Where next for antibiotics?

The immune system and the nature of pathogenicity are providing vital clues in the fight against antibiotic-resistant bacteria

Philip Hunter

Since the discovery of penicillin in the 1940s, antibiotic resistance among bacteria has been a growing problem. It has enabled major killers such as tuberculosis and pneumonia to stage comebacks in developed countries, and has led to multiple drug-resistant strains of bacteria that can be fatal for people with compromised immune systems. The World Health Organization (WHO) believes that the situation is becoming steadily worse. In a keynote speech delivered to the Conference on Combating Antimicrobial Resistance, held in March in Copenhagen this year, the WHO's Director General Margaret Chan warned that the world risked entering into a dark 'post-antibiotic' age unless new drugs become available quickly. Noting that we risk losing our front-line antimicrobials, Chan pointed out that mortality rates are 50% higher among patients infected by drug-resistant bacteria than those who are not.

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Chan's concern was heightened by the discovery in India of resistance among enterobacteria—a family that includes *Salmonella* and *Escherichia coli*—to the broad-spectrum antibacterial group known as the carbapenems [1]. This development could have dire consequences for world health, because carbapenems are often given as the antibiotic of last resort. The bacteria have developed resistance through the production of an enzyme called New

Delhi metallo- β -lactamase-1 (NDM-1), which counters the ability of carbapenems to inhibit bacterial cell wall synthesis. Lhousseine Touqui, from the Innate Host Defense and Inflammation unit at the Pasteur Institute in Paris, France, believes that the resistance mechanisms could now be recruited by other bacteria and might spread to other countries. "These strains are now resistant to almost all available antibiotics," Touqui said, with the implication that the medical community is close to running out of tools to treat diseases caused by such strains.

Another worrying trend is the emergence of bacterial strains resistant to vaccines. The development and widespread administration of a vaccine against invasive pneumococcal bacteria led to a significant decline in pneumonia among children [2], but then resistant strains emerged through genomic recombination [3].

There has also been a commercial factor at play because antibiotics are a difficult market for pharmaceutical companies. Victor Nizet, head of the Laboratory for Bacterial Pathogenesis and Innate Immunity at the University of California at San Diego, USA, explained that, "the issue of resistance itself is also a concern for a manufacturer, because if an organism develops resistance, then the medicine is not useful any more financially." In addition, he noted that, "major pharma has largely abandoned antibiotic development since the heyday of the 1960s and 1970s, partly because [antibiotics] are typically only taken for a few weeks."

Nonetheless, both academia and pharmaceutical companies have noted the impending threat of invincible bacteria and have increased their efforts to find new antibacterials. During the past

two decades, promising approaches have been tried and tested. These have included the use of cationic peptides and renewed attempts to develop bacteriophages into therapies. None of these, however, have made it to widespread clinical use, which leaves clinicians and public health experts with only improved hygiene and better diagnostics as tools to battle drug-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus*, which has wreaked havoc in hospitals around the globe.

Fortunately, there is one big difference between the present day and the pre-antibiotic era: epidemiological and genomics studies have provided valuable knowledge of the mechanisms by which bacteria can evade antibiotics or the host's immune system. This new advantage is attracting pharmaceutical companies back into the field, although at present the focus is still on broad-spectrum antibiotics against a wide range of bacteria. This, Nizet argues, is a backward-looking approach that fails to exploit our molecular knowledge of host-pathogen interactions. Moreover, "[t]he broader spectrum the antibiotic is, the more impact it has on the micro flora known to be critical in immune defence," he explained. "So one avenue now has to be increased specificity of antibiotics, and in order to target a pathogen specifically, we need to understand the molecular basis of host-pathogen interactions, and here we are making a lot of progress."

This will not necessarily lead to the development of new drugs to kill pathogens, but rather to target the specific virulence mechanisms by which bacteria evade host immune responses. These

mechanisms take various forms, including the injection of toxins into host cells to suppress the activity of enzymes, or to disrupt host transcription factors to neutralize pro-inflammatory signalling pathways and repress innate immunity. Such attacks can, for example, inhibit the movement of neutrophils to the site of infection, leading to a dramatic loss of bacterial phagocytosis and digestion. Neutrophils are the most abundant white blood cells and are among the first cells to migrate towards the site of inflammation. They attack pathogens, primarily bacteria, in three ways: through phagocytosis, by producing antimicrobial peptides, and using the generation of extracellular traps comprising fibres made primarily from the neutrophil's DNA to entangle the bacteria. Pathogens can also target or attempt to block these individual mechanisms, for example by inhibiting the production of peptides by neutrophils.

These defence mechanisms against innate immunity can themselves provide avenues to therapies. For example, treatment could involve providing the very peptides that have been suppressed by a particular strain of bacteria. Touqui and

colleagues demonstrated this approach [4] by using a bactericidal molecule known as human group IIA-secreted phospholipase A₂ (sPLA₂-IIA), which is important in innate immune defence against Gram-positive bacteria. They showed that *Streptococcus pyogenes*, a leading human pathogen causing pneumonia and meningitis among other conditions, has evolved a previously unidentified mechanism to resist killing by sPLA₂-IIA. The mechanism involves at least one bacterial surface protein from a family known as LPXTG—referring to an amino acid sequence motif—that protects against sPLA₂-IIA; *S. pyogenes* mutants lacking the gene for this protein are much more susceptible to the bactericidal molecule.

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The team found that although *S. pyogenes* seemed to be resistant to the action of sPLA₂-IIA produced by the innate immune system, it was still susceptible to the molecule when administered as a drug, at least in a mouse model. "We showed that some

bacteria inhibit the expression of sPLA₂-IIA by the host. But, when we add the exogenous sPLA₂-IIA, it is able to kill bacteria and thus compensates for the absence of endogenous sPLA₂-IIA," Touqui explained. "This indicates that exogenous sPLA₂-IIA is able to bypass the repression of the endogenous molecule by the pathogen, and supports the concept of the potential therapeutic use of AMPs [anti-microbial peptides]. This bactericidal activity is effective even against strains resistant to multiple antibiotics. At the same time, our previous studies showed that rhPLA₂-IIA is not toxic to mammalian host cells at concentrations higher than those required to achieve bactericidal effects, making it suitable for therapeutic use."

There have also been instances of drugs developed to treat entirely different conditions that have turned out to have antimicrobial properties. Statins, for example, which were developed to lower cholesterol levels, can be used to combat infections caused by *S. aureus*, one of the bacterial groups most successful at evading natural immune strategies. The key to the natural resistance of the bacteria is

its ability to make strong antioxidant pigments that give it a golden colour and that neutralize several oxidating peptides fired at it by neutrophils. Statins, which had already been through clinical trials as cholesterol-lowering agents, also inhibit bacterial production of the pigment-producing enzyme and prevent them from turning golden. This is not a direct anti-pathogen activity, because the bacteria grow normally, but they become more susceptible to the action of oxidants and are less able to survive in blood [5]. "Here we were fortunate because of an unusual coincidence that the way these bacteria make these colourful pigments has an enzyme that overlaps with the pathway through which humans make cholesterol," Nizet commented. "And that is a pathway drug companies are extremely interested in."

The action of statins does not lead to an increased rate of phagocytosis or a direct attack by oxidants. Instead, it induces phagocytes to release DNA-based filaments that trap the bacteria, and then in effect smother them with antimicrobial peptides to kill them. This is consistent with the anti-inflammatory nature of statins, and shows that it can be possible to stimulate the antibacterial action of white blood cells without upregulating, and in fact perhaps even downregulating, inflammation.

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Unfortunately, statins seem to be specific to *S. aureus* because they counter the bacterial group's use of antioxidant pigments. Yet, there are plenty of other bacteria capable of evading antibiotic therapies for which alternatives need to be found. One of these is *Pneumococcus*, which has acquired resistance to a range of antibiotics and vaccines. So far, it is not known how readily and frequently such adaptations occur—information that would yield important clues for therapies. However, a study by Derek Crook and others at Oxford University in the UK has found that at least some *Pneumococcus* strains are able to diversify very rapidly and easily [6]. The key is the ability of the bacterium to take up and recombine with the chromosomes of other strains to change its capsules, which are polysaccharide

structures on the surface of cell walls. Capsules can act as virulence factors by reducing the surface friction of bacteria, so that they can slip away from the clutches of neutrophils attempting to engulf them.

Adaptive immunity conferred by vaccines and previous infection involves the production of antibodies against specific capsules. The Oxford study showed that *Pneumococcus* can evade this by switching capsules more quickly than had been thought, through recombining with DNA from another strain. "What the paper quite strongly suggested was that there have been periods where there would be a greater extent of recombination and on a larger scale than had been believed," Crook commented.

This, he said, also helps resolve the puzzle of how resistance against penicillin evolved. "In the 1960s and 1970s, it was never understood how resistance to penicillin occurred, because it needed changes in the chromosome at three loci if not four or five, and it was hard to see how this could occur in a straightforward way," Crook explained. "Our work gives a sense of how it might occur, in that you can conceive of acquiring simultaneously at a number of different sites of penicillin capsule variants that confer resistance." In other words, bacteria are capable of acquiring changes at several chromosomal loci simultaneously, as they would need to do to become resistant to penicillin.

The study is good news for vaccine development. The so-called PCV7 vaccine widely used to protect infants and young children against pneumonia is a conjugate or multivalent vaccine incorporating seven different cell membrane sugars. The Oxford study indicates that it takes about eight or nine years for a pneumococcal variant to arise that is resistant to a vaccine such as PCV7. "In terms of vaccine design, you could cope with that," Crook said, comparing the situation with the vaccine against the influenza virus, which is reformulated every year.

"With a pneumococcal vaccine, you have to extract antigens from relevant pneumococcal strains, and conjugate them to proteins," Crook explained. This takes two to three years; thus a vaccine with a useful lifespan of eight to nine years is worth having. According to Bernard Beall from the US Center for Disease Control and Prevention,

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it is also possible to further increase valency by incorporating more serotypes to extend a vaccine's useful life. "The availability of a number of different capsules combined with the immune selection that has existed for undoubtedly thousands of years, and greatly intensified in the vaccine era, also combined with antibiotic selection, has generated a formidable pathogen," Beall admitted. "Conjugate vaccines are winning though. PCV13 (with 13 serotypes) will impressively extend the success of PCV7. PCV15 will extend the success of PCV13. If the technology for making an all-encompassing multivalent vaccine to target perhaps 25–30 capsular serotypes came along, I think we would have it about whipped."

The work on *Pneumococcus* raises the question of why mutations that confer resistance do not spread quickly throughout the bacterial population, given that these occur frequently and spread readily through recombination. One clue might be the fact that *Pneumococcus* is primarily a harmless commensal bacterium that rarely causes disease. As noted by Nicholas Croucher from the Wellcome Trust Sanger Institute near Cambridge, UK, such work implies that there must be selective constraints on the evolution of bacteria that are not yet understood, or else they would adapt to antibiotics and vaccines faster. "Improving our knowledge of the species' genetics can therefore inform the design of future therapeutic interventions in a manner that will hopefully make them effective long-term, guarding them against changes in the bacterium as is best possible," Croucher said.

All of this, however, will take time and significant investment in research to understand both the selective forces that operate on bacteria and the very nature of pathogenicity itself. Armed with such knowledge, scientists might be better able to design long-term, effective antibiotics that specifically and efficiently target virulent strains, whilst leaving most bacteria unharmed. This would drastically decrease the selective pressure for antibiotic resistance.

CONFLICT OF INTEREST

The author declares that he has no conflict of interest.

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EMBO reports (2012) **13**, 680–683; published online 6 July 2012; doi:10.1038/embor.2012.101