

**ANTIBIOTICS RESISTANCE RISK ASSOCIATED WITH
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Abstract: Antimicrobial resistance is a global public health challenge, which has accelerated by the overuse of antibiotics worldwide. Increased antimicrobial resistance is the cause of severe infections, complications, longer hospital stays and increased mortality. Overprescribing of antibiotics is associated with an increased risk of adverse effects, more frequent re-attendance and increased medicalization of self-limiting conditions. Antibiotic overprescribing is a particular problem in primary care, where bacteria cause most infections. About 90% of all antibiotic prescriptions are issued by general practitioners, and respiratory tract infections are the leading reason for prescribing. Multifaceted interventions to reduce overuse of antibiotics have been found to be effective and better than single initiatives. Interventions should encompass the enforcement of the policy of prohibiting the over-the-counter sale of antibiotics, the use of antimicrobial stewardship programmes, the active participation of clinicians in audits, the utilization of valid rapid point-of-care tests, the promotion of delayed antibiotic prescribing strategies, the enhancement of communication skills with patients with the aid of information brochures and the performance of more pragmatic studies in primary care with outcomes that are of clinicians' interest, such as complications and clinical outcomes.

Keywords: -.

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Introduction

Antibiotics resistance is a global public health challenge, which has accelerated by the overuse of antibiotics worldwide. Increased antimicrobial resistance is the cause of severe infections, complications, longer hospital stays and increased mortality. Overprescribing of antibiotics is associated with an increased risk of adverse effects, more frequent re-attendance and increased medicalization of self-limiting conditions. Antibiotic overprescribing is a particular problem in primary care, where bacteria cause most infections(1).

Antibiotics resistance is recognized as one of the greatest threats to human health worldwide. Just one organism, methicillin-resistant *Staphylococcus aureus* (MRSA), kills more Americans every year than emphysema, HIV/ AIDS, Parkinson's disease and homicide combined (2). Globally, 3.7% of new cases and 20% of previously treated cases of tuberculosis are estimated to be caused by strains that are resistant to isoniazid and rifampicin. For decades, these antituberculosis agents have been effective against tuberculosis, but today the effect is insufficient. Nowadays, only one-half of multidrug-resistant tuberculosis is effectively treated with the existing drugs (3). Extensively drug-resistant tuberculosis (defined as multidrug-resistant tuberculosis plus resistance to any fluoroquinolone and any second-line injectable drug) has been identified in 84 countries globally. Carbapenem-resistant Enterobacteriaceae spp. and extended-spectrum beta-lactamase-producing Enterobacteriaceae have been isolated in recent years (4). There is a striking lack of development of new drugs active against these multidrug-resistant Gram-negative bacteria, particularly those producing carbapenemases (5), and none of the antibiotics currently available are now effective (4). While antibiotic resistance has predominantly been a clinical problem in hospital settings recent data show resistant organisms have also been detected in patients in primary care(6).

A recent report from the World Health Organization (WHO) clearly states that this is not a phenomenon occurring in just poor or developing countries; the problem of AMR is now found throughout the world (3). Diseases associated with AMR in primary care include tuberculosis, gonorrhoea (specifically *Neisseria gonorrhoeae*) typhoid fever and Group B streptococcus (7). Community-acquired AMR is of particular concern, as these infections can be common and easily transmitted. The most recent data from the European Antibiotic Surveillance Reports found that antibiotic resistance rates of *Escherichia coli* and/or *Klebsiella pneumoniae* vary markedly between countries. European data from 2011 demonstrate an alarming increase in the resistance of these organisms, with around a third of European countries showing a rise in combined resistance to third-generation cephalosporins, fluoroquinolones and aminoglycosides over the previous 4 years (8). Some of these types of antibiotics are considered by the WHO as 'critically important antimicrobials' in medicine, and these broad spectrum antibiotics should be avoided when narrow-spectrum antibiotics remain effective, as they also increase the risk of *Clostridium difficile* infection, MRSA and resistant urinary tract infections (9).

2.1. History of Antibiotics:

The management of microbial infections in ancient Egypt, Greece, and China is well-documented (10). The modern era of antibiotics started with the discovery of penicillin by Sir Alexander Fleming in 1928 (13). Since then, antibiotics have transformed modern medicine and saved millions of lives (2). Antibiotics were first prescribed to treat serious infections in the 1940s (13). Penicillin was successful in controlling bacterial infections among World War II soldiers (10). However, shortly thereafter, penicillin resistance became a substantial clinical problem, so that, by the 1950s, many of the advances of the prior decade were threatened. In response, new beta-lactam antibiotics were discovered, developed, and deployed, restoring confidence (14).

However, the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) was identified during that same decade, in the United Kingdom in 1962 and in the United States in 1968 (13).

Unfortunately, resistance has eventually been seen to nearly all antibiotics that have been developed. Vancomycin was introduced into clinical practice in 1972 for the treatment of methicillin resistance in both *S. aureus* and coagulase-negative staphylococci. It had been so difficult to induce vancomycin resistance that it was believed unlikely to occur in a clinical setting (10). However, cases of vancomycin resistance were reported in coagulase-negative staphylococci in 1979 and 1983 (10). From the late 1960s through the early 1980s, the pharmaceutical industry introduced many new antibiotics to solve the resistance problem, but after that the antibiotic pipeline began to dry up and fewer new drugs were introduced. As a result, in 2015, many decades after the first patients were treated with antibiotics, bacterial infections have again become a threat (14).

2.2. Benefits of Antibiotics:

Antibiotics have not only saved patients' lives, they have played a pivotal role in achieving major advances in medicine and surgery (12). They have successfully prevented or treated infections that can occur in patients who are receiving chemotherapy treatments; who have chronic diseases such as diabetes, end-stage renal disease, or rheumatoid arthritis; or who have had complex surgeries such as organ transplants, joint replacements, or cardiac surgery (15).

Antibiotics have also helped to extend expected life spans by changing the outcome of bacterial infections (11). In 1920, people in the U.S. were expected to live to be only 56.4 years old; now, however, the average U.S. life span is nearly 80 years (16). Antibiotics have had similar beneficial effects worldwide. In developing countries where sanitation is still poor, antibiotics decrease the morbidity and mortality caused by food-borne and other poverty-related infections (17).

2.3. Causes of Antibiotics Resistance Crises:

2.3.1. Overuse:

As early as 1945, Sir Alexander Fleming raised the alarm regarding antibiotic overuse when he warned that the "public will demand [the drug and] ... then will begin an era ... of abuses." (18) The overuse of antibiotics clearly drives the evolution of resistance (19). Epidemiological studies have demonstrated a direct relationship between antibiotic consumption and the emergence and dissemination of resistant bacteria strains (20). In bacteria, genes can be inherited from relatives or can be acquired from nonrelatives on mobile genetic elements such as plasmids. This horizontal gene transfer (HGT) can allow antibiotic resistance to be transferred among different species of bacteria. Resistance can also occur spontaneously through mutation (19). Antibiotics remove drug-sensitive competitors, leaving resistant bacteria behind to reproduce as a result of natural selection.⁹ Despite warnings regarding overuse, antibiotics are overprescribed worldwide (20).

2.3.2. Inappropriate Prescribing:

Incorrectly prescribed antibiotics also contribute to the promotion of resistant bacteria(13). Studies have shown that treatment indication, choice of agent, or duration of antibiotic therapy is incorrect in 30% to 50% of cases(21). One U.S. study reported that a pathogen was defined in only 7.6% of 17,435 patients hospitalized with community-acquired pneumonia (CAP). In comparison, investigators at the Karolinska Institute in Sweden were able to identify the probable pathogen in 89% of patients with CAP through use of molecular diagnostic techniques (polymerase chain reaction [PCR] and semiquantitative PCR)(18). In addition, 30% to 60% of the antibiotics prescribed in intensive care units (ICUs) have been found to be unnecessary, inappropriate, or suboptimal(21). Incorrectly prescribed antibiotics have questionable therapeutic benefit and expose patients to potential complications of antibiotic therapy(22). Subinhibitory and subtherapeutic antibiotic concentrations can promote the development of antibiotic resistance by supporting genetic alterations, such as changes in gene expression, HGT, and mutagenesis(23).

2.3.3. Extensive Agricultural Use:

In both the developed and developing world, antibiotics are widely used as growth supplements in livestock(20).An estimated 80% of antibiotics sold in the U.S. are used in animals, primarily to promote growth and to prevent infection (24).Treating livestock with antimicrobials is said to improve the overall health of the animals, producing larger yields and a higher-quality product (25).

The antibiotics used in livestock are ingested by humans when they consume food.¹ The transfer of resistant bacteria to humans by farm animals was first noted more than 35 years ago, when high rates of antibiotic resistance were found in the intestinal flora of both farm animals and farmers More recently, molecular detection methods have demonstrated that resistant bacteria in farm animals reach consumers through meat products (18). This occurs through the following sequence of events: 1) antibiotic use in food-producing animals kills or suppresses susceptible bacteria, allowing antibiotic-resistant bacteria to thrive; 2) resistant bacteria are transmitted to humans through the food supply; 3) these bacteria can cause infections in humans that may lead to adverse health consequences (13).

2.3.4. Availability of Few Antibiotics:

The development of new antibiotics by the pharmaceutical industry, a strategy that had been effective at combating resistant bacteria in the past, had essentially stalled due to economic and regulatory obstacles. Of the 18 largest pharmaceutical companies, 15 abandoned the antibiotic field(18). Mergers between pharmaceutical companies have also substantially reduced the number and diversity of research teams. Antibiotic research conducted in academia has been scaled back as a result of funding cuts due to the economic crisis(11).

2.3.5. Regulatory Barriers:

Even for those companies that are optimistic about pursuing the discovery of new antibiotics, obtaining regulatory approval is often an obstacle. Between 1983 and 2007, a

substantial reduction occurred in the number of new antibiotic approvals(12). Difficulties in pursuing regulatory approval that have been noted include: bureaucracy, absence of clarity, differences in clinical trial requirements among countries, changes in regulatory and licensing rules, and ineffective channels of communication(11).

2.3.6. Antibiotic-Resistant Bacterial Infections:

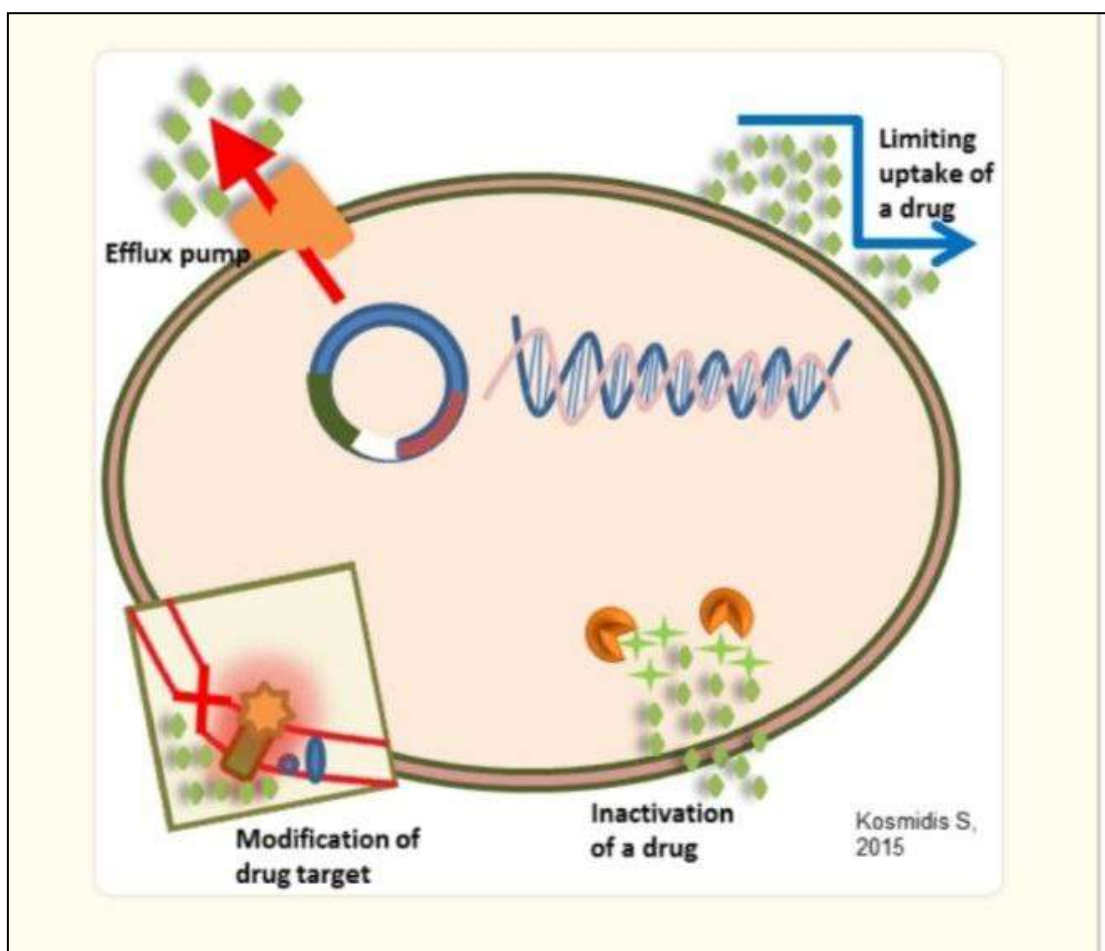
Antibiotic-resistant infections are already widespread in the U.S. and across the globe(26). A 2011 national survey of infectious-disease specialists, conducted by the IDSA Emerging Infections Network, found that more than 60% of participants had seen a pan-resistant, untreatable bacterial infection within the prior year(14). Many public health organizations have described the rapid emergence of resistant bacteria as a “crisis” or “nightmare scenario” that could have “catastrophic consequences”(23). The CDC declared in 2013 that the human race is now in the “post-antibiotic era,” and in 2014, the World Health Organization (WHO) warned that the antibiotic resistance crisis is becoming dire(25). MDR bacteria have been declared a substantial threat to U.S. public health and national security by the IDSA and the Institute of Medicine, as well as the federal Interagency Task Force on Antimicrobial Resistance(26).

Among gram-positive pathogens, a global pandemic of resistant *S. aureus* and *Enterococcus* species currently poses the biggest threat(13). MRSA kills more Americans each year than HIV/AIDS, Parkinson’s enterococci (VRE) and a growing number of additional pathogens are developing resistance to many common antibiotics(26). The global spread of drug resistance among common respiratory pathogens, including

Streptococcus pneumoniae and *Mycobacterium tuberculosis*, is epidemic (17).

2.4. Mechanisms of Antibiotics Resistance:

Antimicrobial resistance mechanisms fall into four main categories: (1) limiting uptake of a drug; (2) modifying a drug target; (3) inactivating a drug; (4) active drug efflux. Intrinsic resistance may make use of limiting uptake, drug inactivation, and drug efflux; acquired resistance mechanisms used may be drug target modification, drug inactivation, and drug efflux. Because of differences in structure, etc., there is variation in the types of mechanisms used by gram negative bacteria versus gram positive bacteria. Gram negative bacteria make use of all four main mechanisms, whereas gram positive bacteria less commonly use limiting the uptake of a drug (don't have an LPS outer membrane), and don't have the capacity for certain types of drug efflux mechanisms (refer to the drug efflux pumps later in this manuscript) [26]. Figure 1 illustrates the general antimicrobial resistance mechanisms.



2.4.1. Limiting Antibiotics Uptake:

As already mentioned, there is a natural difference in the ability of bacteria to limit the uptake of antimicrobial agents. The structure and functions of the LPS layer in gram negative bacteria provides a barrier to certain types of molecules. This gives those bacteria innate resistance to certain groups of large antimicrobial agents [27]. The mycobacteria have an outer membrane that has a high lipid content, and so hydrophobic drugs such as rifampicin and the fluoroquinolones have an easier access to the cell, but hydrophilic drugs have limited access [28].

Bacteria that lack a cell wall, such as *Mycoplasma* and related species, are therefore intrinsically resistant to all drugs that target the cell wall including β -lactams and glycopeptides [29]. Gram positive bacteria do not possess an outer membrane, and restricting drug access is not as prevalent. In the enterococci, the fact that polar molecules have difficulty penetrating the cell wall gives intrinsic resistance to aminoglycosides. Another gram positive bacteria, *Staphylococcus aureus*, recently has developed resistance to vancomycin. Of the two mechanisms that *S. aureus* uses against vancomycin, a yet unexplained mechanism allows the bacteria to produce a thickened cell wall which makes it difficult for the drug to enter the cell, and provides an intermediate resistance to vancomycin. These strains are designated as VISA strains [30].

In those bacteria with large outer membranes, substances often enter the cell through porin channels. The porin channels in gram negative bacteria generally allow access to hydrophilic molecules [31]. There are two main ways in which porin changes can limit drug uptake: a decrease

in the number of porins present, and mutations that change the selectivity of the porin channel [28]. Members of the Enterobacteriaceae are known to become resistant due to reducing the number of porins (and sometime stopping production entirely of certain porins). As a group, these bacteria reduce porin number as a mechanism for resistance to carbapenems [34]. Mutations that cause changes within the porin channel have been seen in *E. aerogenes* which then become resistant to imipenem and certain cephalosporins, and in *Neisseria gonorrhoeae* which then become resistant to β -lactams and tetracycline [36].

Another widely seen phenomenon in bacterial colonization is the formation of a biofilm by a bacterial community. These biofilms may contain a predominant organism (such as by *Pseudomonas aeruginosa* in the lung), or may consist of a wide variety of organisms, as seen in the biofilm community of normal flora in the gut. For pathogenic organisms, formation of a biofilm protects the bacteria from attack by the host immune system, plus provides protection from antimicrobial agents. The thick, sticky consistency of the biofilm matrix which contains polysaccharides, and proteins and DNA from the resident bacteria, makes it difficult for antimicrobial agents to reach the bacteria. Thus, to be effective, much higher concentrations of the drugs are necessary. In addition the bacterial cells in the biofilm tend to be sessile (slow metabolism rate, slow cell division), so antimicrobials that target growing, dividing bacterial cells have little effect. An important observation about biofilms is that it is likely that horizontal transfer of genes is facilitated by the proximity of the bacterial cells. That means that sharing of antimicrobial resistance genes is potentially easier for these bacterial communities [34].

2.4.2. Modification of Antibiotics Targets:

There are multiple components in the bacterial cell that may be targets of antimicrobial agents; and there are just as many targets that may be modified by the bacteria to enable resistance to those drugs. One mechanism of resistance to the β -lactam drugs used almost exclusively by gram positive bacteria is via alterations in the structure and/or number of PBPs (penicillin-binding proteins). PBPs are transpeptidases involved in the construction of peptidoglycan in the cell wall. A change in the number (increase in PBPs that have a decrease in drug binding ability, or decrease in PBPs with normal drug binding) of PBPs impacts the amount of drug that can bind to that target. A change in structure (e.g. PBP2a in *S. aureus* by acquisition of the *mecA* gene) may decrease the ability of the drug to bind, or totally inhibit drug binding [35].

The glycopeptides (e.g. vancomycin) also work by inhibiting cell wall synthesis, and lipopeptides (e.g. daptomycin) work by depolarizing the cell membrane. Gram negative bacteria (thick LPS layer) have intrinsic resistance to these drugs [36]. Resistance to vancomycin has become a major issue in the enterococci (VRE—vancomycin-resistant enterococci) and in *Staphylococcus aureus* (MRSA). Resistance is mediated through acquisition of *van* genes which results in changes in the structure of peptidoglycan precursors that cause a decrease in the binding ability of vancomycin [37]. Daptomycin requires the presence of calcium for binding. Mutations in genes (e.g. *mprF*) change the charge of the cell membrane surface to positive, inhibiting the binding of calcium, and therefore, daptomycin [38].

Resistance to drugs that target the ribosomal subunits may occur via ribosomal mutation (aminoglycosides, oxazolidinones), ribosomal subunit methylation (aminoglycosides, macrolides—gram positive bacteria, oxazolidinones, streptogramins) most commonly involving erm genes, or ribosomal protection (tetracyclines). These mechanisms interfere with the ability of the drug to bind to the ribosome. The level of drug interference varies greatly among these mechanisms [39].

For drugs that target nucleic acid synthesis (fluoroquinolones), resistance is via modifications in DNA gyrase (gram negative bacteria—e.g. gyrA) or topoisomerase IV (gram positive bacteria—e.g. grlA). These mutations cause changes in the structure of gyrase and topoisomerase which decrease or eliminate the ability of the drug to bind to these components [40].

For the drugs that inhibit metabolic pathways, resistance is via mutations in enzymes (DHPS—dihydropteroate synthase, DHFR—dihydrofolate reductase) involved in the folate biosynthesis pathway and/or overproduction of resistant DHPS and DHFR enzymes (sulfonamides—DHPS, trimethoprim—DHFR). The sulfonamides and trimethoprim bind to their respective enzymes due to their being structural analogs of the natural substrates (sulfonamides—p-amino-benzoic acid, trimethoprim—dihydrofolate). The action of these drugs is through competitive inhibition by binding in the active site of the enzymes. Mutations in these enzymes are most often located in or near the active site, and resulting structural changes in the enzyme interfere with drug binding while still allowing the natural substrate to bind [41].

2.4.3. Antibiotics Inactivation:

There are two main ways in which bacteria inactivate drugs; by actual degradation of the drug, or by transfer of a chemical group to the drug. The β -lactamases are a very large group of drug hydrolyzing enzymes. Another drug that can be inactivated by hydrolyzation is tetracycline, via the tetX gene [39].

Drug inactivation by transfer of a chemical group to the drug most commonly uses transfer of acetyl, phosphoryl, and adenylyl groups. There are a large number of transferases that have been identified. Acetylation is the most diversely used mechanism, and is known to be used against the aminoglycosides, chloramphenicol, the streptogramins, and the fluoroquinolones. Phosphorylation and adenylation are known to be used primarily against the aminoglycosides [42].

2.4.4. β -lactamases:

The most widely used group of antimicrobial agents are the β -lactam drugs. The members of this drug group all share a specific core structure which consists of a four-sided β -lactam ring. Resistance to the β -lactam drugs occurs through three general mechanisms: (1) preventing the interaction between the target PBP and the drug, usually by modifying the ability of the drug to bind to the PBP (this is mediated by alterations to existing PBPs or acquisition of other PBPs); (2) the presence of efflux pumps that can extrude β -lactam drugs; (3) hydrolysis of the drug by β -lactamase enzymes [43].

The β -lactamases (originally called penicillinases and cephalosporinases) inactivate β -lactam drugs by hydrolyzing a specific site in the β -lactam ring structure, causing the ring to open. The open-ring drugs are not able to bind to their target PBP proteins. The known β -lactamases are wide-spread, and the group contains enzymes that are able to inactivate any of the current β -lactam drugs. The production of β -lactamases is the most common resistance mechanism used by gram negative bacteria against β -lactam drugs, and the most important resistance mechanism against penicillin and cephalosporin drugs [39].

The β -lactamase enzymes are classified based on their molecular structure and/or functional characteristics. Structurally they are placed into four main categories (A, B, C, or D). There are three functional groupings based on the substrate specificity: the cephalosporinases, the serine β -lactamases, and the metallo (zinc-dependent) β -lactamases. These enzymes may also be commonly known by their enzyme family; for example: the TEM (named after the first patient) family, the SHV (sulphydryl variable) family, and the CTX (preferentially hydrolyze cefotaxime) family. Gram negative bacteria may produce β -lactamases from all four structural groups. The β -lactamases found in gram positive bacteria are mainly from group A, with some from group B [44].

These enzymes may be innately found on the bacterial chromosome or may be acquired via a plasmid. Many members of the Enterobacteriaceae family of gram negative bacteria possess chromosomal β -lactamase genes. Other gram negative bacteria that possess these include *Aeromonas spp.*, *Acinetobacter spp.*, and *Pseudomonas spp.* Plasmid-carried β -lactamase genes are most commonly found in the Enterobacteriaceae, but may also be found in some species of gram positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Enterococcus faecium* [45].

The first β -lactamase to be characterized was from *E. coli* and is chromosomally encoded by the *ampC* gene (so named for ampicillin resistance). This gene is constitutively expressed at a low level, but mutations may result in overexpression of the gene. The AmpC β -lactamases are most effective against the penicillins and some first generation cephalosporins. There are also many plasmid-borne β -lactamases which carry a variety of *bla* genes (β -lactamase genes). If these β -lactamases confer resistance to later generation cephalosporins, they were designated as ESBLs, and include members of the TEM, SHV, CTX-M, and OXA enzyme families. The largest group is the CTX-Ms, which are most commonly found in *E. coli*, especially UTI isolates. The ESBL producers may also be resistant to multiple drug classes, but are generally sensitive to β -lactamase inhibitors. The β -lactamase inhibitors are structurally similar to β -lactamases, have weak antimicrobial ability alone, but work synergistically in combination with a β -lactam drug. Commonly used β -lactamase inhibitor/drug pairings include amoxicillin/clavulanic acid, ampicillin/sulbactam, and piperacillin/tazobactam [46].

Recently there has been an emergence of β -lactamases that are active against the carbapenems (carbapenemases), and are found primarily in the Enterobacteriaceae. There are two types of carbapenemases; the *Klebsiella pneumoniae* carbapenemases (KPCs), and those designated as Carbapenem-Resistant Enterobacteriaceae (CRE) enzymes. The KPCs belong to the serine Class A (functional group 2f) β -lactamases, are resistant to all β -lactam drugs, but may still

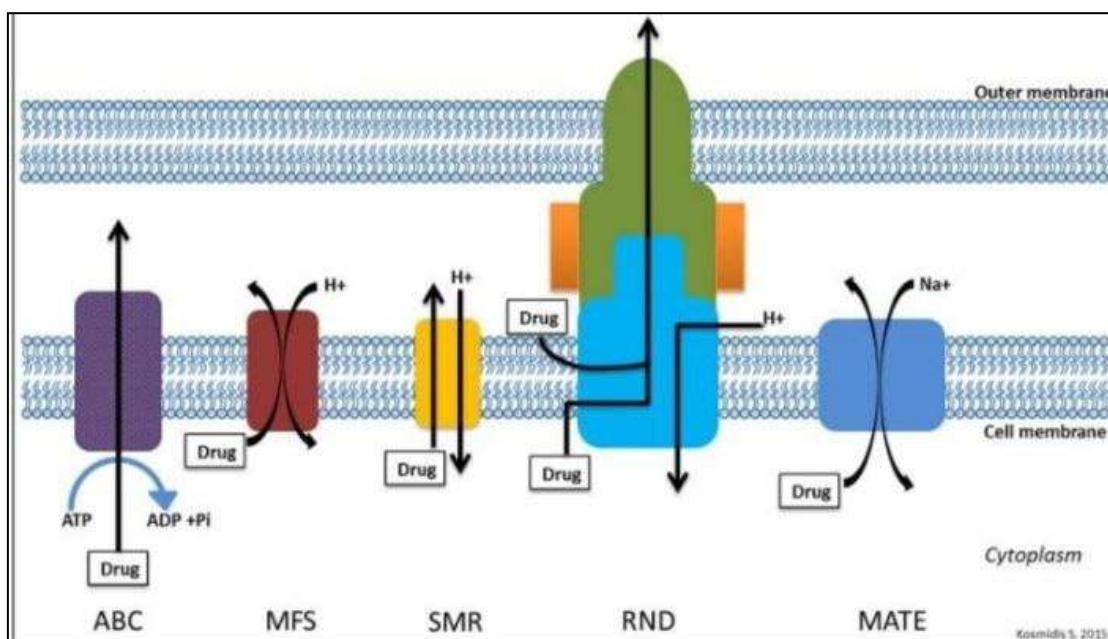
be affected by β -lactamase inhibitors. In bacteria that are CRE strains the carbapenemases are all metallo- β -lactamases (MBLs) in Class B, functional group 3a, and are capable of hydrolyzing all β -lactam drugs, but are not inactivated by β -lactamase inhibitors. The most widely distributed CREs are the IMP-1 (for imipenem resistance) and VIM-1 (Verona integron encoded MBL) types. A new MBL has recently been identified, mainly in strains of *E. coli*. It has been designated as NDM-1 (New Delhi MBL). Infections caused by CRE strains have been associated with in-hospital mortality of up to 71% [47].

There is a lot of emphasis on the development of more effective β -lactamase inhibitor drug combinations, especially in an effort to combat the CRE strains. One newer β -lactamase/drug combination is ceftolozane/tazobactam, which is mainly used against *P. aeruginosa*, and shows promise against gram negative ESBL producing strains. There are also newer β -lactamase inhibitors which do not have a structure similar to the β -lactam drugs. The first one of these to be approved for use is avibactam, and it has been approved for use with ceftazidime against gram negative bacteria. In addition, avibactam is being tested for use with aztreonam against CREs. Another β -lactamase inhibitor which is non β -lactam structured is vaborbactam. It was approved for use with meropenem in 2017 against gram negative bacteria causing complicated urinary tract infections (cUTIs). Unfortunately, so far none of the newer combination drugs is designed to combat the CREs directly. The metallo- β -lactamases are proving difficult to defeat as these enzymes comprise 3 groups that vary greatly in structure and mechanisms [48].

2.4.5. Antibiotics Efflux:

Bacteria possess chromosomally encoded genes for efflux pumps. Some are expressed constitutively, and others are induced or overexpressed (high-level resistance is usually via a mutation that modifies the transport channel) under certain environmental stimuli or when a suitable substrate is present. The efflux pumps function primarily to rid the bacterial cell of toxic substances, and many of these pumps will transport a large variety of compounds (multi-drug [MDR] efflux pumps). The resistance capability of many of these pumps is influenced by what carbon source is available [27].

Most bacteria possess many different types of efflux pumps. There are five main families of efflux pumps in bacteria classified based on structure and energy source: the ATP-binding cassette (ABC) family, the multidrug and toxic compound extrusion (MATE) family, the small multidrug resistance (SMR) family, the major facilitator superfamily (MFS), and the resistance-nodulation-cell division (RND) family. Most of these efflux pump families are single-component pumps which transport substrates across the cytoplasmic membrane. The RND family are multi-component pumps (found almost exclusively in gram negative bacteria) that function in association with a periplasmic membrane fusion protein (MFP) and an outer membrane protein (OMP-porin) to efflux substrate across the entire cell envelope [28]. There are instances where other efflux family members act with other cellular components as multicomponent pumps in gram negative bacteria. One member of the ABC family, MacB, works as a tripartite pump (MacAB-TolC) to extrude macrolide drugs. A member of the MFS, EmrB, works as a tripartite pump (EmrAB-TolC) to extrude nalidixic acid in *E. coli* [49]. Figure 2



Efflux pumps found in gram positive bacteria may confer intrinsic resistance because of being encoded on the chromosome. These pumps include members of the MATE and MFS families and efflux fluoroquinolones. There are also gram positive efflux pumps known to be carried on plasmids. Currently, the characterized pumps in gram positive bacteria are from the MFS family [50]. Efflux pumps found in gram negative bacteria are widely distributed and may come from all five of the families, with the most clinically significant pumps belonging to the RND family [27].

2.5. The Clinical and Economic Burden of Antibiotics Resistance:

Antibiotic-resistant infections are a substantial health and economic burden to the U.S. health care system, as well as to patients and their families. They commonly occur in hospitals, due to the clustering of highly vulnerable patients, extensive use of invasive procedures, and high rates of antibiotic use in this setting. Nearly two million Americans per year develop HAIs, resulting in 99,000 deaths, most due to antibacterial-resistant pathogens.¹ In 2006, two common HAIs (sepsis and pneumonia) were found to be responsible for the deaths of nearly 50,000 Americans and cost the U.S. health care system more than \$8 billion (26).

Antibiotic-resistant infections add considerable costs to the nation's already overburdened health care system. When first-line and then second-line antibiotic treatment options are limited or unavailable, health care professionals may be forced to use antibiotics that are more toxic to the patient and frequently more expensive (22). Even when effective treatments exist, data show that in most cases patients with resistant infections require significantly longer hospital stays, more doctors visits, and lengthier recuperations and experience a higher incidence of long-term disability (13). The duration of hospital stays for patients with antibiotic-resistant infections was found to be prolonged by 6.4 to 12.7 days, collectively adding an extra eight million hospital days (26).

2.6. Procedures for Minimizing for Resistance Resulting from Excessive Use of Antibiotics:

2.6.1. Prescribing Fewer Antibiotics is Needed:

A reduction in antibiotic consumption leads to a reduction of resistance. The classical Finnish study focusing on macrolide resistant *Streptococcus pyogenes* clearly showed how a reduction in macrolide use could lead to a reduction in AMR. Antibiotic resistance dropped from 9.2% in 1997 to 7.4% in 2000, with a statistically significant association between regional macrolide resistance and consumption rates (52). Our goal is not just to reduce the amount of antibiotics. It is also to promote a rational use of antibiotics by prescribing antibiotics only to patients who are expected to benefit from the treatment. Many studies have been performed to determine the effectiveness of different types of intervention in promoting a more rational use of antibiotics. According to the last Cochrane review on interventions to improve antibiotic prescribing, multifaceted interventions combining physician, patient and public education in a variety of venues and formats were the most successful. Interactive educational meetings were more effective than didactic lectures, but levels of improvement were limited. Inappropriate antibiotic prescribing was reduced by less than 20% across a broad range of study populations (53).

2.6.2. Enforcement of Governmental Laws Prohibiting Over-Counter Sale of Antibiotics:

Self-medication with antibiotics is common in many parts of the world. In several countries, antibiotics are sold, illegally, without a prescription. This is particularly common in many countries in Asia, Africa, South and Central America, and even in Southern European countries, such as Italy, Spain, Greece and Malta. In some countries, antibiotics are also available on the free market, i.e. outside pharmacies. Law enforcement to prohibit the illegal over-the-counter sale of antibiotics at pharmacies and the sale of antibiotics for humans and animals on the free market should be promoted worldwide (54).

2.6.3. Antimicrobial Stewardship Programmes, Campaigns and Audits:

In many countries, there have been educational campaigns that aim to change healthcare professional and patient behaviour in antibiotic consumption. Interventions include the publication of guidelines, educational sessions on appropriate prescribing of antibiotics, educational sessions on the diagnosis and management of infectious diseases, review of prescribing data for practices, local interviews by pharmacists, messages included on TV, radio and other mass media, etc. Although the effects of these public campaigns and primary-care projects are positive, they are not sufficient to reduce the problem of AMR. An analysis of 22 national- or regional-level campaigns in high-income countries from 1990 to 2007 did find a reduction in antibiotic use. However, as all but one campaign targeted the patient and healthcare professional simultaneously (55).

2.6.4. Promoting The Use of Valid Point-of Care Tests

If you visit a primary-care consultation in Scandinavian country and compare it with a similar consultation in a Southern European country, you soon realize that the most important difference is the number of diagnostic tools available in Scandinavia. GPs in Northern countries usually use rapid antigen detection testing for the diagnosis of streptococcal pharyngitis, C-

reactive protein (CRP) devices for ruling out serious respiratory tract infections, equipment capable of determining the number and type of leukocytes and agar plates for urine culture and susceptibility testing of bacteria (e.g. Flexicult plates, Petri plates that give clinicians knowledge about the bacterial aetiology of a urinary tract infection and the susceptibility pattern of the involved microorganisms in less than 24 hours). The major contribution of point-of-care tests seems to decrease doctors' uncertainty, adding useful information that helps to identify who to treat or not to treat with antibiotics. However, not all of the rapid tests are useful in primary care; only those that are accurate, precise, easy to use and interpret, fast and affordable for a primary care setting are acceptable (56).

2.6.5. Promote Delayed Prescribing of Antibiotics:

Delayed antibiotic prescribing means that the prescriber delivers an antibiotic prescription, but recommends the patient not to redeem it the same day. The prescription should only be redeemed if the patient feels worse within a few days. If symptoms reduce spontaneously, the prescription should be discarded. Delayed antibiotic prescribing is a widespread practice in the UK and its use is enforced by national guidelines but it has been difficult to implement in other countries. However, recent evidence from Norway also indicates that delayed prescribing may lead to a reduction in antibiotic use, mainly for sinusitis and otitis media (57).

2.6.6. Enhancing Communication Skills with Patients:

Improved communication in primary care can help to bridge this gap between physician and patient expectations. This can be achieved using various approaches. In a pragmatic clinical trial carried out in the Netherlands the authors observed that GPs assigned to CRP testing prescribed fewer antibiotics than those in the control group (30.7% *versus* 35.7%) and those trained in communication skills treated 26.3% of all episodes of respiratory tract infection with antibiotics compared with 39.1% treated by family physicians without (56).

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