Strategies to control the current and continually worsening multi-drug resistant bacterial crisis

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Strategies to control the current and continually worsening multi-drug resistant bacterial crisis

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2004
Dedication

This work is dedicated to, Mr. William Erwin, the gentleman that came into my life in April of 2003. Since that time, he has been a continual source of encouragement and strength. It is his belief in me and love that have guided me and will continue to guide me through the many challenges and joys of life.
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To my friend, Jeff Madden, I owe you so much. Thank you for all that you have done and provided me with over the past two years of PA school. I am proud to call you my friend.

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STRATEGIES TO CONTROL THE CURRENT AND CONTINUALLY WORSENING MULTI-DRUG RESISTANT BACTERIAL CRISIS

During the pre-antibiotic era, bacterial infections, namely tuberculosis, pneumonia, and gastrointestinal tract infections accounted for roughly 30% of deaths in the United States (Walsh, 2003). Alexander Fleming in 1928, with his observation of antibiotic effects of the mold, *Penicillium*, changed the medical world. The product, which later became known as penicillin, was so effective in fighting infections formerly deemed deadly, that scientists labeled it as a “miracle drug” (Andersson, 2003).

The discovery of several other potent new classes of antibiotics flourished from 1930 to 1970 (Table 1). In the 1980s, drug companies shifted resources from antibiotic research to the development of drugs for chronic conditions. Since that time, antibiotics brought to market have mainly been modifications of existing antibiotic classes which have been approved by the Food and Drug Administration (FDA), with one exception (Amyes, 2001). At the beginning of the year 2000, a structurally new antibiotic was approved by the FDA. The antibiotic called linezolid or Zyvox was introduced by Pharmacia. It is active against all enterococci and works by a novel mechanism of action by blocking the formation of the initiation complex in protein synthesis. Discovering and developing safe and effective new medicines is a long and expensive process. The United States system of new drug approvals is one of the most rigorous in the world. According to the FDA, on average, it costs a company $350 million and approximately 12 years to get one new medicine from the laboratory to the pharmacist’s shelf. Five in five-thousand compounds that enter pre-clinical
Antibiotics became widely available for human use in the 1940s and have held onto the image of being the single most important therapeutic discovery in the history of medicine, despite the fact that no infectious disease has been eliminated by their use (Davies, 1999). Antibiotics have saved the lives of millions, reduced illness, and allowed for the development of complex surgical procedures, previously considered too hazardous. In fact, over an eight-decade period (1900-1980) infectious disease mortality rates fell significantly from 797 per 100,000 to 36 per 100,000 in the developed world (Walsh, 2003). Today, however, antibiotics no longer eliminate bacteria as effectively as first introduced (Boodman, 1997). In the 15 year period from 1981 to 1995, the mortality rates due to bacterial infections in the developed world had risen from 36 per 100,000 to 63 per 100,000 (Walsh, 2003). Infectious diseases continue to be one of the leading causes of death in the world, accounting for 13.3 million deaths (Cassell & Mekalanos, 2001). According to the Centers for Disease Control and Prevention (CDC), bacteria are responsible for 90% of hospitalized infections in the developing world.

It is increasingly difficult to treat many infections since therapeutic options are severely limited, and in some cases, non-existent, due to the emergence and spread of resistant bacteria. A recent report states that the first clinical isolate of *Staphylococcus aureus* to be classified as completely resistant to vancomycin was identified (Pearson, 2002). Vancomycin is one of the last lines of defense in the treatment of many bacterial infections. In addition, strains of three bacterial species,
Enterococcus faecalis, Mycobacterium tuberculosis, and Pseudomonas aeruginosa, have the potential for causing life-threatening illnesses and already exhibit resistance to a regiment of more than one hundred antibiotics (Levy, 1992).

The emergence of numerous, unstoppable bacterial killers are looming in the near future. Strategies focused on hastening or better yet, ameliorating this continual increase in antibiotic resistance needs to be employed. Otherwise, we could be forced into living in the pre-antibiotic state described so vividly in an excerpt from Ralph H. Major's "Classic Descriptions of Disease" (1945):

At the end of January in a family composed of a woman and three children, two of the children were attacked and died in less than forty eight hours. Fifteen days later the disease appeared in another family in the neighbourhood composed of a father, mother and five infants, four of whom were attacked almost at the same time, and all died from the tenth to the twelfth of February, after having been sick fourteen to fifteen hours with striking symptoms of malignancy. ... One did not realize how much these rapid and numerous deaths could produce terror...although we did not doubt that there was a malignant contagious fever against which one should take the greatest precautions. As a consequence all the furniture and clothing of the two families were burned. (p. 163)

Antibiotics

Antibiotics are molecules that occur naturally or are synthesized to interfere with the normal cellular functions of bacteria and fungi. Normal cellular functions include cell wall biosynthesis, cell membrane function, DNA replication, DNA transcription, and
RNA translation or protein synthesis. Currently, antibiotics are the second most commonly prescribed class of drugs in the United States (Hughes, 1996). They are classified as either bacteriostatic (inhibiting bacterial growth) or bactericidal (killing bacteria directly). On a yearly basis, 50 million pounds of antibiotics are produced in the United States and roughly half is used in treating human infections, while the remainder is used in non-human applications, such as agriculture and aquaculture (Levy, 2001). According to the researchers at the Centers of Disease Control and Prevention (CDC), one-third of the antibiotic prescriptions written each year for human use are considered unneeded (Levy, 2001). In addition, proper use of the antibiotic does not always occur. Individuals fail to finish the complete course of treatment and often save the remaining prescription to medicate themselves or other family members at a later date (Levy, 2001). People do not realize that a sub-therapeutic dose of an antibiotic fails to eliminate the agent of disease, but rather encourages the growth of more resistant strains (Hughes, 1996).

Antibiotic Resistance

Bacteria develop resistance in several ways. Like all living things, they must cope with and adapt to changing environments in order to survive. Although mutations are rare, occurring at a rate of about 1 in 1,000,000 to 1 in 10,000,000 replications, the rapid reproduction and vast numbers of bacteria increase the frequency of mutations within a population (Alliance for the Prudent Use of Antibiotics, 2003). Once a resistance gene arises, it can be spread between bacteria and even across the species barrier. Furthermore, genes coding for resistance are frequently found on plasmids, transposons, and contained within bacteriophages. Sharing of genetic
material which codes for resistance among bacteria occurs by means of conjugation, transposition, transduction, or transformation.

In a bacterium's fight for survival, there are enzymes that degrade the chemical design of antibiotics. After antibiotics enter the cell, these bacterial enzymes can alter the antibiotic to inactivate its function. These enzymes exhibit high specificity for a particular antibiotic and a broad variety of destructive and modifying enzymes can exist for the same antibiotic. There are more than 90 different kinds of penicillin/cephalosporin-inactivating enzymes specified by resistance genes (Levy, 1992). A well known example of an antibiotic-altering enzyme produced by resistant bacteria is penicillinase, which breaks the B-lactam ring of penicillin.

In order for antibiotics to work, they must penetrate the bacterial cell. Many antibiotics enter the bacterial cell by making use of transport systems that exist within the bacterium. Altered transport of antibiotics into the cell is one resistance mechanism. A more effective resistance mechanism is control of the production of bacterial cellular pumps that expel antibiotics from the bacterial cell. An example of an antibiotic-efflux pump is the pump that expels tetracyclines from bacterial cells. In the case of tetracycline, the antibiotic must bind to the cytoplasmic membrane and then be actively transported into the bacterial cell where it can inhibit ribosomal functioning. Following entry into the bacterial cell, tetracycline-resistant bacteria can quickly expel the drug, preventing it from doing any harm. The efflux of tetracycline is so effective by resistant bacterial cells that they can survive up to 100 times the therapeutic dose of tetracycline (Levy, 1992). Active efflux as a mechanism for bacterial drug resistance
has also been found for other antibiotics, such as erythromycin, chloramphenicol, and ciprofloxacin.

Instead of acting on the antibiotic, alterations or mutations of existing antibiotic target sites of the bacterial cell can be produced. The bacterial cell alters or eliminates the target site. An example is the group of macrolide antibiotics, which include erythromycin, lincomycin, and clindamycin. Bacteria resistant to these macrolides produce methylase, which causes the methylation of the 23S ribosomal RNA in gram-positive bacteria. By methylating the ribosomal RNA, the bacteria make the 50S ribosomal subunit resistant to binding by the macrolide antibiotics. The drugs are no longer able to interfere with ribosomal RNA processes.

Antibiotic resistance is not a new or surprising phenomenon. It has been recognized since shortly after the introduction of penicillin in 1946, but has become serious in the last decades. From observations of bacterial strains in the laboratory, Fleming warned that the misuse of penicillin could lead to the selection of resistant forms of bacteria (Davies, 1999). Penicillin was available orally to the public without a prescription until the mid-1950s. During this period, penicillin was believed by many to be a cure-all medication. It was used for non-bacterial diseases and taken at less than the optimal dose. Today, virtually all important human pathogens treatable with antibiotics have developed some resistance (Table 2 & 3). Antimicrobial drugs today target genes that are essential for survival, creating immense selection pressure for populations to develop resistance.

Resistance occurs rapidly in parallel with the use of antibiotics. An 11-year study of cancer patients at a hospital in Switzerland found no strains of Escherichia
coli to be resistant to any fluoroquinolone antibiotics between 1983 and 1990. However, between 1991 and 1993, 28% of the strains tested were resistant to all five fluoroquinolones administered at the hospital. During the study period, the percentage of patients receiving fluoroquinolone antibiotics rose from 1.4% to 45% (Cometta, Calandra, Bille, & Glauser, 1994).

The World Health Organization (WHO), in The World Health Report 1996 concluded: “Disastrously, [resistance] is happening at a time when too few drugs are being developed to replace those that have lost their effectiveness. In the race for supremacy, microbes are sprinting ahead.” A declaration of antibiotic resistance as a global public health crisis occurred on World Health Day, April 7, 1997 (The World Health Report, 1997). Resistance has adverse effects on public health and contributes to extremely high healthcare costs.

Treating resistant infections often requires the use of more expensive and more toxic drugs and results in longer hospital stays for infected patients. For example, drugs needed to treat multi-drug resistant forms of tuberculosis are over 100 times more expensive than the first-line drugs used to treat non-resistant forms. The Institute of Medicine has estimated that the annual cost of treating antibiotic resistant infections may be as high as $30 billion in the United States. To stop infectious germs from gaining further resistance, doctors and scientists from such organizations as the Food & Drug Administration (FDA), the Centers for Disease Control & Prevention (CDC), and the World Health Organization (WHO) have been focusing on finding ways to prolong the effectiveness of current antibiotics and to encourage the development of new “miracle drugs”.
Biofilms

Within the last decade, it has become clear that in nature, bacteria predominantly exist as sessile, surface-associated communities, known as biofilms. The biofilm mode of bacterial growth is of particular importance, because many chronic bacterial infections are intrinsically linked to the formation of biofilms (Costerton, Stewart, & Greenberg, 1999). Also, conjugation, the mechanism of plasmid transfer, occurs at a greater rate between cells in a biofilm than between planktonic cells (Hausner & Wuertz, 1999). An announcement from the United States National Institutes of Health, states that more than 60% of all microbial infections involve biofilms (Lewis, 2001). Colonization of medical implants and equipment occurs by means of pathogenic bacteria in a biofilm (Costerton et al., 1999). Bacterial biofilm infections are particularly problematic due to the fact that sessile bacteria can withstand host immune responses and are drastically more tolerant to antibiotics than planktonic bacteria (Xu, McFeters, & Stewart, 2000). Biofilm bacterial cells are 100- to 1,000-fold more tolerant to antibiotic treatment than growing, planktonic bacteria (Costerton et al, 1999).

In the initial stages of biofilm development, bacteria attach to a surface, aggregate to each other, and then proliferate to form micro-colonies. The micro-colonies are hydrated structures in which bacterial cells are enmeshed in a matrix of self-produced exopolymeric substance (EPS). The EPS matrix acts as a diffusion barrier and retards the penetration of many antibiotics into the biofilm. Mature biofilms are separated by channels to allow for the transportation of nutrients and removal of wastes from interior parts of the biofilm.
One of the most investigated examples of a disease in which biofilms play a prominent role is the occurrence of chronic lung infections by the opportunistic pathogen *Pseudomonas aeruginosa* in patients with cystic fibrosis (CF) (Tummler & Kiewitz, 1999). CF is the most common lethal, inherited disease among the Caucasian population. During chronic infection, *Pseudomonas aeruginosa* produces copious amounts of alginate, which forms a matrix that completely embeds the bacterial cells and makes them highly resistant to antibacterial treatment. Other pathogens that associate and grow in biofilms include: *Legionella pneumophilia, Staphylococcus aureus, Listeria monocytogenes, Campylobacter species, Escherichia coli O157:H7, Salmonella typhimurium, Vibrio cholerae, and Helicobacter pylori* (Donlan, 2002).

**Problem Pathogens**

*Staphylococcus aureus*

*Staphylococcus aureus* is a common gram-positive bacteria found on the skin or nose of many healthy people. Normally it does not cause illness, however, when the organism enters the body via a wound, the lungs, or bloodstream, infection can occur.

MRSA (Methicillin-resistant *Staphylococcus aureus*) is a subtype of *Staphylococcus aureus*, exhibiting resistance to notably one or more of the following semi-synthetic penicillins: methicillin, oxacillin, or nafcillin. MRSA is neither more infectious nor more virulent than susceptible strains of *Staphylococcus aureus*; it is simply more difficult to treat. Transmission of MRSA primarily occurs by contact with a person who is infected or colonized with the organism. Hands of healthcare personnel are the most likely mode of transmission of MRSA from patient to patient.
It is estimated that as many as 80,000 patients a year get a MRSA infection after they enter the hospital, creating a bill of $122 million for treatment (CDC). According to the CDC’s National Nosocomial Infections Surveillance System (NNIS), the proportion of MRSA in the United States rose from 2% in 1974 to approximately 50% in 1997. Approximately 50% of MRSA isolates identified at NNIS system hospitals are susceptible only to vancomycin. More devastating than this increase in infection rates is the fact that resistant infections have occurred in the general population. In 1999, the CDC reported that four immunocompetent children in the Midwest developed fatal MRSA infections. None of the four children had established risk factors for MRSA infection, so they were all treated with cephalosporins, which are antibiotics ineffective against MRSA. CDC researchers reported, “The delayed use of antibiotics to which MRSA were susceptible may have contributed to the fatal outcomes.”

**Enterococci**

*Enterococci* are a group of bacteria common in the digestive tract of healthy people and do not normally cause illness. They cause infection if they enter the body and invade a wound, the urinary tract, or bloodstream. Individuals with chronic disease such as diabetes and those with a suppressed immune system are more vulnerable to infection due to *enterococci*.

VRE (Vancomycin-resistant *Enterococci*) is a type of *enterococci* that is resistant to several drugs, with vancomycin being the most notable. In 1989, 0.3% of enterococcal isolates were resistant to vancomycin, while in 1993, 7.9% were resistant
(CDC). Resistance further increased between 1994 and 1998, and roughly one in four enterococci were resistant to vancomycin by 1999 (McGeer & Low, 2000).

**Pseudomonas aeruginosa**

*Pseudomonas aeruginosa* is a gram-negative bacterium with minimal nutritional requirements. Among healthy individuals, *Pseudomonas aeruginosa* is found relatively infrequently. The carriage rate on the mucous membranes of the nose or throat occurs among 5% of immunocompetent individuals, while 10% to 30% of immunocompetent individuals carry the bacterium in their stool (Rhame, 1980). Approximately two-thirds of hospitalized patients, particularly the critically ill, become colonized with *Pseudomonas aeruginosa* and various factors are known to increase the likelihood that an infection will occur (Fick, 1993). Immunocompromised patients at the age extremes, those undergoing chemotherapy or antibiotic therapy, those requiring indwelling devices such as a urinary catheter, and those requiring prolonged hospitalization are at a greater than average risk (Emori & Gaynes, 1993). The virulence of *Pseudomonas aeruginosa* depends on an arsenal of extracellular products and is a particular problem in cystic fibrosis patients where it efficiently colonizes host lung tissue causing chronic pulmonary damage. In fact, *Pseudomonas aeruginosa* is the most common pathogen recovered from patients who have been hospitalized for more than a week (Bodey, Bolivar, Fainstein, & Jadeja, 1983).

**Streptococcus pneumoniae**

*Streptococcus pneumoniae* is an agent for causing middle ear infections, sinusitis, bronchitis, meningitis, and is the leading cause of community-acquired
pneumonia (Gonzales, Bartlett, Besser, Cooper, Hickner, Hoffman, et al., 2001). It is spread through respiratory droplets and can cause meningitis and septicemia.

Penicillin resistance in *Streptococci pneumoniae* increased in an epidemic manner during the past 10 years. By 1998, 24% of invasive isolates in the United States were resistant to penicillin and 14% were resistant to three or more drug classes (Whitney, Farley, Hadler, Harrison, Lexau, Reingold, et al., 2000). Resistance to macrolides, doxycycline, trimethoprim-sulfamethoxazole, and second- and third-generation cephalosporins also increased (Gonzales et al., 2001).

**Salmonella Typhimurium**

Infection with *Salmonella* is characterized by sharp stomach pains, fever, and diarrhea that persist from two to five days. It is estimated that 1.4 million people are infected annually and approximately 500 people die as a result of *Salmonella* infection (Mead, Slutsker, Dietz, McCaig, Bresee, Shapiro, et al., 1999). Studies have shown that the organism is present among farm animals and pets and can easily be transmitted to humans. Eating beef, pork, or poultry products have been associated with outbreaks of disease in humans.

A multidrug-resistant strain of *Salmonella typhimurium*, known as *Salmonella typhimurium* Definitive Type 104 (DT 104), has emerged as a severe source of human illness in the United States. This particular type of *Salmonella* accounts for 68,000 to 340,000 infections annually. *Salmonella typhimurium* DT 104 has been present in the United Kingdom since 1984 and is highly resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline.
In October 1996, an outbreak of diarrheal illness associated with fever, headache, nausea and vomiting occurred among elementary school children in a small farming community in Nebraska (Multidrug-resistant Salmonella Serotype Typhimurium – United States, 1996). None of the 19 children required hospitalization and all recovered.

**Mycobacterium tuberculosis**

Concern is mounting over multi-drug resistant strains of *Mycobacterium tuberculosis*. Initially, multi-drug resistant strains were seen only in HIV-infected patients, but it has now spread to the general community. According to the World Health Organization, 7.5 million people are afflicted with tuberculosis (TB) and 2.5 million die as a result each year. TB is the leading cause of death among infectious diseases. New York City was the center for TB infections, with 17.1% of the national cases in 1992 (el-Sadr, Medard, Berthaud, & Barthaud, 1996). It was also the center for multi-drug resistant strains, with 61.4% of the cases in 1991 (el-Sadr et al., 1996). The dramatic rise in drug-resistant TB in New York City in the early 1990s spurred the city to take action. A program known as Directly Observed Therapy (DOT) was initiated. The premise of this program was to watch patients take their medication daily. According to research from 1996, patients from The Harlem Hospital made 91% of their visits to receive medication and 88% completed the entire course of therapy, as compared to 11% in an unobserved therapy study (el-Sadr et al., 1996).

**Nosocomial Infections**

A nosocomial infection develops within a hospital or other clinical care facility, is produced by an infectious organism, and shows no evidence that it was present or
incubating at the time of admission to the hospital (Garner, Jarvis, Emori, Horan, & Hughes, 1988; Jarvis, 1996). Infectious organisms contributing to nosocomial infections most often include bacteria, but viruses and fungi also are known causes of infection. Nosocomial infections, characterized as sporadic, endemic, or epidemic, are not limited only to hospitalized patients, but may occur among doctors, nurses, extended healthcare providers, volunteers, and visitors. Approximately 5% of all infections that occur are epidemic, while the majority of nosocomial infections are endemic (Emori & Gaynes, 1993). The incidence of nosocomial infections varies by site and is mainly determined by underlying disease conditions in the patient and their exposure to high-risk procedures. The most common infections include: urinary tract infections, pneumonias, surgical site infections, and bloodstream infections (Emori & Gaynes, 1993).

Because of the widespread use of antibiotics, advances in hospital practices, and the shift of surgical care to outpatient centers, which leaves extremely ill patients in the hospital, nosocomial infections are a worldwide problem today (Weinstein, 1998). Even though patients are requiring progressively shorter hospital stays, the rate of nosocomial infections per 1,000 patient days has increased by 36%, from 7.2 to 9.8 over the past twenty years (Weinstein, 1998). Approximately 2.4 million people, who stay an average of 5.4 days in the hospital, develop a nosocomial infection in the United States each year and of that number, 100,000 die as a result of infection (Boodman, 1997). Infected patients are subjected to an excess duration of hospitalization to treat nosocomial infections ranging from one to thirty days depending on the site of infection (Jarvis, 1996). In addition, it is estimated that the total cost of
treating nosocomial infections is $4.5 billion, which represents anywhere from $583 to $4,886 per infection (Jarvis, 1996).

**Preventing Emerging Infectious Diseases**

Within the modern health care environment, almost half of patients and almost all patients in ICUs receive antibiotics (Gaynes, 1997). It is not hard to imagine that an organism with a mutation for antibiotic resistance might enjoy a selective advantage to survive, proliferate, and then spread to another patient in this setting. Health care workers spread resistant organisms to patients because their hands, clothing, and equipment become contaminated with resistant organisms as they provide care for infected or colonized patients or as they come into contact with contaminated environmental surfaces (Zachary, Bayne, Morrison, Ford, Silver, Hooper, et al., 2001). One study found that a white coat frequently became contaminated with MRSA when the wearer provided care for a patient colonized or infected with MRSA and that this could result in subsequent contamination of the provider's hands on touching the coat (Boyce & Chenevert, 1998). This study also reported that the use of gowns reliably prevented clinicians' clothing from becoming contaminated (Boyce & Chenevert, 1998).

Preventing infections in the first place may be the best defense against bacterial infections. Optimal control of infection and the transmission of infection involve regular hand washing after contact with patients or surfaces in a hospital, regular disinfection of potentially contaminated surfaces and equipment, and control of patient-to-patient spread through the use of surveillance cultures and contact precautions. It is suggested that the excess costs of infections, particularly, MRSA and VRE, exceed
those of preventing them (Chaix, Durand-Zaleski, Alberti, & Brun-Buisson, 1999).  
Infection control has been convincingly demonstrated in Denmark where the 
nosocomial MRSA infection rate was decreased from 34% to <1% and remained there 
for the past two decades (Monnet, 1999). Efforts to control the prevalence of MRSA 
included antibiotic control and active surveillance cultures with contact isolation for 
colonized patients. Holland and Finland have achieved similarly low MRSA infection 
rates by using this same approach (Jans, Suetens, & Struelens, 2000). Belgium is now 
following this example and reports show that MRSA prevalence is falling (Jans et al., 
2000).

Two studies have reported decreased rates of nosocomial infections associated 
with dramatic increases in hand hygiene compliance (Larson, Early, Cloonan, Sugrue, 
& Parides, 2000; Pittet, Hugonnet, Harbarth, Mourouga, Sauvan, Touveneau, et al., 
2000). Larson et al. (2000) reported that increased hand washing was associated with 
an 85% relative reduction in VRE infection rates. Pittet et al. (2000) reported a 
significant increase in hand disinfection compliance from 48% to 66% over a 3-year 
period, which was associated with a significant decrease in the prevalence of all 
nosocomial infections from 16.9% to 9.9% and a decrease in MRSA transmission 
rates, from 2.16 to 0.93 episodes per 10,000 patient-days.

Decontamination of the environment is also vital in infection control. Wiping 
environmental surfaces with a cloth lightly sprayed with a quaternary ammonium 
compound was reported inadequate for disinfection (Byers, Durbin, Simonton, Anglim, 
Adal, & Farr, 1998). One study found that 15.9% of surfaces were contaminated after 
such routine cleaning, 9.8% were still contaminated after a second cleaning, 10.7%
after a third cleaning, and none after a fourth cleaning (Byers et al., 1998). An alternative “bucket cleaning” method involved dipping a cloth into a bucket of quaternary ammonium compound, and then saturating all environmental surfaces, leaving them wet for 10 minutes, and then drying them with a clean towel. This method was found to remove all contaminating organisms after the first cleaning (Byers et al., 1998).

Using an alcohol pad to wipe the diaphragm of the stethoscope has also been shown to effectively remove resistant pathogens (Marinella, Pierson, & Chenoweth, 1997). Because colonized patients are frequently not recognized in most health care facilities, routine disinfection of stethoscopes between patients has been recommended by the American Medical Association. No compliance surveys or studies to measure the effects of compliance with stethoscope disinfection on the rate of nosocomial infections could be found in the literature.

Quorum Sensing

For hundreds of years, bacteria were believed to exist as individual cells with two goals, finding nutrients and multiplying (de Kievit & Iglewski, 2000). Bacteria, it is now clear, can behave collectively as a group. Hints at the possibility of bacterial group behavior can be found in data obtained over sixty years ago. The luminescent marine bacterium *Vibrio fischeri* was the first to provide solid evidence for a complex bacterial communication system with the objective to coordinate activities of individuals within a population. These organisms express genes controlling light emission when associated with fish or squid symbiotic hosts (Suga & Smith, 2003). Luminescence is initiated by the accumulation of an autoinducer made by the bacteria. The bacteria are
able to sense their cell density by monitoring the autoinducer concentration. Quorum sensing is the name given to this intercellular signaling. The signaling molecule produced by *Vibrio fischeri* was isolated in 1981 and identified as N-(3-oxohexanoyl)-homoserine lactone (OHHL) (Eberhard, Burlingame, Eberhard, Kenyon, Nealson, Oppenheimer, et al., 1981). LuxI is the autoinducer synthase responsible for the synthesis of OHHL. LuxR is a transcriptional activator protein that, when bound to OHHL, promotes transcription of the luciferase structural operon *luxCDABE* (Engebrecht & Silverman, 1984).

In considering an invading pathogen, the ability of a single bacterial cell to communicate with its neighbors is extremely advantageous. Communication among the group allows for the initiation of a unified attack on the host by ensuring that the bacterial cell population has reached a critical mass before individual cells commit to the production of virulence factors. Also, coordinated activity makes certain that the host has insufficient time to mount an effective defense against the invading bacterial population. This cell to cell communication relies upon the production and sensing of small signal molecules, often referred to as autoinducers. Autoinducers, as one would infer, have a positive feedback or regulatory effect in the expression of virulence genes.

Among gram-negative bacteria, several autoinducer families have been identified, however, the most common and understood belong to the N-acylhomoserine lactone (AHL or acyl-HSL) family. Chemically, AHLs consist of a homoserine lactone ring attached, via an amide bond, to an acyl side chain of varying length. All AHLs identified thus far, are 4 to 14 carbons in length, usually in increments
of 2-carbon units, but may be saturated or unsaturated and occur with or without a hydroxy-, oxo-, or no substituent on the third carbon of the N-acyl chain (Figure 1). AHLs with a short acyl side chain diffuse across the bacterial cell membrane, while an AHLs with a long acyl side chain is pumped out of the bacterial cell to accumulate in the extracellular environment. Marine bacteria, several species of proteobacteria, and a number of opportunistic human pathogens, among them Pseudomonas aeruginosa, Aeromonas hydrophila, Burkholderia cepacia, Chromobacterium violaceum, Yersinia pseudotuberculosis, and Serratia marcesens, produce AHLs. Synthesis of the AHL is dependent on a luxI homologue, while a luxR homologue encodes for a transcriptional activator protein.

Gram-positive bacteria are known to most commonly use peptides as autoinducers. They are small and occur in a variety of structures. They are synthesized by precursor peptides and exported out of the bacterial cell via an ATP-binding cassette (ABC) transporter complex (Sturme, Kleerebezem, Nakayama, Akkermans, Vaughan, & de Vos, 2002). Many peptides undergo post-transcriptional modifications in order to enhance functionality and stability (Sturme et al., 2002). They interact with membrane bound sensor kinases that transduce a signal across the cell membrane or they are transported into the cell by oligopeptide permeases, where they then interact with intracellular receptors. Peptide-based signaling molecules are known to be used by several pathogenic bacteria to regulate physiological processes. Bacillus subtilis uses peptides as quorum sensing molecules to regulate development of bacterial competence, while Streptococcus pneumoniae uses peptides for the
process of sporulation. In addition, among *Enterococcus faecalis* and staphylococci, peptide-mediated quorum sensing is involved in virulence gene regulation.

Autoinducer 2 (AI-2) is a furanosyl borate diester with no resemblance to any previously characterized autoinducer. This is the first molecule to have a biological function for boron (Holloway, 2004). AI-2 is believed to allow for interspecies interaction in natural environments. LuxS is the protein responsible for the synthesis of the AI-2 molecule. Recently homologues of LuxS have been found in a remarkably wide range of gram-negative and gram-positive bacteria (Table 4). In every case studied, if a bacterium produced AI-2, it contained a *luxS* gene, and inactivation of *luxS* eliminates AI-2 production. AI-2 is produced from S-adenosylmethionine (SAM) in three enzymatic steps (Schauder, Shokat, Surette, & Bassler, 2001). In *Vibrio harveyi*, AI-2 helps control light production. The role of AI-2 in other species includes: expression of genes required for virulence in *Escherichia coli*, *Vibrio cholerae*, *Clostridium perfringens*, and *Streptococcus pyogenes*; iron acquisition in *Porphyromonas gingivalis* and *Actinobacillus actinomycetemcomitans*; motility in *Campylobacter jejuni*; and mixed-species biofilm formation between *Porphyromonas gingivalis* and *Streptococcus gordonii* (Xavier & Bassler, 2003).

*Pseudomonas aeruginosa* quorum sensing was discovered in 1991 and has gone on to be one of the most studied quorum sensing organisms because of its role as an emerging opportunistic human pathogen. A major contributor to the pathogenesis of *Pseudomonas aeruginosa* is its ability to secrete numerous virulent compounds and degradative enzymes. As *Pseudomonas aeruginosa* enters the stationary phase of growth, these virulent compounds and degradative enzymes are
maximally expressed. Virulence factors include toxins (exotoxin A and exoenzymes), proteases (elastase, LasA protease and alkaline protease) and hemolysins (phospholipase and rhamnolipid). More than six-hundred *Pseudomonas aeruginosa* genes are controlled via quorum-sensing (Schuster, Lostroh, Ogi, & Greenberg, 2003). The late stages of biofilm development are also regulated via quorum sensing.

There are two AHL-dependent quorum sensing systems in *Pseudomonas aeruginosa*. The first is the *las* system, while the second is the *rhl* system (also referred to as *vsm*). Each system has a transcriptional activator protein and an autoinducer molecule. The autoinducers are diffusible molecules produced at a basal level during low cell density and then at higher concentrations as the cell density increases. The *Pseudomonas aeruginosa* autoinducers bind to specific target proteins, the transcriptional activators, and these complexes activate virulence genes.

The *las* system consists of the transcriptional activator, LasR, which is homologous to the LuxR product in the quorum sensing system of *Vibrio fischeri*. GacA, Vfr, and RelA positively regulate the expression of LasR (de Kievit & Iglewski, 2000). The lasI gene codes for a homologous protein to the LuxI protein in *Vibrio fischeri* and directs the synthesis of \(N\)-(3-oxododecanoyl)-L-homoserine lactone (OdDHL) and \(N\)-(3-oxohexanoyl)-L-homoserine lactone (OHHL). At high cell densities, OdDHL, the major autoinducer, reaches a threshold concentration and binds to LasR, creating a transcriptional activator complex that controls the transcription of virulence genes, including lasA (LasA protease), apr (alkaline protease A), toxA (exotoxin A), and lasB (elastase). OdDHL also exhibits immunomodulatory properties. In murine and human leukocyte immunoassays in vitro, OdDHL inhibited lymphocyte
proliferation and tumor necrosis factor alpha production by lipopolysaccharide-stimulated macrophages (Telford, Wheeler, Williams, Tomkins, Appleby, Sewell, et al., 1998). It also down-regulated interleukin-12 production by T-helper cells (Telford et al., 1998) (Figure 2).

The rhl system consists of the transcriptional activator, RhlR, and an autoinducer synthase, RhlI. RhlI directs the synthesis of N-butanoyl-L-homoserine lactone (BHL) and N-hexanoyl-L-homoserine lactone (HHL). BHL, the major autoinducer, binds to RhlR and this complex activates the transcription of rhlA and rhlB, an operon coding for rhamnosyltransferase, which is required for rhamnolipid production and rpoS, a stationary-phase sigma factor important for survival under adverse conditions.

A third LuxR homologue, QscR, was identified by Chugani, Whiteley, Lee, D’Argenio, Manoil, & Greenberg (2001). QscR has been shown to regulate the expression of the genes that code for the LasI and the RhlI AHL synthases (Chugani et al., 2001).

The contribution of quorum sensing to the pathogenesis of Pseudomonas aeruginosa is significant. In a neonatal mouse model of acute pneumonia, a lasR-negative mutant of Pseudomonas aeruginosa showed significantly less virulence than the parent strain. Furthermore, in the same model, a lasI/rhlI double mutant was almost avirulent (Pearson, Feldman, Iglewski, & Prince, 2000). Similar effects were reported in a burned mouse model of infection, where Pseudomonas aeruginosa
strains deficient in \textit{lasR}, \textit{lasI}, or \textit{rhlI}, were less virulent than the parent strain (Rumbaugh, Griswold, Iglewski, & Hamood, 1999).

The two quorum sensing systems of \textit{Pseudomonas aeruginosa} are known to be linked to each other with the \textit{las} system dominant over the \textit{rhl} system. The \textit{las} system controls the \textit{rhl} quorum sensing system in two ways. First of all, LasR and OdDHL positively regulate the expression of rhlR by serving as transcriptional activators of rhlR (Pesci & Iglewski, 1997). LasR and OdDHL also control the activity of RhlR at the post-translational level. OdDHL has been shown to stop BHL from binding to RhlR, therefore inhibiting expression of genes (Pesci & Iglewski, 1997).

There is evidence of interspecies communication via quorum sensing. This is referred to as quorum sensing cross-talk. \textit{Pseudomonas aeruginosa} and \textit{Burkholderia cepacia} are opportunistic pathogens causing chronic infections in cystic fibrosis patients. During chronic infection, the two bacteria were capable of forming mixed biofilms in the lung tissue of patients by communicating via small diffusible molecules (Govan & Deretic, 1996). Communication between the two bacteria occurred in a unidirectional manner, from \textit{Pseudomonas aeruginosa} to \textit{Burkholderia cepacia} (Govan & Deretic, 1996). Cross-talk has also been demonstrated between \textit{Serratia liquefaciens} and \textit{Pseudomonas aeruginosa} (Rasmussen, Manefield, Andersen, Eberl, Anthoni, Christophersen, et al., 2000).

Preserving the Effectiveness of Antibiotics

Antibiotic prescribing is usually empiric, without time-consuming laboratory confirmation of infection. It is irrefutable that antibiotic use promotes resistance development. Not only the amount of an antibiotic used, but also the number of
individuals receiving the drug and the population density, selects for resistance. Giving 1,000 doses of an antibiotic to one individual will have considerably less ecological effect on resistance emergence than giving 1,000 doses to 1,000 individuals (Levy, 2001). Scientists and healthcare professionals are in agreement that a means to decrease antibiotic resistance is through more cautious use of antibiotic drugs and through monitoring outbreaks of drug-resistant infections. Research though is also critical to help understand the various mechanisms that pathogens use to evade drugs. Understanding these mechanisms is important for the design of effective new drugs.

The FDA is working to encourage the development of new antibiotics and new classes of antibiotics. An accelerated approval process exists for drugs that treat severely debilitating or life-threatening diseases and for drugs that show meaningful benefit over existing prescription drugs to cure a disease. Another approach is to reduce the size of the clinic trial program without compromising safety and effectiveness.

Using antibiotics with poor activity or administering them at an inappropriate dosing level, dosing route, dosing frequency, or for prolonged duration increases the opportunity for selection of resistant strains. Guillemot, Carbon, Balkan, Geslin, Lecoeur, Vauzella-Kervroedan, et al. (1998) found that children treated with low daily doses of an oral β-lactam had an increased risk of penicillin-resistant Streptococcus pneumoniae carriage compared to children who did not receive an antibiotic. Another study showed higher penicillin resistance of Streptococcus pneumoniae in children who had taken β-lactam antibiotics for more than 14 days compared with a group
taking no antibiotics or taking them for less than seven days (Nasrin, Collignon, Roberts, Wilson, Pilotto, & Douglas, 2002). Injectable antibiotics can be used to ensure higher and more persistent tissue and nasopharyngeal concentrations than orally administered drugs, as was demonstrated in Italy with a relatively low incidence of bacterial resistance among pathogens responsible for community-acquired infections (Guillemot et al., 1998). The study by Huchon, Gialdroni-Grassi, Leophonte, Manresa, Schaberg and Woodhead (1996) showed that in Italy, 53% of outpatient lower respiratory tract infections were treated with parenteral antibiotics, compared with 10% in France, 8% in Spain, 1.2% in Germany, and 0.2% in the United Kingdom.

Patient compliance is a major contributor to the development of antibiotic resistance. Many people stop taking antibiotics once their symptoms have resolved yet before bacterial eradication is complete. This can lead to re-infection and selection of resistant strains. A recent international survey reported that 69% of patients completed their most recent course of antibiotics and 24% saved part of the antibiotic course for future use (Pechere, 2001).

Every year, tens of millions of prescriptions are written to treat viral illnesses for which antibiotics offer no benefit. Viruses cause the common cold, the flu, and most sore throats. In fact, only 15% of sore throats are caused by the bacterium Streptococcus, which results in strep throat. Physicians’ reasons for over-prescribing antibiotics include diagnostic uncertainty, patient demand, and time pressure. In a study by McFarlane, Holmes, McFarlane, and Britten (1997) patient pressure most commonly influenced the decision to prescribe an antibiotic when a doctor thought it was unwarranted. It was also found that patients who did not receive an antibiotic were
prone to express dissatisfaction and were twice as likely to re-attend for the same episode, as satisfied patients (McFarlane et al., 1997). In a survey of 915 pediatricians randomly chosen by the American Academy of Pediatrics, 40% responded that ten or more times in the past month a parent had requested an antibiotic when the physician did not feel it was indicated (Bauchner, Pelton, & Klein, 1999). According to the CDC, antibiotic prescribing in outpatient settings could be reduced by more than 30% without adversely affecting patient health.

The American Academy of Family Physicians, American College of Physicians, American Society of Internal medicine, Centers for Disease Control and Prevention (CDC), and Infectious Diseases Society of America (IDSA) collaborated in developing clinical practice guidelines for the appropriate use of antibiotics in treating patients with respiratory tract infection. For example, guidelines for when to use antibiotics in the treatment of patients with a sore throat include: 1. Not prescribing an antibiotic for a sore throat unless there is a positive test for strep throat or another positive bacterial culture; 2. Strep throat should be diagnosed with a Rapid Strep A test and not based solely on physical examination of the throat; 3. Penicillin is the best treatment choice for a confirmed infected patient, unless there is an allergy; 4. New, broad-spectrum antibiotics should be avoided.

A report in 2002, showed a dramatic decline in the prescribing of antibiotics to children and adolescents in doctors’ offices as a result of treatment guidelines. Between 1989 and 2000, office-based prescribing to children decreased by 47% (McCaig, Besser, & Hughes, 2002). During this same timeframe, a shift occurred from using targeted therapy to using broad-spectrum agents. By 1999, almost half of all
antibiotics prescribed to adults and 40% of those prescribed to children were broad
presented explanations for the trends they observed, citing in particular the influence
of pharmaceutical marketing on prescribers. In 2001, the 14 largest pharmaceutical
companies spent $9 billion on marketing their products to primary care physicians and
consumers (Petersen, 2002). No marketing was undertaken for targeted agents, such
as penicillin, amoxicillin, and erythromycin, which are no longer under patent
protection (Petersen, 2002).

The Alliance for the Prudent Use of Antibiotics (APUA), an internationally based
group, emerged in 1981 and present membership extends to more than 100 countries
of the world. It is the only organization of its kind that communicates basic tenets of
proper antibiotic usage and the problems of antibiotic resistance. This group is making
people all over the world cognizant of the resistance problem. The Centers for Disease
Control and Prevention (CDC) has also initiated activities for decreasing inappropriate
antibiotic use. One component of the program was the development of practice
guidelines for antibiotics. Another involves providing videos, pamphlets, and other
materials to educate physicians and the general public. In 2000, the CDC launched a
campaign called “GET SMART Know When Antibiotics Work”. Partners of this
program include state and local health agencies, pharmaceutical companies, and
professional organization, just to name a few. Currently, there are 30 federally funded
sites involved in the program.

The CDC estimates that over 40% of the total production of antibiotics in the
United States is used in livestock, whereby 80% of this amount is used in sub-
therapeutic doses as growth promoters. Antibiotics given to food-producing animals for therapeutic, disease prevention, or production reasons, inherently increases the potential for humans to develop microbial resistance to similar antibiotic drugs used to treat human illness. This is especially true for humans who have direct contact with animals or who consume undercooked meat from an antibiotic feed animal. In a study by White, Zhao, Sudler, Ayers, Friedman, Chen, et al. (2001), 200 samples of ground chicken, beef, turkey, and pork were obtained from three supermarkets and tested. Salmonella was present in 20% of the samples and 84% of the salmonella isolates were resistant to at least one antimicrobial agent (White et al., 2001).

At a WHO meeting on the “Medical Impact of the Use of Antimicrobial Drugs in Food Animals” in Berlin, 1997, health officials concluded that the practice of giving antimicrobial agents to food-producing animals for growth promotion should be terminated and that programs of “prudent antimicrobial use in food animals should be developed to reduce the risks of selection and dissemination of antimicrobial resistance” (WHO, 1997). Currently, FDA’s National Center for Toxicological Research (NCTR) is studying the amount of antibiotics that people consume in food from food-producing animals and the effects of these antibiotics on human intestinal bacteria.

Currently, there is speculation of antibacterial resistance increasing as a result of antibacterial substances being incorporated into household products. In June of 2000, the American Medical Association (AMA) began urging people not only to stop over-using antibiotic medications, but to curb buying antibacterial soaps and detergents as well. Only a handful of household products containing antibacterial agents were available in the mid-1990s. Today, several hundred varieties of soaps,
hand lotions, cleansers, and dishwashing detergents are available. One of the common antibacterial agents found in consumer products is triclosan. Research shows that triclosan has the ability to target bacteria and destroy it, which forces the targeted bacteria to mutate and eventually become resistant to triclosan. Using traditional cleaning agents, such as bleach or hydrogen peroxide, to remove dirt is the preferred choice since they do not encourage bacterial mutation.

The above mentioned measures to control the problem of antibiotic resistance has the potential to preserve the life of life-saving gift of antibiotics, but it is believed that pathogens will be victorious in the end. Single-celled organisms have been around for more than three billion years. Their numbers, short generation times, and ability to exchange genes make them flexible.

Interfering with Quorum Sensing

The range of bacterial activities governed by quorum sensing is large and continues to grow. Phenomena regulated by quorum sensing includes: symbiotic associations with multicellular organisms, expression of virulence factors, bacterial motility, entry into the stationary phase of growth, biofilm communities, bacterial mating, and colonization of eukaryotic hosts. It is safe to conclude that quorum sensing is a novel approach to controlling bacterial growth and virulence. Many bacteria have both a species-specific and universal mechanism of quorum sensing. Also, it seems less likely to result in the development of selective resistance. Biotechnology companies, among them QSI Pharma A/S, Microbia, Quorex Pharmaceuticals, MerLion Pharmaceuticals, Athelas, and 4SC AG, have begun to develop anti-quorum-sensing drugs.
Production of acyl homoserine lactones (AHLs) involve a sequentially ordered reaction that uses S-adenosylmethionine (SAM) as an amino donor for generation of the homoserine lactone ring and a charged acyl carrier protein (ACP) as the precursor for the acyl side chain (Figure 3). Reactions are catalyzed by specific AHL synthases, which exhibit acyl chain specificity. The knowledge about signal generation can be exploited to develop quorum-sensing inhibitor molecules that target AHL signal generation. Analogs of SAM, such as S-adenosyl homocysteine and S-adenosyl cysteine, have been demonstrated to be potent inhibitors of AHL synthesis catalyzed by the *Pseudomonas aeruginosa* RhlI protein (Parsek, Val, Hanzelka, Cronan, & Greenberg, 1999). SAM is a necessary and common intermediate in many prokaryotic and eukaryotic pathways. The potential exists for the use of SAM analogs to serve as specific inhibitors of quorum-sensing signal generation without affecting eukaryotic enzymes that use SAM as a substrate.

Quorum sensing genes are positively regulated by GacA, Vfr, and RelA. Deletion of *vfr* significantly eliminated the expression of *lasR* and reduced the production of virulence factors (Whitehead, Barnard, Slater, Simpson, & Salmond, 2001). Additionally, a deletion of GacA, resulted in reduced production of many virulence factors and a decrease in pathogenesis (Whitehead et al., 2001). The use of anti-sense oligonucleotides that specifically bind to *lasR/lasI* or *rhlR/rhlI* transcripts are another approach to the inhibition of quorum sensing. Studies in *Staphylococcus aureus*, *Mycobacterium tuberculosis*, and *Escherichia coli* have successfully demonstrated that anti-sense oligonucleotides can specifically bind to target transcripts and inhibit gene expression (Good, Awasthi, Dryselius, Larsson, & Nielsen,
An approach of this type has several obstacles to overcome if it is going to be used as a therapeutic method, such as cell wall permeability and the efficacy of the delivery mode.

Signaling molecules diffuse or are actively pumped out of the bacterial cell. When they appear in the extracellular environment, they are potential targets for destruction or inactivation. Bacterial quorum sensing can be inhibited by a decrease in the active autoinducer molecule concentration in the environment. Autoinducer degradation could be a result of an enzymatic reaction or due to hydrolysis at an elevated pH or elevated temperature (Yates, Philipp, Buckley, Atkinson, Chhabra, Sockett, et al., 2002). *Bacillus* species produce an enzyme, AiiA, which catalyzes the hydrolysis of AHL molecules (Dong, Xu, Li, & Zhang, 2000). The enzyme acts to cleave the lactone ring (lactonolysis) and thus produce nonfunctional molecules that are unable to activate their transcriptional regulators. Expression of the aiiA gene in the plant pathogen *Erwinia carotovora* resulted in reduced release of AHL signals, decreased extracellular pectolytic enzyme activity, and attenuated soft rot disease symptoms (Dong et al., 2000). Other bacteria, known to produce lactonase enzymes include *Variovorax paradoxus*, *Arthrobacter* species, and *Agrobacterium tumefaciens* (Smith). AHL-degrading enzymes are of great clinical interest for use in the prevention of diseases caused by quorum sensing bacterial populations. Enzymes of this nature could conceivably be used topically on *Pseudomonas aeruginosa* infected burn wounds, but they are unlikely to be useful for systemic administration.

The potential of using AHL-specific antibodies to inhibit AHL activity has been evaluated by Smith and Iglewski (2003). After AHLs diffuse out of the bacterial cell into
the extracellular environment, they can be bound by the antibodies and prevented from interacting with eukaryotic cells or from re-entering the bacterial cell and activating their transcriptional regulators. Preliminary experiments have demonstrated that monoclonal antibodies, made from an OdDHL protein conjugate, were able to inhibit OdDHL activation of a lasB transcriptional reporter in *Pseudomonas aeruginosa* (Smith & Iglewski, 2003).

Blocking of quorum-sensing autoinducer transduction can be achieved by an antagonist molecule capable of competing or interfering with the native AHL signal for binding to the receptor. Competitive inhibitors would be structurally similar to the native AHL signal, in order to bind to and occupy the AHL-binding site, but fail to activate the receptor. Noncompetitive inhibitors would show little or no structural similarities to AHL signals and would bind to different sites on the receptor protein.

Several studies have been performed to demonstrate the likelihood of AHL analogues to inhibit quorum sensing pathways of bacteria. The studies have generated substantial evidence in relation to the structure-function relationships of AHL signals. A strong structure-function relationship exists for AHL molecules and only AHL analogues with conservative changes are capable of binding to the receptor. The acyl side chain has been modified and it has been shown that the length is crucial to activity (Chhabra, Stead, Bainton, Salmond, Stewart, Williams, et al., 1993). In one study of quorum sensing in *Erwinia carotovora*, it was reported that increasing the length of the acyl side chain by one methylene unit reduced activity by 50%, whereas a two-unit extension reduced activity by 90% (Chhabra et al., 1993). Decreasing the chain length by one methylene unit decreased activity by 10% (Chhabra et al., 1993).
Flexibility of the acyl side chain is also important for binding to the receptor. Reduction of the chain rotation by introduction of an unsaturated bond close to the amide linkage almost completely abolishes binding to the receptor (Zhu). No natural AHL signal has been reported to contain a 2,3 unsaturated bond (Zhu).

The homoserine lactone moiety of AHL signals is highly sensitive to modifications. The chirality of the molecule is crucial to biological activity. Natural AHL signals are L-isomers. D-isomers generally are devoid of biological activity (Chhabra et al., 1993). The homoserine lactone moiety also appears to be sensitive to changes in composition and ring size. Conversion of the homoserine lactone ring to a homoserine lactame ring results in a molecule without agonistic or antagonist properties (Chhabra et al., 1993).

Research has lead to the discovery of an algae that produces compounds that mimic quorum sensing autoinducer molecules, but cause inhibition of quorum sensing genes. The marine red algae, *Delisea pulchra*, produces at least 30 different halogenated furanones that are similar in structure to AHLs (Manefield, de Nys, Kumar, Read, Givskov, Steinberg, et al., 1999). These halogenated furanones inhibited expression of quorum sensing genes among gram-negative bacteria most likely by binding to the AHL receptor proteins (competitive antagonist) (Manefield et al., 1999). In the natural environment, these furanone inhibitors of *Delisea pulchra* appear to have a potent effect on the community of bacteria that colonizes the algal surfaces. A shift in the community of bacteria has occurred from gram-negative bacterial species to gram-positive species, which are relatively poor at colonizing marine surfaces (Manefield et al., 1999). Natural halogenated furanones are highly
reactive and too toxic for the treatment of bacterial infections in humans. Currently, numerous analogs of furanone molecules are being synthesized and tested for their effects on pathogenic bacteria such as *Pseudomonas aeruginosa*. A synthetic halogenated furanone compound has been found to penetrate *Pseudomonas aeruginosa* micro-colonies, inhibit quorum sensing regulated virulence factors, and alter the architecture of a biofilm (Hentzer, Riedel, Rasmussen, Heydorn, Andersen, Parsek, et al., 2002).

Hiroaki Suga, associate professor of chemistry at the State University of New York at Buffalo, discovered a compound that blocks biofilm formation among *Pseudomonas aeruginosa* bacteria. Suga and his colleagues synthesized a library of compounds aimed at interacting with *Pseudomonas aeruginosa*’s autoinducer, OdDHL. Among the group of synthetic agonists was an antagonist, which they named compound 3. Compound 3 competes with the natural autoinducer, binds, and inhibits the quorum-sensing system, resulting in inhibition of virulence-factor production. The next step involves testing compound 3 in infected animals.

Anbics, a privately owned biopharmaceutical company founded in 1999, is in the process of developing the first and most advanced quorum sensing inhibitor. This inhibitor, ANB006, is designed for use in humans with *Pseudomonas aeruginosa* related infections. ANB006 works by interfering with the quorum sensing pathways of *Pseudomonas aeruginosa* and therefore reduces its virulence potential. Based on data generated by Anbics, ANB006 is believed to have significant quorum sensing inhibition properties at low drug concentrations and a favorable safety profile. ANB006 is expected to be the first quorum sensing inhibiting drug to market and because it does
not affect vital functions of *Pseudomonas aeruginosa*, resistance is less likely to develop (www.anbics.com). A randomized, double-blind placebo-controlled Phase II clinical trial was initiated in 2002 to investigate the therapeutic potential of ANB006 as a major indication for *Pseudomonas aeruginosa* related infections. The study enrolled approximately 200 patients in 25 centers throughout Europe. If the results support continuation of the study, Anbics will submit an Investigational New Drug (IND) Application with the Food and Drug Administration in the second half of 2004 (www.anbics.com).

**Quorex Pharmaceuticals**, based in Carlsbad, California, was founded on the mission of discovering, developing, and commercializing a new class of broad spectrum antibiotics for the treatment of multi-drug resistant bacterial infections. Quorex technology is based on the discovery from Bonnie Bassler’s laboratory at Princeton University. It was found that a new quorum sensing pathway is used by a broad range of bacteria to regulate gene expression in response to density changes. The bacteria communicate population density information by synthesizing and detecting the accumulation of an autoinducer molecule. At a low cell density and autoinducer concentration, virulence pathways are turned off. As the cell density and autoinducer concentration increases, virulence pathways are turned on. The Quorex anti-infective drug acts to block the receptors for the autoinducer molecule, thereby blocking the mechanism that turns on the virulence factors and rendering the pathogen harmless (www.schuchman.com/Qwebsite/QuorexHTM).

Quorex drug development is structured around the natural ligand, AI-2. Analogue compounds are being designed are screened by a highly sensitive,
luminescent screening system. Some of the bacterial pathogens that carry the signalling pathway targeted by Quorex includes: *Staphylococcus aureus*, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Mycobacterium tuberculosis*, *Escherichia coli* O157:H7, *Haemophilus influenzae*, *Helicobacter pylori*, and *Neisseria meningitidis*. Recent findings indicate that Quorex compounds are effective agents when administered alone, but when combined with existing antibiotics, they significantly potentiate the killing action of the antibiotics, by up to 60 times, in vitro (www.schuchman.com/Qwebsite/QuorexHTM).

MerLion Pharmaceuticals, a privately-owned company, based in Singapore, focuses on the discovery and development of compounds for new drugs. In April of 2003, MerLion entered into a two-year drug discovery and pre-clinical research collaboration to identify compounds for anti-virulence with Athelas, a privately-owned, Geneva-based biopharmaceutical company.

**Conclusion**

Antimicrobial agents were introduced into medical practice approximately 60 years ago. Since then, the prevalence of antimicrobial resistance in community-acquired and hospital-acquired pathogens has increased worldwide. There is a distinct relationship between the consumption of antibiotics and the prevalence of drug resistance. The use of antibiotic in animal, agriculture, and aquaculture should be reduced. High consumption of antibiotics, particularly of broad-spectrum antibiotics, in the community should be avoided. The most common, narrow-spectrum antibiotics should be used whenever possible. Long-term studies that record resistance and antibiotic use patterns should also be performed. Patients should be educated by the
physician to take their antibiotic until the bottle is empty and to never save leftover antibiotics to use at a later time. The invention of new antibiotics that have a new mechanism for fighting pathogens should be encouraged as should the development of vaccines against common microbial diseases.

Several distinct quorum sensing chemical languages are used by bacterial pathogens. Due to the lack of a truly universal bacterial language, therapeutic agents capable of blocking quorum sensing are likely to have a narrower spectrum of activity than conventional antibiotics. It is probable that the selective pressure for the emergency of resistance will be lessened. In theory, these drugs should have the benefit of causing less disturbance in the host’s norma flora. Unlike conventional antibiotics, quorum sensing inhibitors will not kill bacteria or inhibit bacterial growth, but will attenuate virulence, allowing the host’s defense mechanisms to clear the pathogen. Since the mode of action of quorum sensing inhibitors is based on host defenses, it is unlikely that they will be suitable to treat immunocompromised patients. In order for anti-quorum sensing drugs to be successful, they need to demonstrate their efficacy and their advantage over traditional antimicrobials. No such drugs have yet been approved for release, but several drugs are claimed to be in late-phase clinical trials.
References


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Table 1

Introduction of new classes of antibiotics into clinical practice.

<table>
<thead>
<tr>
<th>Year</th>
<th>Antibiotic class</th>
</tr>
</thead>
<tbody>
<tr>
<td>1936</td>
<td>Sulfonamides</td>
</tr>
<tr>
<td>1940</td>
<td>ß-Lactams</td>
</tr>
<tr>
<td>1947</td>
<td>Chloramphenicol</td>
</tr>
<tr>
<td>1948</td>
<td>Tetracyclines</td>
</tr>
<tr>
<td>1950</td>
<td>Aminoglycosides</td>
</tr>
<tr>
<td>1952</td>
<td>Macrolides</td>
</tr>
<tr>
<td>1958</td>
<td>Glycopeptides</td>
</tr>
<tr>
<td>1962</td>
<td>Quinolones</td>
</tr>
<tr>
<td>1962</td>
<td>Streptogramins</td>
</tr>
<tr>
<td>1964</td>
<td>Cephalosporins</td>
</tr>
<tr>
<td>1980</td>
<td>Carbapenems</td>
</tr>
<tr>
<td>2000</td>
<td>Oxozolidinones</td>
</tr>
<tr>
<td>2003</td>
<td>Cyclic lipopeptides</td>
</tr>
</tbody>
</table>

(Information obtained from Levy, 1992; and Amyes, 2001)
Table 2

Development of antibiotic resistance.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Year Deployed</th>
<th>Resistance Observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonamides</td>
<td>1930s</td>
<td>1940s</td>
</tr>
<tr>
<td>Penicillin</td>
<td>1943</td>
<td>1946</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1943</td>
<td>1959</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>1947</td>
<td>1959</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1948</td>
<td>1953</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1952</td>
<td>1988</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1956</td>
<td>1988</td>
</tr>
<tr>
<td>Methicillin</td>
<td>1960</td>
<td>1961</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1961</td>
<td>1973</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>1960s</td>
<td>late 1960s</td>
</tr>
</tbody>
</table>

(Information obtained from Walsh, 2003)
### Table 3

**Overview of the most serious antibiotic-resistant bacteria.**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Resistance to</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococcus</em> species</td>
<td>Aminoglycosides, erythromycin, penicillin, tetracycline, vancomycin</td>
</tr>
<tr>
<td><em>Mycobacterium</em> species</td>
<td>Aminoglycosides, ethambutol, isoniazide, pyrazinamide, rifampin</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Aminoglycosides, β-lactams, ciprofloxacin, chloramphenicol, tetracycline, sulfonamides</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>Ampicillin, chloramphenicol, streptomycin, sulfonamides, tetracycline</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>β-lactams, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, rifampin, tetracycline, trimethoprim, vancomycin</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Chloramphenicol, cefuroxime, erythromycin, penicillin, trimethoprim, tetracycline</td>
</tr>
</tbody>
</table>

(Information obtained from Walsh, 2003)
Table 4

*Bacteria containing a luxS homolog.*

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td><em>Mycobacterium tuberculosis</em></td>
</tr>
<tr>
<td><em>Lactococcus lactis</em></td>
<td><em>Deinococcus radiodurans</em></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td><em>Neisseria gonorrhoeae</em></td>
<td><em>Enterococcus faecalis</em></td>
</tr>
<tr>
<td><em>Neisseria meningitides</em></td>
<td><em>Haemophilus influenzae</em></td>
</tr>
<tr>
<td><em>Pasteurella multocida</em></td>
<td><em>Helicobacter pylori</em></td>
</tr>
<tr>
<td><em>Porphyromonas gingivalis</em></td>
<td><em>Shewanella putrefaciens</em></td>
</tr>
<tr>
<td><em>Proteus mirabilis</em></td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td><em>Salmonella paratyphi</em></td>
<td><em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td><em>Salmonella typhi</em></td>
<td><em>Streptococcus gordonii</em></td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td><em>Streptococcus mutans</em></td>
</tr>
<tr>
<td><em>Bacillus anthracis</em></td>
<td><em>Streptococcus pneumoniae</em></td>
</tr>
<tr>
<td><em>Bacillus halodurans</em></td>
<td><em>Streptococcus pyogenes</em></td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td><em>Vibrio anguillarum</em></td>
</tr>
<tr>
<td><em>Borrelia burgdorferi</em></td>
<td><em>Vibrio cholerae</em></td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td><em>Vibrio harveyi</em></td>
</tr>
<tr>
<td><em>Clostridium acetobolyticum</em></td>
<td><em>Vibrio vulnificus</em></td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td><em>Yersinia pestis</em></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td></td>
</tr>
</tbody>
</table>

(Information obtained from Surette, Miller, & Bassler, 1999)
**Table 5**

*Quorum sensing bacteria and the infections they cause.*

<table>
<thead>
<tr>
<th>Gram-positive Organism</th>
<th>Manifestations</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Cutaneous Lesions</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Food Poisoning</td>
</tr>
<tr>
<td></td>
<td>Bacteremia</td>
</tr>
<tr>
<td></td>
<td>Osteomyelitis</td>
</tr>
<tr>
<td></td>
<td>Endocarditis</td>
</tr>
<tr>
<td></td>
<td>Scalded Skin Syndrome</td>
</tr>
<tr>
<td></td>
<td>Toxic Shock Syndrome</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>Pneumonia</td>
</tr>
<tr>
<td></td>
<td>Pericarditis</td>
</tr>
<tr>
<td></td>
<td>Empyema</td>
</tr>
<tr>
<td></td>
<td>Pleurisy</td>
</tr>
<tr>
<td></td>
<td>Bacteremia</td>
</tr>
<tr>
<td></td>
<td>Otitis Media</td>
</tr>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>Scarlet fever</td>
</tr>
<tr>
<td></td>
<td>Septicemia</td>
</tr>
<tr>
<td>Gram-negative Organism</td>
<td>Manifestations</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>----------------------------------------------------</td>
</tr>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>Food Poisoning</td>
</tr>
<tr>
<td><em>Burkholderia cepacia</em></td>
<td>Opportunistic Pneumonia</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Bacteremia</td>
</tr>
<tr>
<td></td>
<td>Endocarditis</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (O157:H7)</td>
<td>Dysentery</td>
</tr>
<tr>
<td></td>
<td>Traveler's Diarrhea</td>
</tr>
<tr>
<td></td>
<td>Hemolytic Uremic Syndrome (HUS)</td>
</tr>
<tr>
<td></td>
<td>Neonatal Meningitis</td>
</tr>
<tr>
<td></td>
<td>Urinary Tract Infections</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em></td>
<td>Childhood Bacterial Meningitis</td>
</tr>
<tr>
<td></td>
<td>Epiglottis</td>
</tr>
<tr>
<td></td>
<td>Cellulitis</td>
</tr>
<tr>
<td></td>
<td>Bacteremia</td>
</tr>
<tr>
<td></td>
<td>Otitis Media</td>
</tr>
<tr>
<td><em>Helicobacter pylori</em></td>
<td>Peptic Ulcers</td>
</tr>
<tr>
<td><em>Neisseria meningitides</em></td>
<td>Epidemic Bacterial Meningitis</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>Burn Infections</td>
</tr>
<tr>
<td></td>
<td>Urinary Tract Infections</td>
</tr>
<tr>
<td></td>
<td>Opportunistic Pneumonia</td>
</tr>
<tr>
<td></td>
<td>Otitis Externa</td>
</tr>
<tr>
<td></td>
<td>Eye Infection</td>
</tr>
<tr>
<td>Acid-fast Organism</td>
<td>Manifestations</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Tuberculosis</td>
</tr>
</tbody>
</table>
Figure 1

*Acyl homoserine lactone molecules.*

\[ N-(3\text{-oxohexanoyl})\text{-L-homoserine lactone (OHHL)} \]

\[ N-(3\text{-oxododecanoyl})\text{-L-homoserine lactone (OdDHL)} \]

(Figures obtained from http://info.bio.cmu.edu/Courses/03441/TermPapers/99TermPapers/Quorum/nahl.html)
Figure 2

*Pseudomonas aeruginosa* *las* and *rhl* quorum sensing pathways.

(Figure adapted from Whitehead, Barnard, Slater, Simpson, & Salmond, 2001, Figure 6, pp. 376)
Figure 3

*S-adenosyl methionine.*

(Figure obtained from http://www.pdrhealth.com/drug_info/nmdrugprofiles/nutsupdrugs/sad_0231.shtml)
Abstract

Objective

To educate healthcare providers of the current state of antibiotic resistance, the measures needed to prolong the effectiveness of current antibiotics, and the innovative therapeutic measures being developed to treat infections caused by bacteria.

Method

A comprehensive literature review of articles obtained from MEDLINE, PubMed, CDC, FDA, NIH, and WHO.

Results

As the prevalence of bacterial resistance to multiple antibiotics increases it is becoming progressively more difficult to treat infections. There is growing urgency to search for novel targets and the development of new antimicrobial agents. Many gram-positive and gram-negative pathogens communicate via the production and sensing of small, diffusible signaling molecules, to coordinate production of virulence products. This communication, known as quorum sensing, represents a novel therapeutic target to attenuate virulence.

Conclusion

Biotechnology companies have begun to develop anti-quorum sensing drugs that act by inhibiting signaling molecule generation, signaling molecule dissemination, and signaling molecule reception.