Molecular Epidemiology: A Valuable Tool for Determination of Emerging and clonality of Methicillin Resistant \textit{Staphylococcus aureus} (MRSA)

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\textbf{Abstract} – Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is one of the leading nosocomial pathogen that is also emerging as a zoonotic pathogen. In this review, it was observed that rapid emergence of new MRSA clones at a higher frequency has ushered in a new knowledge on the clonality and epidemic potentials of MRSA. Secondly, the success of treatment and management of MRSA infection is threatened by the diversity in the clonal types. This is because different clones harbours different antibiotics resistance characteristics and as such respond differently to treatment. Furthermore, clonal replacement of hospital-acquired MRSA with community-acquired MRSA has also been observed. Thirdly, the transmission of MRSA even though previously thought to be exclusively within the hospital setting through hand contact and nasal colonization has now spread to the community and in addition human to animal and animal to human transmission has also been observed. Similarly, pet owners, veterinarians and farmers have been described as high-risked group with potentials of becoming reservoirs of MRSA. Furthermore, the adoption of hand hygiene in healthcare setting have to a great extent reduced the incidence of MRSA in the hospital. And lastly, the advent of molecular typing such as Pulsed Field Gel Electrophoresis (PFGE), Multi Locus Sequence Typing (MLST), Staphylococcal protein A typing (Spa typing) and Double Locus Sequence Typing (DLST) have proven to be a useful tool in providing valuable information on the evolution and clonal diversity of MRSA. These in turn help researchers to answer some pertinent questions on the epidemiology of MRSA.

\textbf{Keywords:} Methicillin resistant \textit{Staphylococcus aureus}, Clonality, Emergence, Epidemiology, Molecular typing

\textbf{Introduction}

The evolution and worldwide spread of \textit{Staphylococcus aureus} causing nosocomial and community acquired infections is of immense veterinary and public health importance (Ghaznavi-Rad \textit{et al.}, 2010). \textit{Staphylococcus aureus} is the most clinically important and well-studied human pathogen causing nosocomial, community and livestock acquired infections worldwide (Basset \textit{et al.}, 2011). The spectrum of disease caused by these pathogen in humans range from mild uncomplicated superficial skin abscess to a more life threatening infections such as endocarditis, chronic pneumonia, nervous diseases and toxaemia (Grema \textit{et al.}, 2015) whereas in animals, it causes mastitis in cows, septicaemia and arthritis in chickens, pyaemic dermatitis in dogs and botryomycosis in horses (Zunita, Bashir, & Hafizal, 2008).
Methicillin resistant *S. aureus* (MRSA) was first isolated in United Kingdom in the early 1960s when a strain of *S. aureus* developed resistance to a semi-synthetic beta lactams antibiotics a few months after it was introduced for the treatment of *S. aureus* infections (Chambers, 1997; Chen et al., 2014). Development of resistance to methicillin occurs as a result of the acquisition of a genomic island carrying methicillin resistance determinant mecA that codes for an alternative penicillin binding protein (PBP2') with reduced susceptibility to methicillin (Leonard & Markey, 2008). Penicillin binding protein functions in sustained crosslinking of the peptidoglycan layer of the cell wall when methicillin inhibits the penicillin binding protein normally associated with cell wall development (Noto, Fox, Paige & Archer, 2008).

Since then, these early MRSA clones termed the “archaic clone”, were isolated in many countries across Europe, until the early 1970s when a new epidemic clone was identified in the United States, Australia and Ireland (Chen et al., 2014). In the United Kingdom, seventeen epidemic MRSA strains were identified with EMRSA-15 and EMRSA-16 being the most predominant and a major cause of nosocomial infection in countries across Europe and America (Grema, Geidam, Gadzama, Ameh, & Suleiman, 2015). However, researchers have observed that it actually originated from Africa where it has been associated with both nosocomial and community associated MRSA infections in Algeria (Grema et al., 2015). To date, five major pandemic clones have so far been identified. These include the Brazilian or ST239-III clone, the Iberian clone or ST 247-1A clone, the New York/Japanese or ST-II clone, the paediatric or ST-IV clone and the Hungarian clone or ST 239III (Basset et al., 2011; Blanc et al., 2007; Neela et al., 2010). Other clones identified, include EMRSA-15, EMRSA-16 and the Berlin clone which are found in Northern Europe. In Asia, Ghaznavi-Rad et al. (2012) reported the identification of about six major clonal complexes (ST-239-CC8, ST-1-CC1, ST188-CC1, ST-22-CC22, ST-7-CC7 and ST-1283-CC80).

Until early 2000, MRSA was believed to be exclusively a healthcare problem, causing infection among individuals with identifiable risk factors associated with nosocomial infection; however, MRSA strains infecting healthy individuals in the community with no apparent identifiable risk factors associated with hospital acquired MRSA were observed in a local population in Australia and later in cases of severe infection in children in the US, and this new clone is termed CA-MRSA (David & Daum, 2010; Rasigade & Vandenesch, 2014; Witte, 2009). Even though CA-MRSA strains are known to be resistant only to beta lactams antibiotics and causes infection only among healthy individuals living in the community, a new form of CA-MRSA with multiple drug resistance causing infection in hospital setting have been reported (Chen et al., 2014; Witte, 2009). For example, the USA clone (USA300) known to be initially causing infection in the community, is now being isolated in health care setting (Basset et al., 2011; Witte, 2009) However, it could be due to strain replacement as observed in other geographical locations. In Malaysia and other Asian countries, ST 239 is reported as the most predominant clone. This clone was first reported in Brazil in 1995 and later in 1997 in Hungary, and surprisingly from 1998-2003 it disappeared from the Hungarian institution and was replaced by sequence type ST 228-I and ST 5-II (Lencastre et al., 1997; Neela et al., 2010). ST 239 was also identified in Africa, and the Middle East (Grema et al., 2015). Recently, MRSA strain causing infections in livestock, pets and companion animals have also been reported (Fitzgerald, 2012; Graveland, Duim, Van Duijkeren, Heederik, & Wagenaar, 2011; Larsen et al., 2012). This new strain of MRSA was first associated with human disease in 2003, but was actually isolated from animals in 1970s (Graveland et al., 2011; Larsen et al., 2012; Saleha & Zunita, 2010; van Cleef et al., 2011) when a clone of MRSA associated with a reservoir in pigs and cattle were isolated in humans.

These scenarios were created as a result of the frequent change in the epidemiology of MRSA which have been observed to have serious clinical implication. In addition, the epidemiology of MRSA revealed by molecular typing techniques showed that emerging clones were observed to harbour different resistance and virulence marker from the already existing clones (Kuhn, Francioli, & Blanc, 2006; Enright et al., 2000). The sudden drift in the epidemiology of MRSA infections over the past five decades, coupled
with its emergence as a potential zoonosis have been observed to have occurred due to expansion and replacement of the already existing clones. In addition, studies have shown that higher frequency rate of MRSA within a particular geographical area is a reflection of successful spread of one or several clones and this varies depending on the health-care setting, country or region (Basset et al., 2011). Furthermore, it has also been observed that even within a particular region, variation is observed within clones; with some increasing in number while some fading away (Blanc et al., 2007). This review focuses on the importance of molecular epidemiology as a valuable tool in determining the emergence and clonal distribution of MRSA with the intent of providing useful epidemiological data necessary for the prevention and control of MRSA.

Mode of Transmission
Methicillin-resistant *Staphylococcus aureus* also known as the “superbug”, and multi-drug resistant *S. aureus* is one of the most significant pathogens resistant to almost all beta lactams antibiotics (Lindsay & Holden, 2006). Transmission was previously thought to be exclusively hospital-associated through hand contact and nasal colonization, but studies have identified individuals without the identifiable risk factors of prior contact with hospital, antibiotics use, use of invasive surgical devices as well as chronic disease coming down with the disease as a result of community associated MRSA (Chuang & Huang, 2013). Both community-associated and hospital-associated MRSA share some level of similarities; however, they differed in their virulence and antibiotic resistance capabilities (Appelbaum, 2007). Individuals at risk of CA-MRSA infection include prisoners, soldiers, and children living in and overcrowded environment, injection drug users, and men who sleep with fellow men (Charlebois et al., 2004).

In the hospital, MRSA contamination is spread through either contact with infected patients or through aerosols. Areas normally contaminated include, clothing, beddings, furniture, medical instruments, toiletries and contaminated surfaces like door handles, tables, chairs and walls (Grema et al., 2015). In a study to determine the prenatal infant maternal transmission of MRSA, it was observed that 43 of the 304 mothers sampled were colonized by *S. aureus* whereas nine among the 43 colonised women had MRSA. Similarly 25 out of 252 infants sampled were colonized by *S. aureus* while nine had MRSA. Furthermore, DNA finger printing also revealed five isolates from mother and infant pair had similar PFGE pattern indicating the possibility of mother to infant transmission (Pinter, Mandel, Hulten, Minkoff, & Tosi, 2009). In another study demonstrating MRSA transmission in infants from two frequently MRSA colonised nurses in a maternity clinic, genotypic analysis RFLP from PFGE revealed that the persistently colonised nurses had genotypically identical MRSA as those isolated from their children. In addition, nasal decolonization of the nurses also results in decline in MRSA carriage rates among infants in the paediatric unit (Mitsuda et al., 1999).

MRSA was rarely isolated from animals, until recently when high level outbreaks were reported in livestock animals such as pigs, chickens, and cows and which were believed to act as potential reservoirs of MRSA infections to humans (Dressler et al., 2012; Fitzgerald, 2012). A case of bidirectional transmission between animals and humans was also reported (Saleha & Zunita, 2010) indicating the possibility of MRSA being a potential zoonosis. Dressler et al. (2012) also reported that livestock workers, veterinarians, people attending animal exhibition and children as high risk group for colonization with MRSA. Colonization of dogs and cats by *S. aureus* does not normally occur, although transitory association is observed and can sometimes cause very serious infections. There is however, similarities in the genotypes of MRSA isolated from pets and those isolated from humans which are endemic in certain parts of the world such as ST5 in the United States. Not much clinical significance is attached to MRSA colonization in companion animals, however there are some speculations that they can serve as potential reservoirs of infection for humans in contact with them (Fitzgerald, 2012).

Several studies have reported the occurrence of animal to humans MRSA transmission and vice-versa. Weese et al. (2006) described a suspected case of MRSA transmission between household pets to humans
and humans to household pets when one of the eight MRSA isolated from pets were indistinguishable with the human isolates. Similarly, elimination of MRSA colonization in two individuals was achieved only after nasal decolonization of their dog; thus indicating that the dog served as a reservoir for MRSA (Weese et al., 2006). Furthermore, in the Netherlands and Belgium colonization of dog and black rats have been reported as a source MRSA infection (van Duijkeren et al., 2004; Van de Giessen et al., 2009).

**Evolution and Clonal Spread**

The rapid emergence of new clones of MRSA on a frequent basis has ushered in a new knowledge on the clonality and epidemic potentials of MRSA. The successful treatment and management of MRSA infection is threatened by the diversity in the clonal types; this is because different clones have different antibiotics resistance characteristics and as such differ in their response to treatment (Ghaznavi-Rad et al., 2012). In addition, the changing epidemiology and worldwide spread of MRSA over the past decades have constitute a major challenge to effective treatment and management of MRSA infection. This is due largely to the rapid rate of acquisition of resistance determinants to methicillin and other classes of antibiotics (Neela et al., 2010).

During the early 1960s, MRSA was isolated in many countries including Europe and America; however, the incidence was low and only isolated cases were reported. It was not until the 1980s when a change in the prevalence and steady increase were observed worldwide with many epidemic strains causing hospital-associated MRSA infection (Hryniewicz, 1999). For instance, the stock cultures isolated from a case of bacteraemia in the 1950s in Denmark gave a new insight into the origin and evolution of MRSA from MSSA. It was revealed that most MRSA and MSSA isolates belong to the same phage group III (Lindsay & Holden, 2006; Oliveira & de Lencastre, 2002). Furthermore, Stewart & Holt (1963) revealed that the first European MRSA isolate resistant to streptomycin, penicillin, tetracycline and sometimes erythromycin identified in the United Kingdom also belongs to the phage group III. This dynamic change observed in the epidemiology of MRSA was attributed to sustained and indiscriminate use of antibiotics for agricultural use, veterinary and treatment of human infections, mutation, acquisition of antibiotics resistance determinants and lack of proper approach to prevention and control of MRSA spread (Grema et al., 2015; Hryniewicz, 1999).

Molecular genetic information also revealed that the early Denmark and UK isolate also belong to the same clone, both having methicillin resistance determinant mecA and deficient in regulatory gene mecI and transposon Tn554. The recovery of these strains in more than 18 hospitals across Denmark also validates their epidemic potentials (Ito, Okuma, Ma, Yuzawa, & Hiramatsu, 2003). These findings further buttress our knowledge with regard to the first S. aureus progeny that receives heterologous methicillin resistance determinants horizontally from a known donor, a scenario that led to the emergence of MRSA. Furthermore, molecular typing reveals a similarity in genetic make-up between the early MRSA isolates and the widely disseminated multi drug resistant Iberian clone; however, unlike the early “archaic” clone, the Iberian clone carries other plasmids and transposon-borne resistant determinants (De Lencastre, Chung, & Westh, 2000; Katayama, Ito, & Hiramatsu, 2000). In addition, the use of molecular typing techniques from 1994 to 2000 has helped in clonal discrimination and distribution of MRSA isolates across different continents (Tomasz & De Lencastre, 1997). This was depicted in the distribution of the five identified pandemic clones throughout the globe. The Iberian clone was first isolated in Spain in 1989 and was later isolated in Italy, Germany, Switzerland, the United Kingdom, Portugal, Belgium, Czech republic, France and the United States of America, whereas the Brazilian clone which was initially isolated in Brazil in 1992 was later found to be replacing isolates in Portugal, Chile, Argentina, Uruguay and Czech republic. However, the Hungarian clone was widely disseminated in major hospitals across Hungry and Taiwan, while the New York/Japan clone was found dominating hospitals across New York, Pennsylvania, New Jersey, Connecticut and Japan, and lastly, the paediatric clone which was first isolated in Portugal at the paediatric hospital in 1992 was later isolated in Argentina, United states, Columbia and Poland (Oliveira & de Lencastre, 2000). Furthermore, MLST and Staphylococcus
Cassette Chromosome (SCCmec) typing of MRSA strains from 12 different Asian countries revealed clonal complex CCS-2 as the most predominant clones in Korea and Japan while ST 239 appearing as the dominant clones in remaining Asian countries sampled (Chuang & Huang, 2013; Neela et al., 2010). This difference in strain types found in different countries is due to the difference in risk factors associated with infection, clone replacement, horizontal transfer of resistance and virulence determinants, mutation and cross-border migration (Chua et al., 2014).

**Molecular Typing Methods of MRSA**

Molecular typing techniques have greatly facilitated the epidemiological surveillance of MRSA and effective evaluation of prevention and control of infection as well as antibiotic prescribing measures (Murchan et al., 2003). Depending on the method adopted, (PFGE, MLST and Protein A typing Spa-typing), molecular typing techniques normally have helped researchers to group MRSA into clones (Basset et al., 2011). Thus far, MLST has been described as the most robust subtyping system for *S. aureus* and MRSA. These techniques vary in discriminatory power, reproducibility, usage and cost and only give approximation of the strain genome (Chua, Howden, Jiang, Stinear, & Peleg, 2014).

**Pulsed-Field Gel Electrophoresis (PFGE)**

This is the commonest typing method for the analysis of *S. aureus* and MRSA epidemiology and is regarded as the “gold standard” or method of choice because of its’ high discriminatory power (Basset et al., 2011; Chua et al., 2014; Cookson et al., 2007). Digestion of bacterial genomic DNA with Smal restriction enzymes within an agarose plug and electrophoresis to resolve them gives a banding pattern of 8 to 20 bands which can be used to compare with PFGE profiles of other *S. aureus* isolates. PFGE is reproducible and allows for separation of larger fragments of genomic DNA. In addition, it can also detect acquisition or loss of mobile genetic elements and introduction and removal of restriction sites due to mutation or gene deletion (Basset et al., 2011; Blanc et al., 2007; Chua et al., 2014) and also allows for monitoring of MRSA transmission and has the ability to detect MRSA clones with potentials of causing outbreaks at a local and international level (Enright et al., 2000). This technique does not allow for inter-laboratory comparison of MRSA strains and data can be ambiguous, so it cannot be use to study epidemiology of MRSA at global level. However, this technique is still being used by the Centre for Disease Control and Prevention (CDC) in defining MRSA strains in the United States (Chua et al., 2014). Other limitations of PFGE for *S. aureus* typing are that the nature of interpretive criteria is arbitrary, might not be too discriminatory for local or short-term epidemiological studies and the analysis of band pattern is subjective (Cookson et al., 2007).

**Multilocus sequence typing (MLST)**

MLST is a highly discriminatory and widely accepted technique for typing of *S. aureus* and MRSA epidemiology on the basis of sequence of 450bp internal fragment of the seven house-keeping genes and it is basically a sequenced based interpretation of the multilocus enzyme electrophoresis (MLEE) (Chua et al., 2014; Enright et al., 2000; Kuhn et al., 2006). The technique is designed to evaluate and compare genetic polymorphism in a group of bacterial pathogens worldwide. Isolates are defined by distinct alleles assigned for the sequence of each house-keeping gene loci. Since there is more than one allele in each of the sequence of the seven house-keeping genes, the probability of having a sequence with a similar allele by chance is highly unlikely (Enright et al., 2000). MLST provides important information about the variation in the nucleotide sequence of the bacterial core genome, the evolutionary origin of a clone, the phylogenetic relatedness between among strains and recombination rate (Basset et al., 2011). The merit of this technique is that it is reproducible among laboratories, can be used for intercontinental comparison of strains and the data is unambiguous. The main disadvantage however, includes cost and relatively low discriminatory power when use for local epidemiological studies (Basset et al., 2011; Enright et al., 2000).
Staphylococcal protein A (Spa)-typing

This is another widely used sequenced based typing method that employs the use of highly discriminatory genetic marker to characterize bacterial pathogen allowing the identification of closely related isolates with common ancestral origin (Chau et al., 2014; Grundmann et al., 2010). The spa-typing codes for protein A is a specie specific gene known for binding capacity for immunoglobulin G (IgG) (Basset et al., 2011; Grundmann et al., 2010). These loci have a high level polymorphism due to a variation in the internal region of short tandem repeats, which vary in numbers and substitution within individual repeat unit (Basset et al., 2011), a succession of number corresponding to each individual repeat unit of the X region represents the spa profile (Kuhn et al., 2007). This technique provides portable data that are unambiguous and biologically meaningful with great utility for epidemiological studies such as in transmission and outbreak investigation of S. aureus infection different within geographical location (Grundmann et al., 2010). However, Spa typing might reflect homoplasy, the analysis complicated and its discriminatory power lower than PFGE (Basset et al., 2011; Kuhn et al., 2007).

Double Locus Sequence Typing (DLST)

Recently, a new molecular typing method based on the analysis of partial sequences (ca. 500 bp) of the highly variable clumping factor B (clfB) and spa genes called Double Locus Sequence Typing (DLST) was developed (Basset et al., 2011). High discriminatory power ability more than the traditional spa and PFGE have been observed with this method (Kuhn et al., 2007); furthermore, high level of type ability, reproducibility, ease of use, low cost and unambiguous level of type definition makes this technique a promising method for epidemiological studies of MRSA. This method is limited by the fact the spa alleles determined by two methods are not identical; this is because DLST analyses the same region of gene.

Clonal Distribution in Human

In recent years, we have witnessed an increase in the emergence of new and highly pathogenic strains of bacteria that are resistant to almost all known antibiotics with high therapeutic effect. Thus, this lead to increase in health-care cost and prolonged hospital stay, especially in countries where there is poor legislation with regard to the use of antibiotics in the treatment of human infection and in agriculture.

Methicillin Resistant Staphylococcus aureus (MRSA) is a significant problem in health care hospitals and is now commonly recovered in virtually every large hospital in the United States (Musser & Kapur, 1992). This was demonstrated in the work of Zahar & Timsit (2007) where an increase in MRSA infection rate in the intensive care unit from 35.9% to 64.4% was observed between 1992-2003. In Europe, however, the overall prevalence increased from 16% in 1999 to 24% in 2004 which was slightly lower than that of the United States. In 2007, the European Union Surveillance network involving 13 European countries reported that 6.2% of the 54,574 patients admitted in the intensive care unit (ICU) for a period of two days had acquired pneumonia. Furthermore, it was also revealed that the 17% of all ICU cases were caused by S. aureus and 33% of which were MRSA (Kock et al., 2010). In France, Group (1999) reported that 3.7% of patients in intensive care unit had at least one positive MRSA sample in a survey involving 43 hospitals. Similarly, in a survey of 25 university hospitals across 15 European countries, Fluit et al. (2001) revealed that the prevalence of MRSA was high in southern than in northern Europe where the authors observed a higher prevalence of MRSA, 54% in Portugal, 43-58% in Italy while only 2% prevalence was observed in Switzerland and Netherlands.

Recently, MRSA infection have been on the increase in United States, Europe and other parts of the world with 60% of all S. aureus infection in the ICU in United States were MRSA (Boucher & Corey, 2008). According to the 2008 report of the European Antimicrobial Resistance Surveillance system (EARSS), the prevalence of MRSA blood cultures in the Scandinavian countries was 5% whereas a prevalence of less than 10% was reported in Austria, Slovenia and Luxembourg and 10-24% in Czech Republic, France, Belgium, Latvia, Hungary, Poland, Germany and Switzerland. However, the prevalence was high (about 25% and above) in Israel, Portugal, Malta, Romania, Spain, Greece, Croatia, Cyprus,
Bulgaria, Italy, Turkey, Republic of Ireland and the United Kingdom (Kock et al., 2010). The incidence of MRSA was observed to be influenced by geographic location with more outbreaks recorded in the southern part of United States; similar scenario was observed in Europe where incidence of MRSA was 0.5% in Iceland and 44% in Greece from 1999 to 2000 (Boucher & Corey, 2008). Similarly, Grema et al., (2015) in their review also reported a greater than 50% of MRSA rate in the United State, Asia and Malta, 25-50% in Africa, Europe and China while a relatively lower percentage in Europe; however, this result is dependent upon the sample size and area studied.

Molecular typing techniques such as MLST, Spa typing and PFGE have revealed a high level of clonality among the S. aureus population with more than ten major clonal complexes and abundant minor lineage (CCs) CC1, CC5, CC8, CC22, CC30, ST239 and CC45 (Enright et al., 2000; Grema et al., 2015; Purrello et al., 2014). Clonal complexes CC5, CC8, CC22, CC30, ST239 and CC45 lineages, are the sources of hospital associated MRSA with each sequence type having a specific geographical distribution. While the community associated clones arose from clonal complexes (CC) CC1, CC8, CC30, CC59 and CC80 with increasing evidence of its being implicated in nosocomial infections recently in the United States (Basset et al., 2011; Grundmann et al., 2010; Purrello et al., 2014).

The classical example of CA-MRSA clone is the ST-80 SCCmecIV associated with causing infection across Europe in low income earning group (Basset et al., 2011); others include CA-MRSA ST1(USA400) and ST8(USA300) which were reported as dominant clones in the United States. In Asian and the Pacific, the most dominant prevalent clone is the ST59 while ST30 is found almost all over the world (Chuang & Huang, 2013; Grema et al., 2015). CA-MRSA infected cases have been observed to spread across different continents such as Europe, South America, Asia, Africa and Middle East indicating the evidence of transboundary transmission of CA-MRSA (Grema et al., 2015). It is important to also note that, in areas where the prevalence of HA-MRSA is low, studies have shown that it has been replaced by CA-MRSA. The reason for the dominance of a clone in a particular area is poorly understood; however, studies have shown that CA-MRSA carries a small SCCmec type (V and IV) compared to HA-MRSA and does not confer multiple resistance to antibiotics as well as result in a small fitness cost (Basset et al., 2011; Grema et al., 2015; Skov et al., 2006).

The proportion of S. aureus strains resistant to mexitillin causing infections in the community varies across different countries of the globe. Skov et al. (2012), in their review on CA-MRSA revealed that the CA-MRSA carriage rate worldwide ranges from less than 1% in some regions while greater than 50% in other regions; in addition, they also observed that CA-MRSA carriage rate was higher in young children than in adults; this could probably be due to lifestyle and reduced immune response. In Europe, the overall prevalence of CA-MRSA is 1% and 2% in Germany and Spain respectively, 29-50% in Sweden and Denmark, while about 6% in outpatients in Italy, 30% in Greece, 14% in Germany and 18% in France (Kock et al., 2010) while in Asian countries, the incidence of CA-MRSA is between 2.5%-39% which is much lower than the USA300 clone isolated among individuals in the community in the United States (Chuang & Haung, 2013).

Outbreaks of CA-MRSA infected cases were less in some countries while endemic in others. For instance, higher carriage rate of CA-MRSA infected cases were reported in the United States, Taiwan, Australia and Canada. However, in Taiwan alone from 1999 to 2005 there was a significant increase of CA-MRSA carriage rate in children from 9.8% to 56% (Chuang & Huang, 2013; Skov et al., 2012). In Europe, carriage of CA-MRSA infected cases were low compared to the United States and other regions across the globe; however, a significant increase in CA-MRSA was observed in the Netherlands, Denmark and France and even more prevalent and replacing HA-MRSA in Greece (Basset et al., 2011; Chuang & Huang, 2013; Skov et al., 2012). In a study involving 17 hospitals across eight Asian countries it was observed that the prevalence of CA-MRSA varied which could be due to the difference in study design, geographic location and MRSA control programme. The proportion of MRSA in the community
ranges between 2.5% to 39% with four countries (Taiwan, Vietnam, Sri Lanka and Philippines) having CA-MRSA prevalence of greater than 30% (Chuang & Huang, 2013). Similarly, in a review by Grema et al. (2015) high prevalence of MRSA were reported in South Korea (77.6%), Vietnam (74.1%), Taiwan (65%) and Hong Kong (56.8%) with CC8 (ST239-III) as the dominant strain causing infection in the hospital setting.

In Malaysia, Ghaznavi et al. (2010) reported a prevalence of 21% upon reviewing results of microbiology laboratories in all the State hospitals. In another study, Choi et al. (2006) reported a 23.4% prevalence of MRSA after sampling 346 nasal swabs. Similarly, Al-Talib et al. (2013) also reported a 28.7% prevalence of MRSA when they assessed the burden of nasal MRSA among patients and health-care workers in Malaysia. According to the Report of the National Surveillance of Antibiotic Resistance (NSAR) in Malaysia, a total of 37, 341 S. aureus were isolated which was more than the number of S. aureus (34, 492) isolated in 2013; however, about 18% of the S. aureus isolates were from blood samples. Furthermore, the overall numbers of MRSA isolates were almost similar with about 17.2% of the 36,064 S. aureus isolates tested in 2014 and 17.7% out of 31,030 S. aureus isolates tested in 2013 (Norazah, 2014).

Clonal Distribution in Animals
Evidence of isolation of MRSA in animals dates back to the early 1970s even though association with disease in humans was first reported in 2003 (Bosch et al., 2015). The rare isolation of MRSA in animals during this period from 1970 to early 2000 portrayed MRSA as exclusively a human problem (Graveland et al., 2011) until 2007, when transmission of MRSA from cows to humans was reported. In addition, a case of livestock-associated MRSA was reported in a 6 month old girl following invasive surgery in a hospital in the Netherlands even after several decolonization procedures (Voss, Loeffen, Bakker, Klaassen, & Wulf, 2005). Molecular typing shows that the livestock-associated MRSA was non typable by PFGE using Sma1 restriction enzymes and belongs to clonal complex CC398 (Graveland et al., 2011). Isolation of MRSA in pets, companion animals, chickens, and cattle have been reported (Saleha & Zunita, 2010). However, molecular studies have shown that these strains are similar to the strains isolated in humans indicating transmission from humans to animals (Grema et al., 2014). In the Netherlands, about 40% of the MRSA isolated in humans were livestock associated (Bosch et al., 2015). Furthermore, Leonard & Markey (2008) revealed that 14% of the 65 patient attending seven different veterinary teaching hospitals were infected with MRSA and that the MRSA colonization was common in canine and equine, indicating the possibility that dogs, cats and horses can serve as a reservoir for zoonotic staphylococcal infection as reported by Mann in 1959. In another study using specimens from cattle, pigs, and chickens. Lee, (2003) found that, of the 421 S. aureus isolated collected 15 (3.6%) were resistant to oxacillin and positive for mecA. The prevalence of MRSA in China, Japan, Taiwan and South Korea ranges between 70%-80% (Chuang & Huang, 2013). In Korea, Lim et al. (2012) carried out a study in pigs from February 2008 to May 2009 and reported a prevalence of MRSA in pigs at 3.2% and 22.7% respectively. The authors also reported two types of MRSA strain amongst the population of the MRSA isolates that were studied, with 17 strains being livestock-associated type (LA; ST398) and 4 strains being human-associated type (HA; ST72). The most prevalent type strain being ST398 (57%), followed by ST541 (23.8%) and the rest of the isolates were ST72, thus, indicating the existence of not only livestock-associated types (ST398 and ST541) but also human-associated type (ST72) MRSA in pigs.

In Malaysia, studies carried out among pigs and pig handlers by Neela et al. (2009) in 360 pigs and 90 pig handlers from 30 farms identified novel ST9 spa type t4358- V MRSA strains that were found to transiently colonize more than 1% of pigs and 5.5% of pig handlers. In another studies however, Neela et al. (2010) reported the prevalence of another MRSA strain of pig origin with Spa sequencing and MLST and documented t037 and ST 239 (CC8) in 83.3% of the isolates.
MRSA as emerging zoonosis

In the past decades, major changes were seen to have occurred in the epidemiological traits, resistance development, host and environmental interaction of MRSA. Until recently, infection with MRSA was initially thought to be restricted to hospital setting. However, the emergence of a new strain of MRSA with a characteristically unique epidemiology, microbial and clinical identity to the hospital associated was reported (van Cleef et al., 2011). In addition, recent findings also indicate a link between MRSA carriage in livestock and infection in individuals who have close contact with animals (Springer et al., 2009). Furthermore, Petersen et al. (2013) reported that inter-specie transmission of MRSA between human and animal reservoirs may have been achieved by host adaptation as well as to selective pressure to antibiotics. Similarly, isolations of MRSA have been reported in a variety of domestic animals such as cats, dogs pigs, sheep, chickens and horses leading to a sudden increase in reports and interest in colonization and infection with MRSA in animals (Leonard & Markey, 2008; Saleha & Zunita, 2010). Luzzago et al. (2014) reported that t1328 and ST22 isolates obtained from the liver of the chamois kid were methicillin-resistant *S. aureus* (MRSA) harbouring SCCmec cassette type IV. Furthermore, Price et al. (2012) reported that MRSA ST398 initially originated as methicillin susceptible *S. aureus* in a human, which was later transmitted to pigs and in which it acquires methicillin resistance, and is now seen re-infecting humans. In 2011, Petersen et al. (2013) reported the isolation of *S. aureus* in cattle and humans with a new homologue of SCCmec designed as SCCmecC.

Individuals in frequent contacts with farm animals and household pets as well as veterinarians have been described as high risk with potentials of becoming reservoirs of MRSA (Weese et al., 2006). In addition, evidence of transmission of MRSA from pet owners to their pets and subsequent re-infection has been reported to have occurred (Weese et al., 2006). In a study carried out in two veterinary hospitals in the United States and Canada, O’Mahony et al. (2005) reported veterinary staff as the primary source of MRSA infection however the mechanism of which is unknown, while the report from Canada suggested environmental contamination as the main culprit, in particular contamination of stalls housing MRSA positive animals. Studies on livestock-associated MRSA as a cause of infection and colonization of humans by the network of the Dutch German project revealed that although the predominant clone associated with LA-MRSA is the CC 398, the other clonal complexes such as CC5, CC9 and CC97 were also detected in livestock animals. Furthermore, the joint project also revealed that of the 14,036 MRSA isolates obtained between January 2008 to June 2012 from human clinical and screening specimens in an area of high density livestock production, Protein A sequence analysis revealed that 578 staphylococcal protein A Spa types LA-MRSA were among the human isolates which accounted for about 18.6% of all the human isolates.

Prevention and Control

Methicillin resistant *S. aureus* strain is a significant health care problem, resulting in prolonged hospital stay, reduction in the therapeutic value of antibiotics and treatment cost. The reduction and low level maintenance in the incidence of HA-MRSA blood stream infection in many countries across the world has yielded much positive result. The success achieved in these countries is as a result of the implementation of a holistic approach involving the combination of several preventive interventions which include but not restricted to decolonization, screening, prevention of contact, appropriate prescription and use of antibiotics and many other important preventive measures (Kock et al., 2010).

The adoptions of hand hygiene in healthcare setting have been demonstrated to play a crucial role in reducing the incidence of MRSA in the hospital (Allegranzi & Pittet, 2009; Pittet et al., 2006). McBryde et al. (2004) in their studies demonstrated that 17% of contact between a patient colonised with MRSA and a health care worker led to the transmission of MRSA from patients to the gloves of health care worker; thus, further buttressing our understanding on the importance of hand hygiene in the prevention and control of MRSA spread. The adoption of these measures in some European countries has led to the reduction of *S. aureus* and MRSA blood stream infection to the minimum, for instance between 2004 and
2008 in Belgium, a sufficient reduction in the mean incidence of nosocomial associated MRSA from 30 to 25% was observed, whereas in France the adoption of a 16-years national nosocomial infection control programme led to 30% decrease of surgical site infection and 20% reduction in MRSA carriage rates in blood cultures (Kock et al., 2010). Similarly in the UK, the adoption of guidelines for the prevention of MRSA in health care institution between 2004 and 2008 led to halving the incidence of MRSA blood stream infection. The intervention measures adopted include, systematic surveillance, appropriate prescription of antibiotics, screening of MRSA infected patients, nasal decolonization and other valuable measures necessary for the reduction of MRSA spread (Coia et al., 2006).

**Conclusion and Recommendation**

The evolution and worldwide spread of MRSA is a major public health challenge, especially with the emergence of community and livestock- acquired MRSA. The emergence of several pandemic clones coupled with rapid changes observed in their epidemiology on a major scale have important clinical implication on the patient, health care workers and pharmaceutical companies. This is because new clones harbours different determinants for virulence and antibiotic resistance. Furthermore, the advent of molecular typing technologies have proven to be useful in providing valuable insight on the evolution and clonality of MRSA; this in turn help in providing useful information regarding the epidemiology of MRSA. Understanding the factors influencing the emergence and spread of MRSA will help in providing the impetus towards designing an effective model that will help in prevention and control of MRSA infection. This review explore the significance of molecular epidemiology as a valuable tool in determining the emergence and clonal diversity of MRSA. It is therefore recommended that a standard molecular typing protocol be developed to help ensure harmony in strain typing techniques at regional or global level.

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