Pigs carrying methicillin-resistant *Staphylococcus aureus* were found on US farms for the first time in 2007.

DANIEL ACKER/BLOOMBERG/GETTY

FARMING UP TROUBLE

Microbiologists are trying to work out whether use of antibiotics on farms is fuelling the human epidemic of drug-resistant bacteria.

BY BETH MOLE

The sight of just one boot coming through the doorway cues the clatter of tiny hoofs as 500 piglets scramble away from Mike Male. "That's the sound of healthy pigs," shouts Male, a veterinarian who has been working on pig farms for more than 30 years. On a hot June afternoon, he walks down the central aisle of a nursery in eastern Iowa, scoops up a piglet and dangles her by her hind legs. A newborn piglet's navel is an easy entry point for bacterial infections, he explains. If this pig were infected, she would have an abscess, a lump of inflamed tissue, just below the navel. "In human terms, she'd be an outie instead of an innie," he says, rubbing the pig's healthy, pink belly button.

Nearly six years ago, an outbreak of 'outies' at this nursery marked the first known infection with methicillin-resistant *Staphylococcus aureus* (MRSA) in pigs in the United States. MRSA has troubled hospitals around the world for more than four decades and has been infecting people outside of health-care settings since at least 1995 (see *Nature* **482,** 23–25; 2012). It causes around 94,000 infections and 18,000 deaths annually in the United States. In the European Union, more than 150,000 people are estimated to contract MRSA each year. Its first appearance on a US farm signalled the expansion of what many believe is a dangerous source of human infection.

Male investigated the infections with Tara Smith, an epidemiologist at the University of Iowa in Iowa City, who has since launched one of the most comprehensive investigations yet of where MRSA lives and how it spreads into and out of agricultural settings. She has surveyed farms and grocery stores as well as people's homes, noses and pets. Her findings could help to end a raging debate about whether farms' use of antibiotics is contributing to the rise of drug-resistant bacterial infections in humans.

Scientists and health experts fear that it is, and that drug-resistant bacteria from farms are escaping via farmworkers or meat. Last year, the US Food and Drug Administration (FDA) recommended more restraint

in the use of antibiotics in livestock, following the lead of regulatory authorities in other countries (see *Nature* **481,** 125; 2012).

But the meat and agricultural industries are fighting those restrictions. They claim that MRSA and other drug-resistant bacteria that cause human infections arise in hospitals, and that meat production includes safety measures, such as sanitation rules in slaughterhouses, that prevent resistant bacteria from spreading to and infecting people. "There's a long way between the farm and the table," says Ron Phillips, a representative for the Animal Health Institute, a trade organization based in Washington DC that represents veterinary-medicine companies.

The major problem has been lack of data. Many farmers are reluctant to allow scientists access to their facilities, and farmworkers — many of whom, in the United States, are undocumented immigrants — are wary of anyone who might want to sample them. But Smith and a small group of researchers are starting to fill the void. They have "really shaped the state of knowledge in the United States", says Christopher Heaney, an epidemiologist at Johns Hopkins University in Baltimore, Maryland. Smith's current research, says Heaney, could allow officials to "truly say where these bacteria in people's noses are coming from".

PROFIT AND LOSS

At a concentrated animal-feeding operation (CAFO) about an hour's drive west from Ames, Iowa, the usual din of the nursery is punctuated by the sound of piglets sneezing, thanks to an outbreak of H1N2 influenza. Craig Rowles, a veterinarian and the farm's manager, surveys his charges, some of which have mucus dripping from their snouts. "It's just like when you bring kids to a day-care centre," he says. "After a while, they're going to come home with a snotty nose."

Rowles is using a vaccine to fight the outbreak, but he is also dosing the pigs with two antibiotics — chlorotetracycline and Denagard — to prevent secondary bacterial infections. The combination is also routinely used to prevent bacterial diarrhoea and other common ailments in piglets.

Such practices have been common for decades. But few CAFOs have veterinarians on staff to advise on antibiotic use. They are not required to: veterinary antibiotics are generally available over the counter. And some CAFO operators use antibiotics much more liberally than Rowles does. Small doses of antibiotics in feed curb low-grade infections that might otherwise stymie livestock growth. Studies have found that certain antibiotics can increase pigs' growth rate by 2.5%, enough to make the difference for farmers between profit and loss. In the current US market, a farmer might get around US$1 per pound for a pig that costs about $0.94 per pound to produce. Although farm owners do not always reveal the quantities or types of antibiotics they use, an analysis of FDA data by researchers at the Johns Hopkins Center for a Livable Future in Baltimore found that in 2009, some 13.1 million kilograms — 80% of the antibiotics sold in the United States that year — were used on farms.

"IN 2009, 80% OF THE ANTIBIOTICS SOLD IN THE UNITED STATES WERE USED ON FARMS."

Antibiotic use on such a broad scale leads to resistant microbes. In a 1976 study, Stuart Levy, a microbiologist at Tufts University School of Medicine in Boston, Massachusetts, found that when farmers started using tetracycline, the numbers of tetracycline-resistant bacteria on the farms spiked[1]. Within months, resistance had spread to microbes in farmworkers' intestinal tracts. "You don't have to look that far to see resistant bacteria moving to the environment," Levy says.

In humans, *S. aureus* generally lives peacefully on the skin and in the nose. But if the bacterium enters the body through a wound, for example, it can become an aggressive pathogen and eventually make its way into the bloodstream to cause deadly infections. Most infections succumb to antibiotics, but resistant varieties, including MRSA, can be difficult if not impossible to cure. There are 270,000 strains, each potentially harmful.

Smith and her colleagues are distinguishing the strains of *S. aureus* around Iowa City in part by sequence type (ST), a categorization based on DNA sequences from several places in the genome. The sequence type that Male and Smith found in the Iowa nursery in 2007 was ST398 (ref. 2). Before then, researchers had seen ST398 mostly in Europe, where it was found in livestock and farmworkers but usually did not cause infection.

But two years earlier, ST398 had been reported in a hospital in Hong Kong[3], from patient samples dating back to the early 2000s. For Smith and her collaborator Lance Price, an epidemiologist at George Washington University in Washington DC, it was a sign that the boundaries between animal and human infections were blurring.

In February 2012, Price, Smith and their colleagues published a genetic analysis of strains related to ST398 isolated from animals and humans around the world[4]. They found that the lineage that gave rise to ST398 originated in humans. At some point, it crossed into livestock, where it acquired genes conferring drug resistance and a preference for pigs before jumping back to humans. As of 2012, ST398 was the cause of up to 20% of human cases of MRSA in the Netherlands, although the infections are generally mild.

"No one has even looked at these strains in the United States," says Smith. Doctors, she says, often don't determine what strain of MRSA is causing an infection, so it is possible that the bug has been stealthily migrating between farms and hospitals for years.

Smith's next step was to see if there were other ways MRSA might make it off the farm. At a grocery store on the outskirts of Iowa City, Smith pulls a shopping cart from a metal corral, takes a sterile swab from her purse, wipes the cart's handle and deposits the swab in a plastic sheath. Then she heads to the meat case. "I think the average consumer doesn't think of this as a risk," Smith says, picking up a shrink-wrapped tray of bright red steaks. Her swabs have told a different story.

Beginning in January 2012, Smith and her research assistant, Dipendra Thapaliya, spent a year collecting weekly swabs and meat samples from local grocery stores, including this one. They found *S. aureus* on nearly every type of surface. Five per cent of grocery carts carried MRSA. Of meat samples, 30% harboured *S. aureus*, 11% had *S. aureus* resistant to multiple antibiotics and 3% carried MRSA. The data, which have not been published, also showed that pork products had some of the highest levels of MRSA, whereas meat labelled 'antibiotic free' had little or none. This mirrors what Smith and her colleagues found in samples from farms across the state.

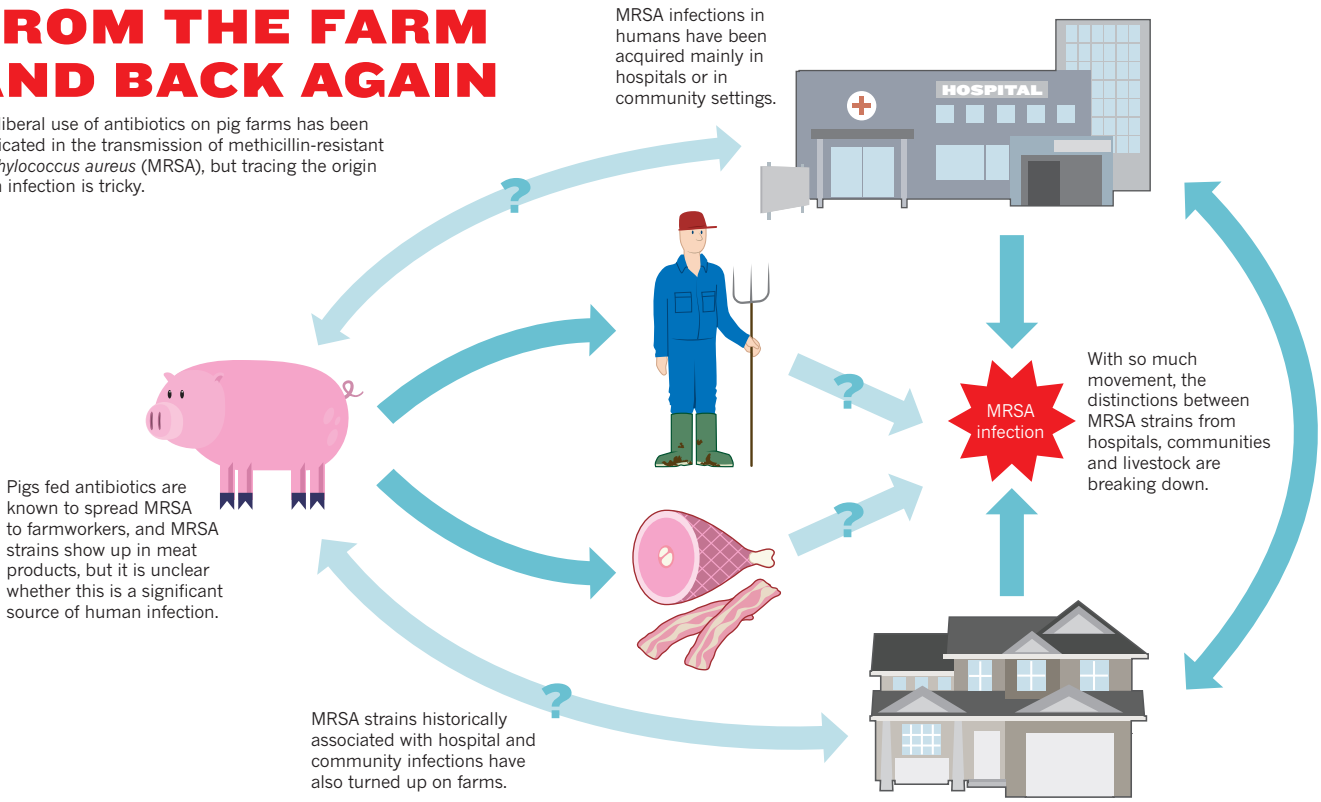
ON THE MENU

Now Smith is conducting detailed genetic analyses of the samples to identify MRSA subtypes and where they might have originated. She and her colleagues have found ST398 in the grocery-store samples. But to their surprise, they also found ST5, which is generally found in hospitals and in 'community' infections with no obvious link to farms or hospitals. Last year, Smith and Timothy Frana, a microbiologist and veterinarian at Iowa State University in Ames, found that veterinary students who carried MRSA in their noses after visiting pig farms picked up mainly ST5, most of which was resistant to tetracycline[5]. The presence of ST5 among livestock suggests that *S. aureus* strains may move easily between pigs and people, and may become resistant on farms. "It's the most interesting finding from our study," says Frana.

In lab tests, Smith and her colleagues have found that 30% of the *S. aureus* harboured in meat is resistant to tetracycline. Given Levy's data from the 1970s, this is not surprising, says Smith. But researchers, including Heaney and his group at Johns Hopkins, are finding that a bacterium's drug-resistance profile can give information about where the bug came from that sequence type and other gene-based

FROM THE FARM AND BACK AGAIN

The liberal use of antibiotics on pig farms has been implicated in the transmission of methicillin-resistant *Staphylococcus aureus* (MRSA), but tracing the origin of an infection is tricky.



NIK SPENCER/*NATURE*

categorizations may not. In a study comparing workers from different farms, tetracycline-resistant MRSA showed up only in workers from farms where antibiotics were used[6].

In the last phase of their research, Smith and her colleagues will try to determine whether MRSA is trafficking between farms, households and clinics (see 'From the farm and back again'). They are taking nasal samples from 1,300 people and swabbing doorknobs, kitchen sinks and even family pets in 96 households around Iowa City. The researchers are comparing the *S. aureus* strains they find in these samples with the MRSA strains they have found in grocery stores and farms and with strains local doctors find in infected patients. If a strain shows up in all the locations, the researchers will sequence the whole genomes of individual isolates to retrace their movement and evolution. The results have the potential to create the first complete link between farms and clinical cases of MRSA.

BAN VERSUS BEAST

CAFO supporters acknowledge that farm strains of drug-resistant bacteria could theoretically spread to people. But "I don't see this equating to human health risk", says Scott Hurd, a veterinarian and epidemiologist at Iowa State University who has conducted multiple studies to assess the risk of drug-resistant bacteria spreading through meat production. He says that the average person has a greater chance of dying from a bee sting than of contracting MRSA from pork. Hurd argues that limiting the use of antibiotics on farms could be harmful to human health. Even Smith's grocery-store study found that meat sold as 'antibiotic free' had the highest levels of garden-variety *S. aureus*, suggesting that untreated animals harbour more pathogens. "Animals really do need to be treated," Hurd says.

Nevertheless, regulatory authorities have clamped down on antibiotic use on farms. The European Union began phasing out antibiotics for growth promotion in the late 1990s. Denmark led the charge with a full ban in 2000. (China, however, which claims half the world's pig population, has yet to rein in antibiotic use.)

The bans' effects on drug resistance and human and animal health have been murky. Levy and other supporters of the bans say that the result in Denmark has been positive, pointing to data showing a drop in the use of antibiotics on farms and an increase in meat production. But opponents, including the Animal Health Institute, point out that the use of antibiotics to treat acute illness in Denmark has increased, as have animal deaths.

Last year, amid mounting pressure from several groups, including the National Resources Defense Council, based in New York, the FDA released new guidance calling for the "judicious use" of antibiotics on farms. The agency discouraged the use of antibiotics for growth promotion and urged label changes to the drugs and more veterinary oversight for their application. Not all the guidelines are yet approved, and compliance is voluntary. Nevertheless, the agency has suggested that it will enforce tougher rules if farmers and drug-makers do not adopt the guidelines within about three years. Few are satisfied with the FDA's policy. Pig farmers and meat-industry representatives consider the move a blow to farmers and animal welfare, and supporters of antibiotic restriction say that the voluntary guidelines do not go far enough. Scientists, meanwhile, have pressed the FDA to reveal more data on how farmers are using antibiotics, so far without success.

Smith, who is concerned that farmers are still overusing antibiotics, hopes that the results of her current research will sway their opinions. Antibiotics on farms can trigger the emergence of resistant strains, she says, and those strains turn up on meat, in grocery stores and in homes, and they can infect people. "For me, that's enough," she says.

At the same time, Smith says that she sympathizes with CAFO operators who are trying to produce meat as safely and efficiently as possible. And although human health should take priority over farm animals, she says, farmers will be reluctant to change until researchers can come up with safe and cost-effective practices to replace the use of antibiotics. For now, Smith says, "we're kind of stumped". ■ SEE EDITORIAL P.379

Beth Mole *is an intern for* Nature *in Washington DC.*

1. Levy, S. B., FitzGerald, G. B. & Macone, A. B. *N. Engl. J. Med.* **295,** 583–588 (1976).
2. Smith, T. C. *et al. PLOS ONE* **4,** e4258 (2009).
3. Ip, M. *et al. J. Clin. Microbiol.* **43,** 5069–5073 (2005).
4. Price, L. B. *et al. mBio* **3,** e00305-11 (2012).
5. Frana, T. S. *et al. PLOS ONE* **8,** e53738 (2013).
6. Rinksy, J. L. *et al. PLOS ONE* **8,** e67641 (2013).