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New ways to treat bacterial infections

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There is an urgent need for fresh approaches to the treatment of bacterial infections because of the changing patterns of infectious disease and the emergence of bacterial strains resistant to current antibiotics. Modification of the cell phenotype to sensitize bacteria to components of the hosts' immune system or to previously ineffective antibiotics could prevent the emergence of the resistant genotype. In addition, the use of light-activated antibacterial agents and lytic bacteriophage specific for key pathogens should be considered as safe and inexpensive alternatives to conventional treatment regimens for certain non-systemic infections.

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▼ The introduction of antibacterial drugs into the clinic, which began in the 1930s, coupled with the use of safe and effective vaccines, gave rise to the belief that infectious diseases could be controlled and, eventually, mastered. Although antibiotics have saved countless lives and transformed the practice of medicine, the initial widespread optimism has proven to be premature and infections remain the leading cause of mortality worldwide [1]. The financial burden placed on the healthcare budgets of developed nations by infectious diseases is huge: for example, in the UK it has been estimated that hospital-acquired (nosocomial) infection costs the National Health Service approximately £1 billion and causes 5000 deaths per annum [2].

Several factors have affected our ability to control nosocomial and community-acquired infectious diseases. There is continuing change in the spectrum of pathogens that cause infections in humans and social, political, economic and environmental factors all impact on the spread of infection. 'New' agents, such as HIV, with the capacity to cause a wide range of apparently novel infections can emerge, although they might have been present for decades until revealed by sophisticated diagnostic procedures. In addition, there are several important diseases in which infectious agents have only recently been implicated, such as *Helicobacter pylori*, now known to have a central role in the aetiology of gastric diseases [3]. Therapeutic advances have increased the potential for the survival of patients with severe underlying disease who have a high susceptibility to infection with opportunistic pathogens. Such immunocompromized individuals are susceptible to infectious agents that would not pose a problem in healthy individuals and are often treated with antibiotics for long time periods. In addition, the introduction of new investigational and therapeutic modalities is often accompanied by infections, some caused by the iatrogenic breaching of host defences during blood transfusion or organ transplantation [4]. Also, there has been a deterioration in social conditions in both developing and industrialized countries that has contributed to the re-emergence of some infectious diseases, such as tuberculosis.

Antibiotic-resistant pathogens pose an enormous threat to the treatment of a wide range of serious infections. Nosocomial and community-acquired agents have developed resistance to a wide range of antibiotics [5] and have proven to be highly successful in their ability to develop resistance mechanisms, often transferable, against virtually all commonly used antibiotics. Currently, the greatest cause for concern is infection by strains of *Staphylococcus aureus,* enterococci and pneumococci, displaying acquired resistance to six or more antibiotics. In these cases, the only established treatment is with vancomycin or teicoplanin and the first report of fully vancomycin-resistant *S. aureus* has recently appeared [6].

This changing pattern of disease and the emergence of bacterial strains resistant to many currently used antibiotics makes the need for fresh approaches to the treatment of infections a medical priority. A recent edition of the British National Formulary [7] lists 63 antibiotics that are available for the treatment of bacterial infections. This apparently large number is deceptive; many of these

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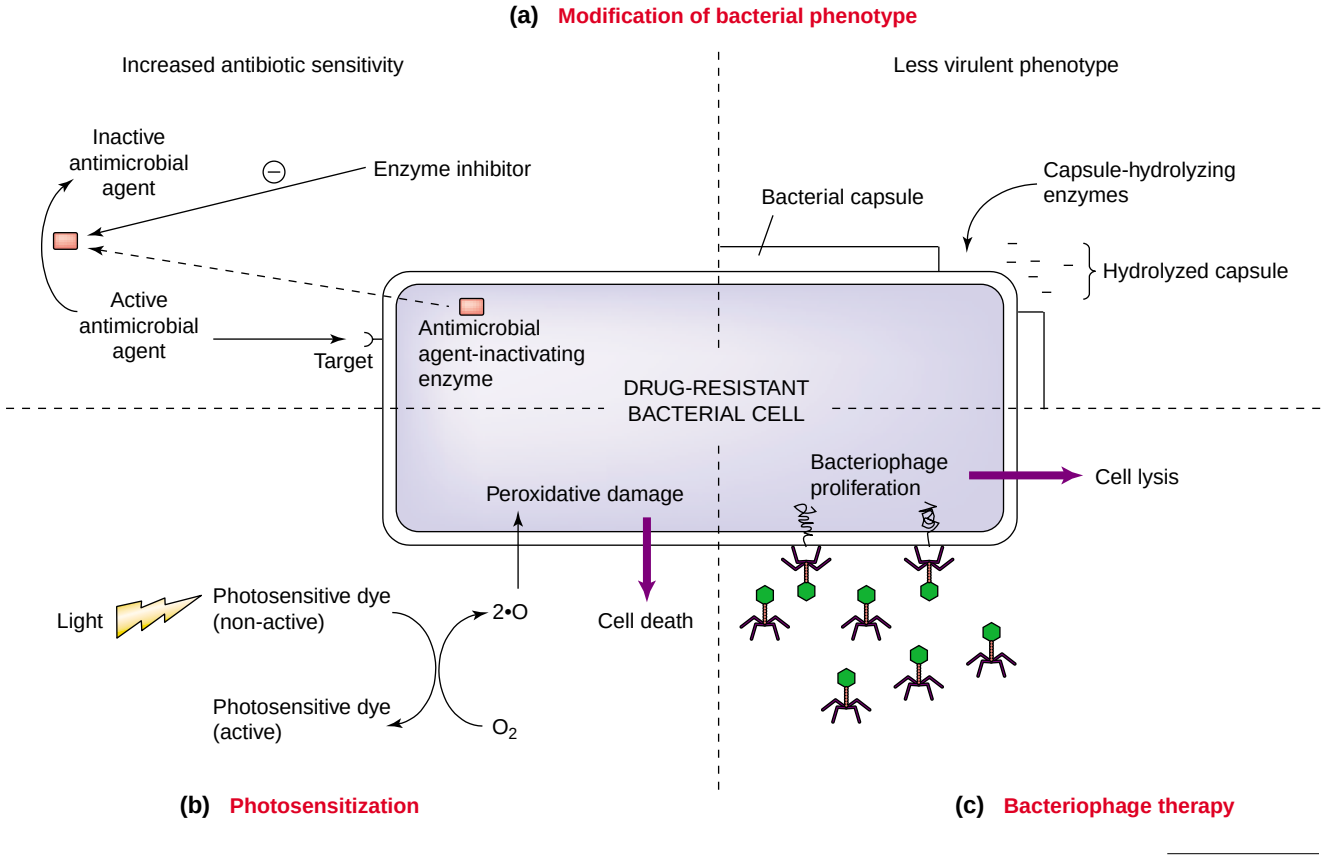

Figure 1. Three approaches to the treatment of infections caused by drug-resistant bacteria: Phenotypic modification, photosensitization and bacteriophage therapy. Increased antibiotic senstivity might result from inhibition of expression of antibiotic resistance determinants in genotypically resistant bacterial cells. Conversion to a less fit phenotype will enable host defences to eliminate the invading pathogen. The combination of light and photosensitive agent could combine to generate lethal singlet oxygen. Bacteriophages specific for invading pathogens can lyse bacteria at the site of infection.

compounds are structurally related, almost half are β-lactams and in total they are directed against only a few biochemical targets within the bacterial cell. The careful management of infections with a larger palette of active molecules, supplemented with drugs having fundamentally different modes of action, could begin to reduce the incidence of antibiotic resistance among the main groups of bacterial pathogens. The major pharmaceutical companies have tended to concentrate their antibacterial drug discovery effort on the improvement of well-established parent structures, and a recent survey [8] has indicated that the major pharmaceutical companies continue to give priority to the search for improved antibiotics in established classes. The problem of antibiotic resistance has been compounded by the development of many broad-spectrum antibiotics, whereas the patient population might be better served by more selective medicines with activity restricted against small groups of pathogens. However, such fragmentation of the antibacterial armamentarium does not represent an attractive commercial scenario and so might not be pursued by the pharmaceutical industry.

Most of the existing classes of antibiotics are bactericidal or bacteriostatic agents that were discovered by systematic screening of natural product libraries within two decades of the introduction of penicillin. In the short term, further chemical modification of existing antibiotics could have a significant impact on antibacterial therapy. The wealth of new information that has emerged as a result of the sequencing of over 40 microbial genomes, together with advances in screening technology and combinatorial synthesis, will provide a future platform for the discovery of new antibiotics. With the dissemination of this technology comes the risk that non-genomic approaches to the therapy of bacterial infections will be cast aside – even though they yielded a stream of effective medicines earlier in the antibiotic era. Some of these non-genomic technologies are considered here (see also Fig. 1).

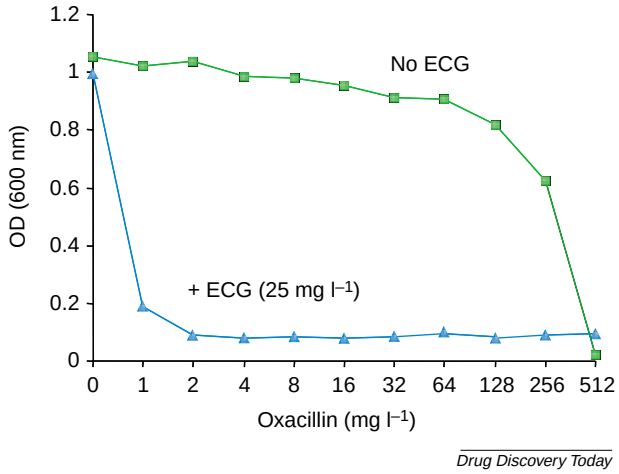

Fig. 2. Modulation of oxacillin resistance in epidemic methicillin-resistant *Staphylococcus aureus* 16 (EMRSA-16) by epicatechin gallate (ECG). EMRSA-16 was grown in Mueller–Hinton broth containing various concentrations of oxacillin and in the presence or absence of a fixed concentration of ECG (25 mg l^{-1}). The ability of ECG to modulate oxacillin resistance was assessed by measurement of the optical density (OD) of the cultures after 18 h growth at 30°C. ECG (25 mg l^{-1}) reduced the minimum inhibitory concentration (MIC) of oxacillin for EMRSA-16 from 512 to 2 mg l^{-1}.

Modification of the bacterial phenotype

The selective pressure imposed by conventional antimicrobial chemotherapy frequently results in the emergence of drug resistance. Bactericidal or bacteriostatic agents facilitate the emergence (by selection) and dissemination (by clonal spread), of antibiotic resistance genes and thus alter the genotypic makeup of bacterial populations. An alternative approach to the treatment of infectious disease could be based on the use of agents that do not kill pathogenic bacteria but modify them to produce a 'less fit' phenotype that is unable to survive under the conditions that pertain at the site of infection. Such agents could render the pathogen susceptible to a previously ineffective antibiotic or to a component of the host's immune system. Because the modifying agent applies little or no direct selective pressure, this concept could slow down or prevent the emergence of resistant genotypes.

Phenotypic conversion to antibiotic sensitivity

The inhibition of expression of antibiotic resistance mechanisms could revive the use of older antibiotics that have become less efficacious because of the emergence and spread of resistance determinants. There is little doubt that compounds that restore the sensitivity of major pathogens to established antibiotics would be invaluable aids to effective therapy and would have a significant impact on the cost of treatment [9]. This strategy has already been successfully exploited through the use of β-lactamase inhibitors in combination with a β-lactam antibiotic; the combination of amoxycillin and clavulanic acid, a 'suicide' inhibitor of class A β-lactamases, has been used clinically since the late 1970s as co-amoxiclav (Augmentin®) and, interestingly in the context of this review, there has been little or no decline in efficacy despite widespread use [10,11]. Other combinations are also available commercially and are widely prescribed. More recently, biphenyl tetrazole- [12,13] and 2,3-(*S,S*)-disubstituted succinate-derived [14] competitive inhibitors of class B metallo-β-lactamases have been assessed *in vitro* in combination with imipenen and were found to restore the antibacterial activity of the antibiotic.

It has been known for many years that tea has significant antibacterial properties against both Gram-positive and Gram-negative bacteria [15]. More recently, Hamilton-Miller and colleagues have demonstrated that extracts of Japanese green tea (*Camellia sinensis*) can reverse methicillin resistance in methicillin-resistant *S. aureus* (MRSA) [16] and they provided some evidence that this phenotypic modification could result from the suppression of penicillin binding protein 2a (PBP2a) – the methicillin-insensitive, *mecA*-encoded peptidoglycan transpeptidase that is responsible for almost all *S. aureus* resistance to methicillin and related β-lactam agents [17]. MRSA-modifying activity is attributable to polyphenolic compounds in tea extracts; as is evident from Fig. 2, relatively low concentrations (25 mg l^{-1}) of epicatechin gallate (ECG) and, to a lesser extent, epigallocatechin gallate (EGCG) sensitize a range of MRSA isolates to methicillin and oxacillin [18,19]. It has been suggested [20] that the modulating activity results from binding of the molecule to peptidoglycan but more recent evidence [21] indicates that ECG exerts its effect by reducing the quantity of proteins targeted from within the cell to the cytoplasmic membrane and external environment. Other compounds are known that potentiate the activity of β-lactams against MRSA: some diterpines have been reported to lower the minimal inhibitory concentration (MIC) of methicillin against *S. aureus* by up to 256-fold and the mechanism has been related to inhibition of PBP2a expression [22].

Several other strategies have been used successfully to re-sensitize Gram-positive and Gram-negative pathogens to antibacterial agents. Vancomycin resistance in enterococci is manifested by peptidoglycan precursors with altered termini that do not bind the antibiotic. Chiosis and Boneca [23] developed N-acetylated prolinol molecules that facilitated selective catalytic cleavage of the terminal depsipeptide moiety of peptidoglycan in the cell wall of the resistant bacteria. These compounds reduced the MIC of *Enterococcus faecium*

to vancomycin from 500 mg l^{-1} to 62.5 mg l^{-1}. Inhibiting the activity of efflux pumps is an attractive strategy to overcome resistance, particularly as multidrug resistance (MDR) pumps expel a variety of compounds from the cell, regardless of their structural class or mechanism of action. Several peptidomimetic compounds have been shown to potentiate the activity of the fluoroquinolone levofloxacin by inhibiting the MDR pump in *Pseudomonas aeruginosa* [24]. More selective pumps, such as the Tet(B) efflux protein in *E. coli*, can also be effectively blocked, as evidenced by the potency of semi-synthetic tetracycline analogues in combination with doxycyline [25]. Phenotypic conversion from chloramphenicol and ampicillin resistance to Cm and Amp sensitivity was achieved under laboratory conditions by employing plasmids carrying external guide sequences (EGSs); these synthetic genes encode small oligoribonucleotides and direct ribonuclease P to selectively cleave mRNAs transcribed from chloramphenicol acetyl transferase and β-lactamase genes. When the plasmids were transferred to strains of *E. coli* harbouring the appropriate antibiotic resistance determinants, the bacteria were efficiently converted to the antibiotic-sensitive phenotype [26]. It is, however, difficult to see how this elegant technology could be harnessed for the treatment of infections.

Conversion to the less virulent phenotype

Pathogenic bacteria, including opportunists, must circumvent the host's innate and adaptive defence mechanisms to cause clinical signs of infection. Antibacterial chemotherapy frequently relies upon an intact immune system to remove invading bacteria after their subjugation by the antibiotic. In immunocompromized individuals, these defences are usually inadequate and judgements about therapy need to be based upon knowledge of these immunological deficits and the timing of the infections; under such circumstances, bacteriostatic antibiotics are only infrequently used. Thus, strategies designed to 'disarm' pathogenic bacteria and so render them more susceptible to components of the host's immune system are unlikely to be successful in severely immunocompromized patients.

There are reports in the literature (reviewed in [27,28]) that several antibiotics, at subinhibitory concentrations, are able to potentiate the ingestion of bacteria by phagocytic cells and also to sensitize refractory organisms to the bactericidal action of complement. There is little evidence presently available to indicate whether these effects contribute towards a successful therapeutic outcome in patients receiving these compounds. Such evidence would be more likely to be obtained from studies involving agents that have no growth-inhibiting capacity but are able to modify the expression of bacterial determinants known to have a role in virulence or survival *in vivo*. The few studies that have been undertaken in this area indicate that reduction in expression of some major virulence determinants can influence the course of the infection. In the pre-antibiotic era, Dubos, Avery and associates used a capsule-depolymerizing enzyme, isolated from cultures of a bacterium found in peat soil [29], to determine the effect of 'capsule-stripping' *in vivo* on the outcome of experimental pneumococcal infection. The enzyme specifically degraded the pneumococcal type III capsular polysaccharide, a major virulence determinant of the ability of strains to cause severe systemic infection. Intraperitoneal administration of the enzyme to mice before challenge with type III pneumococci afforded type-specific protection [30], whereas intravenous administration of the enzyme to rabbits with type III dermal infections resulted in a favourable and early termination of the experimental disease, which is normally fatal [31]. Taylor also noted that removal of virulence capsules impacted on disease outcome. Administration of a bacteriophage-derived endosialidase that specifically depolymerized the *E. coli* K1 capsule, responsible for the invasive potential of these strains in the neonate, resulted in resolution of systemic infection in K1-infected neonatal rats [32]. Similarly, *in vitro* digestion of the alginate mucopolysaccharide of *P. aeruginosa* by bacterial alginase enhanced uptake by human macrophages [33]. Capsules have been shown to enhance the virulence of bacteria that cause neonatal and adolescent meningitis, cystic fibrosis, dental caries, periodontitis and pneumonia, and it would be illuminating to determine the potential impact of capsule depolymerization on the course of several of these infections. The past two decades have seen an explosion in our understanding of the pathogenesis of infection by both Gram-positive and Gram-negative organisms, and a variety of virulence factors have been identified that represent potential targets for therapeutic modulation; it is hoped that further validation of this approach will emerge over the next few years.

Photosensitization of pathogenic bacteria

It is almost a century since Paul Ehrlich demonstrated that bacteria selectively accumulate vital stains, such as methylene blue [34]. Dyes that have the capacity to participate in energy transfer reactions can sensitize these target bacterial cells to destruction by light irradiation. Photosensitive tetrapyrrolic molecules, such as porphyrins, phthalocyanines and bacteriochlorins, are known to accumulate in, and be retained by, a variety of rapidly proliferating tissues and cells, including micro-organisms. Subsequent photoactivation of these molecules with red light (λ650–800 nm) in the presence of oxygen leads to cell death as a result of

singlet-oxygen-mediated peroxidative damage [35]. This concept, photodynamic therapy (PDT), has proven valuable in the treatment of a variety of hyperproliferative conditions, including small solid tumours and macular degeneration of the retina [36]. Pathogenic micro-organisms growing *in vivo* as localized foci of infection, on skin or on accessible mucous membranes, would appear to be good candidates for photodynamic destruction, although it is unlikely that disseminated infections would be amenable to this approach. Some pathogens are known to accumulate a wide range of photosensitive agents [34] and multidrug resistant strains [37] and bacteria in biofilms [38] are known to be susceptible to photodestruction. There is ample evidence in the literature that Gram-positive bacteria can be readily photosensitized but that the outer membrane (OM) of Gram-negative species acts as an effective barrier to the penetration into the cell of many photosensitive dyes [39]. In recent years, a new generation of cationic tetrapyrrolic photosensitizers have been described [40,41] that efficiently sensitize Gram-negatives because of their capacity to traverse the OM.

Some photosensitizers are marketed for a variety of non-microbiological indications and others are in various stages of clinical development [35]. In general, the toxicity of these molecules in the ground state is low but some damage to healthy bystander tissues can occur following photoactivation if host cells accumulate the dye to any extent. Whether this represents a problem for antimicrobial phototherapy remains to be determined. No clinical trials to determine antimicrobial activity have so far been reported, but several studies in animal models illustrate the potential of the technology. Wilson and co-workers, in an extensive series of investigations, have shown that extensive lethal photosensitization of periodontopathogenic species in biofilms and human subgingival plaque can be achieved with a variety of photosensitive dyes [38,42]. Poly-L-lysine–chlorin *e6* conjugates have been shown to kill virtually all bacteria in a mixture of periodontal pathogens while sparing oral epithelial cells following red light illumination from a diode array [43]. Similar selective toxicity was seen when *Helicobacter* on ferret and rat gastric mucosa were exposed to laser light after topical application of photosensitizer; the energy required to kill the photosensitized bacteria was insufficient to damage the underlying mucosa [44]. An arginine haematoporphyrin derivative that has been shown to efficiently photosensitize a variety of drug-resistant Gram-positive and Gram-negative clinical isolates is reported to be under investigation for the treatment of infected wounds [45].

Bacteriophage therapy

The idea that bacteriophages could be used as a biological agent to control bacterial infections was mooted soon after their discovery in the early part of the 20th century and was the subject of intensive research in the pre-antibiotic area. Exaggerated and unsubstantiated claims of efficacy led to a loss of interest in their therapeutic potential in Western countries, although bacteriophages continue to be used in the former Soviet Union and Eastern Europe for prophylaxis and therapy of a variety of non-systemic bacterial infections. This field has been extensively reviewed in the past few years [46–48] and a consensus is emerging that a reappraisal of its potential, particularly in the West, is now due.

The emergence of difficult-to-treat infections, such as those by vancomycin-resistant enterococci and multidrug resistant staphylococci, has undoubtedly driven this renewed interest. Bacteriophages have the potential to be self-replicating and self-limiting medicines and, because they are a complex assemblage of foreign proteins and nucleic acid, evoke a substantial immune response when administered parenterally. They have, therefore, been considered primarily as potential therapeutics for a variety of non-systemic infections, including gastrointestinal, skin, pulmonary, wound, abscess and head and neck infections. The recent successful development of bacteriophage with extended circulating half-lives through serial passage in animals [49] opens up the possibility of extending the repertoire of conditions amenable to this therapy. The narrow host range of bacteriophages represents a significant barrier to their successful therapeutic use, as a comprehensive understanding of the infectious agent becomes a prerequisite for meaningful use, but this stringency determines that it is unlikely that the normal flora will be disturbed in the way seen with chemotherapeutics. Resistance has been repeatedly shown to emerge during treatment with bacteriophage, but in some studies viruses lytic for the mutant have been isolated and successfully used during the course of the study [50]. These extensive studies, ignored for so long and published in inaccessible, non-English language journals, clearly demonstrate the potential for bacteriophage therapy as an inexpensive and safe alternative to conventional treatment regimens.

Conclusions

There is an expectation that the next generation of drugs to combat the increasing threat from multiple antibiotic resistant bacteria will come from the information explosion generated by bacterial genomics and proteomics. There is likely to be a substantial period before new drugs emerge from this source and until promise becomes reality, other approaches, some of which are detailed in this review, should be given serious consideration.

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