I think -- I'm trying to remember. Dr. Karp described the on-farm sampling, and it seems like déjà vu over what Paula Cray was doing with the CAHFES program back in 2004. And it seems like we really can't get these programs off the ground. They're always in a pilot stage.

And, for me, that's deeply disappointing when this has been a priority area for over 10 years. Over a decade this has been -- and we've recognized that we need to do this, but we just don't seem to have -- and for me, it seems to be there is a lack of will within the Agency to actually move forward on substantially collecting data because it's not necessarily going to make everybody happy, and you may have to ask people to do things they don't want to do.

I thought the data from NARMS was good, and it was helpful.

And I also think, again, the sales and distribution data that FDA is reporting is also helpful for us having some understanding of what's happening, though I would like to point out, FDA did not decide to make that data public on its own. Members of Congress and advocacy organizations actually were the ones, through the 2008 ADUFA reauthorization -- actually were the reason that the public has any evidence of the sales data and distribution data that we have.

But that data is clearly not good enough. Dr. Dargatz referred to a paper by Mike Apley, as a lead author -- Randy Singer, who is here, was also one of the authors -- that looked at some of the NAHMS data and came up with some estimates of antimicrobial use. But for me, what that paper showed is that we have a problem in trying to figure out what's actually happening on the farm.

Because if you look at the data that they had for within pigs, which are probably -- again, I don't know, but my assumption is that pigs are probably the highest users of tetracycline in food animals in the U.S. Just for looking at what's happened in other countries and also, if you look at the NAHMS data and the minimal data that we have on poultry, that they're probably not used so much in poultry. They are used a good bit in cattle, but they're probably used more in swine.

But the problem is, in the paper by Dr. Apley, they found less than 700,000 kilograms of tetracycline used annually in the U.S. But if you

look at the sales and distribution data in the U.S. of tetracycline, you have 54.6 million kilograms. There's a lot of tetracycline that we don't understand where it's going, at least from any of the studies that I see. And I think trying to figure out why is there that gap, we need more data to try to figure it out. Where is the tetracycline going?

We've talked a lot about kind of voluntary programs, and I think that's what we tend to move on. And I think there are limitations on those because when you ask people to volunteer, you cannot have -- the worst actors may not be the ones that are volunteering, so you might not get -- you're going to have a selection problem.

So Dr. Lewis talked about how there may be challenges and we can't figure out analytic methods. Some other countries have pretty good data about how antibiotics are used and what they're used for and which animal species they're used for. So our recommendation is to -- you know, we really need to sit down and move away from pilot projects and look at ways to actually -- how we can get this data?

And my organization, Food Animal Concerns Trust, plus Keep Antibiotics Working, has made comments into the USDA suggesting ways that they could collect this data. There are also several members of Congress who have considered doing it as well. And there is some legislation that has been introduced that creates mechanisms for collecting data. And what we have felt is that we have had pushback from the FDA whenever we said maybe you

need more authority, you haven't been able to do this for 14 years, and we really haven't had -- I haven't felt that FDA has really been willing to work with us and see, okay, what type of authority do you really need to get decent drug use data?

And there are two other areas where I think that program is not comprehensive enough. The one I brought up before, it's on primary production. So how do we do the breeder stocks? And there may be a lot of antimicrobial use going on there, and there may be some weird things with antibiotic resistance going on there, but I'm not sure exactly how we can get it. And the other thing is not really part of NARMS because it's not an enteric pathogen, but it would be helpful to have a better idea of resistance among food animal pathogens, and I think some of the programs -- the on-farm sampling, the calves program definitely included that as part of its mandate and I think some other things.

And we periodically hear about resistance in the food animal pathogens. I think where there is a lot of discussion around it, and was at the time of the fluoroquinolone ban by CDC; at that point, the poultry industry said we can't -- you know, if you withdraw the fluoroquinolones from us, the resistance in the *E. coli* and the poultry production is so high, it creates some problems for us.

And I recently heard that in swine dysentery there may be some -- at least in the European populations, there are some problems where

you may have untreatable swine dysentery with the drugs that are available. So I think how would we get some information about that would be helpful.

And I'm going talk a little bit about risk communication. So I'm kind of switching tracks here. Dr. Tate said how can we present the information that we have in the NARMS report so that it can't be misinterpreted? I would say that that is not a very good goal for a risk communicator to try to keep other people from misinterpreting your data. I think if somebody wants to misinterpret your data, there's nothing you can do to keep them -- and if you are withholding data from the public because you're afraid it would be misinterpreted, I think that is a much greater problem. You should be able to explain your data, but to try to keep other people from using it, I think, is very difficult.

But I think the first focus should really be on providing accurate information. And the one thing I would like to say, for years NARMS reported on lincomycin resistance in *Enterococci faecalis* for years. And then each report they would have information on the percent of resistance to this bug/drug combination.

And then an advocacy group, not myself -- and I wasn't involved in the report -- reported that out to the public and said this is a concern. And in responding to it, basically the Center for Veterinary Medicine responded to this advocacy group report very strongly, and in attempting to discredit it, the CVM basically went overboard and made statements that

were inaccurate themselves. Primarily in an attempt to discredit this report that included the amount of resistance in *Enterococci*, the center made statements on its website and to the media that there was no public health risk from resistant *Enterococci* in food or food animals.

Now, there is uncertainty about that clearly, but on the other parts of the FDA's website, it clearly states that *Enterococci*, there is a concern about resistant *Enterococci* in food animals and in food. And also the World Health Organization's AGISAR's list of critical and important drugs considers whether the drug is used to treat *Enterococci* in humans because of the potential for transfer of resistance from resistant *Enterococci* from food animal populations to human.

So what we had hoped is that when you communicate to the public, in risk communication, that accuracy be the highest goal and that risk communication should not -- you know, there are uncertainties. Communicate the uncertainties, but don't make -- you know, don't go overboard and make statements like there is no risk from this when that is clearly not the case.

The other point on risk communication I would like to talk about is that it is important not to equate resistance to "resistance to the drugs of last resort." So I sometimes get frustrated with CDC reports on this because, yes, carbapenem-resistant gram-negatives is very important. Carbapenem-resistant *E. coli* is important. But if you had less resistance to

other drugs, you wouldn't have to use the carbapenems. And one of the cases in terms of the NARMS reports, what I would like to say is, it would be helpful, ampicillin is a potential treatment for *Salmonella* infections.

And I think it would be more helpful for the NARMS reports to communicate this and show charts. And maybe people say that ampicillin is the cows are out of the barn, so we shouldn't really worry about having ampicillin as a treatment for *Salmonella*; we should just always jump to ceftriaxone and fluoroquinolones. And I think that's not a good attitude. I mean, if we can get that amount of resistance in ampicillin down -- I mean, the amount of resistance to ampicillin in *Salmonella* down, then it could it be the first drug of choice. You wouldn't have to go to ceftriaxone or fluoroquinolones. So I think some more communication around that would be helpful.

And I think with that, I'll finish my comments.

Thank you.

(Applause.)

DR. McDERMOTT: Thanks very much.

Our next speaker in the public comment period: Susan Vaughn Grooters, from KAW.

Susan, please come to the podium.

MS. GROOTERS: Hi. I'm Susan Vaughn Grooters. And as many of you know, I am a doctoral student at the George Washington University, as

well as having a work history of working at nonprofit public health organizations that are focused on food safety. And so I am with Keep Antibiotics Working here today, the coalition that Steve spoke about. So I won't go into as much detail. I'm just going to sort of editorialize a little bit about what has happened over the past two days in areas that I think we can improve.

We heard a little bit about trust and timing. So we know that producers sometimes don't trust government, and we know that government sometimes doesn't trust consumer groups, and we know that consumer groups sometimes lose trust in this whole system. And so I think there are some things that happened today and yesterday that are really important. Open access to databases? Open access to anything is always a good thing; transparency is always a good thing for building that trust. It also is important with timing. The more readily data is accessible, the more readily problems can be solved.

So we're all after the same goal. We all want to reduce the burden of illness in human populations while maintaining good animal husbandry and good animal health. So we're all after that same goal. So I think the more we do this, the less we've got to that angle, so I think that that trust is really important. And for consumers, the risk of infections, we're learning, are going way beyond just treatment failure. We're learning that there are more hospitalizations, invasive infections. And so these are issues

that we have to address with a real urgent priority.

So after these two days, I think we are all, I hope, very energized if not a bit tired. So, again, I think there's a lot to be done still. You know, antibiotics are lifesaving in both human and animal medicine, and stewardship and animal production must be part of that solution. It is paramount that it be part of that solution. So hearing about stewardship in both human and animal medicine is important over these past two days.

A production system that relies on antibiotics being used for prevention rather than improving animal husbandry feels like a broken system. So I hope, as we talk about stewardship moving forward, that that can be part of the solution, as sort of really limiting when and where and how we're using antibiotics. So just trust and timing. I think the sooner you can get data out to us, the more we appreciate it and the more we trust.

So thank you.

(Applause.)

DR. McDERMOTT: Thank you, Susan.

Our third speaker is Anna Mazzucco from the Cancer Prevention and Treatment Fund.

Anna.

DR. MAZZUCCO: Hi. My name is Dr. Anna Mazzucco. Thank you very much for the opportunity to speak today. I am speaking on behalf of the Cancer Prevention and Treatment Fund, but also the National Center for

Health Research.

Our center is involved in conducting research and analyzing data and looking at risks and benefits for both policymakers and consumers. And our president is on the board of directors of the Alliance for a Stronger FDA, which is a nonprofit organization dedicated to increasing the resources that FDA needs to do its very important work. So thank you very much for allowing me to make a few comments today.

And I think we all know, and it has been discussed a lot the last two days, that antibiotic resistance and the inability to treat common infections is an increasingly urgent public health crisis which affects everyone. And a CDC report last year estimated that there are at least two million illnesses and 23,000 deaths annually in the United States due to this problem of antibiotic resistance. And so we thank the FDA and the CDC and the USDA for all their joint efforts in this very critical endeavor to address that problem. We just have a few comments on some things that we think could potentially strengthen these important efforts.

As we have said today already, there is growing evidence that supports the link between use of antibiotics in animals and the increase in resistant infections in humans. And we feel that FDA efforts to reduce antibiotic use in animal production could be broadened to not only address the issue of use of antibiotics for growth promotion, but also to address the use of antibiotics for disease prevention, as was also just mentioned,

especially due to the fact that it is often identical drugs or very similar treatment durations that are used for both of those indications. And we also feel that voluntary agreements, while they can be speedy -- you know, further enforcement including phase-outs or even bans could also be really helpful.

We also hope that NARMS sample testing can be even further expanded to more on-farm sites, as was discussed today, and breeding facilities in more geographic areas, as we feel that that information is going to be critical to pinpointing sources of contamination. And as has also been discussed, quantitative data on antibiotic use in animal feed, especially antibiotics that are important for human medicine, would be very, very informative. We also feel that retail product testing could be expanded to include more samples, especially dairy products, ground pork, and turkey. There have been some reports that these products, in particular, have been a more recent source of antibiotic resistant outbreaks.

And we also feel that more comprehensive microbial testing for other strains such *S. aureus* and MRSA could be done. There was a report in 2012 which found that 65% of the pork samples they examined were contaminated with *S. aureus* and 7% of those were MRSA. And we also feel that more sensitive microbial techniques such as selective broth culturing rather than single colony testing might also be helpful.

And we also agree, too, just -- that as much data as possible can be made publicly accessible as quickly as possible will ensure that this

data can really be used by public health and medical workers in order to adequately respond to emerging situations.

So, last but not least, we urge the participating agencies to ensure that NARMS receives all the support and funding that it needs to maintain and build these vital efforts. There is a recent estimate that antibiotic resistant infections cost the U.S. healthcare system between $21 and $34 billion every year, and so all these recommendations that we all want would be cost effective in the end and save millions both for federal programs and for individuals.

So our center will continue to work to educate Congress about the need for these efforts, but we hope that the agencies will also clearly make the case that this is an urgent priority.

So thank you very much.

(Applause.)

DR. McDERMOTT: Thank you.

Our final registered speaker for this period is Gail Hansen from the Pew Charitable Trusts.

Gail, welcome.

DR. HANSEN: Good afternoon. My name is Gail Hansen. I'm with Pew Charitable Trusts. I'm a public health veterinarian and a senior officer with Pew Charitable Trusts.

We applaud the efforts of USDA, FDA, and CDC and the efforts

that they've taken to work with stakeholders to really enhance the reports from NARMS, and I really appreciate the opportunity to speak today. And I'm only going to talk sort of more general, pulling back a little bit. And we talked more about the publicly available reports.

The NARMS data reinforced what decades of scientific research tells us, that routinely using antibiotics for produce, meat, and poultry gives us drug-resistant bacteria that can infect people. However, the data presented in these reports is difficult to interpret, often because important information is omitted and because the findings from each agency really are not comparable. Pew looks forward to this better coordination that we heard about, collaboration and consistency, so that the NARMS reports can become more useful, and more useful public health tools. And they are certainly reports in themselves, but they really are tools.

The coordination of NARMS data and sampling methodologies are critical to making the collected data helpful. Data that CDC, FDA, and USDA collect and convey in their respective NARMS reports are not really a comparable representative. What we suggest is that agencies collect and report the data, and additional information as was recommended by the Government Accountability Office in 2011 and 2007 and 2004 and 1999, specifically that GAO recommends that the NARMS collect more representative data on resistance in food animals and retail meat.

For example, the FSIS, Food Safety Data Inspection Service [sic],

now conducts non-random sampling of animals and animals that are in facilities that are not in compliance with food safety standards. And that results in data that can't really be used for trend analysis. FDA collects samples, as we've heard several times today and yesterday, from 11 states that volunteer, but it is not representative of the entire nation. I lived in a flyover state, so I get that.

It was part of a larger set of recommendations that also included identifying and evaluating approaches to collecting some really detailed data and assessing alternatives to antibiotic use in animals, and figuring out where more research might be needed as we talked about and heard about some of the research today. Both HHS and USDA agreed to these recommendations, the GAO recommendations, but they haven't implemented them yet, and we look forward to seeing that. But we keep looking forward.

Though NARMS does perform minimal susceptibility testing on non-typhoidal *Salmonella*, including clinically important antimicrobial agents, there is no systematic testing of other bacteria from animals and food, testing non-food, aquaculture, or environmental testing in irrigation waters, manure, that kind of thing. Groundwater. Testing for bacteria that have been found in meat and poultry should include *Staph aureus*, not just MRSA; extra-intestinal pathogenic *E. coli*, lincomycin resistant *Enterococcus* and *C. difficile* from retail meat, animals, and people to further integrate this surveillance.

The 2012 Interagency Task Force on Antimicrobial Resistance, ITFAR, the Public Health Action Plan to Combat Antimicrobial Resistance, that report indicated that the data entry and surveillance for NARMS would have a web interface for reporting and that electronic data crews would be available by 2015, and we look forward to that. It's a little unclear as to who will have access to that database. Pew urges that the agencies make that web platform available to as many folks as possible, and as this database is being developed, it's really vital that crews are able to cross databases across the agencies.

Relatedly, FDA currently collects antibiotic sales data. USDA -- we heard about the NAHMS data. But neither one of them is currently linked to NARMS data despite what we heard today is clear benefits. Furthermore, coordinating them with EPA on an environmental surveillance component would really allow these agencies to unravel that interconnected resistance in communities where scientific research clearly indicates that wastewater is a vector for transmission of bacteria, resistant bacteria.

NARMS should adopt the recommendations of the World Health Organization, that data are national representatives, statistically valid, comparable both domestically and internationally. We also heard a little bit about the international. We know that antibiotic use drives resistance, but we don't have detailed data on drug practice patterns in human and animal sectors. NARMS collects data on resistance, but it's important that we do

merge that with usage data. And FDA, USDA, and CDC need to collect that to determine what uses pose the greatest threats to human health and allow for informed policy decisions using that data. As the WHO points out, failure to implement the basics will hinder focused interventions and obstruct any evaluations.

I recognize there will be a change in the Executive Summary that will take time initially to get out to us, but the Agency should consider releasing NARMS data in a little bit more timely and coordinated fashion. For example, the 2012 CDC NARMS data was released in July of 2014, but the USDA-FDA Executive Summary reports for 2012 haven't been put out. In fact, the 2011 executive report just came out on Monday.

One of the NARMS goals is to determine changes in community-associated resistance and develop strategies, so we really do need this One Health approach with collaboration. The 2012 update of the ITFAR Public Health Action Plan to combat resistance summed it up best by their own assessment of the overall task plan by stating that federal accomplishments have been notable but insufficient.

And also Dr. Keiji Fukuda -- who used to be with CDC and is now the Assistant Director-General for WHO for Health Security -- stated in the forward to the release of the WHO report that just came out this past spring, which talked about antimicrobial resistance, and said that determining the scope of this is essential for formulating and monitoring an effective

response. There's a major gap in knowledge about the magnitude of the problem. The surveillance of resistance is generally, and I would say in the U.S., is not coordinated or harmonized. And that really compromises the ability to assess and monitor the situation. The information is vital to these really urgent public health actions.

Thank you.

(Applause.)

DR. McDERMOTT: Thank you, Dr. Hansen.

We have time, if there are others who would like to approach the microphone, that I think we're -- the direction is non-government employees, I believe, for the public comment period. But if others would like to make a few comments, please come forward. And please introduce yourself.

Thank you.

MS. BORRON: Hi. I'm Sarah Borron. I'm one of the food researchers at Food and Water Watch. Food and Water Watch is a consumer advocacy organization that promotes clean and safe food and water for all and works to safeguard our public resources, of which antibiotics are certainly one. I want to thank all the speakers today. This is my first NARMS meeting that I have attended, so I found it interesting and fascinating just to hear more about the depth of what happens with NARMS. I'll try to keep my comments brief so others may speak.

But I come today on behalf of Food and Water Watch with some concerns about FDA's Guidance 213. We're concerned that it restricts growth promotion uses, when so many of the drugs that will lose their growth promotion indications under Guidance 213 can still be used in very similar ways for disease prevention. And we urge those involved with NARMS to carefully consider how this loophole might impact the Guidance 213's effectiveness when it comes to preventing the development and spread of antimicrobial resistance from agriculture. We first see that Guidance 213 is really only one step towards better stewardship of antibiotics in agriculture. As we've seen from our counterparts in Europe, there's still a long way we can go to improving our practices here.

We also -- and I'll echo Susan here -- urge NARMS to make as much of the data publicly accessible. And it was very exciting to hear some of the examples mentioned over the last two days to coordinate data to make data more accessible, both in terms of just access to the data and as well as the interpretation of data in the retail NARMS report.

We're sort of an unusual group in that we actually go out and talk to real people about these issues every day. We have organizers on the ground. And I can say, on behalf of those organizers who have been working all summer in city council resolutions, supporting action on antimicrobial resistance, specifically calling on Congress to pass PAMTA, that there are everyday people out there willing to volunteer to talk about these issues, who

are concerned about these issues, who use the CDC numbers on the number of people infected by antimicrobial resistance, the number of people who have died, to go and be involved in political activism on this topic. There are people out there receptive to making this data more accessible, even for the everyday person, and I urge more of that kind of work.

Finally, we look forward to hearing more about the NIMBioS proposal to collect on-farm antibiotic use data. That's a black hole, it feels like, in terms of this issue, and this effort is really long overdue to try to shed some light in that hole and make the necessary transparency and stewardship of antibiotics.

Thank you.

(Applause.)

DR. McDERMOTT: Dr. Carnevale.

DR. CARNEVALE: Good afternoon. I'm Dr. Rich Carnevale from the Animal Health Institute, and I wasn't going to make any comments today, but I really feel now compelled to make some remarks because there's been a lot of things said that I need to address. And one of those is the fact that people have criticized the voluntary process going on with FDA on Guidance 213. And I'm here to tell you that this process that we have going on with FDA and the industry is the only way this is going to get done.

So if we want to phase out growth promotion and move these products to veterinary control, this is how it's going to happen, with

industry's cooperation. FDA will not be able to do this on their own regardless of how many regulations or guidelines or legislation is crafted, and that's simply the honest truth. And I will say that every single company, not just AHI members, but members of the Generic Animal Drug Association and independent companies that aren't members of either, have committed to doing this. And I am firmly convinced that it will get done, but we need the cooperation of FDA and the cooperation of all the companies to getting it done. There's a lot of work to be done, there's a lot of devil in the details, but honestly, this process will work and will be effective. So I really take exception to the criticisms that a voluntary process is not effective.

I also would like to address this misconception that keeps coming up that prevention doses will be used in place of growth promotion. That simply is not true. If one takes the time to review the currently approved medically important antibiotics that are on the FDA list for GFI 213 action, you will see that the doses are quite different in virtually all cases. So there is not the same dose or the dose regimen being used for growth promotion and disease prevention. So once those growth levels come off those claims, come off those labels, the remaining prevention claims can only be used at the dose levels for those prevention diseases, and a veterinarian would be illegal to use doses any different than that. So that's a misconception that needs to be addressed and needs to addressed so everybody understands that that is simply not true.

Some other things that I'd like to say is that we support NARMS. We always have supported NARMS. Listening the last two days, I'm really impressed with the number of brilliant people that are involved in all this work. This is amazing, what's going on, and there is a lot of detail and a lot of information coming out. As everybody has said, this is a very complex problem, but I do think that we need to step back and wonder and ask the question, are we putting more resources into this than need be? After all, the report just came out yesterday showing that 85% of human *Salmonella* isolates are susceptible to all antibiotics tested. So we're talking about 15% that are resistant. And I think while we all share the concern for that, it is evident that animal antibiotic use is not the prime driver of the biggest problems in human medicine. The CDC report that catalogs 18 pathogens that are of importance to human medicine, only two -- *Campylobacter* and *Salmonella* -- were identified as related to animals.

So we all need to do our part, and we all want to do our part, and industry is doing their part. We should put this problem in perspective, and I hear an awful lot of hand wringing and claims of a crisis when I'm not sure there is a crisis. Is there a concern? Yes. Can we do more? Yes. But let's put the problem in perspective.

With that said, I would also like to congratulate the CVM for putting out the Executive Report, or the FDA putting out that Executive Report. I think it's a great thing that I've used extensively over the last three

years, and I appreciate the changes they're going to make to that. I would caution the Agency that this new cecal sampling needs to be put in perspective, and there needs to be communication to the public as to the difference between cecal sampling and what has been done in carcass sampling over the years, otherwise there's going to be a lot of confusion about how the new cecal sampling matches up with the carcass sampling over the last 15 years.

So with those comments, I appreciate the time.

(Applause.)

DR. McDERMOTT: Thank you, Dr. Carnevale.

Time for another set of comments, if anyone is interested.

(No response.)

DR. McDERMOTT: If not, we can wrap up, and I, at one of the breaks, asked Dr. Tauxe if he wouldn't mind sharing these last 15 minutes we've set aside for sort of wrap-up comments and share some of his thoughts based on what he's heard today and just appreciate your perspective, Dr. Tauxe. And then I'll provide some final comments. I think we'll be out of here a little bit early today.

So Rob.

DR. TAUXE: Well, thanks very much, Pat. And thanks to all of you for your attention, your interest, your concern, your contributions, and all of your thoughts.

NARMS has been quite an adventure. I think it's almost unique as an interagency collaboration, a model program in some ways that actually got started about 18 years ago as an experiment. Let's see. Let's see if we can do this. Let's see if three agencies can come together and agree on basic methods, agree on what we're going to look at, how we're going to look at it, and start down the road together. And I think, as a model of how we can work with our distributed responsibilities in food safety, I think it's been really -- it's been a great experiment.

I think that we continue to plan. I think we continue to think about how to improve. These two days have given you a real sampler of what has been changed; what can be changed; what we would like to expand; what we could do more with in the human side, including more *Salmonella* isolates. And speeding up the identification of MDR, multi-drug resistant *Salmonella* outbreaks is sort of our next step, I think, so that they can be investigated and controlled. And on the retail food side and on the animal side, there are also changes afoot. NARMS is going to continue to evolve. All of that, of course, is somewhat resource dependent, as we make our plans.

We have also, I think, a future ahead of us, not just with the domestic issues, but we have a contribution to make on the global scene. I think we here in the United States, by looking at imported foods coming in, travelers who return who have been exposed to food, water, and people in other parts of the world, can help us map out what the global issues of

resistance look like around the world. And the more we can do that and share that with our partners in other countries, I think the more collaboration we can have in the future, because resistance in many parts of the world is rapidly accelerating.

And I think that the new molecular tools finally offer just tremendous promise, opening the door to tracking individual genes or the plasmids that carry them back to their reservoirs, their sources perhaps, and their points of amplification. And we should prepare to be surprised. A gene might have started out in an aquaculture farm somewhere in another country. It might have joined up with a plasmid and a series of other genes that might have had other origins and that might have shown up now in a turkey farm in Poland. We should prepare to see a lot of the global circulation playing out in what we're looking at and what we find in our own samples: people, food, and animals here in this country.

We can also be looking for the co-traveling genes that have been just a black box, not able to really examine at all, the genes that are participating in the selection process because they happen to be right next door to a resistance gene, and they may be genes that contribute to virulence or they contribute to resistance to disinfectants or thermal resistance. And with the whole genome sequence, we can start pulling apart what are those associations, those related phenomena. And I think that also is going to be just an eye-opening series of explorations.

So, with that, thanks for your support, and thank you, Pat, for your leadership in putting this whole meeting together. I think NARMS will continue to guide research policy and prevention, and I hope we can make it even more useful than it's been. Thank you.

(Applause.)

DR. McDERMOTT: Thank you, Rob, for those comments and those kind words.

I tried to put a few thoughts on paper to just sort of share with you my perspective, and I assure you I thought of this ahead of time, and I don't intend at all to use the advantage of the last speaker to rebut anything that's been said. That's not my intention. In fact, I think we fiercely agree in a lot of ways, and maybe sometimes that's even harder, but we'll see.

So I just wanted to -- you know, when I think of NARMS and I think about what our responsibility is within the context of this really sometimes bewilderingly complex issue that we're trying to address, I think about how much thought -- we put a lot of thought into how to best deploy resources. And a lot of times what we do, we do because that's what we can do. And, you know, in a perfect world we would design things with unlimited resources to be as complete and comprehensive as necessary, and sometimes we can't always do that.

But what we do and what we are able to achieve to help serve the food safety priorities across the government and to help serve

stakeholders and the consumer takes a lot of effort, and it takes a lot of hardworking people, a relatively small number of hardworking people, and a lot of resources. It's expensive and it's laborious to gather this information.

And so, you know, we're fortunate in our country. Not all countries have a burden of illness and an understanding of food consumption in their country to start setting up a program to do this type of surveillance; we at least have that advantage. And we have enough infrastructure and expertise in place that we can change if we need to and if we perceive a hazard is emerging that needs to be better characterized.

But because it is expensive and laborious and difficult, as I noted at the outset, we've put a lot of emphasis on building the best sampling scheme we have because the data, at the end of the day, would only be as reliable as the sampling scheme is sound. So that challenge, I think we've made real progress on, and I appreciate the comments to acknowledge that, and again, kudos to FSIS for really spearheading that effort.

We also recognize that antimicrobial use is an important missing link. We share that desire, we've been saying it out loud, many of us, for a long time, that the data are really difficult to interpret without some understanding of the selection pressures in play.

Another challenge that we continually face is cooperation and good communication between all the stakeholders, and I list here agriculture, public health sectors, and it's really everybody. I do share that sentiment

that we are really each other's stakeholders in this. We're all sharing the same food supply, and we all share the same desire for a safe and wholesome food supply and a situation in which antibiotics are preserved in their effectiveness for humans and animals. And I like the metaphor of looking at them as natural resources. They may not be replenishable forever. We shouldn't operate on the assumption, I think, that they are, and we should treat them as natural resources.

We saw the challenge coming our way from culture-independent diagnostic testing. This is something of cost and market forces that we're simply going to have to grapple with, and CDC pointing that out is worth mentioning, I think, as one of our future challenges that we're going to have to focus on.

Obviously, to sustain the surveillance, we need the political and financial support. Some of the proposals that were brought up, such as CDC's detect program, Detect and Protect, to -- you know, it's dependent on resources to do the work we recognize as being value added to the program. So, again, it's about doing what you can sometimes with what you have and sometimes doing more with the same amount of resources; but that is, no doubt, a perennial challenge and a major limiting feature of the surveillance system itself. And then that's about remaining flexible in order to stay current.

Understanding the implications of the data. This is a big one.

This ties into reporting. It ties into risk communication issues that have been brought up. It points to situations where research is needed to fill the gaps which surveillance by itself and sampling by itself cannot directly address or provide the type of clarity needed to act on the information.

And we've looked at some of the ways in which laboratory technology might help generate data that is more reliable and epi studies that are needed to understand more of the features that are contributing to resistant foodborne infections. We all agree on the challenge of publishing the findings to different audiences in a timely manner. The international harmonization cooperation piece is critical, no doubt. We know this is a global problem and that these organisms spread quite readily and that their resistance is remarkable in how easy they can spread around the globe.

Any surveillance system that can justify its existence needs to have process for review and enhancement. That's one of the reasons we're here today, is to share with you our attempts to make progress, to hear feedback from you on how we're doing and with the context of our strategic plan. We have tried to give it some structure in our conversations over the last two days. And, of course, the ultimate goal, using the data to formulate evidence-based public health policy. So I think this is probably the part that is most obviously controversial and a lot of the comments reflect that.

You know, there's one thing I think we can try to agree on, as scientists and public health officials, is try to follow the data where it does

lead us. And, you know, when we look at the NARMS data, we see good news and bad news. It's a mixed picture. It's always been that way, I think. We see positive trends, which we should be able to say are positive and welcome trends. And since NARMS started in the human isolate data, the proportion of isolates that aren't resistant to any antibiotics is not only 85%, but it's gone up every year almost without exception since NARMS began. That's good news. We should be glad to report that good news.

But we're not Pollyanna-ish, and the reason we do this work is vigilance is the key, and we know there are also issues that are problems, and the ceftriaxone issue is a clear problem, and we shouldn't take it lightly and we should address it with all our might. And how we address it, again, what policies are put in place, what sorts of decisions are made can be the controversial part. But when we look at the data, we know we shouldn't try to do something to address problems on the emergence.

So our role going forward as a surveillance system will be to try to monitor the impact of that extra label use prohibition, for example, and from there on to the new guidance documents that Craig has told us about.

You know, there are other things in the data that aren't as clear, and when we look at the fluoroquinolone issue in *Campylobacter*, you know, it's reasonable. People can stand on either side of that flat line and talk about whether it's a success or a failure. That's great. That's the kind of conversations that we should be able to have. And the role of NARMS is to

make that line really reliable and deep and dark and etched in stone, if you will, or as robust as possible so that we can get close enough to each other to disagree about what we might think it means. So I think, from what we've heard over the last two days, we've made progress to make those lines, those trend lines reliable so that the conversations can be more interesting and more valuable in trying to reach -- I think I put it there -- to get to the green circle, to act on it in ways in which the evidence -- the evidence is going to be as good as our program can make it.

And so, I like some of the things I heard about how well we're doing midway through our strategic plan. We need the input of everybody as we try to even evaluate our own shortcomings and our own successes. And as we go from here to take the new technologies and the new challenges that we face, you know, we'll be devising a new strategic plan, I'm sure, to address those, and we'll value the input of everyone here because, like I said, I think we're all each other's stakeholders as we try to address this issue.

So I would like to end by just thanking everyone for your participation, thanking all of our speakers, those who traveled from far and near, and for your comments and your questions, and we will value all of it and take it to heart, and we will use it to build that next strategic plan.

So thank you very much.

(Applause.)

(Whereupon, at 4:42 p.m., the meeting was adjourned.)

C E R T I F I C A T E

This is to certify that the attached proceedings in the matter of:

2014 SCIENTIFIC MEETING OF THE NATIONAL ANTIMICROBIAL RESISTANCE MONITORING SYSTEM (NARMS)

August 13, 2014

Silver Spring, Maryland

were held as herein appears, and that this is the original transcription thereof for the files of the Food and Drug Administration, Office of Foods and Veterinary Medicine, Center for Veterinary Medicine.

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CATHY BELKA

Official Reporter