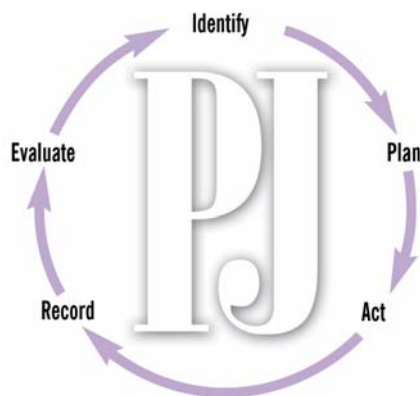


Understanding antibiotic resistance

In this final article in a series of three, **Hayley Wickens** and **Paul Wade** examine the mechanisms by which antibiotic resistance can arise and spread. The article concludes with a look at the main multidrug-resistant organisms causing clinical concern



Identify knowledge gaps

1. How do bacteria develop resistance to antimicrobials?
2. What agents are available to treat multidrug-resistant bacteria?
3. How would you explain the threat of MRSA to a patient?

Before reading on, think about how this article may help you to do your job better. The Royal Pharmaceutical Society's areas of competence for pharmacists are listed in "Plan and record", (available at: www.rpsgb.org/education). This article relates to "drug therapy" (see appendix 4 of "Plan and record").

The treatment of bacterial infections has been described as an evolutionary arms race — as each new class of antimicrobial is introduced, resistance starts to develop in the micro-organisms they are designed to kill. For example, within a few years of the discovery of penicillin there were epidemics of "hospital staphylococcus", a strain of *Staphylococcus aureus* resistant to penicillin, chloramphenicol, erythromycin and tetracycline, among other agents. The development of methicillin followed by flucloxacillin (drugs resistant to the action of the penicillinase enzyme) brought these initial epidemics under control but in the early 1960s the first cases of methicillin-resistant *S aureus* (MRSA) were reported. Since then, the importance of MRSA as a major hospital pathogen and, recently, a political driver has increased exponentially.

Resistance

Antibiotic resistance can be described as microbiological or clinical. Microbiological resistance exists when an organism possesses any resistance mechanism (see Panel 1 for examples). Clinical resistance can be explained as failure to achieve a concentration of antimicrobial that inhibits the growth of the organism in a particular tissue or fluid.

Panel 1: Expression of resistance

A resistance mutation can be expressed in several ways:

Production of an enzyme that destroys active drug

Bacteria can produce enzymes that inactivate antimicrobials. The best known of these enzymes are beta-lactamases. Over 300 different beta-lactamases exist; the most clinically significant of which are produced by Gram-negative organisms. These enzymes can hydrolyse the beta-lactam ring of penicillins, cephalosporins, monobactams and carbapenems. Other agents that can be inactivated by enzymes include aminoglycosides and chloramphenicol.

Changes in cell permeability

Alterations in cell wall constituents can lead to decreased permeability (and therefore decreased sensitivity) to antimicrobials. This resistance mechanism is more common in Gram-negative organisms than in Gram-positive organisms because the latter are readily permeable to most antimicrobials, due to their relatively simple cell wall structure. Some Gram negative organisms (eg, *Haemophilus influenzae*) are more readily permeable than others (eg, *Pseudomonas aeruginosa*).

Changes in the number or structure of porins (protein channels allowing drug penetration) is also an important resistance mechanism. Drugs that require active transport across the cell membrane before they can exert their action can have their effects reduced due to changes in cell wall transporter molecules (this mechanism confers resistance to aminoglycosides in both Gram-positive and Gram-negative organisms).

Mutations in efflux mechanisms

Efflux pumps occur naturally in bacterial cells. They are chiefly concerned with removal of waste products, but changes in their conformation can enable them to remove antimicrobials. Gram-positive organisms can show resistance to macrolides by this mechanism and resistance to tetracyclines is usually mediated via efflux.

Alterations to structural target

Mutations to the molecular target for an antimicrobial can lead to resistance to its effects. This is true for beta-lactams (due to alterations in penicillin-binding proteins), aminoglycosides and macrolides (due to alterations in the 30S and 50S ribosomal proteins, respectively), quinolones (due to alterations in topoisomerases) and rifampicin (due to modification of ribonucleic acid polymerase).

This is the main mechanism by which enterococci become resistant to glycopeptides, through alterations in the peptidoglycan precursors. This resistance can be spread by plasmids (see Panel 3, p502) or transposons (see later) and accounts for the increasing prevalence of glycopeptide-resistant enterococci being seen throughout the world.

Bypass of metabolic pathway

Some antibiotics work on enzymes in metabolic pathways. Microbial cells can develop a novel metabolic pathway that bypasses the effect of the antimicrobial, so rendering it ineffective.

Resistance to sulphonamides and trimethoprim is mediated by metabolic bypass, in this case due to synthesis of altered dihydropteroate synthetase and dihydropteroate reductase. These enzymes have reduced susceptibility and affinity for sulphonamides and trimethoprim, respectively.

Multiple resistance mechanisms

It is not uncommon for organisms to manifest resistance by using a combination of the above mechanisms. For example, macrolide resistance in streptococci may be due to a combination of increased efflux and ribosomal modification, and carbapenem resistance in *Ps aeruginosa* may be due to a combination of beta-lactamase production, increase in efflux pumps and changes to the bacterial cell wall.

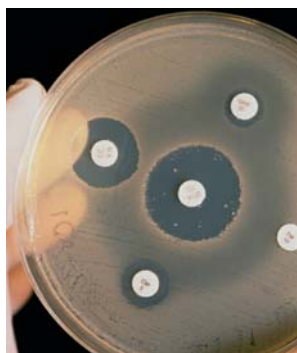
Antimicrobial activity can be described *in vitro* in terms of minimum inhibitory concentration. Bacteria can be described as “sensitive”, “intermediately susceptible” or “resistant” depending on the level of drug required to inhibit growth of the organism. Panel 2 describes how these measures are commonly determined. One of the problems with determining resistance is relating the serum concentration of a drug to that achieved at the site of infection or in tissues. Serum concentration is often used as a surrogate for tissue concentration, but this might not always be reproducible between patients (or even in the same patient) so some consideration of pharmacodynamics is also needed when considering resistance.

How resistance develops

There are two types of microbiological resistance: innate and acquired. Innate (or intrinsic) resistance is the natural resistance bacteria possess to some antimicrobials. For example, organisms may be naturally impermeable to some antibiotics due to their cell structure. Organisms with innate resistance are often of low virulence but, because they are resistant to so many agents, they persist in the environment. In particular, these bacteria can persist in hospitals, where there is a high selection pressure (hospitals use a lot of antibiotics so multiply-resistant bacteria are naturally selected), and can lead to difficult-to-treat nosocomial infections. Species currently causing treatment difficulties include *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, although these problems are also partly due to acquired resistance.

An organism can also acquire resistance to an antimicrobial to which it was previously sensitive. This can be due to chance mutation in the genetic material of the cell, or the acquisition of resistance genes from other drug-resistant cells. Panel 3 outlines bacterial cell structure.

Mutational resistance Changes to DNA bases can lead to changes to RNA and, in turn, to changes in the proteins produced. Chromosomal mutations can, therefore, lead to a change to cell proteins involved in the structure of the site of action of the antimicrobial, or in the metabolic processes by which an antimicrobial is inactivated by the



Hank Morgan/SPL

Disc diffusion allows bacteria to be classed as “sensitive”, “intermediately susceptible” or “resistant”

Panel 3: Bacterial structure

Bacteria are single-celled organisms without a nucleus — prokaryotes. In contrast to eukaryotic (eg, animal) cells bacteria often also contain a cell wall. Most bacteria can be categorised as Gram negative or positive, using the Gram stain, which highlights differences in cell wall structure. Gram negative organisms (eg, *Escherichia* spp, and *Pseudomonas* spp) have a complex lipopolysaccharide-containing outer membrane, and a relatively thin peptidoglycan cell wall. In contrast, Gram-positive organisms (eg, streptococci, staphylococci) lack this outer membrane and have a thick peptidoglycan cell wall. Results from Gram staining can give clues to likely colonisation site, pathogenicity and antibiotic sensitivity.

The bacterial chromosome exists as circular double-stranded DNA, typically around 4,000 kilo-base pairs (kbp) in length. This carries genes essential for bacterial growth.

Plasmids Plasmids are small circles of double-stranded DNA separate from the bacterial chromosome. They carry genes for specialised functions and often carry one or more genes for antimicrobial resistance. Conjugative plasmids are relatively large (usually >30kbp) and cells contain only one or two. Non-conjugative plasmids are small (<10kbp) and several can exist in a cell.

organism. Although the bacterial genome mutates at a low rate (once for every 10^7 cells produced), considering that some bacteria will divide every 20 to 30 minutes, it might only take six hours for one cell to give rise to a mutant daughter cell. Considering that a clinically significant urinary tract infection can be caused by 10^5 colony-forming units per ml of urine, the potential for mutational resistance is huge. It is important to recognise that not all mutations give rise to antimicrobial resistance. However, any slight advantage conferred by a mutation can result in the organism being selected for survival. In the case of drug resistance, sensitive organisms will be killed off in the presence of the antimicrobial, leaving the antimicrobial-resistant clone to multiply and predominate. Fortunately, these mutant organisms are often not as fit for purpose as their wild-type ancestors (eg, they might divide more slowly) and may die out rapidly in the absence of antimicrobial selection pressure. Problems generally occur when secondary mutations confer some stability, allowing persistence of the mutation.

Acquisition or transfer of resistance

Mutational resistance alone does not explain the resistance problems that we face. DNA coding for resistance elements can be transferred from one organism to another. This can occur via conjugation (the passage of a plasmid [see Panel 3] from one cell to another), transformation (the uptake of naked DNA, generated when cells break down) or trans-

Panel 2: Measuring resistance *in vitro*

Disc diffusion Discs containing known concentrations of antibiotic are placed on an agar plate that has been seeded with a pure culture of the bacterium in question. The plate is incubated overnight and examined for zones of inhibition around the antibiotic discs. Inhibited growth indicates susceptibility to the drug. The size of the zone is measured and reference tables are used to classify the organism as sensitive, intermediately susceptible or resistant.

E-test A strip containing an antibiotic at an increasing gradient is placed on an agar plate inoculated with bacteria, and this is incubated overnight. A zone of inhibition will appear as the organism grows around the strip. The minimum inhibitory concentration can be found where this zone intersects the graduated strip.

Panel 4: Hand washing

In its guidance for nursing staff, the Royal College of Nursing recommends a hand washing technique that requires six types of movement. These are illustrated at www.rcn.org.uk/publications/pdf/mrsa.pdf

There have been recent moves in hospitals to replace anti-bacterial scrubs with regular soap. According to the authorities, proper washing with a normal liquid soap should ensure that hands are clean. Following a National Patient Safety Agency alert, alcohol gel dispensers are now being installed at the end of every hospital bed. The gel allows health care professionals to disinfect their hands rapidly between patients but does not replace hand washing.

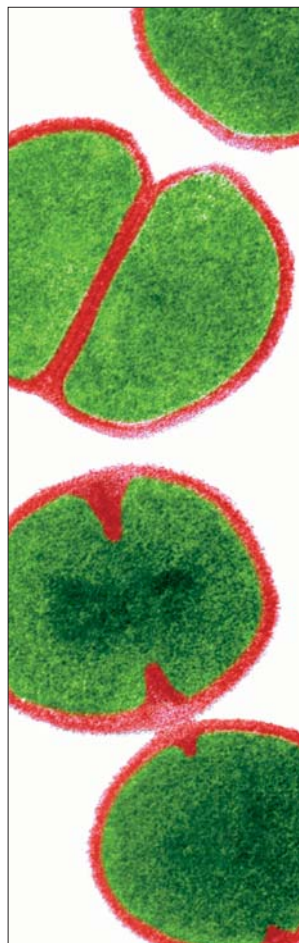
duction (DNA transfer by bacteriophage, a rare but potentially important mechanism).

Large (conjugative) plasmids can often self-transmit to other bacterial hosts in a process known as conjugation, and are common in Gram-negative enteric bacilli. Smaller plasmids, which can only transfer to other bacteria if mobilised by conjugative plasmids, are common in Gram-positive bacteria. Although plasmids are usually restricted in their ability to transfer (eg, Gram-positive plasmids tend to transfer to Gram-positive organisms), conjugation between distinct bacterial groups and between unrelated species, such as bacteria and fungi, has been observed.

Transposons and integrons (sometimes known as “jumping genes”) are short lengths of DNA (up to 5 kilo-base pairs) that can move from one site (which may be a plasmid or a chromosome) to another. They can carry antibiotic resistance genes and may be associated with persistence of antimicrobial resistance long after the selection pressure is removed (ie, after antibiotic use is stopped).

MRSA and multidrug-resistant organisms

The vast majority of clinical *S aureus* strains are penicillin-resistant due to a plasmid-mediated beta-lactamase, which may also confer resistance to other drug classes. MRSA has been around since the 1960s but new epidemic strains in the UK (known as EMRSA-15 and EMRSA-16) have been increasing in prevalence since the 1990s. MRSA prevalence differs from country to country. The UK is not alone in having MRSA bacteraemia prevalence rates of over 30 per cent but rates in Scandinavia and the Netherlands are much lower (under 3 per cent). The reasons for these differences have not been fully established. The UK EMRSA strains that emerged during the 1990s are thought to spread more easily through environmental or personal contacts. These new strains may also be more likely to cause infection than the earlier isolates, and there is evidence that deep infections caused by MRSA have a worse outcome than methicillin-susceptible *S aureus* (MSSA) infections, perhaps due to the treatment difficulties encountered. However, it is important to re-



Biomedical imaging unit Southampton General Hospital (SPL)

Hand washing and cleaner hospitals may not have a significant effect on MRSA

New MRSA strains in the UK (known as EMRSA-15 and EMRSA-16) have been increasing in prevalence since the 1990s

member that, depending on the clinical circumstances, MRSA colonisation might not require treatment (see *PJ*, 26 March, pp365–8).

Factors within hospitals such as inadequate provision of isolation facilities, increased inter-hospital transfers and an increase in the number of sick patients living longer (providing a reservoir for bacteria) assist the spread of MRSA and other resistant organisms. Another factor often forgotten is the effect of increased workload on MRSA spread. In a similar way to medication compliance, the more tasks that are carried out that require hand washing, the less likely it is that full compliance will be achieved. Rapid-acting alcohol gels are being recommended for widespread use due to their simplicity and speed of action. Panel 4 gives guidance on hand washing.

Treatment of MRSA

Resistance within MRSA is mediated by several mechanisms. The *mecA* gene codes for an altered penicillin-binding protein, and plasmid or transposon can confer resistance to a number of antimicrobials, including beta-lactams, aminoglycosides and trimethoprim. Resistance to quinolones has developed due to mutation following selection pressure and, along with potential resistance to rifampicin and sodium fusidate, this leaves a sparse antimicrobial armoury.

Potentially useful agents include the glycopeptides (vancomycin and teicoplanin) and newer agents, such as quinupristin-dalfopristin and linezolid. Of these, only linezolid is available in an oral form. However, recent reports have emerged of MRSA strains that have reduced susceptibility to glycopeptides (known as glycopeptide- or vancomycin-intermediate susceptibility *S aureus*; GISA or VISA), and

Panel 5: OHPAT services

Outpatient home parenteral antibiotic therapy services have been used in the US for many years and are becoming increasingly common in the UK. Patients are given parenteral antibiotic therapy either at home, in a GP surgery or in an outpatient clinic. This enables early discharge of patients who need to complete long courses of intravenous therapy but who are otherwise well.

Patients are typically given a peripherally inserted central venous catheter (PICC line). They can attend a clinic to have their antibiotics administered by a nurse or be trained to self-administer. Patients are reviewed regularly by a clinician and can contact the hospital if they are concerned with their therapy.

Infections treated with OHPAT include osteomyelitis, cellulitis and endocarditis. Patients report improved quality of life and there is a decreased risk of hospital-acquired infection. OHPAT schemes are often cost-saving due to the number of inpatient bed-days avoided. Typical drug regimens include teicoplanin, ceftriaxone or ertapenem, which can be given once daily or, in some cases, three times a week.

there have been rare, but extremely worrying, reports of vancomycin-resistant *S aureus* (VRSA).

Cases of partial or full resistance to quinupristin-dalfopristin have also been reported and the increasing usage of linezolid, driven by capacity pressures within the NHS, may make this agent less useful as time progresses. Alternatives to discharging patients on the expensive, but orally available, linezolid exist such as outpatient home parenteral antibiotic therapy (OHPAT) services (see Panel 5, p503), but these are not always available and require great effort to set up and maintain.

Older oral agents, such as rifampicin, trimethoprim and sodium fusidate (used in combinations such as rifampicin and trimethoprim or trimethoprim and sodium fusidate) can still play a role in the management of MRSA infections where full sensitivity testing has been carried out. Most MRSA strains will be sensitive to these agents and, if an oral agent is required and providing the patient is suitable in terms of potential for interactions or adverse events, they should be carefully considered before linezolid is used.

Further surveillance data on MRSA (and other organisms) can be found at the Health Protection Agency (www.hpa.org.uk) or European Antimicrobial Resistance Surveillance System (www.earss.rivm.nl) websites.

Community-acquired MRSA More recently, community-acquired MRSA (C-MRSA) has become a clinical problem. This is where an MRSA infection occurs in a previously healthy individual without recognised risk factors for MRSA infection, such as previous admission to hospital or prolonged therapy with antimicrobials. The Health Protection Agency has identified over 100 cases in the UK during the past three years. In the US, similar cases have been linked to shared towels, bathing facilities or contact sports, but this has not been mirrored in the UK.

Action: practice points

Reading is only one way to undertake CPD and the Society will expect to see various approaches in a pharmacist's CPD portfolio.

1. Compile an information sheet on MRSA suitable for patient use.
2. Investigate the various surveillance systems available (eg, the European Antimicrobial Resistance Surveillance System).
3. List the infections requiring mandatory reporting used in the UK.

Evaluate

For your work to be presented as CPD, you need to evaluate your reading and any other activities.

Answer the following questions: What have you learnt? How has it added value to your practice? (Have you applied this learning or had any feedback?) What will you do now and how will this be achieved?

Panel 6: Other multidrug-resistant pathogens

Vancomycin-resistant enterococcus

- Enterococci principally colonise the gut
- Vancomycin resistance has been observed in enterococci since the 1990s
- Vancomycin-resistant enterococci chiefly causes problems in immunosuppressed patients (eg, in renal or intensive care units)
- Vancomycin-resistant enterococci is usually susceptible to linezolid or quinupristin-dalfopristin (high-level resistance is less likely in *Enterococcus faecalis*, although this organism is innately resistant to quinupristin-dalfopristin)

Extended-spectrum beta-lactamase (ESBL) producing organisms

- ESBLs hydrolyse most penicillins and cephalosporins and some are active against carbapenems
- Some ESBLs are plasmid-mediated, such as those produced by *Klebsiella* spp (these may be associated with hospital epidemics)
- Some ESBLs can be chromosomally mediated, such as those produced by *Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia* spp, *Citrobacter* spp, and these may be induced by cephalosporin therapy or use of clavulanate
- Metallo-beta-lactamases can hydrolyse carbapenems and may be produced by *Ps aeruginosa* and *Acinetobacter baumannii*

Acinetobacter baumannii

- *Acinetobacter* spp are relatively non-virulent Gram-negative organisms, colonising up to 25 per cent of the population, and usually sensitive to most antimicrobials. (however, *A baumannii* has a propensity to develop antimicrobial resistance rapidly and is increasingly seen in hospitals)
- *A baumannii* is resistant to desiccation and hard to eradicate once established in wards
- Multi-drug resistant *A baumannii* is increasingly a problem in specialist referral hospitals, and is often only sensitive to colistin or occasionally carbapenems
- A novel agent known as tigecycline (a glycylcycline), is currently in phase III trials and may prove useful for multi-resistant cases of *Acinetobacter*

Although C-MRSA is resistant to most of the agents likely to be used in the community, agents used within hospital, such as vancomycin, gentamicin, rifampicin and linezolid, easily treat it. However, this problem may become more significant in the future and is being monitored.

Other organisms There are other drug-resistant organisms which, although they do not have the raised public awareness of MRSA, may be just as significant clinically. Some examples are listed in Panel 6, along with their characteristics.

Government targets The Government has set a target to halve MRSA rates by March 2008, but the achievability of this was questioned at the Clean Hospitals Summit in London last week. According to one speaker, hand washing and cleaner hospitals will not have a significant effect on MRSA because the problem is multifactorial. Isolation of infected patients might help but NHS waiting list targets mean that hospitals are too full for this to be possible.

Hayley Wickens, PhD, MRPharmS, is senior microbiology pharmacist at St Mary's Hospital, London, and **Paul Wade, MSc, MRPharmS**, is a clinical lecturer at Brighton and Sussex University Hospital, Brighton