Journal of Advances in Science and Technology

Vol. IX, Issue No. XIX, May-2015, ISSN 2230-9659

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AN INTERNATIONALLY INDEXED PEER REVIEWED & REFEREED JOURNAL

www.ignited.in

Journal of Advances in Science and Technology
Vol. IX, Issue No. XIX, May-2015, ISSN 2230-9659

The Historical Perspective of Methicillin-Resistant Staphylococcus Aureus (MRSA): Characterization and Quantitative Development

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Abstract – Methicillin-resistant Staphylococcus aureus (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes. The evolutionary origins of MRSA are poorly understood, no rational nomenclature exists, and there is no consensus on the number of major MRSA clones or the relatedness of clones described from different countries. We resolve all of these issues and provide a more thorough and precise analysis of the evolution of MRSA clones than has previously been possible.

Using multilocus sequence typing and an algorithm, BURST, we analyzed an international collection of 912 MRSA and methicillin susceptible S. aureus (MSSA) isolates. We identified 11 major MRSA clones within five groups of related genotypes. The putative ancestral genotype of each group and the most parsimonious patterns of descent of isolates from each ancestor were inferred by using BURST, which, together with analysis of the methicillin resistance genes, established the likely evolutionary origins of each major MRSA clone, the genotype of the original MRSA clone and its MSSA progenitor, and the extent of acquisition and horizontal movement of the methicillin resistance genes. Major MRSA clones have arisen repeatedly from successful epidemic MSSA strains, and isolates with decreased susceptibility to vancomycin, the antibiotic of last resort, are arising from some of these major MRSA clones, highlighting a depressing progression of increasing drug resistance within a small number of ecologically successful S. aureus genotypes.

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INTRODUCTION

Staphylococcus aureus is an opportunistic pathogen often carried asymptomatically on the human body. Methicillin-resistant S. aureus (MRSA) strains have acquired a gene that makes them resistant to all beta-lactam antibiotics. Hospital-associated strains of this organism are serious nosocomial pathogens that have become resistant to most common antibiotics, and treatment can be challenging. Community-associated MRSA strains occur in people who have not been hospitalized or recently had invasive procedures. They first appeared in highrisk populations (e.g., intravenous drug users, people with chronic illnesses), but are now found even in healthy children. Until recently, community-associated strains were susceptible to many antibiotics other than beta-lactams; however, resistance seems to be increasing, and multiple antibiotic resistant strains have started to emerge. Human-adapted MRSA can be transmitted to animals in close contact, which can sometimes act as carriers and re-infect people.

Animal-adapted MRSA strains also exist. The pig-associated lineage MRSA CC398 is a particular concern. This lineage, which apparently emerged between 2003 and 2005, has spread widely among swine in some locations. Colonization with CC398 has also been reported in other species, including veal calves and poultry. Asymptomatic carriage is common among people who work with colonized swine or other livestock, and these organisms can cause opportunistic infections. Other MRSA strains can also affect animals. Outbreaks in horses suggest that MRSA might be an emerging problem in this species. Dogs and cats seem to be infected infrequently, and mainly by human-adapted strains; however, carriage rates can be higher during outbreaks in veterinary hospitals and other facilities. While colonization of pets is often transient, it might contribute to maintaining MRSA within a household or facility.

Methicillin was introduced in 1959 to treat infections caused by penicillin-resistant Staphylococcus aureus. In 1961 there were reports from the United Kingdom

of S. aureus isolates that had acquired resistance to methicillin (methicillin-resistant S. aureus, MRSA) and MRSA isolates were soon recovered from other European countries, and later from Japan, Australia, and the United States. MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community. The methicillin resistance gene (mecA) encodes a methicillin-resistant penicillin-binding protein that is not present in susceptible strains and is believed to have been acquired from a distantly related species. mecA is carried on a mobile genetic element, the staphylococcal cassette chromosome mec (SCCmec), of which four forms have been described that differ in size and genetic composition. Many MRSA isolates are multiply resistant and are susceptible only to glycopeptides antibiotics such as vancomycin and investigational drugs.

MRSA isolates that have decreased susceptibility to glycopeptides (glycopeptide intermediately susceptible S. aureus, GISA), reported in recent years, are a cause of great public health concern.

Many studies have characterized MRSA isolates from individual hospitals or countries and have identified strains that appear to be well adapted to the hospital environment, are established in several hospitals within a country, or have spread internationally (epidemic MRSA, EMRSA). MRSA isolates are generally characterized by pulsed-field gel electrophoresis, a powerful technique for identifying the relatedness of isolates from recent outbreaks within a hospital, but are not well suited to long-term global epidemiology, which requires a procedure that is highly discriminatory but that indexes variation that accumulates slowly. Multilocus sequence typing (MLST) provides such a procedure and characterizes isolates of bacteria unambiguously by using the sequences of internal fragments of seven housekeeping genes. MLST has been developed and validated for S. aureus and provides a discriminatory method that allows related strains recovered in different countries to be readily identified.

The origins of the major MRSA clones are still poorly understood. Kreiswirth et al. proposed that all MRSAs were descended from a single ancestral S. aureus strain that acquired mecA, but more recent studies show that some MRSAs are very divergent, implying that mecA has been transferred between S. aureus lineages. The data from MLST can be used to probe the evolutionary and population biology of bacterial pathogens and to predict ancestral genotypes and patterns of evolutionary descent within groups of related genotypes. We have applied MLST to an international collection of 359 MRSA isolates, which includes examples of the previously described EMRSA and GISA clones, and compare these to a collection of 553 methicillin-susceptible S. aureus (MSSAs). We demonstrate the limited number of major EMRSA genotypes and provide an unambiguous method for characterizing MRSA and GISA clones and a rational nomenclature. We also identify the ancestral MRSA clone and its MSSA ancestor and suggest the evolutionary pathways by which MRSA clones have repeatedly emerged from successful MSSA clones.

Staphylococcus aureus is a gram-positive bacterium of the family Staphylococcaceae. It can be identified by its distinctive yellow-gold pigmentation. The rapid increase of SA strains resistant to methicillin and other antibiotics, from a mere 2% of isolates in 1974 to 64% in 2004, has attracted much attention due to the high rates of human morbidity and mortality associated with these infections. Human infections of Staphylococcus aureus usually affect people who are under hospital care, or who have recently left a hospital setting. Infections that occur while under hospital care, which do not respond to beta-lactam antibiotics, are referred to as nosocomial or hospital-acquired MRSA (HA-MRSA). HA-MRSA typically presents with skin irritation or even more invasive infections in open wounds, the circulation or internal tracks, resulting in acute systemic pathologies. Another form of MRSA is the community-acquired strain (CA-MRSA), which occurs in people who have not been recently hospitalized. This particular strain is “transmitted primarily through direct skin-to-skin contact, but can also be spread through contamination of environmental surfaces such as clothing and towels”. There are many risk factors associated with this bacterial infection aside from a compromised immune system due to openings in the skin. These factors could include crowded living conditions, poor hygiene, and close skin-to-skin contact. Since CA-MRSA attacks populations outside of the hospital, its target populations usually include “athletes, military recruits, children, Pacific Islanders, Alaskan Natives, Native Americans, men who have sex with men, and prisoners”. CA-MRSA most commonly presents with a nonspecific irritation of the skin, and thus is likely widely underreported in compiled epidemiological data. Symptoms such as fevers, chills, and nausea can arise; however, there is a high likelihood that infected persons will not seek treatment, unless the infection becomes highly invasive, leading to septic shock or bacteremia.

STAPHYLOCOCCUS AUREUS – GENERAL DESCRIPTION

Staphylococcus aureus subsp. aureus (S. aureus) belongs to the genus Staphylococcus and to the family Staphylococcaceae. It was firstly described by Sir Alexander Ogston in 1882 and 2 years later Rosenbach isolated it in a pure culture and introduced the name Staphylococcus aureus. The name of the organism is derived from Greek words staphyle (a bunch of grapes) and coccus (grain or berry).

S. aureus is a Gram-positive, facultative anaerobic, catalase-positive, oxidase-negative, nonmotile microorganism that does not form spores. It creates smooth, convex, lustrous, circular colonies reaching a size of 0.5-1.5 μm in diameter and growing in an

irregular threedimensional bunch of grapes-like clusters of cells. In dependence on growth conditions, the colony pigmentation varies from grey, grey-white with yellowish to orange shades with typical β-haemolysis on the blood agar.

For growth it requires B vitamins (thiamine and nicotic acid), inorganic salts and amino acids as a nitrogen source, especially arginine, cystein, proline and valine. Glutamic acid, leucine and tyrosine are not required for growth, but they are essential for enterotoxin production. Deprivation of any amino acid is much less responsive in SEA production than for SEB or SEC production. Arginine seems to be essential for enterotoxin B production.

S. aureus belongs among chemo-organotrophs with a respiratory and fermentative metabolism. Under aerobic conditions, acids are produced from glucose, lactose, maltose and mannitol, under anaerobic conditions acids are produced from many other sugars and alcoholic sugars. Most strains hydrolyse native animal proteins (casein, gelatine, fibrin), lipids, phospholipoproteins and Tween. They also coagulate animal plasma with the assistance of a coagulase and the clumping factor. Besides that, the typical enzymatic activity of S. aureus includes production of coagulase, alkaline phosphatase, proteases, lipases, and esterases and some strains also produce lecithinase.

Staphylococci are very widespread bacteria. Their main representative, Staphylococcus aureus subsp. aureus, is one of the most important and successful human pathogens. According to current knowledge, the Staphylococcus genus has 50 taxons with 39 various types and several subtypes1.

Staphylococcus aureus (S. aureus) is among the most ubiquitous of bacteria. It is highly resistant to adverse environmental conditions and it resists drying as well as high NaCl concentrations. This enables a probably temporary and even permanent colonization of skin and nasal mucosa.

S. aureus has been detected as a carrier strain in the nasal mucosa of the general population with a mean carriage rate of 37.2 %. However, the range of carriage rates is large. This may be due partly to diff erences in the quality of the sampling and of the culture techniques used in these studies. Two billion individuals are estimated to be carrying S. aureus, worldwide. Persons colonized with S. aureus are at increased risk for subsequent infections. Probably 1 % of these are MRSA colonised. S. aureus is also present in the skin and mucosae of various animals, and it is also found in the environment, especially around people, animals, and in food.

The S. aureus strains produce a number of extracellular enzymes (coagulase, hyaluronidase [spreading factor], penicillinase etc.) and toxins (haemolysins, staphylococcal super antigens and leukocidins), which function as virulence factors. Among leukocidins, the Panton-Valentin leukocidin is currently the focus of considerable attention in connection with community-associated strains resistant to methicillin (CA-MRSA) which produce it. The Panton-Valentin leukocidin was described in 1932 and bears the name of its discoverers – Panton and Valentin. In the literature the abbreviations PVL and Luk-PV are also used. In this case, a cytotoxin forms heptameric pores in the leukocyte membrane and this destroys the leukocyte.

PVL consists of two components that are, depending on their relative speed during the chromatographic division, identifi ed as fast (F) and slow (S). PVL increases the virulence of S. aureus. PVL-carrying strains can cause recurrent, chronic and particularly severe skin and softtissue infections as well as rapidly fatal pneumonia which occur notably in previously healthy, immunocompetent individuals9. However, its role as a virulence determinant has recently been disputed. These MRSA strains are called community-associated MRSA (CA-MRSA). PVL production is a common trait among CA-MRSA, it is important to recognise that PVL-negative strains can also occur. Zhang with his colleagues state: ”the specific role that PVL plays in the epidemiological features and pathogenesis of CA-MRSA infections has remained undefined and controversial”.

When the host is weakened, a spectrum of diseases can occur, from minor skin inflammations (furuncles, impetigo), alimentary poisoning, osteomyelitis, toxic shock syndrome (TSS), staphylococcal scalded skin syndrome (SSSS) and bacterial endocarditis to life-threatening sepsis and pneumonia13. S. aureus is one of the major causes of human infections which originate both in connection with staying in a hospital or outside of it. According to the authors Boyle-Vavra and Daum, S. aureus is the most virulent of the Staphylococcus genus, representing the most frequent pathogen in biological material isolated from in-patients, and in out-patients it is the second most frequent isolated pathogen.

RESISTANCE GENES IN METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

Staphylococcus aureus, which was first isolated by Alexander Ogston in 1880s, is known to cause post-operative wound infections. The mortality rate of the individuals, due to S. aureus infections was around 80% before the introduction of penicillin.The first penicillin resistant S. aureus was isolated from clinical environment in 1942. The problem of penicillin resistance was later circumvented by the introduction

of methicillin. In 1961, methicillin resistant Staphylococcus aureus (MRSA) made an appearance, probably due to the acquisition of the mecA gene, leaving vancomycin as the drug-of-last resort to treat it. Since resistance was not because of the antibiotic destruction by enzyme ß-lactamase, the resistance was termed as "intrinsic". Increased outbreaks had subsequently been reported from many countries after the emergence of MRSA as nosocomial pathogen in the early 1960s. There were reports of life-threatening sepsis, endocarditis, and osteomyelitis caused by this organism. Dissemination of clones of various hospital-associated MRSA (HA-MRSA) has been found worldwide during last five decades. The clones of community associated MRSA (CA- MRSA) also increased worldwide, appearing both in the community and healthcare facilities. Introduction of vancomycin to combat MRSA proved ineffective, as strains resistant to this antibiotic emerged rapidly. The quick and high bacterial replication rate was conducive in spreading these "superbugs" everywhere.

The issue of pathogens, continuously developing resistance to various classes of antibiotics can be better understood and addressed at the genetic level. The remarkable observation is that the pathogen resistances, associated with wide varieties of bacterial toxins, especially under clinical settings, are encoded by a set of mobile genetic elements . S. aureus DNA codons, for super antigen toxins, reside as mobile genetic elements in novel pathogenic islands in its genome. The gene for enterotoxins D and A are encoded by plasmids and prophages respectively. In S aureus, scientists have identified mobile genetic elements of 15-20kb which are called staphylococcal pathogenicity islands (SAPIs)[8]. These are mobilized at high frequencies by certain staphylococcal phages. The prototype of this family is SAPII. Its genetic analysis was done by construction of a derivative, with tetM inserted into tst, which is the gene encoding for toxic shock syndrome toxin-1 (TSST-1).

Genetic elements of antibiotic resistance in S. aureus - The genome sequence of MRSA has revealed that it is composed of a complex mixture of genes. Most of the antibiotic resistance genes are carried either by plasmids or by mobile genetic elements which includes a unique resistance island. Pathogenicity islands identified in the genome of S. aureus belongs to three classes, viz., exotoxin islands, toxic-shock-syndrome toxin islands and enterotoxin islands **.** The length of the mecA gene, which is a mobile genomic island, is 2.1kb. The genetic elements of this staphylococcus cassette chromosome (SCC mec) are of types I to VII and ranging from 20.9 to 66.9 kb. The genes of cassette chromosome recombinases (ccr) are located on all types of SCCmec and encode for invertase/resolvase class of enzymes. These are involved in either integration of SCCmec into or excision of SCCmec from, S. aureus genome at the specific site called the SCCmec attachment site (attBscc). These processes occur at the 3' end of an open reading frame (orfX). The types of mec complex and ccr genes determine the class of SCCmec. The regions which are not part of the mec complex and ccr genes are called Junkyard (J) region. Thus, SCCmec element mainly consists of J3-mec-J2-ccr-J1 sequence.

In MRSA strains, near pur-nov-his gene cluster, an additional chromosomal DNA of approximately 30 to 50 kb of mec has been found. mecI and mecR1 are regulatory elements controlling mecA, which is the structural gene encoding for a 76-kDa PBP 2a.

Molecular basis of methicillin resistance - The major PBP types 1, 2, 3 and 4, with approximately 85, 81, 75, and 45 kDa molecular weights respectively are produced by both resistant and susceptible strains of S. aureus. PBPs types 1, 2 and 3 are essential for growth and survival of susceptible strains and they also show high affinity towards ß-lactams. Their binding to PBPs is lethal to the cell. PBP2 functions as both transglycosylase and transpeptidase. In MRSA, there are two mechanisms of resistance to ß-lactams. 95% of S. aureus resistant isolates produce an enzyme ß-lactamase (penicillinase) encoded by the blaZ gene, which hydrolytically cleaves ß-lactams of the penicillin class. The second broader mechanism involves MRSA isolates containing mecA gene which encodes for PBP2a. In these isolates, there is an alteration in the active site of PBP2a due to which there is a decreased affinity for ß-lactams. As a result, the rate of their acylation is significantly reduced. Hence PBP2a is able synthesize the bacterial cell wall, even in the presence of ß-lactam antibiotics, with the help of its ß-lactam-insensitive transglycosylase domain. The distinctiveness of MRSA in their expression of heterogeneous antibiotic resistance is the generation of subpopulations among individual strains with different degrees of higher resistance.

EPIDEMIOLOGY OF MRSA

Resistance to antimicrobial agents is a growing public health concern. There are several ways in which resistance is transmitted between bacteria.

These include transformation, conjugation and transduction. Transformation is the direct uptake of free DNA material through the bacterial membrane. Conjugation occurs as a result of the transferring of plasmids, while transduction is the transfer of genetic material via viruses called bacteriophages. Once the bacteria have acquired the resistance genes, they further spread through clonal expansion.

The emergence of MRSA was first reported in 1961 in the United Kingdom (UK), barely 2 years after the introduction of methicillin in clinical practice. Since then, the organism has spread throughout the world with variable geographical prevalence. Northern Europe has been shown to have the lowest prevalence rates of about 1%, while North-East Asia

shows some of the highest rates of up to over 60%. The European countries showing generally low prevalence rates include Finland, Denmark, Norway, Iceland, Sweden and the Netherlands. This low prevalence rates has been attributed to major 'search and destroy' operations in these countries. However, some European countries such as Portugal, Greece, Italy and Romania have intermediate rates of 49%, 40%, 37%, 34% respectively.

Initially MRSA pathogens were almost exclusively isolated from hospitals, longterm care facilities, or similar institutional settings and were thus called hospital or healthcare-associated or acquired MRSA (HA-MRSA). According to the Centers for Disease Control and Prevention (CDC), an infection is considered hospital-associated, if it occurs more than 48 hours after admission. Thus, an isolate is defined as a HA-MRSA if cultured from a clinical specimen obtained 48 hours after patient's hospital admission or whose sources of isolation were associated with risk factors for HA-MRSA infection within one year of MRSA isolation date.

Risk factors for MRSA colonization or infection in the hospital include prior antibiotic exposure, admission to an intensive care unit or long-term care facility, recent surgery and exposure to an MRSA-colonized patient.

Humans are a natural reservoir for S. aureus, and asymptomatic colonization is far more common than infection. Colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly after birth and may recur anytime thereafter. Family members of a colonized infant may also become colonized.

However, the epidemiology of MRSA has changed with the emergence of MRSA infections in the community and amongst livestock , such as pigs. Community associated or acquired MRSA (CA-MRSA) were first reported in the late 1990s in healthy children and adults in the community without prior exposure to the risk factors associated with MRSA infection. A CA-MRSA isolate is defined as one cultured during the first 48 hours of a patient's hospital admission, or from patient's whose sources of isolation were not associated with risk factors for HA-MRSA infection as mentioned above. CA-MRSA has spread worldwide and is found not only in the community setting but also in healthcare facilities. In fact, some hospitals have reported a predominance of CA-MRSA isolates over HA-MRSA isolates. Thus, the distinction between HA-MRSA and CAMRSA is slowly becoming unclear.

CONCLUSION

The evolutionary origins of MRSA clones were explored with BURST, which, together with an analysis of the distribution and nucleotide sequences of alleles within SLVs and their presumed ancestors, provides a powerful approach to discerning the likely evolutionary relationships among bacterial clones.

Since the time of its discovery, Staphylococcus aureus has been exposed to different environments that persuaded it to undergo genetic modifications like mutation, or acquiesce genes from the resistance organisms. Circumstances, like the use of antibiotics as preservatives, frequent practice of prescribing the same antibiotic to treat the diseases and also incomplete medication are some of major grounds for developing antibiotic resistance.

Resistant strains of Staphylococcus aureus are a growing threat to public health. In less than forty years, the disease has transformed from a nosocomial infection, affecting those who had almost no immunity, to a disease that can kill a healthy person within 72 hours of infection. It is easily transferred from person to person, via skin-to-skin contact or the sharing of contaminated objects. The bacterium has developed sophisticated immune evasion tactics, which often enable it to spread its virulence factors, like PVL and PSM even more effectively, and make eradication of the infection without treatment very difficult. Conventional treatment for MRSA is growing more expensive and less reliable as the rapidly evolving bacterium becomes resistant to one drug after another.

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