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On the Origin, Emergence and Pathogenic Patterns of Acinetobacter baumannii

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ABSTRACT

Acinetobacter baumannii is a deadly Gram-negative species of bacteria whose virulence and resistance to antibiotics is threatening the lives of patients in hospitals across the globe. The examination of Acinetobacter baumannii's virulent behaviour patterns in secluded synthetic, as well as ecological environments and; its acquisition of genes responsible for its persistence in sterile synthetic environments and those responsible for its lethality to humans reveal clear evidence of its recent artificial outbreak origin, virulent emergence and spread within healthcare facility populations around the World. A method of killing Acinetobacter baumannii is proposed based on the information presented herein.

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Pathogenesis and Evolution of Acinetobacter baumannii

Acinetobacter baumannii is a global pathogen causing septicemia, pneumonia and death. As an aerobic bacteria it uses oxygen (02) to grow, which is one of the causes for continued infection in health care facilities. Being pleomorphic, it varies in size, a possible adaptive trait allowing it to respond to diverse environmental conditions. With its many survival tactics in place this bacteria also possesses genetic anomalies that have, not only made it a mystery to researchers but, have guaranteed its survival and its success in becoming increasingly more virulent and lethal. A hypothesis is made here to elucidate the origin and the source of virulence of Acinetobacter baumannii by tracing its emergence and examining its pathogenic patterns.

In the late 1960s the Genus Acinetobacter was determined by Baumann [1]. Acinetobacter baumannii started to be noticed as a dangerous cause of infection in ICUs in the early 1960s [2]. Acinetobacter baumannii is a Gram-negative bacteria without the capacity for motility that has the ability to develop and live in a great temperature range and has been found in natural environments like; soil and water as well as human environments like sewer systems and facilities for healthcare [1]. Juni said that there was genetic evidence that Acinetobacter was a ubiquitous genus [3].

Sahl's study of genes in the complex of species and deadly strains surrounding and focusing on the central Acinetobacter baumannii bacteria is important in understanding this deadly pathogen and may be significant for the study of infectious pathogens in the future [4]. It should be noted that some of the information from that genetic study has been reinterpreted here while strictly keeping with the data provided. One hundred and thirty-six genomes were sequenced using genes from the whole population level to analyze the evolution of the Acinetobacter Genus and create a whole genome phylogeny, an evolutionary tree, which details the branching of species and isolated strains in the Genus Acinetobacter. The whole genome phylogeny allowed a genomic comparison on a global scale and was able to highlight the genes that have been lost and genes that have been acquired over evolutionary time. Two sudden punctuated developments are prominent in the evolution of Acinetobacter with the first occurring long ago [5].

From the earliest common ancestor picture three branches: the first having the species Acinetobacter radioresistens, the second containing most species in the genus and the third; a complex called Acinetobacter calcoaceticus-baumannii (Acb). The Acinetobacter calcoaceticus- baumannii Complex (Acb Complex) has two branches; the first branching off to Acinetobacter pittii and Acinetobacter calcoaceticus; the second branching off to Acinetobacter nosocomialis and Acinetobacter baumannii. This clearly identifies Acinetobacter baumannii as a monophyletic clade within the well-supported monophyletic group Acinetobacter calcoaceticus-baumannii [5]. Within the Acb Complex; Acinetobacter pittii and Acinetobacter calcoaceticus are environmental isolates that are not associated with disease and are not considered to be dangerous [6]. Acinetobacter nosocomialis and Acinetobacter baumannii are deadly.

The second expansion and current rapid evolutionary dispersal of the Acinetobacter baumannii clones has remained unexplained [5]. The strains of community acquired and nosocomial Acinetobacter baumannii differ greatly; with the hospital strains containing more virulent genes [7]. There are a number of genes from the Acinetobacter Genus that are more conserved (slowly evolving)

in the Acb Complex and especially in Acinetobacter baumannii. Researchers have said that the acquisition of specific virulence factors has likely contributed to the widespread persistence and virulence of Acinetobacter baumannii. Antunes suggests that the acquisition of genes causing resistance to antibiotics in Acinetobacter baumannii may have been acquired from other nosocomial bacteria by the horizontal transfer of genes from other strains of Acinetobacter or other deadly bacteria [8]. Transcriptional regulators (proteins that regulate the expression of a gene; turn it "On" and "Off") have been found in the Acb Complex that are unique. Acinetobacter baumannii and the opportunistic pathogen Pseudomonas stutzeri have transcriptional regulators that share distinct characteristics. A number of peptides have also been found in Acinetobacter baumannii that share similarities with genes in numerous other pathogens.

Because of its bacterial hydrophilic proteins Acinetobacter baumannii is desiccation tolerant and able to survive for long periods of time on dry surfaces and thrives in abiotic environments; not only surfaces that are wet or contain organic materials but it persists on dry glass and plastics [9,10]. It makes this species of bacteria difficult to kill on materials that are usually not conducive to biological growth. And it has been proposed that its pathogenicity is due to its adaptation to hospital environments and its resistance to these and other environmental challenges [9]. While Acinetobacter baumannii is non- motile and does not possess flagella, it can spread rapidly through growth. Genes have been found that are important for pilus and biofilm development in Acinetobacter baumannii and Acinetobacter nosocomialis - the infection most often acquired in health care facilities. Similar to the sex pilus used in conjugation, a short pilus, a fimbria, which covers some bacteria, aids in sticking to surfaces and protects the cell from other organisms' defenses. But it is thought that virulence in a number of strains is not due to the development of this biofilm [11]. The biofilm produced by Acinetobacter baumannii and Acinetobacter nosocomialis consist of extra cellular polymers that allow the cells to attach to one another and to surfaces [12]. This biofilm allows Acinetobacter baumannii to attach to human epithelial cells [13]. Putative tip adhesion genes are responsible for pilus and biofilm development [14]. And, a putative tip adhesion gene which is involved in the development of these pilus and biofilms was also found by Brossard and Compagnari [13]. Conservation of this particular gene in the Acb Complex was found to be 92% in Acinetobacter baumannii, 100% in Acinetobacter nosocomialis, 67% in Acinetobacter pittii and 0% in Acinetobacter calcoaceticus and those not in the Acb Complex [4]. The persistence of its growth in sterile medical environments is attributed to the growth of this biofilm [14-16]. Acinetobacter nosocomialis is most closely related to, and probably has evolved from Acinetobacter baumannii. It is probable that, over time, the species Acinetobacter nosocomialis evolved in hospital environments where it required this gene in order to survive and thrive in the abiotic environment. This evolution reveals the origin and reoccurring nature of these closely related infectious strains of bacteria.

Acinetobacter baumannii exhibits robust antimicrobial resistance. Today, some of these strains are resistant to all antibiotics including the Carbapenem group [17,18]. Primaxin, one of the World's strongest antibiotics, does not work against Acinetobacter baumannii strains that have found their way to United States (U.S.) and United Kingdom (U.K.) hospitals. And, this species is more drug resistant than methicillin-resistant Staphylococcus aureus, MRSA [19]. The mechanisms most associated with persistence in infected individuals are multiple iron acquisition systems that are highly toxic and are thought to have a biochemical advantage over other microbes. In addition to these systems are also Beta-lactamase genes which allow the Acinetobacter baumannii strains to persist in the host and resist treatment therapies. These Beta-lactamase genes allow the deadly resistance to broad-spectrum antibiotics [20]. This gene is found in the deadliest infections on the planet. It is also well conserved and unique to Acinetobacter baumannii and has not been found in any other Acinetobacter species.

The bacteria Legionella pneumophila causes Legionnaires disease and, in 1976 one thousand people acquired lung infections from this bacterium at the American Legion Convention. Thirty four of those people died from the infection. Acinetobacter baumannii has acquired genes that are homologous to Legionella pneumophila and Coxiella burnetii type I (type IVB) T4SS virulent secretion systems. These systems mediate horizontal gene transfer which contributes greatly to the evolutionary fitness and pathogenicity of the bacteria [6,11,21]. Nineteen anti-microbial resistant genes have been found by researchers who claim that most of the resistant genes in Acinetobacter baumannii have come from the bacteria Pseudomonas, Salmonella or Escherichia [22]. Twenty eight gene clusters were found to be unique to Acinetobacter baumannii and sixteen that may be a partial cause of its virulence [6,11]. Early clinical isolates of Acinetobacter baumannii were found to share four hundred and seventy five genes that were connected with growth adaptation of the bacteria in humans and were absent in related species of Acinetobacter [23]. The horizontal transfer of virulent genes were found in 2008 along with evidence of other pathogenic systems found in Acinetobacter baumannii and the ability of this bacteria to continued sharing virulent genes will allow an increasing resistance to antibiotics [21,24]. Variations of the Acinetobacter baumannii strain from Iraq have forty five different drug resistant genes in one locus. Its survival and toxicity is also attributed to acinetobactin, a siderophore system, (an iron- acquisition system) which is well conserved in the species Acinetobacter baumannii [25]. And, there are genes that allow the bacterium to invade sterile synthetic and abiotic surfaces like glass and plastics [13-15].

Acinetobacter baumannii Outbreak Strains and Environments

The Genus Acinetobacter is a group of soil and water bacteria found in the environments of humans and animals. These organisms require very little to grow and are ecologically robust in their nutritional and biochemical variability. Henriksen noted that their potential as a pathogen was low but that they were an opportunistic bacteria able to cause infection in humans with low resistance to infection and more often were found in diseased tissues as secondary pathogens [2]. They are also quite able to contaminate pathological material collected from patients.

Being a Gram-negative (containing a thin oily membrane which characterizes most pathogenic bacteria), arid, bacillus (rod-like in morphological structure) and opportunistic pathogen the Acinetobacter baumannii species is found almost solely in medical facility environments, and its species affects individuals whose resistance is very low and are often patients confined to burn and Intensive Care Units (ICUs) [8]. Other Acinetobacter species have been identified as causing human infection, but Acinetobacter baumannii is the most virulent and widespread in the Genus. Acinetobacter baumannii's sudden emergence during the Iraq conflicts is now a number of outbreak strains which have increased the nosocomial (hospital contracted) infections Worldwide. Its infections have caused many amputations and in

2017 alone it has caused 8500 infections and 700 deaths [6,26]. Iraqibacter, as it is called by Military personnel, has spread from Iraq, the Kuwait region and Afghanistan [19]. With its resistance to antibiotics greater than MRSA, Iraqibacter, Acinetobacter baumannii infections began to rise in 2003 in the regions of Iraq and Afghanistan after the start of the Iraq War. The infection and colonization of Acinetobacter baumannii were initially found in military and civilian casualties returning from Iraq and quickly spread to the United Kingdom, the United States and the U.S. Military base Landstuhl regional Medical Center in Germany [27]. Where this organism has come from and its exact source has been unclear. But, its origin could be important in finding a cure and treatment options. Acinetobacter baumannii infections isolated from zones of conflict in and around Iraq have evolved into three or more deadly strains of bacteria which are viewed as having all the characteristics of a persistent outbreak strain. The largest number of cases, with those in the United Kingdom being especially high, is caused by the T strain [28]. T strain isolates in the U.K. come from Iraq region casualties and were not acquired in hospital. The T strain characteristics are similar to the widespread SK clone in South East England and London. Questions about possible contaminants at the scene of infliction have risen. And, it is often thought that these infections are acquired at the health care facilities where patients are treated. The Iraqi soil Acinetobacter baumannii generally has not been isolated from patient's wounds immediately or shortly after injury. It has also been noted that it is unlikely that newly infected individuals injured at various sites would all acquire the same outbreak strain this way. But there is overwhelming evidence that military personnel and civilians have acquired the same outbreak strain in various environments of the Middle East conflicts centered around Iraq [19,20,29]. The problem with this infection's resistance to antimicrobials continues to worsen, and with the high rates of carbapenem-resistant Acinetobacter baumannii, medical practitioners continue to search for medicines to treat Acinetobacter baumannii infections [30].

In the 1960s Acinetobacter infections in intensive care units (ICUs) were first reported. And, over a decade before the Iraq conflict Acinetobacter baumannii had become known as an agent of nosocomial infection. As early as 1990 an outbreak of Acinetobacter baumannii was investigated by researchers in a New Jersey Hospital and traced to hospital transducers. Cultures were taken from blood and vasculature catheter tips of seventy-five patients in five ICUs and it was found that 21% of those in use and 46% of those in storage were positive for the Acinetobacter baumannii bacteria [31]. In 1991 Acinetobacter baumannii was first isolated from a patient at Walter Reed Medical Army Hospital in Washington D.C. Acinetobacter baumannii isolates from opportunistic infections include; septicemia, endocarditis, pneumonia, urinary tract infections and others and was found in the inanimate environments of hospitals beginning in 1991 [32].

In 1993 sixty-five strains of Acinetobacter baumannii were isolated in a neonatal intensive care unit as well as three other wards for comparison in the same health care facility [33]. The samples were taken from patients and the indoor environments. Over several months fourteen different electrotypes were determined, including a predominantly multiple resistant clone. The colonization and infection of neonates in the neonatal ICU spread and was colonized by the use of contaminated incubators, medical equipment, the water used to raise the humidity as well as the hands of staff members. Despite the identification of epidemic clones in the neonatal ICU, the genetic diversity of Acinetobacter baumannii strains from other wards revealed, over the several months taken for this study, that no spread of the epidemic clones to other wards had occurred. While these epidemic clones had not spread over several months in wards of the same hospital where the neonatal ICU was housed, it is remarkable that identical DNA profiles of Acinetobacter baumannii have been found in isolates from the United States (U.S.) and the United Kingdom (U.K.) that are associated with Iraq conflict casualties. In these two sets of isolates with identical DNA profiles, two additional outbreak strains were common and at least one outbreak strain, associated with soldiers returning to the U.S. and U.K. from Iraq is responsible for continued infection and the persistence of these Multi-Drug Resistant (MDR) strains in hospitals. In the conclusion of another clinical study on 27 wards, which included 118 patients, researchers were not able to find the source of the resistant Acinetobacter baumannii strains in Hospital [34].

Bacteria and Genetic Engineering

In the 1950s Joshua Lederberg discovered that bacteria could exchange their genes by using small rings of DNA called plasmids [35]. The gene transfer between bacteria, conjugation, can be initiated by simply introducing two different bacteria that have never been in contact before. Using two completely different species of bacteria, in some cases from different genera, the genome can be altered by conjugation alone, resulting in a Genetically Modified Organism (GMO). An artificial introduction can look like a natural occurrence.

Within the cell and the single celled organism, bacteria, sections of DNA are genes. Inside the bacterium, DNA molecules are cut by restriction enzymes at precise locations and complimentary strands of RNA are composed to govern the synthesis of proteins. The transplantation of genes in an organism alters its protein production. In 1955 Frederick Sanger deciphered the amino acid sequence of a protein [36]. Proteins can control different aspects of an organism's physiology. Changes in an amino acid sequence will alter the production of specific proteins needed within an organism. Sanger's and other researchers' work led to a means of gene assembly from sections of DNA instead of first isolating their proteins [37].

In 1973 restriction enzymes were used to cut precise locations in DNA by Stanley Cohen and Herbert Boyer [38]. This laboratory method, gene splicing, allows specific sections of DNA in bacteria to be cut open. A ligase, also an enzyme, can then be used to bend the gene fragments into curved sections of DNA that will fit into the bacteria's plasmids (loops of DNA). These new sections of DNA can then be used as vectors to combine that specific section of DNA with a second plasmid that has been opened in another bacterium. The vector, new gene, which is the curved plasmid, slips into the opened plasmid of the second bacterium to form recombinant DNA. Other enzymes bond this new gene into the opened plasmid of the second bacterium. When the new recombinant DNA is formed and absorbed into what was a normal bacterium (the Wild Type) it becomes recombinant bacteria. The newly formed recombinant bacterium will then replicate naturally by binary fission, splitting into two identical cells, with exact copies of the altered genetic material. This new organism, like the manipulated parent cell, is a Genetically Modified Organism (GMO).

Cell to cell communication in a bacterial community requires the production of extra-cellular signaling molecules called autoinducers [39]. This allows populations of bacteria to control

gene expression as a community - as if it is one organism. It allows synchronization of its behaviour. This communication, quorum sensing, allows the passage of information between cells. In bacteria it occurs across species (between unrelated species) that are non-dangerous and those that are dangerous. Behaviours coordinated in unison controlled by quorum sensing are only initiated when bacteria has acquired cell populations that are high. Ineffective as one bacterium, specific behaviours require a quorum where the simultaneous action is effective in important behaviours such as bioluminescence, virulence factor expression, bio-film formation, sporulation and conjugation. The sufficient number of cells, a quorum, allows cell to cell communication in Acinetobacter baumannii which allows conjugation; not only between bacteria and across species in its own genera but across species in many genera of bacteria [40,41]. What is most unsettling about Acinetobacter baumannii is its ability to acquire genetic material from other genera including those to which it is not related.

Although bacteria have neither eggs nor sperm it is important to note that Ryuzo Yanagimachi developed a sperm injection technique in the 1990's which led to the in-vitro fertilization techniques in humans used in clinics around the World [42]. Eventually, he used this technique to create the first transgenic Green Florescent Protein (GFP) Mouse [43]. The mouse glows green when the gene responsible for making this protein is transferred from the jelly fish into the mouse egg. This groundbreaking research was accomplished in a small kitchen on the second floor of a modest building on the University of Hawaii campus. All of the techniques described here are used by biologists to create genetically modified organisms and research millions of questions in biology and medicine every day.

Engineered Pathogens

Louis Pasteur's work and the foundation of microbiology led to the affirmation that microorganisms cause disease [44]. Unfortunately, with many great breakthroughs in science there are those who use its insight and power as a destructive means. In the 1980s the hospital use of growth medium in Iraq was claimed to be about 200 kilograms (440 pounds) per year. Yet in 1988, Iraq imported 39 tons (85,980 pounds) of growth medium used for the germination and determination of bacteria. Using this medium the Hussein Regime grew 19000 liters of botulism toxin, 8000 liters of anthrax, 2000 liters of aflatoxins which cause liver cancer, Clostridium perfringens bacteria which causes gas gangrene, ricin which kills by inhibiting protein synthesis as well as cholera, foot and mouth disease, salmonella and camelpox [45].

On August 2nd, 1990 the United States, took retaliatory measures against Iraq when the Hussein regime invaded Kuwait and this "Gulf War" lasted until the end of February of 1991. On departure the U.S. led coalition left Sadam Hussein as the Head of State in Iraq. And, in 1995 an additional 10 tons of biological growth medium was purchased from the company Oxoid of Great Britain by the Hussein regime.

A little over a decade later the United States and the United Kingdom entered into a war with Iraq, after the U.S. homeland was attacked by terrorists on September 11th 2001, on the premise that Sadam Hussein had amassed weapons of mass destruction (nuclear bombs), chemical and biological weapons. Before the start of the war in Iraq in 2003 an Iraqi told German and American Intelligent Services that he had worked as a scientist in Iraq's biological weapons program during the Hussein regime. He later retracted the statement saying it was not true. But, it is probable that he told the truth the first time. The significance of this is that he claimed the biological weapons program in Iraq was housed in mobile laboratories; trucks that could be moved. With the announcements that the U.S. was going to invade Iraq in 2003, these trucks could have been moved out of Iraq before anyone could have found them.

Clear evidence that Sadam Hussein had a biological weapons program has come from United Nations (U.N.) weapons inspectors and confessions by the microbiologist who set up a biological weaponization program in Iraq that made over 200 weapons including; warheads, bombs and missiles [46]. During the Iraq war that began in 2003, neither weapons of mass destruction, chemical nor biological weapons were found. But there is evidence that Sadam Hussein did indeed have a biological weapons program and there is overwhelming evidence that one of these weapons was first deployed during the Gulf War.

The Origin and Dispersal of the Virulent Acinetobacter baumannii Strains

While the genetic mechanisms explaining the emergence of Acinetobacter baumannii as a deadly pathogen and its widespread virulence from the Acinetobacter Genera have not been fully understood, all of the factors outlined here suggest, and it is theorized, that this bacteria has been, during the course of its evolution, artificially modified. The source of these virulent species strains have been a mystery because researchers have been looking in nature for the answer to its sudden emergence and deadly evolution. If examined closely, it is no mystery. The sudden emergence of the deadly pathogen Acinetobacter baumannii during the Iraq conflict is due to its laboratory manipulation as a biological weapon.

The modification of a bacterium through conjugation can make a bacterium resistant to antibiotics. So too in a laboratory would the countless introduction of other bacteria species and growth of a single deadly strain of bacteria cause extreme antibiotic resistance. Bacteria can be modified, not only with disease resistant genes but also with disease causing genes. Using its own unique characteristics and behaviours, the introduction of numerous lethal bacteria would allow the communication and sharing of genetic information. Stock piles of lethal bacteria kept by the Iraqi biological weapons program could have easily been used to manipulate Acinetobacter baumannii. With its growth factor of five billion trillion in twenty four hours from a single bacterium; its adaptation and evolution could have been pushed over a very short period of time. The artificial introduction of genes in Acinetobacter baumannii look natural but there are genes that noticeably should not be present. Engineered with the knowledge of its specific needs for survival in a dry climate; in abiotic environments and within a host, it could have been made in a truck or a small room and later grown in large quantities. The horizontal transfer of genes allow the rapid spread of antibiotic resistance and may be responsible for the emergence of many new antibiotic resistant strains [47]. The horizontal transfer of many genes in Acinetobacter baumannii from unrelated bacteria were probably executed in a laboratory and genes like Beta-lactamase were probably inserted along with other genes and regulators to insure its lethality and survival. It has been grown with adaptations that allow the uncommon ability of a bacterium to stick to metal door knobs, cotton and polyester pillow cases as well as plastics generally found in hospital settings around the World. Its ability to rapidly infect patients and contaminate everything in hospital settings allows it to persist and it can survive

for long periods on clothing. These behaviours are associated with systems in Acinetobacter baumannii that are required for pili formation, attachment to plastics and biofilm development which have been acquired from unrelated sources. And, the system used for the attachment to plastic has been found in food-borne pathogens [15]. It could be widespread among unrelated bacteria but it may have been acquired from these food-borne pathogens in a lab.

After six hundred and nine days of starvation Acinetobacter baumannii was found to adapt to rapid temperature changes and the resources (i.e., food) that are available [48]. These Acinetobacter baumannii strains last for long periods in dry conditions as well as in the human host because it was given these genes. And, this bacteria was specifically designed to spread to the countries of those who invaded Iraq by way of casualties. The Gulf War lasted from 1990 to 1991 and outbreaks in the United States began with an outbreak in New York City in 1991 [49]. Countries, in different areas of the World, who fought in the Gulf War show signs of an outbreak of carbapenem-resistant Acinetobacter baumannii before 2006 [6]. While Acinetobacter baumannii infections have been a problem since the 1980s these new strains and the increase of infections Worldwide are overwhelmingly noticeable after the Gulf and Iraq Wars. And there was a large increase in Acinetobacter baumannii infections after 2003 when the Iraq War began.

With identical DNA profiles of isolated strains spread to two continents this pathogen seems to have been seeded or spread mechanically - as though Iraq, Syria, Afghanistan and their surrounding regions were one origin. This is not possible unless it were moved and transported systematically to each location. Researchers have noted that injured soldiers with Acinetobacter baumannii infections were coming from these conflict areas in Iraq, Syria and Afghanistan [19,20,29].

Some researchers have also questioned whether there are contaminants at the scene where the casualty occurs or whether it is acquired at health care facilities where the injured are treated. The answer seems to be both. Proof of the manipulation and weaponization of Acinetobacter baumannii can be readily identified; in the fragments on the gear, clothing or anatomy of the wounded, as well as the surrounding area, where an Improvised Explosive Device (lED) has injured someone who has also acquire the Acinetobacter baumannii infection shortly after that injury. This means that Acinetobacter baumannii was present at the site. When the infection takes place after hospitalization patients should be screened for Acinetobacter nosocomialis as the primary cause of infection but also examined for Acinetobacter baumannii because its level of contagion may have increased. Evidence of the weaponization of Acinetobacter baumannii can also be found in undetonated IEDs where bacteria is discovered. This is the proof that the Acinetobacter baumannii strains have come from a weaponized biological engineered pathogen.

As early as 1995 the Hussein regime in Iraq had been suspected of biological agent experimentation and biological weapons testing on humans. Clear evidence that these types of agents were used on large animals, including donkeys and beagles was found by United Nations (U. N.) weapon inspectors [46]. With all of the disease causing bacteria produced in Iraq's bioweapons program it is conceivable that the scientists involved took a bacterium, known for causing infections in patients with low resistance, and made it lethal. If the Iraqis wanted to increase the probability of death in soldiers wounded in combat, this is the bacteria they would manipulate and use. An invisible weapon in plain sight - any known biological weapon would have been discovered immediately after deployment. And, infection by any other biological means would alert Military physicians and surgeons, while disease would be widespread, leading Governments around the globe to learn of the use of biological weapons in Iraq over night. It is probable that Acinetobacter baumannii is now and has been a bio-weapon, and it has remained hidden and has continued to evolve and spread. And, the persistence and spread of the Acinetobacter baumannii bacterial infection is due to the persistence of human conflict, including terrorism, in the region, with its Global spread due to the movement of injured humans.

In 2017, when I began this epidemiological study of Acinetobacter baumannii infections, the Center for Disease Control and Prevention (CDC) did not require Acinetobacter baumannii cases to be reported [19]. Reporting Acinetobacter baumannii cases, the collection of contaminated fragment samples, isolation and screening of all incoming wounded for contaminated material (i.e., gear and clothing); to be sent to officials at the CDC and U.N. bioweapons inspectors, allow Acinetobacter baumannii strains to be identified as a bioweapon. Sampling infectious strains received after hospitalization will not only determine if the bacteria is Acinetobacter nosocomialis species, but document the rate of spread within hospital settings for Acinetobacter baumannii. This will also help determine the origin of these species strains of bacteria and answer numerous pressing questions about the Acinetobacter Complex. Acinetobacter baumannii's natural habitat has not been established by researchers [6]. This is because these infectious Acinetobacter baumannii strains began in an artificial setting. It is also likely that Acinetobacter nosocomialis species has evolved over time in the artificial settings of medical facilities across the World. Acinetobacter baumannii's antibiotic resistance, its ability to thrive in sterile environments and the human host, continues today and there are still no answers to antibiotic resistance [50,51].

While treatment options prove to be a great difficulty and the role of all the acquired genes uncovered in Acinetobacter baumannii have not yet been described, there have been numerous research efforts to cure the Acinetobacter baumannii infection. Like Bassler's early suggestion that cell-cell signaling be investigated for targeting virulent bacterial infections, anti cell-cell signaling has recently been suggested as a target for combating this infection [52]. By targeting biofilm production, bacterial hydrophilns (which promote desiccation ability) and the RNase T2 family protein (a positive regulator used to colonize inanimate surfaces and promote motile functionality) it may be possible to stop Acinetobacter baumannii persistence in the human host [9,53,54]. The use of a virus that infects bacteria (a phage) called phage therapy has also been sought in place of antibiotics [55]. Using lytic bacteria phages have shown researchers that treatment of the Acinetobacter baumannii infection in-vitro can lead to positive in-vivo research results [56]. It has also recently been demonstrated that In-vitro phage-antibiotic treatment on Acinetobacter baumannii leads to antimicrobial resensitization. And, the use of phage-antibiotic therapy in animals (in-vivo) with Acinetobacter baumannii infections has allowed phage resistance in 96% of the animals in a study that increased antibiotic sensitivity [57]. But, in the past, it has not been clear if phage therapies, that have been attempted in humans, are working [58]. Caution should also be used when introducing a virus into the cellular environment of an Acinetobacter baumannii infection, especially if this bacteria has been modified artificially. Recently, methods of artificial intelligence (AI) were used to screen over 7000 molecules to find

inhibitors that could stop Acinetobacter baumannii growth in-vitro [59]. Shankar's attempt at bacterial gene transfer therapy is the only treatment to work against Acinetobacter baumannii and has come from another engineered bacterium [29]. The delivery of vectors containing bactericidal genes was initiated and, through bacterial conjugation, the transfer of those genes using attenuated E. coli as a vector to disrupt protein synthesis in Acinetobacter baumannii was successful. By transferring an in-vitro method to an in-vivo burn sepsis model in mice, this treatment led to the death of Acinetobacter baumannii cells, where 90% of the animals under investigation survived with the treatment. Like Shankar's research, it seems that the greatest possibility for a cure will be one that involves the genetic modification of another bacteria that can kill Acinetobacter baumannii — a solution that must be bioengineered.

Killing Acinetobacter baumannii

Vibrio cholerae, the extremely motile marine bacteria, that causes cholera, discovered by Pacini (Type Specimen), and simultaneously by Snow, then confirmed by Koch, has been found to kill strains of Gram-negative bacteria by using protein spikes, a Type VI Secretion System (T6SS) that is active during infection [60-64]. While the Secretion Systems T3 (T3SS) and T4 (T4SS) use proteins and nucleic acids to stop growth or eventually cause death in competitive bacteria (with the exception of Xanthomonas citric T4SS) the Type VI Secretion System (T6SS) in Vibrio cholerae delivers lethal enterotoxins that kill other bacteria [65,66]. Prokaryotic and eukaryotic cells that come into contact with Vibrio cholerae are invaded by the use of the Type VI Secretion System (T6SS) that uses an injection mechanism to puncture the other cell's membranes and deliver these lethal enterotoxins [66-68]. This structure and mechanism, that is quite similar to the tail of the bacteriophage (a bacterial virus), delivers effector proteins into host cells and can attack foreign bacteria that are living and active in mammals that are infected [69,70]. It has been observed in experimental animals that there are Type VI Secretion Systems (T6SS) that can invasively change the structure of the cytoskeleton in a eukaryotic host [71]. The basic function of the T6SS is successful competition, making any species that possesses it robust in habitats where there are multiple competitors [68,72]. The competitive success of non- cholera environmental strains in the microbial environment can be attributed to the Type VI Secretion System (T6SS) [73]. So, it is this system that may account for the persistence of the Vibrio cholerae bacteria in the environment and in humans while infected [74]. The Type VI Secretion System (T6SS) allows for the immediate increase in the fitness of the Vibrio cholerae species. An important aspect of T6SS, found by Bernal while studying the System in plants, is that it is the effector (toxin), used to target cells, that makes these systems lethal to a specific host [75]. Vibrio cholerae has over 200 strains (subspecies) separated by their serogroups, all of which encode for the T6SS gene [76]. There are Vibrio cholerae strains that cause pathogenic cholera and non-cholera Vibrio strains that only cause mild or undetected infections. Vibrio cholerae serogroups 01 and 0139 make the cholera enterotoxin that causes cholera pandemics, (some strains in serogroups 01 and 0139 are also non-cholera bacterial strains and do not make the enterotoxins that cause cholera [77-82]. There are also some non-cholera Vibrios that make the enterotoxins that cause cholera [83]. But, all of the remaining subspecies are non-cholera Vibrio cholerae. The presence of plasmids in nontoxic and toxic species does not determine cholera-causing subspecies. In 1981 Kaper discovered nontoxigenic strains that could not serve as reservoirs for cholera because they did not contain genetic material encoding cholera toxin [84].

MacIntyre's research has shown that the Type VI Secretion System (T6SS) allows the Vibrio cholerae strain V52 (serogroup 037) to kill Salmonella enterica serovar Typhimurium (Salmonella) and Escherichia coli (E. coli) and without the T6SS, the V52 strain does not kill either of these bacteria [63]. Both Salmonella and E. coli have Type VI Secretion Systems (T6SS), yet the Vibrio cholera V52 bacterial strain kills each of them and decreases the growth and survival of E. coli, reducing the multiplicity of infection (MOI) by 100,000-fold. Acinetobacter baumannii has been shown to possess the Type; I (T1SS), II (T2SS), IV (T4SS), V (T5SS) and VI (T6SS) Secretion Systems and has been described by Li [85]. And, Le found that D-Lysine is used by Acinetobacter baumannii to edit its own cellular structure to protect it from other invasive bacteria as well as, alter the external cellular environment of the surrounding cells it is attacking that allows a universal effector, Tse4, to efficiently kill its opponents [86,87]. But attenuated E. coli has been used to deliver vectors with bactericidal genes to disrupt protein synthesis and kill Acinetobacter baumannii in Shankar's study, and Vibrio cholerae strain V52 has been used to kill E. coli and Salmonella, both of which contain the T6SS, then surely an attenuated strain of non-cholera Vibrio cholerae with a Type VI Secretion System (T6SS), that uses non-human virulent effectors, carrying bactericidal genes will kill Acinetobacter baumannii infections. It is also possible that a topical treatment that targets and restricts D-Lysine, in dermal Acinetobacter baumannii infections, will prevent the bacteria from defending itself and halt the initiation of the Tse4 effector. What is most important is finding Vibrio cholerae strains that do not have genes encoding cholera toxin. The use of an attenuated Vibrio cholerae V52 strain or the discovery and attenuation of another non-cholera Vibrio cholerae subspecies, and the elimination of its virulence to humans, will allow in-vitro experiments to be conducted on Acinetobacter baumannii in a laboratory. This could produce positive results that quickly lead to in-vivo experiments where the isolation of Acinetobacter baumannii induced infections in mouse models are killed. These laboratory experiments can lead to successful clinical trials where infected areas are isolated and attenuated non-cholera Vibrio cholerae bacteria are introduced to severe Acinetobacter baumannii infections in humans. Cholera is a water-borne disease, and the primary transmission of Vibrio cholerae bacteria is from the environment - through the contamination of food and water, and its secondary route is the human-fecal contamination of food and water [88]. Isolating external or topical infections (i.e., dermal wounds) and using sterilized oxygen masks and delivery systems should also protect experimental animals and humans from ingesting the non-cholera Vibrio cholerae bacterial strain. After the significant reduction in the Acinetobacter baumannii bacteria infection, antibiotics, used in the treatment of cholera, can then be administered to the patient to clear the presence of Vibrio cholerae bacteria.

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