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Antibiotic Resistance: An Important Issue for Public Health Safety

Micheal Savage[1], Elaine Meade[2], Mark Anthony Slattery[2,3] and Mary Garvey[1,2,3]*

[1]*Lir Analytical LTD, Athlone Rd, Longford, Ireland*

[2]*Department of Life Science, Cellular Health and Toxicology Research Group, Institute of Technology Sligo, Ireland*

[3]*Mark Anthony Slattery, Veterinary Practice, Manorhamilton, Leitrim, Ireland*

Abstract

Antibiotic resistance has emerged as one of the greatest difficulties facing drug therapy in recent years. Initially, the mass production and therapeutic use of antibiotic agents lead to a reduced rate of morbidity and mortality globally. The increasing and often inappropriate use of these drugs however, has meant that pathogenic bacterial species have emerged with resistance to one or more of the antibiotic types. Furthermore, the ability of species to share this resistance and provide acquired resistance to other species means that the problem is ever expanding. The issue has become so great that the treatment of such antibiotic resistance infections is proving difficult and costly to health care systems. Furthermore, WHO have established a list of priority pathogens categorising bacterial species based on the urgent need for novel treatment options. This review aims to provide a summary of the key methods of antibiotic resistance and an overview of healthcare acquired infections currently proving difficult to eradicate with antibacterial therapy.

Keywords

Antibiotic resistance, ESBL, Pathogens, Morbidity, WHO

Introduction

Antibiotic resistance has emerged as one of the toughest challenges facing modern man in recent years. The appearance of an ever increasing range of microorganisms showing resistance to a collective array of antimicrobial drugs presents a serious threat to public health safety. The first antibiotic was marketed by Hoechst in the early 1900s under the name Salvarsan for the treatment of syphilis, a sexually transmitted disease caused by *Treponema palladium*. This was soon followed by Neosalvarsan which was more tolerable while remaining highly effective [1]. The discovery of *Penicillium notatum* by Fleming in 1928 was a landmark finding which eventually led to the mass production of the antibiotic penicillin from the 1940s on [2]. The production of antibiotics as pharmaceutical agents increased significantly in the 1940s during the pharmaceutical industrial boom. Since then, antimicrobials have undoubtedly remained one of the most useful and beneficial methods of chemotherapy in use, reducing morbidity and mortality rates globally. To date there are numerous antibiotics available which generally fall into one of the following classes: Penicillins, cephalosporins, macrolides, fluoroquinolones, sulphonamides, tetraclines and aminoglycosides. Unfortunately, the rapid emergence of Antimicrobial Resistance (AMR) expressed by pathogenic species to one or more of these antibiotics has meant that there is a significant decrease in their efficacy. Additionally, there is a marked increase in the rate of persons non responsive to antibiotic therapy for infections of the Urinary Tract (UTIs) and upper respiratory tract [3] due to the presence of antibiotic resistant bacteria. AMR has major consequences for public health safety as well as a significant economic impact, where approximately 25,000 people in Europe die from HAIs annually caused by resistant bacteria cost-

ing the healthcare system 1.5 billion euros [4]. Resistance amongst the Gram negative species is especially worrying as they display resistance to most antibiotics currently in use. The situation has become so dangerous that the World Health Organisation (WHO) now lists AMR as top of the danger list and has established a list of 12 families of bacterial species classified as priority pathogens. This pathogen list has a subdivision of 3 categories according to the urgency for novel drug therapy options: 1) Critical priority including HAI relevant strains such as *Acinetobacter, Pseudomonas* and *Enterobacteriaceae;* 2) High priority including VRE, MRSA and *Salmonella* and 3) Medium priority strains such as *Streptococcus* and *Shigella*. Due to the enormous threat and clinical significance relating to this issue, this review aims to provide an outline of AMR mechanisms and current pathogenic species associated with HAIs.

Mechanisms of resistance

Antibiotics have the effect of interfering with cell wall synthesis making them bacterial specific (penicillin's and cephalosporins), protein synthesis (tetracyclines, macrolides and aminoglycosides), nucleic acid synthesis (fluoroquinolones), inhibition of metabolic pathways (sulphonamides) or disrupting the bacterial cell membrane (polymyxin) [5]. Certain bacterial and fungal species possess the ability to synthesis and secret antibacterial agents to prevent the growth of other bacterial species in their close proximity. For this reason, resistance to antibiotics is something that bacterial species display as survival mechanisms in response to a threat in their environmental habitat. Such organisms are deemed "intrinsically" resistant to one or more antimicrobial agents [6]. It is when bacterial species not previously displaying resistance to drug compounds start to display "acquired resistance" to the man-made antibacterial agents that issues with antibiotic therapy arise. Furthermore, these defensive traits are often shared between microbes by Horizontal Gene Transfer (HGT) of plasmid DNA, an evolutionary process of transferring genetic information between species. These resistance mechanisms can be developed by a bacterium through mutations that modify the gene targets of antibiotics, by limiting entry of the antimicrobial substance into the cell, removing the antibiotic compound following entry to the cell or by deactivating the antibiotic [7] via enzymatic degradation.

Modifying gene targets

There are two methods by which bacteria modify their genes to prevent cell death: 1) A mutation in a gene which will affect the mode of action of the antibacterial agent, for example, the antibiotic rifampicin targets the RNA polymerase enzyme b subunit, rpoB, and resistance to this antibiotic is acquired through mutations to the

gene encoding rpoB; and 2) Obtaining foreign DNA which codes for resistance mechanisms via HGT. Resistance resulting from mutations in bacterial genes is often a by-product of a survival mechanism of that species. For example, Studies report how mutations in the rpsL, gryA and rpoB genes confer resistance to streptomycin, quinolones and rifampicin and also aids bacterial survival in the presence of macrophages [7]. Mutations of the rpsL and rpoB genes in *E. coli* code for hyperaccurate ribosomes which are aid the survival of non-dividing cells during starvation by allowing them to survive in nutrient poor and reactive oxygen dense macrophages [8]. Furthermore mutations in the gene rpsL leads to resistance to streptomycin while also allowing for better growth of species in conditions with low carbon sources. Gryase is an enzyme coded by the gryA gene plays essential roles in most nucleic acid processes including the formation of double strand breaks in bacterial DNA. Quinolone antibiotics have effect by increasing the concentration of enzyme-DNA cleavage complexes in the DNA. Mutations in this gene lead to AMR as it results in increased drug protein interactions which stabilise the DNA and limits the number of breaks induced [9]. The AMR genes are transferred between species by transduction, conjugation or transformation of either plasmids (circular double stranded DNA) or transposons (pieces of DNA which can move to different positions on the genome) [4]. In biofilm communities where gene sharing is a common phenomenon [10], resistance can be spread within the community conferring multiple species with resistance to both antibiotic and chemical disinfection solutions. Following exposure to antibiotics, a survival pressure is put on the bacterial cells where those possessing AMR mechanisms have a distinct advantage over non-resistant cells, survival of the fittest resulting in the elimination of the weaker strain and proliferation of the AMR cell type.

Antibiotic inactivation

Beta-lactam antibiotics have a mode of action where they inhibit the cell wall synthesis by preventing structural cross linking of peptidoglycans and are currently the most common therapeutic agent for treating bacterial infections [5]. The first bacterial enzyme reported to inactivate penicillin was an Amp C beta-lactamase produced by *E. coli* having a degradation effect on the beta lactam ring, rendering the drug ineffective. In Gram positive species, resistance to the beta-lactams is by modification of the target site of the lactam ring, the Penicillin-Binding Proteins (PBPs). Typically, carbapenem antibiotics were the mainstay for treatment of such infections as they were unaffected by beta lactamase enzymes. The production of carbapenemase enzymes which degrade the carbapenem antibiotics by certain species means that

the antibiotic is rendered ineffective [6]. Amino glycoside antibiotics are used to treat many Gram-negative, Gram-positive and multidrug-resistant tuberculosis infections [11]. Resistance to amino glycoside antibiotics comes from the production of Amino Glycoside Modifying Enzymes (AMEs) that covalently modify the hydroxyl or amino groups of the amino glycoside molecule [6]. The chloramphenicol antibiotics are highly specific and excellent inhibitors of protein synthesis in both Gram negative, Gram positive, aerobic and anaerobic bacteria [12]. Enzymatic alteration of this antibiotic involves the chemical modification of chloramphenicol by the expression of acetyltransferases known as CATs (Chloramphenicol Acetyltransferases). These CAT genes have been identified in both gram-positive and gram-negative bacteria and are classified in two main types: Type A, which results in high-level resistance and type B that confers low-level chloramphenicol resistance [6].

Active efflux

Energy dependent drug efflux systems are increasingly being recognized as mechanisms of antibiotic resistance, having either limited or broad spectrum activity [13]. Efflux pumps can be either intrinsic (chromosomally located) or acquired by bacteria. A multidrug transporter known as AcrAD is the main efflux pump in Gram-negative bacteria including *E. coli* and *S. enterica* [11]. AcrAD spans the cell envelope, the innermost cellular membrane and the periplasm, where it binds drug molecules and transports them to the outer surface by use of energy (ATP). AcrAB efflux system has shown to be responsible for the removal of a broad range of antibiotics such as fluoroquinolones, cephalosporins, tetracyclines as well as other drug compounds [14]. Indeed, active efflux is another mode of resistance displayed by some amino glycoside resistant species, where the drug molecule is actively transported out of the cell. Similarly, macrolide resistance displayed by *S. pneumoniae* occurs primarily via active drug efflux [15].

AMR relating to nosocomial infections

Due to the close proximity of patients and turnover of staff, clinical settings such as hospitals are high risk environments for the spread of pathogenic species and AMR bacteria. Particularly members of the *Enterobacteriaceae* group are amongst the Gram negative species commonly associated with resistance and nosocomial infections [16], perhaps because conjugation and gene sharing between species co habiting the intestinal tract is common. These species may express Extended-Spectrum Beta Lactamases (ESBLs), which result in resistance to lactams. Importantly, ESBL *Enterobacteriaceae* also demonstrates resistance to aminoglycosides, sulfonamides and fluoroquinolones [17]. Drug therapy for such pathogens relies

on the use of carbapenem antibiotics; however ESBLs now resistant to carbapenem have now emerged and are a major concern [16]. For example, studies show that infections caused by carbapenem-resistant *Klebsiella pneumoniae* have up to a fivefold higher risk of mortality than infections caused by carbapenem-susceptible strains [18]. The Gram negative *Pseudomonas aeruginosa* is another such pathogen frequently associated with mutlidrug resistant nosocomial infection and patient morbidity [4]. Indeed, *P. aeruginosa is* the second most common cause of pneumonia and third most common cause of UTIs [19] in admitted patients. *Staphylococcus aureus* is a Gram positive opportunistic and nosocomial pathogen which was treated with the beta-lactam antibiotic penicillin G prior to the 1950s, after which beta-lactamase producing resistant strains emerged. Methicillin was utilised as the main treatment option for this *beta aureus* strain, resulting in the clinical emergence of Methicillin Resistant *S. Aureus* (MRSA). For this strain, resistance resulted from the expression of an additional Penicillin-Binding Protein (PBP2a), acquired from another species, having a higher affinity for the drug than the normal PBP resulting in resistance to the action of the antibiotic [20]. Vancomycin was the drug option of choice for MRSA strains however, resistance to vancomycin soon emerged in *Staphylococcus* species from 1979 with Vancomycin Resistant *Enterococcus* (VRE) currently setting challenges for effective drug therapy. Treatment of VRE typically consists of amino glycoside antibiotics gentamicin and streptomycin. In recent years issues have arisen where High-Level Gentamicin Resistance (HLGR) and High-Level Streptomycin Resistance (HLSR) have emerged [21], resulting in difficult to treat infections and patient morbidity. Due to the high occurrence of Multidrug Resistant (MDR) and Extensively Drug-Resistant (XDR) isolates of *Acinetobacter baumannii*, this species has also been identified was one of the main pathogens threatening the healthcare system. In this species antibiotic resistance is primarily the consequence of genetic transfer of resistance genes via plasmids, and the mutation of target genes [3]. *Clostridium difficile* is now the most common organism to cause healthcare associated infections, where prolonged infection may result in patient mortality [22]. Resistance has emerged due to increased use of broad-spectrum antibiotics against *C. difficile* such as cephalosporins and fluoroquinolones. Metronidazole and vancomycin is often prescribed for *C. difficile* infections.

Combating resistance

Methods employed to combat antibiotic resistance typically rely on the modification of existing antimicrobials with the aim of producing more potent and patient friendly drug compounds. Although initially beneficial,

the reality of this approach means that antibacterial resistance to these new compounds will emerge due to the rapid evolution of resistant strains and gene sharing. This scenario has occurred since the mass production of antibiotics [23] and will undoubtedly continue to do so. Limiting the use of antibiotics and preventing the spread of pathogens seems to be an obvious solution to the problem. Indeed, in developing countries such as China and India where the use of antibiotics is not regulated and antibiotics are supplied as Over The Counter (OTC) medicines the rates of resistance are high [16] Such self-medication and prescription of antibiotics in situations where a bacterial pathogen is not the cause of disease is a major factor in stimulating resistance. Additionally, the use of antibacterial drugs in treating domesticated and non-domesticated animals must be reduced as resistance organisms can pass from animal to human hosts via zoonotic transmission. The use of antibiotics as growth promoters and for prophylactic purposes in food animals, as well as for a broader and less-targeted treatment in aquaculture and horticulture [1] must also be examined if resistance is to be limited. Current research focuses on the use of nanoparticles such as Titania Dioxide (TiO$_2$) and graphene as antimicrobial surface coatings [24]. Studies are also being conducted into the use of phages as antibacterial agents, where bacteriophages cause cell destruction and a reduction in viable bacterial numbers as a result. Bacteriophages have the advantage to specifically target pathogenic bacteria while not negatively affecting the normal microbiota [25]. Efflux pump inhibitors which can be used in conjunction with antibiotics also show potential for the treatment of AMR strains. Efflux pumps are a common resistance mechanism particularly amongst gram negative species, pump inhibitors have effect by interfering with the proton motive force mechanism or by competing with the binding site of the pump itself [14]. Public awareness, better hygiene practices and disinfection of surfaces are all preventative measures which can reduce the numbers of bacterial species present and infection rates of persons in healthcare systems.

Conclusion

The drug therapy options for resistant, emerging resistant and multidrug resistant bacterial species are currently limited, resulting in high rates of morbidity and mortality. There is a need to develop novel ways of preventing the spread of AMR species and of treating incidences of infection. At present research focuses on the use of nanoparticle agents as surface coatings in medical settings and on the use of bacteriophages as antibacterial agents. Issues arise however in terms of the mammalian cytotoxic effect of nanoparticles whereas bacteriophages require highly specific diagnostic procedures. Areas

where efforts can be made to prevent the exposure of vast numbers of species to antibiotic agents and the transfer of such species to the environment should be assessed. One such area is the veterinary industry where food producing animals are given growth promoting antibiotic drugs on a daily basis which are subsequently excreted onto land and into water ways resulting in the presence of AMR species in environmental settings. To reduce this proliferation of AMR the European Union banned the use of antibiotics as growth promoters in 2006. In countries such as America, Canada and Asia however, this ban has not been applied where antibiotics are continually used for agricultural purposes. In order to make serious progress in this area increased efforts are needed to slow the emergence of resistant strains and to protect the environment from antibiotic pollution at a global level.

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