Microbes – Friend or Foe?

AGNES DAY: The bacteria, especially the resistant ones, are the recapitulation of Darwin's theory of Survival of the Fittest. If you have the capacity to be resistant, then you are going to be the population that survives, while all the sensitive ones die off.

LUCY SHAPIRO: And, so what you want to do is design double-headed antibiotics, that not only knock out the target but, knock out a mechanism for drug resistance.

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LUCY SHAPIRO: I think that After World War II, when we built up our whole arsenal our whole armamentarium of incredible antibiotics, we also built up with that a sense of security and, we were able to deal with all manner of infectious bacteria that were, heretofore, killing off lots of people. And, suddenly, we had a way of dealing with it, and it was remarkable. And we didn't pay attention to the fact that, even as early as the early fifties, antibiotic resistance was building and building and building, because I don't think we had the kind of ability to understand how clever these bugs are. And now, now we're in full trouble. There isn't a single antibiotic now that there isn't some bug that's resistant to it, and that's the drug of last resort is Vancomycin and there are staph and strep that are resistant to Vancomycin now.

AGNES DAY: We've gotten our wake-up call, we really have. And the indiscriminate use of antibiotics has led a lot to this situation.

ROBERT L. KUHN: How come these bugs are so smart?

PAUL EWALD: They have a great evolutionary potential, they have very short generation time, they have high mutation rates, but most importantly, I think, is you've got vast numbers of these microbes. So, when you use an antibiotic you can knock down the microbe population by 99.99%. If you just have one in 10000 or one in 1000,000 microbes that's a bit resistant, that is the microbe of the future.

ROBERT L. KUHN: How long does it take them to reproduce?

AGNES DAY: About 22 minutes, if you're talking about *E. coli*, but that's interesting that Paul should say that, because the bacteria, especially the resistant ones are the recapitulation of Darwin's theory of Survival of the Fittest. If you have the capacity to be resistant, then you are going to be the population that survives, while all the sensitive ones die off. If you look at a single-celled microbe and his environment changes and he knows that he has 22 minutes to change or die…

ROBERT L. KUHN: …her environment. [THEY LAUGH]

AGNES DAY: If it's the bad bacteria, it's him. But it's a form of intelligence that they can mutate or change to protect themselves and survive and give rise to a whole new species.

ROBERT L. KUHN: It sounds like we've gotten into an arms race we cannot possibly get out of, and we are on a scientific treadmill.

LUCY SHAPIRO: I don't like the word treadmill. Why are you using the word treadmill? I don't know what you mean by that.

ROBERT L. KUHN: It's going faster and faster.

LUCY SHAPIRO: Faster, treadmill comes back around, it's a wheel, this is expanding…

PAUL EWALD: It actually comes back to the arms race metaphor, another metaphor is the Red Queen, which is, you're walking just to stay in place. Whether this expansion allow the key question is whether the expansion associated with new technologies, allows us to stay ahead of the pathogens and, because, if we still, even with all of these examples, we're still talking about getting involved in some sort of a race with the pathogens. And so I think that the treadmill analogy, actually I think it does work although it's a different metaphor.

ALICE HUANG: But you know we are always discovering new antibiotics. Every time we sort of go into the soil and, and screen that or, if we isolate new bacteria that we haven't seen before, we find that they make antibiotics against other bacteria.

LUCY SHAPIRO: There's another thing that's happening, as well, and that is now that we are able to understand, because we have sequences and because we have genetic engineering and we can actually take these cells apart as though we are systems engineers, we can design double-headed antibiotics, that not only knock out the target but, knock out a mechanism for drug resistance. So, I think that the additional knowledge we have now is allowing us to design things in such a way that we're trying to keep up with the way these bugs can evolve and change.

ALICE HUANG: Of course, we have some of these newer antibiotics that people are making, the peptide antibiotics, which can drill through the membrane of the bacteria, and this is a whole new class now that offers us some hope

PAUL EWALD: I think a more general way of approaching this problem is to change the environment so that you favor the mild organisms You can make vaccines in ways that favor the mild organisms, by selectively knocking out the harmful organisms

ROBERT L. KUHN: So it's reversing the natural selection because you're selecting for the less virulent strain.

PAUL EWALD: Well, if you favor mild strains of organisms for, let's say, an organism like the one that causes cholera, that means, instead of having half or three-quarters of the people showing severe infection, you may have only one percent, of the people who are infected, showing symptoms of disease.

LUCY SHAPIRO: But, somebody in that part of a country can get on a plane and be in Chicago in 17 hours. And they can travel asymptomatically, and then you're taking pathogens and putting them in completely different environments than they're used to, and then they very rapidly evolve into doing something else. And, so I see, in a sense, that we'll be putting out brush fires, and I worry about that.

PAUL EWALD: I like your point, I mean, it's a point that we really need to be concerned about. The way we evaluate it is to take a look at what happens when we get these nasty strains, for example, of diarrheal diseases imported in the United States, something that's occurring every day. Right? And the studies that have been done suggest that, when these nasty strains get in the United States where we've got protection against waterborne transmission, they can't make it, they die out. So, for example, there's this horrible outbreak of dysentery that occurred in the early seventies in Latin America, killing thousands and thousands… the worst *Shigella dysenteriae* type 1. When it got into Los Angeles, where the water supplies were protected, the number of new infections was about one-half, that means it just died out on its own, even without any controlled measures. Even though this a global village, even though we're concerned about things coming in all the time, if we, on a local or regional scale, if we adjust the infrastructure so that we disfavor the harmful strains, that's an extra layer of protection.

LUCY SHAPIRO: But if we come back to the issue of what happens with antibiotic resistance when we all get panicked, like happened with anthrax in New York. And, in fact, the, the very bad outcome of this particular anthrax attack that really just killed a very few number of people, is that the collateral damage was enormous. Number one, it disrupted our entire way of living and thinking. We had 24/7 barrage on TV about how horrible it was. But the real villain was the antibiotic resistance to Cipro. And with respect to Cipro, just several years ago, there was a big chicken flu scare, and so much Cipro was used in zillions of chickens, it would blow your mind. It managed to bring down the chicken infection epidemic and the resistance to Cipro went from very small number to, I think, 15%. Now, I don't even know what the numbers are after this last barrage of using Cipro. We're going to make Cipro useless, and that was the big danger of this particular, very mild attack with anthrax.

AGNES DAY: Cipro is not only active against anthrax, but there are about 30 or 40 different bacteria that it is also effective against. And, once you were giving Cipro

prophylactically to people who may have been exposed, you're also killing off those friendly microbes and other microbes that are living in your gut that you wanted to keep, and it's called microbial antagonism, where you want to keep the good guys and the bad guys sort of equal.

LUCY SHAPIRO: The same things that have to be done to protect our populations from infectious disease – globally, not necessarily for malevolent intent, are the same things that are going to help us understand how to deal with bioterrorism and bioterrorist threats. And it's tremendously important that we understand how bacteria can evolve and change and do things that we are not happy with and things we're happy with and, at the same time, the knowledge that we have there, can be applied to how to deal very rapidly with both infectious disease that's natural, and bioterrorism.

ALICE HUANG: Let me tell you about some recent, findings which I think is very encouraging for us, and that is that, when we look at bacteria, we've often, in the laboratory, looked at them as single-cell animals. In nature, they actually exist in communities, in very complex communities. And there are rules in that community, so that no one really takes over. In fact, they need each other, sometimes some bacteria will provide food for another organism and *vice versa*. Well, as we understand these situations, we also begin to realize that there are many microbes that are very good and that we actually need them and that they can be useful, in fact, in our own gut, we find that there's a nice mixture of microbes and generally, if we disturb them, and if that balance is not correct, all sorts of terrible things happen. And a good example of that is that, I don't know how many people here live on farms, but, actually, cows are known to pass gas a great deal, and the reason that they do so is because they have a methane creating bacteria which does this. Now, one third of the human population has the same bacteria, predominantly in their gut. And so those are the friends that you sort of want to avoid. [THEY LAUGH] But two-thirds have a different kind of bacteria, so, each of our guts are actually quite different from the other.

LUCY SHAPIRO: There's, there's an incredible story about a good bacterium that lives inside what's called an eyespot on a squid that lives in very shallow waters off, in Hawaii, and this bacterium sends off photons, so it causes light to be generated. It does this at night. And, what it does is protect this squid from predators because, when there's a full moon shining down here and you've got this squid in shallow water, the light turns on and obliterates the shadow, and, so, it becomes invisible to predators. And then, in the morning, this particular population goes away, and then it comes back in again, cyclically. And there are many, many examples of bacteria living with other living organisms providing critical functions, not just for our own metabolism in what we do, but, many other critters on this globe really need the bacterial population.

ROBERT L. KUHN: How do the different microbial strains work together, in terms of beneficial ones versus harmful ones, in this case?

AGNES DAY: One way that the do work together in bringing about drug resistance is the sharing of extrachromosomal material that will have the genes' encoding for drug resistance. In the past, we thought that only like species could exchange this DNA, through conjugation where the cells come in contact with each other, now we know that they have something called promiscuous plasmids, who will also effect other species. They have now pieces of DNA called integrons, that have sometimes up to six genes that are encoding for enzymes that will destroy six different drugs. And, so, with the rise of these types of resistance mechanisms in the bacteria, I think that drug discovery is going to have to be focused on a little bit more.

ROBERT L. KUHN: With that, we're going to switch to our next subject which is the question of whether microbes can cause diseases beyond the normal things we think of as infectious diseases. Most people don't even realize that cancers can be caused by external, infectious microbes.

PAUL EWALD: The figures now are about, depending on how you count it, about 15% to 20% of all human cancers are caused by infection, which is an astonishing number. If you were to ask people in 1975, how many human cancers, what proportion of all human cancer is caused by infection, the, people would say, maybe a 10^{th} of a percent, max. And, so, what's happened, over the last 25 years, is every five years we've been discovering more and more cancers being caused by infection.

ROBERT L. KUHN: Is this discovery of what always was or an increase…

PAUL EWALD: A discovery of what always was.

ROBERT L. KUHN: So you don't you think the percentage has been increasing?

PAUL EWALD: No, it's just, what we had 25 years ago is virtually 100% of the cancers had causes that we didn't understand well. Okay? And, now we understand some of those causes a bit better, we understand them enough to know that cervical cancer is caused by infectious agent, liver cancers are triggered by infectious agents

AGNES DAY: And then there's the *Heliocobacter pylori*, which has a strong association with gastric cancer

PAUL EWALD: We do have an awful lot of very interesting data which are experimental data such as curing stomach cancers with antibiotics that knock out *Helicobacter,* and a recent study from Japan, in which experimental and controls were followed longitudinally in the control population, there were 33 cases of stomach cancer, in the experimental, which had the *Helicobacter* knocked out, there were zero. You know, we just have to think more cleverly about what the evidence suggests.

LUCY SHAPIRO: But, I think critically, the operational word there is triggering something in the host. So, this is not a simple chain of events that causes a cancer to be a consequence of a bacterial or viral infection. In most instances, what you're doing is having a tissue attacked in a person that then elicits a whole series of biochemical reactions, the cytokines go rolling in, everything goes charging in, and especially in tissues that turn over rapidly. It's not that the bug itself is causing the cancer, we are over-responding to the infection, trying to get rid of it and, especially in cells that keep recycling. So, you get mutations, these mutations build up, and then you get an oncogenic response.

PAUL EWALD: But I would say, sometimes yes, sometimes no. In other words, there are two categories, two ways in which infections can cause cancer , one is the sort of the irritation mechanism, which is, I think, the one that you're talking about. But there are other mechanisms in which you actually have the pathogen encoding a compound that goes in and knocks out one of the cancer-prevention compounds. So, for example, Human *Papilloma* viruses produce E6 protein, which knocks out p53, which is something that is one of our barriers against cancer, and the organism isn't doing this to get cancer, and this may be probably what you're referring to. The organism is doing this because it allows the organism to reproduce by allowing, by causing the cell it's in to reproduce a little bit more, and that allows the organism to avoid destruction by the immune system, the organism is very secretive in this case. But, what that means is that it pushes the cell one step closer to cancer.

ROBERT L. KUHN: What is the percentage of cancers that are caused, in some way, by infection?

PAUL EWALD: Less than 5%, for less than 5% of human cancers can we exclude a role for infection. For about 15 to 20% we say infections are playing a triggering or primary role. And the other ones we don't know yet.

LUCY SHAPIRO: And, and it's also more than cancers. In other words, these infectious agents can come in and cause some effect in the host and then our immune system overreacts. And so many, many, many of our diseases are auto-immune diseases that are triggered initially by either a viral or a bacterial infection.

ALICE HUANG: Well, a great example of this is when *Helicobacter* was discovered to be causing ulcers that everyone then thought, okay, for the inflammatory bowel diseases, it must be a bacteria and we're going to go after it and we'll treat it all with antibiotics, and then it didn't work because it was really the inflammatory response of the host.

LUCY SHAPIRO: But they can also be, antibiotics can also be, not only useless, but dangerous. And the case, again, is *E. coli* 0157, in which you have the genes for a toxin

that came from another kind of bacterium called *Shigella,* and that *Shigella* is sitting within a piece of DNA that came from a bacteriophage, a bacterial virus. And, so, anything that you do to that *E. coli* 0157 that's harboring this latent virus in its chromosome, will cause the viral genes to turn on the toxin gene and then the toxin kills you, or makes you very ill. And, so, what you have here is a situation, if you give antibiotics, you stimulate the production of the virus that turns on the genes for the toxin. So, you can't give antibiotics in this case.

ROBERT L. KUHN: With that, we're going on to another subject, which is the other side of microbes, which is probiotics. Can microbes be utilized to make us better?

PAUL EWALD: This kind of research has been going on for quite a long time, all the way back into the sixties and even late fifties, people were studying the possibility that, if you had a nasty infection, let's say with staph, maybe you could get a milder staph and introduce that and have that interference occur.

AGNES DAY: Well, the poster child for probiotics would be the l*actobacillus* or the active yogurt cultures that you see now in grocery stores, you see the LactAid Milk for people who are lactose intolerant. This bacterium will break down the milk sugar, as it were, so that you don't get the stomach cramping and thing. Now, that's just one example the good that probiotics can do.

PAUL EWALD: With probiotics things like vaccination to get the mild organisms but let natural selection, in the human population, do that evolution, we do it in the lab when we make live vaccines, we get mild organisms, those mild strains are like probiotic strains. Then the vaccines knock out the harmful strains and they leave the mild strains. And, in fact, the second most successful vaccination program in history, has used just this strategy without really knowing it, this is the diphtheria vaccination program, and the success of that program is second only to the smallpox vaccination program. which eradicated it.

ALICE HUANG: Well, the one that's probably least familiar to the public is the current idea of putting anaerobic bacteria into tumors, so that it would destroy the inside of the tumor, but, once it get into an oxygenated environment, it can no longer spread and grow uncontrollably.

ROBERT L. KUHN: That's fascinating. That's like controlling tumors by shrinking the blood vessels, a similar effect. Is that showing promise?

LUCY SHAPIRO: I don't know what the results are yet, it's too early.

ALICE HUANG: These are just beginning to be done. Let me get back to an issue, which I think that we've dealt with somewhat simplistically and that is thinking that, just

by cleaning up the water supply, that we will get rid of all the bad microbes. Very often, when you deal with one population of microbes in a situation that you change and you get the desired result, it doesn't mean that other populations, other kinds of microbes don't change, as well. In fact, you might get good ones that change into bad ones under the same circumstances. So, even though we all agree that having clean water is a good thing, you know that in instances as we've become more hygienic, we've changed our cultural lifestyle. In that way we've become more susceptible as host to certain other agents, Homeovirus for example, which really has been around since the Egyptian days, we have recordings of *Poliomyelitis* going on. And we were able to cause that to become an epidemic, only because we developed much better water supplies.

PAUL EWALD: That's right, we know that the success associated with cleaning up of water supplies has been something that dwarfs most other success stories in medicine, but yet, there's still, there are one or two potential problems and, in this case, with polio, we have to come back with a good vaccine program and knock out this straggler that sort of got in the back door that was a little, had some characteristics that made it a little bit unusual compared to other waterborne pathogens. So, I think that it is terribly important to do this, to take into account what's the whole balance sheet. And the same thing, the same kind of argument's been made for peptic ulcers, for example. If we knock out *Heliocobacter pylori*, we have this tremendous positive effect in reducing the frequencies of peptic ulcers, and also reducing the frequencies of some stomach cancers. Some people have argued, but it looks like it might be associated with an increase in esophageal cancer, which may or may not be true, we have to look hard at those data. But, if we actually look at the numbers, the positive effect associated with knocking out *Heliocobacter pylori* is so great that it dwarfs the potential negative effect.

ROBERT L. KUHN: So we always have to be on the lookout for unintended consequences.

PAUL EWALD: THAT'S RIGHT. That's right, we do and I think that's your point.

AGNES DAY: But in cleaning up the water supply, you would probably have to use various types of chemicals and things to do that. Isn't that a type of selective pressure that you're applying so that that one percent that does survive might be even more detrimental?

PAUL EWALD: Luckily, in a *a priori*, you wouldn't know whether or not, let's say the use of chlorine was going to be selecting for chlorine resistant organisms that could be more resistant to the chlorine than we are, in which case, we're in trouble. Luckily it turns out that there're only a few of these organisms that look like that they can generate resistance to chlorine.

LUCY SHAPIRO: That we know about.

PAUL EWALD: That we know about. But we do have some pretty good evidence on this because we can look and see whether we get infections of dangerous organisms in water supplies that are chlorinated. But we can also use things like filtration, there are a lot of other mechanisms for cleaning up water supplies, if we're worried about chlorine.

LUCY SHAPIRO: But, I think that we have to continually remind ourselves that the water supply is just one way. And that most likely I think that there's great history of epidemics being spread through water supply, but there's also great history of epidemics being spread just by aerosol, and just through the air, and certainly, my feeling about bioterrorist attacks will be that it will be most likely not through the water, but aerosol.

PAUL EWALD: That's right, waterborne pathogens are lousy terrorist weapons so do microbes.

LUCY SHAPIRO: And, I think that one way of looking at this that's very pertinent to everything we're doing, is we turn back to the bioterrorism issue, and the bad news is that we can now genetically manipulate organisms to change their host range, to make them resistant to drugs, to make it more of an aerosol, to make it more able to transmit from person to person.

ROBERT L. KUHN: "Weaponize" as they say.

LUCY SHAPIRO: Weaponize. On the other hand, the good news is that we have learned so much about these bugs that now we can design detectors. We can now design ways of saying we know what that bug is and we know how to stop it now.

ROBERT L. KUHN: And also the science of doing this will accelerate our knowledge in general that might be applied to other areas.

LUCY SHAPIRO: Yes.

AGNES DAY: But on the flipside of that example that you just gave for biowarfare, there are also some bioweapons that utilize the toxins from the organism. And so if we know the gene sequence for these toxins, we can put these genes into bacteria that we would normally identify as being benign or nonpathogenic, but under certain conditions if you turn on that toxin gene, then now you have your warfare agent.

LUCY SHAPIRO: So you have both plus and minus, so we can manipulate these clearly, but on the other hand we can find out what they are and we can design things that will stop them.