

# Antimicrobial agents targeting bacterial cell walls and cell membranes

K. Bush

Biology Department, 1001 East Third Street, Indiana University Bloomington, IN 47405, United States of America

## Summary

Antimicrobial agents that target the bacterial cell wall or cell membrane have been used effectively for the past 70 years. Among the agents that inhibit bacterial cell wall synthesis, the  $\beta$ -lactam antibiotics have emerged into broad-spectrum agents that inhibit most pathogenic bacteria, but are now being threatened by the rapid spread of drug-inactivating  $\beta$ -lactamases. Glycopeptides still retain high activity against staphylococci, but resistance among the enterococci has become a major problem. Recently, fosfomycin has been used in the treatment of multidrug-resistant Gram-negative bacteria. Daptomycin, which targets both membrane function and peptidoglycan synthesis, is especially effective in treating staphylococcal infections. The polymyxin antibiotics that target cell membranes are being used more frequently to treat multidrug-resistant Gram-negative infections. The ionophore antibiotics, used in veterinary medicine, target membranes in many microbial and animal species. Although increasing resistance is a continuing concern, these classes of bactericidal agents can provide highly effective antibiotics.

## Keywords

Cell membrane – Cell wall – Daptomycin – Fosfomycin – Glycopeptide – Ionophore – Lipopeptide – Polymyxin – Vancomycin –  $\beta$ -lactam.

## Introduction

Antimicrobial agents that function by interacting with bacterial cell walls or cell membranes include some of the most frequently used antibacterial agents in human medical and veterinary practice. Agents that target these essential features of pathogenic bacteria are often considered to be bactericidal agents, because their action is so disruptive to the cell that viability is curtailed (88, 90). This makes them highly attractive for therapeutic purposes.

Among the agents that target the cell wall are the common  $\beta$ -lactams, which include penicillins, cephalosporins, carbapenems and monobactams, the glycopeptides and fosfomycin. Membrane-active agents include the multi-targeted lipopeptide daptomycin, the peptidic antibiotics such as colistin and polymyxin B and the ionophore antibiotics monensin and salinomycin. As seen in Table I,  $\beta$ -lactams and glycopeptides have been used in both human and veterinary medicine, whereas daptomycin,

fusidic acid and the polymyxins have established themselves in human chemotherapy and the ionophore antibiotics are restricted to animal use. In this review, the various agents in each of these classes will be presented with respect to their structure, mechanism of action and resistance profiles.

## Cell wall active agents

Bacterial cell wall synthesis involves a complex metabolic pathway, which begins with precursors in the cytoplasm that are linked enzymatically in a series of chemical reactions before being transported to the outer surface of the cytoplasmic membrane. The resultant N-acetylmuramyl-pentapeptide containing a D-Ala-D-Ala terminal dipeptide is then incorporated into the growing peptidoglycan structure that forms the cell wall by utilising specific transglycosylases and transpeptidases to facilitate cross-linking of the cell wall components in replicating bacteria (9).

**Table I**  
**Representative antibacterial agents used in animals or humans to kill bacteria by targeting the bacterial cell wall or cell membrane**

Class and subclass	Target	Animal or human use	Representative drugs
<b><math>\beta</math>-lactams</b>			
Penicillins	Cell wall	Animal	Benzylpenicillin, ticarcillin, amoxicillin +/- clavulanic acid
		Human	Penicillin, ticarcillin, piperacillin, amoxicillin +/- clavulanic acid, piperacillin +/- tazobactam
Cephalosporins	Cell wall	Animal	Cephalexin, cephalothin, ceftiofur
		Human	Cephalexin, cephalothin, cefotaxime, ceftazidime, cefepime
Carbapenems	Cell wall	Human	Doripenem, ertapenem, imipenem, meropenem
Monobactams	Cell wall	Human	Aztreonam
<b>Glycopeptides</b>	Cell wall	Animal	Avoparcin <sup>a)</sup>
		Human	Vancomycin, teicoplanin, telavancin
<b>Phosphonic acids</b>	Cell wall	Human	Fosfomicin
<b>Lipopeptides</b>	Cell wall and membrane	Human	Daptomycin
<b>Peptide antibiotics</b>	Membrane	Human	Polymyxin B, colistin (polymyxin E)
<b>Ionophores</b>	Membrane	Animal	Monensin, salinomycin

a) Withdrawn from the market

## $\beta$ -Lactams

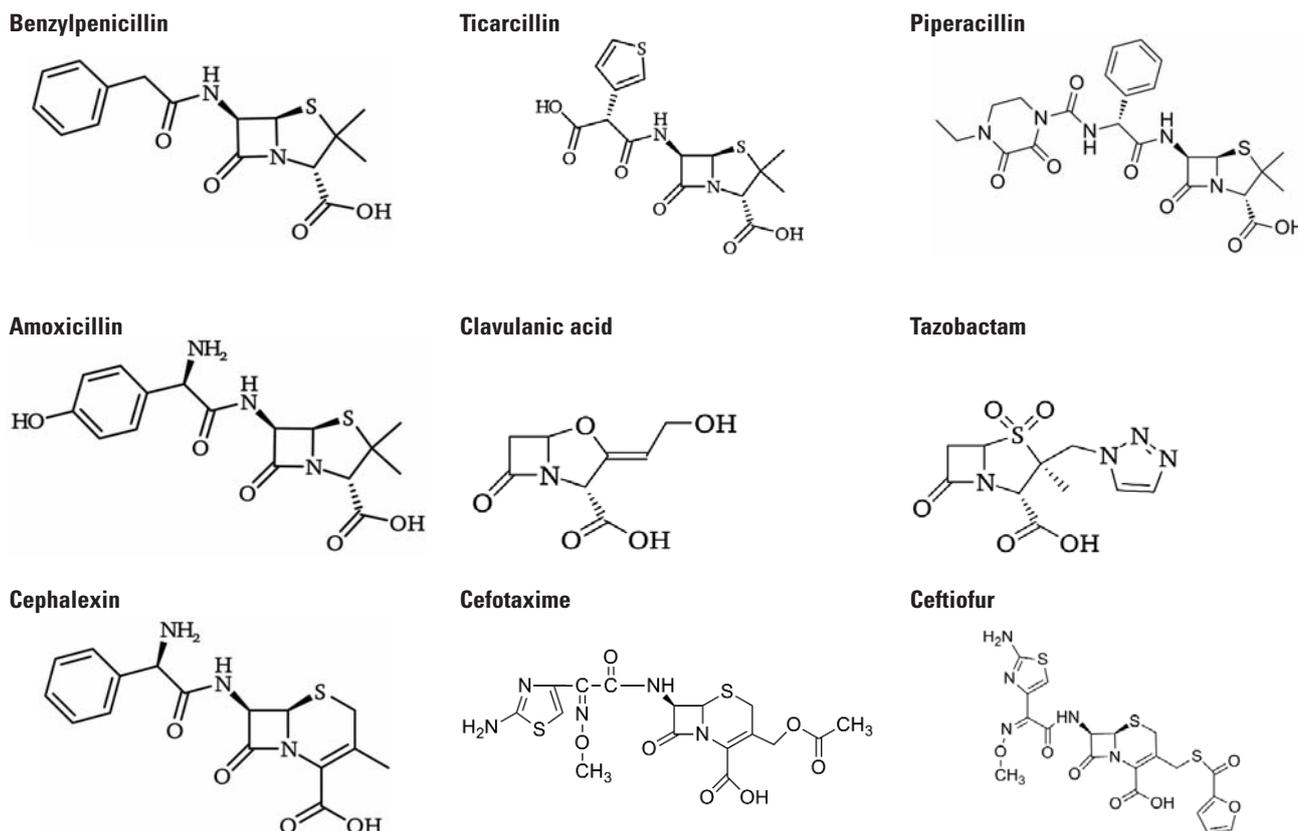
$\beta$ -Lactam antibiotics have been used safely and effectively for over 70 years, initially to treat infections caused by Gram-positive cocci (28). With the introduction of broad-spectrum  $\beta$ -lactams, infections caused by Gram-negative anaerobic and aerobic bacteria can also be treated with this family of agents (60). Structures of some commonly used  $\beta$ -lactams are shown in Figure 1. Among these agents are narrow-spectrum penicillins and cephalosporins, expanded-spectrum cephalosporins, the potent broad-spectrum carbapenems, and the monobactam aztreonam, which has activity against Gram-negative pathogens only. These antibiotics, particularly the early penicillins and cephalosporins, have been used extensively to treat infections not only in humans but also in domestic animals (82) (Table I). Although benzylpenicillin is a natural product that can be isolated directly from fermentation broths, the other commonly used  $\beta$ -lactam antibiotics are semi-synthetic molecules that originate from 6-aminopenicillanic acid (79) or 7-aminocephalosporanic acid (2).

All the  $\beta$ -lactams utilise a similar strategic mechanism to produce their antibacterial activity. The enzymatic targets for these antibiotics operate in the final stage of bacterial cell wall synthesis, and include multiple species-specific proteins named penicillin-binding proteins, or PBPs (37). Those membrane-bound PBPs that serve an essential function in cell wall biosynthesis often appear as bifunctional enzymes with a combination of transpeptidase and transglycosylase activities, or transpeptidase and carboxypeptidase activities (64). The  $\beta$ -lactam antibiotics interfere with cell wall synthesis by binding to the terminal D-Ala-D-Ala in the lengthening peptidoglycan architecture, thereby preventing cross-linking of the cell wall (90). Bacterial PBPs all possess a serine in the enzymatic active

site that can be acylated by  $\beta$ -lactams during bacterial growth (64). Rapid acylation of the active site serine, followed by very slow deacylation, results in virtual inactivation of the PBPs, leading to bacterial cell death.

Two major resistance mechanisms for  $\beta$ -lactams are operative, which vary with the organisms involved (13). In Gram-positive bacteria, the most common mechanism is acquisition of new PBPs that have low affinity for common  $\beta$ -lactams (19). The pre-eminent example of this is in *Staphylococcus aureus*: the presence of the PBP2a-encoding *mecA* gene results in a PBP with low affinity for all but a few recently developed  $\beta$ -lactams, leading to methicillin-resistant *S. aureus*, or MRSA (81). Before the recognition of staphylococci that produced PBP2a, however, penicillin resistance in the staphylococci was more often due to the production of penicillinase activity (54). Today, both mechanisms are operative in MRSA (72), although penicillinase production is superfluous in the presence of the *mecA* gene.

The pneumococci have also demonstrated the ability to acquire low affinity PBPs, although the range of  $\beta$ -lactam resistance is more restrictive than in *S. aureus*. Isolates of penicillin-resistant *Streptococcus pneumoniae* (PRSP) can exhibit a broad range of minimum inhibitory concentrations (MICs) for different  $\beta$ -lactams (23). This means that some PRSP isolates may be susceptible to selected cephalosporins, whereas MRSA has been considered to be uniformly resistant to all  $\beta$ -lactams (24). That dogma is changing, however, with the development of new 'anti-MRSA  $\beta$ -lactams', such as the anti-MRSA cephalosporin ceftaroline that was recently approved by the United States Food and Drug Administration (FDA) (61). Notably, no  $\beta$ -lactamases have been reported in the streptococci.



**Fig. 1**  
**Representative  $\beta$ -lactam antibiotics used in veterinary and human medicine**

Enterococci have also acquired low affinity PBPs, notably PBP5 in *Enterococcus faecium* (39, 58). Although enterococci were reported in the 1980s to produce a penicillinase that was identical to a staphylococcal  $\beta$ -lactamase (96), recent reports have indicated that this enzyme is now rarely seen in surveillance studies (1).

In Gram-negative bacteria, the major resistance mechanism is production of  $\beta$ -lactamases rather than modifications of PBP (13). In addition, efflux and modification or deletion of porin function can play a role in resistance to specific  $\beta$ -lactams. Loss of porin function may occur in concert with  $\beta$ -lactamase production, which results in high levels of resistance to virtually all  $\beta$ -lactams (11). Development of  $\beta$ -lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam has been a major tactic of the pharmaceutical industry to counteract the activity of these enzymes (26).

More than 1,000 unique  $\beta$ -lactamases have been described, with varying molecular and functional properties, on the basis of their substrate preference and inhibitor profile (14, 49). On a molecular level,  $\beta$ -lactamases can be divided into those that utilise serine at

the active site (Classes A, C and D) and those that require one or two zinc ions to facilitate  $\beta$ -lactam hydrolysis (Class B) (7, 47, 50). Functional grouping of these enzymes allows the correlation of molecular structure with biochemical characteristics, as seen in Figure 2 (15). The groups are defined according to the distinguishing  $\beta$ -lactam(s) hydrolysed by that enzyme family, and by the inhibitor profile. For example, clavulanic acid is a good inhibitor of many  $\beta$ -lactamases in class A, but is not very active against class B or C enzymes, or most class D  $\beta$ -lactamases. Metal chelators such as ethylene diamine tetra-acetic acid (EDTA) inhibit the metallo- $\beta$ -lactamases (MBLs) but have no effect on serine enzymes.

The enzyme families that are currently of most concern are:

- the extended-spectrum  $\beta$ -lactamases (ESBLs), which hydrolyse penicillins, early cephalosporins and expanded-spectrum cephalosporins, and monobactams such as aztreonam
- serine carbapenemases, which hydrolyse all  $\beta$ -lactams, including the monobactams
- the MBLs, which hydrolyse all  $\beta$ -lactams except the monobactams (13).

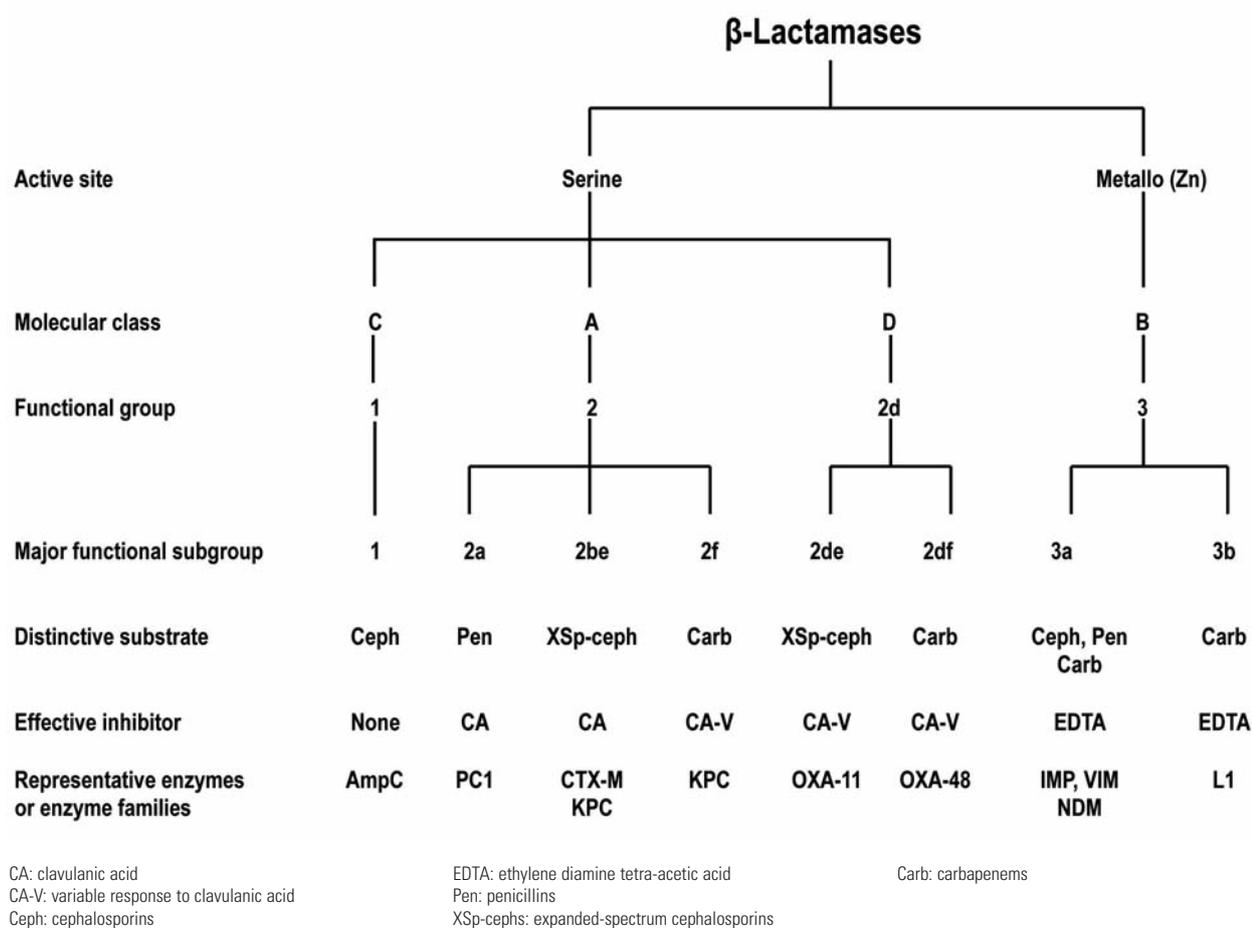


Fig. 2

### Correlation of β-lactamase structure and function

These three families of enzymes have been spreading among Gram-negative bacteria throughout the world on mobile elements that involve integrons and transposons, with transferable plasmids that convey resistance determinants for multiple antibiotic classes. Although ESBLs were the major β-lactamases of interest in the 1990s, the plasmid-encoded carbapenemases with a broader substrate spectrum have now become prominent. Examples of these enzymes include the KPC serine carbapenemases originally identified from *Klebsiella pneumoniae* (13) and the NDM-1 family of MBLs (89) that often appear in multidrug-resistant Enterobacteriaceae.

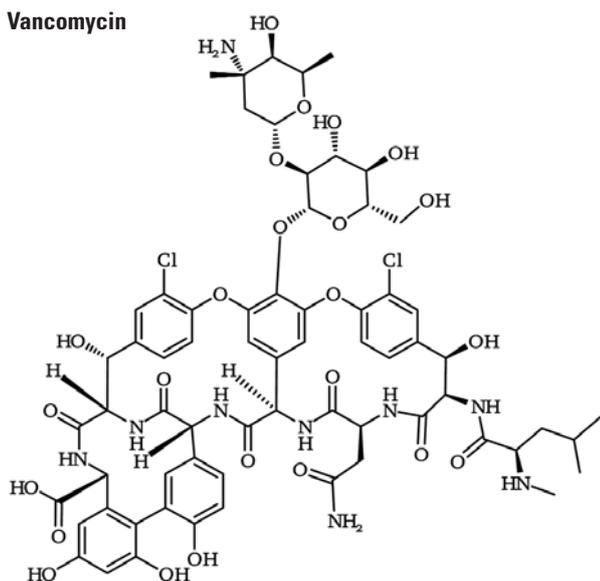
### Glycopeptides

Vancomycin, the first glycopeptide to be introduced into medical practice to treat infections caused by Gram-positive cocci, was not widely accepted at first. When this natural product was initially isolated from fermentation broths of *Streptomyces orientalis* (now *Amycolatopsis orientalis*) for therapeutic use, the isolation procedures were inadequate and did not remove impurities from the preparations. This resulted in material affectionately

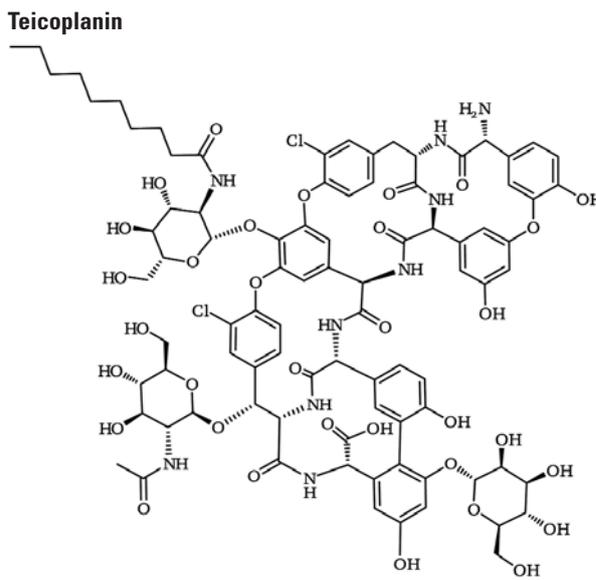
named 'Mississippi Mud', and, as a result, dose-limiting toxicity, including nephrotoxicity, restricted the utility of this agent (41). However, over time, greater purification of the material has resulted in a minimally toxic antibiotic that is used extensively for the treatment of MRSA infections (29). Unfortunately, its activity against enterococci has been compromised by several elaborate resistance mechanisms.

Other glycopeptides that have been introduced include teicoplanin and avoparcin, neither of which was approved for use in the United States, and telavancin (Fig. 3). Teicoplanin, like vancomycin, is widely used therapeutically to treat MRSA infections, but only outside the United States. Compared with vancomycin, teicoplanin generally has greater potency against streptococci and enterococci, while retaining activity against some vancomycin-resistant enterococcal isolates (see below) (71, 75). The once-a-day dosing regimen of teicoplanin is particularly attractive for outpatient use (87). Telavancin was recently approved by the FDA for treatment of skin infections caused by susceptible Gram-positive pathogens, including MRSA, but not vancomycin-resistant enterococci, and has lower MICs against most of these

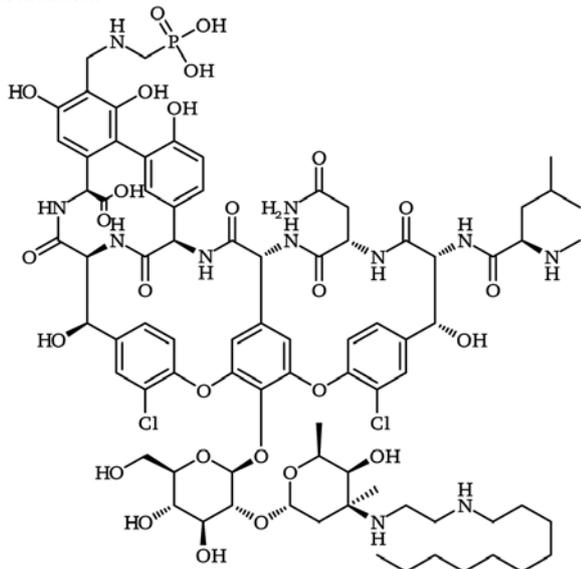
Vancomycin



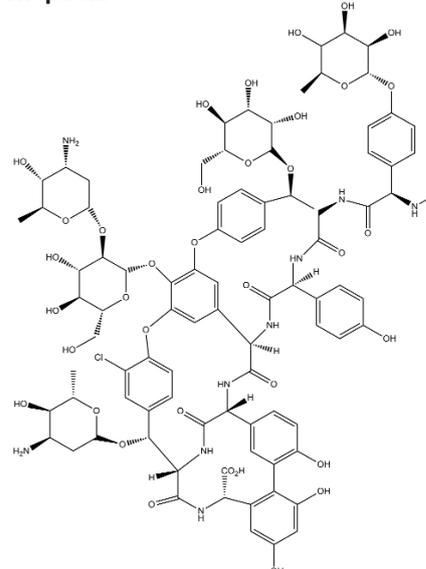
Teicoplanin



Telavancin



Avoparcin



**Fig. 3**  
Structures of glycopeptides

pathogens than vancomycin or teicoplanin (25). The veterinary glycopeptide avoparcin was approved for use as an antimicrobial growth promoter in Europe in 1974, but by 1997 it had been banned throughout the continent because of the association of avoparcin with vancomycin resistance in *E. faecium* isolates from veterinary patients (91). Concerns were raised because of the ability of the VanA phenotype to be transmitted to humans from animal reservoirs, which resulted in an increase in glycopeptide resistance in human populations (91).

Glycopeptides inhibit the growth of bacteria by forming a complex between the antibiotic and the C-terminal D-Ala-D-Ala dipeptide of the nascent peptidoglycan on the outer

surface of the cytoplasmic membrane (9). The formation of this complex prevents the transglycosylation and transpeptidation reactions that are necessary for completion of the peptidoglycan chain, resulting in an incomplete cell wall and subsequent cell death. In addition to its interaction with D-Ala-D-Ala, telavancin binds to lipid II, a cell wall precursor on the cytoplasmic side of the cell membrane. This interaction results in membrane depolarisation and eventual membrane disruption, thereby providing a second mechanism that leads to cell death (63).

Vancomycin resistance was initially thought to be difficult, if not impossible, to attain. Glycopeptides, with their

primary killing targets on the outer surface of the cell membrane, have no membrane barrier to overcome. Notably, these agents do not interact with enzyme targets but with their substrates. Thus, unsurprisingly, the major glycopeptide resistance mechanisms that have been identified are associated with structural modifications in the substrates for the enzymes that incorporate the final amino acids in the pentapeptide precursors. The most important of these involve replacement of the C-terminal D-Ala by D-lactate or D-serine, resulting in D-Ala-D-Lac or D-Ala-D-Ser depsipeptides with reduced binding affinities for vancomycin (9, 20).

Resistance to vancomycin and teicoplanin was first reported as transposon-related resistance in a variety of enterococci in the late 1980s (9). Glycopeptide resistance was initially characterised by phenotypes, using letter names for each of the profiles. The VanA and VanB phenotypes are associated with gene clusters that may be transferred on large plasmids, or from chromosome to chromosome, whereas the other vancomycin resistance mechanisms are related to chromosomal mutations that may be either inducible or constitutive (20). Isolates that exhibit the VanA phenotype are typically resistant to all glycopeptides, although telavancin seems to be less commonly affected than vancomycin or teicoplanin (25). Teicoplanin and telavancin are both active against vancomycin-resistant isolates with the VanB phenotypes (25, 75), while teicoplanin also exhibits activity against VanC, VanE and VanG strains (20).

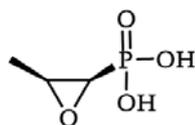
Full resistance to vancomycin in the staphylococci has been reported exceedingly rarely, with vancomycin-resistant *S. aureus*, or VRSA, reported in only about a dozen cases worldwide (70). High-level glycopeptide resistance is generally due to the *vanA* gene cluster that has been

acquired from vancomycin-resistant enterococci (76). More frequent is the reporting of staphylococci with reduced susceptibility to glycopeptides, although these strains are still not a large proportion of the staphylococci encountered in therapeutic practice. These strains, known as vancomycin-intermediate *S. aureus* (VISA), or GISA (glycopeptide-intermediate *S. aureus*), include a subset known as hVISA (heterogeneously resistant VISA) that are difficult to identify using standard clinical microbiology methodology (86). The strains are identified under a microscope by their prominent thickened cell wall. The characteristics of these strains are varied, and include reduced autolysis, lower levels of lysostaphin, and changes in cell wall teichoic acids; contributions from point mutations in global regulatory genes, such as *agr*, are thought to be important (46, 70).

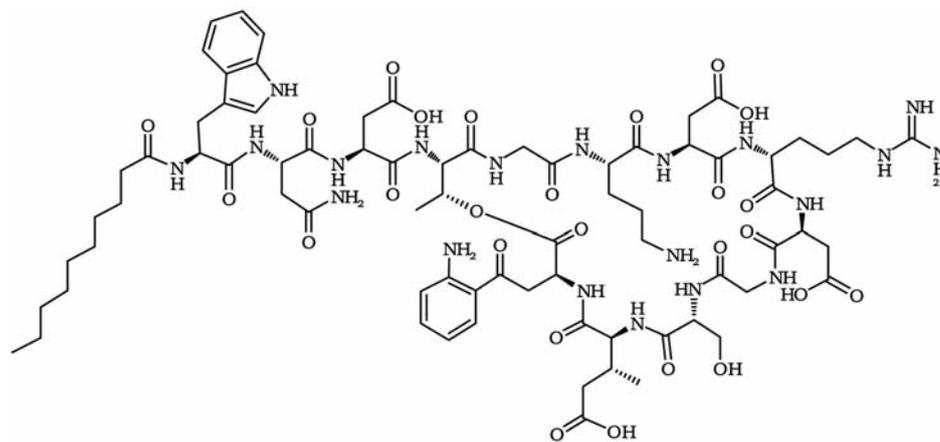
### Phosphonic acids

Fosfomicin, or, as it was first known, phosphonomycin, is a simple epoxide-containing antibiotic (Fig. 4) isolated from streptomycetes (66), and related to phosphoenolpyruvate. It has generated increased attention over the past few years due to its bactericidal activity against a wide range of microorganisms, including multidrug-resistant Gram-positive and Gram-negative bacteria such as *P. aeruginosa* (38). Susceptibility to fosfomicin among recent Gram-negative isolates that produce KPC carbapenemases or the ubiquitous CTX-M ESBLs has been reported to be from 54% to 90% (78, 84). Interestingly, a clinical isolate of *E. coli* that produced the rapidly disseminating NDM-1-metallo- $\beta$ -lactamase was recently shown to be susceptible only to fosfomicin, colistin and tigecycline among all the antibiotics tested (77).

**Fosfomicin**



**Daptomycin**



**Fig. 4**  
**Structures of fosfomicin and daptomycin**

Fosfomycin is active because it inhibits the cell wall synthesising enzyme MurA, the enolpyruvyl transferase that catalyses the first committed step of peptidoglycan synthesis (52). Because this enzyme is essential for formation of the bacterial cell wall, fosfomycin inhibits the growth of a broad range of bacterial species. In Gram-negative bacteria, only one gene encodes an enzyme with MurA functionality (12), whereas in Gram-positive bacteria two *murA* genes exist with different nucleotide sequences, but similar biochemical characteristics. At least one MurA protein must remain functional for the cell to survive (27). Thus, it is possible that one *murA* gene product may be inactivated, but the cell may retain viability.

Resistance to fosfomycin may involve several different mechanisms (69):

- the *murA* gene can mutate to produce an enzyme with reduced affinity for fosfomycin (92)
- increased levels of MurA can be produced, requiring higher concentrations of fosfomycin for growth inhibition
- more general resistance mechanisms can emerge that involve increased efflux or decreased cellular uptake, due to alterations in the *glpT* and/or *uhp* transport systems (45, 74).

In addition, enzymatic inactivation of the antibiotic can occur (8, 85). Inactivation by the enzyme FosA occurs in a reaction that involves Mn<sup>2+</sup>-dependent covalent addition of the glutathione cysteinyl sulfhydryl to the C1 of fosfomycin (10, 62). A homologous thiol transferase inactivation reaction is catalysed by FosB (95), which adds cysteine to the C1 oxirane (33). In *Listeria monocytogenes*, the enzyme FosX that hydrates fosfomycin, also at the oxirane C1 position, can exist as multiple variants in different *Listeria* strains (34). Other fosfomycin-inactivating enzymes, FomA and FomB, are capable of mono- and di-phosphorylation of the phosphonate functionality of fosfomycin, thereby providing another resistance mechanism (55). Of these mechanisms, those most commonly reported are combinations of *murA* gene overexpression with transport defects (69).

## Membrane-active agents

### Lipopeptides

The only lipopeptide currently approved for therapeutic use is daptomycin, a cyclic lipopeptide of high molecular weight (Fig. 4) that has rapid bactericidal activity against Gram-positive cocci (6). Daptomycin has been approved for the treatment of complicated skin and skin structure infections (cSSSI), and for therapy of bloodstream infections (bacteraemia) caused by *S. aureus*, including

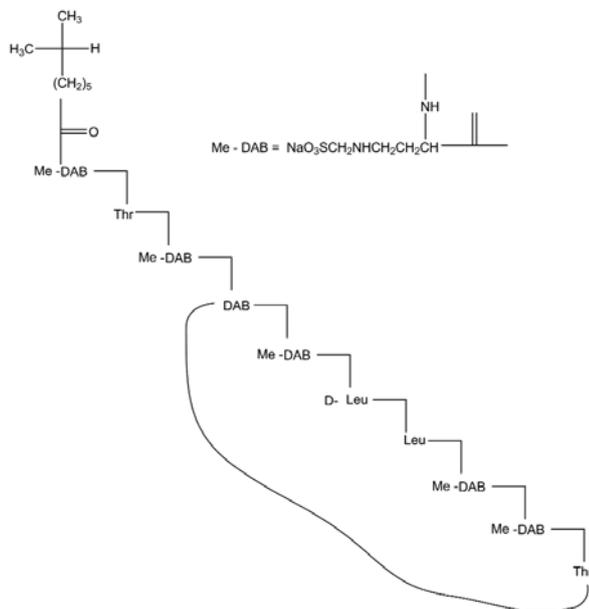
those in patients with right-sided infectious endocarditis ([www.accessdata.fda.gov/scripts/cder/drugsatfda](http://www.accessdata.fda.gov/scripts/cder/drugsatfda)). It is not used to treat pneumonia, because the molecule is inactivated by pulmonary surfactant (83).

Originally described as an inhibitor of peptidoglycan synthesis (6), daptomycin was later demonstrated to cause calcium-dependent membrane depolarisation (5), which results in the cessation of macromolecular synthesis and disruption of the cellular membrane in the dying bacteria. These results were recently confirmed through transcriptional profiling studies in daptomycin-treated *S. aureus*, in which genes in the cell wall stress stimulon were affected, as well as genes affecting membrane depolarisation (68).

Clinical resistance to daptomycin is relatively rare (80), but it has been observed among occasional enterococcal and staphylococcal clinical isolates. Recent studies indicate that < 0.1% of staphylococci and < 2% of enterococci are non-susceptible to daptomycin (53, 65). Decreased susceptibility to daptomycin in *S. aureus* has been associated with point mutations in MprF, a lysylphosphatidylglycerol synthetase, and with a nucleotide insertion in YycG, a histidine kinase (35). In a study of sequential clinical *S. aureus* isolates from an endocarditis patient treated with daptomycin, resistant isolates exhibited increased membrane fluidity, reduced polarisation and permeability, and an elevated net positive surface charge (51). Although some staphylococcal isolates that are not susceptible to daptomycin exhibit a thickened cell wall, this is not a universal characteristic (93). In addition, staphylococcal strains can be generated in the laboratory with an RpoB mutation, which results in heteroresistance to daptomycin and vancomycin (21). In the enterococci, non-susceptibility to daptomycin has been studied less extensively and appears to be due to other mechanisms (18, 67).

### Cyclopeptide antibiotics

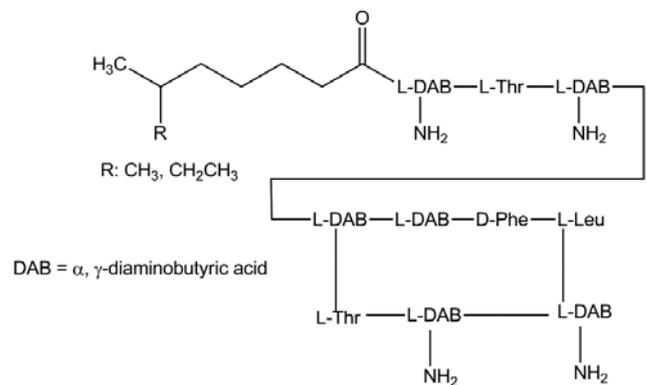
Polymyxin B and colistin (polymyxin E), the two peptide antibiotics that are commercially available, are similar in their spectrum of activity, mechanism of action, resistance characteristics and toxicity (56). However, their antimicrobial potency, pharmacodynamic and pharmacokinetic properties, and chemical structures (Fig. 5) are distinct. These antibiotics, first identified in the late 1950s, were used in both human and veterinary medicine when they were first introduced (44). Their primary role was to treat serious infections caused by Gram-negative bacteria (40). Toxicity, reported as early as 1965 (43), limited the use of these agents for several decades. However, recent data analyses demonstrate less renal and central nervous system toxicity than previously

**Colistin sulphate, polymyxin E**

**Fig. 5**  
**Structures of colistin (polymyxin E) and polymyxin B**

reported (30, 57), and the drugs are now used as a treatment of last resort for infections caused by multidrug-resistant Gram-negative pathogens, particularly *P. aeruginosa*, *Acinetobacter* spp. and carbapenemase-producing *K. pneumoniae* (59).

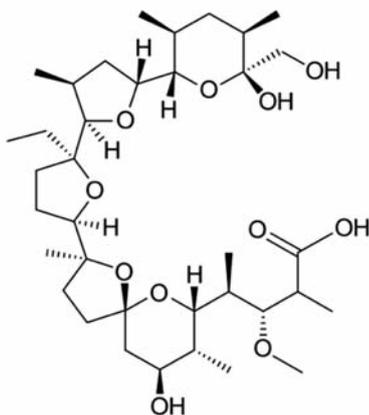
The polymyxins are bactericidal antibiotics that bind to lipid A of the lipopolysaccharide in the bacterial membrane, resulting in membrane disintegration (31). Resistance is relatively rare, with current estimates of < 10% worldwide (31), but is increasing as these agents are used more frequently; a 12% rate of resistance to colistin has been reported for recent isolates of *Acinetobacter* from Kuwait (4). Resistance most frequently involves modifications of the outer membrane (31). These mechanisms may be either intrinsic or adaptive, involving

**Polymyxin B**

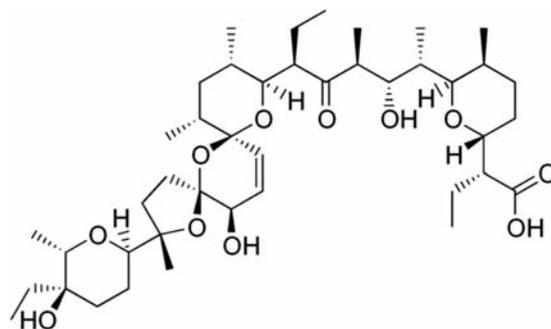
numerous regulatory systems (32). Mechanistic studies have demonstrated that intrinsic resistance occurs when the phosphate groups on the lipid A anchor in the membrane lipopolysaccharide are modified in polymyxin-resistant Gram-negative bacteria (94). Other studies have demonstrated that mutations in the PmrAB and PhoP/PhoQ two-component regulatory systems are correlated with colistin resistance (3, 36, 42).

**Ionophore antibiotics**

The polyether (carboxylic) ionophores, monensin and salinomycin (Fig. 6), which are used exclusively in veterinary medicine, were initially isolated as fermentation products from *Streptomyces* spp. (22). These ionophores

**Monensin**

**Fig. 6**  
**Structures of monensin and salinomycin**

**Salinomycin**

have activity against both protozoa and bacteria (16). As a result, they are effective as prophylactic and therapeutic agents in the control of coccidiosis (22), and in controlling or preventing swine dysentery (16). Monensin (in cattle) and salinomycin (in pigs) are also used as growth promoters; they alter the gut flora of the animal, resulting in more efficient food utilisation due to a variety of proposed mechanisms (17). Quasi-ionophore antibiotics that include channel-forming agents such as gramicidin and the polyene antibiotics (16) will not be discussed in this review.

Monensin and salinomycin are monovalent ionophore antibiotics that transport Na<sup>+</sup> and K<sup>+</sup> ions across cell membranes, not only in microbes but also in birds and mammals, organisms in which high doses are toxic, and sometimes lethal (48, 73). They perturb normal ion transport by lowering the energy barrier for cell transport and disrupting the ion gradient across the membrane, resulting in cell death (16). Resistance to these agents has been reported in Gram-positive cocci, but no mechanism has been defined, although the resistance did not appear to be transferable (16).

## Conclusion

Antimicrobial agents that act against cell wall or membrane targets are among the most commonly prescribed agents in the antibiotic armamentarium. Included are the broad-spectrum, safe and effective,  $\beta$ -lactams, which are now suffering from an overabundance of  $\beta$ -lactamases. For infections caused by Gram-positive bacteria, the glycopeptides and lipopeptides are highly effective, even though resistance is a problem in the enterococci. The peptide antibiotics colistin and polymyxin B have become the agents of last resort for the treatment of many multidrug-resistant Gram-negative infections, but concerns abound related to perceived toxicity and an increase in resistance. The less popular fosfomycin is experiencing a resurgence in its use for the treatment of some Gram-negative pathogens, although resistance is also a concern. As the emergence of resistance continues to drive the selection of therapeutically useful antibiotics, bactericidal agents that target the bacterial cell wall and membrane may remain attractive drug choices. ■

## Les agents antimicrobiens ciblant la paroi cellulaire et la membrane cellulaire des bactéries

K. Bush

### Résumé

Les agents antimicrobiens ciblant la paroi ou la membrane cellulaires des bactéries sont utilisés avec succès depuis 70 ans. Parmi les agents inhibant la synthèse de la paroi cellulaire bactérienne, les antibiotiques  $\beta$ -lactame jouent un rôle intéressant, car ils constituent une famille d'agents à spectre large inhibant la plupart des bactéries pathogènes, mais ce rôle est désormais menacé par la propagation rapide de  $\beta$ -lactamases qui inactivent la molécule active. Si les glycopeptides présentent toujours une bonne efficacité contre les staphylocoques, l'apparition de résistances chez les entérocoques est devenue un sujet majeur de préoccupation. La fosfomycine a récemment été introduite afin de traiter les bactéries à Gram négatif multirésistantes. La daptomycine, qui

cible en même temps les fonctions exercées par la membrane et la synthèse du peptidoglycane est particulièrement indiquée pour traiter les infections à *Staphylococcus*. Les polymyxines ciblent les membranes cellulaires et sont utilisées habituellement pour traiter les infections dues à des bactéries multirésistantes à Gram négatif. Les antibiotiques ionophores utilisés en médecine vétérinaire ont pour cible la membrane de nombreuses espèces microbiennes et animales. Bien que l'augmentation des résistances doive faire l'objet d'une vigilance constante, ces classes d'agents bactéricides peuvent constituer des antibiotiques efficaces.

**Mots-clés**

Bêta-lactame – Daptomycine – Fosfomicine – Glycopeptide – Ionophore – Lipopeptide – Membrane cellulaire – Paroi cellulaire – Polymyxine – Vancomycine.



## Agentes antimicrobianos que atacan la pared y la membrana celulares de las bacterias

K. Bush

**Resumen**

Hace setenta años que se vienen utilizando con buenos resultados agentes antimicrobianos que atacan la pared o la membrana celular de las bacterias. Entre los agentes que inhiben la síntesis de la pared celular figuran los betalactámicos, antibióticos de amplio espectro que son capaces de inhibir el crecimiento de la mayoría de las bacterias patógenas, aunque ahora su eficacia empieza a peligrar debido a la rápida diseminación de betalactamasas que inactivan el fármaco. Los glucopéptidos aún siguen siendo muy activos contra los estafilococos, pero en los enterococos han aparecido resistencias que plantean graves problemas. En los últimos tiempos se ha utilizado fosfomicina para combatir bacterias gram negativas multirresistentes. La daptomicina, que ataca tanto la función de membrana como la síntesis de peptidoglucano, resulta especialmente eficaz para tratar las infecciones estafilocócicas. Las polimixinas, que atacan la membrana celular, se vienen empleando con mayor frecuencia para combatir las infecciones por bacterias gram negativas multirresistentes. Los ionóforos, utilizados en medicina veterinaria, son antibióticos que atacan las membranas de muchas especies microbianas y animales. Aunque la proliferación de resistencias sigue generando inquietud, estos grupos de antibacterianos pueden constituir antibióticos de gran eficacia.

**Palabras clave**

Betalactámico – Daptomicina – Fosfomicina – Glucopéptido – Ionóforo – Lipopéptido – Membrana celular – Pared celular – Polimixina – Vancomicina.



## References

- Abbassi M.S., Achour W., Touati A. & Ben Hassen A. (2009). – *Enterococcus faecium* isolated from bone marrow transplant patients in Tunisia: high prevalence of antimicrobial resistance and low pathogenic power. *Pathol. Biol.*, **57** (3), 268–271.
- Abraham E.P. (1987). – Cephalosporins 1945–1986. *Drugs*, **34** (Suppl. 2), 1–14.
- Adams M.D., Nickel G.C., Bajaksouzian S., Lavender H., Murthy A.R., Jacobs M.R. & Bonomo R.A. (2009). – Resistance to colistin in *Acinetobacter baumannii* associated with mutations in the PmrAB two-component system. *Antimicrob. Agents Chemother.*, **53** (9), 3628–3634.
- Al-Sweih N.A., Al-Hubail M.A. & Rotimi V.O. (2011). – Emergence of tigecycline and colistin resistance in *Acinetobacter* species isolated from patients in Kuwait hospitals. *J. Chemother.*, **23** (1), 13–16.
- Alborn W.E., Jr., Allen N.E. & Preston D.A. (1991). – Daptomycin disrupts membrane potential in growing *Staphylococcus aureus*. *Antimicrob. Agents Chemother.*, **35** (11), 2282–2287.
- Allen N.E., Hobbs J.N. & Alborn W.E., Jr. (1987). – Inhibition of peptidoglycan biosynthesis in Gram-positive bacteria by LY146032. *Antimicrob. Agents Chemother.*, **31** (7), 1093–1099.
- Ambler R.P. (1980). – The structure of  $\beta$ -lactamases. *Philos. Trans. roy. Soc. Lond., B, biol. Sci.*, **289**, 321–331.
- Arca P., Reguera G. & Hardisson C. (1997). – Plasmid-encoded fosfomycin resistance in bacteria isolated from the urinary tract in a multicentre survey. *J. antimicrob. Chemother.*, **40** (3), 393–399.
- Arthur M. & Courvalin P. (1993). – Genetics and mechanisms of glycopeptide resistance in enterococci. *Antimicrob. Agents Chemother.*, **37** (8), 1563–1571.
- Bernat B.A. & Armstrong R.N. (2001). – Elementary steps in the acquisition of Mn<sup>2+</sup> by the fosfomycin resistance protein (FosA). *Biochemistry (Wash.)*, **40** (42), 12712–12718.
- Bradford P.A., Urban C., Mariano N., Projan S.J., Rahal J.J. & Bush K. (1997). – Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid-mediated AmpC  $\beta$ -lactamase, and the loss of an outer membrane protein. *Antimicrob. Agents Chemother.*, **41**, 563–569.
- Brown E.D., Vivas E.I., Walsh C.T. & Kolter R. (1995). – MurA (MurZ), the enzyme that catalyzes the first committed step in peptidoglycan biosynthesis, is essential in *Escherichia coli*. *J. Bacteriol.*, **177** (14), 4194–4197.
- Bush K. (2010). – Alarming  $\beta$ -lactamase-mediated resistance in multidrug-resistant Enterobacteriaceae. *Curr. Op. Microbiol.*, **13** (5), 558–564.
- Bush K. & Fisher J.F. (2011). – Epidemiological expansion, structural studies, and clinical challenges of new  $\beta$ -lactamases from Gram-negative bacteria. *Annu. Rev. Microbiol.*, **65**, 455–478.
- Bush K. & Jacoby G.A. (2010). – Updated functional classification of  $\beta$ -lactamases. *Antimicrob. Agents Chemother.*, **54**, 969–976.
- Butaye P., Devriese L.A. & Haesebrouck F. (2003). – Antimicrobial growth promoters used in animal feed: effects of less well known antibiotics on Gram-positive bacteria. *Clin. Microbiol. Rev.*, **16** (2), 175–188.
- Callaway T.R., Edrington T.S., Rychlik J.L., Genovese K.J., Poole T.L., Jung Y.S., Bischoff K.M., Anderson R.C. & Nisbet D.J. (2003). – Ionophores: their use as ruminant growth promotants and impact on food safety. *Curr. Issues Intestinal Microbiol.*, **4** (2), 43–51.
- Canton R., Ruiz-Garbajosa P., Chaves R.L. & Johnson A.P. (2010). – A potential role for daptomycin in enterococcal infections: what is the evidence? *J. antimicrob. Chemother.*, **65** (6), 1126–1136.
- Chambers H.F. (1999). – Penicillin-binding protein-mediated resistance in pneumococci and staphylococci. *J. infect. Dis.*, **179** (Suppl. 2S), 353–359.
- Courvalin P. (2006). – Vancomycin resistance in Gram-positive cocci. *Clin. infect. Dis.*, **42** (1), S25–34.
- Cui L., Isii T., Fukuda M., Ochiai T., Neoh H.M., Camargo I.L., Watanabe Y., Shoji M., Hishinuma T. & Hiramatsu K. (2010). – An RpoB mutation confers dual heteroresistance to daptomycin and vancomycin in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.*, **54** (12), 5222–5233.
- Danforth H.D., Ruff M.D., Reid W.M. & Miller R.L. (1977). – Anticoccidial activity of salinomycin in battery raised broiler chickens. *Poult. Sci.*, **56** (3), 926–932.
- Davies T.A., He W., Bush K. & Flamm R.K. (2010). – Affinity of ceftobiprole for penicillin-binding protein 2b in *Streptococcus pneumoniae* strains with various susceptibilities to penicillin. *Antimicrob. Agents Chemother.*, **54** (10), 4510–4512.
- Diederer B., van Duijn I., van Belkum A., Willemsse P., van Keulen P. & Kluytmans J. (2005). – Performance of CHROMagar MRSA medium for detection of methicillin-resistant *Staphylococcus aureus*. *J. clin. Microbiol.*, **43**, 1925–1927.
- Draghi D.C., Benton B.M., Krause K.M., Thornsberry C., Pillar C. & Sahn D.F. (2008). – Comparative surveillance study of telavancin activity against recently collected Gram-positive clinical isolates from across the United States. *Antimicrob. Agents Chemother.*, **52** (7), 2383–2388.

26. Drawz S.M. & Bonomo R.A. (2010). – Three decades of beta-lactamase inhibitors. *Clin. Microbiol. Rev.*, **23** (1), 160–201.
27. Du W., Brown J.R., Sylvester D.R., Huang J., Chalker A.F., So C.Y., Holmes D.J., Payne D.J. & Wallis N.G. (2000). – Two active forms of UDP-N-acetylglucosamine enolpyruvyl transferase in Gram-positive bacteria. *J. Bacteriol.*, **182** (15), 4146–4152.
28. Duemling W.W. (1946). – Clinical experiences with penicillin in the Navy. *Ann. N.Y. Acad. Sci.*, **48**, 201–220.
29. Elting L.S., Rubenstein E.B., Kurtin D., Rolston K.V., Fangtang J., Martin C.G., Raad I.I., Whimbey E.E., Manzullo E. & Bodey G.P. (1998). – Mississippi mud in the 1990s: risks and outcomes of vancomycin-associated toxicity in general oncology practice. *Cancer*, **83** (12), 2597–2607.
30. Evans M.E., Feola D.J. & Rapp R.P. (1999). – Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant Gram-negative bacteria. *Ann. Pharmacother.*, **33** (9), 960–967.
31. Falagas M.E., Rafailidis P.I. & Matthaïou D.K. (2010). – Resistance to polymyxins: mechanisms, frequency and treatment options. *Drug Resist. Updat.*, **13** (4–5), 132–138.
32. Fernandez L., Gooderham W.J., Bains M., McPhee J.B., Wiegand I. & Hancock R.E. (2010). – Adaptive resistance to the ‘last hope’ antibiotics polymyxin B and colistin in *Pseudomonas aeruginosa* is mediated by the novel two-component regulatory system ParR-ParS. *Antimicrob. Agents Chemother.*, **54** (8), 3372–3382.
33. Fillgrove K.L., Pakhomova S., Newcomer M.E. & Armstrong R.N. (2003). – Mechanistic diversity of fosfomycin resistance in pathogenic microorganisms. *J. Am. Chem. Soc.*, **125** (51), 15730–15731.
34. Fillgrove K.L., Pakhomova S., Schaab M.R., Newcomer M.E. & Armstrong R.N. (2007). – Structure and mechanism of the genomically encoded fosfomycin resistance protein, FosX, from *Listeria monocytogenes*. *Biochemistry (Wash.)*, **46** (27), 8110–8120.
35. Friedman L., Alder J.D. & Silverman J.A. (2006). – Genetic changes that correlate with reduced susceptibility to daptomycin in *Staphylococcus aureus*. *Antimicrob. Agents Chemother.*, **50** (6), 2137–2145.
36. Fu W., Yang F., Kang X., Zhang X., Li Y., Xia B. & Jin C. (2007). – First structure of the polymyxin resistance proteins. *Biochem. Biophys. Res. Comm.*, **361** (4), 1033–1037.
37. Georgopapadakou N.H. & Liu F.Y. (1980). – Penicillin-binding proteins in bacteria. *Antimicrob. Agents Chemother.*, **18**, 148–157.
38. Giamarellou H. (2010). – Multidrug-resistant Gram-negative bacteria: how to treat and for how long. *Int. J. antimicrob. Agents*, **36** (2), S50–54.
39. Grayson M.L., Eliopoulos G.M., Wennersten C.B., Ruoff K.L., De Girolami P.C., Ferraro M.J. & Moellering R.C., Jr. (1991). – Increasing resistance to beta-lactam antibiotics among clinical isolates of *Enterococcus faecium*: a 22-year review at one institution. *Antimicrob. Agents Chemother.*, **35** (11), 2180–2184.
40. Green L.J. & Hall W.H. (1961). – Treatment of serious *Pseudomonas* infections with colistin sulfate. International Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Boston.
41. Griffith R.S. (1981). – Introduction to vancomycin. *Rev. infect. Dis.*, **3** (4), 200–204.
42. Gunn J.S., Lim K.B., Krueger J., Kim K., Guo L., Hackett M. & Miller S.I. (1998). – PmrA-PmrB-regulated genes necessary for 4-aminoarabinose lipid A modification and polymyxin resistance. *Molec. Microbiol.*, **27** (6), 1171–1182.
43. Hansen J.L., Levinsen C., Schmidt A. & Eriksen K.R. (1965). – Toxic effect of polymyxin: experiences with clinical material on 307 patients [in Danish]. *Ugeskr. Laeger*, **127** (44), 1411–1414.
44. Hirsch H.A., McCarthy C.G. & Finland M. (1960). – Polymyxin B and colistin: activity, resistance and crossresistance *in vitro*. *Proc. Soc. experim. Biol. Med.*, **103**, 338–342.
45. Horii T., Kimura T., Sato K., Shibayama K. & Ohta M. (1999). – Emergence of fosfomycin-resistant isolates of Shiga-like toxin-producing *Escherichia coli* O26. *Antimicrob. Agents Chemother.*, **43** (4), 789–793.
46. Howden B.P., Davies J.K., Johnson P.D., Stinear T.P. & Grayson M.L. (2010). – Reduced vancomycin susceptibility in *Staphylococcus aureus*, including vancomycin-intermediate and heterogeneous vancomycin-intermediate strains: resistance mechanisms, laboratory detection, and clinical implications. *Clin. Microbiol. Rev.*, **23** (1), 99–139.
47. Huovinen P., Huovinen S. & Jacoby G.A. (1988). – Sequence of PSE-2 beta-lactamase. *Antimicrob. Agents Chemother.*, **32**, 134–136.
48. Islam K.M., Klein U. & Burch D.G. (2009). – The activity and compatibility of the antibiotic tiamulin with other drugs in poultry medicine: a review. *Poult. Sci.*, **88** (11), 2353–2359.
49. Jacoby G.A. & Bush K. (2011). –  $\beta$ -Lactamase classification and amino acid sequences for TEM, SHV and OXA extended-spectrum and inhibitor resistant enzymes. Available at: [www.lahey.org/Studies](http://www.lahey.org/Studies) (accessed on 28 December 2011).
50. Jaurin B. & Grundstrom T. (1981). – *amp C* cephalosporinase of *Escherichia coli* K-12 has a different evolutionary origin from that of  $\beta$ -lactamases of the penicillinase type. *Proc. natl Acad. Sci. USA*, **78**, 4897–4901.

51. Jones T., Yeaman M.R., Sakoulas G., Yang S.J., Proctor R.A., Sahl H.G., Schrenzel J., Xiong Y.Q. & Bayer A.S. (2008). – Failures in clinical treatment of *Staphylococcus aureus* infection with daptomycin are associated with alterations in surface charge, membrane phospholipid asymmetry, and drug binding. *Antimicrob. Agents Chemother.*, **52** (1), 269–278.
52. Kahan F.M., Kahan J.S., Cassidy P.J. & Kropp H. (1974). – The mechanism of action of fosfomycin (phosphonomycin). *Ann. N.Y. Acad. Sci.*, **235**, 364–386.
53. Kelesidis T., Humphries R., Uslan D.Z. & Pegues D.A. (2011). – Daptomycin nonsusceptible enterococci: an emerging challenge for clinicians. *Clin. infect. Dis.*, **52** (2), 228–234.
54. Kirby W.M.M. (1945). – Bacteriostatic and lytic actions of penicillin on sensitive and resistant staphylococci. *J. clin. Invest.*, **24**, 165–169.
55. Kobayashi S., Kuzuyama T. & Seto H. (2000). – Characterization of the *fomA* and *fomB* gene products from *Streptomyces wedmorensis*, which confer fosfomycin resistance on *Escherichia coli*. *Antimicrob. Agents Chemother.*, **44** (3), 647–650.
56. Kwa A., Kasiakou S.K., Tam V.H. & Falagas M.E. (2007). – Polymyxin B: similarities to and differences from colistin (polymyxin E). *Expert. Rev. anti-infect. Ther.*, **5** (5), 811–821.
57. Levin A.S., Barone A.A., Penco J., Santos M.V., Marinho I.S., Arruda E.A., Manrique E.I. & Costa S.F. (1999). – Intravenous colistin as therapy for nosocomial infections caused by multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. *Clin. infect. Dis.*, **28** (5), 1008–1011.
58. Ligozzi M., Pittaluga F. & Fontana R. (1996). – Modification of penicillin-binding protein 5 associated with high-level ampicillin resistance in *Enterococcus faecium*. *Antimicrob. Agents Chemother.*, **40** (2), 354–357.
59. Lim L.M., Ly N., Anderson D., Yang J.C., Macander L., Jarkowski A., 3rd, Forrest A., Bulitta J.B. & Tsuji B.T. (2010). – Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. *Pharmacotherapy*, **30** (12), 1279–1291.
60. Livermore D.M. (2002). – The impact of carbapenemases on antimicrobial development and therapy. *Curr. Opin. Investig. Drugs*, **3** (2), 218–224.
61. Livermore D.M. (2009). – Has the era of untreatable infections arrived? *J. antimicrob. Chemother.*, **64** (Suppl. 1), i29–i36.
62. Llanea J., Villar C.J., Salas J.A., Suarez J.E., Mendoza M.C. & Hardisson C. (1985). – Plasmid-mediated fosfomycin resistance is due to enzymatic modification of the antibiotic. *Antimicrob. Agents Chemother.*, **28** (1), 163–164.
63. Lunde C.S., Hartouni S.R., Janc J.W., Mammen M., Humphrey P.P. & Benton B.M. (2009). – Telavancin disrupts the functional integrity of the bacterial membrane through targeted interaction with the cell wall precursor lipid II. *Antimicrob. Agents Chemother.*, **53** (8), 3375–3383.
64. Massova I. & Mobashery S. (1998). – Kinship and diversification of bacterial penicillin-binding proteins and  $\beta$ -lactamases. *Antimicrob. Agents Chemother.*, **42** (1), 1–17.
65. Mendes R.E., Moet G.J., Janecek M.J. & Jones R.N. (2010). – *In vitro* activity of telavancin against a contemporary worldwide collection of *Staphylococcus aureus* isolates. *Antimicrob. Agents Chemother.*, **54** (6), 2704–2706.
66. Miller T.W., Chalet L., Kahan F.M., Foltz E.L., Woodruff H.B., Mata J.M., Hernandez S. & Mochales S. (1969). – Phosphonomycin, a new antibiotic produced by strains of *Streptomyces*. *Science*, **166**, 122–123.
67. Montero C.I., Stock F. & Murray P.R. (2008). – Mechanisms of resistance to daptomycin in *Enterococcus faecium*. *Antimicrob. Agents Chemother.*, **52** (3), 1167–1170.
68. Muthaiyan A., Silverman J.A., Jayaswal R.K. & Wilkinson B.J. (2008). – Transcriptional profiling reveals that daptomycin induces the *Staphylococcus aureus* cell wall stress stimulon and genes responsive to membrane depolarization. *Antimicrob. Agents Chemother.*, **52** (3), 980–990.
69. Nair S.K. & van der Donk D.W.A. (2011). – Structure and mechanism of enzymes involved in biosynthesis and breakdown of the phosphonates fosfomycin, dehydrophos, and phosphinothricin. *Arch. Biochem. Biophys.*, **505** (1), 13–21.
70. Nannini E., Murray B.E. & Arias C.A. (2010). – Resistance or decreased susceptibility to glycopeptides, daptomycin, and linezolid in methicillin-resistant *Staphylococcus aureus*. *Curr. Opin. Pharmacol.*, **10** (5), 516–521.
71. Nichol K.A., Sill M., Laing N.M., Johnson J.L., Hoban D.J. & Zhanel G.G. (2006). – Molecular epidemiology of urinary tract isolates of vancomycin-resistant *Enterococcus faecium* from North America. *Int. J. antimicrob. Agents*, **27** (5), 392–396.
72. Norris S.R., Stratton C.W. & Kernodle D.S. (1994). – Production of A and C variants of staphylococcal  $\beta$ -lactamase by methicillin-resistant strains of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.*, **38** (7), 1649–1650.
73. Novilla M.N. (1992). – The veterinary importance of the toxic syndrome induced by ionophores. *Vet. hum. Toxicol.*, **34** (1), 66–70.
74. O'Hara K. (1993). – Two different types of fosfomycin resistance in clinical isolates of *Klebsiella pneumoniae*. *FEMS Microbiol. Lett.*, **114** (1), 9–16.
75. Parenti F., Schito G.C. & Courvalin P. (2000). – Teicoplanin chemistry and microbiology. *J. Chemother.*, **55**, 4.
76. Perichon B. & Courvalin P. (2009). – VanA-type vancomycin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.*, **53** (11), 4580–4587.
77. Pfeifer Y., Witte W., Holfelder M., Busch J., Nordmann P. & Poirel L. (2011). – NDM-1-producing *Escherichia coli* in Germany. *Antimicrob. Agents Chemother.*, **55**, 1318–1319.

78. Prakash V., Lewis J.S., 2nd, Herrera M.L., Wickes B.L. & Jorgensen J.H. (2009). – Oral and parenteral therapeutic options for outpatient urinary infections caused by Enterobacteriaceae producing CTX-M extended-spectrum beta-lactamases. *Antimicrob. Agents Chemother.*, **53** (3), 1278–1280.
79. Rolinson G.N. & Geddes A.M. (2007). – The 50th anniversary of the discovery of 6-aminopenicillanic acid (6-APA). *Int. J. antimicrob. Agents*, **29** (1), 3–8.
80. Rossolini G.M., Mantengoli E., Montagnani F. & Pollini S. (2010). – Epidemiology and clinical relevance of microbial resistance determinants versus anti-Gram-positive agents. *Curr. Op. Microbiol.*, **13** (5), 582–588.
81. Ryffel C., Kayser F.H. & Berger-Bachi B. (1992). – Correlation between regulation of *mecA* transcription and expression of methicillin resistance in staphylococci. *Antimicrob. Agents Chemother.*, **36** (1), 25–31.
82. Shryock T.R. & Richwine A. (2010). – The interface between veterinary and human antibiotic use. *Ann. N.Y. Acad. Sci.*, **1213**, 92–105.
83. Silverman J.A., Mortin L.I., Vanpraagh A.D., Li T. & Alder J. (2005). – Inhibition of daptomycin by pulmonary surfactant: in vitro modeling and clinical impact. *J. infect. Dis.*, **191** (12), 2149–2152.
84. Souli M., Galani I., Antoniadou A., Papadomichelakis E., Poulakou G., Panagea T., Vourli S., Zerva L., Armaganidis A., Kanellakopoulou K. & Giamarellou H. (2010). – An outbreak of infection due to beta-lactamase *Klebsiella pneumoniae* carbapenemase 2-producing *K. pneumoniae* in a Greek university hospital: molecular characterization, epidemiology, and outcomes. *Clin. infect. Dis.*, **50** (3), 364–373.
85. Suarez J.E. & Mendoza M.C. (1991). – Plasmid-encoded fosfomycin resistance. *Antimicrob. Agents Chemother.*, **35** (5), 791–795.
86. Tenover F.C. (2010). – The quest to identify heterogeneously resistant vancomycin-intermediate *Staphylococcus aureus* strains. *Int. J. antimicrob. Agents*, **36** (4), 303–306.
87. Torok M.E., Chapman A.L., Lessing M.P., Sanderson F. & Seaton R.A. (2010). – Outpatient parenteral antimicrobial therapy: recent developments and future prospects. *Curr. Opin. Investig. Drugs*, **11** (8), 929–939.
88. Van Bambeke F., Mingot-Leclercq M.P., Struelens M.J. & Tulkens P.M. (2008). – The bacterial envelope as a target for novel anti-MRSA antibiotics. *Trends pharmacol. Sci.*, **29** (3), 124–134.
89. Walsh T.R., Weeks J., Livermore D.M. & Toleman M.A. (2011). – Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet infect. Dis.*, **11**, 355–362.
90. Waxman D.J., Yocum R.R. & Strominger J.L. (1980). – Penicillins and cephalosporins are active site-directed acylating agents: evidence in support of the substrate analogue hypothesis. *Philos. Trans. roy. Soc. Lond., B, Biol. Sci.*, **289** (1036), 257–271.
91. Wegener H.C., Aarestrup F.M., Jensen L.B., Hammerum A.M. & Bager F. (1999). – Use of antimicrobial growth promoters in food animals and *Enterococcus faecium* resistance to therapeutic antimicrobial drugs in Europe. *Emerg. Infect. Dis.*, **5** (3), 329–335.
92. Wu H.C. & Venkateswaran P.S. (1974). – Fosfomycin-resistant mutant of *Escherichia coli*. *Ann. N.Y. Acad. Sci.*, **235**, 587–592.
93. Yang S.J., Nast C.C., Mishra N.N., Yeaman M.R., Fey P.D. & Bayer A.S. (2010). – Cell wall thickening is not a universal accompaniment of the daptomycin nonsusceptibility phenotype in *Staphylococcus aureus*: evidence for multiple resistance mechanisms. *Antimicrob. Agents Chemother.*, **54** (8), 3079–3085.
94. Yokochi T., Inoue Y., Jiang G.Z., Kato Y., Sugiyama T., Kawai M., Fukada M. & Takahashi K. (1994). – Increased phosphodiesterase in lipopolysaccharide prepared from the polymyxin B-resistant isolate of *Klebsiella pneumoniae*. *Microbiol. Immunol.*, **38** (11), 901–903.
95. Zilhao R. & Courvalin P. (1990). – Nucleotide sequence of the *FosB* gene conferring fosfomycin resistance in *Staphylococcus epidermidis*. *FEMS Microbiol. Lett.*, **56** (3), 267–272.
96. Zscheck K.K. & Murray B.E. (1991). – Nucleotide sequence of the beta-lactamase gene from *Enterococcus faecalis* HH22 and its similarity to staphylococcal beta-lactamase genes. *Antimicrob. Agents Chemother.*, **35** (9), 1736–1740.