Genomic Epidemiology of Global Carbapenemase-Producing *Enterobacter*

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

[Sarah Gregory] Today, we're talking with Dr. Johann Pitout, a microbiologist and professor of microbiology, immunology, and infectious diseases, as well as pathology and laboratory medicine, at Calgary University. We'll be discussing bacterial species that are resistant to antibiotic carbapenem. Welcome, Dr. Pitout.

[Johann Pitout] Thank you, Sarah, for the invitation.

[Sarah Gregory] Okay, to start, what are Enterobacter and are they harmful to humans?

[Johann Pitout] So, Enterobacter species belong to the family Enterobacteriaceae, and are actually environmental bacteria. That means that their normal habitat, where they live and breathe, are in the soil, the water, and plants. They are actually a very common causes of bulb rot in onions and potatoes. So, the next time you see an onion that is. . .that is brown inside, that is Enterobacter.

[Sarah Gregory] Oh!

[Johann Pitout] As far as human pathogens go, there are two species of Enterobacter species that are the most clinically relevant—Enterobacter aerogenes and Enterobacter cloacae complex. Enterobacter aerogenes has recently been moved back to the Klebsiella genus, and is now named Klebsiella aerogenes. It's not an Enterobacter anymore.

Enterobacter cloacae complex cause infections, such as urinary tract infections, bloodstream-associated infections, intra-abdominal infections, and respiratory tract infections, in immuno-compromised patients, especially those admitted to hospitals in ICUs or high-care units. Because they have the ability to survive on surfaces of inanimate objects, Enterobacter cloacae complex are often responsible for nosocomial outbreaks requiring stringent infection prevention and control procedures to control such outbreaks.

[Sarah Gregory] So, sorry, backing up to the onion, is that. . .so, is that dangerous?

[Johann Pitout] Well, if your immune system is normal, which I assume most people outside the hospital would be, you don't have much of a problem. So these bacteria really only affect people that somehow the immune system is not functioning normally, either being admitted to a hospital Intensive Care Unit or they are having some anti-cancer therapy that suppresses their immune system. For them it can be, could be more dangerous.

[Sarah Gregory] Ah! Okay, well, I'm going to start throwing them out more regularly anyway.

[Johann Pitout] Yeah.

[Sarah Gregory] How are Enterobacter infections normally treated then?

[Johann Pitout] So, Enterobacter species are inherently resistant to most of the beta-lactam antibiotics, and for serious infections, most physicians will use either a carbapenem, such as imipenem or meropenem, or a fluoroquinolone, such as ciprofloxacin, or a fourth generation

cephalosporin, such as cefepime, to treat such infections. For nonserious infections, such as a lower urinary tract infection, trimethoprim sulfamethoxazole is another option, if the bacteria tests sensitive to this antibiotic.

[Sarah Gregory] Tell us about using genomic sequencing to discover different Enterobacter.

[Johann Pitout] So, the taxonomy of Enterobacter cloacae complex is confusing, and uncertainty still remains about what species make up this complex. In the early 2000s, two researchers, named Hoffman and Roggenkamp, sequenced the gene called hsp60, and established 12 genetic clusters in the Enterobacter cloacae complex. In 2005, the same authors further defined the taxonomy of this Enterobacter cloacae complex and added three species to the list. And in 2014, some Chinese colleagues described a novel Enterobacter that was obtained from Chinese sourdough. An additional identification methodology, for example, sugar fermentation, and a newer multitask mass spectrometry have problems in identifying these different species with Enterobacter complex. Therefore, most clinical laboratories will report Enterobacter species as Enterobacter cloacae complex.

[Sarah Gregory] So, where are the geographic locations of the Enterobacters that were discovered?

[Johann Pitout] So, we at this study had access to bacteria obtained from two global surveillance programs. The first program is called the SMART program, which was established in 2008 and ran until 2014. And this included bacteria from intra-abdominal and urinary tract infections from 55 countries in Africa, Asia, Europe, Latin America, North America, Middle East, and the South Pacific. The second surveillance program is referred to as the INFORM Program. This program started in 2012 and ran until 2014, and this included bacteria from intra-abdominal, urinary tract, blood, skin, and soft tissue, and lower respiratory tract infections from 42 countries in Africa, Asia, Europe, Latin America, North America, Middle East, and the South Pacific. Both programs collected 100 consecutive clinically relevant, nonrepeat, gram-negative bacteria per annum from each of the institutions that were enrolled in this program.

[Sarah Gregory] So, apparently, many of these Enterobacter are developing resistance to carbapenem. How do bacteria develop drug resistance?

[Johann Pitout] So, for an antibiotic to reach its target and kill the bacteria, it needs to penetrate the outer membrane of that bacterium, using a structure called a porin. Bacteria, such as Enterobacters as well, can prevent the antibiotics from reaching the target by doing the following. The first thing, what they can do is they can close some of these porins. This will prevent the antibiotic from penetrating the outer membrane and is referred to as impermeability. Enterobacters, specifically, stop making a porin named OmpF that prevents carbapenems or other beta-lactam antibiotics from penetrating into the outer membrane. The second thing bacteria can do is they can pump out the antibiotic as it reaches the inside of the bacteria. This is referred to as efflux. And, interestingly enough, this mechanism is not well defined as an important course among Enterobacter species.

And the last thing that they can do is they can produce an enzyme that binds to and inactivates the antibiotic. One of such class of enzymes are called the beta-lactamases that imply they have activity against the beta-lactam antibiotics. And carbapenemases are a type of beta-lactamase that has specific activity against the carbapenems. Of those three mechanisms, carbapenemases

are considered to be the most clinically important cause of carbapenem resistance. There are two reasons for that. The first, they are associated with certain genetic elements, referred to as mobile genetic elements, and therefore can be transferred between different members of the Enterobacteriaceae. And secondly, Enterobacter species with carbapenemases are important causes of nosocomial outbreaks, while porin mutants rarely cause such outbreaks. And we still don't understand why carbapenemase-producing Enterobacters are such an important cause of nosocomial outbreaks, but not the porin mutants.

[Sarah Gregory] Okay, are there different types of carbapenemases?

[Johann Pitout] Yes, there are. There are various beta-lactamases with activity against the carbapenems, hence the name carbapenemases. But from a global and a clinical point-of-view, there are really only five that are relevant. They are named the KPCs, NDMs, IMPs, the VIMs, and the OXAs.

[Sarah Gregory] Do you want to tell us a little bit more about each of those?

[Johann Pitout] So, ND. . .KPC stands for Klebsiella pneumoniae carbapenemase. This was first described as a carbapenemase in Klebsiella from North Carolina during the late 1990s. And today it is considered in the USA as the, . . .and other areas in the world, as the most common and most important carbapenemase among the Enterobacteriaceaes.

NDM stands for New Delhi metallo-beta-lactamase. This enzyme was first described in the late 2000s, round about 2008, from a Swedish patient that has just been transferred from India. This enzyme is endemic in the Indian subcontinent that includes Pakistan, India, and Bangladesh. And it's often the second most carbapenemase among global surveillance studies.

IMP stands for imipenemase, which implies that it inactivates imipenem, which is a carbapenem. They are probably the oldest of the group of carbapenemases, and not that common among the Enterobacteriaceae, more an important cause among Pseudomonas.

VIM stands for Verona integron-associated metallo-beta-lactamase. This was first described, as the name suggests, in Italy around about the early 2000s, and again, this is an important carbapenemase among Pseudomonas and not found that often in Enterobacteriaceae.

And then the last group is the oxacillins. This is a very large and confusing group of carbapenemases, even for those people that study them. However, among the Enterobacteriaceae and Enterobacters, specifically, OXA-48, which is just a number—these enzymes have different numbers as they are being characterized—OXA-48 is the most common one. And, interestingly enough, countries in North Africa, the Middle East, and Turkey is endemic for Enterobacteriaceae with OXA-48.

[Sarah Gregory] Okay, your study looked at Enterobacter from around the world in the years from 2008 through 2014. What were you looking for?

[Johann Pitout] So, the objective of this study was to take these two global collections, as I mentioned before, of Enterobacter cloacae complex, just about 160 of these bacteria, and we were going to identify the following. First is we wanted to determine the species of the Enterobacter cloacae that were the most common. Secondly, we wanted to define if there's any dominant clones among these species. And what I mean by that is, is there a certain clone among

these species that are more common than other clones? And then we wanted to take these clones, if there were dominant clones, and we wanted to further identify them into sub-clones, which is referred to as clades. Then we wanted to look at if there's any specific geographical distribution associated with these different species, the different clones, and if we have find. . .if we found clades among the clones, were there different geographic distribution of these clades, as well. And the fourth thing that we would like. . .wanted to do is we wanted to take the carbapenemase gene and look at mobile genetic elements that is surrounding this carbapenemase gene. And the reason for that is mobile genetic elements are responsible for moving carbapenemase genes within bacteria, and sometimes between bacteria. And for Enterobacter, that type of information is still not that common, so we wanted to look at these type of elements and characterize them for future researchers, if they wanted to do something similar.

[Sarah Gregory] And what did you find?

[Johann Pitout] So, as far as the first objective goes, we identified eight different species among our collection, but these consisted mainly of two species: *Enterobacter xiangfangensis*, and I'm pretty sure I'm pronouncing that completely different, because it's a Chinese word, and *Enterobacter hormaechei*, subspecies steigerwaltii, which obviously comes. . .sounds like it comes from Germany.

The most common carbapenemase among these groups was actually the VIMs, which was a bit of a surprise to us because, I mentioned before that the VIMs are common among Pseudomonas. It's not really a common carbapenemase among the Enterobacteriaceae. So, that was. . .that's something that we did not expect when we started out with this study.

And then the second most carbapenemase was NDMs, followed by KPCs, OXA-48, and the IMPs. The different species were associated with different types of carbapenemases. What I mean by that is there wasn't one specific carbapenemase that was associated with a specific species. So, the carbapenemases tend to be present in all the different species. And they showed a global distribution, the different species. But we did see some interesting global distribution of some of the species. For example, the Chinese one, the *Enterobacter xiangfangensis*, was frequent in the Balkans (and the Balkans is Croatia, Romania, and Serbia), while *Enterobacter hormaechei*, subspecies steigerwaltii, this is the second most common species, was most prevalent in Greece and in Vietnam.

As far as the second objective, this was trying to identify if there was any dominant clones among this collection of ours; we found four clones. The first clone, and this is just a number, it's just as a different clone is being identified, among a species, as they come into this centralized website that is responsible for maintaining these clones, they give them a number. So, as you start, it's ST-1 would be the first one, and then it carries on as people send their sequencing into this website. So the most common clone that we found was numbered ST-114. This was present among the species *xiangfangensis,* and had a global distribution. There wasn't one specific country where this clone was more common than, say. . .than, it was just a global; it was all over the world. We could divide that clone into four different clades, and it was interesting to note that the clades tended to have a certain global. . .a certain global restriction. For example, the clade which we numbered clade B, this was specifically a Greek clone, while the clone. . .or the clade A was a global clone. So that was another interesting thing. It seems like

certain clades within this clone tendeda to be more global, while other clades tended to be more restricted to certain countries.

Then, the second most common clone that we found was ST-93. This consisted only of one clade, so we were not able to differentiate them into different clades. This suggests that this clone is the more closer to each other, these different members of the clone, while within the previous one, like ST-114, the members tend to be a little bit more separate; then you can divide them into sub-clones or clades, but not for this specific clone. This had a global distribution. We could not find that there was any country where it specifically was more prevalent, and also, it had different carbapenemases. There wasn't one specific carbapenemase associated with this clone. The other two clones that we found was ST-90 and ST-78. And here what's interesting is that ST-90 was mostly a Greek clone, while ST-78 tend to have a global distribution.

So, what does it really mean? We're not really sure yet, but it's important to document that you have certain clones, and certain clades within these clones, there are certain. . .some of them are global. There has to be a reason why they are global, while others tend to be more restricted to certain countries, so more of localized type of approach.

And then, as far as our third objective, I wanted to mention that we were able to characterize the immediate genetic environments of these carbapenemases genes in the majority of isolates, and we also described, then, 14 novel structures that were associated with KPCs, NDMs, the VIMs, and OXA-48 carbapenemases.

[Sarah Gregory] What do these results mean in the larger trend of antibiotic resistance? Is this another. . .another area we should be worried about?

[Johann Pitout] So, this study identified, then, the different species and described the important global clones and their respective clades associated with carbapenemases. We were able to describe the global distribution and the association with different mobile genetic elements. We noticed that some clones, with the same carbapenemase, can move between certain countries. But then, they are associated with different mobile genetic elements within each country. It's sort of, as people immigrate to a country, a new country, after a while, they tend to pick up local accents. We see the same with antimicrobial-resistant clones. They tend to pick up the local genetic element as they move between different countries and they establish themselves in such a new country. These associations demonstrate that certain mobile genetic elements have the ability to easily move between clones and clades of Enterobacter species on a global scale. And this is highlighted by one clone, numbered ST-78, with a VIM type of carbapenemases, that circulated. . .we clearly showed that circulated between Greece, Italy, and Spain. And I assumed this is due to the fact that these countries must share patients in hospital settings. There obviously is transfer of patients between. . .between these three countries.

Then, a special concern that we found was we described a common NDM genetic structure. We just named it NDM-GEUS because it sort of. . .it sounds good and it makes sort of sense to name it that. But this genetic element was first found on a plasmid from a *Klebsiella pneumoniae*, in 2010, in the USA, from a patient that just returned from India. And we found this NDM genetic structure, NDM-GEUS, among six different species, 14 different clones, and obtained from six countries across four continents, showing that certain genetic elements can easily move between different species, because it was first described in *Klebsiella pneumoniae*, then found its way

among the Enterobacter species, and then moved between different countries. But not using a clone, so this is a mobile genetic element that moves between different species, different clones, different clades, across the world. Didn't really expect that, to be honest. That was quite an exciting finding of this. . .in this study.

So, to summarize, this study support the current understanding that the carbapenem-resistant pandemic is both a consequence of circulating clones, as well as the spread of mobile genetic elements. And we showed that using surveillance programs that uses the latest molecular technique, provides insight into the characteristics of the global distribution of clones, clades, and their associated. . .and their association with mobile genetic elements.

[Sarah Gregory] So, it's exciting find, you said, but should we be worried?

[Johann Pitout] Well, we don't really know yet what it means. Just, so. . .the biggest surprise that we found here, is that it seems that among Enterobacter species, this pandemic of carbapenemases is due to the mobile genetic elements that surrounds the carbapenemase gene, and basically take these type of carbapenemase genes from one species, one clone, one clade, to another, and then travels across the globe, it looks like it.

So, how do you stop such a spread of carbapenemase genes that can move so seemingly effortlessly between. . .between different species? And it'll be very, very difficult to do. We always assumed that this carbapenemase pandemic is due to bacteria that spread, but it looks like, although bacteria plays an important role, it's more a mobile genetic element that's spreading between different bacteria. And our whole approach to this, as far as infection prevention and control, might have to take a different approach, because you, now you don't have a clone that sort of moves, it's more of a genetic element that moves between different clones. And we might have to look at this and see if this. . .if our traditional infection prevention and control procedures will be able to efficiently really stop this spread. Because at the moment, we're not stopping any spread; this is just continuing and spreading across the globe.

[Sarah Gregory] Yikes! Okay, so along those lines, are there any next steps that you're going to take or that you suggest taking?

[Johann Pitout] So, one of the limitations of our study was that we were not able to characterize the plasmid. So, a plasmid is, if I can put it to you this way. The mobile genetic elements can be classified into two different groups. The first group has the ability to move genes within the bacteria, and these are the ones that we were able to describe. However, there's another group that takes those genes and move it between bacteria, and these are plasmids, are probably the most important of these. . .this group. And due to the fact that we used a technique called short-read sequencing, we were not able to characterize the plasmids. And we need to do that because we've showed now that those genetic elements within the bacterium, that can move genes within bacteria, seem to be widespread among different bacteria, and different clades and clones within Enterobacter. However, we don't really yet understand how they move in between these two. . . these different clades and clones. And for that we need to characterize the plasmids that probably were taking these genes from one bacterium to another bacterium. And so, we are in the process, actually, of now using a different technique called long-read sequencing, and now we're going to be characterizing and creating a library of these different plasmids and the other mobile genetic elements that are responsible for moving carbapenemases in Enterobacter on a global scale.

[Sarah Gregory] Okay, tell us about your job and how it relates to this study and how you became interested in this research.

[Johann Pitout] I'm a medical microbiologist, as you mentioned up front. I have a dual appointment. I work for Calgary Laboratory Services, where I do 50 percent of my time, as a medical microbiologist. And as this implies, this is my clinical job. And I sort of service the. . .one of the microbiologists that service the Calgary region as a microbiologist. And then the second applic. . .I have a second appointment. This is at the University of Calgary, where I do research and teaching. And so this study was basically part of that, part of my. . .of my job description where we do a little bit of research and do molecular epidemiology. I come from a beta-lactamase background. I did my post-doc in beta-lactamases back, a long time ago, in the U.S., when I qualified as a medical microbiologist. And then, was always been really interested and fascinated by bioresistance in gram-negative. So I really only work in gram-negatives, I don't really work in gram-positive bacteria, and find gram-negatives fascinating. So, we'll be continuing this for sure.

[Sarah Gregory] Well, thank you so much, Dr. Pitout. Listeners can read the June 2018 article, Genomic Epidemiology of Global Carbapenemase-Producing *Enterobacter* spp., 2008–2014, online at cdc.gov/eid. I'm Sarah Gregory for Emerging Infectious Diseases.

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