

University of Nevada, Reno

**Threat of Carbapenem Resistant Enterobacteriaceae and the Rise of
Antibiotic Resistance**

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Abstract

Antibiotic resistance is no new phenomenon. Evidenced by the presence of intrinsic resistance, microbes have had mechanisms to subvert antimicrobials for a very long time. Improper and poor use of antibiotics has further exacerbated the prevalence of antibiotic resistance in the microbial world, posing a significant threat to human health. Among these antibiotic resistant microbes, Carbapenem Resistant *Enterobacteriaceae* (CRE) are of particular concern. CRE infections have a relative mortality rate of 50% (Guh et al., 2013). While containing Carbapenem inactivating enzymes, horizontal gene transfer is a powerful force influencing virulence of CRE. Several other factors have led to the current, dire state of antibiotic resistance: frequent and improper use of antibiotics, minimal understanding of fundamental microbiological principles, and lack of focus on unification under a common purpose. International impact necessitates a multifaceted approach focusing on cooperative, and unified efforts.

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Introduction

Antibiotics have been an essential tool for fighting bacterial infections ever since Sir Alexander Fleming discovered the first modern antibiotic, *penicillin*, in 1928 (Ventola, 2015). Over the last sixty years, since the discovery of antibiotics, millions of lives have been saved as a result (Ventola, 2015). Despite these advancements in combating bacterial infections, bacteria have evolved to thwart the effects of antibiotics. Antibiotic resistance is not a new problem (Bhullar, 2012). However, this threat towards humanity is increasing as these drugs are prescribed more frequently (Ventola, 2015).

Gram negative organisms are of large significance in the medical field and a focus for controlling the spread of disease (Logan and Weinstein, 2017). Gram negative organisms are often resistant to antibiotics through the use of a β -lactamase, thus rendering antibiotics containing β -lactams useless (Logan and Weinstein, 2017). Plasmids further complicate the problem because they can contain the genes responsible for the microbe's antibiotic resistance and can migrate to other bacteria, transferring antibiotic resistance (Logan and Weinstein, 2017). *Enterobacteriaceae* are within the classification of Gram-negative organisms. Among the family of *Enterobacteriaceae*, the most notable are those that have developed a resistance to the Carbapenem class of

antibiotics (Logan and Weinstein, 2017). For years, Carbapenems were reserved as a last line of defense against persistent bacterial infections. However, certain microbes have become resistant to this class of antibiotics over the last couple of decades (Logan and Weinstein, 2017).

In a world where many are encouraged to self medicate with over-the-counter medicine, we have elevated the risk of overuse. This mentality rooted in a desire for a healthy lifestyle reflects a misunderstanding deeply ingrained in popular culture (Michael et al., 2014). This paper is focused on the problem of antibiotic resistance in *Carbapenem Resistant Enterobacteriaceae* and the risks they pose to world health. Creating more antibiotics to reverse the antibiotic resistance problem is not the most effective strategy. Instead it requires a multifaceted approach which spans political borders through a combined effort in order to slow the progression of antibiotic resistance.

Development of Antibiotic Resistance

Antibiotic resistance is often thought of as a new phenomenon, but research shows that development of antibiotic resistance genes has been a trend throughout history (Bhullar, 2012). This research showed that

bacterial isolates, from a cave thought to have little human interference, were resistant to a wide range of frequently used current antibiotics (Bhullar, 2012). This provided evidence for evolutionary selective pressures, revealing that antibacterials are no new human invention. This study discusses intrinsic resistance which is naturally acquired, chromosomally encoded and highly species specific (Sengupta, 2013). While antibiotic resistance is no new phenomenon, it is clear that the prevalence of antibiotic resistant microbes is on the rise, resulting from human actions or factors (Charani, 2010). In the past years, an increased prevalence in the number of microbial species with antibiotic resistant genes has been significant (Sengupta, 2013). There are a number of factors contributing to this surge: incorrect usage and prescribing of antibiotics, lack of proper sanitation and other factors, which will be discussed later.

In order to understand the problem, we must broadly understand the possible mechanisms underlying its development. A bacterial genome, which includes all genes encoded by the bacterial DNA sequences, can encode resistance genes (Nordmann et al., 2011). Plasmid encoded antibiotic resistance genes is another common way these genes can be introduced into the bacterial chromosome. This is different from the genome encoding it naturally or as a result from mutations because the

plasmid horizontally transfers the gene into the bacterial chromosome. Horizontal transfer is a general term for any gene which is acquired by transfer from another microbe. This does not include acquisition through bacterial replication. Along with plasmids, genes can be horizontally transferred via conjugation, transformation of bacteria from the environment, transduction mediated by bacteriophage, transposons, integrons and recombination. Plasmid encoded antibiotic resistance genes and naturally occurring resistance genes are found to be two common mechanisms for the existence of antibiotic resistance in certain species of *Carbapenem Resistant Enterobacteriaceae* (Nordmann et al., 2011). The proteins encoded are often enzymes which hydrolyze β -lactams present in the carbapenem class (Nordmann et al., 2011). By hydrolyzing these β -lactams, these enzymes render the antibiotic useless against the bacteria.

Carbapenem Resistant Enterobacteriaceae

In 2013, the US Centers for Disease Control and Prevention declared that we are now in the “post-antibiotic era” (Michael et al., 2014). Within the same year, the CDC first published a report on antimicrobial resistance and associated dangers. Due to the significant increased prevalence of antibiotic resistance, the CDC released an updated report in

2019. In this new report, Carbapenem Resistant *Enterobacteriaceae* (CRE) were listed in the top five urgent threats (Antibiotic resistance threats in the United States, 2019). This is alarming as the carbapenem class of antibiotics has historically been reserved as a last resort. With resistance to this class of antibiotics growing and spreading, it is necessary to develop a better understanding of these microorganisms considered to be CRE.

Enterobacteriaceae are microbes which colonize the human gastrointestinal tract, such as *Klebsiella pneumoniae* (Nordmann et al., 2011). Among all the pathogens included in the distinction of *Enterobacteriaceae*, each pathogen has different mechanisms of causing disease. Most of these pathogens can lead to more severe complications such as systemic sepsis, peritonitis, and pneumonia (Nordmann et al., 2011). The mechanism of disease is not of concern as much as the severity of the illness caused by the pathogen in regards to antibiotic resistance. CRE have devastating effects on those infected, with severe complications leading to death.

CRE Resistance Mechanism

As previously mentioned, carbapenems were historically used last until specific CRE isolates developed different mechanisms to thwart their effectiveness. The first carbapenemase was found in an

Enterobacteriaceae isolate in 1993 (Nordmann et al., 2011). CRE have developed β -lactamases which hydrolyse the lactam ring in β -lactams (Nordmann et al., 2011). There are three classes of carbapenamases. They are the Ambler class A, B, and D β -lactamases (Nordmann et al., 2011). The carbapenamases are found more commonly in *K. pneumoniae* isolates compared to *E. coli* (Nordmann et al., 2011).

The carbapenamases vary slightly among each other. Class A Carbapenamases can be encoded by a plasmid (*Klebsiella pneumoniae* carbapenamases or KPC) or by the bacterial chromosome (Nordmann et al., 2011). All Class A carbapenamases hydrolyze carbapenems (Nordmann et al., 2011). These carbapenamases are ineffective in the presence of clavulanic acid (Nordmann et al., 2011). About a 50% mortality rate is associated with KPC infections (Nordmann et al., 2011).

Class B metallo- β -lactamases (MBLs) are found around the world and are common in Greece, Taiwan and Japan (Nordmann et al., 2011). All β -lactams can be hydrolyzed by this class of lactamases, excluding aztreonam (Nordmann et al., 2011). Similar to the KPC associated mortality percentage, around 18%-67% of patients infected with bacteria containing MBL's result in death (Nordmann et al., 2011). Most notable

among the MBL's is NDM-1 which is found everywhere except in Central and South America (Nordmann et al., 2011). This protein is unique because it is often found on plasmids that have more than one resistance gene (Nordmann, 2011).

Lastly, Class D Enzymes of the OXA-48 Type are encoded on a plasmid (Nordmann et al., 2011). This was first isolated in Turkey being identified as the cause of several hospital acquired infections (Nordmann et al., 2011).

Isolates containing these virulence factors, factors contributing to effectiveness of causing disease, are more commonly isolated in hospital settings compared to community acquisition (Nordmann et al., 2011). This could be associated with the higher prevalence of selective pressures present in a hospital setting including sanitization, sterilization and antibiotic regimens. Selective pressures are forces exerted on a microbe by its environment which influence evolutionary changes. Mutations are a natural process within the bacterial life cycle, and certain mutations are favored over others based on environmental factors. (Michael et al., 2014). The bacteria do not create these mutations as a result of the environmental needs, but stemming from the rapid doubling time and high number of bacterial generations, mutations arise, and the best adapted mutants survive (Michael et al, 2014).

Mutations are not solely responsible for the presence of new phenotypes. Horizontal gene transfer is a common mechanism by which *Enterobacteriaceae* gain resistance to carbapenems (Nordmann et al., 2011). Both plasmids and integrases are involved in horizontal gene transfer among CRE (Nordmann et al., 2011). Genes can be acquired from the same species of the target bacteria, or even other bacterial species present within the environment (Michael et al., 2014). Selective pressures also play a role in horizontal gene transfer because they can stress the microbe (Michael et al., 2014). Stress in many bacterial species can increase the transference of genes through horizontal gene transfer (Michael et al., 2014). Many of the genes transferred often favor the survival of the bacteria at the expense of human health. Antibiotics have been our first and last line of defense ever since clinical introduction in the 1900's (Michael et al, 2014). Before this, less effective measures like debridement were used (Michael et al, 2014). Professionals may need to start reverting back to these methods in an attempt to curb *Enterobacteriaceae* Carbapenem resistant infections (Michael et al., 2014).

CRE are currently identified using Polymerase Chain Reaction (PCR) based techniques (Nordmann et al., 2011). Results can be seen within a period of 4-6 hours, allowing for relatively quick identification (Nordmann et al., 2011). While this is a relatively short period of time, a patient's condition could become critical within this time. Along with PCR tests being expensive, technical and long, rapid tests are going to be a necessity in the coming future to prevent rapid spread (Nordmann et al., 2011).

CRE also place a major burden on the economy. The cost varies, but on average it costs hospitals between \$22,484 to \$66,031 per patient infected by CRE, and \$1.4 billion for every 15 cases out of 100,000 (Bartsch et al., 2017). Society costs would amount to \$2.8 billion dollars for every 15 cases out of 100,00 (Bartsch et al., 2017). CRE are a major threat to the international healthcare system, economy and overall well being of the society.

Factors that lead to Development of the Problem

A host of possible factors have contributed to the problem we are facing now with CRE, leading the CDC to declare this group of organisms as the top priority to neutralize among antibiotic resistant pathogens (Antibiotic resistance threats in the United States, 2019). Antibiotics serve to limit the growth and spread of harmful bacteria in a given environment.

While it is beneficial to the human population to destroy these bacteria, the selective pressure placed on the bacteria poses a threat to humanity.

These selective pressures which come from an effort to ameliorate the problem posed by bacteria, inadvertently undo the progress made in the long run.

While antibiotics are extremely useful in limiting or stunting bacterial growth, more is not better. The rise in antibiotic resistance is attributed to the significant and prolonged practice of overprescription (Sengupta, 2013). Additionally, several factors are associated with the overuse of antibiotics.

Inadequate knowledge of Microbiological Principles

Two specialties within medicine are properly equipped with the tools to correctly identify a situation in which antibiotics are appropriate and prescribe a proper antibiotic regimen to treat the infection (Esmita, 2010). These are Infectious Disease physicians and Medical Microbiology physicians (Esmita, 2010). These specialties are not included among those with the highest numbers of prescribed antibiotics (Esmita, 2010). The majority of specialties do not have the adequate knowledge in microbiological principles to understand the double-edged sword of

antibiotics. On one edge of the sword, is the power to eradicate a large majority of pathogens propagating infectious disease worldwide. The sword seems to be unevenly weighted, favoring the other edge. Overprescription of antibiotics is leading to resistance to all classes of known antibiotics, leaving us empty handed, in a worse situation than we started.

To properly identify an infectious agent, tests are required to empirically determine a particular microbes role during infection (Michael et al., 2014). While this is the best approach, laboratory tests often take days to complete. While a number of cases are not emergencies, conversely many require urgent treatment (Michael et al., 2014). This often leads to a rushed prescription, not always of one, but several antibiotics. While this approach can work, it often does not and necessitates further antimicrobial intervention (Michael et al., 2014). Further antimicrobial intervention may successfully eradicate the targeted microbe, but these pharmaceuticals are nonspecific. Commensal bacteria in our gastrointestinal tract are directly affected. Scientific data shows the important role played in our physiological health by these gut microbes (Clemente et al., 2012). These microbes have been shown to affect our mood as well as metabolism and proper nutrient digestion (Huang et al., 2019). These findings suggest the important role microbes hold in our

overall health. A depletion of these beneficial bacteria commonly leads to gut dysbiosis, an imbalanced, chaotic state of gut health (Clemente et al., 2012). This depletion of healthy gut microbes allows non-native microbes to grow with lack of competition which prevent their growth under normal circumstances. *Clostridioides difficile* is a commonly seen infection in the hospital and is more prevalent in patients who have taken recurrent antibiotic (Clemente et al., 2012).

In non-emergency cases, doctors tend to prescribe based on what is commonly seen in their area and based on particular protocols (Esmita et al., 2010; Michael et al., 2014). These protocols are outdated and do not provide sufficient advice about prescribing for physicians (Esmita et al., 2010). With a majority of physicians lacking proper training in microbiological concepts and outdated, insufficient protocols in place, the question is not how we got here, but how did we let it get so out of control?

Lack of public education regarding proper use of antibiotics is a large contributor to the problem we face. Public ignorance to the gravity of improper use of antibiotics further aggravates this dire situation. The number of citizens worldwide largely outnumbers physicians,

microbiologists and epidemiologists. While some individuals understand the gravity of this situation, current systems in place have failed to convey this to the group which has the most power in decisions affecting the situation. Those privy to the knowledge of antibiotic resistance have far less weight when voting for policies that dictate how hospitals and pharmaceutical companies operate, directly affecting the situation. The masses have the most influence when voting for public health policies which can determine how our hospitals are run and who gets funding for new drugs. This is another way in which lack of public awareness has led to our current situation.

Antibiotics have been publicly deemed as the “silver bullet” to cure common ailments (Michael et al., 2014). While it is commonly understood among the scientific community that these drugs work to curb the negative effects of bacteria, the general public is ignorant to this simple fact, due to no fault of their own. The scientific community has made few efforts to educate the masses until recently, almost before it is too late. The idea that antibiotics can cure any ailment is comforting to the human psyche. This misconception could explain the general acceptance and popularity of this misconstrued understanding.

It is extremely difficult to erase incorrect information from the public mind, evidenced by the Wakefield article used by antivaxers to support

their decision to abstain from vaccinations. While this paper has been retracted and the science proven incorrect and invalid, the information still circulates in this age of scientific information. Incorrect information is extremely difficult to correct when people believe it is true. This is exacerbated by people spreading this information. For instance, if one individual becomes infected with bacteria and is prescribed antibiotics, they associate antibiotics to be used for the symptoms they were presenting. The problem arises when realizing that viral infections can present with similar symptoms. While scientists and some physicians know this, the individual thinks all cases with these symptoms need antibiotics to clear the infection. So, if they have a friend with the same or very similar symptoms, they will suggest they ask their doctor for antibiotics because that is what they received in a similar situation. This new patient then goes to the doctor demanding antibiotics, and is dissatisfied because they do not receive them. This then puts the doctor in a tricky situation because he is left to question whether his decision is correct, and even if it is, the patient may not be compliant with the non-antibiotic prescribed.

Agricultural Impact

The United States has a consumer base focused on eating quantity, not quality. In order to rise to these demands, meat and egg producers have moved from the classical free-range farming model to a more “efficient,” large production model. Cattle and swine have been moved from large pastures and pens to stables just big enough to fit the animal. Chickens are no longer in coops where they are allowed to roam during the day, but cooped up in tight confines. This move to closer quarters has created a breeding ground for bacteria, so to speak. Being in closer proximity to each other, animals have a higher risk of passing microbes along, and this is significantly amplified by the mass number housed in these close quarters. This large population of animals in one location can serve as a large reservoir of bacteria containing antibiotic resistant genes (Chang et al., 2014). As a solution, farmers administer antibiotics prophylactically to avoid high numbers of mortality from microbial infection (Chang et al., 2014). Along with preventing disease, antibiotics increase the body mass of these animals (Khachatourians, 1998).

Agriculturally used antibiotics are similar to antibiotics used in human treatment (Chang et al., 2014). When these zoonotic pathogens

develop antibiotic resistance to the antibiotics, the problem then arises if they cross the species barrier and develop the ability to infect humans (Chang et al., 2014). With frequent human-to-animal contact in close quarters on these farms, there is ample opportunity for these pathogens to jump into the human population (Chang et al., 2014). These bacteria which have developed resistance to agricultural antibiotics then can transfer these genes horizontally through plasmids and other mechanisms (Chang et al., 2014). Change is fast and frequent in the microbial world, and it does not take long for these genes to confer resistance to the human antibiotic analogues. During the transition from livestock to food products, possible contamination is another route by which these drug resistant pathogens can enter the human population (Chang et al., 2014). These strong selective pressures present in this environment elicit a necessary, urgent response to curb the rise of antibiotic resistance.

How do we go about solving the problem?

There is no clearcut, easy solution to the problem posed by Carbapenem Resistant Enterobacteriaceae, but it is certain that a multi-faceted approach combined with communication and cooperation is necessitated. To develop an effective plan, it is necessary to recognize

shortcomings of the past, and use these to better inform our future approach.

Antibiotic Resistance Education Programs

As was discussed earlier, many physician specialties and the general public lack proper education in microbiological principles pertaining to antibiotics and resistance. Antibiotic stewardship refers to proper education and promotion of proper antibiotic use (Wong, 2016). Antibiotic stewardship assists in education on proper dosage and regimen length (Wong, 2016). No one antibiotic stewardship program would be effective in all scenarios, but there are a few general components needed in each program. Most importantly in all proposed remedies, communication and cooperation are key to proper functioning and efficacy. An education component tailored to the target group is also essential. For instance, if the target group is general physicians, the education program would need to include basic principles of bacterial replication and functions such as respective bacterial doubling times, lag phase in growth, and mechanisms of attachment in humans. Due to the important role held by plasmids, conjugation, transformation, pathogenicity islands and other mobile genetic elements, in depth understanding of these elements should be incorporated into continuing education course for physicians. Employers could incentivize physicians with a bonus upon

completion of the program, seeing as aversion of treatment for CRE infected patients saves the hospital around \$60,000 (Bartsch, 2017).

Additionally, education programs for the public would need to take a much different approach. To be effective, research would need to be done to determine a standard level to achieve universal comprehension. Instead of a focus on harder scientific facts, these programs could focus on explaining negative selective pressures placed on these microbes by incorrect antibiotic use. Further, the programs could further provide ways in which everyone could contribute to amelioration of the problem by creating a sense of community. In an effort to be efficacious for the entire public, insurance companies could offer a reduction in insurance costs upon completion of the program.

Inter healthcare Cooperation

Mentioned previously, cooperation is extremely important in this effort. The healthcare system is often discussed as a single organism that functions together, when in reality, it does not. Hospitals are, in the end, businesses in the United States. Through a rewards and penalty system, hospitals and other healthcare facilities are often pitted against each other through competition to be the best. While it is necessary to recognize

these shortcomings, penalizing a hospital does not promote cooperation and willingness to improve for the health of the community. This continues to promote competition along with seeking accolades and financial reimbursement. Being as this is not the reason hospitals and healthcare settings exist, the illformed rewards system currently in existence serves as a detriment to combating problems such as Carbapenem Resistant *Enterobacteriaceae*, perpetuating the corrupt system from which it originated. Instead of penalizing hospitals with fines and tarnishing its reputation, programs should be set in place where professionals in health policy and microbiology instruct these individuals, providing guidance on how to better prevent antibiotic resistant infections, markedly CRE, and encourage antibiotic stewardship. These programs would aim to set facility specific protocols in order to meet international standards.

Competition is commonly thought to foster an environment of development and growth. In a healthcare setting, competition solely serves to shift the focus away from patients in need of care to aforementioned accolades and monetary acquisition. Competition also pits facilities against other 'competitors' providing similar, or the same, services. In this situation, competition undermines safety, cooperation and interaction among facilities needed to form a solid front to effectively address the threat of CRE. Presence of CRE at several locations in a

community proves much more difficult than one case in one location (Lee, 2016). Screening of patients upon transfer from one facility to another, combined with specified contact precautions can significantly reduce transmission (Lee, 2016). Communication among healthcare facilities in a given area about CRE cases allows for a conjoined effort to combat its spread (Lee, 2016). Antibiotic stewardship programs aimed at educating the public sponsored by several community healthcare facilities would also help create a feeling of community. Creating a sense of community is important because it creates a sense of responsibility for your neighbor, generally speaking.

Microbial surveillance has become a common practice for problematic pathogens as technology has advanced to give us this ability (Ramsamy et al., 2013). Through the use of whole genome sequencing, online databases, and standardized protocols for isolating these microbes, we are able to track movement and evolution of CRE worldwide. To streamline this effort, a task force of professionals from every country would help in consolidating data retrieved regarding CRE prevalence. This task force would also help establish protocols for identifying and tracking CRE to avoid misinformation. This would provide a unified front providing

the most up to date information regarding prevalence of CRE per region broken down into community acquired versus hospital acquired infections, comparisons of different isolates, and other relevant data.

Clinical Measures

As of now, a majority of CRE cases are hospital acquired (Tang et al., 2016). As with any hospital acquired infection, the risk of contracting CRE is increased the longer an individual stays in the hospital (Tang et. al, 2016). While this sounds like common sense, it is necessary that physicians aim to minimize the stays of their patients to decrease occurrence of these hospital acquired infections. Patients can bring in microbes from the community with the ability to transfer genes horizontally, and combined with extended stays, the likelihood of this occurring becomes very high. This is of concern because it could contribute to the difficulty to treat CRE, possibly introducing other resistance genes, resulting in a pan-resistant microbe (resistant to all known drugs). Measures to limit this from occurring could include a mandatory consultation by the attending physician with other physicians after a set number of days a patient is in the hospital. Consultation would ensure accountability as well as place the focus on the goal of getting the patient home in a stable, healthy condition.

Other common sources of hospital acquired infections are any foreign medical device placed within the human body such as IV's, Foley catheters, central lines, and especially ventilators (Tang et. al, 2016). Currently, hospitals have in place measures to track the number of indwelling devices so as to account for possible cases of infection. Minimizing indwelling time of these devices is paramount in decreasing occurrence of CRE infections.

In addition, it is important to educate patients on these risks. We should encourage patients to advocate for themselves by educating them on the pros and cons. While this could be frustrating for nurses and physicians, these patients deserve to understand the risks with certain interventions. Considering CRE infections are most prevalent in the hospital from indwelling devices, they should be encouraged to ask whether implementation of an indwelling device is truly necessary for their plan of care.

Indwelling devices are a common entry point of infection by CRE (Tang et. al, 2016). Despite protocols for sterilization measures in place, individuals continue to develop these infections. In an attempt to lower infection incidence, current protocols need to be reassessed, taking into

account new technologies and published studies on the subject. These protocols must also be standardized universally, eliminating variation among hospitals or healthcare establishments.

Agricultural Interventions

As mentioned earlier, agriculture serves as a large reservoir for bacteria containing antibiotic resistance genes (Chang et al., 2014). Instead of prophylactically administering antibiotics to livestock, it is necessary to find other alternatives. While the world is becoming more populated and inhabiting more acreage every year, a return to the old, free range method of farming is not necessarily feasible. An intermediate option would be best in which livestock have the ability to graze and roam around 2-3 times a week. Along with this, they would reside in spaces tailored as best as possible to a natural habitat. This new habitat must be a sufficient amount of space determined through studies analyzing overall health of the animal in the prescribed environment.

Along with an alteration in living conditions, regular inspection would be conducted. This would fall under the duties of the microbial surveillance task force for that region of the world suggested above. Thorough and frequent analyses of the overall health of the livestock would be prophylactic, yet with a goal to cause no unnecessary harm. The

measures of surveillance would need to be constructed in order to contribute as minimally to selective pressures as possible.

Drug Treatments

As the CDC declared in 2013, we may be in the 'post-antibiotic era' (Michael et al., 2014). Antibiotics have proved to be very effective in controlling bacterial infections, both bacteriostatic and bactericidal antibiotics. Both types can act as selective pressures, making mutations favorable for the survival of the bacteria. Research is being done specifically looking at trying to reduce the virulence of the pathogen, thus reducing the selective pressures influencing mutations (Antibiotic resistance threats in the United States, 2019). We have attenuated pathogens and used them to vaccinate, but by attenuating these pathogens in vivo, this allows the immune response to rid the microbe, creating natural immunity (Antibiotic resistance threats in the United States, 2019).

With the development of whole genome sequencing we now know the genes of almost every microbe. Reverse vaccinology uses information gained from whole genome sequencing to identify epitopes and other

targets that could be used for a vaccine (Kelly, D. F., & Rappuoli, R, 2005). This method differs significantly from typical vaccine development because traditional methods relied primarily on wet-bench techniques to identify a target. This was very tedious and only identified a few possible targets. Bioinformatics capabilities have advanced our ability to identify possible targets. For example, when applied to meningitidis Serogroup B, reverse vaccinology identified several times more targets than by traditional methods, which researchers had spent years finding (Kelly, D. F., & Rappuoli, R. 2005). This success could be achieved for CRE using reverse vaccinology and is a viable investment.

Conclusion

These days the world's population is more connected than ever (Michael et al, 2014). Anyone can be halfway across the world in just a few hours. We will not be able to enjoy these luxuries of travel and international connectedness if Carbapenem Resistant *Enterobacteriaceae* infections deplete our financial resources, spread internationally, and result in a pandemic. As with all pathogens, this is the ultimate threat. This pandemic is not yet upon us, yet the threat necessitates an immediate, uniform response to avoid an international crisis. As described thoroughly, a multifaceted approach combining new drug therapies, antibiotic resistance education programs, revised CRE protocols and coordinated

efforts to slow the spread of CRE among healthcare facilities are the most important ways to slow the rise of Carbapenem Resistant Infections and antibiotic resistance.

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