Before the widespread use of antibiotics in the 1940s, it was much more common for women to die from post-childbirth infections, and diseases such as tuberculosis were rife. In addition, farmers often faced losing vast numbers of crops and animals to infectious diseases, leading to serious food shortages, even famine. The discovery and introduction of antibiotics gave us the ability to prevent these tragedies. However, as microorganisms become resistant to antimicrobial treatments, including antibiotics, there is a very real possibility that the drugs we have come to rely upon may become obsolete.

Since 1928, when Sir Alexander Fleming accidentally discovered penicillin growing on a petri-dish of bacteria, antibiotics have saved the lives of millions of people and animals. Their discovery is seen as one of the most important scientific achievements of the 20th century. But overuse and misuse of antibiotics has contributed to the emergence of resistance. Sir Alexander Fleming himself, on collecting a Nobel Prize for his discovery, predicted the dawn of this battle, saying, “It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them.”

England’s Chief Medical Officer Professor Dame Sally Davies warned in 2013 of the “catastrophic effect” of antimicrobial resistance and urged immediate action from global leaders before deaths from routine surgery once again become a common occurrence. The World Economic Forum has suggested that antimicrobial resistance (AMR) be added to the global risk register, and the World Health Organization has highlighted the serious implications for global public health in its AMR Global Report on Surveillance. Antimicrobial resistance is one of the Innovative Medicine Initiative’s priorities and a Joint Programming Initiative on antimicrobial resistance was set up in 2011 to streamline European research efforts in AMR.

The UK Research Councils support research, capability and training to pursue a range of strategies to tackle this global problem. Years of research mean that we are now in a better position than ever to understand microbes such as bacteria, viruses and fungi, how they interact with their hosts, and to identify possible routes for alternative diagnostics and treatments. New technologies which could help prevent the spread of bacteria and infections, including smart surfaces and medical dressings, are also being developed. This timeline and series of case studies showcase some of these advances, supported by the Biotechnology and Biological Sciences Research Council (BBSRC), Engineering and Physical Sciences Research Council (EPSRC) and Medical Research Council (MRC). This work lays the groundwork for the cross-Council antimicrobial resistance initiative that was launched in July 2014. This will see all seven Councils working together to tackle AMR. A joined-up, multi-disciplinary approach is essential and so the initiative will coordinate the work of medical researchers, biologists, engineers, vets, economists, social scientists, mathematicians and designers. It is only through tackling the problem at every level and in every environment that we will be able to take the next steps towards a solution.

References
1. Chief Medical Officer annual report: volume 2
2. Antimicrobial resistance: global report on surveillance 2014
1. Understanding resistant bacteria in context of the host

**2007:**
University of Newcastle spin-out company e-Therapeutics Ltd identifies three drugs that are effective against antibiotic-resistant superbugs, including MRSA, using Grid computing and e-science techniques developed during research funded by EPSRC and the Department of Trade and Industry. The company searched through tens of millions of compounds for any that showed action against superbugs in a fraction of the time it would take using conventional drug discovery methods.

**2008:**
The first case of a bacterial infection with resistance caused by NDM-1, a powerful enzyme that gives bacteria resistance to most antibiotics, is discovered. MRC-funded researcher Professor Tim Walsh was part of the group that identified the enzyme, which is commonly produced by *Escherichia coli* and *Klebsiella pneumonia*, but can also spread between different strains of bacteria.

**2010:**
The EU uses the results of research by BBSRC David Phillips Fellow Dr Mark Webber in two reports on the use of common biocides. During his fellowship, Dr Webber characterised the genetic changes that grant Salmonella resistance to the biocide triclosan and others. There were around 9,000 cases of *Salmonella* food poisoning in the UK in 2010, although three quarters of cases may go unreported.

**2011:**
Scientists at the MRC Research Complex at Harwell determine the structure of NDM-1 using the STFC's Diamond Light Source crystallography facility. Understanding the structure will help researchers develop drugs that could inactivate the enzyme or that are not susceptible to it.

**2012:**
Bacteria transmit resistance genes to other bacterial strains by way of plasmids — small loops of DNA. Carrying these plasmids is commonly thought to reduce a bacterium's fitness, so removal of antibiotic pressure should reduce the number of resistant bacteria. However, in a BBSRC and MRC-funded study, Professor Laura Piddock and Dr Mark Webber at the University of Birmingham discover that the plasmid pCT persists in the absence of antibiotics because it has evolved to have little impact on the host. They conclude that resistance genes will persist even with careful rationing of antibiotics.

Changes from its normal form to a slow-growing antibiotic-resistant form as part of its natural lifestyle to ensure its survival.

Bacteria transmit resistance genes to 'community-acquired' MRSA strains, which can both resist antibiotics by making changes to the bacterial cell wall and maintain high levels of toxin production.

Dr Andrew Edward at the MRC Centre for Molecular Bacteriology and Infection (CMBI), Imperial College London, demonstrates that *Staphylococcus aureus* changes its normal form to a slow-growing antibiotic-resistant form as part of its natural lifestyle to ensure its survival.

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In an MRC-funded study, Professor Gad Frankel at Imperial College London uses a mouse infected with bacteria genetically modified to produce light to show how an infection moves around the body in real time\textsuperscript{12,13}. Regular CT scans of the mouse could show how different vaccines and antibiotics change the way bacteria take over parts of the body.

\textbf{2013:}

A research team from the Universities of Nottingham, Birmingham and Newcastle, funded by EPSRC and BBSRC, discover that artificial materials based on simple synthetic polymers can disrupt the way in which bacteria communicate with each other. The findings\textsuperscript{14} open up the possibility to influence microbial behaviour by controlling their ability to form productive communities, which could be exploited to prevent the release of toxins during the spread of infection.

MRC-funded Professor Guy Frankel at Imperial College shows how enteropathogenic \textit{Escherichia coli} (EPEC), a pathogenic strain of \textit{E.coli} which is a common cause of infant diarrhoea in the developing world, interferes with the host cell's normal antimicrobial response\textsuperscript{16}. EPEC injects a toxin into host cells during infection. This blocks the cell's ability to send messages to the immune cells, preventing a response and subsequent death of the infected cells, allowing the bacteria to survive and spread.

A common mutation in \textit{Salmonella} grants the bacteria resistance to an important class of antibiotics, the fluoroquinolones, and also increases its resistance to many other antibiotics and the biocide triclosan, according to research by Professor Laura Piddock and Dr Mark Webber at the University of Birmingham\textsuperscript{15} and supported by BBSRC and the MRC.

\textbf{2014:}

BBSRC-funded researchers at the MRC Centre for Molecular and Biomolecular Informatics (CMBI), identify the pathway behind the 'stringent response', the mechanism \textit{E.coli} use to survive when under stress, such as when deprived of nutrients or in the presence of antibiotics\textsuperscript{18}. When under stress, bacteria produce guanosine tetraphosphate (ppGpp), which instructs the bacteria to stop growing and to use minimal resources. The CMBI researchers show that a protein called NtrC plays a central role in the process by controlling the level of ppGpp.

BBSRC-funded researchers at the London Centre for Nanotechnology, University College London, show how drug-binding mechanically weakens bacterial cells and leads to their death, whilst unravelling how the antibiotic vancomycin works. Vancomycin is one of the few effective treatments for MRSA. The study was funded by EPSRC, BBSRC and the Royal Society.

Researchers at the MRC Centre for Molecular Bacteriology and Infection (CMBI) study ‘persister’ cells in \textit{Salmonella}, visualising them for the first time using a fluorescent protein produced by the bacteria. Persister cells are a non-replicating form of the bacteria and ‘lie low’ to evade antibiotic action\textsuperscript{19}. See ‘Antibiotic-evading bacteria’.

Professor Laura Piddock at the University of Birmingham sequences the plasmid pCT, which confers antibiotic resistance to bacteria carrying it. She concludes that the plasmid’s success lies in its stability in a range of hosts, the lack of a fitness cost to the host bacteria — meaning that carrying the plasmid has no detrimental effect — and efficient transfer between bacterial hosts\textsuperscript{20}. 

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2. Accelerating therapeutic and diagnostics development

1985: Researchers at the John Innes Centre (JIC), which receives strategic funding from BBSRC, led by Dr David Hopwood, are the first to produce a ‘hybrid’ antibiotic using genetic engineering, alongside colleagues from Japan and the USA. The researchers transferred genes associated with antibiotic production between strains of Streptomyces bacteria, enabling the bacteria to produce an entirely new antimicrobial compound.

2002: The Streptomyces genome, sequenced by BBSRC- and Wellcome Trust-funded researchers, is published in the journal Nature. Researchers subsequently discover a large number of previously-unknown gene clusters in the Streptomyces genome that produce ‘specialised metabolites’, potentially including previously-unknown antimicrobials.

2005: Dr Curtis Dobson founds spin-out company A2i2 to develop an anti-infective coating for contact lenses. The anti-infective arose from Dr Dobson’s BBSRC-funded research at the University of Manchester into a protein that could help protect against the viral infections associated with Alzheimer’s disease.

2008: Procarta Biosystems is co-founded by Professor Mervyn Bibb and Dr Michael McArthur at JIC to develop and commercialise a new class of antibiotics, DNA-based transcription factor decoys (TFDs), to combat infections caused by drug-resistant bacteria. TFDs work by blocking the action of ‘transcription factor’ proteins that control the expression of large numbers of genes within the bacterial cells.

1998: Professor Jeff Errington founds spin-out company Prolysis to develop and commercialise screening techniques to find novel antibiotics to tackle drug-resistant bacterial infections. The company is based on fundamental bacterial cell biology research supported by BBSRC at the University of Oxford. In 2009 Prolysis is acquired by Australian drug development firm Biota.

2003: JIC spin-out company Novacta Biosystems is founded to discover and develop potential treatments for infectious diseases, particularly those caused by antimicrobial-resistant bacteria. Their lead product, based on a long history of Streptomyces research at JIC, is designed to treat infections caused by the bacterium Clostridium difficile, which was involved in 2,704 deaths in the UK in 2010. See ‘New antibiotics from bacterial bioscience’.

2007: Professor Simon Foster and Dr Jorge Garcia-Lara at the University of Sheffield create spin-out company Absynth Biologics to develop vaccines against S. aureus infection, including MRSA. Absynth arose from Professor Foster’s BBSRC and MRC-funded research into S. aureus, and in particular the genes essential for its survival.

2009: Professor Jeremy Lakey co-founds spin-out company Di-Bio, based on BBSRC-funded research at Newcastle University, to develop miniature wireless sensors that can be used to test for a diverse range of infectious diseases in humans. The devices are currently being evaluated by commercial partners in the healthcare industry to test for infectious diseases including flu, HIV and gum disease. Further funding has been provided by the EPSRC and Technology Strategy Board (now Innovate UK) to develop the technology.
MRC and BBSRC-funded researcher Professor Adam Cunningham at the University of Birmingham begins development of a vaccine against Salmonella. The vaccine development has been licensed to Novartis Vaccines Institute for Global Health.

**2010:**
A team of researchers at the MRC Centre for Molecular Bacteriology and Infection (CMBI), with funding from BBSRC, reveal the structure of a protein called Gp2, produced by the ‘bacteriophage’ virus T7, which disables E.coli cells. Bacteriophage viruses infect and kill many bacterial species, including those that cause human and animal diseases. See ‘Bacteria-eating viruses’.

Researchers at the MRC Laboratories in The Gambia, in collaboration with scientists in the US, find that infection with H.pylori—the bacterium responsible for gastritis and gastric ulcers—may protect the host against other pathogens, such as tuberculosis.

Funded by the MRC, Dr Andrew Gorringe at the Health Protection Agency develops a vaccine against bacterial meningitis. The vaccine is currently being developed with funding from the Biomedical Catalyst by ImmBio, a vaccine development company based at the Babraham Research Campus.

BBSRC-funded researchers begin to develop a new type of vaccine to protect chickens against coccidiosis, based on a single protein that plays a vital role in the early stages of infection. The coccidiosis parasite, which is widely resistant to antimicrobials, is the most important parasite of poultry globally.

Predatory bacteria with the potential to be used as ‘living antibiotics’ are safe when ingested by chickens, according to BBSRC-funded researchers from the University of Nottingham. When given to live, Salmonella-infected chickens, Bdellovibrio bacteria reduced the number of Salmonella cells by 90 per cent while leaving the birds unharmed.

**2011:**
A Sheffield University research team produce a gel containing molecules that bind to bacteria and activate a fluorescent dye. The gel will be used in wound dressings to indicate when an infection has developed and will help clinicians to make rapid, informed decisions about wound management as well as reduce the overuse of antibiotics. The research team was funded by the EPSRC and Ministry of Defence. See ‘Wound dressing provides glowing evidence of infection’.

Funded by the MRC, a vaccine against bacterial meningitis developed by Dr Andrew Gorringe at the Health Protection Agency is currently being developed with funding from the Biomedical Catalyst by ImmBio, a vaccine development company based at the Babraham Research Campus.

BBSRC-funded researchers from the University of Nottingham are using synthetic biology approaches to alter antibiotic production in marine bacteria to produce new hybrid antibiotics. The BBSRC- and EPSRC-funded researchers, from the University of Birmingham and working with others in the UK and Japan, found that the marine bacteria could combine two antibiotic molecules to produce a much more effective antibiotic, which works against MRSA.

**2012:**
BBSRC-funded researchers at the MRC Centre for Molecular Bacteriology and Infection (CMBI) demonstrate how Gp2 interacts with the bacteria’s RNA polymerase—an enzyme that enables the instructions in the bacteria’s genes to be read and turned into proteins—to stop it from functioning. The scientists now plan to identify small
molecules that mimic the structure and function of Gp2 and use these as the basis for new drugs to combat bacterial infections.

Novacta Biosystem’s lead product, NVB302, which is being developed to treat C. difficile infections, completes phase I clinical trials, showing it is safe when administered to healthy people42.

JIC and University of Oxford researchers begin a BBSRC-funded project to investigate whether they can use synthetic biology to remove the toxic side effects of tunicamycin; an antibiotic produced by the soil bacterium Streptomyces43. See ‘New antibiotics from bacterial bioscience’.

MRC-funded researchers at the Wellcome Trust Sanger Institute demonstrate that treatment of C. difficile-infected mice with faeces from healthy mice rapidly restores a diverse, healthy microbiota and subsequently cures the disease and removes its contagiousness44.

MRC-funded researcher Professor Robert Akid at the University of Manchester patents an antimicrobial coating for cementless prostheses, such as hip and knee replacements, to prevent infection45. The controlled release ensures the antimicrobial is released only at the appropriate time (during and after surgery).

The world’s largest antibody search engine, CiteAb47, is founded by EPSRC-funded Dr Andrew Chalmers at the University of Bath. The service, which allows researchers to find antibodies for use in their research, is the largest antibody search engine in a $2Bn antibody industry, and ranked number one by Google. Ranking antibodies by academic citations means CiteAb provides an independent, verifiable guide as to whether an antibody is likely to work in the laboratory, saving both time and money.

Imperial College London scientists, with support from BBSRC, identify how a protein, called P7, produced by a certain bacteriophage virus disables an essential bacterial enzyme called RNA polymerase48. The viral protein uses a previously-unknown method to disable the RNA polymerase, which is involved in bacterial gene expression, by preventing it from identifying target genes.

Absynth Biologics receives more than £2M through the Technology Strategy Board- and MRC-funded Biomedical Catalyst to take forward to a pre-clinical stage its vaccine against MRSA.

A team led by MRC-funded researcher Dr Martha Clokie at the University of Leicester isolates 40 different bacteriophages — viruses that ‘eat’ bacteria — against hospital superbug C. difficile. US pharmaceutical company AmpliPhi Biosciences Corporation are funding the further development of these phages. See ‘Bacteria-eating viruses’.

2013:

An EPSRC Interdisciplinary Research Centre is established at University College London to create a new generation of early-warning sensing systems to diagnose, track and prevent the spread of infections, including influenza, antimicrobial resistance and HIV, using mobile communication, nanotechnology, genomics and big data analysis to actively manage outbreaks and prevent infectious diseases.

2014:
Antimicrobial resistance

3. Understanding the real world interactions

2007:
A peptide molecule found in American Bullfrogs is being developed to treat wounds infected with MRSA\(^{49}\). Researchers led by Dr Peter Coote at the University of St Andrews find that the bullfrog peptide ranalexin can inhibit MRSA growth when combined with another antimicrobial, lysostaphin. The researchers patent the discovery and aim to develop effective treatments for MRSA-infected wounds.

2010:
Bacteria carried on the surface of leafcutter ants produce a range of antimicrobial compounds, according to a study by BBSRC and MRC-funded researchers from the University of East Anglia, JIC, and The Genome Analysis Centre (TGAC), which receives strategic funding from BBSRC\(^{50}\). The antimicrobials help the ants cultivate a fungus that provides them with food, protecting their nest against infection and controlling competing strains of fungi\(^{51}\).

2011:
Materials scientists at the University of Birmingham, funded by EPSRC, devise a way of making stainless steel surfaces resistant to bacteria by introducing silver or copper into the surface rather than applying it as a coating. The technique could prevent the spread of superbug infections on stainless steel surfaces in hospitals as well as medical equipment such as instruments and implants, the food industry, and domestic kitchens.

2012:
Researchers at the University of Nottingham, funded by BBSRC and the Wellcome Trust, discover a new class of material that resists colonisation by bacteria\(^{52}\). The materials, known as synthetic acrylate polymers, have been licensed to UK company Camstent Ltd, which is now working with the academics to develop coated urinary catheters.

In an MRC-funded study, Professor Timothy Walsh sequences \(K.\) pneumonia containing NDM-1 from three different countries and shows that there is great diversity between the strains. He finds that one of the most common strains, ST14, is associated with the most invasive form of the disease\(^{53}\).

Researchers from Bristol’s Frenchay hospital and Bedfordshire based AmpliPhi Biosciences, uses dye-filled nanocapsules that burst open in the presence of disease-causing bacteria. Using a UV light, clinicians can quickly check whether there is any infection by seeing if the dressing lights up.

Researchers at the University of Sheffield discover that combinations of bacteria, commonly found in water pipes, can form a ‘biofilm’ that enables other, potentially harmful, bacteria to thrive\(^{55}\). The EPSRC-funded study isolated four types of bacteria and found that when any of them grew alongside bacteria called Methylobacterium, they formed a biofilm within 72 hours. The findings mean it should be possible to control the creation of biofilms in water supplies by targeting particular bacteria.

Researchers at the University of Warwick adopt a DNA-based approach to understand the community of microbes that live in a chicken’s gut\(^{56}\). Chickens and other farm animals can act as a reservoir of human pathogens and of microbes that carry antimicrobial resistance genes. The researchers, with support from BBSRC, used high-throughput sequencing to rapidly sequence the DNA of microbes in the chicken gut to identify which bacterial species were present.

Some strains of MRSA that cause disease in humans originate in livestock, according to research led by Professor Ross Fitzgerald at the Roslin Institute, which receives strategic funding from BBSRC\(^{57}\). The findings suggest that livestock can act as a potential reservoir of new human epidemic strains of the bacteria. See ‘Making the leap’.

2013:
EPSRC-funded researchers develop a method for quickly detecting infections in children with serious burns\(^{54}\). Children are at higher risk than adults from the effects of subsequent infection. The dressing, developed at the University of Bath with researchers from Bristol’s Frenchay hospital and Bedfordshire based AmpliPhi Biosciences, uses dye-filled nanocapsules that burst open in the presence of disease-causing bacteria. Using a UV light, clinicians can quickly check whether there is any infection by seeing if the dressing lights up.

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3. Understanding the real world interactions
MRC-funded Professor Sharon Peacock at the University of Cambridge uses whole-genome sequencing to analyse an outbreak of MRSA. Whole genome sequencing of bacterial samples could lead to fewer antibiotics being used as a more specific diagnosis would allow the targeted use of specific antibiotics to treat it. This sequencing also means that researchers can track the spread of infection, helping with infection control and prevention. See 'Whole-genome sequencing'.

The Chief Medical Officer publishes the second volume of her annual report, focusing on infection and antimicrobial resistance. Professor Peacock writes a section on the use of whole genome sequencing to track the transmission of infections to improve surveillance and control.

An MRC-funded team at the University of Oxford, led by Dr David Eyre and Dr Sarah Walker, use whole genome sequencing to show that many cases of C. difficile infection are caused by bacteria transmitted from people who show no sign of infection, or from environmental sources such as water, animals, or food, rather than from symptomatic patients. See 'Whole-genome sequencing'.
Antimicrobial resistance

4. Behaviour within and beyond the health care setting

2005:
With up to 50 per cent of antibiotic prescribing inappropriate\(^\text{61}\), Professor Peter Davey at the University of Dundee looks at interventions to improve prescribing, such as education, restriction of drugs, guideline implementation and expert approval in an MRC-funded study\(^\text{62}\).

2007:
In an MRC-funded study, Professor David Mant at the University of Oxford shows that antibiotic-resistant bacteria are present in children prescribed the common antibiotic amoxicillin, which although transitory in the children is sufficient to sustain a high-level of antibiotic resistance in the population\(^\text{63}\). The findings provide clinicians with guidance on which antibiotics should be used if a patient requires a second course of antibiotics within 12 weeks of the first.

It was previously thought that using less active antibiotics was the best first defence in order to reserve more active antibiotics for more resilient bacteria. In an MRC-funded study, Professor Sebastian Amyes at the University of Edinburgh concludes that using less active antibiotics first — which are generally more likely to cause resistance to develop — results in resistance to the whole class of antibiotics, rendering even the more active types unusable\(^\text{64}\).

2011:
Pigs on farms with access to the outdoors and a clean, enriched environment are less likely to suffer Post Weaning Multi-systemic Wasting Syndrome (PWMS), which is associated with a certain virus, than those on other farms, according to BBSRC-funded researchers from the Royal Veterinary College\(^\text{65}\). The researchers are now working with the British Pig Executive to develop monitoring systems to help farmers identify animals at risk of PWMS.

The Imperial Antibiotic Prescribing Policy (IAPP) smartphone app is developed by Imperial College Healthcare NHS Trust’s antibiotic review group and the UKCRC Centre for Infection Prevention and Management\(^\text{66}\). The app helps healthcare professionals choose the most appropriate course of treatment to ensure antimicrobials are prescribed appropriately. The app is used over 4,800 times in the first month. 85 per cent of users responding to a post-implementation survey considered that the IAPP added to their knowledge base regarding antimicrobial prescribing and 96 per cent found that it influenced their prescribing practice.

Professor Ian Chopra, director of the Antimicrobial Research Centre at the University of Leeds advocates that new business models for antibiotic development are required. He suggests new methods of screening for compounds, delinking product sales from the companies’ return on investment and financing incentives for drug development\(^\text{67}\).

2012:
EPSRC-funded researchers at the University of Leeds discover that superbugs, such as MRSA and C. difficile, not only spread through contact, but they also float on air currents and contaminate surfaces far from infected patients’ beds\(^\text{68}\). Coughing, sneezing or shaking bedclothes can send superbugs into the air, allowing them to contaminate recently cleaned surfaces. This may explain why, despite strict cleaning regimes and hygiene controls, some hospitals still struggle to prevent bacteria moving from patient to patient.

2013:
The Joint Programming Initiative on Antimicrobial Resistance publishes its Strategic Research Agenda\(^\text{69}\). MRC-funded Professors Tim Walsh and Paul Williams were involved in its development.

2014:
An international team of researchers, including MRC-funded researcher Dr Tim Felton, recommend individualised antibiotic dosing for critically-ill patients\(^\text{70}\). These patients often exhibit different responses to antibiotic treatment; dosing that does not take this into consideration can lead to sub-optimal treatment and increase antibiotic resistance.
EPSRC-funded researchers at Newcastle University and the Indian Institute of Technology in Delhi reveal that the spread of antibiotic-resistance at sacred sites along the Ganges is linked to annual human pilgrimages. When thousands of visitors travelled to the sacred sites, levels of resistance genes in bacterial populations were about 60 times greater than other times of the year. The study is helping to understand how resistance gene blaNDM-1 spreads through specific human activity.

The World Health Organization publishes its report *Antimicrobial resistance: global report on surveillance 2014*\(^2\). Professor Tim Walsh was part of the review group.
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Front cover
Petri dishes with cultures of bacteria grown on agar jelly. Credit: M J Richardson. CC BY 3.0 (<http://creativecommons.org/licenses/by/3.0/>)

Understanding resistant bacteria in context of the host
Image 1: Salmonella invading cultured human cells. Credit: NIAID. CC BY 2.0 (<https://creativecommons.org/licenses/by/2.0/>)
Image 2: NDM-1 was first identified in Klebsiella pneumonia bacteria. Public domain.
Image 3: Pills. Credit: Thinkstock
Image 5: A scanning electron micrograph of MRSA and a dead human white blood cell. Credit: NIAID. CC BY 2.0 (<http://creativecommons.org/licenses/by/2.0/deed.en>)

Accelerating therapeutic and diagnostics development
Image 2: Contact lenses. Credit: Thinkstock
Image 4: Vaccine. Credit: iStock
Image 5: A chicken. Credit: Liz West CC BY 2.0 (<https://creativecommons.org/licenses/by/2.0/>)
Image 6: The connectedness of today's society. Credit: Thinkstock.

Understanding the real world interactions
Image 1: American Bullfrog Rana catesbeiana. Credit: Fr0002. CC BY-SA 2.5 (<http://creativecommons.org/licenses/by-sa/2.5/deed.en>)
Image 2: Surgical instruments. Credit: Thinkstock
Image 3: Cows on Eifee Hill. Credit: John Comloquoy CC BY-SA 2.0 (<http://creativecommons.org/licenses/by-sa/2.0/deed.en>)

Behaviour within and beyond the healthcare setting
Image 1: Pigs. Credit: Hadyn Blackey. CC BY-SA 2.0 (<http://creativecommons.org/licenses/by-sa/2.0/deed.en>)
Image 2: The Imperial Antibiotic Prescribing Policy smartphone app. Credit: Imperial College London.
Researchers at the MRC Centre for Molecular Bacteriology and Infection (CMBI) at Imperial College London are studying dormant ‘persister’ cells produced by *Salmonella* bacteria. These cells are formed by bacteria when they are exposed to stresses such as antibiotics. By studying persister cells, the researchers hope to understand the link between these dormant cells and antibiotic resistance, as well as develop treatments that target persister cells directly.

Most antibiotics act only on active bacteria. But nearly all bacterial pathogens produce a small sub-population of dormant cells that can evade antibiotics. These cells — called persisters — tolerate antibiotics and other environmental stresses, such as nutrient depletion or host cell acidity. Once the stress has been removed, for example, by the completion of a course of antibiotics, the dormant cells are able to revert back to the active, disease-causing form. These cells are thought to be the cause of many persistent or recurrent infections.

This antibiotic ‘tolerance’ is temporary and reversible, unlike resistance, which is caused when the bacteria acquire stable genetic traits. However, it is thought that prolonged and repeated treatment of persistent infections may lead to genetic drug resistance, and so it is important that the mechanisms behind this evasion are identified to help develop appropriate strategies to treat these persisters. Despite their discovery by Joseph Biggar more than 70 years ago, persister cells are still poorly understood.

**Persisters and resistance**

Up until now, persister cells have only been studied in test tubes. However, in 2014, a team led by Professor David Holden at the CMBI used a technique they had developed to visualise persisters for the first time at the single-cell level as they are consumed by white blood cells.

Using a fluorescent protein, Professor Holden and colleagues showed that the bacteria produced persister cells when consumed by white blood cells at a much greater rate than when grown in laboratory media. The researchers demonstrated that the bacteria formed persisters immediately after being attacked and consumed by the host’s white blood cells in response to the levels of acidity and lack of nutrients inside the cells.

These stresses also cause some bacterial cells to start replicating rather than form persister cells, and this dual response allows bacteria to ‘hedge their bets’ to gives them a selective advantage.

Professor Holden is now hoping to use these approaches to study how persister cells might lead to resistance. "It is widely thought that the multiple courses of antibiotics made necessary by persistent infections leads to resistance. However, this has not been tested experimentally. Since the genetic basis of persister formation has been worked out in recent years, we
Antimicrobial resistance

can make bacterial mutants with enhanced or reduced persister frequency and use these in conjunction with our techniques to determine if and how persisters contribute to emergence of resistance during infection,” says Professor Holden.

Surviving adverse conditions

Part of Professor Michael Barer’s research at the University of Leicester looks at the transmission and persistence of Mycobacterium tuberculosis. In 2008 he, together with colleagues at the MRC unit, The Gambia, demonstrated that the tuberculosis bacteria in samples of sputum — the mucus and other matter brought up from the lungs by coughing, and which helps transmit the disease between people — were likely to be in their persistent state. These samples contained a fat called triglyceride, produced by the bacteria when they form persister cells. This suggests that formation of the persisters might help the bacteria survive the adverse conditions that M. tuberculosis encounters when it is transmitted between people.

Targeting persisters

Pyrazinamide is the only drug that specifically targets persister cells. In 1970 researchers at the MRC Tuberculosis and Chest Diseases Unit demonstrated for the first time that the inclusion of pyrazinamide in an antibiotic regimen for the treatment of Mycobacterium tuberculosis substantially reduced the relapse rate. Certain antibiotics however do have limited action against the persisters and REMoxTB, a clinical trial involving several MRC researchers, is currently underway to determine whether the inclusion of the antibiotic moxifloxacin can shorten the duration of treatment.

Enhancing the host’s immune response is another method of targeting persister cells. The bacillus Calmette-Guerin (BCG) vaccine has limited success as a preventative measure. However, researchers at the MRC’s National Institute of Medical Research (NIMR) showed that the use of the M. tuberculosis Hsp60 DNA vaccine, in combination with antibacterial treatment, was successful in treating heavily infected mice. The DNA vaccinations can switch the immune response from one that is relatively inefficient to one that kills these persistent bacteria.

“Another possibility is to work out what triggers the persister cells to start growing again — give someone with a persistent infection a drug that induces this and then attack the bacteria as they come out of hiding,” says Professor Holden.

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Natural products from certain bacteria are forming the basis of promising new antimicrobials being developed to tackle drug-resistant infections. Researchers led by Professor Mervyn Bibb at the John Innes Centre, which receives strategic funding from BBSRC, are studying a group of bacteria called actinomycetes, that produce unique ‘specialised metabolites’. These compounds are not vital to the bacteria’s immediate survival, but can give them a long-term advantage in their natural environment. Many of these specialised metabolites inhibit the growth of rival microbes, and so could potentially be used to develop new human or animal antimicrobials.

Professor Bibb and his group have been studying a specialised metabolite, which acts as a potent antimicrobial compound, called NAI-107 (also known as microbisporicin). It is from a class of antimicrobials called ‘lantibiotics’ that are not currently used clinically, and is produced by the actinomycete Microbispora. In 2010, the researchers cloned the gene cluster that makes NAI-107 and developed a comprehensive understanding of how the bacteria synthesise the compound and control the amount that is produced.

Around the same time, Italian company NAICONS began developing NAI-107 commercially. An EU-funded project then brought the company, the JIC researchers, and scientists from Germany, Denmark, Italy and Switzerland together to develop it further. The JIC researchers are helping to increase the amount of the lantibiotic produced by the bacteria. “A big issue for pharma companies, when they proceed towards clinical trials, is getting enough of the natural product, because often these compounds are made in very small amounts,” says Professor Bibb. “By understanding how the gene cluster is regulated we’ve been able to manipulate the natural producer and make significantly more.”

NAI-107 is now on the verge of entering phase I clinical trials to treat MRSA. The market for MRSA therapeutics was estimated to be worth around $2.78bn in 2012, growing to $3.48bn in 2019.

Fundamental bacterial biology

The researchers at JIC are interested in the fundamental biology of actinomycetes – how natural products such as NAI-107 are made and regulated by the bacteria. However, Professor Bibb is also keen to ensure his work is of use to industry, and collaborates with researchers from pharmaceutical companies. “We develop a lot of technology, and fundamental understanding which we feed in to pharma and to small biotech companies. Additionally, two start-up companies have resulted from work carried out in our group,” he explains.

One of those companies, Novacta Biosystems, was established in 2003 based on intellectual property developed by JC researchers studying the lantibiotic cinnamycin, from Streptomyces cinnamoneus bacteria. The group, in collaboration with Novacta,
developed a method using synthetic biology to construct ‘arti-
ficial’ genes to generate variants of cinnamycin, based on their
understanding of how the bacteria produce and regulate the
compound. Novacta adopted this technology to develop and screen around 170 variants of cinnamycin for their antimicrobial properties.

The same technology was later used by Novacta during their in-house programme to develop an antibiotic based on the lantibiotic actagardine, which can be used to treat Clostridium difficile infections. A semi-synthetic variant of actagardine called NVB302 has successfully passed phase I clinical trials and is now waiting to enter phase II.

A potent antibiotic
Professor Bibb is also using synthetic biology to develop improved variants of the antibiotic tunicamycin, produced by the actinomycete Streptomyces chartreusis. Working with Professor Ben Davis’ group at Oxford, Professor Bibb and colleagues are investigating whether it is possible to use synthetic biology to modify tunicamycin to make it more suitable for use as a human antimicrobial.

“It’s a very potent antibiotic,” says Professor Bibb. “The attractive thing from an antimicrobial perspective is that it has a clinically unexploited target. It targets the production of lipid I, which is used in the production of the bacterial cell wall. No one else has used that as a target, so there is no resistance out there in the clinic at the moment.”

“The bad thing is that it also inhibits [a vital biological process called] protein glycosylation in people, so it is toxic.” The aim of the latest project is to use synthetic biology to modify tunicamycin so that it loses its toxic effects in people while retaining its antimicrobial properties.

BBSRC and its predecessors have funded research into the biology of the actinomycetes, and in particular a species called Streptomyces coelicolor, since the 1960s. Much of this research was conducted at JIC, and in 2002 resulted in the first sequence of an actinomycete genome; that of S. coelicolor.

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Wound dressing provides glowing evidence of infection

Fundamental research in polymer physics, jointly supported by the Engineering and Physical Sciences Research Council (EPSRC) and Ministry of Defence (MoD), led to the development of wound-healing technology and collaboration between researchers at the University of Sheffield and medical technology company Smith & Nephew Wound Management. Wound dressings which will accurately and quickly detect the presence of bacteria in wounds and help reduce the overuse of antibiotics are being developed.

Bacteria detecting technology

When Professors Stephen Rimmer¹, Sheila MacNeil² and Ian Douglas³ presented the results of their EPSRC/MoD supported research into branched polymers to military scientists at Porton Down, it was clear the next stage would be to develop a fast, accurate and possibly life-saving technique for detecting the presence of bacteria in wounds.

Professor Rimmer, who heads an interdisciplinary team of polymer scientists, microbiologists and tissue engineers at the university, says: “The polymers we have developed incorporate a fluorescent dye and are engineered to recognise and attach to bacteria, collapsing around them as they do so. The level of fluorescence detected will alert clinicians to the nature and the severity of infection. We were the first people to propose this theory.”

The team’s work also makes for a much more efficient use of antibiotics. “When the polymer collapses it traps the bacteria around it, allowing us to pull the whole thing out without releasing any antibiotics into the wound. This means the bacteria do not develop any antibiotic resistance – which is crucial for patients suffering from chronic wounds who need long-term care,” says Professor Douglas.

From fundamental science to real application

Having published papers describing the research in prestigious journals, the team were looking for sponsorship to take the technology closer to real application when Professor MacNeil was invited to a national science conference and the team’s work started to gain wider public recognition.

Dr Mark Richardson, Vice President of Research and Technology at Smith & Nephew Wound Management⁴, had been following the team’s work. He says: “We knew the team’s research had been well funded; that it was innovative, of the highest quality and of global significance for the treatment of wounds. While we would not normally get involved at the applied research stage, because
of the EPSRC funding and the possibility of Technology Strategy Board’s support, we could see the benefits of collaborating with the Sheffield research team to help develop and build their technologies into some of our existing products.”

With follow-on funding from the Technology Strategy Board, a joint University of Sheffield and Smith & Nephew team is now developing the technology that will provide enhanced care for patients suffering from chronic wounds such as diabetic foot ulcers and venous leg ulcers.

Dr Richardson adds: “Chronic wounds such as these are major health and economic burdens in most developed countries and are primarily wounds of the elderly. With the rise in the levels of obesity/diabetes this problem can only get worse. These are critical wounds. If they become infected they can be very problematic for the patient, in some cases leading to the amputation of digits or limbs. The early and accurate detection of infection is very important, but at the moment we have no point-of-care diagnosis for wounds. Clinicians can take swabs, but this can mean a delay of up to 48 hours to get a result, during which time the patient is potentially at risk.”

Rapid response

The new wound dressings will look very much like conventional wound dressings, but will contain a hydrogel membrane. A handheld device will be able to detect changes in the colour of the dressing, indicating the presence of bacteria and how best to treat it.

Providing the clinician and the patient with a tool that alerts them early to a potential infection – and which also reassures them when there is no infection – could transform the care of wounds and reduce the unnecessary use of antibiotics. By highlighting the presence of an infection at an early stage, it could also help prevent wounds becoming colonised by an established layer of bacteria (biofilms) which are more resistant to normal antibiotic treatment and can lead to protracted care.

In the UK alone there are over 200,000 patients suffering from chronic foot ulcers, with up to 60 per cent of these being infected. By finding a way of detecting and treating these cases earlier, and more effectively, the team are confident their research will improve patient care and reduce the cost burden on the National Health Service. The aim is to have the new technology available commercially within the next four years.

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Antimicrobial resistance

Bacteria-eating viruses

With the ever-growing threat of antimicrobial resistance, there is a critical need for alternatives to antibiotics. MRC-funded researchers at the University of Leicester are pursuing one such route. A team led by Dr Martha Clokie has isolated bacteriophages — viruses that ‘eat’ bacteria — targeting the hospital superbug *Clostridium difficile* or *C. difficile*.

Bacteriophages were discovered and used as a therapy for bacterial infections almost 100 years ago, long before the development of antibiotics. Dr Frederick Twort, a British bacteriologist and later recipient of MRC funding, is credited with their initial discovery in 1915. French-Canadian scientist Felix d’Herelle later developed them to treat infections following his independent discovery of them in 1917. To date however, they are not in widespread use. Although phages did reach commercial production in the 1940s, and have been used to treat several bacterial infections, treatment does not produce consistent results. In the pre-antibiotic area, many aspects of phage biology were not well understood. Doses of phages often did not contain enough viable viruses to be effective, and viruses were used that did not kill the intended bacteria. There were also problems with the production of a stable contaminant-free phage stock. Perhaps the greatest barrier to phage acceptance in the west was the inadequate scientific methods used by researchers, such as the exclusion of placebos in trials. With the advent of the antibiotic dawn, phage research and production were all but shelved, with the exception of Eastern Europe and the former Soviet Union where they continue to be used therapeutically.

Renewed interest

Now the threat of widespread antimicrobial resistance has sparked a renewed interest in phages. Dr Clokie has been studying phages for 14 years. She says, “As their natural enemy, phages specifically target and kill bacteria. They encode a diverse set of gene products that can potentially be exploited as novel antimicrobials. They have the advantage over antibiotics of being much more specific and, as they can self-replicate at the site of an infection, they are able to clear infections that antibiotics can’t reach.” Over the past few years, Dr Clokie has isolated and characterised 40 different phages that infect *C. difficile* — the largest known set of these phages. Of these, she has developed a specific mixture that has proved to be effective against 90 per cent of the most clinically relevant *C. difficile* strains seen in the UK. The US pharmaceutical company AmpliPhi are funding the further development of these phages, with the aim of testing them in Phase I and Phase II trials. This will involve optimising phage preparations for maximum effectiveness against *C. difficile* infections and establishing production, storage and delivery systems for the phage mixture. Dr Clokie will evaluate the effectiveness of the therapy and dosing regimes in collaboration with Dr Gill Douce at the University of Glasgow.
Dr Clokie says, “The number of bacteriophages that exist on Earth, combined with their vast genetic diversity and exquisitely specific interactions with bacterial hosts means that they have the potential to offer a real solution for the treatment of a range of human pathogens. A lot of fundamental science needs to be carried out in order to ensure that we understand how to best exploit them.”

**Phage products**

A potential problem with systemic phage use is the possibility that they may be seen as foreign by the body’s immune system and be destroyed. Delivery of phages also needs to be investigated. To prevent them being damaged by the acidity of the digestive system when ingested, phages would need to be encapsulated or stabilised. A way around these problems might be to use the products of phages rather than the whole organism.

In 2010, a team of researchers at the MRC Centre for Molecular Bacteriology and Infection (CMBI), also funded by BBSRC, determined the structure of Gp2 — a protein produced by the phage T7 that disables *E. coli* cells. In 2012, they demonstrated how Gp2 blocks the action of the bacteria’s RNA polymerase — an enzyme that enables the instructions in the bacteria’s genes to be read and turned into proteins. The researchers now plan to identify small molecules that mimic the structure and function of Gp2 and use these as the basis for new drugs to combat bacterial infections.

Different bacterial infections will require different treatment solutions, but it is hopeful that both whole phage particles and their products can be developed as important alternative treatments for human infection.

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Antimicrobial resistance

Making the leap: Cross-species transmission of *Staphylococcus aureus*

The disease-causing bacterium *Staphylococcus aureus*, which is carried by and causes serious infections in both humans and livestock, can be transmitted between different host species, providing a source of new infectious strains in people and animals.

Research led by Professor Ross Fitzgerald from the Roslin Institute at the University of Edinburgh, and others, has found that *S. aureus* has made numerous leaps between host species; from humans to animals such as dairy cattle and pigs and vice versa. In particular, a 2013 study by Professor Fitzgerald and colleagues showed that a bovine strain called CC97 had made two separate leaps to humans. “There may be a lot more cross-species transmission than we anticipated,” says Professor Fitzgerald.

Following these transmissions, CC97 spread to people on four continents over a forty year period. During that time, the strain also acquired resistance to common antibiotics, becoming methicillin-resistant *S. aureus*, or MRSA.

The findings suggest that farm animals can provide a ‘reservoir’ of *S. aureus* and MRSA strains that can spread to and cause disease in human populations.

The emergence of resistance

Antibiotic use is widespread in animal farming, including the dairy industry and pig farming, as well as in human medicine, so researchers might have expected to see resistance evolving in strains of *S. aureus* present in dairy cattle, as it does in people. However, Professor Fitzgerald found that strains of CC97 *S. aureus* in cattle were not resistant to the antibiotic methicillin. Only once CC97 strains had crossed to humans and pigs did they acquire resistance to methicillin, and further work is needed to understand why resistance arose in some strains of the bacteria but not others.

“There may be something about the pig farming industry that lends itself to the emergence of antibiotic-resistant strains of *S. aureus*,” speculates Professor Fitzgerald. “We’ve seen that for several different strains [from pigs] now – they acquire methicillin resistance. We don’t see that to the same level in dairy cows.”

A widespread pathogen

*S. aureus* is a widespread pathogen of humans and of livestock. In 2013-14, the NHS reported 826 cases of MRSA infection, and 9,290 cases of infection by *S. aureus* susceptible to the antibiotic methicillin. *S. aureus* is also the leading cause of bovine mastitis, a painful inflammation of the mammary tissue, which costs the UK dairy industry £200M a year. The bacteria also cause mastitis in sheep and goats, and various conditions in broiler chickens, including septic arthritis.

As a result, the livestock industry relies on antibiotics to prevent and treat the infection, which can result in the emergence of antimicrobial resistance. Globally, around 70 per cent of antimicrobial use is in farm animals.

Image: Cows on Eefie Hill.
Credit: John Comloquoy CC BY-SA 2.0, http://creativecommons.org/licenses/by-sa/2.0/deed.en
Almost nine hundred strains

Previous studies by Professor Fitzgerald and others found that different strains of *S. aureus* are associated with different host species, and have become adapted to the conditions those hosts provide. The researchers wanted to understand where the ancestor of these strains came from, and when and how *S. aureus* made the leap between host species.

To do so, they previously used a technique called ‘multi-locus sequence typing’ to identify genetic changes that had occurred in the strain at certain locations, or loci, within their genomes. This could tell the researchers which strains were closely-related and enabled them to estimate when two strains shared a common ancestor. Genetic changes accrue over time, so strains that have been separated for a long time have more genetic differences than strains that have only recently evolved from a common ancestor.

The subsequent development of whole-genome sequencing gave researchers a powerful tool to look for genetic changes in the entire genome of *S. aureus* strains. Professor Fitzgerald is now involved in a collaborative project using whole genome sequences of almost 900 *S. aureus* strains. The researchers will study how the bacteria have jumped between hosts across an entire species, rather than focussing on a single *S. aureus* strain such as CC97. They also plan to look at the acquisition of antibiotic resistance across all of these strains, and whether it is more likely to appear in certain hosts.

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Whole genome sequencing

Following recent improvements in sequencing technologies, whole genome sequencing (WGS) is set to become a crucial tool in the control of antimicrobial resistance. WGS has already shown considerable promise for the surveillance of infection, the development of new diagnostic tests and the identification of resistance.

Infectious diseases are often transmitted globally. So rapid detection and identification of outbreaks, and the exchange of information between different authorities and research facilities, are essential to identify trends and control spread. WGS could have a major part to play in this process.

Impact on patient care
Professor Sharon Peacock at the University of Cambridge specialises in the role of sequencing technologies in diagnostic microbiology and public health. In 2013 she provided the first evidence that bacterial WGS could be used in clinical practice to impact on patient care. The infection control team at the study hospital had identified several infants in a special care baby unit (SCBU) infected with superbug methicillin-resistant Staphylococcus aureus (MRSA) over a six-month period. Although a link was suspected, a persistent outbreak could not be confirmed with conventional methods. The use of WGS confirmed the outbreak, and also identified a larger population of 26 related cases. Analysis showed that transmission had occurred within the SCBU, between mothers on a post-natal ward, and in the community. WGS data were used to propose and confirm that infection by a staff member had enabled the infection to persist during periods without known infection on the SCBU and after a deep clean. This individual was successfully treated, after which the outbreak ceased. This demonstrated that healthcare and community-associated infection should no longer be regarded as separate entities.

Professor Peacock says, “This study demonstrates that sequencing of microbial pathogens can influence the quality of infection control and patient care.”

Better antimicrobial stewardship
MRC-funded researchers at the University of Oxford have also used WGS to assess the transmission of fellow superbug Clostridium difficile (C.difficile). Dr David Eyre and Professor Sarah Walker demonstrated that far fewer cases of C. difficile infection were transmitted from symptomatic patients than expected, with other cases mostly likely coming from asymptomatic individuals or an environmental source such as water or animals, and food. They analysed whole genome sequences of samples obtained from all patients with C. difficile infection in Oxfordshire over 3.6 years and found that 45 per cent were sufficiently genetically diverse to suggest transmission from sources other than symptomatic patients. However, the whole genome sequences were also used to show that the incidence of cases transmitted from other symptomatic patients and cases from other sources both declined similarly over the study. These results demonstrate the importance of interventions to reduce susceptibility to disease in
Exposure of patients, such as better antimicrobial stewardship, rather than just reducing transmission from symptomatic patients. They also illustrate the value in combining information from whole genome sequencing with traditional epidemiology. The use of rapid benchtop sequencing\(^5\) again allowed the identification of genetically related cases in almost real time so that cases clearly linked by a hospital or community contact can be targeted to prevent further spread.

**100,000 genomes project**

Other MRC-funded researchers in Oxford have also demonstrated the value of using whole genome sequencing to investigate clusters of cases of Mycobacterium tuberculosis\(^6\,7\). Professors Derrick Crook and Tim Peto found that whole genome sequencing could identify previously unrecognised links between cases, more than doubling the number of tuberculosis transmissions previously identified through standard methods. It was also able to refute the possibility of transmission between other cases, saving hours of work trying to work out how transmission could have happened. The technique could also identify super-spreaders and predict the existence of undiagnosed cases, potentially leading to early treatment of infectious patients and their contacts. This work has led to whole genome sequencing being adopted by Public Health England, initially in a pilot study within the “100,000 genomes” project, working towards widespread implementation in English tuberculosis reference laboratories from 2016.

Professor Peacock has also successfully used WGS to investigate a case of multi drug-resistant (XDR) Mycobacterium tuberculosis\(^8\). This proved more accurate than standard methods, with WGS detecting mixed infection by two distinct strains of *M.tuberculosis*, which was not identified by standard genotyping. This has important implications for distinguishing relapse from reinfection and for identifying secondary cases of infection. The study also highlighted the potential of WGS to predict the antimicrobial resistance of *M.tuberculosis*, which could reduce the time taken to implement effective antimicrobial therapy for XDR *M.tuberculosis*. This would benefit individual patient care and could help to contain the spread of infection.

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<tr>
<th>Antibiotic class</th>
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## Antimicrobial resistance

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