DRUGS FOR DIFFICULT BUGS: AN OVERVIEW OF NEW ANTIBACTERIAL AND ANTIVIRAL AGENTS

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Despite repeated predictions of the demise of infectious diseases, most human pathogens have stubbornly resisted attempts aimed at their control and eradication. Concern has been expressed at the highest level over the rapid rise in the prevalence of drug-resistant microbes in recent years.[1-3] The inappropriate use of antimicrobial agents, in both human and veterinary medicine, needs to be addressed urgently to delay the further emergence of organisms which are resistant to the drugs currently in use. However, there is also an urgent need for new antimicrobial agents. Besides a requirement for effective therapies for multi-drug resistant pathogens, a need exists for new antimicrobial drugs with improved efficacy, better pharmacokinetic characteristics and reduced toxicity. Furthermore, effective treatment options have yet to be discovered for many viral infections.

BACTERIAL INFECTIONS: WHAT ARE THE PROBLEMS?

Although some bacteria have remained fully sensitive to antimicrobial agents over a prolonged period of time, including *Streptococcus pyogenes* to penicillin and *Chlamydia trachomatis* to tetracyclines, resistance to antibiotics is now widespread. The mechanisms underlying bacterial resistance are complex, and several different mechanisms may contribute to resistance to a given drug.[4-6] Table 1 shows some important examples. Multi-drug resistance is an increasing problem, and can be passed between bacteria on mobile genetic elements such as plasmids and transposons. Site-specific recombination of resistance genes contained in elements called 'gene cassettes' is now recognised as an important mechanism for resistance transfer.

The prospect of 'untreatable' multi-drug resistant bacterial infections is a frightening one and, not surprisingly, infection with such multi-drug resistant bacteria can be associated with a poor clinical outcome,[7] although extensive co-morbidity often makes such outcome assessments difficult.[8] However, *in vitro* resistance does not inevitably translate into an impaired treatment outcome, an important example being intermediate penicillin resistance and response to penicillin therapy in pneumococcal pneumonia.[9] Invasive infection and colonisation also need to be carefully distinguished, such as when a drug-resistant organism is obtained from a critically unwell patient in an intensive care unit setting.

Several national and international surveillance schemes of antimicrobial resistance are now in place, including the SENTRY bacteraemia survey[10] and the Alexander project[11] for respiratory pathogens. Major areas of current concern are summarised in Table 2 and discussed below.

Streptococcus pneumoniae

Penicillin resistance is increasing worldwide, with multi-drug resistant strains also being detected increasingly. Penicillin-resistance rates exceeded 40% in ten countries in 1998.[12] In the UK, the prevalence of penicillin resistance (intermediate or full) increased from 1.5% to 3.9 % between 1990 and 1995.[13] Erythromycin resistance increased from 2.8% to 8.6 % and cefotaxime resistance from 1.1% to 2.9 % during the same period. However, even the highly resistant strains remained sensitive to vancomycin and rifampicin.[13]

Neisseria

Penicillin resistance rates in *Neisseria gonorrhoeae* in the UK, unlike other parts of the world, were relatively low and stable until recently. However, since the mid-1990s the incidence of gonorrhoea has been climbing, and penicillin resistance also appears to be increasing.[14, 15] Fluoroquinolone resistance is also a growing concern with this organism. Clinically significant resistance to *N. meningitidis* is not a problem in the UK as yet, but the sensitivity of the organism to penicillin is steadily declining.[16]

Haemophilus influenzae

Ampicillin resistance, which is usually due to ß-lactamase production, was observed in 15.1% of UK isolates in 1995–6,[17] whilst ß-lactamase production rates of 20–30% have been found in Spain, Hong Kong, and North America.[11] Ciprofloxacin resistance is now also a threat. Besides clinical concerns regarding possible treatment failure, the increasing rate of resistance may also encourage the overuse of new, extended-spectrum antibiotics empirically.

Escherichia coli

Resistance to antibiotics such as ampicillin, trimethoprim, gentamicin and ciprofloxacin increased in UK hospital isolates during the 1990s, and increasing rates of resistance in community isolates endanger the use of traditional antibiotics, such as trimethoprim for urinary tract infection.

Enteric pathogens

Multi-drug resistant *Salmonella typhi* is now a major international health problem.[18] In the UK, non-typhoidal salmonella infection usually causes self-limiting disease, but can be life-threatening. Multi-resistant *S. typhimurium* DT-104 (resistant to fluoroquinolones such as ciprofloxacin and other antibiotics) is now widespread in the UK and elsewhere;[19] treatment failure and fatalities have been reported in human infection with this organism in Denmark.[20] Quinolone resistance in *Campylobacter* species is also an increasing problem.[21]

Staphylococcus aureus

Methicillin resistance has been reported in an alarming 34–47% of the *S. aureus* isolates in recent bacteraemia surveys in UK hospitals.[22, 23] Furthermore, MRSA isolates with intermediate resistance to vancomycin (VISA) have been reported from several countries since 1996,[24, 25] generating worldwide concern. The extent to which VISA organisms (also referred to as glycopeptide-intermediate *S. aureus* (GISA), a term which also encompasses teicoplanin

TABLE 1
Mechanisms of antibiotic resistance.

MECHANISM	CLINICALLY IMPORTANT EXAMPLES
ENZYMATIC MODIFICATION OF DRUG	
1. ß-lactamases	
Chromosomal ß-lactamases:	
Constitutive	Intrinsic resistance of Klebsiellae to ampicillin.
Inducible organisms and hyper-producing mutants	AmpC plasmid mediated cephalosporin resistance may be selected by cephalosporin exposure in enterobacteraciae including *Enterobacter cloacae*, *Citrobacter freundii*, and *Serratia marcescens*. *Pseudomonas aeruginosa* has a similar enzyme. *Stenotrophomomas maltophilia* possess broad, intrinsic, inducible ß-lactam resistance.
Plasmid or transposon mediated:	
'Classical' ß-lactamase	Staphylococcal penicillinase: widespread in *S. aureus* and coagulase-negative isolates. TEM-1 plasmid: the usual cause of ampicillin resistance in *E.coli*; it has minimal activity against third generation cephalosporins. SHV-1 is similar but less widespread.
Extended spectrum ß-lactamases (ESBLs)	Mutants (mainly TEM and SHV mutants) which may cause resistance to third generation cephalosporins in enterobacteraciae; *Klebsiellae pneumoniae* is a particular problem. Emerging ESBLs (e.g. IMP-1) confer resistance to imipenem and meropenem; still rare.
2. Aminoglycoside-modifying enzymes	
Acetylation, adenylation or phosphorylation of the drug	Important mechanism of plasmid or transposon mediated resistance in G- organisms including various enterobacteraciae and *P. aeruginosa*, as well as G+ cocci such as staphylococci and enterococci.
ALTERED DRUG TARGET	
1. Altered penicillin binding proteins (PBPs)	Penicillin resistance in *Streptococcus pneumoniae* occurs due to PBP modification. Methicillin resistance in *S. aureus* (MRSA) and coagulase-negative staphylococci occurs due to a *mecA* encoded PBP (PBP 2a) with decreased affinity for methicillin and other ß-lactams.
2. Cell-wall precursors with low affinity for glycopeptides	Five types (VanA, VanB, VanC, VanD and VanE) of vancomycin resistance now described in enterococci. Potential for future transfer to *S. aureus* a major concern.
3. Quinolone resistance: DNA gyrase and/or topoisomerase IV alterations	Mutations in the *gyr* and *par* genes alter DNA gyrase and topoisomerase IV enzymes respectively. Quinolone resistance has emerged in many organisms, including *S. aureus*, *P. aeruginosa*, and enteric pathogens such as *Salmonella* spp. and *Campylobacter* spp.
4. Rifampicin resistance: altered RNA polymerase	Mutations resulting in alterations in the ß-subunit of RNA polymerase result in rifampicin resistance in *S. aureus*, *Neisseria meningitidis* and *Mycobacterium tuberculosis*.
DECREASED UPTAKE OR INCREASED EFFLUX OF DRUG	
1. Reduced permeability	Reduced uptake may contribute to aminoglycoside resistance in *P. aeruginosa*, and loss of the specific OprD porin results in carbapenem resistance.
2. Increased efflux	One of several mechanisms of tetracycline resistance e.g. in enterobacteraciae. Macrolide resistance in *S. pneumonia* and *S. pyogenes* can result from *mefA* or *mefE* encoded efflux pumps (one of several mechanisms). Efflux operons encoded by Multiple EffluX (*mex*) genes, such as the *mexAmexB-oprM* operon in *P. aeruginosa,* are important for fluoroquinolone resistance in particular.

TABLE 2
Bacterial infections: current therapeutic concerns.

	ORGANISM	DRUG RESISTANCE CONCERN
Community acquired infections		
Gram-positive	*Streptococcus pneumoniae*	Penicillin, macrolides, (fluoroquinolones) and multidrug resistance (MDR)
	Streptococcus pyogenes	Macrolides
Gram-negative	*Neisseria gonorrhoea*	Penicillin; fluoroquinolone resistance widespread in some countries
	Neisseria meningitidis	Penicillin (concern for future)
	Haemophilus influenzae	Ampicillin; ciprofloxacin (now emerging)
	Moraxella catarrhalis	Ampicillin
	Escherichia coli	Ampicillin, trimethoprim, and fluoroquinolones
	Salmonella spp.	Fluoroquinolone and MDR
	Campylobacter spp.	Fluoroquinolone and MDR
	Shigella spp.	Fluoroquinolone and MDR
Mycobacteria	*Mycobacterium tuberculosis*	Isoniazid, rifampicin and MDR-TB
Hospital acquired infections		
Gram-positive	*Staphylococcus aureus*	Methicillin (MRSA) and MDR, including vancomycin intermediate MRSA (VISA)
	Coagulase negative staphylococci	Methicillin, vancomycin and MDR
	Enterococci	Vancomycin resistant enterococci (VRE)
Gram-negative	*Klebsiella pneumoniae* and *K. oxytoca*	Cephalosporins, aminoglycosides, fluoroquinolones, and MDR
	Other Enterbacteraciae, including *Escherichia coli*, *Serratia* spp., *Proteus* spp. and *Enterobacter* spp.	Cephalosporins, aminoglycosides, fluoroquinolones, and MDR
	Pseudomonas aeruginosa and other *Pseudomonas* spp.	Cephalosporins, aminoglycosides, fluoroquinolones, carbapenems, and MDR
	Acinetobacter baumannii	Cephalosporins, aminoglycosides, fluoroquinolones, carbapenems, and MDR

resistance) are likely to become a major clinical problem remains unclear,[26] but the possibility of high-level glycopeptide resistance and 'untreatable' *S. aureus* infections is a major concern.

Enterococci

Enterococcus faecalis and *Enterococcus faecium* are common causes of hospital-acquired bacteraemia. Vancomycin-resistant enterococci (VRE) produce modified cell-wall precursors with a reduced glycopeptide affinity by several different mechanisms.[27] A bacteraemia survey performed during 1997–9 in English hospitals found vancomycin resistance in 26% of *E. faecium* strains.[22] Vancomycin resistance in *E. faecium* increased almost four-fold between 1990 and 1998 in England and Wales.[23] Treatment options for invasive VRE infection had therefore become very limited until recently.

Klebsiella species and other enterobacteraciae

Reports of multi-drug resistant klebsiellae (MDRK) with plasmid-mediated extended spectrum ß-lactamases (ESBLs), which confer resistance to third-generation cephalosporins, first appeared in the mid-1980s. Many ESBLs have now been described in klebsiellae and other enterobacteria.[5] Outbreaks of MDRK, including a large outbreak in the north-east of Scotland,[28] typically start in ITU environments, where selection pressure from antibiotic use is an important factor. In a recent bacteraemia study in England, 12% of Klebsiella isolates were ceftazidime resistant whilst 8% were gentamicin-resistant and 14% ciprofloxacin resistant.[22] Much more alarming figures have been reported from hospitals in Europe.[10] Isolates of MDRK showing resistance to carbapenems, such as imipenem, have also been reported,[29, 30] and are a major concern for the future.

Pseudomonas aeruginosa

Drug-resistance can be a major problem in certain settings and patient groups, including intensive care units and cystic fibrosis patients. Encouragingly, resistance rates to aminoglycosides, ciprofloxacin and ceftazidime in the UK are still quite low by international standards and were stable during the 1990s, although resistance to carbapenems (imipenem and meropenem) increased significantly.[31] Carbapenem resistance is usually due to reduced bacterial uptake (due to the loss of the OprD2 porin), or increased efflux, and is often low-level. However, a plasmid transferable ß-lactamase (IMP-I) which confers high-level resistance to all ß-lactams including carbapenems was

identified in Japan in 1996,[32] and a similar enzyme has been isolated in the UK.[33]

Acinetobacter baumannii

This organism has become an important cause of hospital bacteraemia,[23] and drug-resistance is now a major problem. Acinetobacter species show the highest rates of antimicrobial resistance amongst Gram-negative bacteraemia isolates in English hospitals, and are commonly resistant to ceftazidime (35%), and ciprofloxacin (27%), aminoglycosides (12–14%) and also carbapenems (12%).[22] Even higher rates of resistance, with aminoglycoside resistance of around 50%, have been documented elsewhere in Europe,[10] prompting the re-evaluation of toxic and 'obsolete' drugs such as colistin for therapy.

Mycobacterium tuberculosis

Drug toxicity and drug interactions (particularly with rifampicin) are significant problems with the current first-line anti-tuberculous drugs. Drug-resistance has also become a major problem globally.[34] Outbreaks of multi-drug resistant tuberculosis (i.e. resistant to at least isoniazid and rifampicin) in the US in the early 1990s attracted widespread attention.[35] Multi-drug resistant tuberculosis (MDRTB) is also well recognised in the UK, with 60 cases recorded in 1996 (1.7% of all isolates).[36] Cases of MDRTB are exceptionally difficult and costly to treat, and clinical outcome is very poor even in immuno-competent patients.[37]

NEW ANTIBACTERIAL AGENTS

Quinupristin-dalfopristin

Streptogramin antibiotics have two structurally unrelated antibacterial compounds (streptogramins A and B) which act in synergism. Pristinamycin is an oral streptogramin which has been available in France for over two decades. Quinupristin and dalfopristin were derived from pristinamycin IA and IIB respectively, the two components of naturally occurring pristinamycin, and are combined in a 30:70 ratio to form a new antibiotic for intravenous use.

Quinupristin-dalfopristin is principally active against Gram-positive bacteria. The two drug components bind irreversibly to different sites on the bacterial 50s ribosomal sub-unit, thus inhibiting protein synthesis.[38] *In vitro*, quinupristin-dalfopristin is active against drug-resistant bacteria, including MRSA, penicillin-resistant pneumococci and many VRE isolates (*E. faecium* is usually sensitive whilst *E. faecalis* is generally resistant).[39, 40] Although cross-resistance between macrolides, lincosamides (such as clindamycin) and streptogramin B antibiotics occurs (so-called MLS_B resistance), streptogramin A antibiotics are not affected and quinupristin-dalfopristin remains effective.[38] However, in the face of MLS_B resistance, bacteriostatic rather than bacteriocidal activity against vancomycin resistant *E. faecium* occurs. Other mechanisms of streptogramin resistance are recognised,[41] but are currently uncommon.

Interest in quinupristin-dalfopristin, which is now licensed in the UK, has centred around its role in difficult Gram-positive infections. In randomised trials of its use in complicated Gram-positive skin and soft tissue infection (mainly staphylococcal, including MRSA), equivalent responses were observed with quinupristin-dalfopristin compared to controls (controls received vancomycin and/or a ß-lactam, depending on pathogen sensitivity).[42] Quinupristin-dalfopristin was also found to be equivalent in efficacy to vancomycin in Gram-positive nosocomial pneumonia.[43]

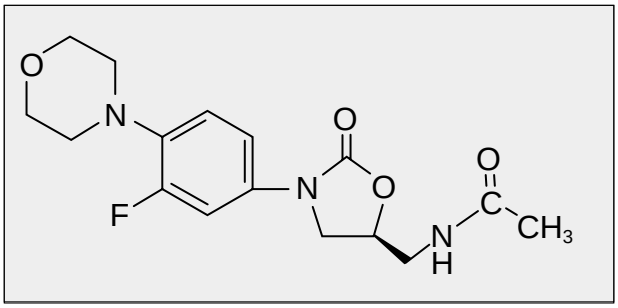

FIGURE 1
Linezolid structure.

Quinupristin-dalfopristin is one of the few available treatment options for vancomycin resistant *E. faecium* (VREF) infections. In two open-label studies of nearly 400 seriously ill patients with VREF infections, a successful overall treatment outcome was observed in 65% of the patients included in the analysis.[44] Of concern, drug-resistance has emerged during treatment of VREF infection, and super-infection with *E. faecalis* has also been observed.

The safety profile of quinupristin-dalfopristin is reasonable with regards to serious toxicity, but arthralgia, myalgia, gastro-intestinal upset and infusion-related phlebitis (necessitating administration using a central venous catheter) have emerged as problems.[44, 45]

Linezolid and the oxazolidinones

The oxazolidinones are a new class of antimicrobial agents with a unique chemical structure (Figure 1). Oxazolidinones appear to block protein synthesis by preventing the formation of the initiation complex in bacterial translation systems.[46] Whilst early oxazolidinones were limited by toxicity, the drug linezolid has a favourable pharmacokinetic and toxicity profile. Linezolid is virtually 100% bioavailable, and can be given either orally or intravenously. It was recently licensed in the UK.

Linezolid has bacteriostatic activity against a variety of Gram-positive organisms, but minimal Gram-negative cover. *In vitro* studies demonstrate that linezolid remains active against resistant Gram-positive pathogens including MRSA, penicillin-resistant pneumococci, macrolide resistant streptococci and VRE (both *E. faecalis* and *E. faecium*).[47, 48]

Clinical experience with linezolid is still limited. In a non-randomised study where linezolid was given on a compassionate use basis to patients with a variety of resistant Gram-positive infections (mainly VRE and MRSA), treatment success was reported in around 75% of the patients analysed.[49] The preliminary reports of randomised clinical trials where linezolid has been used in settings including nosocomial pneumonia and complicated soft tissue sepsis have also been encouraging.[50, 51] Linezolid is generally well tolerated[49, 52] and appears to be a genuine advance in the treatment of Gram-positive bacteria. However, there have been recent reports of drug-resistance emerging during the treatment of VRE infection.

New fluoroquinolone agents

A rather bewildering array of new fluoroquinolone agents

TABLE 3
Fluoroquinolone agents.

GROUP	AGENTS	COMMENTS
1. Older non-fluorinated quinlolone agents		
	Cinoxacin	Older agents with limited potency; use restricted to uncomplicated urinary tract infections.
	Nalidixic acid	
2. Current fluoroquinolones		
	Ciprofloxacin	The most potent current agent for *P. aeruginosa* infection, but limited activity against *S. pneumoniae* and other G+ bacteria. Good safety record.
	Grepafloxacin	Recently withdrawn in the UK (toxicity).
	Levofloxacin	The pure L-enantiomer of ofloxacin (active form of drug) so double the potency of ofloxacin. Active against *S. pneumonia*.
	Norfloxacin	The first fluoroquinolone. Limited potency.
	Ofloxacin	Similar to ciprofloxacin in most respects.
	Others	Perfloxacin, fleroxacin, enoxacin, lomefloxacin, rufloxacin (none currently used in UK).
3. The newer fluoroquinolones		
	Clinafloxacin	Broad spectrum, with improved G+ and G- cover (*P. aeruginosa* activity similar to ciprofloxacin). Toxicity a concern: phototoxicity and CNS effects.
	Gatifloxacin	Broad spectrum, with improved G+ cover. In late stages of clinical development. Licensed in the US.
	Gemifloxacin	Similar to gatifloxacin in most respects. License application recently rejected in the US.
	Moxifloxacin	Similar to gatifloxacin in most respects. Licensed in the US, but UK license application recently rejected.
	Sitafloxacin	Potent G+, G-, and anaerobic activity *in vitro*. Now undergoing clinical evaluation.
	Sparfloxacin	Broad spectrum, with improved G+ cover. Toxicity concerns: phototoxicity and QTc-prolongation. Licensed in the US, but not in the UK.
	Trovafloxacin	Broad spectrum, with, improved G+ cover and good anaerobic cover. Licensed in US, but hepatic toxicity has led to strict FDA restrictions on use.
	Others	Tosufloxacin, pazufloxacin.

is emerging, many of which are now licensed in the US, although it is not clear at present which of these drugs will gain license in the UK. Table 3 attempts to summarise the principal agents. The newer fluoroquinolones typically have enhanced action against Gram-positive organisms and pharmacokinetic characteristics which allow for once-daily dosing.

There is little to choose between many of the new agents on the basis of *in vitro* activity. Most have been targeted at respiratory tract infections and possess excellent activity against *S. pneumoniae* (including penicillin resistant strains), *H. influenzae*, *M. catarrhalis*, *L. pneumophilia* and *M. pneumoniae*.[53, 54] Multiple mutations in both the *gyrA* and *parC* genes of *S. pneumoniae* are needed for significant resistance to develop to the newer fluoroquinolones,[55, 56] with isolates which are resistant to older fluoroquinolones usually remaining sensitive.[54-56] Other Gram-positive bacteria including staphylococci also show increased sensitivity to the newer fluoroquinolones compared to their predecessors, although MRSA isolates (which are typically ciprofloxacin and ofloxacin-resistant) do show reduced sensitivity to newer drugs such as gemifloxacin and moxifloxacin.[54]

The newer agents typically have similar or slightly less activity against Gram-negative bacteria as compared to ciprofloxacin, although clinafloxacin and sitafloxacin are very active against Gram-negatives including *P. aeruginosa*.[54, 57] Several newer fluoroquinolones, including trovafloxacin, clinafloxacin and sitafloxacin,[57] show good activity against anaerobic bacteria.

Numerous randomised clinical trials of the newer fluoroquinolones have been reported or are ongoing. In general, they have performed at least as well as comparison drugs in the treatment of community-acquired pneumonia[58-60] and acute exacerbations of chronic bronchitis.[61, 62] Trovafloxacin has also been shown to be effective in the treatment of intra-abdominal and pelvic infection. To date there are disappointingly few clinical data regarding their efficacy in the more challenging drug-resistant bacterial infections.

Toxicity is an issue which has plagued the development of new fluoroquinolones. Whilst CNS side-effects, rash, phototoxicity, and tendon inflammation are well recognised with ciprofloxacin and ofloxacin, these drugs have had a fairly good safety record overall. However, infrequent but

serious side-effects (prolonged QTc and *torsades de pointes*) led to the withdrawal of grepafloxacin soon after its release. QTc prolongation also occurs with other fluoroquinolones (sparfloxacin in particular, but also moxifloxacin[63] and gemifloxacin[64]), and they should be avoided if prolonged QTc is present or where other drugs which cause QTc prolongation are used. Hepatotoxicity related to trovafloxacin use emerged during post-marketing surveillance in the US; its FDA licence has now been restricted to in-hospital treatment of life- or limb-threatening sepsis. Phototoxicity occurs with increased frequency with sparfloxacin, and also appears to be common with the potent new agent clinafloxacin.[65] The new agents gatifloxacin, gemifloxacin and moxifloxacin appear to be relatively well tolerated overall,[63, 64] but clinical experience is still limited.

How the new fluoroquinolones will be used remains to be seen, but one fears that they may be over-utilised as first-line agents. Agents will probably be chosen by their safety record as much as by consideration of efficacy. Meanwhile, research into new quinolone agents continues, with considerable current interest in a group of novel non-fluoroquinolone (NFQ) compounds.

New ß-lactam agents

Relatively little recent development has occurred in this field as compared to other areas. The so-called fourth generation cephalosporins have been introduced, with cefpirome and cefepime (not licensed in the UK) being the principal agents. They are characterised by their zwitterionic properties, conferring improved ability to rapidly cross the outer membrane of Gram-negative bacteria. Their antimicrobial spectrum is broader than third-generation agents such as cefotaxime: activity against *P. aeruginosa* and other Gram-negatives is enhanced (including strains which hyper-produce chromosomal ß-lactamase, but not ESBL-producing strains), whilst good Gram-positive cover is maintained.[66] Clinical trials confirm that they are effective in a variety of infections, including community- and hospital-acquired pneumonia,[67, 68] bacteraemia,[69] febrile neutropenia,[70] and intra-abdominal infection.[71] However, the clinical benefit of using these newer agents over the third-generation cephalosporins remains rather unclear.

Much of the recent research into cephalosporin agents has focused on developing agents which are effective against resistant Gram-positive organisms including MRSA, GISA, and VRE. A promising compound, RWJ-4428, is currently undergoing pre-clinical evaluation.[72]

The carbapenem agents, imipenem/cilastatin and meropenem, were important additions to our antimicrobial armementarium, with broad Gram-positive and Gram-negative activity. However, gaps in cover exist, including a lack of activity against MRSA and certain other organisms such as *Stenotrophomonas maltophilia*. Furthermore, the prospect of widespread future carbapenem resistance developing in organisms such as *P. aeruginosa*, *A. baumanii* and *K. pneumoniae* is a significant concern. Advances have been made in the development of new carbapenems (reviewed elsewhere),[73] but new drugs with significant advantages in cover have proved elusive.

The use of ß-lactamase inhibitors to 'protect' a ß-lactam compound has been successfully exploited in several compounds. Piperacillin-tazobactam, introduced relatively recently, is active against most Gram-positive and Gram-negative bacteria, including anaerobes. Piperacillin-tazobactam in combination with amikacin has proved a successful alternative to ceftazidime with amikacin in the treatment of nosocomial pneumonia[74, 75] and febrile neutropenia.[76] Piperacillin-tazobactam is also an effective agent for severe intra-abdominal sepsis.[74]

New drugs for tuberculosis

This area has been reviewed in detail elsewhere.[77] Two new rifamycin agents, rifabutin and rifapentine, have emerged recently. Rifabutin offers several potential advantages over rifampicin, including *in vitro* efficacy against some strains of *M. tuberculosis* which display low-grade rifampicin resistance.[78] Clinical trials have confirmed that rifabutin can be successfully substituted for rifampicin within a standard four-drug regimen,[79] but its role in the treatment of rifampicin-resistant tuberculosis is probably limited.[80] The long half-life of rifapentine is the principal attraction of this drug, allowing once- or twice-weekly administration. It is now licensed in the US, despite concerns that relapses may be more frequent when rifapentine is substituted for rifampicin.[81]

Ciprofloxacin and ofloxacin have been extensively used in TB treatment over the last decade, despite very limited clinical data to support such use.[80, 82, 83] Levofloxacin and newer fluoroquinolones, such as sparfloxacin[84] and moxifloxacin,[85] are more active against *M. tuberculosis*, but concerns over toxicity have halted the clinical assessment of sparfloxacin. Pre-clinical studies suggest a potential role for various other drugs, including linezolid and other oxazolidinones.[86]

Other emerging antibacterial agents

Many novel macrolides and ketolide agents (the 3-keto derivatives of macrolides) have been synthesised (summarised by Bryskier).[87] Ketolides such as telithromycin (HMR 3647) and ABT-773 retain activity against erythromycin resistant bacterial strains and look promising,[88, 89] but are still at an early stage of development. Everninomicin, a novel agent with activity against a variety of Gram-positive organisms including drug-resistant isolates, is another promising drug.[90] Other drugs under investigation include daptomycin (a unique semi-synthetic peptolide agent),[91] new glycopeptides, and the glycylcyline agents (tetracycline derivatives).

VIRAL INFECTIONS: WHAT ARE THE PROBLEMS?

Unlike bacterial infections, effective treatment options have yet to be developed for many viral infections. However, in infections where antiviral agents have been widely used, drug-resistance has become an important issue. Furthermore, the dynamics of many viral infections such as herpes virus infections and HIV, where treatment suppresses but does not eradicate the infection, may predispose to the development of resistance. Important current concerns are summarised in Table 4 and discussed below.

Influenza

Influenza is a major cause of morbidity and mortality worldwide. In England and Wales, an estimated average excess of 420,000 consultations with flu-like illness occur

TABLE 4
Viral infections: current therapeutic issues.

Viral respiratory tract infections		
	Influenza A and B	Major cause of morbidity and mortality, with pandemic potential (influenza A). Effective drugs now available, but controversial.
	Rhinoviruses	Significant morbidity and societal costs. No treatment currently available.
	Respiratory syncytial virus (RSV)	Nebulized ribavirin used in context of severe bronchiolitis, but is teratogenic. Palivizumab (monoclonal antibody) now available for RSV prevention in high-risk infants.
Blood-borne viruses		
	Human immunodeficiency virus (HIV)	Many drugs now available. Viral resistance, toxicity, drug interactions, 'pill-burden' and cost remain important issues.
	Hepatitis B virus (HBV)	Major cause of chronic liver disease and hepatoma worldwide. Response to interferon alpha monotherapy relatively poor; alternatives are evolving.
	Hepatitis C virus (HCV)	As for HBV.
Herpes viruses		
	Herpes simplex virus (HSV)	Cause of significant morbidity, particularly in the immunocompromised. Aciclovir effective, but poorly absorbed. Alternative agents now available.
	Varicella-zoster virus (VZV)	As for HSV.
	Cytomegalovirus (CMV)	Major cause of morbidity and mortality in the immunocompromised. Ganciclovir and foscarnet effective but toxic, and resistance a problem. Alternative agents now available.
Other		
	Enteroviruses	Very common. Usually cause mild illness, but can be life-threatening. No effective treatment available at present.
	Haemorrhagic fever viruses (Lassa fever, hantavirus)	Ribavirin shown to be of benefit in these severe illnesses.

annually during the winter influenza epidemic period, with over 9,000 hospital admissions and 12,500 excess deaths.[92] Pandemic influenza A also occurs at regular intervals, with truly devastating consequences: over 20 million deaths occurred during the 1918 pandemic. An influenza outbreak in 1997 in Hong Kong due to a virulent avian influenza A virus caused widespread concern about a possible influenza pandemic.

Interest in new drugs for influenza chemoprophylaxis and/or treatment has been intense. Amantadine and rimantadine (not used in the UK), the only anti-influenza drugs available until recently, suffer from several limitations, including their lack of activity against influenza B virus, poor side-effect profile, and the relative ease with which drug-resistant mutants emerge during therapy.

Human immunodeficiency virus (HIV)

The tragic consequences of the global spread of HIV, and the lack of affordable treatment options for the vast majority of the world's population who are infected by the virus, dominated the discussion at the recent XIII International AIDS Conference in Durban (July 2000). In Western nations, by contrast, recent advances in antiretroviral therapy have significantly improved the outlook for people living with HIV and AIDS.

Important advances in our understanding of the dynamics of HIV replication have occurred in parallel with major therapeutic advances. The rapid turnover of HIV, with some ten^{10} viruses being produced daily in an error-prone replication cycle, implies that combination drug therapy is essential to suppress viral replication effectively and prevent drug-resistant mutants from emerging.[93, 94] Potent new drug regimens (see below) and the introduction of quantitative plasma HIV RNA determination has allowed us to set the ambitious goal of suppressing plasma HIV viraemia to undetectable levels.[95, 96]

Resistance to zidovudine (AZT) was detected soon after its introduction, and drug-resistant HIV emerges despite the use of potent regimens. Mutations conferring resistance emerge under the selective influence of treatment, and correlate with treatment failure.[97] A sequential accumulation of resistance mutations over time

may be needed for high-grade resistance, but for some drugs only a single mutation is needed: resistance to the drug lamivudine and to the non-nucleoside reverse transcriptase inhibitors are important examples. Furthermore, partial or complete cross-resistance between drugs of the same class frequently occurs,[98] driving the constant search for new drugs and drug classes.

Hepatitis B and C

Chronic hepatitis B (HBV) infection affects in excess of 300 million people worldwide. Until recently, interferon alpha was the only licensed treatment option for HBV. Interferon therapy only rarely eradicates HBV infection, but does result in the loss of hepatitis B e antigen (HBeAg) and HBV DNA in some 25–40% of recipients,[99] and this correlates with improved prognosis[100] and reduced infectivity. However, interferon therapy typically causes significant side-effects, including fatigue, myalgia, mood disturbance, neutropenia and thrombocytopenia.

Chronic hepatitis C (HCV) infection is another enormous international health problem, with an estimated 170 million people infected worldwide. Like HBV, chronic HCV infection may cause cirrhosis, hepatocellular carcinoma and death. HCV is the cause of 70% of hepatocellular carcinoma in Japan, and 30–50% in the US.[101] Interferon alpha was the only licensed treatment of chronic HCV infection until recently. Response to interferon monotherapy is poor: using three mega-units thrice weekly for six months, the sustained virological response rate is only 10–15%, although this can be improved a little by prolonging the treatment period to 12–18 months and/or giving higher doses.[102]

Herpes simplex virus (HSV) and varicella zoster virus (VZV)

These viruses cause significant morbidity. Treatment is not necessary for mild infections such as minor cold sores and uncomplicated childhood chickenpox, but is essential for life-threatening disseminated infection. Aciclovir, an analogue of 2'-deoxyguanosine, remains a very useful drug for both HSV and VZV, although it is significantly less active against the latter virus. Intravenous aciclovir is still the drug of choice for severe infections and treatment of the immunocompromised. The five times daily dosing schedule and limited bio-availability are drawbacks of the oral formulation.

Aciclovir requires intracellular phosphorylation to generate its active form, aciclovir triphosphate, with the first phosphorylation stage being catalysed by a thymidine kinase (TK) induced by viral infection. Drug-resistance is uncommon in immunocompetent individuals even after prolonged therapy for HSV.[103] However, aciclovir-resistant HSV is not uncommon in the immunocompromised, where resistance is usually caused by TK-deficient mutants.[104] Aciclovir resistant mutants show cross-resistance with ganciclovir, but generally still respond to the pyrophosphate analogue foscarnet, as this drug (which has to be given intravenously) does not require intracellular activation.

Cytomegalovirus (CMV)

The prophylaxis and treatment of CMV infection in immunocompromised hosts is a difficult area. The two drugs for which there is most clinical experience, intravenous ganciclovir and foscarnet, are both toxic agents with a propensity to cause bone-marrow and renal toxicity respectively. Furthermore, drug resistance and treatment failure are well recognised, although treatment failure is not always related to acquired resistance.

Ganciclovir requires phosphorylation to a triphosphate form to be active, with the initial phosphorylation stage being mediated by a unique CMV viral kinase, the UL97 gene product. Ganciclovir resistance is most commonly related to mutations in the UL97 gene; these mutants remain sensitive to foscarnet. Foscarnet resistance occurs with certain mutations in the UL54 gene encoding DNA polymerase, which may confer cross-resistance to ganciclovir as well.[105]

Picornaviruses

The picornaviruses encompass both the enteroviruses and the rhinovirus group. They are the commonest cause of human viral infection. Rhinovirus infection causes much minor morbidity in the form of the common cold, and may also cause serious illness where there is underlying cardiopulmonary disease.[106] The enteroviruses are a large and diverse group, which can produce a variety of infections ranging from mild respiratory tract infection to severe illnesses such as myocarditis and meningo-encephalitis. No treatment for picornaviral infection has been available until recently, although a cure for the common cold has been a sought after goal for many years.

NEW ANTIVIRAL AGENTS

The neuraminidase inhibitors: new agents for treating influenza

Neuraminidase cleaves terminal sialic acid residues from glycoproteins, which constitutes an essential step in influenza virus replication and pathogenicity. Determination of the three-dimensional structure of influenza virus neuraminidase allowed for the development of potent and specific competitive inhibitors. Zanamivir and oseltamivir are the principal current agents. Zanamivir is administered by inhalation as it has poor oral bio-availability, whereas oseltamivir, an ethyl ester prodrug of the active compound, can be given orally. Both drugs are administered twice daily and are effective against both influenza A and B viruses.

In clinical trials both zanamivir and oseltamivir (commenced within 36–48 hours of symptom onset and continued for five days) were shown to be effective in significantly reducing the duration and severity of symptoms of acute influenza.[107, 108] They are also effective when administered as chemoprophylactic agents over a four to six week period, with a protective efficacy of around 70%.[109, 110] Both drugs are generally well tolerated, although bronchospasm can be precipitated by zanamivir therapy, whilst nausea occurs in around 12% of patients receiving oseltamivir.[108] Viral resistance has been much less of a problem than with earlier drugs such as amantadine.

The appropriate use of neuraminidase inhibitors has been the subject of much debate. In 1999 the National Institute for Clinical Excellence (NICE) in England and Wales considered the available data on zanamivir to be insufficient to conclude that treatment reduced the frequency of serious complications in high-risk patients, but revised this opinion in November 2000. The current NICE guidance recommends the targeted use of zanamivir for at-risk adults only (chronic respiratory or cardiac disease, immunocompromised, or aged >65), but only when influenza is circulating in the community and where

treatment can be commenced within 48 hours of symptom onset. This approach to zanamivir use is also currently adopted in Scotland.

Therapy for HSV and VZV infection

Two new drugs, famciclovir and valaciclovir, were introduced during the 1990s. Famciclovir is the oral prodrug of penciclovir, whilst valaciclovir is an ester prodrug of aciclovir, which has better pharmacokinetic properties than the parent compound. Both are now widely used in the UK for the treatment of herpes zoster and mucocutaneous HSV infection. They are both effective in the treatment of acute herpes zoster in immuno-competent individuals,[111, 112] as well for the treatment and suppression of genital herpes.[113, 114] They offer more convenient dosing schedules than aciclovir, but are also more expensive. Drug-resistance in immuno-competent individuals appears to be very uncommon, even after prolonged suppressive treatment for genital HSV infection.[113] Laboratory studies demonstrate cross-resistance between famciclovir (penciclovir) and valaciclovir (aciclovir) in TK-deficient HSV mutants, although partial mutants may remain penciclovir-sensitive.[115]

New options for CMV infection in the immuno compromised

Several new options for treating CMV disease became available during the 1990s. Cidofovir, a nucleotide analogue, is an effective alternative to ganciclovir and foscarnet in acute CMV retinitis in AIDS patients. It only needs to be given weekly (induction) or every two weeks (maintenance), thus avoiding the need for long-term central venous access. Unfortunately, it may cause severe nephrotoxicity, although intravenous hydration and the use of probenecid reduce the risk of this complication.[116] Ganciclovir-resistant CMV isolates with UL54 mutations show cross-resistance with cidofovir,[105] although at present such isolates are uncommon clinically.

Formavirsin is an interesting new drug which was recently licensed for the treatment of CMV retinitis. It is an antisense oligonucleotide which specifically inhibits CMV replication by binding to complementary sequences of viral immediate-early (IE) RNA. It has to be administered by direct injection into the vitreous humor. Preliminary studies, summarised elsewhere,[117] indicate that it is generally well tolerated and can be effective even in refractory disease.

Alternative methods for ganciclovir administration are now also available. In CMV retinitis, ganciclovir may be given by regular intraocular injection or by an intraocular ganciclovir implant. The implant is highly effective in acute CMV retinitis and works for a six-month period. However, ocular complications including retinal detachment can occur, and extraocular CMV disease may develop.[118]

Oral ganciclovir is now available for primary and secondary CMV prophylaxis (not acute treatment), but is poorly absorbed. Its efficacy in HIV-infected patients is unclear as studies have produced conflicting findings.[119, 120] Oral ganciclovir has been used successfully in patients with solid-organ transplants.[121] However, breakthrough disease with resistant virus can occur in transplant patients, and low drug levels may be an important risk factor.[122] A better absorbed valyl ester prodrug of ganciclovir, valganciclovir, is currently being developed.

Antiretroviral drugs

Advances in antiretroviral therapy have been taking place at a breathtaking pace in recent years, and it would be beyond the scope of this article to review these in detail. Current antiretroviral agents (most of which are now licensed in the UK or widely available through expanded access programmes) are summarised in Table 5.

The nucleoside analogue reverse transcriptase inhibitors (NRTIs) were the first antiretroviral class to be developed. Zidovudine (AZT), the first agent, was shown to prolong survival in AIDS patients in 1987. Didanosine (ddI) and zalcitabine (ddC) were the next NRTI agents to be developed. Clinical trials reported in the mid-1990s demonstrated that using zidovudine, in combination with either didanosine or zalcitabine, slowed disease progression and improved survival significantly compared to zidovudine monotherapy.[123, 124]

Drugs which inhibit the HIV protease enzyme became available in the mid- to late-1990s. The protease inhibitors (PIs) are potent agents, typically reducing HIV RNA levels by a factor of 100- to 1,000-fold when given as mono-therapy, with a parallel rise in CD4+ cell count being observed.[125] Predictably, treatment responses to PI monotherapy are not sustained. However, the important ACTG 320 study demonstrated that giving the protease inhibitor indinavir in combination with two NRTI drugs (lamivudine and zidovudine) not only produced a sustained virological and immunological response, but also significantly improved clinical outcome (time to clinical progression or death) when compared to using the two NRTIs alone.[126] Other studies have confirmed the efficacy of combination regimens containing one or more protease inhibitor.[127, 128]

The third class of agents are the non-nucleoside reverse transcriptase inhibitors (NNRTIs), of which two drugs (nevirapine and efavirenz) are currently licensed in the UK. There is now good clinical trial evidence, including clinical end-point data, to demonstrate that the NNRTI efavirenz is as effective as the PI agent indinavir when given in combination with two NRTI agents.[129] Nevirapine is also an effective antiviral,[130] and is emerging as an important agent for the prevention of perinatal HIV transmission.

Aggressive combination therapy (often referred to as HAART – highly active anti-retroviral therapy) has resulted in a dramatic fall in HIV-related morbidity and mortality in Western countries.[131, 132] Sustained viral suppression is now an appropriate and realistic therapeutic goal,[95, 96] and maximal suppression correlates with long-term response.[133] Whilst previous mathematical models suggested that maximal suppression for around three years might eliminate HIV infection,[134] it is now clear that viral persistence occurs even after prolonged, suppressive treatment.[135]

HAART has its problems. Besides cost, these include a large 'pill-burden' with many regimes, food restrictions, drug toxicity, and drug interactions (see Table 5). The occurrence of body fat distribution abnormalities (lipodystrophy) and raised blood lipids in patients receiving HAART is a particular concern,[136] although the cause of this syndrome(s) remains controversial. In the face of complex regimens and possible toxicity, adherence to therapy can be poor, with sub-optimal adherence (<95%) predisposing to therapeutic failure.[137] Strategies aimed at improving adherence, which include the use of regimens aimed at minimising toxicity and reducing the 'pill-burden', are essential.

TABLE 5
Current anti-retroviral agents.

CLASS	ACTION	AGENTS	COMMENTS ON DRUG CLASS	MAJOR SIDE-EFFECTS*
Nucleoside reverse transcriptase inhibitors (NRTIs)	Inhibit reverse transcriptase	Zidovudine (AZT) Didanosine (ddI) Zalcitabine (ddC) Lamivudine (3TC) Stavudine (d4T) Abacavir	The first available drug class (AZT) Low 'pill burden' Usually once- or twice-daily administration Only ddI has significant food interactions Few drug interactions Side-effect profiles vary considerably for the different drugs, but overlap. Mitochondrial toxicity may underlie many forms of toxicity.	Gastrointestinal upset (AZT, ddI) Neuropathy (ddI, ddC, d4T) Pancreatitis (ddI, ddC) Stomatitis (ddC) Bone-marrow suppression (AZT) Hypersensitivity (Abacavir, in ~4%) Lactic acidosis and hepatic steatosis (all: a rare but potentially fatal class effect). Lipodystrophy (d4T, ? all)
Non-nucleoside reverse transcriptase inhibitors (NNRTIs)	Inhibit reverse transcriptase	Nevirapine Efavirenz Delavradine	Emerging as effective alternatives to PIs Low 'pill burden' Once- or twice-daily administration Minor food interactions (efavirenz only) Significant drug interactions (P450 mediated) Single mutation confers NNRTI-resistance	Rash (all, but commonest with nevirapine; usually mild, but rarely Stevens-Johnson Syndrome). Neuro-psychiatric effects (Efavirenz) Hepatotoxicity (all) Overall, less long-term toxicity than with PIs (?).
Protease inhibitors (PIs)	Inhibit viral protease enzyme	Saquinavir Indinavir Ritonavir Nelfinavir Amprenavir Lopinavir/ritonavir	Clinical end-point data confirm the efficacy of several of these potent drugs. High 'pill burden' (up to 18 tablets/day) Strict 8- or 12-hrly administration needed Significant food restrictions Significant drug interactions (P450 mediated) Toxicity a problem. Profiles vary for individual drugs, but overlap. PIs can be combined for pharmacokinetic and anti-viral enhancement.	Gastrointestinal upset (all) Rash (amprenavir, indinavir) Paresthesias (ritonavir, amprenavir) Renal stones (indinavir) Taste perversion (ritonavir) Glucose intolerance (all) Hepatotoxicity (all) Bleeding tendency in haemophiliacs (all) Lipodystrophy (all) Blood lipid abnormalities (all, particularly ritonavir): hypercholesterolaemia, hypertriglyceridaemia +/- lipodystrophy.

*Drugs in parentheses indicate the commonest cause(s) of a particular side-effect, rather than that side-effect is necessarily confined to the given drug.

Many factors need to be taken into consideration when planning initial treatment. These include the stage of the disease, previous treatment history, co-medications, potential for toxicity and drug interactions, and the patient's lifestyle and wishes. Choosing an appropriate second- or third-line regimen following treatment failure is even more difficult. Recent studies have highlighted the benefit of viral-resistance determination,[138, 139] potentially combined with therapeutic drug level monitoring,[140] in this situation.

Antiretroviral therapy will undoubtedly continue to evolve rapidly. A number of new NRTI, NNRTI and PI agents are being developed, and new classes of drugs such as fusion inhibitors[141] are being evaluated. Other areas of interest include the simplification of PI-containing regimens by pharmacokinetic means, 'class-sparing' regimens,[142] the development of cytokine therapy[143] as well as the use of therapeutic vaccines.[144]

New drugs for chronic hepatitis B and C

HBV has an unusual replication cycle which involves DNA synthesis from RNA by reverse transcription, rendering the virus susceptible to HBV reverse transcriptase inhibitors. Lamivudine (3TC), a nucleoside analogue which is also used in HIV treatment, is now licensed for chronic HBV infection. Oral lamivudine therapy for a 12-month period was associated with histological improvement in around half of the treated patients, compared to a quarter of controls, in clinical trials of chronic HBV infection.[145, 146] Improvements in liver function tests, suppression of HBV DNA, and higher rates of HBeAg loss compared to placebo (16–17% vs. 4–6%) also occurred, and the drug was well tolerated.[145, 146] Unfortunately, HBV viraemia tends to recur after lamivudine therapy has been discontinued,[145] and treatment also results in the selection of drug resistant viral mutants.[145, 146] Other treatment options for HBV are currently under evaluation, with a particular focus on other nucleoside and nucleotide analogues. As in HIV disease, combination drug therapy and/or immunotherapy are likely to be the way forward.

In HCV infection, ribavirin, a synthetic guanosine nucleoside analogue previously used for RSV infection, has recently established itself as an important 'new' therapeutic agent. Randomised clinical trials have shown that combining thrice-weekly subcutaneous interferon injections with the daily oral ribavirin, for a six to 12 month period, significantly improves the sustained virological response rate to around 30–40%.[147, 148] Additional toxicity is generally limited, although ribavirin-induced haemolysis can occur. Ribavirin is also potentially teratogenic. International guidelines support the use of ribavirin-interferon combination therapy over interferon monotherapy,[149] and this position was endorsed in Scotland in the recent Scottish Needs Assessment Programme (SNAP) report on HCV[150] and a report by NICE.[151]

Another advance in HCV therapy has been the development of pegylated interferons. Attachment of a polymerized polyethylene glycol (PEG) chain to an interferon results in a protein with a much longer half-life, which allows for weekly injections rather than the thrice-weekly regime needed with conventional interferons. Two pegylated interferons are currently being evaluated: an interferon alpha-2b molecule attached to a 12-kD PEG chain and an interferon alpha-2a molecule attached to a 40-kD branched PEG molecule. Treatment responses with both of these PEG-interferon compounds are significantly improved compared to conventional interferons.[152–154]

Clinical trials are in progress to examine the combination of PEG-interferon with ribavirin. The results of a large, recent multi-centre trial of PEG-interferon alpha-2b and oral ribavirin found an overall sustained response rate of 54% using PEG-interferon plus ribavirin, compared to 47% using standard interferon plus ribavirin.[155] PEG-interferon alpha-2b has now been licensed in the UK for use either as monotherapy or in combination with ribavirin.

A number of other strategies for HCV therapy are also at various stages of development. These include combination therapy using amantidine,[156] immunotherapy with cytokines such as IL-10,[157] antisense oligonucleotides,[158] and research into novel inhibitors of the enzymes involved in viral replication such as helicase, protease and polymerase.[159]

Therapy for picornaviral infections

Pleconaril is a new, oral, antiviral drug, which is active against enteroviruses and rhinoviruses. It blocks viral uncoating, viral attachment to host cell receptors, and transmission of infectious virus particles.[160] Oral pleconaril reduced symptom severity and viral shedding in experimental coxsackie A21 respiratory infection.[161] Clinical trials of pleconaril therapy for viral meningitis and respiratory tract infections, summarised by Rotbart,[162] are underway.

CONCLUSION

An overview of the important problem areas in the treatment of bacterial and viral infections has been provided in this article, and recent therapeutic advances have been reviewed. Several promising new drugs have emerged or are emerging, and it is clear that the 'post-antimicrobial era' predicted by Cohen in 1992 has not come to pass.[162] Nevertheless, it could be argued that no really major breakthroughs in our ongoing battle against drug-resistant bacterial and viral infections have emerged. The importance of conserving our current agents by appropriate and targeted use cannot be overstated, and a responsible and conservative approach to the introduction of the new drugs is also crucial.

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