CHAPTER **14**

Antimicrobial Resistance in Gram Positive and Gram Negative Bacteria Progress and Challenges

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ABSTRACT

Serious infections caused by bacteria that have become resistant to commonly used antibiotics have become a major global healthcare problem in the 21st century. Antibiotic resistance, initially a problem of the hospital setting associated with an increased number of hospitals acquired infections usually in critically ill and immuno-suppressed patients, has now extended into the community causing severe infections difficult to diagnose and treat. In hospitals, most common resistant bacteria include methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and gram-negative rods including Enterobacteriaceae and *Pseudomonas aeruginosa*. Vancomycin intermediate and resistant *S. aureus*, represent a new treatment challenge. In the community, penicillin and macrolide-resistant pneumococci developed several decades ago and are now present all over the world. More recently, community-acquired methicillin-resistant *S. aureus* has become a problem in several countries causing skin infections but also severe diseases. The molecular mechanisms by which bacteria have become resistant to antibiotics are diverse and complex. Bacteria have developed resistance to all different classes of antibiotics discovered to date. The most frequent type of resistance is acquired and transmitted horizontally via the conjugation of a plasmid. In recent times new mechanisms of resistance have resulted in the simultaneous development of resistance to several antibiotic classes creating very dangerous multidrug-resistant (MDR) bacterial strains, some also known as ''superbugs''. In many cases the use of

antibiotics is unnecessary or questionable. The indiscriminate and inappropriate use of antibiotics in outpatient clinics, hospitalized patients and in the food industry is the single largest factor leading to antibiotic resistance. In recent years, the number of new antibiotics licensed for human use in different parts of the world has been lower than in the recent past. In addition, there has been less innovation in the field of antimicrobial discovery research and development. The pharmaceutical industry, large academic institutions or the government are not investing the necessary resources to produce the next generation of newer safe and effective antimicrobial drugs. In many cases, large pharmaceutical companies have terminated their anti-infective research programs altogether due to economic reasons. The potential negative consequences of all these events are relevant because they put society at risk for the spread of potentially serious MDR bacterial infections.

Key words: antimicrobial resistance, antibacterial agents, antibiotic, antimicrobial activity

1. INTRODUCTION

The term "antibiotic" originally referred to a natural compound produced by a fungus or another microorganism that kills bacteria, which cause disease in humans or animals. Some antibiotics may be synthetic compounds (not produced by microorganisms) that can also kill or inhibit the growth of microbes. Technically, the term "Antibiotic" refers to synthetic compounds as well as natural compounds, and to refer to antiviral as well as antibacterial drugs.

The first antibiotic, penicillin, was discovered in 1929 by Sir Alexander Fleming who observed inhibition of staphylococci on an agar plate contaminated by a *Penicillium* mold. In World War II the inevitable bacterial infections that occurred in war-related wounds was an important impetus to study the chemotherapeutic value of penicillin. Penicillin became generally available for treatment of bacterial infections, especially those caused by staphylococci and streptococci, about 1946. Initially, penicillin was effective against all sorts of infections caused by these two gram-positive bacteria. A significant fraction of all human infections are caused by these two bacteria (i.e., strep throat, pneumonia, septicemia, skin infections, wound infections, scarlet fever, toxic shock syndrome). Penicillin had unbelievable ability to kill these bacterial pathogens without harming the host that harbored them. Resistance to penicillin in some strains of staphylococci was recognized almost immediately after introduction of the drug. Surprisingly, *Streptococcus pyogenes* (Group A strep) have never fully developed resistance to penicillin and it remains a reasonable choice antibiotic for many types of streptococcal infections. Interestingly, penicillin has never been effective against most gram-negative pathogens (e.g. *Salmonella*, *Shigella*, *Bordetella pertussis*, *Yersinia pestis*, *Pseudomonas*) with the notable exception of *Neisseria gonorrhoeae*.

The list of bacteria developing resistance is impressive, from sulfonamide and penicillin-resistant *Staphylococcus aureus* in the 1930s and 1940s (Levy, 2002;

Rammelkamp, 1942) to penicillin-resistant *Neisseria gonorrhoeae* (PPNG), and β-lactamase-producing *Haemophilus influenzae* in the 1970s, (Jaffe *et al.*, 1981; Lind, 1990; Williams and Moosdeen, 1986; Jorgensen, 1993) methicillin resistant *Staphylococcus aureus* (MRSA) and the resurgence of multi-drug resistant (MDR) *Mycobacterium tuberculosis* in the late 1970s and 1980s, (Lowy, 1998; Deresinski, 2005; Lowy, 2003; Foster, 2004; Pablos-Mendez *et al.*, 1998; Espinal *et al.*, 2001) and several resistant strains of common enteric and non-enteric gram-negative bacteria such as *Shigella* sp., *Salmonella* sp., *Vibrio cholerae, E. coli, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa*, some of these associated with the use of antimicrobials in animals grown for human food consumption in the 1980s and 1990s (Waterer and Wunderink, 2001; Rupp and Fey 2003; White *et al.*, 2001; Smith *et al.*, 1999; Wegener, 1999; Fey *et al.*, 2000). Recently we have also witnessed the report of very worrisome cases of previously unthinkable resistance as well as the spread of resistant bacteria outside the hospital causing community-acquired infections. Such is the case or strains of Group A, Streptococcus becoming resistant to macrolide antibiotics (Seppala *et al.*, 1995; Cizman *et al.*, 2001; Martin *et al.*, 2002; Huovinen, 2002), *Streptococcus pneumoniae* developing resistance to different antibiotic classes, including penicillin, and causing serious infections (Nuorti *et al.*, 1998; Whitney *et al.*, 2000; Hofmann *et al.*, 1995; Tomasz, 1995; Amsden, 2004; Vanderkooi *et al.*, 2005; File, 2004; Jacobs, 2004) and more virulent strains of MRSA (due to the expression of certain toxins such as the so-called Panton-Valentine leukocidin) spreading to the community (Saravolatz *et al.*, 1982; Robinson *et al.*, 2005; Kaplan *et al.*, 2005), as well as *Staphylococcus aureus* and Enterococci becoming resistant to vancomycin (Smith *et al.*, 1999; Sieradzki *et al.*, 1999; Waldvogel, 1999).

The period of the late 1940s and early 1950s saw the discovery and introduction of streptomycin, chloramphenicol, and tetracycline, and the age of antibiotic chemotherapy came into full being. These antibiotics were effective against the full array of bacterial pathogens including gram-positive and gram-negative bacteria, intracellular parasites, and the tuberculosis bacillus. However, by 1953, during a *Shigella* outbreak in Japan, a strain of the dysentery bacillus was isolated, exhibiting resistances to chloramphenicol, tetracycline, streptomycin, and the sulfanilamides. There was also evidence mounting that bacteria could pass genes for multiple drug resistance between strains and even between species.

Antibiotic resistance, initially a problem of the hospital setting associated with an increased number of hospital acquired infections usually in critically ill and immunosuppressed patients, has now extended into the community causing severe infections difficult to diagnose and treat. In hospitals, most common resistant bacteria include methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci and gram-negative rods including Enterobacteriaceae and *Pseudomonas aeruginosa*. Gram-positive bacteria are common causes of bloodstream and other infections in hospitalized patients, and the percentage of nosocomial bloodstream infections caused by antibiotic-resistant gram-positive bacteria is increasing. Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant

enterococci (VRE) are of particular concern. Vancomycin is the standard treatment for serious MRSA infections, but a few cases of vancomycin-resistant *S. aureus* (VRSA) have recently emerged, represent a new treatment challenge. More recently, community-acquired methicillin-resistant *S. aureus* (CA-MRSA) has become a problem in several countries causing skin infections but also severe diseases. Resistance to co-trimoxazole in *Escherichia coli* has changed empirical treatment of urinary tract infections.

It was also apparent that *Mycobacterium tuberculosis* was capable of rapid development of resistance to streptomycin, which had become a mainstay in tuberculosis therapy. It is estimated that one third of the world's population is infected with TB, with about 8 million new cases reported annually (National Institute of Allergy and Infectious Diseases (NIAID), Fact sheet on tuberculosis. www.niaid.nih.go.factsheets/tb.htm). India is one of the developing countries having TB and HIV infection on rise in an alarming speed. Still worse is the situation due to the emergence of multi-drug-resistant (MDR) strains of *M. tuberculosis* along with human immunodeficiency virus (HIV) infection. Moreover, secondary fugal infections that occur towards the later part of medication, among the TB patients aggravate the situation. In July 2003 report, the WHO has called TB (not AIDS) is the biggest health concern of India (Gupta, 2003). This reflects the magnitude and the seriousness of the problem.

In recent times new mechanisms of resistance have resulted in the simultaneous development of resistance to several antibiotic classes creating very dangerous multidrug-resistant (MDR) bacterial strains, some also known as "superbugs". The indiscriminate and inappropriate use of antibiotics in outpatient clinics, hospitalized patients and in the food industry is the single largest factor leading to antibiotic resistance. In recent years, the number of new antibiotics licensed for human use in different parts of the world has been lower than in the recent past. In addition, there has been less innovation in the field of antimicrobial discovery research and development. The pharmaceutical industry, large academic institutions or the government are not investing the necessary resources to produce the next generation of newer safe and effective antimicrobial drugs. In many cases, large pharmaceutical companies have terminated their anti-infective research programs altogether due to economic reasons. The potential negative consequences of all these events are relevant because they put society at risk for the spread of potentially serious MDR bacterial infections.

2. MECHANISMS OF RESISTANCE TO ANTIBACTERIAL AGENTS

Bacteria may manifest resistance to antibacterial drugs through a variety of mechanisms (Table 1). Of greater concern are cases of acquired resistance, where initially susceptible populations of bacteria become resistant to an antibacterial agent and proliferate and spread under the selective pressure of use of that agent. Several mechanisms of antimicrobial resistance are readily spread to a variety of bacterial genera-

(1) The organism may acquire genes encoding enzymes, such as β-lactamases, that destroy the antibacterial agent before it can have an effect.

(2) Bacteria may acquire efflux pumps that extrude the antibacterial agent from the cell before it can reach its target site and exert its effect.

(3) Bacteria may acquire several genes for a metabolic pathway, which ultimately produces altered bacterial cell walls that no longer contain the binding site of the antimicrobial agent, or bacteria may acquire mutations that limit access of antimicrobial agents to the intracellular target site via down regulation of porin genes.

Thus, normally susceptible populations of bacteria may become resistant to antimicrobial agents through mutation and selection, or by acquiring from other bacteria the genetic information that encodes resistance. The last event may occur through one of several genetic mechanisms, including transformation, conjugation, or transduction. Through genetic exchange mechanisms, many bacteria have become resistant to multiple classes of antibacterial agents, and these bacteria with multidrug resistance have become a cause for serious concern, particularly in hospitals and other healthcare institutions where they tend to occur most commonly. As noted above, susceptible bacteria can acquire resistance to an antimicrobial agent via new mutations. Such spontaneous mutations may cause resistance by-

➢ altering the target protein to which the antibacterial agent binds by modifying or eliminating the binding site (e.g., change in penicillin-binding protein 2b in pneumococci, which results in penicillin resistance),

➢ upregulating the production of enzymes that inactivate the antimicrobial agent (e.g., erythromycin ribosomal methylase in staphylococci),

➢ down regulating or altering an outer membrane protein channel that the drug requires for cell entry (e.g., OmpF in *E coli*), or upregulating pumps that expel the drug from the cell [efflux of fluoroquinolones in *S. aureus* (McManus, 1997)]. In all of these cases, strains of bacteria carrying resistance-conferring mutations are selected by antimicrobial use, which kills the susceptible strains but allows the newly resistant strains to survive and grow.

Acquired resistance that develops due to chromosomal mutation and selection is termed vertical evolution. Vertical evolution is strictly a matter of Darwinian evolution driven by principles of natural selection: a spontaneous mutation in the bacterial chromosome imparts resistance to a member of the bacterial population. Bacteria also develop resistance through the acquisition of new genetic material from other resistant organisms. This is termed horizontal evolution, and may occur between strains of the same species or between different bacterial species or genera.

Bacteria are able to exchange genes in nature by three processes: conjugation, transduction and transformation (McManus, 1997). For each of these processes, transposons may facilitate the transfer and incorporation of the acquired resistance genes into the host's genome or into plasmids. Conjugation: Conjugation is the most important and the most common mechanism of transmission of resistance in bacteria. During conjugation, a gram-negative bacterium transfers plasmid-containing

resistance genes to an adjacent bacterium, often via an elongated proteinaceous structure termed as *pilus*, which joins the two organisms. Conjugation among gram-positive bacteria is usually initiated by production of sex pheromones by the mating pair, which facilitate the clumping of donor and recipient organisms, allowing the exchange of DNA. Transformation: Transformation is another form of transmission of bacterial resistance genes and takes place when there is direct passage of free DNA (also known as ''naked DNA'') from one cell to another. The ''naked DNA'' usually originates from other bacteria that have died and broken apart close to the receiving bacteria. The receiving bacteria then simply introduce the free DNA into their cytoplasm and incorporate it into their own DNA. Transduction: Transduction is a third mechanism of genetic transfer and occurs via the use of a ''vector'', most often viruses capable of infecting bacteria also known as ''bacteriophages'' (or simply ''phages''). The virus containing the bacterial gene that codifies antibiotic resistance (the ''resistant DNA'') infects the new bacterial cell and introduces this genetic material into the receiving bacteria. Most times, the infecting bacteriophage also introduces to the receiving bacteria its own viral DNA, which then takes over the bacterial replication system forcing the cell to produce more copies of the infecting virus until the bacterial cell dies and liberates these new bacteriophages, which then go on to infect other cells.

Mutation and selection, together with the mechanisms of genetic exchange, enable many bacterial species to adapt quickly to the introduction of antibacterial agents into their environment. Genetic recombination can follow the transfer of DNA from one cell to another leading to the emergence of a new genotype (recombinant). It is common for DNA to be transferred as plasmids between mating bacteria. Since bacteria usually develop their genes for drug resistance on plasmids (called resistance transfer factors or RTFs), they are able to spread drug resistance to other strains and species during genetic exchange processes.

2.1 Biological Mechanisms of Resistance

Whichever way a gene is transferred to a bacterium, the development of antibiotic resistance occurs when the gene is able to express itself and produce a tangible biological effect resulting in the loss of activity of the antibiotic. These biological mechanisms are many and varied but they can be summarized as follows.

2.1.1 Antibiotic Destruction or Antibiotic Transformation

This destruction or transformation occurs when the bacteria produces one or more enzymes that chemically degrade or modify the antimicrobial making them inactive against the bacteria. This is a common mechanism of resistance and probably one of the oldest ones affecting several antibiotics but especially b-lactam antibiotics via the bacterial production of β-lactamases (Jacoby and Munoz-Price, 2005).

2.1.2 Antibiotic Active Efflux

Efflux was first described for tetracycline and macrolide antibiotics (Roberts, 1996; Leclercq, 2002) but is now common for many other antibiotics such as

fluoroquinolones (Sefton, 2002; Hooper, 2005). Antibiotic active efflux is relevant for antibiotics that act inside the bacteria and takes place when the microorganism is capable of developing an active transport mechanism that pumps the antibiotic molecules that penetrated into the cell to the outside milieu until it reaches a concentration below that necessary for the antibiotic to have antibacterial activity. This means that the efflux transport mechanism must be stronger than the influx mechanism in order to be effective (Hooper, 2005).

2.2 Receptor Modification

Receptor modification occurs when the intracellular target or receptor of the antibiotic drug is altered by the bacteria, resulting in the lack of binding and consequently the lack of antibacterial effect. Examples of this mechanism include modifications in the structural conformation of penicillin-binding proteins (PBPs) observed in certain types of penicillin resistance, ribosomal alterations that can render aminoglycosides, macrolides or tetracyclines inactive, and DNA-gyrase modifications resulting in resistance to fluoroquinolones (Levy and Marshall, 2004; Sefton, 2002). It is likely that more and newer biological mechanisms of resistance will develop in the future.

TABLE 1. Mechanisms of resistance

Mechanism	Class of antibiotic
1. Reduced uptake into cell	Chloramphenicol, β-lactams
2. Active efflux from cell	Tetracycline, Quinolones Sulfonamides, Trimethoprim
3. Modification of target to eliminate or reduce binding of antibiotic	β-lactams, Erythromycin Rifampicin, minoglycosides
4. Detoxification of antibiotic by enzyme modification: i. Hydrolysis ii. derivatization	β-lactams, Erythromycin Aminoglycosides, Chloramphenicol Fosfomycin, Lincomycin
5. Sequestration of antibiotic by protein binding	β-lactams, Fusidic acid
6. Metabolic bypass of inhibited reaction	Sulfonamides, Trimethoprim
7. Binding of specific immunity protein to antibiotic	Bleomycin
8. Overproduction of antibiotic target (titration)	Sulfonamides, Trimethoprim
9. Failure to active prodrug	Isoniazid

3. MECHANISMS OF ACTION OF ANTIBACTERIAL AGENTS

Most antimicrobial agents used for the treatment of bacterial infections may be categorized according to their principal mechanism of action. There are 4 major modes of action (Table

2): (1) interference with cell wall synthesis, (2) inhibition of protein synthesis, (3) interference with nucleic acid synthesis, and (4) inhibition of a metabolic pathway (Neu, 1992).

Antibacterial drugs that work by inhibiting bacterial cell wall synthesis include the β-lactams, such as the penicillins, cephalosporins, carbapenems, and monobactams, and the glycopeptides, including vancomycin and teicoplanin (Neu, 1992; McManus, 1997). β-Lactam agents inhibit synthesis of the bacterial cell wall by interfering with the enzymes required for the synthesis of the peptidoglycan layer (McManus, 1997). Vancomycin and teicoplanin also interfere with cell wall synthesis, but do so by binding to the terminal D-alanine residues of the nascent peptidoglycan chain, thereby preventing the cross-linking steps required for stable cell wall synthesis (McManus, 1997). Macrolides, aminoglycosides, tetracyclines, chloramphenicol, streptogramins, and oxazolidinones produce their antibacterial effects by inhibiting protein synthesis (McManus, 1997). Bacterial ribosomes differ in structure from their counterparts in eukaryotic cells. Antibacterial agents take advantage of these differences to selectively inhibit bacterial growth. Macrolides, aminoglycosides, and tetracyclines bind to the 30S subunit of the ribosome, whereas chloramphenicol binds to the 50S subunit. Fluoroquinolones exert their antibacterial effects by disrupting DNA synthesis and causing lethal double-strand DNA breaks during DNA replication, (Drlica and Zhao, 1997) whereas sulfonamides and trimethoprim (TMP) block the pathway for folic acid synthesis, which ultimately inhibits DNA synthesis (Yao and Moellering, 2003; Petri, 2006). The common antibacterial drug combination of TMP, a folic acid analogue, plus sulfamethoxazole (SMX) (a sulfonamide) inhibits 2 steps in the enzymatic pathway for bacterial folate synthesis. Disruption of bacterial membrane structure may be a fifth, although less well characterized, mechanism of action. It is postulated that polymyxins exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial contents (Storm, 1977). The cyclic lipopeptide daptomycin apparently inserts its lipid tail into the bacterial cell membrane, (Carpenter and Chambers, 2004) causing membrane depolarization and eventual death of the bacterium.

TABLE 2. Mechanisms of action of antibacterial agents

Mechanisms	Antibiotic class
Interference with cell wall synthesis	β-Lactams: penicillins, cephalosporins, carbapenems, monobactams Glycopeptides: vancomycin, teicoplanin
Protein synthesis inhibition Bind to 50S ribosomal subunit Bind to 30S ribosomal subunit Bind to bacterial isoleucyl-tRNA synthetase	macrolides, chloramphenicol, clindamycin, quinupristin-dalfopristin, linezolid aminoglycosides, tetracyclines mupirocin
Interference with nucleic acid synthesis Inhibit DNA synthesis Inhibit RNA synthesis	fluoroquinolones rifampin
Inhibition of metabolic pathway	sulfonamides, folic acid analogues
Disruption of bacterial membrane structure	polymyxins, daptomycin

4. ANTIMICROBIAL RESISTANCE IN GRAM-POSITIVE BACTERIA

Gram-positive bacteria—particularly gram-positive cocci like coagulase-negative staphylococci, *Staphylococcus aureus*, and *Enterococcus* spp—are extremely important pathogens in the hospital environment. Data from the Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) project, which monitors significant bloodstream infections in hospitalized patients in the United States, indicated that 60% of nosocomial bloodstream infections for the 3-year period from April 1995 through April 1998 involved gram-positive bacteria (Edmond *et al.*, 1999). Coagulase-negative staphylococci were the causes of 31.9% of monomicrobial nosocomial bloodstream infections, followed by *S. aureus* in 15.7 %, enterococci species in 11.1%, and viridans streptococci in 1%. Updated figures from the SCOPE project covering March, 1995 through September, 2002 revealed similar findings (Wisplinghoff *et al.*, 2004). Increasing resistance in gram-positive bacteria can be expected to complicate treatment and potentially lead to increased morbidity and mortality.

4.1 Methicillin-resistant *S. aureus*

The first of these problematic bacteria was *S. aureus*. Virtually all *S. aureus* strains were susceptible to penicillin G when the latter was initially introduced in the early 1940s, but by 1944 the first reports of penicillin-resistant *S. aureus* had already appeared, and today virtually all strains of *S. aureus* are resistant to natural penicillins, aminopenicillins, and antipseudomonal penicillins (Chambers, 2001; Neu, 1992). Resistance to these drugs occurs because of the acquisition of genes that encode drug-inactivating enzymes, initially known as penicillinases and now called β-lactamases. At first, cases of penicillin resistant *S. aureus* were limited and appeared only in healthcare settings, but over time resistant species were observed and became increasingly prevalent in the wider community as well (Chambers, 2001). Methicillin and other penicillinase-resistant penicillins were developed to treat infections caused by penicillin-resistant *S. aureus* and met with initial success. MRSA is currently recognized as a major problem in hospitals and the broader community in the United States and throughout the world (Diekema *et al.*, 2001). The most recent SCOPE project report showed that methicillin resistance was present in 44% of *S. aureus* bloodstream isolates from ICU infections (Wisplinghoff *et al.*, 2004).

Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant *Staphylococcus aureus*. In 1961, there were reports from the United Kingdom of *S. aureus* isolates that had acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) (Jevons, 1961), and MRSA isolates were soon recovered from other European countries, and later from Japan, Australia (Palavecino, 2004). The acquisition of methicillin resistance gave these bacteria the name methicillin-resistant *S. aureus* (MRSA), but in reality these were multidrug-resistant *S. aureus* (Wadsworth *et al.*, 1992). MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community (Hussain *et al.*, 2000). Periodic outbreaks of MRSA were observed in various countries throughout the 1970s and were typically associated with high methicillin use in intensive care units

(Palavecino, 2004), but it was not until the 1980s that MRSA began to become a really significant problem in United States hospitals—first in hospitals with a large number of beds and then in community hospitals (Panlilio *et al.*, 1992).

The methicillin resistance gene (*mecA*) encodes a methicillin-resistant penicillin-binding protein that is not present in susceptible strains and is believed to have been acquired from a distantly related species (Hiramatsu *et al.*, 2001). Expression of the *mecA* gene encoding low-affinity penicillin-binding protein PBP2a confers resistance to other β-lactams in addition to methicillin (Utsui and Yokota, 1985), but the resistance pattern of MRSA typically includes other classes of antibiotics as well.

Nosocomial MRSA is remarkable for its clonal pattern of spread. A recent study looking at 359 MRSA isolates collected from 20 countries from 1961 to 1999 identified 11 major MRSA clones within 5 groups of related genotypes (Enright *et al.*, 2002). Results from a recent study in the United Kingdom examining a new epidemic strain of MRSA, designated EMRSA-17, illustrate multidrug resistance (Aucken *et al.*, 2002). EMRSA-17 expressed resistance to methicillin, fluoroquinolones (ciprofloxacin), macrolides (erythromycin), aminoglycosides (gentamicin, kanamycin, neomycin, and streptomycin), tetracycline, rifampin, and fusidic acid. Virtually all variants of this strain were multidrug resistant. In particular, fluoroquinolone resistance is a hallmark of nosocomial MRSA, although this was not always the case. When ciprofloxacin was first introduced, it was recommended as the first orally administered treatment effective against MRSA. However, within one year many hospitals observed dramatic increases in the rate of ciprofloxacin resistance in MRSA. High-level ciprofloxacin resistance was observed within 3 months of ciprofloxacin introduction, and within one year, 79% of all MRSA from hospitalized patients exhibited resistance (Blumberg *et al.*, 1991). For many years, vancomycin was the only effective treatment for serious MRSA infections. But in the past 4 years, 4 new agents with anti-MRSA activity have been introduced (quinupristin-dalfopristin, linezolid, daptomycin, and tigecycline). These new agents are most welcome, since the past decade has seen the emergence of vancomycin resistance in *S. aureus*. *S. aureus* with intermediate resistance to vancomycin (vancomycin-intermediate *S. aureus* [VISA]; minimum inhibitory concentration [MIC], 8 to 16 mg/L) was first observed in 1996 in a strain isolated from a hospitalized patient in Japan (Hiramatsu *et al.*, 1997). The mechanism of resistance in VISA has been linked to cell wall thickening, which may cause vancomycin molecules to become trapped in the outer layers of the cell wall, thereby limiting access to the cytoplasmic membrane where the functional targets of vancomycin are located (Cui *et al.*, 2003). The mechanism for this high-level vancomycin resistance involves the horizontal transfer of a transposon containing *vanA* and associated genes from VRE (Rice, 2006).

Many studies have characterized MRSA isolates from individual hospitals or countries and have identified strains that appear to be well adapted to the hospital environment, are established in several hospitals within a country, or have spread internationally (epidemic MRSA, EMRSA). MRSA isolates are generally characterized by pulsed-field gel electrophoresis, a powerful technique for

identifying the relatedness of isolates from recent outbreaks within a hospital, but are not well suited to long-term global epidemiology, which requires a procedure that is highly discriminatory but that indexes variation that accumulates slowly. Multilocus sequence typing (MLST) provides such a procedure and characterizes isolates of bacteria unambiguously by using the sequences of internal fragments of seven housekeeping genes (Enright and Spratt, 1999). MLST has been developed and validated for *S. aureus* (Enright *et al.*, 2000), and provides a discriminatory method that allows related strains recovered in different countries to be readily identified. The origins of the major MRSA clones are still poorly understood. Kreiswirth *et al.* (1993) proposed that all MRSAs were descended from a single ancestral *S. aureus* strain that acquired *mecA*, but more recent studies (Musser and Kapur, 1992) show that some MRSAs are very divergent, implying that *mecA* has been transferred between *S. aureus* lineages.

4.2 Multidrug-Resistant (MRD) Enterococcal Species

Enterococcus faecalis and *E. faecium* are species normally found in human gut flora and, apart from their rare occurrence as the cause of urinary tract infections, they were not usually considered as pathogenic. However, when patients were given immunosuppressive therapy, enterococci were increasingly found as the causative organism of infection. Like MRSA, the final defense against these gram-positive bacteria was considered to be vancomycin, but, by the end of the 1980s, they had acquired the ability to resist high levels of vancomycin (Woodford *et al.*, 1993). Despite the fact that vancomycin has been in clinical use since the late 1950s, VRE were not observed until the mid-1980s, and in the United States, VRE were virtually non-existent as recently as 1989 (Martone, 1998). During the 1990s, however, a dramatic rise in VRE occurred—first in Intensive Care Units (ICUs), then essentially throughout hospitals. The latest NNIS report indicated that nearly 30% of all enterococci isolated from patients infected in ICUs are now resistant to vancomycin. Perhaps an even more remarkable aspect of this outbreak is the fact that an overwhelming majority of VRE are *Enterococcus faecium*. The Surveillance Network Database –USA showed that resistance to both vancomycin and ampicillin was much more prevalent among *E. faecium* than among *Enterococcus faecalis* in patients with nosocomial bloodstream infections in 1995 and 1997 (Sahm *et al.*, 1999).

The mechanism of vancomycin resistance is particularly sophisticated and, although there are different variations of it, they all involve the reversal of the bacterium's normal manufacture of the cell wall. This is accompanied by the synthesis of a modified cell wall, which includes D-alanyl-D-lactate, or D-alanyl-D-serine terminal dipeptides, which are less capable of binding vancomycin than the normal bacterial dipeptide D-alanyl-D-alanine (Arthur *et al.*, 1996). Vancomycin resistance is mediated by either of 2 classes of related gene clusters: one class contains *vanA* and other class contains *vanB* (Arthur *et al.*, 1996). Both produce resistance by altering the target for vancomycin from D-alanine-D-alanine to D-alanine- D-lactate.

5. ANTIMICROBIAL RESISTANCE IN GRAM-NEGATIVE BACTERIA

Pseudomonas aeruginosa, *Acinetobacter baumannii* and *Klebsiella pneumoniae* are among the bacteria that readily develop multiple resistance mechanisms to various classes of antibiotics (Hanlon, 2005; Helfand and Bonomo, 2005; McGowan Jr, 2006; Ramphal and Ambrose, 2006; Rossolini and Mantengoli, 2005). In addition, they are important nosocomial pathogens affecting both immunocompetent and immunocompromised patients and are responsible for a considerable proportion of infections in patients in ICUs worldwide. Thus, infections by multidrug-resistant (MDR) *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* strains have become common in healthcare institutions. The continuously evolving resistance to antibiotics of *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* has led to the emergence of clinical isolates susceptible to only one class of antimicrobial agents and eventually to pandrug-resistant (PDR) isolates, i.e. resistant to all available antibiotics (Kuo *et al*., 2004; Marais *et al*., 2004; Yoon *et al*., 2004). Polymyxins, an old class of polypeptide cationic antibiotic that was abandoned during the 1980s and 1990s in most parts of the world, have been used as the last class of available antibiotics to which some of these bacterial isolates were susceptible *in vitro*. Several recent clinical studies have reported on the therapeutic use of polymyxins in patients with infections by organisms with such a phenotype (i.e. susceptible only to polymyxins) Garnacho-Montero *et al*., 2003; Michalopoulos *et al*., 2005; Falagas *et al*., 2006; Levin *et al*., 1999). In addition, there have been some recent clinical studies that reported on infections with isolates of *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* that were characterized as PDR. However, in some of these studies either the isolates were not tested *in vitro* against polymyxins or they were tested and found to be susceptible to them (Kuo *et al*., 2004; Marais *et al*., 2004). PDR infections due to the aforementioned gram-negative bacteria represent a fearful clinical situation with tremendous public health implications in which the clinician is left with practically no rational choice of antibiotic treatment. Thus, data regarding the frequency of PDR clinical isolates, the morbidity and mortality related to infections by these isolates and the therapeutic options, if any, are of great clinical and public health importance.

6. STRATEGIES FOR REDUCING THE IMPACT OF RESISTANCE

Antibiotic resistance is driven by antibiotic use. When antibiotics are superseded and therefore used less, strains resistant to these tend to disappear.

6.1. In the Community

In the UK, more than 80% of human use of antibiotics occurs in the community, mostly for respiratory tract infections. The Standing Medical Advisory Committee, in its report *The Path of Least Resistance*, made recommendations to reduce inappropriate prescribing (Standing Medical Advisory Committee, Department of Health. *The path of least resistance*. London: HMSO, 1998).

• No antibiotics should be given for simple coughs and colds.

- Antibiotics should not be routinely prescribed for sore throats, unless there is evidence of streptococcal infection.
- Antibiotics are not routinely required for acute otitis media and sinusitis-like symptoms; if given, courses can be limited to 3 days. In addition, 3 days' treatment should suffice in otherwise healthy women with uncomplicated cystitis. This strategy has been useful in reducing prescribing by GPs, but anxiety has resulted from anecdotal reports of an increase in bacterial respiratory infections. In hospital, antibiotic use can be reduced by several means.
- Routine use of antibiotics for surgical prophylaxis should be reduced to a minimum.
- Antibiotics should not be started immediately in all suspected infections. Certain patients (e.g. those with febrile neutropenia or evidence of septicaemia) require urgent antibiotic therapy, but in many other cases (e.g. mild pyrexia postoperatively), it is safe and ultimately preferable to withhold antibiotics until culture results are known or there is clear evidence of bacterial infection.
- An alternative is to discontinue antibiotics as soon as information is available suggesting that the problem is not bacterial or has resolved itself. This is termed an 'antibiotic-stop' policy, and the aim is to encourage doctors to actively review the need for antibiotics after, say, the second day, given information on cultures and surrogate markers that has by then become available.
- Certain antibiotics can be withheld from the hospital formulary. This is the main benefit of an agreed antibiotic policy. The choice of restricted antibiotics may be decided on the basis of cost rather than likely selection of resistance (these antibiotics must be used occasionally, however, when resistance to all other available drugs has been selected).
- In theory, antibiotics can be rotated such that, for example, predominantly penicillins are used at some times, and cephalosporins or quinolones at others. There is little clear scientific evidence that this has any effect, and major, complicated studies would be needed to determine the effect of change in use on both normal and infecting flora. However, it has been shown that, in a setting of heavy cephalosporin use, discontinuation of use of this class of drugs leads to a reduction in the risk of colonization with glycopeptide-resistant enterococci and *Clostridium difficile* associated diarrhoea.

6.2 Other Strategies:

Development of resistance in human pathogens might be delayed if antibiotics were not used so widely in animal husbandry, particularly for growth promotion.

7. THE FUTURE

Resistance to antibiotics is one of the greatest threats to the success of modern medicine. It has recently become more serious because we can no longer be sure that any antibiotic chosen empirically will work, and because of the emergence of totally resistant bacteria. How to reduce resistance without simply discontinuing use of all

antibiotics is a dilemma. We do not know to what degree antibiotic use must be reduced to decrease the selective pressure and allow reversion to sensitivity, nor do we know how to protect the few remaining drugs that can be used to treat resistant infections. Doctors and vets should overcome the view that they have an inalienable right to prescribe empirical antibiotics, and should set targets for reduction of their personal prescribing.

8. NOVEL AGENTS FOR RESISTANT BACTERIAL INFECTIONS

Emerging bacterial resistance to many available antimicrobial agents has led to a recent surge of pharmaceutical development, which has resulted in production of several new antibiotics. Novel agents, almost all derivatives of old compounds, have recently been introduced into clinical practice.

8.1 Linezolid

The oxazolidinones are a unique family of antimicrobial agents, first developed in the late 1970s for agricultural use (Brickner *et al.*, 1996). The agents are effective against a wide range of gram-positive bacteria, anaerobes and *Mycobacterium tuberculosis*. Linezolid was the first derivative with acceptable tolerability in man to advance to clinical trials, and is the first Federal Drug Administration approved oxazolidinone for the treatment of pneumonia and skin and soft tissue infections caused by susceptible organisms, and infections caused by VRE. It has not yet been approved for the treatment of penicillin-resistant *Streptococcus pneumoniae* (Cammarata *et al.*, 2000).

Linezolid possesses activity against staphylococci, including *Staphylococcus aureus*, irrespective of its oxacillin susceptibility (Cercenado, 2001; Gemmell, 2001), and against the recently isolated glycopeptide-intermediately-resistant *S. aureus* (GISA) (Rybak, 2000). Linezolid is active against glycopeptide resistant coagulase-negative staphylococci (Cercenado, 2001). In addition, activity against other major multidrug-resistant Gram positive pathogens, including VRE and penicillin resistant Streptococcus pneumoniae has also been demonstrated, independent of their resistance profile to other antibacterials (Jones *et al.*, 1999; Noskin *et al.*, 1999).

8.2 Daptomycin

Daptomycin is the first in a new class of cyclic lipopeptide drugs derived from the fermentation of *Streptomyces roseosporus*. It was first discovered in the 1980s and was shown to be effective in the treatment of skin and soft tissue infection (SSTI). However, because of muscle toxicity, which appeared at higher doses as used in the treatment of endovascular infections and endocarditis, and because of clinical failures (Garrison *et al.*, 1989) clinical trials were suspended. The emergence of infections caused by resistant gram positive bacteria led in 1997 to renewed interest in daptomycin, with new data obtained in the laboratory and in animal and human studies.

Daptomycin is active solely against gram-positive bacteria. Daptomycin was also active against betahaemolytic streptococci, viridans streptococci, as well as against gram-positive rods such as Leuconostoc, which are characteristically resistant to glycopeptides (Jones and Barry 1987; Barry, 2001; King and Phillips 2001). The effect of calcium in the medium was especially evident when enterococcal susceptibility was evaluated. Daptomycin was active against most strains, including VRE. However, added calcium increased the activity of daptomycin two- to fourfold (Barry, 2001; King and Phillips 2001). Unlike most other antibiotics, which are only bacteriostatic against enterococci, daptomycin exhibits rapid, concentration dependent bactericidal activity against these pathogens, as well as against other bacteria (Snydman, 2001).

8.3 GAR-936

Tetracyclines have been important broad-spectrum antimicrobial agents for the last 40 years. In order to overcome resistance to these drugs, modification of their structures was undertaken. Glycylcyclines are obtained by modification of the 9-position of chlortertracycline, minocycline, or doxycycline. GAR-936 is a novel derivative of minocycline, active against tetracycline-resistant organisms (Sum and Petersen, 1999)

GAR-936 is generally less active than its parent compound against minocycline sensitive, oxacillin susceptible and -resistant staphylococci, but it does show *in vitro* activity against minocycline-resistant strains of *Staphylococcus aureus* and CONS. However, mean MIC against these isolates is higher than the MIC against the susceptible strains (Boucher, 2000; Petersen, 1999).

The activity against aerobic gram-negative rods is fair. Many enterobacteriaceae, including *Escherichia coli* were inhibited by GAR-936 (*Proteus* spp. excluded). However, other strains were resistant, with the potency of other antibiotics, including ciprofloxacin, ceftazidime and imipenem, being significantly better (Petersen, 1999; van Ogtrop, 2000). In addition, GAR-936 retains its activity against some nonfermentative bacteria such as *Stenotrophomonas maltophilia*, although it is not active against *P. aeruginosa* (van Ogtrop, 2000; Gales and Jones, 2000).

Most strains of *Neisseria gonorrhoeae* are susceptible to GAR-936, including penicillin- or tetracycline resistant strains (Whittington, 1995). In addition, GAR-936 is highly active against other sexually transmitted diseases pathogens, including *Chlamydia trachomatis* (Roblin and Hammerschlag, 2000), as well as against *Mycoplasma* spp. and *Ureaplasma urealyticum* (Kenny GE, Cartwright, 2001).

8.4 LY-333328 (Oritavancin)

Vancomycin was introduced in 1956 because of its effectiveness against penicillin-resistant staphylococci. Approximately 30-years later, acquired glycopeptide resistance in *Enterococcus* spp. was reported followed by clinical isolates of GISA in 1997 (Fekety, 2000). However, to overcome resistance, vancomycin derivatives that has hydrophobic substituents on the vancosamine nitrogen have been developed.

LY-264826 is a naturally occurring glycopeptide with the same core structure as vancomycin but with differences in associated sugars present. Alkyl modification of the disaccharide amino led to several compounds, the most active of which is LY-333328 (Nicas *et al.*, 1996).

LY-333328 proved effective against the major multi-drug-resistant gram-positive bacteria (Zeckel *et al.*, 2000). LY-333328 was equally effective against oxacillin susceptible and methicillin-resistant *Staphylococcus aureus* (MRSA). However, its activity for most strains was either identical or less efficient than other glycopeptides (vancomycin and teicoplanin) (Zeckel *et al.*, 2000; Biavasco, 1997). LY-333328 possesses good *in vitro* activity against *Enterococcal* spp., including glycopeptide-resistant strains of the three major phenotypes (VanA, VanB, and VanC), with MICs significantly lower than the other glycopeptides (Zeckel *et al.*, 2000; Garcia-Garrote *et al.*, 1998; Biavasco *et al.*, 1997; Fraise *et al.*, 1997).

9. CONCLUSION

Development of new antibiotics has not kept pace with the emergence of antibiotic resistance. In the past few decades, pharmaceutical companies have shifted their developmental efforts to drugs for chronic diseases such as heart diseases and high blood pressure and anti-viral instead of antibiotics. The steady increase in resistant organisms is related to the widespread use of antibiotics in community and hospital settings. New therapeutic options are needed, including treatments for infections caused by antibiotic-resistant gram-positive organisms. Moreover, resurgence of TB in an epidemic form due to HIV infection especially in both developed and underdeveloped countries together with the development of multi-drug resistant strains among the patients have assumed a serious situation in health care sector. Now, the only answer is to search for novel and effective drug regime to fight this menace.

Today, intensive programmes seeking for antibiotics are running worldwide. In our laboratory (NEIST, formerly RRL (CSIR), Jorhat, India), mainly, by screening soils and water, and testing the activity of the isolated organisms against specific pathogenic bacteria or fungi that are resistant to several known antibiotics. In our laboratory, a bacterial strain, namely, *Streptomyce*s sp. 201 was isolated from the tea garden soil of Jorhat, Assam, India, (26.44 N and 94.10 E) exhibited promising anti-fungal and anti-bacterial activity against a wide range of pathogens including *M. tuberculosis (*Bordoloi *et al.*, 2001; Bordoloi *et al.*, 2002). Absolute stereochemistry of the new antifungal and antibacterial antibiotic produced by *Streptomyces* sp.201 has been established by achieving the total synthesis of the product (Boruwa *et al.*, 2004).

The vast resources of microorganisms have received increasing attention from chemists and pharmacologists and played an important role in the explosive growth of biomedical science during the past two decades. The recent advent of sophisticated chromatographic (HPLC, gel filtration etc.), high field NMR and mass spectroscopic techniques coupled with force field calculations and X-ray crystallography have markedly enhanced the isolation and structure elucidation of

more complex and diverse natural products and along with advanced bioassay methods has opened new possibilities in discovery of drugs and agrochemicals from natural sources.

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