GENETICS OF ANTIBIOTIC RESISTANCE
IN STAPHYLOCOCCUS SPECIES

A SCHOLARLY PAPER PRESENTED TO
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Genetics of Antibiotic Resistance

In *Staphylococcus* Species

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SCHOLARLY PAPER APPROVED

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Scholarly Advisor

Date

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Dean of Graduate School

Date
I. Introduction

A. General Problem of Antibiotic Resistance to Medicine

Antibiotics are chemical substances used to prevent and treat infections caused by microorganisms, such as bacteria, parasites and fungi. Antibiotic medications play a role by suppressing or inhibiting the growth of microorganisms (Sanders et al. 2011). There are more than 150 antibiotics currently available, but only 125 are effectively used for treatment (Zuchora-Walske 2014). Antibiotics have benefitted society (Fabbretti et al. 2011) by providing treatments for tuberculosis, gonorrhea, methicillin-resistant *Staphylococcus aureus* (MRSA), bronchitis, urinary infections, and many others (Tindall et al. 2013). Yet, antibiotic resistance has become a worldwide issue that has expanded and persisted among all nations on earth. New strains of antibiotic-resistant microbes migrate easily, and they often create a “worldwide threat” to society (Levy 2002).

Developing countries have been impacted greatly due to malnutrition, poor living conditions, and lack of medical sources (Alanis 2005). Over-the-counter antibiotics can be obtained more easily in developing countries than in developed nations where their access is more restricted (Alanis 2005). Resistant strains evolved during times of environment instability, such as war, and migrations introduced new strains in communities. Some antibiotics are no longer effective or efficient to treat infections (Baquero and Campos 2003). The biggest challenge is when the most clinically significant pathogens become resistant to most widely effective drugs (Lee et al. 2013).

One of the settings in which antibiotic resistance is most concerning is a hospital. In the United States (US), numerical data for antibiotic resistance have been collected from
hospitalized patients since 2002 (Collins 2008). Yearly, more than 35 million US patients require hospital care due to infection or disease (Wenzel and Edmond 2001). Additionally, exposure to microorganisms in intensive care units presents a 60% risk of acquiring nosocomial infections. In other words, these infections that are caused by pathogens that acquired resistance to antibiotics (Haddadin et al. 2002). Nearly 2 million patients develop hospital-acquired infections during treatment, 55% of which involve antibiotic-resistant bacteria (Stone 2009).

Some bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), have become resistant to antibiotics and spread throughout hospitals, clinical settings, and other areas. Nowadays, there are only a few effective drugs that can control MRSA (David and Daum 2010). In the U.S., National Nosocomial Infections Surveillance (NNIS) stated that approximately 60% of *S. aureus* infections derived from intensive care units were methicillin resistant, and the number of infections rose from 29% in 2005 to 49% in 2009 (Sydnor and Perl 2011). Vancomycin resistance in *Enterococcus faecium* infections increased from 9,829 infections in 2000 to roughly 22,000 infections in 2006 (Sydnor and Perl 2011). With restricted options to cure infections, healthcare providers propose to increase medical fees and introduce patients to medications with potentially harmful? side effects (David and Daum 2010). Without effective antimicrobial agents, any individual with an infection is approximately 70% more likely to die (Frieden et al. 2013).

Researchers have seen a consistent and rapid increase in antibiotic resistance, especially in recent years. Sydnor (2011) stated that hospital epidemics can be minimized if accurate surveillance of nosocomial infections are installed, best practices
are implemented to prevent and treat them, and health care personnel are trained to avoid transmission of infectious microbes from patient to patient (Sydnor et al. 2011). As of 2011, the Netherlands was reported to have the lowest antibiotic consumption rate in Europe, and this country believes antibiotic resistance can be prevented by reducing the dosage of antibiotics prescribed to a patient (Vandenbroucke - Grauls 2014).

Community environments have also shown signs of bacterial infections that are resistant to antibiotics. Vancomycin resistance in *Enterococcus faecium* infections have shown an increase percentage of 124% between 2000 to 2006 (Sydnor and Perl 2011). For instance, *Mycobacterium tuberculosis* is a community-acquired pathogen responsible for tuberculosis (TB), which infects 30% of the human population and accounts for nearly 2 million deaths annually (Jensen et al. 2005). Until 2002, streptomycin was routinely used to treat patients with tuberculosis (Gillespie 2002). Drug resistance developed because *M. tuberculosis* was continuously exposed to a single drug. Gillespie studies (2002) showed that a combination of isoniazid, pyrazinamide, rifampin, and ethambutol drugs were able to control and prevent tuberculosis from expanding (Gillespie 2002). As of 2014, a combination of multiple drugs diminished the number of spontaneous mutations and increased the treatment rate by 48% (Fonseca et al. 2015). Hence, antibiotic-resistant pathogens have become a major concern not only in the healthcare facility, but also in community settings (Tomasz 1994).

**B. Mortality Rate**

Mortality and morbidity rates are escalating due to the prevalence of antibiotic resistance strains in intensive care units (Hanberger et al. 2014). Non-resistant strains are nonpathogenic, while antibiotic resistant strains are pathogenic, leading to an
increase death rate (Hanberger et al. 2014). In the US, about 2 million people developed bacterial infections from 2005 through 2008 that were resistant to more than one antibiotic, resulting in an estimated 99,000 deaths (Kallen et al. 2010). In 2011, the EU had nearly 2 million people with nosocomial infections that lead to 200,000 deaths. In the EU, housing of vulnerable patients within the same area increases the probability of resistant strains to pass from one person to another (Guggenbichler et al. 2011).

Based on Table 1, multi-drug resistance (MDR) *M. tuberculosis* (TB) is one of the three organisms that causes the highest mortality in humans. Highly diverse populations and poor sanitation leads to development of multidrug resistance, which contributes to 95% of mortality rates in low- and middle-income states (Drobniewski et al. 2015). As of 2007 in India, MDR TB was twice as common in TB patients living with HIV versus in TB patients without HIV because patients were showing spontaneously resistant mutants in reserve drugs, such as ofloxacin (Isaakidis et al. 2011).

Despite the fact that MDR TB is curable, its treatment depends upon extensive chemotherapy that is exceptionally costly for low-income nations. Efforts to prevent the spread of the infection highly depend on the socioeconomic status of the country (Olson et al. 2012). Implementation of new programs, effective screening tests, and efficient therapy options assist reducing the mortality rates in US patients. Maintaining the low mortality of TB in the U.S. will demand continued prevention and control efforts, specifically fast diagnosis, guaranteed accomplishment of treatment, and efficient and complete reporting (Geiter 2000).

Vancomycin-resistant *Enterococcus* (VRE) infections are also emerging as serious health risks. VRE is the fourth most common antibiotic-resistant pathogen with
the highest expected annual causes in the US and the third most common with the highest annual deaths. Several drugs have been used effectively against VRE, but its mortality rate (Table 1) still spiked (Cho et al. 2013). A patient who contracts VRE is considered immunosuppressed because he/she has been previously exposed to the organism during a major surgery or other medical procedures, which have been treated with multiple antibiotics (Collins 2008). These infections pose a 10% risk of death in patients with graft transplant, but almost 70% in those with endocarditis, tumors, and liver transplants (Kapur et al. 2000). Nonetheless, some authorities are more skeptical and tend to admit that patients might have other medical conditions that could lead to the increase of mortality and not necessarily attribute all mortalities to VRE infections (Cho et al. 2013). A potential reason for high mortality rates among VRE infections is because VRE can potentially transfer genetic traits to *S. aureus*, another organism with high fatality rates (Cetinkaya et al. 2000).

According to Table 1, drug-resistant *Campylobacter jejuni* and *Neisseria gonorrhoeae* infections are the most common cases of antibiotic resistant infections, followed by MRSA which continues to be the number one antibiotic resistant pathogens with highest mortality rate in the US. Carbapenem-resistant *Enterobacteriaceae* (CRE) infections are relatively more predominant than MDR *Acinobacter infections* by showing 100 more annual deaths in the US (Gupta et al. 2011). Vancomycin-resistant *Staphylococcus aureus* (VRSA) infections have shown no mortality rate in the US in comparison with drug-resistant *Candida*. Drug-resistant *Candida* infections contribute to the leading cause of invasive candidiasis due to the delays on finding appropriate and efficient antifungal therapeutic agents (Pfaller and Diekema 2007).
<table>
<thead>
<tr>
<th>Antibiotic-Resistant pathogens</th>
<th>Expected annual cases of antibiotic resistant infections in the US</th>
<th>Expected annual deaths in the US</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin-resistant <em>Staphylococcus aureus</em> (VRSA)</td>
<td>N/A</td>
<td>0</td>
</tr>
<tr>
<td>Drug-resistant <em>Mycobacterium tuberculosis</em></td>
<td>9,582</td>
<td>1,631</td>
</tr>
<tr>
<td>Drug-resistant <em>Candida</em></td>
<td>3,400</td>
<td>220</td>
</tr>
<tr>
<td>Multidrug-resistant <em>Acinetobacter</em></td>
<td>7,300</td>
<td>500</td>
</tr>
<tr>
<td>Carbapenem-resistant <em>Enterobacteriaceae</em></td>
<td>9,000</td>
<td>600</td>
</tr>
<tr>
<td>Vancomycin-resistant <em>Enterococcus</em></td>
<td>20,000</td>
<td>1,300</td>
</tr>
<tr>
<td>Methicillin-resistant <em>Staphylococcus aureus</em> (MRSA)</td>
<td>80,461</td>
<td>11,285</td>
</tr>
<tr>
<td>Drug-resistant <em>Neisseria gonorrhoeae</em></td>
<td>246,000</td>
<td>N/A</td>
</tr>
<tr>
<td>Drug-resistance-resistant <em>Campylobacter Jejuni</em> <em>(Gillespie et al. 2006)</em></td>
<td>310,000</td>
<td>120</td>
</tr>
</tbody>
</table>
Table 1 - Comparison of annual antibiotic resistant infections with annual deaths in U.S patients due to several types of bacteria (most data condensed from text in (Frieden 2013)).

C. Health Care Costs

Antibiotic resistance is a financial burden on the healthcare system. Physicians are obligated to treat individual patients with the most effective drugs for an infection. Health care institutions believe that hospital expenses are a form of reimbursing physicians for their service to society (Roberts et al. 2009). Patients, on the other hand, demand better healthcare service and better quality of life for them and for those around them (Roberts et al. 2009).

Antibiotics for bacterial infections are costly, and sometimes hospitalization is required until the infection is cleared, further raising costs (Roberts et al. 2009). In the US, Ventola’s (2015) statistics showed that antibiotic-resistant infections incur roughly $20 billion in annual healthcare costs and estimated that patients with antibiotic-resistant infections cost from $19,000 to $29,000. According to a study by Dellit (2007), VRE infections required up to 17 days of hospital care with an average cost of $27,000. Antibiotic-resistant *Pseudomonas aeruginosa* infections incurred hospital fees of $54,081 per patient, compared to $22,116 for those infected with antibiotic-susceptible strains (Slama 2008).

*S. aureus* infections are expensive to treat, and MRSA infections are more costly than those that are methicillin-sensitive (Singh et al. 2006). Inpatient treatment, which includes overall/basic hospitalization costs, antibacterial drugs, preliminary exams, and imaging will total close to $35,000 for patients with resistant strains (Filice 2010).
Assuming that 15% of the US patients become infected annually, costs for only MRSA infections would total approximately $45 million (Filice 2010).

Currently, several elements have played a vital role in the cost of controlling infection. The cost to create a new antibiotic is recently calculated at $1 billion (Slama 2008). Not only are novel antibiotics needed, but surveillance within each hospital must be augmented (Slama 2008). Hospital administration plays a vital role on controlling resistant pathogens, as well as enforcing rules to limit resistant strains from spreading. Hospitals or medical centers can only initiate surveillance programs or policy changes to improve infection control if there is financial support. Without financial support, resistant pathogens will continue to develop and spread, and the quality of health care worldwide will be drastically affected (Slama 2008).
II. Methods Used by Bacteria to Acquire Antibiotic Resistance

A. Basis for Antibiotic Resistance

Numerous mechanisms (illustrated in Figure 1) are used by bacteria to avoid antibiotic treatment, such as reducing drug uptake, actively pumping antibiotics outside of the cell, enzymatic modification of antibiotics, altering antibiotics’ target sites, overproduction of antibiotic’s target, and metabolic bypass of the antibiotic’s target (Wilcox 2004).

Figure 1 - Different antibiotic-resistance strategies (Wilcox 2004).
Decreased drug intake is an antibiotic-resistance mechanism used by bacteria (Lee et al. 2013). Aminoglycosides are one of the main antimicrobial classes that are addressed by reduced drug uptake (Lee et al. 2013). Resistance to aminoglycosides, which block protein synthesis, can be acquired by modifying the number and structure of the porin channels that allow passage of the antibiotic into the cell (Bonomo and Szabo 2006). Bacteria tend to be resistant to the number of aminoglycosides molecules present in the cytoplasm (Ramirez and Tolmasky 2010). As a result, aminoglycoside uptake will decrease, and they cannot enter the cytoplasm, bind to ribosomes and inhibit protein synthesis (Ramirez and Tolmasky 2010).

Actively pumping drugs outside of the cell is accomplished by efflux pumps, as demonstrated in Figure 1 (Webber and Piddock 2003). Efflux pumps are energy-dependent (active transport) mechanisms that move antibiotics and other elements outside of the cell (Ramirez and Tolmasky 2010). Pumps can be specific to a single antibiotic or function with multiple targets (Nikaido 2009). Pumps that are functional against multiple antibiotics are called multidrug efflux pumps and can lead to multidrug resistance (Nikaido 2009). Genetic elements encoding efflux pumps may be expressed from chromosomes and plasmids (Weinstein 2005). As an example, quinolones are a class of antibiotics responsible for inhibiting DNA gyrase and type IV topoisomerase - two enzymes important for bacterial DNA replication (Collin et al. 2011). Efflux pumps can remove quinolones from the cell before their concentration is sufficient to inhibit DNA metabolism (Drlica et al. 2009). Often times, this mechanism is employed with both Gram-positive (e.g. *Staphylococcus sp.*) and Gram-negative (e.g. *Pseudomonas aeruginosa*) bacteria (Drlica et al. 2008).
Enzymatic modification of antibiotics utilizes enzymes that are capable of altering or destroying the antibiotic's structure before it reaches its target (Figure 1). Enzymes are selective due to their different specificities for their target (Mahon et al. 2014). A common example of enzymatic modification of an antibiotic is found with aminoglycoside resistance. Enzymes in aminoglycoside-resistant bacteria catalyze the modification at different hydroxyl and ammonium groups of the 2-deoxystreptamine nucleus (nucleus linked to various sugars through glycosidic linkages) by the addition of an acetyl, a nucleotidyl, or phosphate group by acetyltransferases, nucleotidetransferases, or phosphotransferases, respectively (Ramirez and Tolmasky 2010). Addition of these functional groups (illustrated on Figure 2) to an antibiotic blocks their interaction with ribosomes, leaving the bacterium drug resistant (Ramirez and Tolmasky 2010).

Figure 2 – Aminoglycoside Resistance: Mechanism of Action (Jia et al. 2013)
Bacteria may also alter target sites of antibiotics. Isolates of *Mycobacterium tuberculosis* have become resistant to ciprofloxacin due to mutations of *gyrA* and *gyrB* that combine to form DNA gyrase (Aubry et al. 2006). DNA gyrase has a crucial function in DNA replication, where it nicks DNA, introduces negative supercoils, then re-joins DNA (Collin et al. 2011). Quinolones, such as ciprofloxacin bind to GyrA and interfere with its ability to cut DNA strands and reconnect them (Collin et al. 2011). However, some strains of *Mycobacterium tuberculosis* produce mutant proteins that have changed their physical structure, blocking ciprofloxacin from binding and inhibiting its function (Collin et al. 2011).

Another example of altering antibiotics’ target sites to gain resistance can be found with enzymes responsible for peptidoglycan synthesis. Peptidoglycan synthesis during cell growth requires the activity of penicillin binding proteins (PBPs) that break and reform peptide interbridges between peptidoglycan polymers (Kohanski et al. 2010). PBPs can be targeted by β-lactam antibiotics (e.g. penicillin and methicillin) as an effective means for halting cell growth (Kohanski et al. 2010). Upon structural modification of the PBPs through mutations, PBPs have low affinity for these antibiotics, making them useless (Kohanski et al. 2010).

Resistance to carbapenems can occur when bacteria produce carbapenem-hydrolyzing enzymes (carbapenemases) that fall into two categories (serine carbapenemases and metallo-β-lactamases) based on the reactive site of the enzymes (Bush 2010). Serine carbapenemases also known as class A carbapenemases, undergo a substitution that changes the active site (Queenan and Bush 2007). On the other hand,
metallo-β-lactamases (class B carbapenemases) cleave the beta-lactam ring of the antibiotic and disrupt its activity (Queenan and Bush 2007).

Overproduction of an enzyme that is targeted by an antibiotic is a fourth resistance mechanism employed by antibiotic-resistant bacteria (Nikaido 2009). Enzyme overproduction can be used in response to sulfonamides, which mimic p-aminobenzoic acid (PABA), a substrate of dihydropterate synthetase (Behera 2010). If PABA conversion to folic acid by dihydropterate synthetase (DHPS) is blocked by sulfonamides, biosynthesis of purines and several amino acids is blocked (Behera 2010). A cell can create an overabundance of PABA, diluting the antibiotic’s concentration against its target molecule (Behera 2010). Therefore, a smaller fraction of dihydropterate synthetase molecules will bind PABA, instead of the sulfonamide antibiotic, preserving some enzyme function (Behera 2010).

A gene’s promoter site (sequence that initiates a gene’s transcription into mRNA) can be altered to increase production of an antibiotic’s substrate (Nikaido 2009). For example, trimethoprim functions by obstructing a different step in folic acid production (Vilcheze and William 2012). Trimethoprim binds to an enzyme known as, dihydrofolate reductase, which suppresses the reduction of dihydrofolic acid to tetrahydrofolic acid (Vilcheze and William 2012). Trimethoprim resistance results from overproduction of the enzyme dihydrofolate reductase (DHFR). If the promoter is altered, more DHFR gene will be transcribed and translated into above-normal levels of DHFR (Vilcheze and William 2012). This excess of DHFR enzyme compensates for those enzyme molecules inhibited by trimethoprim and retains net activity in the cell (Vilcheze and William 2012).
B. Transfer of Antibiotic Resistance

Genetic diversity in bacterial populations is a result of mutations, vertical gene transfer, and horizontal gene transfer (Bennett 2008). Specific mechanisms that allow the horizontal transfer of DNA material between bacteria are transformation, transduction and conjugation (Bennett 2008).

1. Vertical gene transfer

The evolution of pathogenic bacteria is associated with innumerous influencing factors dictated by their population genetics. Vertical gene transfer (VGT) or vertical evolution is a mechanism in which genetic material is transmitted from a mother cell to daughter cells during cell division (Vogan and Higgs 2011). VGT and mutation are the mechanisms responsible for Darwin’s principles of evolution and natural selection and the basic principles of Mendel’s work: spontaneous mutations on the bacterial chromosome confer resistance to a member of the bacterial population (Gogarten et al. 2002). Mutations are considered rare, but bacterial growth rates are so rapid that resistance can become dominant in a population very quickly (Davies and Davies 2010).

2. Horizontal gene transfer

Horizontal gene transfer (HGT) is defined as the transfer of genetic material between individual bacteria of the same or different species (Aminov 2011). HGT relies
on three fundamental mechanisms in terms of genetic exchange: transduction, transformation or conjugation (Aminov 2011).

3. Transduction

Transduction is the passage of genes from one bacterium to another via bacteria-specific viruses called bacteriophages (Huddleston 2014). Bacteriophages replicate by infecting bacterial cells and using them as a host to produce more virus particles. Upon multiplication, virus particles accumulate, burst the cell and are released into the surrounding environment to begin another round of infection (Huddleston 2014). Bacteriophages are generally very host-specific, often infecting only small numbers of strains within a species and rarely infecting more than one species. Therefore, transduction is an HGT method that is viable for only closely related species.

Transduction occurs within the lytic cycle, which could be delayed due to a lysogenic cycle. During a lysogenic cycle, phage DNA is combined into the bacterial chromosome (known as a prophage) and remains dormant for some length of time (Blackstock 2014). Usually, due to environmental cues (UV damage to the cell, starvation, etc.), the phage genome will undergo excision from the bacterial chromosome and begin the lytic cycle (Russell et al. 2013). Defective excision steps play a crucial role because assembling phage heads can accidentally and randomly package host DNA, instead of its own DNA (Koneman 2006). Subsequent infection of host cells by these phage particles would transfer DNA from the previous host to the new host. This also means that
this phage particle is defective for replication, and the next host cell will not die as a result of infection. Therefore, transduction can be associated with antibiotic resistance because antibiotic resistance genes are just as likely to be accidentally packaged and transduced as any other gene (Koneman 2006).

4. Conjugation

Plasmid-mediated conjugation takes place in *Bacillus subtilis*, *Streptococcus lactis*, and *Enterococcus faecalis* but is not present as frequently in Gram-positive as Gram-negative bacteria (Mayer 2010). Plasmids can carry genes needed for formation of a sex pilus, and, therefore, conjugation. Overall, conjugation is the most common mechanism of horizontal gene transfer in bacteria (Huddleston 2014). Conjugation, as the term indicates, is a physical linkage between two bacteria by direct cell-to-cell contact. A sex pilus (tube-like appendage) is required for bacterial conjugation in Gram-negative bacteria (Bennett 2008). Conjugation takes place when DNA is transferred directly from a donor to a recipient bacterium through the pilus (Parija 2009). Transferred genetic material can contain not only the genes required to make pili, but other segments of the donor chromosome, as well. If antibiotic-resistance genes are among those transferred, conjugation will lead to the spread of antibiotic resistance (Griffiths et al. 2000). Not all bacteria are capable of producing sex pili, but conjugation can exist between bacteria of different species (Griffiths et al. 2000).

Gram-positive cells are not known to produce sex pili, but conjugation is still possible. In Gram-positive bacteria sticky surface molecules are formed in order to bring the two bacteria together in what is known as a mating bridge (Grohmann et al. 2003). This bridge allows cells to aggregate, and DNA can then be transferred from the donor to the recipient cells.
5. Transformation

Transformation is a natural process of HGT, during which genetic material in the environment is transferred across the cytoplasmic membrane, leading to integration of foreign DNA within the host chromosome (Huddleston 2014). The ability to acquire naked, foreign DNA is referred to as natural competence (Chen and Dubnau 2004). During the natural transformation process, foreign DNA links to a DNA translocase protein made by the host and enters the host cell (Domingues et al. 2012). Only one single-stranded DNA molecule is retained by the host cell, while the other is degraded by nucleases. The translocated, single-stranded DNA may then be integrated into the bacterial chromosome by a RecA-mediated transformation, if the foreign DNA was linear (Domingues et al. 2012). However, plasmid DNA (small, closed-circular DNA; discussed later) may be maintained as a plasmid in the host’s cytoplasm. Acquired plasmid or chromosomal DNA will continue to replicate and be expressed along with the host’s original DNA (Bennett 2008). If the foreign DNA contained genes that encode antibiotic resistance, the “transformed” cell is now resistant and can transfer this gene to its daughter cells (Giedraitiene et al. 2011).

6. Transposons

Transposons are also important sources of antibiotic resistance acquired via horizontal gene transfer (Huddleston 2014). Transposons are mobile DNA elements that integrate into the chromosomal or plasmid DNA of prokaryotes (Lodish 2000). In other words, transposons have the ability to move a short piece of DNA from one location to another within a cell. Inverted repeats (IR) are short, palindromic sequences of
nucleotides that read in reverse direction and flank the gene(s) within the transposon to facilitate recombination into the host DNA (Bousios et al. 2010). Between the inverted repeats is the gene for transposase, whose protein product detects the ends of the insertion sequences (Bousios et al. 2010). Transposases do not usually move transposons to specific sequences in the chromosome; therefore it moves (hops) the transposon into random positions (Alberts et al. 2002). In some transposons, the original transposon leaves a copy of itself at the progenitor site while replicating itself in other locations in a process called, replicative transposition (Skipper et al. 2013). Gene sequences disrupted by transposition are no longer capable of forming their intended products and are “knocked out” (Hickman et al. 2011).

The simplest transposable elements are known as insertion sequences, which carry only the transposase genes necessary to insert into and excise itself from a molecule of DNA (Hickman et al. 2011). However, standard transposons typically contain inverted repeats, a transposase gene and unrelated genes encoding metal-antibiotic resistance markers, proteins needed for causing disease, or simply metabolic genes (Juhas et al. 2009). Insertion sequences can also lead to formation of composite transposons (Wagner 2006). Composite transposons are normally a pair of insertion sequence elements that flank a sequence of DNA composed of one or more genes, often coding for antibiotic and/or metal resistance (Wagner 2006). The terminal insertion sequence is able to transpose by itself, which can be seen in transposon Tn5, Tn9 and Tn10 families (Wagner 2006). Also, the Tn3 family of transposases contains genes for a transposase, β-lactamase and resolvase (enzyme used for excising the transposon from the host DNA) in which all are located in between the inverted terminal repeats (Mayers...
β-lactamase is a resistance gene used for the β-lactam antibiotics, including penicillin and its derivate (Mayers 2009).

7. Bacterial plasmids

Plasmids are independently-replicating, double-stranded DNA molecules that are separate from chromosomal DNA in prokaryotic cells (Murray et al. 2012). Plasmids are generally up to approximately 10 kilobases in size and are often considered mini-chromosomes (Bennett 2008). Plasmids can be described as having narrow- or broad-host range (Gelder et al. 2008). Narrow-host range (NHR) plasmids are the most common plasmids in nature. A limited number of NHR plasmids have been developed in specific hosts to be second chromosomes due to their stability in a particular strain and an inability to replicate in other strains or species. This stability allows NHR plasmid size to successfully increase by acquiring genes from the host’s chromosome due to transposition and recombination events (Gelder et al. 2008). Broad-host-range (BHR) plasmids can be transferred to and replicate in relatively diverse and distant bacterial species than can NHR plasmids (Gelder et al. 2008). Hence, NHR plasmids seemed to play a role as genetic storage for the same group of species, while BHR act as an active gene transporter between diverse species (Gelder et al. 2008). Occasionally, plasmids possess characteristics that provide some selective advantage to the host bacterium, such as virulence elements, adherence and antibiotic resistance genes (Mahon et al. 2014). The expansion of plasmids is definitely something that the public health sector wants to avoid (Gelder et al. 2008). With better technology, researchers will be able to analyze the epidemiology of the plasmids.
C. Development of Antibiotic Resistance

Antibiotics function either by altering the bacterial structures or by interfering with fundamental physiological mechanisms of the bacteria (e.g. growth, DNA replication, gene expression, etc.) (Kohanski et al. 2010). Bacteria may acquire or be innately resistant to an antibiotic because they have no sites where molecules can enter the cell, because efflux pumps expel the antibiotic, or the cell is capable of producing enzymes that interfere with and destroy the antibiotics (Fernandez and Handock 2012). There are numerous reasons that can lead to the survival of the bacteria, regardless which drug is used to prevent infections from developing (Davies and Davies 2010).

The use and misuse of antibiotics is another factor that allows the expansion of resistant organisms. To make matters worse, clinicians prescribe antibiotics that perhaps are unnecessary, and patients increase the problem by requesting drugs for every infection (Weckx 2012). Diseases for which antibiotics are prescribed, like sinus infections, are expanding, especially at day-care settings (Oxford et al. 2013). With an increase in day-care environments, infections will surge; therefore, more antibiotics will be used, leading to an increased spread of drug resistance. Viral infections (e.g. influenza or cold) are often treated with unnecessary drugs at patients’ demands, increasing chances of creating resistant bacterial strains that can evolve. Even when drugs are prescribed correctly, patients fail to use them appropriately. Therefore, the weak organisms are killed, but the strongest will persist and propagate within the population (Weckx 2012).

Even when antibiotics are used correctly, problems can arise. Travel increases the probability of transferring drug resistance from one country to another at jet speed. Hospitalized patients often times are being medicated with several drugs, which create a
favorable situation for multi-resistant strains to develop and thrive. Hospitalized patients often have compromised immune systems, which increases their vulnerability to many infections, in particular those who are antibiotic-resistant (Oxford et al. 2013).

To conclude, a variety of factors such as population growth, usage and misuse of antibiotics have all contributed to development of antibiotic resistance. Hopefully, the evolution of resistance can be stopped eventually, if society begins to change its behavior and attitude towards infectious diseases. Precautions should be taken serious for not only the patients’ benefit, but for the good of all humankind (Davies and Davies 2010).
III. The Genus *Staphylococcus*

A. Natural Habitat and Host Range

Worldwide, *Staphylococcus* species are notorious human pathogens and are the focus of comprehensive studies among researchers. More than 40 *Staphylococcus* species cause diseases known to be clinically and economically significant (Lindsay 2008). Public health has become threatened by antibiotic-resistance strains that pass among humans, and it is thought that species or their habitats might correlate with type of infection (Lindsay 2008). There is a wide host range among species that belong to the genus *Staphylococcus*. For instance, *S. aureus* can be isolated from horses, rabbits, humans, and ruminants and cause skin infections, septic arthritis, and others (Lindsay 2008). Overall, the pathogens among the *staphylococcus* species can be colonize among man and other mammals. Depending upon the host, infections can range from a mild skin infections to a more severe type of syndrome. Table 2 shows the host range and infections caused by several species of staphylococci.
Table 2: Hosts of *Staphylococcus* species according to the type of infections.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Pathogen</th>
<th>Host</th>
<th>Associated Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mahon et al. 2011</td>
<td><em>S. aureus</em></td>
<td>Humans and Animals</td>
<td>Skin and wound infections, scalded skin syndrome, toxic shock syndrome, toxic epidermal necrolysis, and food poisoning</td>
</tr>
<tr>
<td>Raz et al. 2005</td>
<td><em>S. saprophyticus</em></td>
<td>Humans</td>
<td>Cystitis an urinary tract infections</td>
</tr>
<tr>
<td>Lindsay 2008</td>
<td><em>S. delphini</em></td>
<td>Horse and cows</td>
<td>Pyoderma</td>
</tr>
<tr>
<td>Lindsay 2008</td>
<td><em>S. hyicus</em></td>
<td>Pig</td>
<td>Exudative dermatitis</td>
</tr>
<tr>
<td>Lindsay 2008</td>
<td><em>S. sciuri</em></td>
<td>Pig</td>
<td>Greasy Pig syndrome</td>
</tr>
<tr>
<td>Vela et al. 2012</td>
<td><em>S. xylosus</em></td>
<td>Gerbils and birds</td>
<td>Nasal dermatitis in gerbils, avian staphylococcosis and Pyelonephritis</td>
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<tr>
<td>Tibra et al. 2009</td>
<td><em>S. gallinarum</em></td>
<td>Chickens</td>
<td>Endophthalmitis</td>
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<tr>
<td>Hoffman et al. 2007</td>
<td><em>S. auricularis</em></td>
<td>Humans (infants)</td>
<td>Opportunistic infections</td>
</tr>
<tr>
<td>Bocher et al. 2009</td>
<td><em>S. lugdunesis</em></td>
<td>Humans</td>
<td>Endocarditis, septicemia, meningitis, skin and soft tissue infections</td>
</tr>
<tr>
<td>Wang et al. 2013</td>
<td><em>S. intermedius</em></td>
<td>Canines</td>
<td>Pyoderma and skin tissue infections in dogs</td>
</tr>
<tr>
<td>Lindsay 2008</td>
<td><em>S. epidermidis</em></td>
<td>Cows and goats</td>
<td>Mastitis</td>
</tr>
</tbody>
</table>
B. How are *Staphylococcus* Infections Acquired?

An individual is exposed to diverse strains of microorganisms daily without causing any infections. Human microbiota are usually nonpathogenic, as long as they remain within their normal habitat (Reid et al. 2011). When normal microbiota are displaced, infections can occur. Human skin is one of the favorable sites for pathogens to establish a direct interaction and potentially cause infections (Ki and Rotstein, 2008). Infections associated with staphylococci can range from minor skin problems to life-threatening systemic infections (Ki and Rotstein, 2008). A variety of conditions may be associated with staphylococcal infections. Therefore treatment depends on the site and severity of the illness (Ki and Rotstein, 2008).

1. Bacteremia

Bacteremia, the presence of bacteria in the blood, is one of the main routes taken to destabilize the immune system of an individual (Delost 2014). Bacteremia cases begin showing signs of fever, chills and hyperventilation but at late stages can travel through the internal organs (heart, brain and lungs), catheters and implanted devices, such as pacemakers (Delost 2014). Bacteria enter the bloodstream through small cuts and wounds, following by the adhesion to inner membranes of the tissues, which subsequently triggers a cascade of infections (Cabell et al. 2003).

2. Food Poisoning

Food poisoning is derived from ingesting contaminated products. Symptoms such as nausea, vomiting, diarrhea, dehydration, and low blood
pressure can present (Argudin et al. 2010). Often times, negligent food workers contract staphylococcal infections and continue to process food without further precautions. Once *S. aureus* is present inside of the human body, it can trigger infections (Argudin et al. 2010). *S. aureus* is capable of forming a broad variety of toxins, such as staphylococcal enterotoxins (SEs) responsible for creating potent biological effects, such as damage of gastrointestinal tract (Dinges et al. 2000). Usually SEs are active in low amounts and are capable of resisting heat and cannot be eliminated by cooking. Acid-resistant SEs can withstand the pH of the digestive tract (Loir et al. 2003).

3. Skin

Staphylococcal infections are normally skin infections, such as boils, impetigo, and staphylococcal scalded skin syndrome (SSSS) (Proft 2013). Usually, bacteria invade superficial layers of the skin via open wounds or cuts. Boils, for instance, are skin infections created by pockets of pus that developed in a hair follicle or oil gland (Murray et al. 2008). The skin over the infected site normally shows some redness and swelling (Murray et al. 2008). In this case, the boil breaks open and pus drains and causes other infections. Boils generally develop under the arms or around the groin (Ibler and Kromann 2014). SSSS forms blisters due to the production of toxins that disrupts the epidermis, leaving the affected area exposed to harmful pathogens. Clinically, SSSS is seen to spread throughout large portions of the body (Bukowski et al. 2010). A defined area of infection created by SSSS is commonly diagnosed as impetigo (Bukowski et al. 2010).
C. Risk Factors for Infections

1. Recent Hospitalization

Regardless of intense efforts implemented by infection control programs, bacteria remain persistent in hospital settings where they can infect susceptible people, primarily those with weakened immune systems (Sydnor and Perl 2011). Infections can occur before, during, or after surgery if certain precautions are not followed (Sydnor and Perl 2011). In a hospital setting, staphylococci infect critically ill patients more than any other organism because of the way they spread. Generally staphylococci, in particular *S. aureus*, diffuse by skin-to-skin contact between doctors, nurses, or visitors (Barnes 2010). Normally, someone who contracts a staphylococcal infection, such as MRSA, can stay hospitalized for up to 10 days (Barnes 2010). Therefore, precautions such as maintaining proper personal protective equipment to protect patients and physicians must be followed (Barnes 2010). However, what makes staphylococci significant is that several species have become resistant to antibiotics. Methicillin-resistant *S. aureus* (MRSA) is a prime example of antibiotic resistance to methicillin and penicillin (Nikaido 2009). With limited treatments, it becomes extremely difficult to control the spread of infection. The best way to minimize the problem is to maintain good hygiene between health care workers and extending these protocols to visitors and patients (Barnes 2010).

2. Residing in a Long-Term-Care Facility

MRSA is 20% more common in long-term-care facilities (LTCFs) than it is in hospitals (Mazur and Gudiol 2009). Urinary tract infections are the number one
risk found among elderly that reside in the LTCFs, followed by respiratory and gastrointestinal infections (Bonono 2000). As a whole, these diseases comprise 94% of the infections in a LTCF due to the lack of training among staff and proper care of residents (Engelhart et al. 2005). Some individuals admitted to a care facility are likely to carry MRSA and have the ability to spread it to other patients (Bonono 2000). As the population ages and technology advances to provide better care for patients, both the number and proportion of people residing in LTCFs will increase (Bonono 2000). Hospital-acquired infections differ from those contracted in community settings because it is an older population of patients with a broad range of disabilities (Mathei et al. 2007). Some characteristics of a nursing-home setting promote the growth of infectious diseases. Residents tend to be clustered in confined areas and daily activities usually involve groups (Mathei et al. 2007). Also, healthcare workers might have limited training in how to control infectious diseases. Understaffing is also a common problem in LTCFs, and caregivers are often too occupied to worry about infectious control (Mathei et al. 2007).

3. **Indwelling devices**

As opportunistic pathogens, staphylococci can migrate from medical equipment to an individual’s internal organs (Otto 2008). Dialysis, catheters, pacemakers and prosthetic devices are examples of invasive, indwelling devises that are used routinely in hospital facility (Guggenbichler et al. 2011). As an example, _S. epidermidis_ can directly and indirectly cause infections, despite its role as a constituent of human skin’s normal microbiota. Patients with weakened and debilitated immune systems are more vulnerable to _S. epidermidis_ infections when introduced to the organism via the implantation of medical devices (Otto 2008).
According to Baddour, in 2003 roughly 53% and 20% of the patients became infected with *S. aureus* and coagulase-negative staphylococci (CoNS), respectively, as a result of prosthetic vascular graft. Indwelling devices side-step the normal defense mechanism of the epidermal surface and provide a habitat in which pathogens can proliferate protected from the patient’s immune defenses (Mehta et al. 2014). Therefore, proper maintenance and cleaning of these devices could reduce the risk of staphylococcus infections (Mehta et al. 2014).

**D. Physiology of How Staphylococci Cause Disease**

1. **Human Pathogens**

   Staphylococci are known to be opportunistic pathogens, meaning that they take advantage of a wound or weakened immune system within a host. Infections are predominantly found in hair follicles, sutures, and even along the respiratory tract (Mahon et al. 2011). Upon entering skin or mucous membranes, staphylococci are able to produce and secrete several components that allow them to survive in the host and cause damage to host tissues (Coates et al. 2014). Pathogenicity of staphylococci can be attributed to various virulence factors, including surface proteins (Protein A), exfoliatin toxins, and biofilm formation (Crossley et al. 2009).

   *Staphylococcus aureus* has on its cell wall a surface protein that is referred to as Protein A, which is capable of attaching to a fragment of the antibody Immunoglobulin G (Mahon et al. 2011). IgG is a protein synthesized by B cells that protects the internal tissues and binds to foreign substances upon entering the
body (Janeway et al. 2001). Once bound by IgG, foreign bodies are typically engulfed and destroyed by phagocytic white blood cells. However, Protein A of *S. aureus* binds in the wrong orientation to the IgG surface (Tattlybaeva et al. 2013). As a result, white blood cells show decreased ability to ingest and kill them, and potential for diseases increases (Tattlybaeva et al. 2013).

Exfoliatin Toxins (ETs), also referred to as epidermolytic toxins, are known to cause SSSS (affecting large parts of the skin) and can have implications in bullous impetigo (affecting limited areas of the skin) (Bukowski et al. 2010). Serine proteases are enzymes present in ETs responsible for cleaving desmosomal cadherins on the extracellular layer of the skin (Bukowski et al. 2010). Desmosomes are cell junctions composed of cadherins linked to catenins that interact to form strong mechanical attachments between basement membrane and epithelial cells (Garrod and Chidgey 2007). Disruption of desmosomal cadherins causes cell attachments to weaken and, as a result, clinical manifestation of SSSS ensues (Garrod and Chidgey 2007). During early stages of SSSS, fever, fatigue and malnutrition are often followed by erythematous rash (large fluid blisters). Bullous impetigo refers to large liquid filled blisters, specifically present at the site of infection (Bukowski et al. 2010). Diagnosis involves PCR or randomly amplified polymorphic DNA analysis (Bukowski et al. 2010). Strains producing ETs are strictly treated intravenously with antibiotics. ET-producing strains contain the *eta* gene, which encodes for exfoliative toxin A (Magni 2010), is only positive in 10% of MRSA cases (Bukowski et al. 2010). Hence, a vast range of antibiotics can still be effective, according to the patient’s needs (Bukowski et al. 2010).
Biofilms formed by staphylococci are responsible for the microorganisms’ adherence to each other in an aqueous matrix (Schommer et al. 2011). Adherence takes place due to a secreted matrix, referred to as extracellular polymeric substance (EPS). EPS can be made of extracellular components such as DNA, proteins, and polysaccharides, although polysaccharides and proteins are most common and abundant (Flemming et al. 2007). Biofilms on living or nonliving surfaces show increased resistance to antibiotics and disinfectants. Infections in cystic fibrosis patients and indwelling medical devices become problematic when biofilms are involved (Joo and Otto 2012).

Development of bacterial pathogenicity depends greatly upon the formation of the biofilm and the interaction between bacteria and the host. Biofilms are capable of hiding bacteria from antimicrobial agents and affect the host immune system during and after the infection (Joo and Otto 2012). Diagnoses of biofilms can be accomplished by performing microscopy and culture studies (Hassan et al. 2011). Current treatment involving a combination of a StaphVAX (polysaccharide conjugate vaccine) and vancomycin usually shows effectively reduced signs of infection. However, further treatments must be studied to provide a better and efficient approach (Brady et al. 2011).

2. Non-Human Pathogens

As referred to in Table 2, several species of *Staphylococcus* are animal pathogens or promote infections that impact humans and animals. Pathogenicity of these species is also due to a variety of opportunistic infections including skin lesions, pyoderma, and mastitis.
S. hyicus is found predominantly associated with pigs, particularly along nasal and vaginal mucosa (Dworkin et al. 2006). S. hyicus causes exudative epidermatitis, more commonly referred to as Greasy Pig Disease (Casanova et al. 2011). Greasy Pig Disease, as the name suggests, causes greasy, peeling skin due to the production of an ET that forms blisters that disrupts spinosum epithelium cells. The spinosum epithelium becomes fragile and ruptures, allowing entry of pathogens (Fudaba et al. 2005). To control the spread of infections, this disease can be treated using a combination of procaine penicillin G and novobiocin for a period of 5 days (Park et al. 2013).

S. intermedius is naturally present among skin microbiota of all canines, and no pathogenicity is known for humans (Tanner et al. 2000). S. intermedius directly affects canine skin flora but indirectly impacts their central nervous system (Koneman 2006). Infections that occur at the central nervous system level are poorly understood. The brain barrier is an extremely complex membrane capable of preventing the entry of invasive pathogens. Toxins and other organisms may enter through the skull and the selective membranes, but that rarely occurs because of the brain membrane isolation (Durdik et al. 2009). Development of pyoderma occurs when S. intermedius invades the superficial layer of the skin and hair follicles without formation of a scab (Futagawa-Saito et al. 2006). Without a scab or coating, the epidermis becomes too fragile, superficial scale is produced, and debris will accumulate in and around hair follicles, leading to hair loss (Zachary and McGavin 2013). Infections normally respond well to cefotaxime therapy (Durdik et al. 2009).
S. lentus is primarily found in animals that are used as a source of aliment, such as cattle, poulty, and goats (Schwendener and Perreten 2012). Rarely, S. lentus can transmit infections to humans, but most commonly this organism can inflame the breast tissues of sheep and goats, leading to pain, redness and soreness (Schwendener and Perreten 2012). Once the breast is infected, milk becomes very cloudy due to the presence of clots. Normal milk contains predominately epithelial and white blood cells (Rupp et al. 2008). If mammary gland infection occurs, the number of neutrophils will increase in the bloodstream (Rupp et al. 2008). Hence, by performing a somatic cell count it is possible to determine that cows and/or goats with high cell counts will have greater risk to contract clinical mastitis in comparison to those with lower somatic cell counts (Rupp et al. 2008). Treatment should be focused on culture results obtained from milk samples (Pieterse and Todorov 2010). Dry-off (non-lactating) period is an efficient method to cure mastitis (Pieterse and Todorov 2010). Antibacterial agents may be utilized during the non-lactating period in the infected animal. Once the dry-off period is completed, prophylactic antibiotics can be administered to prevent subsequent infections (Pieterse and Todorov 2010).
E. Diagnosis

1. Selective Testing for Identification of *Staphylococcus* species

Staphylococci testing is accomplish by doing several and selective laboratory examinations. The schema for identification of *Staphylococcus* species relies primarily on microscopic confirmation of a Gram-positive coccus, followed by a catalase-positive test (Mahon et al. 2011). Catalase-positive cultures then undergo coagulase testing. Coagulase-positive results are seen mainly in *S. aureus*, whereas coagulase-negative cultures (CoNS) are seen mostly in other staphylococcus species, such in *S. epidermidis* (Foster 1996). CoNS can then be examined for oxidase/bacitracin susceptibility. Cultures resistant to oxidase/bacitracin will proceed with novobiocin susceptibility test (Mahon et al. 2011). Most CoNS are novobiocin-sensitive, whereas *S. saprophyticus* is known to be novobiocin-resistant (Mahon et al. 2011).

2. Salt Tolerance and Mannitol Fermentation

Many media are utilized for identification of staphylococci, but certain media are more useful than others (Tille 2013), such as Mannitol Salt Agar (MSA), Blood Agar (BA), and Muller-Hinton Agar (MHA). MSA is a selective and differential media with 7.5% of NaCl concentration that favors the growth of Gram-positive bacteria. Any isolates forming colonies on MSA are considered salt-tolerant. MSA also differentiates mannitol fermentation to acids by changing color from red to yellow. Non-fermenters of mannitol leave the medium red (Mahon et al. 2011). *Staphylococcus aureus* ferments mannitol and the medium turns into
yellow color while other non-pathogenic staphylococci will not ferment mannitol and the media will remain red in color (Mahon et al. 2011).

3. Catalase

Catalase is an enzyme that catalyzes the conversion of hydrogen peroxide into water and oxygen. Detection of catalase is a key feature for differentiation of staphylococci from streptococci. Bacteria are characterized as catalase positive when bubbles are formed when cells contact hydrogen peroxide. *Staphylococcus* species are catalase-positive, whereas species of *Streptococcus* are catalase-negative (Mahon et al. 2011).

4. Coagulase Reaction

*Staphylococcus* and *Yersinia* are two of the few genera that can produce coagulase (Lackie 2010). Coagulase is an enzyme that converts fibrinogen to fibrin, which forms clots that encapsulate cells. Coagulase occurs when clumping factor binds to the cell walls of bacteria that react with fibrinogen. Then fibrinogen dissolves, allowing cells to clump together (Kateete et al. 2010). Coagulase differentiates staphylococcal isolates, and it can be classified as CoNS or coagulase-positive staphylococcus (CPS) (Foster 1996). CoNS isolates are the most frequent in medical microbiology laboratory because they survive in dead or decomposing matter, but CPS isolates are considered to be the most pathogenic (Foster 1996).

5. Clumping Factor

Clumping factors are heat-stable proteins that reside in the cell wall of *S. aureus* (Tille 2013). These proteins cause clumping of fibrinogen or fibrin.
Disruption of the bacterial cell wall allows the release of clumping factor, which can then act upon the fibrinogen in the plasma, allowing blood cells to aggregate (Tille 2013). Despite the fact that clumping factor and coagulase might have similarities, they prove to be distinct from each other. Coagulase is an enzyme created by many bacteria that distinguishes S. aureus from CoNS species (Becker et al. 2014). The confusion occurs because clumping factor is a portion of coagulase that is bound to the bacterial cell wall and links directly to fibrinogen. Clumping factor and coagulase act independently from each other (Josefsson et al. 2001).

6. Hemolysins

Hemolysins are one of many features utilized to differentiate staphylococcal species from other organisms. Hemolysins are microbial proteins that can lyse red blood cells (RBCs) by disrupting their cell membrane. Many hemolysins are pore-forming toxins which induce lysis of RBCs by creating pores in their cytoplasmic membrane (Thompson et al. 2011). This can be visually seen by performing a blood agar test (Nayak et al. 2013).

Blood Agar (BA) is a nutritive medium composed of tryptic soy agar amended with 5-10% of sheep, rabbit, or horse blood and is used to grow a variety of bacteria. The identification of each species would depend on the hemolytic activity (Mahon et al. 2011). If RBCs are completely destroyed by a hemolysin and a clear zone forms around the colonies. This is referred to as β-hemolysis. In the agar, partial destruction shows a greenish color underneath the colony because bacteria contain hydrogen peroxide that converts hemoglobin (red) into methemoglobin (green). Non-hemolytic or γ-hemolysis is the result of no
destruction of RBCs and therefore, the agar does not undergo any changes (Engelkirk et al. 2011).

*S. haemolyticus, S. lugdunensis* and *S. aureus* can produce hemolytic zones around the colonies (Mahon et al. 2011). Others such as, *S. epidermidis* are referred to be non-hemolytic (Mahon et al. 2011). However, *S. epidermidis* shows partial destruction of hemoglobin in the RBCs, referred to as α-hemolysis (Engelkirk et al. 2011).

7. Production of Deoxyribonucleases

Testing for enzymes which degrade DNA, deoxyribonucleases (DNAses), is essential to the identification of potentially pathogenic bacteria (Kateete et al. 2010). Testing involves streaking the organisms on an agar medium that also contains DNA and a dye which changes color in the presence of the degraded DNA (Tille 2013). Several staphylococcal species are able to produce this enzyme, such as *S. aureus, S. epidermidis, S. intermedius* (Pammi et al. 2013), and *S. hyicus* (Bhatia and Zahoor 2007).

8. Thermostable Nuclease (TNase)

TNase is an enzyme that is produced only by a few species of *Staphylococcus* (Enoch et al. 2008), including *S. aureus, S. hyicus and S. intermedius*. TNase testing is performed by placing blood broth from the blood culture in heated environment (60 °C) and then transferring the suspension to an agar medium for up to 4 hours at room temperature. If the DNA has been degraded a clearing zone will form, demonstrating the TNase activity (Enoch et al. 2008).
9. Sensitivity to Novobiocin and Methicillin

Mueller-Hinton Agar (MH) with 2% NaCl makes the agar selective to staphylococcus species (Mahon et al. 2011). MH contains acid digest of Casein, beef extract, starch and agar, specifically to select pathogenic *Neisseria* species (Mahon et al. 2011). The translucent appearance of the agar helps determine the susceptibility to antimicrobial agents. The Kirby-Bauer method continues to be a very common method to measure bacterial resistance. If bacteria growth is inhibited in the presence of a certain antibiotic, a zone of inhibition (no visible growth) is formed (Mahon et al. 2011).

Novobiocin is an antimicrobial agent discovered in *Streptomyces niveus* that disrupts the normal ATP formation and its activity (Leboffe and Pierce 2012). Most CoNS can be distinguished by using the novobiocin test. Novobiocin sensitivity is tested by placing a 5-μg disk containing the antibiotic onto Muelher-Hinton agar (MH) for 24 hours at room temperature (Leboffe and Pierce 2012). A novobiocin-susceptible isolate forms a zone of inhibition greater than 16 mm in diameter around the disk. If the organism is resistant to novobiocin, no zone would be formed. *S. saprophyticus* is often used as a positive control because it is resistant to novobiocin (Leboffe and Pierce 2012).

The Kirby-Bauer test differentiates from novobiocin testing by using a choice of antibiotics that should be directly applied into the MH and then classified as being Resistant (R), Intermediate (I) or Susceptible (S) according to the organism in the study.
S. aureus is said to be resistant to methicillin upon determining that no visible growth occurred in the agar (Mahon et al. 2011). Based on Clinical Laboratory and Standards Institute guidelines, S. aureus is resistance to methicillin when the minimal inhibitory concentration (MIC) is $> 4 \text{ mg/L}$ and susceptible when the MIC is $\leq 4 \text{ mg/L}$ (Brown et al. 2005).

F. Treatment

Staphylococci are considered to be the most pathogenic organisms in healthcare facilities, as well as in society (Mahon et al. 2011). Without prevention, they can impact the health of an individual at any age. The choice of an antimicrobial agent should follow certain criteria, such as drug sensitivity, site of infection and disease condition. The two main types of treatment used are surgery or antibiotic chemotherapy (Leekha et al. 2011). In the majority of cases, patients that require surgical treatment also need antibiotics. However, each individual requires personalized treatment based upon their medical condition (Leekha et al. 2011).

Some CoNS, such as S. epidermidis, are known to cause UTIs and serious inflammation but have become resistant to drugs such as methicillin, gentamicin, ampicillin, and erythromycin (Villari et al. 2000). Currently, S. epidermidis is treated by using vancomycin or rifampin (Villari et al. 2000). Nearly 20% of UTIs are derived from S. saprophyticus, which are novobiocin resistance, and are treatable with multiple antibiotics (Raz et al. 2005). S. haemolyticus is considered to be the most antibiotic-resistant organism among the CoNS family (Barros et al. 2012). Strains have become resistant to multiple drugs such as penicillins, ampicillin,
gentamicin, and ciprofloxacin (Barros et al. 2012). Vancomycin was reported to treat patients that were infected with *S. haemolyticus* (Biavasco et al. 2000).

CoNS tend to create problems when it comes to animal infections, as well (Mahon et al. 2011). Pigs typically contract *S. hyicus*, which causes exudative epidermidis (Casanova et al. 2011). This disease is highly contagious to other pigs and potentially to humans through skin exfoliation (Casanova et al. 2011). No satisfactory treatment has yet been determined (Park et al. 2013). However, the use of topical antibiotics (procaine penicillin G and novobiocin) appeared to be the most efficient way to prevent infection from spreading (Park et al. 2013).
IV. Pathogenesis of *Staphylococcus aureus*

A. Mode of Pathogenicity

*Staphylococcus aureus* is a well-known bacterium for its commensal role in animal health. It is commensal because it can harmlessly colonize a host’s nasal cavities, armpits, vagina, or pharynx (Jenkins et al. 2015). Commensal bacteria protect the superficial layer of our body from colonization of potentially pathogenic bacteria. For instance, *S. aureus* produces bacteriocins (toxins created by bacteria that target other bacteria) that impede pathogenic *Staphylococcus epidermidis* from increasing in number (Chiller et al. 2001).

A versatile, pathogenic bacterium is formed when an organism successfully overcomes the normal host defense protection and causes disease (Chiller et al. 2001). *S. aureus* causes superficial lesions, such as boils or furuncles, systemic infections (endocarditis and osteomyelitis), food poisoning, and toxic shock syndrome (Jenkins et al. 2015). Infections may result from a combination of actions between multiple genetic factors or single virulence factors (Jenkins et al. 2015). Infections commonly result from wounds that allow staphylococci to access neighboring tissues and the bloodstream. Infections can remain localized or become systemic, depending upon the interaction between virulence factors and host-defense mechanisms (Anderson et al. 2012).

1. Adherence to Host Tissue

*S. aureus* is found primarily in the nares, and nearly 20% of the human population is considered to be carriers for this pathogen, which increases risk of contracting an infection (Naber 2009). Cells are capable of binding to host cells via extracellular-matrix elements with the support of microbial-surface-components
recognizing adhesive-matrix molecules (MSCRAMM), which are a collection of adhesive molecules (Clarke and Foster 2006). MSCRAMM adhere to fibrinogen (protein that assists with the formation of blood clots) via different binding sites (Cognasse et al. 2015). Consequently, MSCRAMM function during early stages of infections. Due to the broad range of MSCRAMM types, a variety of S. aureus strains may initiate an array of diseases (Bien et al. 2011). For instance, coagulase is a type of adhesive molecules in the MSCRAMM which links to prothrombin to produce staphylothrombin (Cognasse et al. 2015). Staphylothrombin converts fibrinogen into fibrin, leading to platelet aggregation and allowing adherence of S. aureus to platelets (Vanassche et al. 2012). Fibrin clots protect cells from phagocytosis, and, as a result, they will persist and cause staph infections (Cheng et al. 2010). All clinical isolates of S. aureus are known to be coagulase-positive, making it a classical phenotypic marker for identifying this bacterium (Cheng et al. 2010).

2. Invasion of Host Tissues

The invasion of S. aureus into host tissues activates production of a wide variety of extracellular proteins, each with a distinct role (Bien et al. 2011). Alpha-toxin, also referred to as alpha hemolysin, is one of several pore forming toxins and a critical virulence factor for S. aureus against platelets, erythrocytes, and monocytes (Berube and Wardenburg 2013). S. aureus produces alpha-hemolysin as a single molecule to facilitate the linkage with eukaryotic cell membranes (Frank et al. 2012). On target membranes, its subunits oligomerize to create heptameric rings with a central pore (Berube and Wardenburg 2013). The central pore formed on the target membrane can now permit leakage of simple ions (e.g. Ca$^{2+}$) that would result in apoptosis (Berube and Wardenburg 2013).
Beta-toxin is composed of sphingomyelinase, a fatty-acid enzyme responsible for catalyzing sphingomyelin into ceramide and phosphorylcholine (Kurek et al. 2013). Sphingomyelinase is found in animal cell membranes capable of targeting membranes rich in this lipid (Kurek et al. 2013). Beta-toxin lyses erythrocytes by crossing the lipid membrane and promoting the release of their contents (Fournier and Philpott 2005). Normally, beta-toxin is expressed by \textit{S. aureus} strains in non-human infections to a greater extent than in human isolates.

Delta-toxin, despite its small size, is produced by 97\% of strains of \textit{S. aureus} (Dinges et al. 2000). It is also speculated that nearly 60\% of coagulase-negative staphylococci (CoNS) form delta-toxin, especially \textit{S. epidermidis} (Noble 2004). Delta-toxin is said to be lytic to a variety of cells by the disintegration of their membranes. However, its pathogenicity remains unknown (Turnidge et al. 2002).

Gamma-toxin and Panton-Valentine-Leukocidin (PVL) are additional toxins produced by \textit{S. aureus}. Each individual toxin creates secreted proteins, called S and F subunits.

S subunit stands for slow-eluting proteins during purification on an ion exchange column responsible for identifying the cell specificity of the cytolysin (Sabour and Griffiths 2010). S class is created as a single molecule that ended up linking tightly to other S molecules, forming a multi-complex structure. This complex structure can then attach and produce tiny pores on the membranes of the neutrophils (Sabour and Griffiths 2010). F subunit refers to fast-eluting proteins during purification on an ion-exchange column. F subunit attaches primarily to red blood cells and then to S subunits to perforate their membranes (Fluit and Schmitz 2003). The difference between the
Gamma toxin and PVL toxin is that gamma toxin binds strongly to red blood cells, while PVL binds to white blood cells, specifically neutrophils (Nilsson et al. 1999). Overall, S and F subunits act together in the destruction of membranes on both toxins (Fluit and Schmitz 2003). Gamma-toxin is present in 99% of *S. aureus* strains whereas only 2%-3% of strains produce PVL toxins (Lydyard et al. 2009).

3. **Avoidance of Host Defenses**

*S. aureus* can interfere with innate and adaptive immune responses in numerous ways (Liu 2009). Capsular polysaccharide (CP) is composed of a variety of polysaccharides present on the surface of *S. aureus*, and the majority of strains express CPs *in vivo or in vitro* (Turnidge et al. 2002). In the presence of CPs, phagocytosis is inhibited by preventing cell-surface structure identification by receptors on the surface of a phagocyte. Therefore, CPs prevent complement activation and prevent *S. aureus* from being recognized by the host’s immune system (Mahon et al. 2014). As a result, the host defense mechanism no longer fights against the infection (Moroni et al. 2011).

Superantigens belong to a class of antigens that initiate a non-specific T-cell response to release cytokines, causing severe inflammation (Proft and Fraser 2003). Enterotoxins belong to the family of superantigens that stimulate T-cell production due to the interaction of the major histocompatibility complex class II molecules on antigen presenting cells and T cell receptors (Proft and Fraser 2003). Among numerous enterotoxins, staphylococcal superantigens A, B, and C contain superantigenic features. Enterotoxins have been associated with autoimmune diseases, such as systemic lupus erythematosus, and rheumatic arthritis, and are involved in abnormal immunologic stages, such as psoriasis and atopic dermatitis (Turnidge et al. 2002). *S. aureus* avoids the immune system because it expresses different types of superantigens that corrupt
the normal humoral immune response, leading and immunosuppression (Foster 2005). Superantigens help *S. aureus* avoid the host immune system by stimulating a large amount of T cells, which secrete large amount of interleukin-2. Interleukin-2 is a protein that, in large amounts, can induce vomiting. Overstimulation by superantigens is not always beneficial to the host and can lead to shock and death (Freeman-Cook et al. 2006).

Toxic shock syndrome toxin is caused by a superantigen known to be produced by *Staphylococcus aureus* (Mahon et al. 2014). These toxins are not processed by APCs, but instead TSS toxins attach directly to the outside portion of an MHC II antigen on APCs and to the outside portion of the T cell receptor of the T helper cells. Superantigens are non-specific, so they can bind without antigen specificity and stimulate many helper T cells. Excessive amounts of interleukin-2 produced by T cells will enter the bloodstream, instead of only acting locally as they normally do. Overproduction of interleukin-2 circulating in the bloodstream initially induces vomiting and nausea, but left untreated can become fatal (Freeman-Cook et al. 2006).
A. Modes of Antibiotic Resistance

The spread of antibiotic resistance and the occurrence of multiple drug resistant pathogenic bacteria have negatively impacted the medical field due to the lack of effective therapeutic treatment for bacterial infections, such as *S. aureus* (Lowry 2003). *S. aureus* can develop resistance to antibiotics by altering the antibiotic target, trapping the antibiotic, or through efflux pumps (Pantosti et al. 2007).

1. Alteration of the Antibiotic Target

*S. aureus* tolerates the cytotoxic activity of β-lactam antibiotics by modifying the normal penicillin-binding proteins, such as PBP2a (Stapleton and Taylor 2002). PBP2a modification is best seen in methicillin-resistant *S. aureus* (MRSA) due to the *mecA* gene that is responsible for encoding PBP2a. MRSA forms PBP2a as a fifth PBP, in addition to four PBPs found in all *S. aureus* strains (Zapun et al. 2008). β-lactams have a reduced affinity for PBP2a, and enzyme function will not be disturbed even if it is in the presence of methicillin or any other β-lactam (Drawz and Bonomo 2010).

Vancomycin-resistant strains can also modify antibiotic targets D-Alanyl-D-Lactate of peptidoglycan precursors (Kwun et al. 2013). According to Figure 3, D-Ala-D-Lac production requires VanS, a membrane detector of vancomycin that regulates the levels of phosphorylation of VanR. VanR is a transcriptional inducer of the *van* operon composed of membrane bound histidine kinase and cytoplasmic response regulator *vanHAX* (Depardieu et al. 2007). VanH is a dehydrogenase that converts pyruvate to D-Lac, while VanA acts as a ligase which assembles the formation of an ester bond between D-Ala and D-Lac precursors (Kwun et al. 2013). The resistance takes place because vancomycin does not interact with D-Ala-D-Lac in peptidoglycan (Courvalin
2. Exclusion of the Antibiotic

Daptomycin is an antibiotic created by *Streptomyces roseosporus*, a soil actinomycete, that is used to treat systemic infections with Gram-positive bacteria (Steenbergen et al. 2005). Daptomycin is composed of a 13-amino-acid-lipopeptide chain that is trapped by cytoplasmic membranes, which leads to membrane perforation, K⁺ efflux (Steenbergen et al. 2005), and apoptosis of the cell (Straus and Hancock
It is believed that resistance to daptomycin arises due to a modification of bacterial cell walls and might be correlated to vancomycin resistance in vancomycin-intermediate \textit{S. aureus} (VISA) strains (Camargo et al. 2008). VISA strains show peptidoglycan layers that are joined together to prevent vancomycin and daptomycin access to the cell membrane (Yu et al. 2012). Daptomycin is a large molecule and is unable to cross through the porin channels of modified walls (Kohanski et al. 2010).

3. \textbf{Efflux Pumps}

Efflux pumps are mechanisms that transport elements, like antibiotics, from the interior to the exterior of the cell (Webber and Piddock 2003). Some efflux pumps are specific to one antibiotic while others can accommodate a variety of antibiotics (multidrug) and be responsible for a multitude of bacterial resistance (Nikaido 2009). \textit{S. aureus} is known to have 30 efflux pumps for quinolones (Costa et al. 2013). NorA is an efflux pump for fluoroquinolones in \textit{S. aureus} (Costa et al. 2013). \textit{norA} is a chromosomal gene that encodes for three alleles that differ by up to 10% in their nucleotide sequences (Costa et al. 2013). According to the literature, fluoroquinolone resistance has increased with the overexpression of \textit{norA} gene. The resistance to fluoroquinolones can be due to mutations in the promoter region of the \textit{norA} gene through the action of regulatory proteins (Costa et al. 2003). Thus, if mutations take place at the \textit{norA} promoter region, transcription level is affected and consequently, overexpression of the gene associated with a mutation will occur. As a result, the efflux pump suffered a misfolding due to the inability of the regulator protein to bind to the promoter and act against \textit{S. aureus} (Couto et al. 2008).
4. Mode of *mecA* gene

SCCmeC stands for staphylococcal cassette chromosome *mec*, which is part of a family of mobile genetic elements that encode genomic islands with a methicillin-resistance gene known as *mecA* (Katayama et al. 2000). SCCmeC elements that do not have *mecA* gene have cassette chromosome recombinase (*ccr*) gene instead (Ito 2009). *ccr* genes code for recombinases that allow deletions or insertions to take place in the SCCmeC chromosome in order to obtain the correct orientation for transcription (Zong 2013). *mec* complex carries copies of plasmids with resistant genes while *ccr* complex is responsible for the mobility of SCCmeC. Five types of *ccr* and four classes of *mec* complex (*mecA* being the most important) work together to form SCCmeC cassette (Katayama et al. 2003). All methicillin-resistant *S. aureus* (MRSA) strains harbor at least one SCCmeC element in their chromosome, some of which include a *mecA* gene that encodes PBP2a (Lowry 2003). PBP2a is a protein which binds to peptidoglycan and disrupts the peptide bond in cross-linkages of peptidoglycan chains. Penicillin-binding proteins have a natural affinity for penicillin, methicillin, and other β-lactam/β-lactam-like antibiotics (Drawz and Bonomo 2010). When PBPs are bound to one of these antibiotics, they no longer catalyze cross-linkage of peptidoglycan in the cell wall. This weakens the cell walls, and cells are more likely to lyse (Drawz and Bonomo 2010). However, PBP2a shows a decreased affinity to these drugs, conferring antibiotic resistance (Stapleton and Taylor 2002).
MRSA strains have the ability to activate mecA expression in the presence of β-lactam antibiotics and repress mecA expression in their absence (Stapleton and Taylor 2002). In reference to Figure 4, if methicillin, penicillin or any other β-lactam is not present, mecA expression is repressed by MecI, which binds to the promoter of mecA and inhibits transcription (Stapleton and Taylor 2002). In the presence of β-lactams, expression of mecA is activated by MecR1, a cell surface protein whose role is to detect β-lactams. Upon detection, the MecR1 inhibitor is disrupted by deoxyribozymes which reduce mecRI and PBP2a mRNAs expression, and thus facilitate expression of mecA (Meng et al. 2009).
Figure 4- Mechanism of action of mecA gene in the presence and absence of beta-lactam antibiotics (Arede et al. 2012).
5. Specific Treatment for Staphylococcus aureus

S. aureus infections are the most alarming of all staphylococci. In the US, nearly 60% of infections with S. aureus are caused by methicillin-resistant strains, and this bacterium is thought to be the most difficult to cure (Sakoulas and Moellering 2008). As it was previously stated, strains can cause food poisoning, toxic shock syndrome, MRSA, MSSA, and skin infections (Turnidge et al. 2002). Despite the fact that these diseases are serious, they are worsened because they are also resistant to a number of antibiotics. Treatment options are limited, complex, and expensive (Sakoulas and Moellering 2008). Without effective antibiotics, it becomes nearly impossible to treat such complicated infections (Davies and Davies 2010). Forrest and Tamura (2010) believe that a combination of antibiotics can improve the efficacy of the treatment against MRSA versus the use of a single monotherapy.

1. Methicillin-Resistant Staphylococcus aureus (MRSA)

To effectively reduce and stop MRSA development, it is imperative to test clinical isolates against antibiotics using disk-diffusion (Kirby-Bauer) tests on agar plates (Skov et al. 2006). Kirby-Bauer tests determine an isolate’s sensitivity to a certain antibiotic. In the presence of an efficient antibiotic, a zone of inhibition will appear on agar plates (Skov et al. 2006). Vancomycin, for example, is considered an efficient antibiotic to treat MRSA infections (Appelbaum 2007). On the other hand, methicillin disks show no zone of inhibition, indicating that bacteria are uninhibited (Skov et al. 2006). Antibiotic therapy should be continually administered for 72 hours to MRSA patients until multiple sets of blood cultures are obtained, even if the symptoms have improved. Early stoppage of therapy can allow MRSA to survive and develop further antibiotic resistance (Hughes
2008). Overall, each patient may require a personalized approach based on the type and severity of infection and status of the individual (Hughes 2008).

For patients with severe MRSA infections (more prone to death), a combination of two or more drugs, like vancomycin, linezolid, and sulfamethoxazole-trimethoprim is considered to be the most efficient treatment (Deresinski 2009). Minor skin infections may respond well to mupirocin. Mupirocin is utilized mainly as a topical agent that specifically and irreversibly binds to isoleucyl tRNA synthetase, thus preventing protein formation (Gisby and Bryant 2000). The effectiveness of mupirocin has been observed in surgical and bronchopulmonary infections (Watanabe et al. 2001).

For patients with endocarditis, teicoplanin is known to be the most efficient treatment. The recommended target is more than 20 mg/L of plasma reached by teicoplanin prior to the next dose administered, which is referred to as trough concentration (Ueda et al. 2014). Long-term use of teicoplanin does not lead to resistance, but isolates can become resistance to vancomycin (Cetinkaya et al. 2000). Alternative drugs are available for patients with less severe infections. Normally, a combination of oral therapy with two active agents, such as rifampin or fluoroquinolones, can be prescribed (Leekha et al. 2011). Oral therapeutic antimicrobial agents are discouraged for long-term use because of risk for occurrence of resistance and adverse effects of the antibiotics (Turnidge et al. 2002).

2. Methicillin-Susceptible *Staphylococcus aureus* (MSSA)

In the 1990s, methicillin-susceptible *S. aureus* (MSSA) strains were studied and known from infections in previously healthy patients. (David et al. 2011). Currently, MSSA infections are treated with penicillinase-resistant penicillins, such as dicloxacillin
and oxacillin in oral or injectable form (Rayner and Munckhof 2005). A wide range of researchers have adopted these drugs because penicillinase-resistant penicillins destroy bacteria and show a reduced incidence of adverse reactions (Fair and Tor 2014). Studies have shown that outpatients with serious staphylococcal infections have presented satisfactory outcomes (Turnidge et al. 2002).

Cephalosporins have been a useful alternative to penicillinase-resistant penicillins due to their stability against staphylococcal β-lactamase. Patients with a history of mild allergies or intolerance to penicillins have been able to use cephalosporins as a treatment (Turnidge et al. 2002). Nonetheless, cephalosporins are not considered a safe choice for patients who suffer from severe allergies, such as angioedema or anaphylaxis. This can be due to partial cross-allergenicity between penicillins and cephalosporins which can create severe hypersensitivity (Rayner and Munckhoff 2005). In these cases, clindamycin or vancomycin are known to be most effective treatments (Rayner and Munckhof 2005).

Currently, β-lactams combined with β-lactam inhibitors, such as amoxicillin/clavulanic acid, or ampicillin/sulbactam are effective against Gram-negative anaerobes. This combination of drugs gives a broader spectrum, a considerable advantage when staphyloccoci are present in mixed infections with Gram-negative bacteria, including anaerobes (Turnidge et al. 2002).
V. Conclusion

Antibiotics developed to answer the demands of the global society. Antibiotics became one of the resources used to treat and prevent diseases. During World War II, penicillin saved millions of soldiers and ultimately changed the course of history (Ventola 2015). Until 1950s, antibiotics were responding well to the demands of society and controlled nearly all major bacterial infections during WWII (Ventola 2015). Bacteria began to survive in the presence of the most common antibiotics used in the medicine era. Today, several studies have shown and prove that inappropriate and unnecessary use of antibiotics plays a very crucial role in the expansion of antibiotic resistance (McEachran et al. 2015).

Bacteria are capable of passing genetic material from one bacterium to another, leading to the appearance of mutations. Mutations when developed can confer resistance to antibiotics. Despite warnings from Ventola and other researchers, antimicrobial agents continue to be misused and overprescribed all over the world (Ventola 2015). In the U.S., data collected in 2010 states that up to 50% of antibiotics where inappropriately prescribed to the patients (Ventola 2015). Zdziarski study shows that unnecessary drug prescription has led to foodborne and waterborne infections, gastrointestinal complications, antibiotic oversensitivity, ecosystem modification, and disturbances in human and animal evolution (Zdziarski et al. 2003).

Further studies should investigate how to minimize antibiotic resistance by controlling the over-prescription of drugs. Not every disease requires antibiotics. In the UK and Thailand, self-medication is easily available over the counter, and without
evaluation people continue to abuse antibiotics (Pechere 2001). However, the Netherlands is one of the northern European countries with the lowest incidence of antibiotic resistance because laws have been implemented prohibiting over the counter drugs. For instances, physicians are the only ones allowed to prescribe drugs that are used to treat the infections. If a patient has common flu or cold, antibiotics cannot be prescribed (Rossignoli and Calvenna 2007). In livestock settings, the European Union committee implemented drug prescription forms in order to control the usage of antibiotics in food animals and farms. A threshold limit determined by the European committee is controlled to ensure that the usage of antibiotics are been met in farm animals (Bartlett et al. 2013). Also, educating the general public of the risks associated with misuse of drugs can facilitate and control the bacterial spread (Grigoryan et al. 2010). By following hygiene guidelines and effective disinfection procedures in the community and in the hospital, the Netherlands believes that it can play a vital role in minimizing the risks of antibiotic resistance (Deurenberg et al. 2006).

In the US, staphylococcal infections have imposed severe health conditions and economic burden in over 2 million people every year (Stone et al. 2005). In 2005, after hand hygiene compliance was implemented, successful results arose in the US hospitals for MRSA but have not yet been seen in community settings (Carlet et al. 2012). On the other hand, Netherlands communities found that MRSA skin abscesses less than 5 cm in size are effectively treated with an incision and drainage instead of using antibiotics. Scandinavia has followed similar measures to control antibiotic resistance and has the lowest MRSA prevalence of all European countries, at only 0.6% (Deurenberg et al. 2006). Even though in the US laws have been implemented, guidelines have been established and educational programs have been campaigned, society still has
preconceived habits concerning antimicrobial agents and their side effects. The scientific community would highly benefit if further research could be done on how to effectively educate society, pharmacists, physicians, nurses, and parents of every nation (Carlet et al. 2012). National and international committees should together propose effective strategies to address antibiotic resistance among humans and animals, in particular the interaction between livestock feed and the environment (McEachran et al. 2015).
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VII. References


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