PJSRR (2016) 2(2): 121-134
eISSN: 2462-2028 © Universiti Putra Malaysia Press

Pertanika Journal of Scholarly Research Reviews
http://www.pjsrr.upm.edu.my/

Molecular Epidemiology: A Valuable Tool for Determination of Emerging and clonality of Methicillin Resistant *Staphylococcus aureus* (MRSA)

Asinamai Athliamai BITRUS[a], Zunita ZAKARIA[a*], Siti KHAIRANI-BEJO [a], Sarah OTHMAN[b], Abdul-Nasir TIJJANI[a]

[a]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

[b]Department of Cell and Molecular Biology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Malaysia

[*]zunita@upm.edu.my

Abstract – Methicillin-resistant *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.

Keywords: Methicillin resistant *Staphylococcus aureus*, Clonality, Emergence, Epidemiology, Molecular typing

Introduction

The evolution and worldwide spread of *Staphylococcus aureus* causing nosocomial and community acquired infections is of immense veterinary and public health importance (Ghaznavi-Rad *et al*., 2010). *Staphylococcus aureus* is the most clinically important and well-studied human pathogen causing nosocomial, community and livestock acquired infections worldwide (Basset *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 *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 *mec*A 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-IIIA clone, the Iberian clone or ST 247-IA 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 pathogen 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 *mec*A and deficient in regulatory gene *mec*I 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 only 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 CC5-II 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 *Sma*I 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, Hungry, 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 methicillin 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 *Sma*1 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 *mec*A. 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 and infection 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 16years 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.

Acknowledgements: This scholarly research review has been supported by Universiti Putra Malaysia Research University Grant (9428800) and Fundamental Research Grant Scheme, Ministry of Higher Education Malaysia (5524643).

Author's contribution: The design and execution of this research study is a collective effort of all the authors. All authors were also involved in the critical analysis and review of the draft manuscript. There is no conflicting interest.

References

Allegranzi, B., & Pittet, D. (2009). Role of hand hygiene in healthcare-associated infection prevention. *Journal of Hospital Infection*, *73*(4), 305-315.

Al-Talib, H., Yean, C.Y., Hasan, H., Nik Zuraina, N.M., & Ravichandran, M. (2013). Methicillin-resistant *Staphylococcus aureus* nasal carriage among patients and healthcare workers in a hospital in Kelantan, Malaysia. *Polish Journal of Microbiology*, *62*(1), 109-112.

Appelbaum, P .C. (2007). Microbiology of antibiotic resistance in *Staphylococcus aureus. Clinical Infectious Diseases*, *45*(Supplement 3), S165-S170.

Basset, P., Feil, E. J., Zanetti, G., & Blanc, D .S. (2011). 25 - The Evolution and Dynamics of Methicillin-Resistant *Staphylococcus aureus*. In M. Tibayrenc (Ed.), *Genetics and Evolution of Infectious Disease* (pp. 669-688). London: Elsevier.

Blanc, D. S., Petignat, C., Wenger, A., Kuhn, G., Vallet, Y., Fracheboud, D., Siegrist, H.H. (2007). Changing molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in a small geographic area over an eight-year period. *Journal of clinical microbiology*, *45*(11), 3729-3736.

Bloemendaal, A.L.A., Brouwer, E.C, & Fluit, A. C. (2010). Methicillin resistance transfer from *Staphylocccus epidermidis* to methicillin-susceptible *Staphylococcus aureus* in a patient during antibiotic therapy. *PLoS One*, *5*(7), e11841.

Boerema, J.A., Clemens, R., & Brightwell, G. (2006). Evaluation of molecular methods to determine enterotoxigenic status and molecular genotype of bovine, ovine, human and food isolates of

Staphylococcus aureus. *International Journal of Food Microbiology, 107*(2), 192-201. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2005.07.008

Bosch, T., Verkade, E., van Luit, M., Landman, F., Kluytmans, J., & Schouls, L.M. (2015). Transmission and Persistence of Livestock-Associated Methicillin-resistant *Staphylococcus aureus* among Veterinarians and Their Household Members. *Applied and environmental microbiology, 81*(1), 124-129.

Boucher, H.W., & Corey, G.R. (2008). Epidemiology of methicillin-resistant *Staphylococcus aureus*. *Clinical infectious diseases, 46*(Supplement 5), S344-S349.

Chambers, H.F. (1997). Methicillin resistance in s*taphylococci*: molecular and biochemical basis and clinical implications. *Clinical microbiology reviews, 10*(4), 781-791.

Charlebois, E.D., Perdreau-Remington, F., Kreiswirth, B., Bangsberg, D.R, Ciccarone, D., Diep, B.A., Chambers, H.F. (2004). Origins of community strains of methicillin-resistant *Staphylococcus aureus*. *Clinical infectious diseases, 39*(1), 47-54.

Chen, C.J., Huang, Y.C, Su, L.H, Wu, T.L, Huang, S.H, Chien, C.C, Ko, W.C. (2014). Molecular epidemiology and antimicrobial resistance of methicillin-resistant *Staphylococcus aureus* bloodstream isolates in Taiwan, 2010.

Chiang, Y.C., Liao, W.W., Fan, C.M., Pai, W.Y., Chiou, C.S., & Tsen, H.Y. (2008). PCR detection of Staphylococcal enterotoxins (SEs) N, O, P, Q, R, U, and survey of SE types in *Staphylococcus aureus* isolates from food-poisoning cases in Taiwan. *International Journal of Food Microbiology, 121*(1), 66-73. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2007.10.005

Choi, C.S., Yin, C.S., Bakar, A.A., Sakewi, Z., Naing, N.N., Jamal, F., & Othman, N. (2006). Nasal carriage of *Staphylococcus aureus* among healthy adults. *Journal of microbiology, immunology, and infection Wei mian yu gan ran za zhi, 39*(6), 458-464.

Chua, K.Y.L., Howden, B.P., Jiang, J-H., Stinear, T., & Peleg, A.Y. (2014). Population genetics and the evolution of virulence in *Staphylococcus aureus*. *Infection, Genetics and Evolution, 21*(0), 554-562. doi: http://dx.doi.org/10.1016/j.meegid.2013.04.026

Chuang, Y.Y., & Huang, Y.C. (2013). Molecular epidemiology of community-associated meticillin-resistant *Staphylococcus aureus* in Asia. *The Lancet Infectious Diseases, 13*(8), 698-708.

Coia, J.E., Duckworth, G.J., Edwards, D.I., Farrington, M., Fry, C., Humphreys, H. (2006). Chemotherapy, Joint Working Party of the British Society of Antimicrobial. Guidelines for the control and prevention of meticillin-resistant *Staphylococcus aureus* (MRSA) in healthcare facilities. *Journal of hospital infection, 63*, S1-S44.

Cookson, B.D., Robinson, D.A., Monk, A.B., Murchan, S., Deplano, A., De Ryck, R., Salmenlinna, S. (2007). Evaluation of molecular typing methods in characterizing a European collection of epidemic methicillin-resistant *Staphylococcus aureus* strains: the HARMONY collection. *Journal of clinical microbiology, 45*(6), 1830-1837.

Cuny, C., Nathaus, R., Layer, F., Strommenger, B., Altmann, D., & Witte, W. Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs.

David, M.Z., & Daum, R.S. (2010). Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clinical microbiology reviews, 23*(3), 616-687.

Davis, S.L., Perri, M.B., Donabedian, S.M., Manierski, C., Singh, A., Vager, D., Robinson-Dunn, B. (2007). Epidemiology and outcomes of community-associated methicillin-resistant *Staphylococcus aureus* infection. *Journal of clinical microbiology, 45*(6), 1705-1711.

De Lencastre, H., Chung, M., & Westh, H. (2000). Archaic strains of methicillin-resistant *Staphylococcus aureus*: molecular and microbiological properties of isolates from the 1960s in Denmark. *Microbial Drug Resistance, 6*(1), 1-10.

Diep, BA., Carleton, H.A., Chang, R.F., Sensabaugh, G.F., & Perdreau-Remington, F. (2006). Roles of 34 virulence genes in the evolution of hospital-and community-associated strains of methicillin-resistant *Staphylococcus aureus*. *Journal of Infectious Diseases, 193*(11), 1495-1503.

Dressler, A.E., Scheibel, R.P., Wardyn, S., Harper, A.L., Hanson, B.M., Kroeger, J.S., Diekema, D.J., Bender, J.B., Gray, G.C. and Smith, T.C., (2012). Prevalence, antibiotic resistance and molecular characterisation of Staphylococcus aureus in pigs at agricultural fairs in the USA. *Veterinary Record: Journal of the British Veterinary Association*, *170*(19).

Enright, M.C., Day, N.P.J., Davies, C.E., Peacock, S.J., & Spratt, B.G. (2000). Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus. Journal of clinical microbiology*, *38*(3), 1008-1015.

Fitzgerald., J. R. (2012). Livestock-associated *Staphylococcus aureus*: origin, evolution and public health threat. *Trends in Microbiology*, *20*(4), 192-198. doi: http://dx.doi.org/10.1016/j.tim.2012.01.006

Fluit, A.C., Wielders, C.L.C., Verhoef, J., & Schmitz, F-J. (2001). Epidemiology and susceptibility of 3,051 *Staphylococcus aureu*s isolates from 25 university hospitals participating in the European SENTRY study. *Journal of clinical microbiology*, *39*(10), 3727-3732.

Ghaznavi-Rad, E., Neela, V., Shamsudin, M.N., Moghaddam, H.G., Tavakol, M., van Belkum, A., Andar-Ali, A.F. (2012). Diversity in the antimicrobial susceptibility patterns of methicillin-resistant *Staphylococcus aureus* clones. *European journal of clinical microbiology & infectious diseases*, *31*(12), 3317-3321.

Ghaznavi-Rad, E., Shamsudin, M.N., Sekawi, Z., Khoon, L.Y, Aziz, M.N., Hamat, R.A., Ghasemzadeh-M, H. (2010). Predominance and emergence of clones of hospital-acquired methicillin-resistant *Staphylococcus aureus* in Malaysia. *Journal of Clinical Microbiology*, *48*(3), 867-872.

Gosbell, I. B., & van Hal, S.J. (2013). *Staphylococcus aureus* colonisation: some questions answered. *The Lancet Infectious Diseases*, *13*(5), 380-381. doi: http://dx.doi.org/10.1016/S1473-3099(13)70048-3

Graveland, H., Duim, B., Van Duijkeren., E, Heederik, D., & Wagenaar, J A. (2011). Livestock-associated methicillin-resistant *Staphylococcus aureus* in animals and humans. *International Journal of Medical Microbiology*, *301*(8), 630-634.

Grema, H., Geidam, Y., Gadzama, G., Ameh, J., & Suleiman, A. (2015). Methicillin resistant *Staphyloccus aureus* (MRSA): a review. *Adv. Anim. Vet. Sci*, *3*(2), 79-98.

Group, H.P II Study. (1999). Methicillin-resistant *Staphylococcus aureus* in French hospitals: A 2-month survey in 43 hospitals, 1995. *Infection control and hospital epidemiology: the official journal of the Society of Hospital Epidemiologists of America*, *20*(7), 478.

Grundmann, H., Aanensen, D.M., Van Den W., Cees C., Spratt, B.G., Harmsen, D., Friedrich, A.W., & Group, European *Staphylococcal* Reference Laboratory Working. (2010). Geographic distribution of *Staphylococcus aureus* causing invasive infections in Europe: a molecular-epidemiological analysis. *PLoS medicine*, *7*(1), 49.

Hryniewicz, W. (1999). Epidemiology of MRSA. *Infection*, *27*(2), S13-S16.

Ito, T., Okuma, K., Ma, X. X., Yuzawa, H., & Hiramatsu., K. (2003). Insights on antibiotic resistance of *Staphylococcus aureus* from its whole genome: genomic island SCC. *Drug Resistance Updates*, *6*(1), 41-52.

Katayama, Y., Ito, T., & Hiramatsu, K., (2000). A new class of genetic element, staphylococcus cassette chromosome mec, encodes methicillin resistance in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy*, *44*(6), 1549-1555.

Köck, R., Becker, K., Cookson, B., van Gemert-Pijnen, J.E., Harbarth, S., Kluytmans, J., Struelens, M.J. (2010). Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe.

Kuhn, G., Francioli, P., & Blanc, D.S. (2006). Evidence for clonal evolution among highly polymorphic genes in methicillin-resistant *Staphylococcus aureus*. *Journal of bacteriology*, *188*(1), 169-178.

Kuhn, G., Francioli, P., & Blanc, D.S. (2007). Double-locus sequence typing using clfB and spa, a fast and simple method for epidemiological typing of methicillin-resistant Staphylococcus aureus. *Journal of clinical microbiology*, *45*(1), 54-62.

Larsen, J., Imanishi, M., Hinjoy, S., Tharavichitkul, P., Duangsong, K., Davis, M.F., Nelson, K.E., Larsen, A.R. and Skov, R.L., (2012). Methicillin-resistant *Staphylococcus aureus* ST9 in pigs in Thailand. *PloS one*, *7*(2), p.e31245.

Lencastre, H., Severina, E., P, Milch., Hedda, T., Marianne, K., & Tomasz, A. (1997). Wide geographic distribution of a unique methicillin-resistant *Staphylococcus aureus* clone in Hungarian hospitals. *Clinical Microbiology and Infection*, *3*(3), 289-296.

Leonard, F.C., & Markey, B.K. (2008). Meticillin-resistant *Staphylococcus aureus* in animals: A review. *The Veterinary Journal*, *175*(1), 27-36.

Lindsay, J.A., & Holden, M.T.G. (2006). Understanding the rise of the superbug: investigation of the evolution and genomic variation of *Staphylococcus aureus*. *Functional & integrative genomics*, *6*(3), 186-201.

Luzzago, C., Locatelli, C., Franco, A., Scaccabarozzi, L., Gualdi, V., Viganò, R., Sironi, G., Besozzi, M., Castiglioni, B., Lanfranchi, P. and Cremonesi, P., (2014). Clonal diversity, virulence-associated genes and antimicrobial resistance profile of Staphylococcus aureus isolates from nasal cavities and soft tissue infections in wild ruminants in Italian Alps. *Veterinary microbiology*, *170*(1), pp.157-161.

McBryde, E.S., Bradley, L.C., Whitby, M, & McElwain, D.L.S. (2004). An investigation of contact transmission of methicillin-resistant Staphylococcus aureus. *Journal of Hospital Infection*, *58*(2), 104-108.

Mitsuda, T., Arai, K., Ibe, M., Imagawa, T., Tomono, N., & Yokota, S. (1999). The influence of methicillin-resistant *Staphylococcus aureus* (MRSA) carriers in a nursery and transmission of MRSA to their households. *Journal of Hospital Infection*, *42*(1), 45-51. doi: http://dx.doi.org/10.1053/jhin.1998.0551

Murchan, S., Kaufmann, M.E, Deplano, A., de Ryck, R., Struelens, M., Zinn, C.E., El Solh, N. (2003). Harmonization of pulsed-field gel electrophoresis protocols for epidemiological typing of strains of methicillin-resistant *Staphylococcus aureus*: a single approach developed by consensus in 10 European laboratories and its application for tracing the spread of related strains. *Journal of clinical microbiology*, *41*(4), 1574-1585.

Musser, J.M., & Kapur, V. (1992). Clonal analysis of methicillin-resistant *Staphylococcus aureus* strains from intercontinental sources: association of the mec gene with divergent phylogenetic lineages implies dissemination by horizontal transfer and recombination. *Journal of clinical microbiology*, *30*(8), 2058-2063.

Neela, V., Moghaddam, H.G., Van Belkum, A., Horst-Kreft, D., Mariana, N.S., & Rad, E.G. (2010). First report on methicillin-resistant *Staphylococcus aureus* of Spa type T037, Sequence type 239, SCCmec type III/IIIA in Malaysia. *European journal of clinical microbiology & infectious diseases*, *29*(1), 115-117.

Noto, M.J., Fox, Paige M., & Archer, G L. (2008). Spontaneous deletion of the methicillin resistance determinant, mecA, partially compensates for the fitness cost associated with high-level vancomycin resistance in *Staphylococcus aureus*. *Antimicrobial agents and chemotherapy*, *52*(4), 1221-1229.

O'Mahony, R., Abbott, Y., Leonard, F.C., Markey, B. K., Quinn, P.J., Pollock, P. J., Fanning, S., Rossney, A.S. (2005). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. Veterinary microbiology. 30; 109(3):285-96.

Oliveira, D. C., & de Lencastre, H. (2002). Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother*, *46*(7), 2155-2161.

Pinter, D.M., Mandel, J., Hulten, K.G., Minkoff, H., & Tosi, M.F. (2009). Maternal-infant perinatal transmission of methicillin-resistant and methicillin-sensitive *Staphylococcus aureus*. *American journal of perinatology*, *26*(2), 145-151.

Pittet, D., Allegranzi, B., Sax, H., Dharan, S., Pessoa-S., Carmem L., Donaldson, L., & Boyce, J.M. (2006). Evidence-based model for hand transmission during patient care and the role of improved practices. *The Lancet infectious diseases, 6*(10), 641-652.

Price, L.B., Stegger, M., Hasman, H., Aziz, M., Larsen, J., Andersen, P.S., Pearson, T., Waters, A.E., Foster, J.T., Schupp, J. and Gillece, J., (2012). *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio*, *3*(1), pp.e00305-11.

Purrello, S.M., Daum, R.S., Edwards, G.F.S., Lina, G., Lindsay, J., Peters, G., & Stefani, S. (2014). Meticillin-resistant *Staphylococcus aureus* (MRSA) update: New insights into bacterial adaptation and therapeutic targets. *Journal of Global Antimicrobial Resistance, 2*(2), 61-69. doi: http://dx.doi.org/10.1016/j.jgar.2014.02.003

Rasigade, J-P, & Vandenesch, F. (2014). Staphylococcus aureus: A pathogen with still unresolved issues. *Infection, Genetics and Evolution, 21*(0), 510-514. doi: http://dx.doi.org/10.1016/j.meegid.2013.08.018

Saleha, A., & Zunita, Z. (2010). Methicillin resistant Staphylococcus aureus (MRSA): An emerging veterinary and zoonotic pathogen of public health concern and some studies in Malaysia. *J Anim Vet Adv, 9*(7), 1094-1098.

Skov, R., Christiansen, K., Dancer, S.J, Daum, R.S., Dryden, M., Huang, Y-C., & Lowy, F.D. (2012). Update on the prevention and control of community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA). *International journal of antimicrobial agents, 39*(3), 193-200.

Tomasz, A., & De Lencastre, H. (1997). Molecular microbiology and epidemiology: coexistence or alliance. *prevention and control of nosocomial infections. Baltimore: Williams and Willkins*, 309-321.

Van Cleef, B.A., Monnet, D.L., Voss, A., Krziwanek, K., Allerberger, F., Struelens, M., Zemlickova, H., Skov, R.L., Vuopio-Varkila, J., Cuny, C. and Friedrich, A.W., (2011). Livestock-associated methicillin-resistant Staphylococcus aureus in humans, Europe. *Emerging Infectious Diseases*, 17(3), 502-505

Van de Giessen, AW., van Santen-Verheuvel, M.G., Hengeveld, P.D., Bosch, V.D.T., Broens, E.M., & Reusken, C.B.E.M. (2009). Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. *Preventive veterinary medicine, 91*(2), 270-273.

van Duijkeren, E., Box, A., Heck, M., Wannet, W., Fluit, A., (2004). Methicillin-resistant staphylococci isolated from animals. Veterinary Microbiology.103, 91–97.

Voss, A., Loeffen, F., Bakker, J., Klaassen, C., & Wulf, M. (2005). Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerging Infectious Diseases, 11*(12), 1965-1966.

Weese, JS., Dick, H., Willey, B.M., McGeer, A., Kreiswirth, B.N., Innis, B., & Low, D.E. (2006). Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. *Veterinary microbiology, 115*(1), 148-155.

Witte, W. (2009). Community-acquired methicillin-resistant *Staphylococcus aureus*: what do we need to know? *Clinical Microbiology and Infection, 15*(s7), 17-25.

Zahar, J-R., & Timsit, J-F. (2007). How To Control MRSA Spread in the Intensive Care Unit *Infectious Diseases in Critical Care* (pp. 226-232): Springer.

Zunita, Z., Bashir, A., & Hafizal, A. (2008). Occurrence of Multidrug Resistant *Staphylococcus aureus* in horses in Malaysia. *Veterinary World, 1*(6), 165-167.