ISSN 0975-3303
Mapana J Sci, **11**, 1(2012), 31-48
https://doi.org/10.12725/mjs.20.3

Multidrug Resistant Bacteria - the Fatal Menace in Healthcare

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Abstract

The "antibiotic era" saw the discovery of a lot of wonder drugs which killed pathogenic bacteria without significantly harming the host. Never before had nature and sickness seemed so much within the control of mankind. The hopes soon died down with the reports of antibiotic resistant bacteria. Microbes, such as bacteria, viruses, fungi and parasites are living organisms that evolve over time. Their primary function is to reproduce, thrive, and spread, quickly and efficiently. Therefore, microbes adapt to their environment and change in ways that ensure their survival. If something stops their ability to spread, such as an antimicrobial, genetic changes can occur that enable the microbe to survive. This survival mechanism is transferred to other bacteria mainly by plasmids through vertical and horizontal gene transfer. The extensive overuse and misuse of antibiotics by human beings has given the problem a larger dimension in that now we have superbugs resistant to a variety of antibiotics. People need to be informed of the dangers of antibiotics and educated on the forms of illnesses that antibiotics cannot treat.

Keywords: multidrug resistance, MRSA, plasmid, receptor, superbug.

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Received: Nov. 2011, Reviewed: Dec. 2011

1. Introduction

It is estimated that 90% of the cells in the human body belong to non-human organisms, mostly bacteria. Some 100 trillion microbes nestle in niches from our teeth to our toes. Without microbes human beings would not have been alive. This is just one side of the coin. On the other side, there are bacteria which cause life threatening diseases to human beings. Man started exercising his control over bacteria way back in 1928 when Alexander Fleming discovered the first antibiotic, Penicillin. Since then, antibiotics have been critical in the fight against infectious diseases caused by bacteria. In fact, antimicrobial chemotherapy has been a leading cause for the dramatic rise of average life expectancy in the twentieth century. However, disease-causing microbes have been gaining resistance to these antibiotics with the result that scientists had to discover newer and newer antibiotics to kill these resistant bacteria. The problem has now grown into a global threat with the emergence of super bugs, resistant to more than ten antibiotics.

2. History of Antibiotics

The antibiotic era was initiated when Paul Ehrlich first coined the term ‘magic bullet’, or chemotherapy, to designate the use of antimicrobial compounds to treat microbial infections. In1910 Ehrlich discovered the first antibiotic drug, Salvarsan, which was used against syphilis.[1] The accidental discovery of Penicillin by Alexander Fleming in 1928 opened new avenues in the treatment of bacterial diseases.[2] 1940s saw the extensive usage of Penicillin and by the end of the Second World War, Penicillin became widely available and won widespread acceptance. In1935, Gerhard Domagk discovered the sulfa drugs[3], thereby paving the way to the discovery of the anti-TB drug Isoniazid. Finally, in 1943, the first TB drug, Streptomycin, was discovered by Selman Waksman[4] who coined the term ‘antibiotics’. Thus antibiotics have been used to treat bacterial infections since the 1940s.

3. Basic features of antibiotics

The definition of an antibiotic as proposed by Waksman is simply a description of a use or a laboratory effect or an activity of a chemical compound.[5] Today, there are about 4,000 compounds with antibiotic properties which are used to treat and prevent infections, and to promote growth in animals. Antibiotics are derived from three sources: fungi, bacteria and synthetically derived. Antibiotics are more effective against actively growing bacteria, than against non-growing persisters or spores. When two antibiotics are used in combination, the effect could be additive, synergistic, or antagonistic. Antibiotics can also be divided into broad-spectrum and narrow-spectrum antibiotics. For example, Tetracycline, a broad spectrum antibiotic, is active against Gram positive bacteria, Gram negative bacteria and even against mycobacteria; whereas Penicillin, which has a relatively narrow spectrum, can be used mainly against Gram positive bacteria. Other antibiotics, such as Pyrazinamide, have an even narrower spectrum, and can be used merely against *Mycobacterium tuberculosis.*[6]

3. Mode of action of Antibiotics

Bacterial cells grow and divide, replicating repeatedly to reach the large numbers present during an infection. To grow and divide, organisms must synthesize or take up many types of biomolecules. Antimicrobial agents interfere with specific processes that are essential for growth and/or division. Based on these processes antibiotics can be divided into five major classes - Cell wall inhibitors, such as Penicillin and Vancomycin, inhibitors of nucleic acid synthesis, such as fluoroquinolones, which inhibits DNA synthesis, Rifampin, which inhibits RNA synthesis, protein synthesis inhibitors, such as aminoglycosides, anti-metabolites, such as the sulfa drugs and cell membrane damaging antibiotics such as Polymyxin B and Gramicidin. Elucidation of the structure and function of an antibiotic compound is extremely daunting. Even though Penicillin was discovered in 1928, the complete structure of this relatively simple molecule was not revealed until

1949, by the X-ray crystallographic studies of Dorothy Crowfoot Hodgkin[7] and was confirmed by total synthesis in 1959.[8] Studies of modes of action of antibiotics have provided biochemical information on ligands and targets throughout antibiotic history.[9, 10]

4. History of antibiotic resistance

Several years before the introduction of Penicillin as a therapeutic, a bacterial penicillinase was identified by two members of the penicillin discovery team.[11] In his 1945 Nobel Prize lecture, Alexander Fleming himself warned of the danger of resistance – "It is not difficult to make microbes resistant to Penicillin in the laboratory by exposing them to concentrations not sufficient to kill them and the same thing has occasionally happened in the body…." Indeed, it took little time for *Staphylococcus aureus* to develop resistance to penicillin. In 1947 just four years after the drug started being mass-produced, physicians observed the first case of clinical resistance.

Methicillin was then the antibiotic of choice, but has since been replaced by oxacillin due to significant kidney toxicity. MRSA (methicillin-resistant *Staphylococcus aureus*) was first detected in Britain in 1961 and is now quite common in hospitals. MRSA was responsible for 37% of fatal cases of blood poisoning in the UK in 1999, up from 4% in 1991. Half of all *S. aureus* infections in the US are resistant to penicillin, methicillin, tetracycline and erythromycin. This left vancomycin as the only effective agent available at the time. However, strains with intermediate levels of resistance (GISA- glycopeptide intermediate *Staphylococcus aureus* or VISA -vancomycin intermediate *S. aureus*), began appearing the late 1990s. The first identified case was in Japan in 1996[12] and strains have since been found in hospitals in England, France and the US. The first documented strain with complete (>16ug/ml) resistance to vancomycin, termed VRSA (Vancomycin-resistant *S. aureus*) appeared in the United States in 2002. VISA-type resistance has now been identified in each of the globally spread pandemic

clones of MRSA..[13] Oxazolidinones, a new class of antibiotics became available in the 1990s. The first commercially available oxazolidinone, linezolid, is comparable to vancomycin in effectiveness against MRSA. Linezolid-resistance in *S. aureus* was reported in 2003.[14] CA-MRSA (Community-acquired MRSA) is especially troublesome in hospitals and nursing homes, where patients with open wounds, invasive devices, and weakened immune systems are at greater risk of infection than the general public.

5. Causes of antibiotic resistance

A majority of the antibiotic resistance cases are caused by the improper use of antibiotics by humans. These include incorrect usage of antibiotics, noncompliance with treatment regimes and inappropriate usage of antibiotics in animal feed. Recent studies have uncovered the presence of antibiotic resistance genes and even resistance-encoding integrons in the gut flora of people who live in isolated areas apparently untouched by modern civilization and not exposed to antibiotic therapies.[15],[16]

5.1. Incorrect usage of antibiotics

When an antibiotic is used inappropriately, for too short a time, at too low a dose or at inadequate potency—or for the wrong disease, microbes are more likely to develop resistance to that drug. According to the U.S. Centre for Disease Control and Prevention (CDC), one large part of the problem, is the tendency for people to take antibiotics to fight viruses, which they cannot do. Antibiotics fight bacteria, not viruses and will not fight colds, flu, bronchitis, runny noses, or sore throats not due to *Streptococcus*. Nonetheless says CDC, “more than 10 million courses of antibiotics are prescribed each year for viral conditions that do not benefit from antibiotics.”

5.2. Inadequate compliance with treatment regimens

High levels of antibiotics in the body over a certain period of time are necessary to wipe out the entire population of bacteria. But since patients often discontinue treatment after just a few pills when they start to feel better, with only a partial dose of the medicine, only the weakest bacterial populations in the body are destroyed. Meanwhile, the surviving bacteria may develop resistance to the antibiotic, and will continue to multiply, thereby passing on the trait to future generations and even to other microorganisms in the body. Resistant bacteria can then be passed to other human hosts.

5.3. Antibiotic use in animal feed

Many developed countries use antibiotics for veterinary purposes—to accelerate weight gain and to prevent and treat diseases in animals. Farmers and ranchers use antibiotics heavily in beef, pig and poultry industry. In fact, the Union of Concerned scientists (UCS), a non-profit research and advocacy group, estimates that 70 percent of all antibiotics are used as additives in the feed given to healthy pigs, poultry and cattle. These drugs leave the animals' bodies as waste and work their way into local water supplies, as well as right into the food chain. Use of antibiotics in farm animals promotes the development of drug-resistant microbes in those animals. Drug resistance in animals may lead to drug resistance in humans, because the drug-resistant bacteria can be transmitted from animals to humans. Apart from the resistance problem, this can also cause physiological damage to local resident populations of insects, birds, animals, and humans.[17]

6. Mechanisms of resistance

Some of the important mechanisms by which a microorganism can resist an antimicrobial agent are (1) to alter the receptor for the drug (the molecule on which it exerts its effect); (2) to decrease the amount of drug that reaches the receptor by altering entry or

increasing removal of the drug and (3) to destroy or inactivate the drug. Bacteria can possess one or all of these mechanisms simultaneously. A brief summary of the important resistance mechanisms of a few antibiotics are listed in table 1.

Antibiotic	**Method of resistance**
Chloramphenicol	reduced uptake into cell
Tetracycline	active efflux from the cell
β-lactams, Erythromycin, Lincomycin	eliminates or reduces binding of antibiotic to cell target
β-lactams, Aminoglycosides	enzymatic cleavage to inactivate antibiotic molecule
Sulfonamides, Trimethoprim	metabolic bypass of inhibited reaction
Trimethoprim, sulphoninamides	overproduction of antibiotic target (titration)

Table 1. Mechanisms of Antibiotic Resistance in Bacteria

6.1. Resistance due to altered receptors

The β-lactam ring is part of the core structure of several families of antibiotics, the principal ones being the penicillins, cephalosporins, carbapenems and monobactams which are, therefore called β-lactam antibiotics. Nearly all of these antibiotics work by inhibiting bacterial cellwall biosynthesis. Some bacteria are capable of making changes in receptors for β-lactams. In 1977, *Streptococcus pneumoniae* strains resistant to Penicillin G were encountered in South Africa.[18] Plasmids were not the cause of the resistance. Penicillin-resistant *S pneumoniae* cells have altered penicillin-binding proteins, which bind penicillin less well. Resistance of *S pneumoniae* to penicillin has been increasing and there are now relatively resistant isolates in many parts of the world. Altered penicillin-binding proteins also account for the resistance of some *Staphylococcus aureus* strains to β-lactamase-stable penicillins (methicillin-resistant strains). Staphylococcal organisms resistant to

methicillin are resistant to all penicillins, cephalosporins, and carbapenems. Enterococci are resistant to all cephalosporins because of failure to bind to the penicillin-binding proteins. The β-lactams induce synthesis of a new penicillin-binding protein, PBP2a, which does not bind any β-lactam.

Vancomycin is a glycopeptide antibiotic which acts by inhibiting cell wall synthesis in Gram-positive bacteria. Due to the different mechanism by which Gram-negative bacteria produce their cell walls and the various factors related to entering the outer membrane of Gram negative organisms, Vancomycin is not active against Gram-negative bacteria (except some non-gonococcal species of *Neisseria*). It has traditionally been reserved as a last resort drug, used only after treatment with other antibiotics had failed Some transposon encoded enzymes alter the side chains in the cell wall peptidoglycan thereby preventing the binding of vancomycin which results in resistant enterobacteria.

Rifampicin is a semisynthetic compound derived from *Amycolatopsis rifamycinica.* It inhibits DNA-dependent RNA Polymerase in bacterial cells by binding its beta-subunit, thus preventing transcription to RNA and subsequent translation to proteins. Rifampicin is used against a number of bacteria, but it is best known for activity against Mycobacteria responsible for causing tuberculosis and leprosy. Rifampicin can be used as monotherapy for a few days as prophylaxis against meningitis, but resistance develops quickly during long treatment of active infections, so the drug is always used against active infections in combination with other antibiotics. The resistance of bacteria to rifampin is caused by an alternation of one amino acid in DNA-directed RNA polymerase, which results in reduced binding of rifampin. The degree of resistance is related to the degree to which the enzyme is changed. This form of resistance occurs at a low level in any population of bacteria so that resistance develops by natural selection during a course of therapy.[19]

Quinolones are a family of synthetic broadspectrum antibiotics. The first generation of the quinolones began with the introduction

of nalidixic acid in 1962 for treatment of urinary tract infections in humans. These drugs interfere with DNA replication by inhibiting bacterial DNA gyrase or the topoisomerase II enzyme, thereby inhibiting DNA replication and transcription. Quinolones can enter cells easily via porins and therefore, are often used to treat intracellular pathogens such as *Legionella pneumophila* and *Mycoplasma pneumoniae.* For many Gram-negative bacteria, DNA gyrase is the target, whereas topoisomerase IV is the target for many Gram-positive bacteria. Resistance to quinolones can be caused by mutations in DNA gyrase subunits A or B, reduced outer membrane permeability in gram-negative cells, or to active efflux transporters found in many bacteria. The highest level of resistance to the newer fluoroquinolones is most frequently associated with chromosomal mutations, causing amino acid substitutions in a highly conserved region in the A subunit of DNA gyrase. Multiple-mechanisms of resistance can occur in a single isolate of bacteria, leading to a higher level of resistance to many fluoroquinolones. .[20]

6.2. Resistance Due to Decreased Entry of a Drug

Tetracycline is a broad-spectrum polyketide antibiotic which binds to the 30S subunit of microbial ribosomes. It inhibits protein synthesis by blocking the attachment of charged aminoacyl-tRNA. Thus it prevents the introduction of new amino acids to the nascent peptide chain. The action is usually inhibitory and reversible upon withdrawal of the drug. Resistance to tetracyclines results from changes in permeability of the microbial cell envelope. In susceptible cells, the drug is concentrated from the environment and does not readily leave the cell. In resistant cells, the drug is not actively transported into the cell or leaves it so rapidly that inhibitory concentrations are not maintained. This is often plasmid-controlled. It is a protein synthesis inhibitor. Tetracycline resistance is common in both Gram-positive and Gram-negative bacteria. In most cases it is plasmid encoded and inducible; however, chromosomal, constitutive resistance is found in some organisms such as *Proteus* species.

Tetracycline resistance is a major concern because it is located on plasmids near insertion sites and these plasmids readily acquire other genetic information to enlarge the spectrum of resistance. The widespread use of tetracycline in animal feeds may be a factor in the extensive, worldwide resistance of members of the Enterobacteriaceae, particularly enteric species such as *Salmonella*, to tetracyclines and subsequently to many other drugs.[21] Not only can tetracycline resistance move among members of the Enterobacteriaceae on plasmids, but plasmids mediating tetracycline resistance have moved between *S aureus, S epidermidis, S pyogenes, S pneumoniae*, and *S faecalis*.

6.3. Due to Destruction or Inactivation of a Drug

Chloramphenicol is a broadspectrum antibiotic effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms. The most serious adverse effect associated with chloramphenicol treatment is bone marrow toxicity, which may occur in two distinct forms: bone marrow suppression - a direct toxic effect of the drug and is usually reversible - and aplastic anemia.[22] Many Gram-positive and Gram-negative bacteria, including some recently discovered *H influenzae* strains, are resistant to chloramphenicol because they possess the enzyme chloramphenicol transacetylate, which acetylates hydroxyl groups on the chloramphenicol structure. This enzyme, unlike the aminoglycoside-inactivating enzymes and β-lactamases, is an intracellular enzyme of higher molecular weight and subunit structure. Acetylated chloramphenicol binds less well to the 50S ribosome.[23]

7. Superbugs

The increasing emergence of superbugs is a direct consequence of antibiotic misuse. Resistance of microbes to drugs can be classified into MDR, XDR and PDR. MDR or multiple drug resistant bacteria are those which are resistant to antibiotics of at least three different classes. XDR or extensively drug resistant bacteria are those which

are resistant to all antibiotics except only one class of antimicrobial agent used to treat the infections caused by this.PDR or pan-drug resistant bacteria are those which are resistant to antibiotics of all classes of antimicrobial agents used to treat the infections.

The term "superbugs" refers to microbes with enhanced morbidity and mortality due to multiple mutations endowing high levels of resistance to the antibiotic classes specifically recommended for their treatment; the therapeutic options for these microbes are reduced, and periods of hospital care are extended and more costly. In some cases, super resistant strains have also acquired increased virulence and enhanced transmissibility. Superbugs can be dangerous because of the limited number of treatment options available. Among some of the deadly superbugs are methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumonia, Clostridum difficile Acinetobacter baunammii, Pseudomonas aeruginosa* and Vancomycin-resistant *Enterococcus faceium*.[24],[25],[26]

The development of MDR *Staphylococcus aureus* well illustrates the battle between the agile pathogens and drugs. *S. aureus* is a bacterium that harmlessly lives in the human body but can cause various kinds of infections. After the clinical application of Penicillin in the 1940s, *S. aureus* soon adapted to the treatment mechanism of penicillin, and by the 1950s, almost half of *S. aureus* strains had become resistant to Penicillin. A new antibiotic, Methicillin, developed in the 1960s kills *S. aureus* by interfering with the bacterium's ability to form a cell wall. Methicillin was a huge success in the initial years of its discovery, but within a few years, some strains of *S. aureus* germ picked up a gene called *mecA* which reduced the ability of Methicillin to interfere with the *S. aureus* cell wall by a thousand fold.

Tuberculosis is the world's number one killer among infectious diseases. Combined with HIV, MDR TB has become a real menace especially in African countries. MDR-TB is caused by strains of *Mycobacterium tuberculosis* that are resistant to at least the antibiotics isoniazid and rifampicin. A subset of MDR-TB, extensively drug-resistant tuberculosis (XDR-TB), caused by rare

strains that are resistant to isoniazid and rifampicin, as well as second-line (or follow-up) medications have spread rapidly in the last decade or so.[27],[28] And now there are TDR strains, which are totally drug resistant.[29]

8. NDM-1

NDM-1 stands for *New Delhi metallo-beta-lactamase*, which is an enzyme produced by certain strains of bacteria that have recently acquired the genetic ability to make this compound. The enzyme is active against compounds that contain the beta-lactam ring. Unfortunately, many antibiotics contain this ring, including the penicillins, cephalosporins and the carbapenems. Bacteria that produce NDM-1 are resistant to all commonly used beta-lactam antibiotics. Some antibiotics like aminoglycosides and fluoroquinolones do not contain beta-lactam rings. Unfortunately, the bacteria that have acquired NDM-1 have also acquired other resistance factors and most are already resistant to aminoglycosides and fluoroquinolones. NDM production has enabled these bacteria to transform into true superbugs which are resistant to virtually all commonly used antibiotics. Cases of NDM-1 infection are usually caused by Gram negative bacteria from the Enterobacteriaceace family (*Klebsiella* and *Escherichia coli.).* The plasmid-mediated bla_{NDM-1} gene encodes a metallo-β-lactamase (MBL) with high carbapenemase activity and was first identified in *Escherichia coli* and *Klebsiella pneumoniae* in Sweden from a patient transferred from India.[30] It was later identified in different enterobacterial species from a series of patients in the United Kingdom, India, and Pakistan[31] and also from Australian and U.S. patients.[32]

9. Future of Antibiotics

If mankind has to win over microbes in the race, extremely strategic and diversified measures have to be adopted in tackling the bugs. These include discovery of novel antimicrobial compounds and new targets for antimicrobials. The antibiotic treatment choices for already existing or emerging hard-to-treat

multidrug-resistant bacterial infections are limited, resulting in high morbidity and mortality rates. Majority of antimicrobials in use today have been isolated in the golden era of antibiotic discovery from a limited number of ecological niches and taxonomic groups, mainly from soil *Actinomyces*. Some possible approaches to tap the novel antimicrobial diversity is the exploration of ecological niches other than soil, such as the marine environment[33],[34], borrowing antimicrobial peptides and compounds from animals and plants[35], mimicking the natural lipopeptides of bacteria and fungi[36], accessing the uncultivated portion of microbiota through the metagenomic approach[37] and finally finding drugs possessing dual target activities, such as a rifamycin–quinolone hybrid antibiotic, CBR-2092.[38]

Almost all the antibiotics discovered in the past decades target the same cellular processes like translation, cell wall biosynthesis, DNA/RNA metabolism etc. Since we have a sufficient amount of genome sequence data available presently, it's possible to have more precise drug targets. The comparison of metabolic pathways in commensal and pathogenic bacteria may help to identify the novel drug/target combinations in pathogens.[39] Successful implementations of this approach have already been demonstrated in the suppression of an important virulence factor, type III secretion system[40] and in the inhibition of the QseC-mediated activation of virulence gene expression in several pathogens.[41] The drugs initially designed for a different purpose may find application as antimicrobials. For example, BPH-652, a phosphonosulfonate, which was previously tested for cholesterol-lowering activity in humans as targeting the enzyme in cholesterol biosynthesis pathway, squalene synthase also inhibits an important enzyme involved in *Staphylococcus aureus* virulence, dehydrosqualene synthase and thus may be considered as a candidate drug to control MRSA.[42] Combination therapy like coupling antibiotics with an antibiotic-enhancing phage, has demonstrated the potential to be a promising antimicrobial intervention.[43]

10. Conclusion

The war between bugs and man had begun centuries back. Every time humans emerge with a drug to combat microbes, within a few years bugs outsmart the humans and become resistant to it. This chase has been going on for decades. If man doesn't keep pace with the bugs by emerging with newer and stronger drugs, we are sure to lose the battle with the bugs. The need of the hour is to prevent antibiotic resistance by adhering to strict norms during its prescription and usage and also to devise novel strategies for equipping ourselves with newer and more efficient drugs to launch the defense during future outbreaks of drug resistant microbes.

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