



CRE (Carbapenem Resistant Enterobacteriaceae) and the Globalization of Antimicrobial Resistance: Problems and Solutions

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Abstract

The decreasing effectiveness of antibiotics in treating common infections results from the spread of antimicrobial resistance (AMR), and is building up to become an epic global public health crisis. Extended periods of antibiotic overuse and misuse since their introduction have applied strong selective pressure towards high-level AMR and multiple drug resistance (MDR), rendering entire classes of antibiotics ineffective. The primary driving force for this global AMR pandemic is the widespread misuse and overuse of antibiotics, in both medical and non-medical applications. The introduction of every antibiotic product has been closely followed by emerging resistance to that antibiotic. Levels of antibiotic consumption correlate with levels of AMR.

Keywords: Carbapenem resistant enterobacteriaceae; Antimicrobial resistance

Introduction

Antibiotics have been misused in all of their applications, including [1]:

- Hospital and outpatient use by physicians through unnecessary, indiscriminate or incorrect prescribing;
- By patients, through incorrect dosing and course durations;
- Large-scale use in agriculture for disease treatment, prophylaxis and growth promotion in animal husbandry and food production.

These actions not only have provoked the emergence of resistant microbes, but also have provided optimal environments for the spread of and selection of resistance determinants. It has been established in many countries that the levels of antibiotic consumption consistently correlate with levels of antibiotic resistance (i.e. the more antibiotics are being used in a population, the more resistance to antibiotics there will be in bacteria responsible for infections in that population). The increase in

resistance from overuse of antibiotics in turn leads to cross transmission of AMR microbes between humans, between animals, and between humans and animals and the environment [2]. Almost two million Americans per year develop hospital-acquired infections (HAIs), resulting in 99,000 deaths per year. The vast majority of these HAI related deaths are due to AMR infections [3]. Based on studies of the costs of infections caused by antibiotic resistant pathogens vs. antibiotic susceptible pathogens, the annual cost to the US health system of antibiotic resistant infections is \$21 to \$34 billion, and eight million additional hospital days [3]. Of particular concern is the development and spread of Carbapenem - resistant Enterobacteriaceae (i.e., CRE). The global emergence of carbapenemase - producing organisms is a public health emergency because these enzymes confer resistance to both carbapenems and nearly all β -lactam antibiotics, and are often associated with multidrug or pandrug resistance [3,4]. Resistance

to antibiotics mediated by acquired carbapenemase enzymes in gram - negative bacteria - principally the Enterobacteriaceae species is a serious concern. Most carbapenemase producing isolates of Enterobacteriaceae are resistant to multiple other classes of antibiotics, limiting therapeutic options to patients. Since the carbapenem antibiotics are the last line of defense against multidrug-resistant gram-negative bacterial infections, their vulnerability represents a real public health crisis. In addition, the cost of CRE infection is higher than the cost of many chronic and acute diseases.

Antimicrobial Resistance (AMR): A Global Public Health Crisis

The age of antibiotics

Prior to the discovery of penicillin as the first available antibiotic, infectious disease had been the leading cause of death throughout history. Penicillin was the first successful chemotherapeutic agent produced by microbes and it initiated the age of antibiotics. It represents the first therapeutic agent that destroyed bacteria in vivo, was not destroyed in the body, and was non-toxic to humans. Penicillin belongs to the beta-lactam class of antibiotics, which are the most successful natural product group used in chemotherapy. Later developed members of this antibiotic class which are widely used in medicine today include the orally active semisynthetic penicillins (ampicillin, amoxicillin) and the cephalosporins. It has been estimated that at the end of the nineteenth century, nearly one third of all deaths were due to infectious disease. By the end of the twentieth century, the death rate from all sources of infection dropped to levels well below 10% [3]. No other class of medicine has had a comparable cumulative impact on reducing death rates and increasing life spans [4].

The spread of antimicrobial resistance (AMR)

The world is now entering the post antibiotic age because of the growing problem of antimicrobial resistance (AMR). Infections from resistant bacteria have become more common and some pathogens have become resistant to multiple classes of antibiotics. The spread of AMR threatens to compromise the treatment of all infectious disease and is one of the most serious problems confronting both contemporary and future global public health.

The global struggle against AMR

The loss of effective antibiotic treatment will not only compromise the ability to control routine infectious disease, but will also prevent the treatment of infectious complications in patients with other disease states. The following advanced medical treatments are dependent on antibiotics to fight infections [5,6]:

Cancer chemotherapy: Cancer patients are at risk to develop serious infections when their white blood cell count is low. Such infections can be serious, and effective antibiotics are needed to protect cancer patients from complications and death.

Surgery: Patients are at risk for infection from many surgeries including joint replacements, etc. Antibiotics are routinely given before surgery to prevent infection.

Rheumatoid arthritis (RA): This disease reduces the patient's immune system and increases the risk of infection. Since many medicines used to treat RA can weaken the immune system, effective antibiotics are needed to ensure that arthritis patients can continue receiving treatments.

Dialysis for end Stage renal disease: Such patients have a weakened immune system and a higher risk for blood stream infections. Such infections are the leading cause of death in dialysis patients. Effective antibiotics ensure that dialysis patients will continue to receive life-long treatment.

Organ and bone marrow transplant: These patients receive complex surgery and have weakened immune systems. They are at high risk for infections and the use of effective antibiotics is essential. Without effective antibiotics, all of these procedures would have to be reduced. The reduction in antibiotic effectiveness from resistant pathogens leads to more difficult and costly treatments as well as greater morbidity and mortality and death.

The Center for Disease Control (CDC) reports that some 2 million people per year in the USA are contracting infections that are resistant to antibiotic treatment. And among such infections, there are more than 23,000 reported deaths per year as a result of AMR. It is estimated that health-care-associated infections (HAI) is reported at 5% in the USA. However, the infection rates in the developing world are much higher. The pooled presence of HAI in the developing world is estimated at 15.5% [7]. AMR has been observed to emerge in the clinic within a decade or less after development of a new antibiotic [8-11].

AMR Driving Forces

AMR as natural evolution

AMR is the acquired ability of pathogens to withstand the actions of an antibiotic that kills all of its sensitive counterparts. This feature originally arises from random mutations in existing genes or from intact genes that serve a similar purpose. Exposure to antibiotics and other antimicrobial products (biocides) in humans and animals applies pressure that encourages resistance to emerge, and favoring naturally resistant strains and strains that have acquired resistance [12].

The specific meaning of the term 'AMR' depends on the context. The clinical definition used refers to the ability of a microbe (bacteria, virus, fungus or parasite) to survive concentrations of



antibiotics that kill sensitive cells of the same strain. For every antibiotic there are sensitive microbial strains which are killed or inhibited by the drug, and there are naturally resistant strains. Bacterial species that are not susceptible to a particular drug are 'naturally resistant.' But species that were once sensitive to an antibiotic but eventually became resistant to it have 'acquired resistance'. Acquired resistance affects a subset of the strains in the entire species, and varies with location. When a sensitive strain gains the ability to withstand the antibiotic, it is resistant to that antibiotic [12,13]. Some of the mechanisms that bacteria acquire to become resistant to antibiotics include; (i) Acquisition of genes coding for enzymes that destroy antibiotics (e.g: betalactamases); (ii) Acquisition of efflux pumps that expel antibiotics from the bacterial cell; (iii) Mutations that produce altered cell walls with dysfunctional antibiotic binding sites; and (iv) Mutations that result in a decrease in the outer membrane channels antibiotics need to enter the bacterial cell [12,13].

In biochemical terms, AMR means that a pathogen is less susceptible than its counterparts and may not respond to the antibiotic. The evolution of microbes is Darwinian; only the fittest survive change. Antibiotics represent an evolutionary challenge to microbes, which if not overcome will kill them. Resistance is not an on and off condition. Resistance exists as a gradient that reflects phenotypic and genotypic variations in large microbial populations. Different resistance mechanisms confer different levels of resistance. Low resistance levels are often overcome, but can also play an important role in the emergence of resistance. Currently used definitions of AMR do not take such diversity into account [12].

AMR arises by chance through mechanisms that may represent a history of natural competition among microbes. The mechanisms, genes, and pathways of antibiotic production and resistance help microbes compete for niches in nature. Therefore, AMR is a normal component of microbial life and represents a normal evolutionary phenomenon. However, these natural evolutionary phenomena are amplified by the use, both appropriate and inappropriate, of antimicrobials (antibiotics, antifungals, antivirals and biocides).

Most microbes can be a source of resistant genes, but selection for AMR often takes place in non-pathogenic microbes, since they make up the majority of the microbial world. Resistant genes are often derived from existing essential genes. Resistant genes may also originate from antibiotic producing strains that are used to protect themselves from their own harmful products, or from natural protection mechanisms. Developing resistance to antibiotics increases each of the genes available to microbes to also import other genes. And this causes their evolutionary approximation. Once a microbe derives genetic tools from resistance, it can pass that gene onto its progeny by clonal replication; or to other microbes through horizontal gene transfer.

Horizontal gene transfer – the movement of genetic material from one organism to another- is the primary mechanism by which bacteria acquire antibiotic resistance. Antibiotics promote this genetic exchange by inducing the transfer of conjugative elements. [2,12-14]. Indeed, for any Gram negative resistance issue, and especially for Enterobacteriaceae, one must also consider not just the spread of resistant strains but also the spread of their resistance genes between plasmids, and the spread of those plasmids between strains, species and genera. There is significant potential for transfer of bacteria and their resistance elements between reservoirs, as well as both to and from man, and from animal and environmental sources [8].

Antibiotic misuse as a primary driver for AMR

The largest driver for development and spread of AMR is the overuse and misuse of antibiotics in both medicine and in agriculture. Overuse includes use of broad spectrum antibiotics in varied practice settings when the pathogens that cause the infection are not known. Such misdiagnosis is caused by a number of factors in healthcare settings, including: lack of knowledge by prescribers; prescriber attitudes; lack of effective diagnostics; and lack of current treatment guidelines. Overuse and misuse of antibiotics occur in both the hospital setting as well as in community / primary care and long-term care settings [2,6,12,13]. Studies indicate that nearly 50% of antibiotic use in hospitals is unnecessary or inappropriate [5]. Antibiotic misuse is defined in the study as an absence of an indication for such antibiotics [15,16]. Misuse of broad spectrum antibiotics may be the largest single factor for the spread of AMR.

Inappropriate use also includes the use of sub inhibitory concentrations of antibiotics. Low concentrations of antibiotics can enrich for resistance genes in a population while having little effect on overall bacterial mortality. The tendency to mutate also increases upon exposure to sub-inhibitory concentrations of antibiotics. Low concentrations of antibiotics can also select for strains that increase expression of their existing resistance genes, further enhancing their resistance [12]. Although antibiotic resistance is mainly considered to be a clinical problem, antibiotic use and overuse is not restricted to clinical settings. The majorities of antibiotics consumed in the world are used in farming and animal agriculture and related settings (aquaculture). Overuse of antibiotics in agriculture leads to the spread and cross-transmission of antimicrobial-resistant microbes between humans, between animals, and between humans and animals and the environment.

AMR is most often portrayed as an undesirable consequence of antibiotic abuse or misuse. But this explanation is not a complete picture. The rate of AMR emergence is related to all uses of these drugs, and not just to their misuse. The total quantity of antibiotics put out into the environment also plays a role in AMR.

Selection for AMR is not confined to the human body, and is not limited to hospitals, clinics and farms. Selection takes place anywhere an antibiotic is present in natural environments, and especially in sewage and surface water sediments. Antibiotics are often found in the latter places coupled with high densities of large microbe populations. Large amounts of antibiotics and biocides end up in sewage sludge, which make them primary sources for development of AMR. Increasing amounts of antibiotics and biocides that are found in waste water, sediment and sludge discharges originate from agricultural applications. The stability of an antibiotic is one key as to how it will impact development of AMR in the environment.

Stable antibiotics are more likely to persist long enough to select for resistance [12,13].

(Figure 1) shows the effect of direct selective pressure as a primary driving force for AMR.

The figure illustrates that β -lactamases evolve with the appearance and use of each new antimicrobial class.

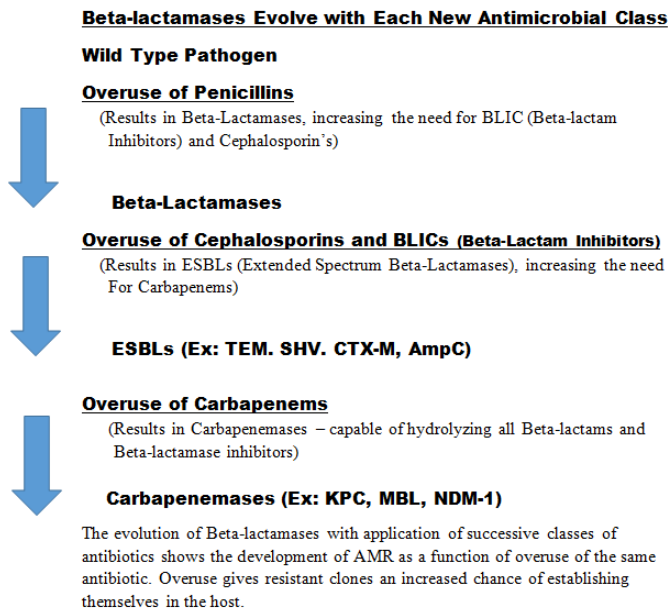


Figure 1: Selective Pressure as a Primary Driving Force for Antimicrobial Resistance (AMR)

Center for Disease Control (CDC) Threat Assessment for Healthcare Acquired Infections (HAI)

The decreasing effectiveness of antibiotics in treating common infections results from the spread of antimicrobial resistance (AMR) and is building up to become an epic global public health crisis. The crisis of reduced effectiveness of antibiotics to treat infections because of increasing AMR have been dramatically publicized in recent years by international health organizations (WHO) [6] as well as Central Govt. Agencies (NHS in UK [10],

CDC in USA [1]. Extended periods of antibiotic overuse and misuse since their introduction have applied strong selective pressure towards high level AMR and multiple drug resistance (MDR), rendering entire classes of antibiotics ineffective. The traditional response to AMR had been the introduction of new classes of antibiotics, a strategy which did not solve the problem but only bought a brief reprieve. However, over the last twenty years, there has been a significant decline in development and clinical introduction of new antibiotics to keep pace with the escalation of global AMR [14]. The rapid evolution and spread of AMR is illustrated in the case of the Beta-Lactam class of antibiotics. There are nearly 1,000 resistant related Beta lactamases that inactivate these antibiotics that have been identified, and have spread worldwide. The primary driving force for this global AMR pandemic is the widespread misuse and overuse of antibiotics, in both medical and non-medical applications. The introduction of every antibiotic product has been closely followed by emerging resistance to that antibiotic [6]. Levels of antibiotic consumption correlate with levels of AMR. Antibiotics have been misused in all of their applications, including:

- Hospital and outpatient use by physicians through unnecessary, indiscriminate or incorrect prescribing
- Out patients, through incorrect dosing and therapy course durations
- Large scale use in agriculture for disease treatment, prophylaxis and growth promotion in animal husbandry and food production

These actions not only have provoked the emergence of resistant microbes, but also have provided optimal environments for the spread of and selection of resistance determinants. It has been established in many countries that the levels of antibiotic consumption consistently correlate with levels of antibiotic resistance (i.e. the more antibiotics are being used in a population, the more resistance to antibiotics there will be in bacteria responsible for infections in this population). The increase in resistance from overuse of antibiotics in turn leads to cross transmission of AMR microbes between humans, between animals and between humans and animals and the environment. The two major areas for managing control and prevention of AMR are: (i) Prudent use of antibiotics: Use antibiotics only when needed, and with the correct dose, at the correct dose intervals, and for the correct duration; and (ii) Hygienic precautions for control of infections [12].

In September 2013, the Center of Disease Control (CDC) in the USA published a lengthy study describing the scope of AMR in the USA. The CDC estimates that more than 2 million people per year contract AMR infections and resulting in at least 23,000 direct deaths, and another 100,000 deaths from related



complications. Similarly the European Center for Disease Prevention and Control reports that 25,000 people die each year in Europe from antibiotic resistant bacteria. This problem is also present in other parts of the world. For example, in India over 58,000 people die in one year from antibiotic resistant infections. In Thailand, antibiotic resistance causes more than 38,000 deaths per year and 3.2 million hospitalization days [17,18].

The cost of AMR infections goes beyond patient deaths. The consequences include greater morbidity and healthcare expense. The impact of AMR on the American healthcare system is enormous. Almost two million Americans per year develop hospital acquired infections (HAI), resulting in 99,000 deaths, most of which are due to antibiotic resistant pathogens. It has

been estimated that the annual cost to the American healthcare system of antibiotic-resistant pathogens versus antibiotic-susceptible pathogens is \$21 to \$34 billion/year and more than 8 million additional hospital days [15,19,20]. The vast majority of these HAI related deaths are due to AMR infections. HAI incidence is reported at approximately 5% in the USA and 7.1% in Europe [13], and pooled infection rates in the developing world are estimated at 15.5% [14]. The CDC has listed the hazard level of threat for AMR into three categories: Hazard Level Urgent; Hazard Level Serious; and threat Level Concerning. The pathogens covered in these threat levels are summarized in (Table 1).

Table 1: CDC Levels of Concern - Prioritization of Resistant Bacteria.

Urgent Threats	Serious Threats	Concerning Threats
<i>Clostridium difficile</i>	Drug-resistant <i>Acinetobacter</i>	Vancomycin-resistant <i>Staphylococcus aureus</i> (VRSA)
Carbenapenem-resistant Enterobacteriaceae (CRE)	Drug-resistant <i>Campylobacter</i>	Erythromycin-resistant <i>Streptococcus</i> Group A
Drug-resistant <i>Neisseria gonorrhoeae</i>	Fluconazole-resistant <i>Candida</i>	Clindamycin-resistant <i>Streptococcus</i> Group B
	Extended-spectrum, cephalosporin-resistant <i>Enterobacteriaceae</i>	
	Vancomycin-resistant <i>Enterococcus</i> (VRE)	
	Drug-resistant <i>Pseudomonas aeruginosa</i>	
	Drug-resistant nontyphoidal <i>Salmonella</i>	
	Drug-resistant <i>Salmonella typhi</i>	
	Drug-resistant <i>Shigella</i>	
	Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)	
	Drug-resistant <i>Streptococcus pneumoniae</i>	
	Drug-resistant tuberculosis (MDR & XDR)	

Notes: MDR = Multiple Drug Resistance
XDR = Extensive Drug Resistance
Reference: [1]

ESKAPE pathogens & ESBL

A select group of pathogens are responsible for the majority of HAI; acronymically termed the ESKAPE pathogens by IDSA (Infectious Disease Society of America [15-23]. The term ESKAPE indicates that these pathogens are capable of escaping the biocidal actions of antibiotics and collectively represent new

paradigms in pathogenesis, transmission, resistance and severity of their infections. The ESKAPE acronym consists of: [16]

- E - *Enterococcus faecium*
- S - *Staphylococcus aureus*
- K - *Klebsiella pneumoniae*
- A - *Acinetobacter baumannii*
- P - *Pseudomonas aeruginosa*



- *E - Enterobacter Species and includes ESBL (Extended-Spectrum beta lactamase)*

ESKAPE indicates that these bacteria have developed defenses that permit them to escape the biocidal actions of available and effective antibiotic therapies. All of the ESKAPE pathogens produce beta lactamase enzymes and have developed resistance against carbapenems. A brief highlight of the more important carbapenemase producing pathogens follows:

Klebsiella pneumoniae: This pathogen is a member of the Gram-negative Enterobacteriaceae family, and is prevalent worldwide in both hospital and community infections. This pathogen is recognized for accumulation and rapid dissemination of MDR determinants. And over the past decade, it has acquired an extensive range of β lactamase enzymes capable of hydrolyzing the β -lactam ring common in penicillins, cephalosporins and also carbapenems. The development and spread of carbapenem-resistant *K. pneumoniae* (CRKP) threatens the clinical effectiveness of β -lactams, fluoroquinolones and aminoglycosides [19].

Acinetobacter baumannii: This is a Gram-negative opportunistic pathogen most often encountered in intensive care units and surgical wards, where extensive antibiotic use has enabled selection for AMR. This pathogen can grow across a range of temperatures, pHs and nutrient levels which make it highly adapted to survival in both human and environmental vectors and carries high rates of nosocomial cross-contamination. This pathogen is intrinsically resistant to antibiotics. It has acquired a broad range of β lactamases, including carbapenemases. The broad acquisition of ESBLs gives some isolates resistance to all antibiotics, including imipenem and colistin [20].

Pseudomonas aeruginosa: This pathogen is a Gram-negative facultative anaerobe. It preferentially colonizes immunocompromised patients and is known as an opportunistic pathogen associated with cancer patients and burn victims. It is resistant to fluoroquinolones through point mutations. In addition, this pathogen harbors broadspectrum ESBLs including carbapenemases, which make it difficult to employ suitable empirical antibiotic therapies [20].

Enterobacter species and includes ESBL (Extended-Spectrum beta lactamase): These species most commonly infect the urinary and respiratory tracts, but also cause bloodstream infections. They are shown to display broad MDR via plasmid-encoded ESBLs and carbapenemases. Beside colistin and tigecycline, few antibiotics are effective against these resistant pathogens [23].

The ESKAPE organisms are responsible for a substantial percentage of nosocomial infections in hospitals and represent the vast majority of isolates whose resistance to antibiotics present serious treatment limitations. Within the ESKAPE pathogens, the

problem of carbapenem resistant Enterobacteriaceae (CRE) infections are becoming the leading healthcare infection problem, and are referred to as “nightmare bacteria” by CDC director Dr. Tom Frieden [1]. The CRE pathogens include carbapenem resistant *Klebsiella* species and carbapenem resistant *E. coli*, resulting in 9,000 drug resistant infections/year and 600 deaths. CRE infections have become resistant to nearly all antibiotics in use today, including carbapenems - the antibiotic of last resort. CRE infections most commonly occur among patients who are receiving treatment for other conditions and whose care requires devices like ventilators, urinary catheters or intravenous catheters, and also patients taking long courses of antibiotics. Up to one half of patients die from CRE bloodstream infections [22].

Extended spectrum β -lactamases (ESBLs) are enzymes produced by a variety of gram negative bacterial which confer an increased resistance to commonly used antibiotics [22,23]. β -lactamases are hydrolytic enzymes which cleave the β -lactam ring and the primary mechanism of conferring bacterial resistance to β -lactam antibiotics such as penicillins and cephalosporins. These enzymes can be carried on bacterial chromosomes or may be plasmid mediated with the potential to move between bacterial populations. This ability of ESBL genes to jump between organisms leads to outbreaks of infections where easily transmissible pathogens are involved. Moreover, organisms that produce ESBLs have the capacity to acquire resistance to other antimicrobial classes such as quinolones, tetracyclines, cotrimazole, trimethoprim and aminoglycosides, which further limits therapeutic options. ESBLs have become a major cause of hospital-acquired infections and particularly in the intensive care unit (ICU).

CRE – Definition and Classification

Introduction

Carbapenemases represent the most versatile family of β -lactamases, with the widest spectrum of activity among all the β -lactam-hydrolyzing enzymes. Although known as “carbapenemases” many of these enzymes recognize almost all hydrolysable β -lactams, and most are resistant against inhibition by all commercially viable β -lactamase inhibitors [24,25]. Carbapenemases are produced by Enterobacteriaceae bacteria (Carbapenem resistant Enterobacteriaceae = CRE), which include more than 70 different genera covering many different mechanisms causing carbapenem resistance. CRE are included in a broader category of CP-CRE- standing for carbapenemase-producing CRE. CP-CRE are a subset of all CRE. All CRE are likely multidrug-resistant organisms for which interventions are required in healthcare settings to prevent transmission [24]. Carbapenemase-producing pathogens cause infections that are difficult to treat and have high mortality rates, due to their



appearance in multidrug resistant pathogens such as *K. pneumoniae*, *P. aeruginosa*, and *Acinetobacter* spp, It has been found that carbapenemase genes are easily transferred on mobile elements among bacterial species, leading to the spread of infections. It should be noted that CRE that do not produce carbapenemases are generally still resistant to multiple antibiotics and are a serious health problem.

CDC Definition of Multiple Drug resistant Organisms (MDRO) [24,25]

The expert consensus of MDRO as microbial species resistant to multiple antimicrobial agents contains the following subsets and applies to CP and CRE infections:

- PDR: (Pan Drug resistance) non susceptible to all agents in all antimicrobial drug categories
- MDR: (Multi Drug Resistance) an isolate non susceptible to a minimum of one agent in at least three drug categories
- XDR- (Extensive Drug Resistance) an isolate is non-susceptible to at least one agent in all but two or less antimicrobial drug categories

CDC Definition of Carbapenemases

Previously, the CDC defined CRE as “Being non-susceptible to the carbapenems ‘imipenem,’ ‘meropenem’ or ‘doripenem,’ AND resistant to all third generation cephalosporins tested.” The CDC has adopted a new definition since January 2015 for CRE to read: “Being non-susceptible to the carbapenems ‘imipenem,’ ‘meropenem’ or ‘doripenem,’ or ertapenem, or documentation that the isolate possesses a carbapenemase.” [24].” The previous CDC CRE definition was designed to be more specific for CP-CRE. However, that definition was found to be complicated, difficult to implement and also missed some CP-CRE. Other reasons for the change in the CDC definition were (i) ertapenem was included to increase the ability to detect carbapenemase-producing strains compared to the previous CDC CRE definition; (ii) third generation cephalosporins are not included from the current CDC definition to simplify the definition, facilitate application and also accommodate the emergence of OXA-48-type producing CRE which might not be resistant to this class of antimicrobials [24].

Classification of β -lactamases

More than 1,000 β -lactamases exist in gram-negative bacteria. Production of β -lactamases is the most widespread cause of carbapenem resistance. Two classification schemes exist for β -lactamases. One scheme is based on molecular classification and

puts all β -lactamases into four distinct classes (A through D) based on amino acid sequence homology- the Ambler classes, also called ‘Molecular Classes.’ The other scheme is based on functional classification using substrate and inhibitor activity (classes 1 through 4) – the Bush-Jacoby classes, also called ‘Functional Classes.’ [26-28]. This article will use the Ambler classification scheme and is depicted in (Table 2).

Molecular classes A, C and D contains serine in their active site, while Group B contains zinc in their active site. Carbapenemases are found in Classes A, B & D.

- **Class A β -lactamase:** They have serine active sites. The more important enzyme groups are: NMC, IMI, SME, and KPC & GES. KPC is the most common carbapenemase in the USA. While first isolated from *K. pneumoniae*, it has since spread to other Enterobacteriaceae.
- **Class B Beta Lactamases:** They are also referred to as MBL (metallo- β -lactamases because they require the presence of zinc to function. The more important enzymes are: IMP, VIM, GIM and SPM. Until 2009, VIM subtype was the most widespread MBL. It has since been displaced in ranking by NDM-1 which is now the most globally prevalent MBL subtype.
- **Class C β - lactamases:** They have a serine active site. The important enzymes are AmpC. AmpC genes on the bacterial chromosomes produce low levels of β - lactamases (repressed). When “de-repressed” the BL is hyper produced. AmpC BL has minimal activity against carbapenems and monobactams. However, when AmpC is combined with other mechanisms for reduced cell susceptibility, clinically significant levels of resistance are achieved Class C β -lactamase are not generally classified as carbapenemases [28].
- **Class D β -lactamases:** This class has a serine active site. The important β -lactamases are of the oxacillinase (OXA) enzyme type. They have weak activity against carbapenems and are found primarily in *P. aeruginosa* and *Acinetobacter*. The major concern with OXA carbapenemases is their ability to rapidly mutate and expand their spectrum of activity [29,30].
(Table 2) shows substrate and inhibition profiles of the more important carbapenemases - based on both the Ambler and Bush-Jacoby classification schemes (S-6-4) Clinically Important Carbapenemases: The most common carbapenemases in Enterobacteriaceae are displayed in (Tables 3,4). They are: KPC; VIM; NDM and OXA-48 [31,32].

Table 2: Groupings of carbapenemases and AmpC Beta-lactamases within Beta-lactamase classifications.

Ambler Group	Bush-Jacoby Group	Common Name	Mediates Resistance to	Representative Enzymes
A	2f	Serine carbapenemase	Carbapenems; Penicillins; Cephalosporins; Aztreonam	KPC, GES, SME1
B	3a	Metallo-β-lactamases (MBLs)	All β-lactams except aztreonam	IMP, NDM, VIM
C	1	Serine cephalosporinase	Penicillins; Cephalosporins	AmpC
D	2df	Carbapenemase	Carbapenems; Penicillins; Cephalosporins; Aztreonam	OXA

Based on table in Ref [27][28][29][48]

Notes:

KPC: Klebsiella pneumoniae carbapenemase

GES: Guiana extended spectrum

AmpC: Cephalosporinase

SMEI: *Serratia marcescens* enzyme

IMP: Imipenem-hydrolyzing-β-lactamase

VIM: Verona integron-encoded metallo-β-lactamase

NDM: New Delhi metallo-β-lactamase

OXA: Oxacillinase-hydrolyzing-β-lactamase

Table 3: Therapeutic treatment for CRE infections.

Several therapeutic agents have been used to treat serious CRE infections, and have been use both as monotherapy, and with additional agents as combination therapy.

MONOTHERAPY

- Carbapenems
- Fosfomycin
- Gentamycin
- Polymyxins (Colistin & Polymyxin B)
- Tigecycline

COMBINATION THERAPY

The following antibiotics are used in combination to treat CRE infections:

- Colistin + Meropenem
- Colistin + Tigecycline + Meropenem
- Colistin + Tigecycline
- Meropenem + Aminoglycosides
- Colistin + Aminoglycosides

Ref: Tabled assembled by author based on data from Ref [29].

KPC: Klebsiella pneumoniae carbapenemase is a class “A” β-lactamase. It can hydrolyze penicillins, cephalosporins and carbapenems. KPC was first reported in North Carolina in 1996, and has since spread globally. The KPC gene can be acquired by other species of Enterobacteriaceae including Enterobacter spp. and Escherichia coli and on rare occasions Pseudomonas aeruginosa and Acinetobacter baumannii as well. The potential of

E. coli acquiring KPC is concerning because of its community-wide distribution as a commensal organism.

VIM: Verona Integron-encoded-metallo-β-lactamase. This is a class “B” β-lactamase first identified in Verona Italy in 1997 in a P. aeruginosa clinical isolate. This family has 10 members and is found mostly in P. aeruginosa. VIM-2 is the most reported metallo-βlactam worldwide.

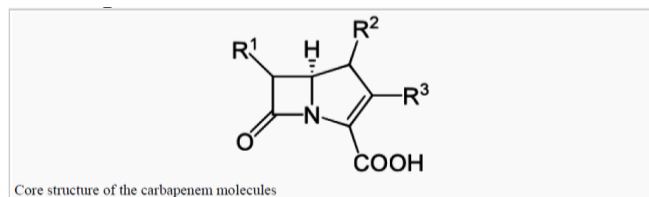
NDM: New Delhi metallo- β -lactamase is a class “B” β -lactamase capable of hydrolyzing penicillins, cephalosporins and carbapenems. The Indian subcontinent is the primary reservoir. This pathogen was first reported in Indian hospitals in 2006, and has since spread globally.

OXA-48: Oxacillin-hydrolyzing- β -lactamase is a class “D” serine β -lactamase, and OXA-48 is the carbapenemase of concern. It was first isolated in Turkey in 2001 from an Enterobacteriaceae strain. Most cases have been reported in *K pneumoniae* and are resistant to all β -lactams including carbapenemases. Most reports of this strain are in Turkey, North Africa and India, but rarely in the USA.

CRE – Treatment and Carbapenem Antibiotics

Carbapenem antibiotics

Of all the different classes of β -lactamases, the carbapenemases have the broadest activity spectrum and greatest potency against gram-positive and gram-negative, aerobic and anaerobic bacteria. They all have low oral availability and so must be administered parenterally. All carbapenem antibiotics are eliminated by renal excretion.



Principal Carbapenems

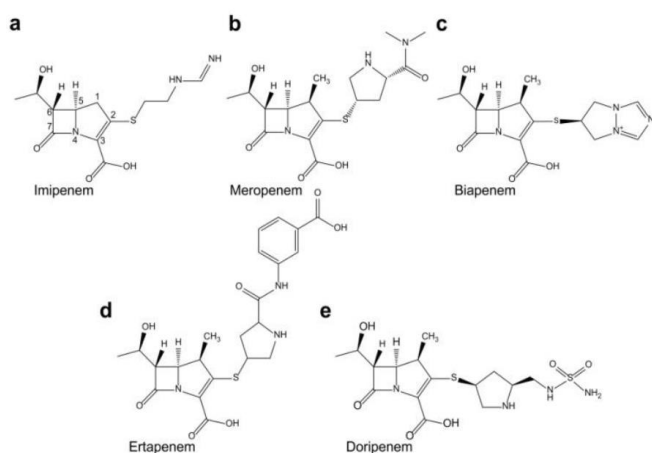


Figure 2: Structure of carbapenem antibiotics.

The carbapenems that are available in the USA are: Imipenem (FDA approved 1985; Meropenem (FDA approved 1996); Ertapenem (FDA approved 2001) and Doripenem (FDA approved 2008). In addition, another carbapenem – Biapenem- was approved for use in Japan, China and Korea (2002). (Figure 2)

shows the chemical structure of these five carbapenems [33-35] (Figure 2).

Treatment of ESBL infections

ESBLs are primarily produced by the *Enterobacteriaceae* family of Gram-negative organisms, especially from *K pneumoniae* and *E. coli*. They are also produced by the gram-negative bacteria *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. ESBLs are classified as Class A β -lactamases. They are plasmid mediated enzymes that hydrolyze cephalosporins and monobactams but not cephamycins nor carbapenems. Treatment options are [30,33,34-37].

Carbapenems: The antibiotic of choice against severe ESBL infections. They are rapidly bactericidal and have time dependent killing. They are also effective against other β -lactamases. They also offer low resistance rates and reduced mortality to patients.

Fluoroquinolones: Recommended for treatment of urinary tract infections (ESBL).

Piperacillin- Tazobactam: This is not a first line treatment, and presents lower susceptibility rates for ESBL infections. It is not used for empirical coverage when ESBL rates are high.

Cefepime: Is a fourth generation cephalosporin. Its use leads to selection for resistant strains, and is less effective than carbapenems. Cefepime is not a first line treatment and is not used as monotherapy. It is used in combination with other antibiotics such as aminoglycosides and fluoroquinolones.

Fosfomycin: While an old drug, it lacks cross resistance with other antibiotics. It has a wide spectrum of activity covering many gram-negative and gram-positive pathogens including ESBL and CRE. It is available in the USA only as an oral formulation and used to treat urinary tract infections. In Europe an intravenous formulation is available.

SMX – TMP (Sulfamethoxazole – Trimethoprim): This class can be used to treat ESBL infections in patients that are allergic to β -lactams. However, high resistance rates to ESBL and CRE limit their effectiveness.

Aminoglycosides: This class can be effective if lab testing shows that isolates are sensitive to aminoglycosides. This class should not be used as mono therapy against ESBL infections.

Treatment options for CRE infections: Tigecycline

Aminoglycosides; Polymyxin B and Colistin; Fosfomycin For treatment of CRE infections and carbapenemase producing infections, these antibiotics can be used as monotherapy or in combination therapy [30,29,35]. (Table 5) shows the various treatment options with these antibiotics – used in both monotherapy and in combination therapy. Because of limited clinical data, the choice of treatment for CPE and CRE infections is controversial. The extensive use of combination therapy remains under debate, as well as the optimal choice of drugs when

combinations are used. Most clinical studies have involved patients with KPC and VIM producing strains (Table 4).

The treatment options for CRE infections are fewer than for ESBL infections. The primary elements for this application are [28,33].

Table 4: Antibiotic usage in U.S. hospitals (2006 to 2012).

Antibiotic Class	2006	2007	2008	2009	2010	2011	2012	Change 2006 to 2012 %
All	53.8	54.0	54.9	55.7	55.7	56.3	55.6	+ 2.8
1 st & 2 nd Generation Cephalosporins	20.4	20.3	20.1	20.2	20.1	19.5	18.9	- 7.4
3 rd & 4 th Generation Cephalosporins	10.9	10.9	11.1	11.6	12.1	13.3	13.4	+ 32.1
Fluoroquinolones	16.8	16.7	16.9	16.4	15.8	15.7	15.0	- 10.7
Glycopeptides	8.2	8.9	7.9	10.7	11.3	12.3	12.9	+ 57.3
B-Lactamase Inhibitors	7.5	8.0	8.6	9.1	9.5	10.2	10.4	+ 38.7
Carbapenems	1.7	2.0	2.3	2.6	2.7	2.9	3.0	+ 76.5
Penicillins	6.0	5.7	5.5	5.1	5.2	5.3	5.3	-11.7

Notes: (i) DOT = Days of therapy; PD = Patient Days
 (ii) Overall use of antibiotics from 2006 to 2012 = 2.8% increase
 (iii) Use of carbapenem antibiotics from 2006 to 2012 = 76.5% increase
 Ref: Table assembled by author based on data from Ref [50].

Table 5: Carbapenem retail sales in Selected countries: 2005 – 2010.

Country	Sales 2005	Sales 2010 (i)	% Sales Increase 2005 – 2010
	Standard Units (SU) per 1,000 population		
Netherlands	9.5	18.5	95
USA	18.0	21.0	17
Brazil	2.5	4.0	60
Vietnam	1.0	9.0	800
Indonesia	3.5	10.0	186
India	11.0	63.5	477
Pakistan	37.0	90.0	143
Egypt	24.5	82.0	237

Table assembled by author from data in Ref. [13].

Carbapenems: Using carbapenems as monotherapy is discouraged, as it leads to increased resistance. However carbapenems are an essential element of combination therapy.

Polymyxins (Colistin and Polymyxin B): This is an older drug and is active against most gram-negative bacteria and CPE isolates. It is usually used in combination with other agents. Some adverse effects are nephrotoxicity (reversible) and neurotoxicity (rare).

Tigecycline: It has a broad spectrum of activity against gram-positive and gram-negative bacteria including CPE. Non-susceptibility to tigecycline against KPC-producing *K. pneumoniae* is becoming more common in patients who have been treated with this agent.

Aminoglycosides: Is effective against KPC-pneumoniae producing strains, and is always used in combination therapy. It is not effective against NDM-producing Enterobacteriaceae.

Fosfomycin: The oral formulation is used to treat urinary tract infections. For systemic infections, intravenous fosfomycin is

used in combination with another agent. Combinations of carbapenem and fosfomycin are an option for treating CRE strains that are resistant to colistin.

Monotherapy vs combination therapy

The preferred clinical practice is to treat invasive infections with a combination of two active agents based on susceptibility patterns of the infection strain. This approach results in lower patient mortality compared to monotherapy treatment. The dosing for each agent should be optimized by using high doses. Uncomplicated urinary tract infections can be managed with a single agent. Clinical data suggests that combination treatment (two or more agents) that is active against the infecting bacterial strain is superior in outcomes compared to monotherapy. It has been shown that combination therapy is more effective against most CPE infections even when the bacteria are resistant to an individual drug [30,32]. Most combination treatments utilize colistin. It is believed that colistin acts to increase the



permeability of the bacterial outer membrane. This effect in turn facilitates action by the other agents used [33].

Empirical therapy considerations for patients

For patients suffering from severe septic infections and septic shock, use of empirical antibiotic therapy should cover all of the patient's suspected bacterial infections. Empirical treatment with colistin and carbapenems or aminoglycosides is justified for treating severely ill patients with a suspected CRE infection. However, exclusive use of a broad spectrum antibiotic should be avoided to prevent further selection of resistant bacteria and also prevent fungal super infections and *C. difficile* outbreaks in hospitals [34-38].

Risk Factors for Development and Spread of CRE

General risk factors

CREs are easily introduced into the population because they are highly transmissible, resulting in colonization or infection of patients. Dissemination of mobile genetic elements coding for resistance and especially with multidrug resistant strains has been the cause of many reported outbreaks in hospitals [39]. The European Center for Disease Prevention has listed general risk factors associated with colonization or infection with CRE, including: the length of hospitalization (time at risk); severity of illness; mechanical ventilation; admission to the ICU; presence of wounds; positive blood culture; prior surgery; transfer between hospital units; prior hospital stay; presence of catheters and intubation [39].

Prior antimicrobial use

Prior antibiotic exposure has been found to be a risk factor for colonization and infection with CRE and especially for *K. pneumoniae* (KPC) [30,39].

Carbapenems: Prior use of carbapenems is identified as an independent risk factor for the acquisition of KPC producing *K. pneumoniae* and for carbapenem resistant KPC and carbapenem resistant *E. coli* [40].

Cephalosporins: Prior use of an extended spectrum cephalosporin is identified as a risk factor for the acquisition of KPC producing *K. pneumoniae*, and also as an independent risk factor for the acquisition of KPC producing *K. pneumoniae* for carbapenemase resistant *K. pneumoniae* and also for carbapenem resistant *E. coli*.

Fluoroquinolones: Prior use of a fluoroquinolone antibiotic is identified as a risk factor for the acquisition of KPC producing *Klebsiella pneumoniae*. Use of fluoroquinolones is also an independent risk factor for the acquisition of carbapenemase resistant *K. pneumoniae*.

Other antibiotics: Other antibiotics associated with risk of acquiring carbapenemase resistant enterobacteriaceae are the anti-pseudomonal penicillins and metronidazole.

Cross border transmission and patient mobility

Cross border transfer of patients is a documented risk factor for the introduction of carbapenemase producing *Enterobacteriaceae* into healthcare settings and systems. When patients are infected or colonized with carbapenemase-producing *Enterobacteriaceae* are transferred across borders, the risk of CRE being introduced and spread into healthcare facilities in the country of destination is increased. The risk is higher when patients are transferred from areas with high rates of CRE to healthcare facilities in another country, or if such patients have received medical care abroad in areas with high rates of CRE [39]. Cross-border transmission has been reported in Asia, Europe and North America involving carbapenemase producing *K. pneumoniae*.

Infection control measures

Carbapenemase - producing *Enterobacteriaceae* can colonize and infect not only patients who are debilitated, immunocompromised or critically ill, but also that patients that were previously healthy and became colonized or infected in healthcare settings that practice poor infection control [39]. CREs and other ESBL producing organisms can easily spread within the hospital environment. Preventing spread of these organisms from patient to patient is the main focus of infection control. A major issue is hand hygiene for healthcare professionals. The cleaning of medical equipment and prevention of colonization of the hospital environment are also important infection control measures. It is important to screen patients being admitted or transferred from other institutions including from nursing homes. Surveillance of infected and high risk patients is an important action for monitoring an outbreak and also to prevent one [40-42]. Small hospital outbreaks tend to be caused by a single clone and usually occur in high risk areas such as the ICU, neonatal units and hematology-oncology units. Large outbreaks usually involve several circulating strains of organisms at one time and in several different areas of a healthcare setting [43]. The prevention of the spread of carbapenemase producing pathogens relies on early detection [44]. Patients who undergo screening should include: (i) patients who were hospitalized while abroad and then transferred to another country; (ii) patients at general risk – intensive care patients, immunocompromised patients). Screened patients should be kept in strict isolation before obtaining results of the screening (at least 24 to 48 hours). Because the reservoir of carbapenemase producers remains in the intestinal flora, use of rectal swabs for screening are adequate to perform this screening measure- and then plated directly onto screening media. After this screening procedure, carbapenemase producers may be identified through a



variety of techniques including antibacterial drug susceptibility testing and molecular & PCR based techniques [22,29].

CRE – Extent of the Problem (USA and International)

The economic burden of CRE

A study done by several medical schools in the USA in 2016 (i.e. Johns Hopkin School of Public Health; UCLA Medical Center; Torrance Memorial Medical Center; Univ. CA Irvine Medical School) developed a CRE clinical and economic outcomes model using Monte Carlo type simulations to determine the cost of CRE infection from the hospital, third-party payer and societal perspectives to evaluate the economic burden of CRE to the USA [41]. Depending on the infection type, the median cost for a single CRE infection can range from \$22,484 to \$66,031 for hospitals, \$10,440 to \$31,621 for third-party payers, and \$37,778 to \$83,512 for societal costs (greater mortality and reduced productivity). An incidence of 15 infections per 100,000 population would cost hospitals \$1.2 billion, third-party payers \$0.8 billion, and societal costs of \$2.4 billion per year [41]. CRE infections are more costly than episodes of other infectious diseases. For example, in 2016 values, the costs for the following infectious disease to society are as follows: (i) one influenza case is \$2,807 to \$8,889; (ii) one pertussis case is \$600 to \$1,169; (iii) one food borne salmonella case is \$3,899 [41].

Increasing global prevalence of CRE

The first Carbapenemase-resistant *Enterobacteriaceae* (CRE) - K pneumoniae- was identified in North Carolina in the 1990s. Since then, CRE have spread globally and are endemic in some countries, including USA, Italy, Greece, Israel, China and India among others. Infections with CRE are being increasingly reported from healthcare facilities in the developed world, and with a higher prevalence over the past five years. Moreover, the presence of CRE infections are being increasingly reported in LMI countries as well. For example, in U.S. hospitals 11% of K pneumoniae and 2% of E. coli were resistant to carbapenems in 2013. As a comparison, in India 13% of E. coli were resistant to carbapenems in 2012 and 57% of K pneumoniae were resistant to carbapenems in 2014 [36,45].

Global reporting of extended spectrum β -lactamase (ESBL) producing strains:

ESBLs can inactivate all penicillins and cephalosporins, including third generation cephalosporins and monobactams. In Europe, 17 of 22 countries reported that 85% to 100% of E.coli isolates were ESBL positive. In the USA, healthcare-associated ESBL-producing *Enterobacteriaceae* made up 14% of E. coli isolates and 23% of K. Pneumoniae isolates. ESBL-producing

Enterobacteriaceae are increasing in Asia. In China in 2011, ESBL-producing E. coli accounted for 71% of E. coli isolates, and more than 50% of K. pneumoniae strains produced ESBL. In Latin America, ESBL-producing *Enterobacteriaceae* prevalence is rising. Rates of ESBL in E. coli in Mexico were as high as 41% in 2009. In 2014, resistance of K. pneumoniae isolates to third-generation cephalosporins ranged from 19% in Peru to 87% in Bolivia. In North Africa, ESBL prevalence ranged from 12 to 99% in hospitals and from 1 to 11% in communities.

Global reporting of CRE

Surveys by WHO (2014) & CCDEP (2015): Countries surveyed submitted at least 30 isolates showing resistance to at least one carbapenem. Those countries reporting CRE K. pneumoniae in the range of 50% to 61% included: India, Pakistan, Bangladesh and Greece. Those countries reporting CRE K pneumoniae in the range of 20.1% to 49.9% included: Vietnam, Iran, Romania, Israel, Guatemala, and Nicaragua. Those countries reporting CRE K. pneumoniae in the range of 6% to 20% included: China, Burma, Serbia, Argentina, Ecuador, Colombia, Venezuela, and USA [31].

CRE prevalence in Europe

A multi country survey was carried out in Europe in 2012 with 33 countries reporting data [46,47]. The prevalence of CRE is variable across Europe, but is higher in Greece and Italy and lower in the Nordic countries. It was found that K pneumoniae was the most prevalent type of *Enterobacteriaceae* species harboring CRE in those countries. The five most common CRE among *Enterobacteriaceae* reported by these countries are the metallo- β -lactamases IMP, VIM, NDM- from Amber Molecular Class B; OXA-48 and its derivatives from Amber Molecular Class D; KPC from Molecular Class A [29]. Overall, KPC producing *Enterobacteriaceae* are the most frequently detected among CRE in Europe. The major concern with OXA carbapenemases is their ability to rapidly mutate and expand their spectrum of activity. In the United States, >50% of A. baumannii are resistant to carbapenems due to production of OXA class carbapenemases [48]. It appears that prevalence of CRAB in Europe is under reported. This is because surveillance and reporting of CRAB are not performed routinely and there are fewer reference laboratories for CRAB in Europe.

CRE – Problem: Hospital Overuse of Antibiotics

General considerations

There is a causal association between use of antimicrobial drugs and the emergence of AMR. Changes in antibiotic use are also paralleled by changes in the prevalence of resistance. AMR is more prevalent in healthcare associated bacterial infections than



with community acquired infections. Patients with healthcare associated infections caused by resistant strains are more likely than control patients to have received prior antibiotic treatment within the hospital. Those patients with the highest rates of resistant strains also have the longest duration of exposure to antibiotics. The longer exposure period increases the likelihood of colonization with resistant organisms [13,30,41]

Overuse of broad spectrum antibiotics

It is the practice in hospitals to give patients broad spectrum antibiotics, even when a specific pathogen is identified. Such practices contribute to the spread of resistant strains to many non-target organisms. In a survey of 605 hospitals in the USA during 2009/2010, only 59% of patients received appropriate antibiotic treatment. In this survey, during the 5th day of therapy, 6% of antibiotic treatments regimens were unchanged despite there being negative bacterial cultures in 58% of the patients on therapy. The survey indicated that broad spectrum antibiotics were commonly prescribed to patients even when the signs of infection were not present – including negative cultures – and the antibiotic treatment was not discontinued. Another survey done in 2010 of 323 hospitals in the USA 56% of patients received an antibiotic during their hospital stay, and primarily with broad spectrum antibiotics. Among the patients receiving antibiotics, 37% of antibiotic treatments given were judged as needing improvement. The use of diagnostic tests in these cases would have resulted in more appropriate antibiotic regimen to those patients. The trend for antibiotic usage in U.S. hospitals is illustrated (Table 4). The rates of inappropriate prescribing of antibiotics is also common in international hospitals. For example, In Vietnam, it was found that ½ of hospital prescriptions for antibiotics were inappropriate [48-50].

Antibiotic use for surgery prophylaxis

In high income countries, pre surgical antibiotics are standard treatment to prevent post-surgical infection. However, in many LMICs (Low & Middle Income countries) antibiotics are commonly given after surgical procedures. This practice presents a higher risk of surgical site infections. It is found that LMIC hospitals used seven times more antibiotics when they are given post-surgery rather than pre surgery [49]. This practice increases costs and also increases AMR potential. Even when antibiotics are given before surgery, the regimen or duration of the treatment may be suboptimal. A survey of hospitals in India [51] showed a range of 19% to 86% of patients received inappropriate antibiotic prophylaxis. Proper antibiotic prophylaxis improves both hygiene, better surgical techniques and lowers the rate of surgical site infections.

Suboptimal use of antibiotics in hospitals

The suboptimal use of broad spectrum antibiotics and also suboptimal use of post-surgical antibiotics is prevalent both in North America, Europe and the LMIC, and so represents an opportunity to improve antibiotic use in hospital settings. Estimates have been made that a range of 20% to 50% of total antibiotic use in hospital settings are inappropriate [50,52]. Inappropriate antibiotic use includes: (i) Use of antibiotics when there is no benefit from its use, such as treating urinary tract infections caused by virus and (ii) incorrect antibiotic selection, incorrect dosage, incorrect duration of treatment the patient receives.

CRE – Problem: Outpatient and Nursing Home Overuse of Antibiotics

Antibiotic use in the community

Overuse and misuse of antibiotics in outpatient settings is a major driver of AMR. An estimated 80% of all antibiotics are consumed outside of hospitals in outpatient settings [49]. These healthcare settings include: i) self-medication; (ii) outpatient clinics and private physician offices; (iii) nursing homes.

Antibiotic overuse by self-medication

Outpatient use also includes antibiotics purchased by consumers directly and without a prescription. Although most countries require a prescription as condition for purchase of an antibiotic, these regulations are not enforced in most LMIC, or do not exist. Nonprescription use of antibiotics can range from 19% to over 90% outside of the USA and Europe. For example, in rural and urban pharmacies in Vietnam, 88% to 91% of all antibiotic sales in a survey made were without a prescription. Similarly, most antibiotics sales in Saudi Arabia (78%) and Syria (87% to 97%) were dispensed without a prescription [49].

Antibiotic overuse by prescribers

Healthcare providers also play a role in driving inappropriate antibiotic use in the community. They routinely prescribe antibiotics for infections that are not caused by bacteria. A major factor for overprescribing is the pressure that patients put onto prescribers for an antibiotic prescription. In a survey done in the USA, 48% of respondents indicated that they expected an antibiotic when they visited a doctor [49]. In a survey done in France, 50% of interviewees expected an antibiotic for treatment of influenza like illness [49]. Other factors leading to high rates of antibiotic prescriptions by prescribers include diagnostic uncertainty. Since most diagnostic lab methods are based on the culture of pathogens that require 36 to 48 hours to provide results, few infections are accurately diagnosed in these practice settings. In the absence of a clear diagnosis, the prescriber often feels pressured to prescribe antibiotics to be on the safe side or to



prevent secondary bacterial infections [49,53]. A recent study commissioned by the Pew Charitable Trusts in May 2016 [54] found that 13% of all out patient office visits in the USA (154 million visits/year) resulted in an antibiotic prescription. About 31% of these antibiotic prescriptions (47 million) are considered to be unnecessary. The Pew study also found that 44% of outpatient antibiotic prescriptions (68 million) were written to treat patients with acute respiratory conditions (including: sinus infections, middle ear infections, pharyngitis, viral upper respiratory tract infections -common cold; bronchitis; bronchiolitis; asthma; allergies; influenza and pneumonia). Half of these prescriptions are unnecessary because many of these conditions are viral illnesses or other conditions that do not respond to antibiotics. In addition to using medications that will not affect the illness, such improper use of antibiotics also carries the risk of adverse drug effects and other side effects. It is estimated that improper use of antibiotics in the USA results in 140,000 emergency room visits per year [54]. (Table 5) compares the retail sales of Carbapenem antibiotics in selected countries for the period of 2005 to 2010 and showing the growth in antibiotic sales increase during that period [13,55-57].

Antibiotic overuse in nursing homes

In the USA, 1.6 million people live in nursing homes. Out of that population, about 250,000 people will acquire infections every year. And out of that population, nursing home residents will acquire 27,000 antibiotic resistant infections. It is further estimated that up to 70% of nursing home residents are prescribed an antibiotic every year. And of these antibiotic prescriptions, from 40% to 75% are prescribed incorrectly – either being unnecessary or else incorrectly for the drug prescribed, or the dose, or the duration of therapy [58]. Of particular concern is the widespread use of broad spectrum oral antibiotics such as quinolones for which overuse can be a major driver of AMR. Nursing home residents are more vulnerable to infections because of biological factors (reduced immune system, prevalence of chronic diseases, use of invasive devices such as urinary catheters and feeding tubes) and environmental (crowding, sanitation issues). Nursing homes are being increasingly identified as important reservoirs for the development of multidrug-resistant (MDR) organisms and their transmission into the community. The three most frequently reported infections in nursing homes are: Urinary tract infections (UTI), Respiratory tract infections (RTI) and Skin and soft tissue infections (SSTI) [58]. These same groups of infections are the leading causes for antibiotic prescribing in nursing homes. Some studies also report that UTIs and RTIs are the most commonly observed causes for hospital admissions among the elderly from nursing homes. Overuse of antibiotics in nursing homes not only produces UTI and RTI that are resistance to antibiotic treatment, but are also a major cause of

hospital admissions of nursing home residents. Studies have demonstrated that nursing homes are a reservoir for carriage of three major groups of MDR organisms (i.e. MRSA, VRE and MDR GNB (Gram-negative bacteria). However in recent years there has been a shift towards greater colonization with MDR GNB. Some studies show MDR GNB colonization far exceeding that of MRSA and VRE. It has been found that prior exposure to antibiotics is a prominent risk factor associating with both colonization and infection of both MDR gram-positive and gram-negative organisms [39,41,58]. Moreover, repeated antimicrobial utilization, and particularly the repeated use of broad-spectrum antibiotics will increase the risk of *Clostridium difficile* infection – a leading cause of hospitalization of nursing home residents [58].

Areas of potential antibiotic misuse in nursing homes

Major areas of potential antibiotic misuse in nursing home that lead to development of AMR are listed below [58-63].

- **Prophylactic antibiotics for UTI:** There is little evidence to support the use of long-term urinary prophylaxis. However, there is strong evidence showing that prolonged antibiotic use in the absence of infection will always select for resistant organisms.
- **Empiric prescribing without microbiological investigation:** Causative etiologic agents should be identified through microbiological testing, especially for symptomatic UTIs, to guide the adjustment of empiric antibiotic therapy.
- **Treatment of asymptomatic bacteriuria:** This condition is common with chronically cauterized patients. However, antibiotic treatment will not prevent recurring bacteriuria or symptomatic infections. The right strategy is to change indwelling catheters prior to starting antibiotic therapy and taking a urine sample collected from the newly placed catheter. Discontinuation of catheter use and proper aseptic technique on catheter changing are the keys to preventing UTIs and other urinary complications [58].
- **Widespread prescribing for upper RTIs or acute bronchitis:** Among elderly nursing home residents, upper RTIs are usually caused by viral pathogens. Therefore, empiric antibiotic treatment is both unnecessary and ineffective. It is necessary to differentiate between bacterial or viral origin to reduce inappropriate use of ABS to treat RTS.
- **Prolonged duration of antibiotic treatment:** It is generally considered that antibiotic courses of 7 days or less are as effective as longer treatment duration for the majority of common bacterial infections. In contrast, unnecessary



prolonged antibiotic treatment increases patient risk for side effects and AMR.

- **Widespread prescribing of quinolones as empiric treatment for UTIs:** The quinolone antibiotics are frequently used to treat both low and complicated UTI because of their high bioavailability, long half-life, and broad-spectrum activity spectrum. Consequently a high rate of quinolone-resistant gram negative organisms is often observed in nursing homes with a high use of quinolones [58-108].

CRE-Solution: Prudent Use of Antibiotics in Healthcare

General considerations

The consensus of experts is that prior use of all antimicrobial products, and more specifically the carbapenems, 3rd and 4th generation cephalosporins and fluorquinolones increases the risk of infection or colonization with CRE [79]. High rates of multi drug resistant organisms (MDROs, ESBL producing bacteria are considered to be independent risk factors for the spread of carbapenemase resistant mechanisms. The intensity and duration of antibiotic treatment are the most important variables for the selection of CRE producing bacteria [109]. A review of the medical literature indicates there is an important relationship between prior antimicrobial therapy and the subsequent identification of carbapenemase-producing bacteria. In a four year case control study of 102 patients, the only covariate independently associated with CRE in all multivariate analyses was the cumulative number of prior antibiotic exposures [109,110]. In another case-control study performed in Greece and lasting 26 months (96 ESBL-carbapenem-resistant *K. pneumoniae* and 55 ESBL-carbapenem-sensitive *K. pneumoniae*) identified the key risks factors as: (i) prior cumulative exposure to antibiotics and (ii) increasing duration of prior antibiotic treatment [109] [111]. The antibiotic treatments covered in these studies included use of a β -lactam or β -lactamase inhibitor or a combination of fluoroquinolone and carbapenem. The most important variables responsible for selection of carbapenemase – producing bacteria are the intensity and duration of antibiotic therapy [109]. Controlling the spread of CRE infections requires both the prudent use of antibiotics plus infection control. Additional clinical studies suggest that the patient's cumulative exposure history is likely to be a greater risk factor than any one specific antibiotic exposure to determine a patient's chance of acquiring a resistant pathogen [112].

Reducing overuse of antibiotics

From 20% to 50% of all antibiotic use is estimated to be inappropriate [110], covering both (i) the use of antibiotics when

there is no possible health benefit, such as treating upper respiratory tract infections caused by virus origin, and (ii) the suboptimal use of antibiotics such as incorrect choice of prescribed drug (i.e.- unnecessary broad spectrum antibiotic), incorrect dosage or duration, or poor patient adherence, substandard quality of antibiotic product – all of these factors contribute to the development and spread of AMR. It has been found in Europe that regional and national campaigns to educate healthcare workers and patients about the danger of AMR can help change behavior to reduce inappropriate prescribing. Two such effective campaigns took place in Belgium and France [49]. Prior to this national education/awareness campaign, both countries had the highest rate of antibiotic consumption in Europe. The French campaign started in 2001 and achieved a reduction of 27% for antibiotic prescribing over five years. The Belgian national media campaign achieved a 36% reduction in antibiotic prescriptions over a seven year period. Both countries also showed a corresponding reduction in antibiotic resistant pneumococci following these campaigns.

Prudent use recommendations

- **Rapid diagnostics:** Patients with sepsis must be treated quickly, but empirical antibiotic treatment is often started before reliable microbiology assessments are available. This results in some patients receiving incorrect antibiotic treatment. More rapid diagnostics are needed so that the appropriate antibiotic treatment can be administered more quickly and using broad spectrum antibiotics for the shortest duration to reduce selection pressure for resistance [111,112].
- **Colonization prevention:** The prevention of colonization can have considerable benefit in the era of CRE infections. Hospitals should take rectal samples of patients at risk. If the isolates are KPC positive, the patient should be isolated in a single room and bathed daily with chlorhexidine gluconate
- **Heterogeneity of antibiotic usage:** Patterns of antibiotic use in which the same antibiotic given is given repeatedly (homogeneity) is associated with higher rates of resistance than when there is variability in antibiotics prescribed for the patients (heterogeneity). It has been shown that monthly cycling of four antibiotics (PTZ, imipenem/cilistin, ceftazidime, ciprofloxacin) as the primary antibiotic to treat suspected Gram-negative infections was associated with an overall improvement in the antibiotic susceptibility profile of Gram – negative organisms compared with the medical ICU in the same hospital where cycling was not performed. These results suggest that increased diversity of prescribing may correlate with reduced levels of resistance.

The avoidable costs of antibiotic misuse



In addition to the rise of antimicrobial resistance (AMR), the misuse of antibiotics for medical applications represents large avoidable healthcare costs in the USA \$35 billion/year [113]. Within that amount, most of that amount (\$23 billion) is incurred through inpatient care (hospital use). The avoidable costs are defined as the added cost of treating a patient with an antibiotic resistant infection relative to a patient with an antibiotic susceptible infection. The estimated avoidable costs for the inpatient setting include longer medical treatments, expensive second-and third-line antibiotic therapies, and screening and diagnostics to detect and prevent the spread of bacterial strains. The cost of excessive antibiotics prescribed in outpatient settings in the USA is \$1 billion/year [113].

CRE-Solution: Antibiotic Stewardship Programs

Basis for antimicrobial stewardship (AMS) program

An AMS program is a set of coordinated strategies to optimize the use of antimicrobial medications in order to improve patient safety and outcomes, decrease and prevent the development of antimicrobial resistance, and decrease costs [114]. Recent data [115] suggests that up to 55% of all hospital patients in the USA receive an antibiotic, and that between 30% to 50% of the prescribed antibiotics may not be appropriate. The inappropriate prescribing of antibiotics leads to potential complications and adverse drug events. It also may cause hospital acquired infections. Comprehensive AMS programs have been shown to decrease antibiotic use by 22% to 36% and halt the development and spread of antimicrobial resistant bacteria [115]. Core members of a multidisciplinary AMS team may include: (i) Infectious disease physician; (ii) Clinical pharmacist with infectious disease training; (iii) Clinical microbiologist; (iv) Information system specialist; (v) Infection control professional; and (vi) Hospital epidemiologist. The CDC reported that in 2014 nationally, 39.2% of all hospitals had AMS programs (1,642 out of 4,184 hospitals). The national goal is for 100% of hospitals to have an AMS program by 2020.

The Centers for Medicare and Medicaid Services (CMS) will require hospitals to reduce antibiotic use starting in January 2017 [116].

Core strategies for an AMS program

There are two core strategies that provide the foundation for an AMS program. These strategies are not mutually exclusive [117].

- **Prospective audit with intervention and feedback strategy:** This strategy involves a prospective audit of antimicrobial use with direct interaction and feedback to the prescriber, performed by either an infectious disease physician or a clinical pharmacist. This strategy can result in both reduced inappropriate use of antibiotics and also the

improved use of antibiotics. Effective auditing with intervention and feedback can be facilitated through computer surveillance of antimicrobial use. .

- **Formulary restriction and preauthorization strategy:** Formulary and preauthorization requirements can lead to immediate and significant reductions in antimicrobial use and cost, and may be beneficial as part of a multifaceted response to a nosocomial outbreak of infection. The use of preauthorization requirements as a means of controlling AMR is less clear, because a long-term beneficial impact on resistance has not yet been established. And in some circumstances, this policy may just shift to using an alternative agent with resulting increased resistance. Preauthorization use policy requires monitoring overall trends in antibiotic use in order to assess and respond to such shifts in use. The effectiveness of a preauthorization process depends on who is making the recommendations. One example is a study [118] done in a hospital that experienced an increasing incidence of cephalosporin resistant *Klebsiella*. A preapproval policy was implemented for cephalosporins, resulting in an 80% reduction in hospital-wide cephalosporin use and a subsequent 44% reduction in the incidence of ceftazidime-resistant *Klebsiella* throughout the medical center.

CDC's core elements of antibiotic stewardship programs

In 2014, CDC recommended that all acute care hospitals implement AMS programs. The CDC published seven core elements of successful hospital antibiotic stewardship programs. These elements include the pharmacist's role in antibiotic stewardship [119]. The CDC core elements are the following:

- **Leadership Commitment:** Dedicating necessary human, financial and information
- **Accountability:** Appointing a single leader responsible for program outcomes. Experience with a successful programs show that a physician leader is effective.
- **Drug Expertise:** Appointing a single pharmacist leader responsible for working to improve antibiotic use.
- **Action:** Implementing at least one recommended action, such as systemic evaluation of ongoing treatment need after a set period of initial treatment (i.e; "antibiotic time out" after 48 hours)
- **Tracking:** Monitoring antibiotic prescribing and resistance patterns
- **Reporting:** Regular reporting information on antibiotic use and resistance to doctors, nurses and relevant staff
- **Education:** Educating clinicians about resistance and optimal prescribing.



Supplementary elements of an AMS program

The Infectious Disease Society of America (IDSA) and the Society for Healthcare Epidemiology of America have also recommend supplemental strategies [117]:

- **Antimicrobial cycling and scheduled antimicrobial switch:** “Antimicrobial cycling:” refers to the scheduled removal and substitution of a specific antimicrobial or antimicrobial class to prevent or reverse the development of AMR within an institution. In true cycling, there is a return to the original antibiotic after a defined time as opposed to a simple switch of antibiotics. Antimicrobial cycling is an attempt at controlled heterogeneity of antimicrobial use to minimize antimicrobial selection pressures. There is insufficient data to recommend the routine use of antimicrobial cycling as a means of preventing or reducing AMR over a prolonged period of time. Substituting one antibiotic for another may transiently decrease selection pressure and reduce resistance to the restricted agent. However unless the resistance determinant has been eliminated from the bacterial population, the reintroduction of the original antibiotic will likely select for the expression of the resistance determinant in the exposed bacterial population.
- **Combination therapy-prevention of resistance versus redundant antimicrobial coverage:** The rationale for combination therapy includes broad spectrum empirical therapy for serious infections, improved clinical outcomes and the prevention of resistance. These recommended situations include the use of empirical therapy for critically ill patients at risk of infection with multidrug resistant pathogens in order to increase the breadth of coverage and the likelihood of adequate initial therapy. However in many situations, combination therapy is redundant and unnecessary, and there is insufficient data to recommend the routine use of combination therapy to prevent the emergence of resistance.
- **Streamlining or de-escalation of therapy:** Efforts to optimize empirical initial antimicrobial therapy may conflict with good AMS to promote judicious use of antibiotics, because continuing excessively broad therapy contributes to the selection of AMR pathogens. This conflict can be resolved when culture results become available by streamlining or de-escalating antimicrobial therapy to more targeted therapy that decreases antimicrobial exposure and contains cost. The elimination of redundant combination therapy can result in reduced antimicrobial exposure and resistance.
- **Dose optimization:** Optimization of antimicrobial dosing based on individual patient characteristics, causative

organism, site of infection, and pharmacokinetic and pharmacodynamics characteristics of this drug is an important part of AMS.

- **Conversion from parenteral to oral therapy:** A systematic plan for parenteral to oral conversion of antimicrobials is important.
- **Coordination with microbiology laboratory:** The clinical microbiology laboratory plays a critical role in AMS by providing patient-specific culture and susceptibility data to optimize individual antimicrobial management and by assisting infection control efforts in the surveillance of resistant organisms and in the molecular epidemiologic investigation of outbreaks.
- **Monitoring of process and outcome measurements:** Both process measures (did the intervention result in the desired change in antimicrobial use?) and outcome measures (did the process implemented reduce or prevent resistance or other unintended consequences of antimicrobial use?) are useful in determining the impact of AMS on antimicrobial use and resistance patterns [118-124].

CRE Conclusion

Antimicrobial resistance (AMR) among gram-negative bacteria has reached critical levels on a global basis. The rise of carbapenemase resistance in Enterobacteriaceae carrying additional resistance genes for multiple antibiotic classes has created a phalanx of organisms that are resistant to all available antimicrobial treatment [122]. Carbapenem Resistant Enterobacteriaceae (CRE) infections are known to be associated with significant morbidity and mortality. Some experts have suggested that the global population will face two concomitant and worldwide epidemics of carbapenemase producers [43]. The first epidemic will cover carbapenemase producers as a source of community acquired infections. The second epidemic will cover nosocomial carbapenemase producers in *K. pneumoniae* of all types. (KPC, IMP, VIM, NDM and OXA-48). For the first epidemic, community acquired infections for these carbapenemases are primarily of the NDM and OXA-48 types. In contrast to a viral epidemic (i.e. - pandemic H1N1 in 2009), an epidemic of carbapenemase producers cannot stop spontaneously. This is because there are multiple factors involved that favor propagation, including: lack of hygiene, overuse of antibiotics and increased global travel. In addition, there are many carbapenemase producing organisms that carry unrelated drug-resistance determinants resulting in their spread to both β -lactam antibiotics and to other antibiotic that are not structurally related to β -lactams. The actual prevalence of carbapenemase producers is not clear because many countries that are likely to be their main reservoirs have poor detection and reporting systems in place. For the second epidemic – nosocomial infections, the likely cause will



be carbapenemase producers in *K. Pneumoniae* of all types (KPC, IMP, VIM NDM1 and OXA-48). In certain countries, high rates of various types of carbapenemase producers already exist: Greece (VIM, & KPC) and the Indian subcontinent (NDM, KPC, and OXA-18). It is believed that *K. pneumoniae* will be a likely carrier of carbapenemase infections because it has been repeatedly cultured in the most common Enterobacteriaceae species for spreading ESBL genes in healthcare facilities during the past 30 years. It is therefore expected that *K. Pneumoniae* will be the most likely carbapenemase producer found in patients with identical risk factors for ESBL infections: It is therefore an urgent task for early identification of carbapenemase producers (CRE) in clinical infections at the carriage stage – in order to prevent the development of hospital outbreaks of infection [43]. The lack of new antibacterial drugs in the development pipeline of pharmaceutical companies is a serious global problem. Carbapenemase producers in Enterobacteriaceae are different from other multi-drug resistant bacteria because they are susceptible to few if any antibiotics. Therefore, it is essential that prudent use of antibiotics in clinical and outpatient practice settings be done in order to preserve the therapeutic efficacy of the existing arsenal of antibiotics for as long as possible [11]. Fundamental changes are required in the application of antibiotics for both medical and non- medical applications. A recent study by the British Govt [11] has recommended the following steps to reduce antibiotic demand and consumption:

- A massive global public awareness campaign to cover both patients and farmers to reduce their demand; and prescribers and clinicians to not prescribe antibiotics when they are not needed.
- Improve hygiene and prevent the spread of infection. This effort includes improvement in access to clean water and better sanitation in developing countries, and reducing infections in healthcare settings in all countries.
- Reduce unnecessary use of antibiotics in agriculture and their dissemination into the environment.
- Improve global surveillance of drug resistance and antimicrobial consumption in humans and animals. While surveillance is a cornerstone of infectious disease management, it has been under-resourced in the effort to reduce AMR.
- Promote new rapid diagnostics to reduce unnecessary use of antibiotics.
- Promote the development and use of vaccines as alternatives. By reducing and preventing infections, demand for therapeutic treatments and also antibiotic use will be reduced. These steps are expected to slow the development and spread of AMR.

- Increase the number of effective antimicrobial drugs to defeat infections that have become resistant to existing medicines.

In conclusion, the world faces serious global public health problems from a post antibiotic world. The problem of AMR and CRE infections in particular put a high economic burden onto the healthcare system. The cost of CRE infection is higher than the annual cost of chronic diseases and of many acute diseases. Costs rise proportionately with the incidence of CRE infection [41]. Many of the drivers of AMR have a common origin in inappropriate use of antimicrobials in both human and animal health care or in agriculture or from environmental contamination. From a more immediate tactical view, key steps to prevent the establishment of CRE are early detection through good diagnostic practices and containment of spread through patient and contact screening as well as infection prevention and control measures including antibiotic stewardship [47].

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