The health detectives

Doctors are becoming medical sleuths in the race to contain antibiotic-resistant bacteria.

Annabel Stafford reports.

It was the kind of call that an infectious diseases expert dreamed of. It was a morning in March, two years ago, when Rebecca Davis took the call from the head nurse of her hospital's neonatal intensive care unit.

"There was something worrying on the routine swabs taken from the babies - could Davis please review it as soon as possible?"

Davis, an infectious diseases physician and microbiologist at Sydney's Royal Prince Alfred Hospital, hurried to the lab to look at the Petri dish that had been incubating overnight. In five separate dishes, the clear agar jelly had spread a nasty green mucous. It was unmistakable: *Pseudomonas aeruginosa*.

A few months later, in mid-2015, a routine swab of a patient at Monash's St Vincent's Hospital grew a bacterium that sent chills down the spine of another infectious disease physician, Professor Kumar Visvanathan. It was a deadly CRE, a carbapenem-resistant Enterobacteriaceae, a gut-dwelling bacterium resistant to most antibiotics that kills between 40 and 50 per cent of those it infects.

There had been an outbreak of a carbapenem-resistant bug at St Vincent's dating back to 2012 that had been linked to at least 12 deaths.

But after an extensive program of discrimination, patient quarantine, and screening of patient contacts, they thought they had the bug licked. So how had this patient been colonised?

Visvanathan needed to find the source. And fast.

In the mid-1990s, Dr John Snow became the father of epidemiology by tracing the streets of London to pinpoint the source of a cholera outbreak to a single well.

Now, with the World Health Organization warning of catastrophic consequences from the rise of antibiotic-resistant superbugs, a new breed of detective is tracking killer infections by tracing them back to the gut of an individual patient or hospital sink.

By mapping the entire genome of lethal superbugs, these medical detectives can follow the bug's evolution to find out where it started - and work out how it is spreading.

The *Pseudomonas aeruginosa* bug is harmless in most of the population - it's carried around on skin "without causing infection" - but Rebecca Davis knew that for a baby in intensive care it could be deadly.

A baby in neonatal unit has caught a superbug from *Pseudomonas aeruginosa* earlier in the year.

She survived, but Davis had watched her struggle for breath and knew it could have come either way.

None of the other babies who had been swabbed were infected - they were simply carrying the bug.

"But if the bacteria spread, "it was only a matter of time before a baby did get ill," Davis says.

"We needed to find out if it was an outbreak."

But traditional testing wouldn't work. All it would do was show that the babies had the bacteria, not whether they had got it from one another or from a common source.

In Melbourne, Visvanathan, too, was faced with a potential outbreak.

During 2015 a further three patients tested positive for the carbapenem-resistant bug in routine swabs that were introduced after the earlier outbreak.

The first patient, they were simply colonised, a colonisation patient in poor health and it is unlikely to get sick; they simply carry the bacteria in their gut. CRE generally only infects those with weak immunity, but the more it of about, the more likely a sick person will be exposed.

Still, Visvanathan and the infection control team knew what CRE could do if it did cause an infection - and they didn't want it rearing its head.

Because of the bug's resistance, the only optimal doctors had to treat it were ampicillin.

But back in 2015, Visvanathan and his team were concentrating on another puzzle. The four patients who carried CRE had all been in the hospital at different times, so they couldn't have passed the bug to one another - at least, not directly.

The only thing they had in common was that they'd stayed in the same ward.

"Either there was a big group of people outside in the community all walking around with CRE who had come in random, or there was some other source in the hospital."

"It was only a matter of time before a baby did get ill."

Dr Rebecca Davis

To prove there was a hospital source, Visvanathan and the infection control team would have to show that the bacteria isolated from the four patients were from a related strain. But traditional testing only told them what kind of bug it was and what antibiotics it was resistant to.

It wasn't enough. So the samples were sent to Professor Ben Howden, director of Melbourne's Microbiological Diagnostic Unit Public Health Laboratory. There, Howden and his team could do something - back then, CRE didn't have the genome to become CRE.

"We're in trouble."

Dr Howden, director of Melbourne's Microbiological Diagnostic Unit Public Health Laboratory, sent the samples to his team to study.

By looking at these microscopic differences between bacteria, they could follow the evolution of a particular branch of the bacterium - the passage of an outbreak - right back to its origins. And they can do it within days.

They can even prevent an outbreak from occurring.

"We can find out if a new patient is carrying the carbapenem-resistant bacteria and put in infection control measures to stop the bug spreading from that patient," Howden says.

In a recent case, scientists at the Microbiological Diagnostic Unit traced a local case of infection back to a stone fruit imported from California by comparing it with genomes held by the US Food and Drug Administration.

"In the future," Howden says, "scientists will be able to do this in real time, finding the strain as soon as it is found and preventing infections in other people by removing the source."

Dr Teresa Anderson, chief executive of the Sydney Local Health District that oversees Royal Prince Alfred Hospital, says whole genome sequencing has the potential to totally change the tracking of infections in a hospital.

"This is particularly important with the increased worldwide emergence of superbugs and other drug-resistant infections," Anderson says.

In Snow's time, finding the well was the only hope for halting a cholera outbreak because there was no cure for the disease.
tracking superbugs

In 2014 Dr Rebecca Davis used genome mapping to trace the source of a potentially deadly outbreak to a sink in the RPA Hospital's neonatal ward in Sydney. Inset, right to left: Professors Sebastiania van Hal, Kumar Visvanathan and Bell Howden. Photos: Kate Geraghty, Paul Jeffers, Pat Scia, Anabel Stafford

And with resistance to antibiotics growing – in May it was reported that a woman in the US was infected with a bug that unknown antibiotic could kill – today's doctors may soon find themselves in a similar position.

Monitoring the outbreak of a disease and preventing transmission may become the only way to save patients.

Antibiotic resistance - the Australian experience

■ In 2014, 46 per cent of the population were prescribed antimicrobials (overwhelmingly antibiotics but also include other substances that kill microorganisms).
■ More than 50 per cent of people with colds or upper respiratory tract infections were inappropriately prescribed antibiotics.
■ On any one day in 2014, about 38 per cent of hospital patients were being treated with antimicrobials.

Twelve years ago, he spent six months at Oxford University learning how to map superbug genomes.

When Davis came to him with her green mucousy Petit dishes, van Hal suggested she sequence the entire genome of the bacteria found in her samples as well as samples swabbed from the sinks and taps in the neonatal intensive care unit (pseudomonas is a hardy bug that thrives in moist environments).

Davis, together with a registrar and infection control nurse, took samples from across the unit with a growing sense of urgency.

"There were babies there that were very tiny and very premature," Davis remembers.

"I had some conjunctivitis, so I wanted to make sure she wasn't going to get infected. It tears at the heartstrings a bit.

The samples were returned to van Hal. To put a complicated process very simply: van Hal extracted DNA from each sample, broke the circular DNA chromosome into multiple pieces so they could be read by the genome-sequencing machine, and fed them into the machine.

When the results came back, he looked at where each sample differed from the reference genome.

It was an outbreak. Eleven of the samples had “no significant differences whatsoever,” van Hal says.

"In a core genome level, they were identical sequences.

A further sample taken from one sink differed from the cluster by a mere nine mutations. The two strains were related.

Much like Snow had done, van Hal and Davis plotted the bacteria sample on a map of the intensive care unit and found the identical samples came from beds clustered around the sink. They had their culprit.

The sink was knocked out and all the ward’s grouted-tile splashbacks were replaced with smooth ones. None of the babies got sick and no further babies were infected.

In Melbourne, too, the bacteria samples in Vincenat’s had sent to Howden were closest to another. But the patient’s hospital stays hadn’t overlapped, there had been no direct path of patient transmission. Where was the bug hiding?

After the previous outbreaks, St Vincent’s had cleaned and sampled carpets, sinks and beds and found nothing. "It was our registrar who thought of the toilet water," Visvanathan says.

Samples taken from the toilet bowl contained the same antibiotic-resistant genes that had been found in bacteria collected from the patients. Indeed, the same resistance gene was found in several different bacteria in the toilet, suggesting it was being swamped between bugs.

Still the mystery was not solved.

After the earlier outbreak, the hospital had sealed the roof around the water tank, the water supply and other parts of the sewage system and found no sign of the resistance gene. It wasn’t until they broke the toilet apron that they found the bug hiding in a biofilm within the porcelain pan of the toilet bowl near the rim. It may have been brought there by a colonised patient, then colonised others when the toilet had been flushed without the seat cover down. "There’s some data that suggests that a splash from a toilet flush can go 20 metres," Visvanathan says. "People have been in contact with other fecal organisms through using a toothbrush near a toilet flush in the family home."

Clearly, it is wise to put the cover down before flushing.

"It’s Vincenat’s replaced the toilets and since then, there have been no further sightings of this CRE superbug. But Visvanathan is too much of a realist to think he’s seen the last of it. "Patients will come from overseas, there will be potential for new sources of infection," he says. This time, though, the superbug detectives will be ready.

How it spread

Royal Prince Alfred neonatal units

Infection traced to this sink

Feb 25-Mar 17 2014

Mar 18-Apr 29 2014

SCN Special Care Nursery

NICU Neonatal Intensive Care Unit

HDU High Dependency Unit

SCN Special Care Nursery

SCN Special Care Nursery

SCN Special Care Nursery