A Review of *Staphylococcus aureus* Antibiotic Resistance: Geographical, Molecular, and Evolutionary Patterns in Recent Years

Roger Christopher Hseih

Writer's Comment: Prior to taking UWP 102B, I had worked as a research assistant for UC Davis Medical Center's Infectious Diseases Division. The patients we saw often faced extended stays and were debilitated from sepsis due to strains like Staphylococcus aureus. *Combination antibiotic therapy was frequently prescribed to those who were initially hospitalized for unrelated reasons, indicating the prevlence of antibiotic resistance. This was frustrating to witness. Surprisingly enough, I had recently accepted a postgraduate position to understand immune interactions with this microbe's evasion mechanisms. So, when Dr. Brenda Rinard encouraged us to write reviews on a biological topic that we wanted to master and were intrigued by, I knew exactly what to do. Creating my review not only provided a foundational understanding of the work I would be involved with, but it also enhanced my appreciation for the breadth of medical research on systemic issues in healthcare delivery. With hospitalization rates as high as they've ever been, I hoped to emphasize this issue's importance so that future generations will not lack necessary treatments we somewhat take for granted.*

Instructor's Comment: Roger wrote this review article in my UWP 102B: Writing in the Biological Sciences course. I encouraged the students to research a topic they were curious about, or one that would help them understand the work that was being done on campus. They first found peer-reviewed sources, wrote an annotated bibliography, and then organized the sources in the review article with a focus on synthesis

and theme. Roger's article, "A Review of Staphylococcus Aureus *Antibiotic Resistance: Geographical, Molecular, and Evolutionary Patterns in Recent Years," illustrates the best of this genre. His sources, taken from a variety of credible journals, show that even a niche topic in the biological sciences can be aproached from a multidisciplinary perspective.*

—Brenda Rinard, University Writing Program

Abstract

Staphylococcus aureus presents a large problem for present-day scientists, clinicians, and epidemiologists due to its quick adaptations to pharmacological development, increasing virulence, and rapid spread across the globe. The geographical effects of *S. aureus* are notable both within the hospital environment and the community. Thus, there is a dilemma of preventing this intertwining genetic transfer of strains carried out by workers who inhabit both settings. This dynamic presents a challenge, as the majority of procedures are largely associated with postoperative *S. aureus* infection complications, which can lead to death in those with many comorbidities or weakened immune systems. HA (hospital-associated) and CA-MRSA (community associated, methicillin-resistant *Staphylococcus aureus*) must be typed and tracked closely to both derive treatments and also prevent the future production of new superbugs. In addition to geographical patterns, molecular mechanisms must be well understood to produce new antibiotics. Resistance to penicillin in the mid-1940s has resulted in new indirect methods of attacking gram-positive *S. aureus* primarily through inhibitory mechanisms, but they are slowly becoming obsolete due to the elusive mechanisms of *S. aureus*. As a result, tracking *S. aureus* lines, comparing their relative genomes, and tracing their evolutionary patterns are essential to deriving new antimicrobial strategies. Relevant progress to combat this issue includes focusing on universal bacterial mechanisms such as quorum sensing or shared genetic characteristics, as well as recognizing the importance of utilizing the most conservative antibiotic treatment plans for patients.

Introduction

Staphylococcus aureus is a gram-positive bacterial strain commonly found in human microbiota with a commensal relationship for human

metabolic function, but it also often acts as a potent opportunistic pathogen that is the leading cause of hospital-acquired infections (Klein et al., 2007). This microbe presents a global problem due to the additional expenditure required for treatment of hospitalized patients, increased mortality and complication rates, and also allocation of needed medical resources for longer stays (Zhen et al., 2020). A surge of antibiotic resistance by *Staphylococcus aureus* since the mid-1940s has caused much concern both within the scientific and clinical communities, prompting researchers to not only understand the source and carriers of new pathogenic strains but also the molecular mechanisms that are driving the continuation of these small epidemics (Demerec, 1945).

Despite many publicized therapeutic discoveries and antimicrobials such as penicillin and vancomycin on the market today, the elusiveness of *S. aureus* has outpaced pharmaceutical progress. As a result, it has forced scientists to establish a better understanding of its unique ability to acquire virulence factors that are far more potent than those of other strains (Spellberg et al., 2004). Its speed in changing has been attributed to horizontal gene transfer, antibiotic selection, and chromosomal mutations in important genes, which occur in select strains at a far more rapid pace than others. Resistance has also recently been attributed to normal bacterial survival mechanisms such as cell synthesis, quorum sensing, and new protein functions (Hanaki et al. 1998; Rudkin, 2012; Ubukata et al., 1989). Some are more prevalent in hospital infections than community spread infections due to overuse of common antibiotics. However, the unique method by which *S. aureus* excels at these common bacterial processes is yet to be determined, and has not been fully elucidated in recent research. There is also a lack of understanding as to how certain bacterial resistance mechanisms can be adapted to multiple types of antibiotics.

This review will outline the epidemiological variation in *S. aureus* and transmission patterns of both hospital-associated and community associated strains. Understanding the specific demographic of *S. aureus* carrier patients helps identify the origins of these infections and allow scientists to implement a "search and destroy" initiative to reduce epidemic occurrences (Chatterjee & Otto, 2013). In addition, this paper also examines the evolutionary patterns and traits specific to methicillin-resistant *S. aureus* strains in more recent years. These include methicillin, vancomycin, and rifampicin resistance that accounts for research

developments being made and their effectiveness in mitigating the burden of disease. *S. aureus*' constantly changing abilities requires a timely and concerted multidisciplinary effort to continue antibiotic development, identify factors that contribute to resistance, and understand the patterns of strain transmission.

Epidemiology of *S. aureus* Infection Types

S. aureus has long been recognized as the major cause of skin and soft tissue infections but is also a large contributor to systemic infections. Due to its clinical significance and invasion in pathology involving lung infections and sepsis, *S.* aureus was originally thought to be solely a burden in the clinical setting. The rise of MRSA had been previously established as a result of improper dosing regimen due to weakened immune systems and other chronic disorders. Hospital-associated (HA) infections were much more apparent and a result of direct patient contact, open wounds, procedures, and airborne transmission due to the presence of nosocomial infections (Solberg, 2000). However, sampling within the past few decades has proven the large presence of community associated (CA) infections. In addition, these infections were observed in seemingly healthy and nonsusceptible patients who had no clinical exposure, emphasizing the pathogenicity of these strains. Approximately 65 percent of MRSA epidemic infections sampled from three different locations were revealed to be CA infections (Dukic et al., 2013). This drastic shift in dominant MRSA strains from HA to CA suggests a difference in virulence factors and fitness properties that had not been previously observed in the already potent methicillin resistant microbes. Toxins exclusive to CA strains, such as particular SCC*mec* elements like type IV, are a source of difference from the original HA type I-III toxins and indicate a target for future therapeutics. In addition, the higher level of expression of Panton-Valentine leukocidin (PVL) toxins in CA-MRSA allows evasion from neutrophil immune response (Otto, 2013). Based on this molecular typing, CA-MRSA can thus be inferred to be much more pathogenic than HA-MRSA. While none of the features are exclusive to its origin of discovery, it is important to continue whole-exome sequencing of these different strains to establish new virulence factors in strains from different locations. These efforts are essential to creating new broad-spectrum antibiotics that could be potentially effective against MRSA and other similar up-and-coming microbes.

The prominence and increased pathogenicity of these strains raises the question of transmission methods within the community. Direct skin-to-skin contact and hygiene in specific settings such as gyms, daycare facilities, and prisons and among specific demographics such as cultural minorities have been established as the most common forms of spread outside of the clinical setting (Dukic et al., 2013). Targeting these strains and treating infected patients before requiring acute medical attention is a priority. These measures are intended to prevent pathogen dominance in the clinical setting due to their increased virulence and spread to other patients who may be more susceptible to worse clinical outcomes. As a result, identification of strain origins has been conducted in those who may be present in both the community and healthcare settings to elucidate whether those entering the workforce may acquire HA-MRSA and spread it into the community, or possess CA-MRSA and spread it into the clinic. Of the medical students at Galilee of Bar-Ilan University in Safed, Israel, 12 percent were revealed to have CA-MRSA with characteristic SCC*mec* type IV elements prior to even entering clinical education (Orlin et al., 2017). Students also acquired MRSA after exposure to the clinical setting going from 33 percent carriers to 41 percent, but acquisition of any new strain presents the unique problem of genetic transfer between strains of both settings.

Due to the interaction between CA-MRSA and HA-MRSA, risk factors for infection and morbidity must be delineated for effective treatment. Prevention of HA-MRSA is essential to avoid worsening outcomes due to the threat of community infection encroachment. Within the hospital setting locally in Wales, risk factors for invasive MRSA infection are greatest for urinary catheterization, central lines, and surgical procedures, with 51 percent, 39 percent, and 16 percent association, respectively (Carnicer-Pont et al., 2016). These are necessary focal points prior to medical intervention in order to reduce the economic burden and mortality rates of patients. Data from Novant in the southeastern United States has shown that hand hygiene may be associated with all these procedures and can drastically reduce HA-MRSA infections by half over the span of three years, from 2005 to 2008 (Lederer et al., 2009). The prevention and isolation of MRSA strain geographical overlap is essential not only for treatment but potential future eradication of other antibiotic-resistant strains.

Molecular mechanisms of antibiotic resistance in *S. aureus*

Decoding the molecular mechanisms behind *S. aureus* resistance is essential not only to understanding potential therapeutic candidates, but also for identifying unique behavioral patterns within the species. Resistance in common bacterial strains has been established as a result of incomplete therapy, but a large portion of *S. aureus*'s effects have been attributed to its unique evasion molecules encoded by its genome (Chatterjee & Otto, 2013). Resistance of *S. aureus* has been noted since the inception of penicillin production, but primarily due to SCC*mec* elements. These mobile genetic elements may be transferred from strain to strain and encode for penicillin binding proteins (PBPs). Since penicillin binds to the peptidoglycan layer of most gram-positive bacteria and disrupts cell wall synthesis, particular *mec* genes have been sought to understand its evasion of common antibiotics. PBPs were shown to be produced at high amounts by *mec*A, which could be potentially induced into non-resistant strains. This process required recombinant plasmid vectors, transformed *mec*A cassettes, and a variable amount of penicillin-derived antibiotics containing B-lactam. Destruction of penicillin by exogenous penicillinase and increased antibiotic resistance with a MIC (minimum inhibitory concentration) was found to be four times as high as the baseline as well (Ubukata, 1989). This further proved that penicillinase may be a translational product that could assist MRSA in its PBP efficacy. These statistically significant results point to horizontal or lateral resistance as a large source of resistance in various cell lines both *in vitro* and in clinical subjects. The ease by which *S. aureus* obtains these resistance factors is alarming, as it indicates the abundance and combination of *mec* elements can produce strains that may be even more difficult than current MRSA strains to treat. *mecA*'s prevalence worldwide is a prime example of this universal resistance that occurs rapidly regardless of geographical location (Hiramatsu et. al, 2002).

The ubiquity of MRSA resistance is likely due to its primal status in resistance development relative to other bacterial strains, but evasion is not solely isolated to methicillin-derived products. Development of newer MRSA-susceptible antibiotics, including vancomycin, have been required to combat widespread bacterial resistance. These therapeutics have also resulted in newer strains of vancomycin-resistant *S. aureus* (VRSA) as a result of agricultural antibiotic usage (Bager et al. 1997). The mechanisms of vancomycin resistance have been evaluated in MRSA

strains, particularly with relation to cell-wall synthesis. While penicillin serves to destroy gram-positive bacteria through direct interaction with cellular components, newer antibiotics serve as inhibitory factors. Vancomycin had been previously shown to interfere with hydrogen bonds with N-acetylglucosamine in the cell wall, preventing further cross-linkage of more glucose polymers (Chatrchai, 1984). Certain *S. aureus* strains, such as Mu3 and Mu50, were used in vancomycin treatment studies to identify N-acetylglucosamine synthesis and incorporation. As the Mu50 strains had inherently larger cell walls, it was established to have a statistically significant correlation to increased vancomycin resistance (Hanaki et al. 1998). This is opposed to Mu3, whose slower incorporation of cell-wall components and smaller cell walls have been shown to result in lower fitness. Due to the exogenous nature of most therapeutics, this mediation of antibiotic resistance through released toxins or altered membrane features is common. This suggests that continued incorporation of mobile genetic elements can adapt to different external environments and provide better survival despite antibiotics with different mechanisms of action.

Evolution of *S. aureus* resistance

Evolution of resistance mechanisms is largely unknown in *S. aureus* due to the variability of toxins and virulence factors *S. aureus* is capable of producing. To attempt to understand this variability, genome sequencing for strains from different environments such as hospitals and community locations was conducted (Holden et al. 2004). These were compared to previously known MRSA strains to understand recurrent genetic elements, insertion sequences, protein-encoding sequences, and chromosomal genes. Through hospital-associated strains such as MRSA252 and community-associated strains like MSSA476, a set of criteria can be compared. Such criteria include mobile cassettes like *mec*A which are present in hospital-associated MRSA, but absent in community-associated methicillin-susceptible strains like MSSA476. MRSA strains like MRSA252 have more resistance determinants, virulent homologues, and encoding transposons, indicating that many hospital-associated strains are still more virulent. However, the similarities between MRSA252 and MSSA476 represent a case study in which their similarities suggest genetic relationship and familiar derivation from one to another (Holden et al. 2004). This data suggests that there is a high

but not statistically significant chance that many MRSA strains are born from MSSA strains through biofilm interaction and symbiosis between one another. Due to the variability within hospital environments, it is possible that there is an increased evolution of resistance in particular MRSA strains.

This interaction between strains and abundance of *S. aureus* in the clinic however, may lead to negative consequences for MRSA strains. The *agr* quorum sensing system, which is intended for MRSA to employ a virulent response, was observed to be inhibited by *mec*A mobile elements (Rudkin, 2012). This toxicity reduction is dose dependent, suggesting that the presence of more HA strains may reduce its virulence overall and cause self destruction despite resistance. This was noted with *in vitro* studies where rifampicin-resistant strains led to lower fitness when exposed to increasing rifampicin levels (Wang et al. 2019). However, despite a shorter lifespan, the same strains were still incredibly pathogenic at its initial MIC. Antibiotic resistance may come with a fitness cost to certain bacteria in the absence of antibiotic overuse, but the relative threshold can not be determined *in vitro* and will vary between patients due to prior exposure and different immune systems. *S. aureus* may capitalize, proliferating at this MIC threshold and triggering a storm of pathogen-specific toxins that could be resistant to a singular commercial antibiotic and immune responses. As a result, it is important for certain antibiotics such as rifampicin to not be used alone and recklessly, and it is essential to employ complex antibiotic regimens in certain patients. Due to the constant evolution of existing and new strains and our knowledge on its diverse mechanisms, it is important to maintain a wide arsenal of differing treatment plans when combatting *S. aureus*.

Conclusion

Recent data suggests a significant relationship between *S. aureus* prevalence in geographical communities and the constant quest to develop more therapeutics for nonexistent resistant mechanisms. The elusive nature of certain strains requires sequencing, identification of genetic elements, and observation of molecular phenotypes. However, reduction of transmission highly depends on geographical origin and is essential to delay an imminent need for pharmacological breakthrough. Thus, HA and CA infections must be identified and epidemiologically traced. Discovery of particular strains has revealed newer antibiotic resistance

mechanisms that may now be more prevalent within communities, but these observations are definitely not an endpoint indication of mechanism prevalence. These strains have exhibited increased cell-wall synthesis mechanisms as well as SCC*mec* and *mec*A elements that specifically target current broad-spectrum antibiotics like methicillin and penicillin. These antibiotic resistance mechanisms have evolved rapidly in *S. aureus* through horizontal gene transfer and chromosomal mutations within biofilms over the past seventy-five years. However, some mutations may cause a decrease in fitness cost. Due to the rapidly changing schematic of *S. aureus* virulence factors and the rise of MRSA, much work and clinical studies should be conducted for more effective pharmaceutical solutions. Further studies on strain-specific characteristics are essential for treatment of patients, due to the highly variable combination of genetic elements, in order to reduce economic burden and mortality rates.

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