Livestock, Phages, MRSA, and People in Denmark

[Announcer] This program is presented by the Centers for Disease Control and Prevention.

[Sarah Gregory] Hello, I'm Sarah Gregory, and today I'm talking with Dr. Jesper Larsen, a senior researcher at the Statens Serum Institut in Denmark. We'll be discussing the potential spread of livestock-associated MRSA into community and healthcare settings.

Welcome, Dr. Larsen.

[Jesper Larsen] Thank you Sarah, and thanks for having me here.

[Sarah Gregory] We've got a lot of complicated things going on in this study, so let's start with some explanations. What is methicillin-resistant *Staphylococcus aureus*, more commonly known as MRSA?

[Jesper Larsen] Yeah. So in the U.S., you prefer to pronounce it "mur-*suh*", and in Europe we pronounce it "M-R-S-A." So, please forgive me if I call it "M-R-S-A" once in a while. So, MRSA refers to *Staph aureus* strains that have become resistant to methicillin. And *Staph aureus* (the sensitive one) is actually part of our normal skin and nose flora, and around 30–50% of all people in the world actually carry *Staph aureus*. And it's also present in many different animals. So, the gene responsible for methicillin resistance is called *mecA*, and it's actually located on a mobile genetic element called staphylococcus cassette chromosome *mec* (or just SCC*mec*). And *Staph aureus* probably acquired SCC*mec* multiple times from other staph species. So, if we stick with the MRSA now, we...it's commonly known that basically anybody can get MRSA, and it spreads through skin to skin contact and shared equipment or supplies. And CDC has estimated that around 5% of patients in the U.S. hospitals actually carry MRSA. So, sometimes MRSA carriers can go on and get a MRSA infection, and fortunately most of these infections are in the...in the skin. They are quite mild, and they occur among young and healthy persons. But sometimes they can also cause more severe infections, such as bloodstream infections, pneumonia, and surgical site infections among the elderly or immunocompromised persons. And MRSA actually accounts for 9,000 deaths in the U.S. and 7,000 deaths in Europe, so it's definitely an important pathogen.

[Sarah Gregory] Excuse me a second, is that 9,000 and 7,000 annually? Or…

[Jesper Larsen] Annually, yeah. So, the *mecA* can not only come from resistance to methicillin but also to all other β-lactam antibiotics, including penicillin, cephalosporins, and carbapenems. And it's actually very important because methicillin resistance...it actually causes that relevant treatment of the infection is delayed, and that leads to longer hospital stays and increased mortality. Now, alternative antibiotics are very expensive and may have unwanted site effects and are often less potent than...than the β-lactam antibiotics we usually use to treat staph infections with. And furthermore, if you have to use other antibiotics, that leads to increased use of broad-spectrum antibiotics which can actually cause resistance to emerge in *Staph aureus*, but also in...in other bacteria in that individual.

So, if we look at it historically, MRSA has been around us for almost precisely 60 years. It was first discovered in 1960 in a London-based hospital where they found it among the elderly and immunocompromised persons. Since then, it has spread worldwide in the healthcare setting. And for a long time, that was the only place where we could find it. But then something happened in the 1990s, when new clones appeared in the community (we call those community-associated

MRSA). And they were a little bit different because they could survive outside the hospitals, and they actually also caused infections among young individuals and also previously healthy individuals. So, it's a very different behavior than the hospital-associated MRSA. Then something new happened in the early 2000s, because new clones again emerged this time in livestock. And...so we have basically three different types of MRSA—we have the healthcare-associated MRSA, the community-associated MRSA, and the livestock-associated MRSA. But there are no thick boundaries between these settings and the MRSA strains can easily spread between them.

[Sarah Gregory] Ok. So, tell us what a phage is now.

[Jesper Larsen] So, a phage...phages are viruses that can infect bacteria, just like a coronavirus and the flu virus can infect us, and usually the phages kill the bacteria. And we actually have exploited that behavior since 1919, when we started to use phage therapy to treat bacterial infections. And that was actually nine years before Alexander Fleming discovered penicillin, so it was the first antibacterial treatment. But some phages can also infect bacteria without killing them. And in fact, they can also benefit the bacteria because they can contain genes that code for useful functions such as antibiotic resistance and immune evasion.

[Sarah Gregory] So, are they...they're not a virus, and they're not a bacteria. What exactly...what exactly are they?

[Jesper Larsen] So, we do call them viruses. But it's a special kind of virus that only infects bacteria.

[Sarah Gregory] Now, what's an immune evasion cluster?

[Jesper Larsen] So, an immune evasion cluster (or IEC) is a cluster of genes that provide protection from neutrophils and complement, which are actually part of our first-line defense against invading microorganisms. The genes are located on a phage that is present in virtually all human *Staph aureus* clones, but not in livestock-associated *Staph aureus* clones. So we think it's really important for...for the human...for the human staph.

[Sarah Gregory] And what does the enzyme TarP do?

[Jesper Larsen] So, TarP is a phage-encoded enzyme that changes the surface structure of *Staph aureus*. As a consequence, the antibodies that usually recognize and inactivate *Staph aureus* become ineffective. But in contrast to IEC, the *TarP* gene is located on many different phages in both human and livestock-associated *Staph aureus* clones.

[Sarah Gregory] How is livestock involved in MRSA in Demark and Europe generally?

[Jesper Larsen] So, livestock-associated MRSA emerged around 15 years ago in both Europe and China, and especially in pigs. And since then, if we talk about Denmark, the...the herd prevalence has increased from 3.5% to 90% over a 10-year period from 2008 to 2018. So, virtually all of our pig herds have MRSA today. It doesn't cause infection in pigs but it's quite important pathogen in humans, mainly for farmers and their family members. So, these people are very often young and healthy and we mostly see skin infections in this group of people. Unfortunately, we have also seen that livestock MRSA can cause community-associated infections in the general population. Again, we mostly see skin infections in...in young people, but they can also cause bloodstream infections in elderly and immunocompromised people. Finally, we have also seen that few cases of healthcare-associated infections—that's infections

that are going on in...in hospitals and nursing homes—but they are...they are relatively rare, fortunately. But a high proportion of them are quite severe. And if you talk about Europe…well, some countries have more human MRSA than others. So, livestock-associated MRSA is most important in those countries with low levels of human MRSA, like the Netherlands and Denmark. If we take Denmark, we are a rather small country. We are 6 million people, but we produce more than 30 million pigs. So, we have a huge MRSA reservoir in pigs. And today, livestock-associated MRSA is responsible for 20% of all human MRSA infections.

[Sarah Gregory] Why pigs? What happened? Do we know why they carry more and why this huge increase in 10 years?

[Jesper Larsen] Yeah, that's a really good question. And I can't give you the final answer, but we have some really good clues. So, the SCC*mec* element—which also carries the *mecA* gene that actually confers methicillin resistance—the SCC*mec* element we find in Danish livestock-associated MRSA contains additional resistant genes against tetracyclines and zinc, and these antibiotics are among the most commonly used in the Danish pig production system. So, we actually think that the use of these antimicrobials are selecting for MRSA in pigs. And that might also explain why we find a very low frequency of livestock-associated MRSA in organic pig farms. On the other hand, Denmark is actually one of the pig-producing countries with the lowest use of antibiotics. So there must be other factors involved and we are currently investigating what that could be.

[Sarah Gregory] I know listeners will want to know this. Can you get MRSA in your bloodstream from eating the pigs?

[Jesper Larsen] So, we have actually conducted a lot of studies in Denmark and it seems that almost all of our cases are in rural areas where pigs are raised. And that really suggests to us that it's...it's not foodborne. We do find it in our...in...in pork, for example, but it's not really an important transmission route for MRSA. And for example, in Copenhagen where one fifth of our population lives, we have very few cases with a LA....a livestock-associated MRSA. So, that just confirms that foodborne transmission (if it occurs) is very, very rare.

[Sarah Gregory] Ok. So, clarify for us again how does this pathogen get from the pigs into people?

[Jesper Larsen] So, I want to go back a little bit in time because it's...it's quite fascinating history. So, livestock-associated MRSA actually originates from a human *Staph aureu*s clone. And when it made the host jump from humans to...to animals, it lost IEC but later acquired the SCC*mec* element and became resistant to methicillin. So, we think that it has a kind of a memory of its time in humans and that's probably why we see transmission to humans and quite some spread of it in the human population. So, because it has lost the IEC element, we are actually using IEC as a marker to differentiate between livestock-associated and human *Staph aureus* clones. So, I...I think that's really important because it probably didn't happen so many years ago (this host jump), and it...it probably has a memory of its time in humans. And we do see a lot of animal to human transmission in the pig farms. It probably occurs through direct contact of via aerosols—we find a lot of MRSA in the air inside the pig stables. And...but from there, we believe that it's the farmers that actually transmit or bring the MRSA bacteria outside of the stables and cause transmission to their household members, and from the households further into the community and healthcare facilities.

[Sarah Gregory] Why did you do this study?

[Jesper Larsen] We did the study because we started to observe that some patients were infected with IEC-positive LA-MRSA. And as I mentioned before, IEC is usually absent in livestock-associated MRSA and is known to protect human *Staph aureus* clones from our immune system. So, we wanted to find out if the livestock-associated MRSA strains that have reacquired IEC might be better adapted for...for a life in humans and for human to human transmission.

[Sarah Gregory] You used whole-genome sequencing and epidemiology to study the effects of IEC- and TarP-harboring phages on household transmission of livestock-associated MRSA in the North Denmark Region during 2004–2011. So tell us about that.

[Jesper Larsen] Well, we basically looked for human to human transmission of IEC-positive and IEC-negative strains to family members of pig farmers in 36 different households. And we were actually able to observe transmission in 80% of the IEC-positive households but only in 32% of the IEC-negative households. In addition, we also found LA-MRSA in 65% of the family members in the IEC-positive households but only in 22% of the family members in IEC-negative households. We did the same thing with...with TarP. We analyzed the households with TarP-positive and TarP negative strains, but we got completely different results. We observed transmission in 42% of the TarP-positive households and in 36% of TarP-negative households—so, not a big difference. And we found LA-MRSA in 30% of the family members in the TarP-positive households and 27% of the family members in the TarP-negative households. So, these results led us to conclude that IEC, but not TarP, is associated with increased household transmission.

[Sarah Gregory] You went on to review information about all patients throughout Denmark who had experienced livestock-associated MRSA infection during 2007–2018 to determine whether IEC is associated with increased spread into the general population. So tell us how that was done.

[Jesper Larsen] We...we started by searching the national MRSA registry for patients who had...who had had a livestock-associated MRSA infection in that period. And we divided around 1,500 cases into four groups representing the different steps in the transmission chain between farmers and healthcare facilities. So, 50% of the cases were farmers, 70% were family members of farmers, 25% were community residents, and the last 10% were in…in a healthcare facility at the time of diagnosis. We then determined for each group the proportion of IEC-positive strains, and the proportion actually increased steadily along the transmission chain from 3% in farmers to 6% in their family members, 7% in community residents, and finally 11% in patients with healthcare-associated infections. And these results really confirmed to us that IEC facilitates human to human transmission not only at the household level, but also into the community and healthcare settings.

[Sarah Gregory] MRSA apparently uses a diverse range of immune-evasive strategies to maintain a lifelong relationship with its human host. Tell us how this works.

[Jesper Larsen] Well, most of our knowledge actually comes from laboratory experiments, so we actually don't know much about the exact roles of the different immune evasion strategies in the human host. And we really can't use animal models because the immune evasion factors only interact with the human immune system, not with the animal immune system. But I believe we can learn a lot from studies like ours, as they allow us to see how uptake of certain genes by

livestock-associated MRSA clones affect their potential for human to human transmission and also virulence.

[Sarah Gregory] How did you use sequencing and epidemiology to determine structure and the contribution of IEC and TarP to household transmission?

[Jesper Larsen] We were very lucky because we had access to an extremely detailed information about all persons who tested positive and negative for livestock-associated MRSA in North Denmark region during 2004 to 2011. We had exact information about where they lived and with whom, and if they had livestock contact or not. We also had access to all the corresponding livestock-associated MRSA isolates. And we then used the whole-genome sequencing to detect both IEC and TarP and to divide the strains into genetically related groups, which allowed us to check if all the persons in a given household carried the same strain.

[Sarah Gregory] Where did the data that you used come from?

[Jesper Larsen] So, the data came from two sources. The human isolates and patient information came from the National Reference Laboratory for Antimicrobial Resistance at Statens Serum Institut (where I work), and the animal isolates came from the Danish Veterinary and Food Administration.

[Sarah Gregory] What's the good news in all of this? What did you find? Is it relatively limited in its spread in Denmark?

[Jesper Larsen] Well, there are...there are two good news. One is that the proportion of IEC-positive strains has remained relatively stable over the years, so there are no signs to indicate that they have become fully adapted to humans. And the other one is that IEC-positive strains only contribute marginally to the overall disease burden of livestock-associated MRSA. But our results also...also shows the importance of surveillance in both animals and humans if you want to be able to detect evolutionary as well as epidemiological changes that might affect the public health in the future.

[Sarah Gregory] Were there any particular challenges in doing this study?

[Jesper Larsen] So, the biggest challenge in this kind of study is often to establish and get access to large and representative strain collections from both humans and animals. But we are very privileged in Denmark because we have a very good and active collaboration between the National Reference Laboratory for Antimicrobial Resistance on the human side, and the Danish Veterinary and Food Administration and the farmers themselves on the animal side. As part of this collaboration, we...we performed surveillance of antibiotic-resistant bacteria—not only MRSA but other antibiotic-resistant bacteria as well—in both humans and animals. So we have a huge nationwide strain collection that we can use in studies like ours. And this data are published...the surveillance data are published together in an annual report called DANMAP, which is freely available on the internet if you'd like to read more about it. We have also access to related patient records and to information about the farms, the use of antibiotics, and animal movements between them.

[Sarah Gregory] Tell us about your job. Where you work, what you do, and what you most enjoy about it.

[Jesper Larsen] So, I'm a veterinarian at Statens Serum Institut where I work as a senior researcher. And my main focus is to improve our understanding about the evolution and spread

of antibiotic-resistant bacteria at the human-animal interface, which can then be used to implement effective infection control strategies. At the risk of sounding like a nerd, I've always been interested in the evolution of host parasites and the interactions, and I work with that every day. It's like being a detective, and I even get paid for it.

[Sarah Gregory] Tell us again the name of that website that people can go and read more about this you mentioned?

[Jesper Larsen] It's DANMAP (danmap.org).

[Sarah Gregory] Ok, thank you. What are things like now in Denmark with COVID-19? And what do you like to do for fun and relaxation?

[Jesper Larsen] So, we had the first wave in...in March and April like the rest of Europe, but only very few new cases over the summer. And we are now unfortunately experiencing an increasing number of new cases. In fact, we have more new cases now than during the first wave. But the number of associated hospitalizations and patients in intensive care and deaths are actually much lower this time, probably because many of the new cases are relatively young and healthy. My hobbies....well, I like to travel, read, cook, and to spend time with my family and friends. I also enjoy fishing and road cycling when I have the time. And these are, by the way, great ways to clear your mind off that day in front of the computer.

[Sarah Gregory] Well thank you so much for taking time to talk with me today, Dr. Larsen.

[Jesper Larsen] It was my pleasure. Thanks again for having me.

[Sarah Gregory] And thanks for joining me out there. You can read the November 2020 article, Phage-Mediated Immune Evasion and Transmission of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* in Humans, online at cdc.gov/eid.

I'm Sarah Gregory for *Emerging Infectious Diseases*.

[Announcer] For the most accurate health information, visit cdc.gov or call 1-800-CDC-INFO.