Review Article

Dissemination of Resistance and Virulence Determinants in Methicillin-Resistant *Staphylococcus aureus* During Colonization and Disease: A Review

Asinamai Athliamai Bitrus[1,2*], Zakaria Zunita[1], Mohammed Dauda Goni[3], Innocent Thlama Mshelia[4]

[1]Research Unit, Microbial food safety and Antimicrobial resistance, Department of Veterinary Public health, Faculty of Veterinary Sciences, Chulalongkorn University, 10330 Pathumwan, Bangkok, Thailand; [2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor Malaysia; [3]Unit of Biostatistics and Research Methodology,School of Medical Sciences, Health Campus,Universiti Sains Malaysia,16150 Kubang Kerian, Kelantan, Malaysia; [4]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri, P.M.B 1069 Maiduguri, Borno State, Nigera.

Abstract | The successful in vivo horizontal transfer of mobile genetic elements carrying resistance and virulence determinants have contributed immensely to a global dissemination of virulent and multi-drug resistant pathogens. The pathogenesis of MRSA infection is enhanced via initial colonization of the skin through the component of the microbial surface antigen recognizing adhesive matrix molecules and by their ability to evade host immune response. Furthermore, it was also observed that the genetic diversity of pathogenic MRSA is due to its' ability to rapidly acquire resistance and virulence determinants. A characteristic feature that made it one of the most important nosocomial pathogen worldwide. Similarly, the expression of virulence gene in MRSA has been observed to be regulated by the accessory gene regulator system (*agr*). These system is made up of a series of genes whose product build up quorum-sensing regulatory mechanisms that is growth dependent. At a certain growth stage, the *agr* systems triggers a pronounced changes in the expression of genes called the quorum sensing. The findings of this review affirmed the importance of horizontal gene transfer in the dissemination of resistance and virulence determinants and as well as the genetic diversity of MRSA.

Keywords | Accessory gene regulators, Colonization, Horizontal gene transfer, Resistance, Virulence

Editor | Kuldeep Dhama, Indian Veterinary Research Institute, Uttar Pradesh, India.
Received | December 18, 2017; **Accepted** | January 06, 2018; **Published** | January 18, 2018
***Correspondence** | Asinamai Athliamai Bitrus, Research Unit, Microbial food safety and Antimicrobial resistance, Department of Veterinary Public health, Faculty of Veterinary Sciences, Chulalongkorn University, 10330 Pathumwan, Bangkok, Thailand; **Email**: abasinamai@gmail.com
Citation | Bitrus AA, Zunita Z, Goni MD, Mshelia IT (2018). Dissemination of resistance and virulence determinants in methicillin-resistant *Staphylococcus aureus* during colonization and disease. A Review. Adv. Anim. Vet. Sci. 6(1): 44-54.
DOI | http://dx.doi.org/10.17582/journal.aavs/2018/6.1.44.54
ISSN (Online) | 2307-8316; **ISSN (Print)** | 2309-3331

INTRODUCTION

The emergence and worldwide spread of methicillin resistant *S. aureus* strains is largely due to horizontal transfer of mobile genetic elements carrying resistance and virulence determinants (Haaber et al., 2017; San et al., 2017). Horizontal gene transfer (HGT) provided the basis for the expansion of previously unsuccessful clones (Kelly et al., 2009; Haaber et al., 2017). Interspecies transfer of Staphylococcal cassette chromosome *mec* (SCC*mec*) have been reported to contribute to the spread of resistance and virulence gene (Bloemendaal et al., 2010) . The clinical importance of MRSA is due to its' ability to rapidly acquire and loss resistance and virulence determinants (Lamy et al., 2012). Thus, leading to an increased burden on health care setting due to a limited treatment options. Because of its frequent association with mobile genetic elements, natural resistance genes can be spread rapidly among pathogenic strains and therefore impedes the clinical value of many drugs (Toh et al., 2007).

The emergence of methicillin- resistant *S. aureus* (MRSA)

in the late 1950s to early 1960s when a strain of *S. aureus* acquired a genomic island called SCC*mec* carrying methicillin resistance determinants *mec*A ushered in a new era in the epidemiology, disease severity, and antibiotic resistance characteristic of *S. aureus* (Noto et al., 2008). Methicillin resistant *Staphylococcus aureus* being a frequent colonizer of the skin and mucous membrane of the nares is known to cause a number of diseases with varying degree of severity in humans and animals respectively. Host colonization has been observed to predispose an individual to infection especially when there is compromise or break in the integrity of the skin or immune suppression. Methicillin Resistant *Staphylococcus aureus* (MRSA) is known to produce a number of potent virulence factors and toxins. In addition, the pathogen has the potentials of acquiring resistance to almost all classes of antibiotic, a feature that makes MRSA one of the most important nosocomial pathogen worldwide. Currently, MRSA is recognized as one of the most leading cause of blood stream infection and a major cause of nosocomial, community and livestock associated infection worldwide (Kock et al., 2013).

Nasal colonization is the most important means of disseminating MRSA to other body parts (Al-Talib et al., 2013). This is because evidence abounds that after topical treatment of nasal colonization, corresponding decrease in colonization of other body parts was also observed (Liu, 2009; Kluytmans et al., 1997). Furthermore, Kluytmans et al. (1997) described three types of nasal colonization and these include; persistent carriers which carry a particular strain of MRSA and are observed in about 20-25% of the human population, the intermittent carriers which were found in about 60% of the population and the strains change at varying degree and finally the non-carriers or non-colonised which were observed in 20% of the population. Similarly, it was also observed that persistent carriage was more common in children than adults and the pattern of carriage was observe to change in most individuals between the ages of 10 and 20 years. A number of studies have revealed that quite a large number of the human populations are at great risk of *S. aureus* infection with some persistently colonized while others intermittently colonized (Oliveira et al., 2002; Sakwinska et al., 2009). For instance, investigation of pig farm dominated areas for nasal colonization of MRSA between farm owners, veterinarians and their non-exposed families as well as 462 pupils revealed that 86% of farmers sampled where exposed and only 4.3% of their family members were observed to carry MRSA with similar spa types belonging to that of clonal complex CC398 found in Pigs. The authors further reported that nasal colonization was observed in 45% of the veterinarians who had contact with pig farms and in 9% of their family who had no history of exposure to pigs. Similarly, 3 pupils out of the 462 pupils sampled were observed to be colonized by CC398 and all were observed to have had history of contact with pigs or living on pig farms. The health implication of this finding is that, CC398 can serve as a potential reservoir for MRSA infection to humans (Cuny et al., 2016).

Higher carriage rates of about 25% to 50% MRSA were reported in persons with skin conditions, patients with the history of prolong hospital admission and indwelling intravascular devices, health-care workers, young children and injection drug users (Chambers, 2001).

It is difficult to ascertain the primary source of infection and the role of a particular virulence factor in the pathogenesis of disease caused by MRSA. This is because virulence determinants present in MRSA have been shown to spread across different patients, health care settings and personnel. In addition, virulence determinants have been observed to work synergistically in initiating a disease process (Mainous et al., 2006). The spectrum of infection associated with MRSA ranges from mild uncomplicated superficial abscess to a more life- threatening conditions such as central nervous disease, necrotic pneumonia, necrotic fasciitis, meningitis, cerebritis, osteomyelitis, pericarditis, myocarditis, urinary tract infections and infection associated with the use of an invasive device (Lindqvist, 2014). Furthermore, MRSA is frequently implicated in cases of skin and subcutaneous infections such as Furunculosis, bulos impetigo, cellulitis, mastitis, and folliculitis. Similarly, it is also associated with post- operative wound infection, toxic shock syndrome, scalded skin syndrome and staphylococcal food poisoning (Cuny and Witte, 2017). The overall mortality as a result of MRSA blood stream infection was 30%. However, the severity of the infection was observed more in children and adults or individuals with immune-related diseases (Liu, 2009). Individuals at risk of infection with hospital acquired-MRSA includes, persons in contact with health care setting, prolong admission, persons with invasive surgical devices and intensive care unit, indiscriminate and prolong use of antibiotics and patients with weak immune system (Salgado et al., 2003). However, a new strain of MRSA without known identifiable risk factors associated with the hospital associated MRSA was found causing severe infection in healthy individuals in the community (Leiber et al., 2017). In the United states, studies have shown that these strains were replacing the hospital acquired MRSA (Chua et al., 2014) . Community- acquired MRSA (CA-MRSA) is the cause of skin and soft tissue infection in children and adults (Maree et al., 2007). In addition, CA-MRSA infection was observed in individuals in overcrowded place, army recruits in training facilities, athletes, and prisoners and in men sleeping with men (Maree et al., 2007). Methicillin resistant *Staphylococcus aureus* is a highly versatile human pathogen than is also emerging as a potential zoonosis with an incredible adaptive powers (Bitrus et al., 2017). Transfer of resistance and virulence determinants occurs through the horizontal

transfer of mobile genetic elements (MGEs). That is commonly shared between isolates from same clonal lineage. These have resulted in rapid changes seen in the epidemiology of MRSA strains (Bitrus et al., 2016). Recent reports have also shown that, MRSA is no longer restricted to the hospital setting. Additionally, evidence of clonal replacement at a higher frequency is reported, this is because, community acquired MRSA are now been reported as the major cause of hospital acquired infections and in some cases evidence of human colonialization with livestock associated MRSA have been reported (Bitrus et al., 2017; Haaber et al., 2017). This wouldn't have been possible without the active role of horizontal gene transfer. Thus, the need for a review on the dissemination of resistance and virulence determinants in MRSA during colonization and disease.

MATERIALS AND METHODS

This review employed the collection of published articles on the "the transfer of resistance determinants and the evolution of new MRSA clones". The articles were retrieved from Thompson Reuter's web of science, Springer, Lancet, Scopus, Science direct, Pub Med and Wiley. The search was also conducted on other scientifically relevant database. Words and phrases such as Methicillin resistant *S. aureus*, Clonal evolution of MRSA, Pathogenesis of MRSA and gene acquisition in MRSA were used. All articles used in these review were appropriately cited in the bibliography.

Acquisition of Resistances Determinants and the Evolution of New MRSA Clones

Proliferation of bacterial pathogens from a previously unexploited habitat leading to emergence of new strains and diversification of the natural population was made possible through Horizontal gene transfer (HGT) (Kelly et al., 2009; Haaber et al., 2017). The concept of horizontal gene transfer was first demonstrated by Griffiths in 1928 where he transferred virulent determinants between pneumococci in mice (Kelly et al., 2009). The emergence of MRSA from MSSA through horizontal acquisition of SCC*mec* carrying methicillin resistance determinants *mec*A has greatly increase research interest in the study of MSSA (Lindqvist, 2014; Bitrus et al., 2017). Horizontal transfer of mobile genetic elements carrying resistance and virulence gene occurs at a higher frequency between strains of the same cluster while limited between strains of different clusters (Rapacka-Zdonczyk et al., 2014). In addition, successful in vivo horizontal transfer of mobile genetic elements carrying resistance and virulence determinants have contributed immensely to a global dissemination of virulent and multi-drug resistant pathogens (Bloemendaal et al., 2010; Corvaglia et al., 2010).

In *S. aureus,* generalized transduction, conjugation and transformation are the processes involved in the transfer of bacterial DNA from one cell to another. However, generalized transduction is likely to be the main medium of transfer of DNA between strains of *S. aureus* as it possess many phage required for transfer and it is very efficient in the laboratory (Lee, 1995; Corvaglia et al., 2010; Lindsay et al., 2014; Bitrus et al., 2017; Haaber et al., 2017; Rahimzadeh et al., 2017). Host specificity have been reported in the bacteriophage of *S.aureus* and this explains why *S. aureus's* mobile genetic elements are rarely found in other species or genera. The phage genomes is 45kb in size and are known to code for bacterial virulence determinants such as enterotoxins A and Panton valentine leucocidin while others encode chemotaxis inhibitory protein or complement inhibition proteins a strategy that helps the bacteria escape host immunity (Lindsay, 2014; Stanczak-Mrozek et al., 2017; Cafini et al., 2017; Rahimzadeh et al., 2017). Central to evolution of virulent strain, is the transfer of toxin genes phage conversion or lysogenic bacteriophage (Kelly et al., 2009). Restriction and modification system most especially type I are the barriers to gene transfer in MRSA, even though CRISPR (clustered, regularly interspaced palindromic repeats) also played a role but at a lower frequency (Lindsay, 2014). New clones of MRSA will continue to evolve because of selective pressure exerted upon them by the environment and horizontal gene transfer. Methicillin resistant *Staphylococcus* aureus is constantly evolving and increasingly becoming a global threat, the pathogen is no longer restricted to the hospital setting as previously reported Aires-de-Sousa, (2017). This is because, MRSA has been isolated among individuals without the apparent identifiable risk factors of MRSA acquisition. Additionally, colonization with Livestock Associated MRSA (LA-MRSA) have been reported among Veterinarians, farmers and personnel working in close contacts with animals (Kwoji et al., 2017). The reservoir of MRSA is also expanding and cases of colonization of animals and other food sources from animals have also been reported (Ge et al., 2017). Recently, there is an increasing report of human colonization and infection with LA-MRSA. Even though this is commonly reported among veterinarians and other personnel working in close contact with animals, LA-MRSA colonization of individuals without prior contact with animals have been reported (Doulgeraki et al., 2017). The major clonal complex commonly shared between animals and humans is the CC 398 which was predominantly reported in pigs. However, Poultry and other animals have been reported to serve as reservoirs of colonization and infection with CC 398. In a study conducted by Tang et al. (2017) using n= 145 meat samples consisting of n= 102 from chickens, n=23 from turkey and n= 20 from pork meat showed that 13% (19/145) of the isolates were MRSA which corresponds to the prevalence of 52% in Turkey, 15 % in pork and 4 % in chickens. Genetic profiling of the isolates showed that 68%

of the MRSA belongs to CC 398, 1% of which were from chickens. Spa typing also showed that 16% of samples collected from Turkey belongs to t1430 CC9. Additionally, 16 % of the samples collected from chickens are associated with spa type t008 belonging to clonal complex CC8. The isolation of MRSA from food animals poses a serious health risk. This also indicated how horizontal transfer of mobile genetic element and transposon between bacterial cells contribute to the spread of MRSA. 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). Similarly, 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. Isolation of MRSA have also been reported in variety of domestic animals such as cats, dogs pigs, sheep, chickens and horses leading to a sudden increase in reports and interest in colonization with MRSA and infection in animals (Leonard and Markey, 2008; Saleha and Zunita, 2010; Aires-de-Sousa, 2017). Luzzago et al. (2014) reported that t1328 and ST22 isolates obtained from the liver of the chamois kid was a methicillin-resistant *S. aureus* (MRSA) harbouring SCC*mec* cassette type IV. Furthermore, Price et al, (2012) reported that MRSA ST398 initially originated as methicillin susceptible *S. aureus* in humans which was later transmitted to pigs where it acquires methicillin resistance and is now seen re-infecting humans. In 2011, García-Álvarez et al. (2011) reported the isolation of *S. aureus* in cattle and humans with a new homologue of SCC*mec* designed as SCC*mec*C. Subsequently, increasing numbers of reports have been published on MRSA infection and colonization in both companion and food-chain animals showing MRSA as an important veterinary and zoonotic pathogen (Obaidat et al., 2017). Molecular typing showed that some animal lineages are host specific while others are able to colonize or infect a wide variety of animals including humans. Studies on the clonal evolution of MRSA has shown that some MRSA lineages are host specific while others are not (Bitrus et al., 2016; Aires-de-Sousa, 2017). The isolation of MRSA from pets in the late 1990s provided sufficient evidences in the clonal dissemination of the pathogen, this is because, prior to this, MRSA is hardly isolated from pets. However, cases of human to pet transmission at a high frequency have been reported. In Australia, Malik et al. (2006) reported the isolation of HA-MRSA (ST239-III) in dogs. In another studies conducted in UK and Portugal, the authors reported the occurrence of EMRSA-15, ST22-IV isolates that is commonly associated with human nosocomial infection. This is suggestive of human to animal transmission. (Coelho et al. , 2011; Wedley et al., 2014). Interestingly, Lin et al. (2011) reported that more than 50% of the MRSA strains isolated from pets in the North-eastern and Midwestern states of the United States of American belong to the HA-MRSA (USA100,ST5-II). The occurrence of MRSA 398 from pets have also been reported in Germany, France and UK (Witte et al., 2007; Loeffler et al., 2009). In horses, MRSA colonization differ from those isolated in humans and pets. For example, CC8 consisting of ST8-IV and ST254 is the most predominantly isolated MRSA isolates from horses in Canada, while in other countries horse colonization and infection is through ST1,ST22 and ST254 (Cuny et al., 2009). In Malaysia, Zunita et al. (2017) reported the first occurrence of ST 177 from horses.

Host Colonization, Disease Pathogenesis and Gene Regulation in MRSA

Methicillin resistant *S. aureus* being an important pathogen of veterinary and public health significance and regarded as one of the most frequent causes of hospital, community and livestock associated infection. The pathogen is highly versatile and is considered as a potential emerging zoonosis (Saleha and Zunita, 2010; Obaidat et al., 2017). The diversity and emergence of pathogenic methicillin resistant *S. aureus* is due to its' ability to rapidly acquire resistance and virulence determinants. This characteristic feature, is what gives MRSA the potentials of causing a wide range of infections in the host in different habitats worldwide (Bitrus et al., 2016). The successful adaptation and difference in pathogenic potentials is largely due to the presence of a variety of coordinated virulence gene (Shaw et al., 2004). To establish or initiate a disease process the virulence of *S. aureus* functions basically to promote adherence and invasion of host tissue, evasion of the host immune systems and direct damage and induction of inflammatory process by the actions of the toxins they elicit (Lindqvist, 2014). Survival and multiplication inside the hosts is enhanced through formation of small colony variants, biofilm and production of antiphagocytic microcapsule which prevent opsonisation (Liu, 2009). Furthermore, bacterial surface proteins are produced at the early growth stage while secretion of toxins and enzymes is at the late phase of growth mostly when the growth rate declines due to depletion of resources (Lindqvist, 2014). In most cases, it is difficult to ascertain the role of single virulence determinants in disease pathogenesis, their effect were however cumulative indicating the possibility of the virulence determinants working synergistically (Peacock et al., 2002).

Host colonization provides the basis for initiation of disease process, MRSA are frequent colonizers of the nostrils (Gordon and Lowy, 2008; Doulgeraki et al., 2017). Nasal carriage contributes to dissemination of MRSA to other body parts, this was evident when topical mupirocin was used to treat nasal colonization of MRSA which was followed by a subsequent decolonization of other body parts. Similarly, studies on bacteraemia revealed that 82% of *S. aureus* isolated from blood samples were identical to those

isolated from the nostrils (Gordon and Lowy, 2008; Kluytmans et al., 1997). In MRSA, invasion and adherence to nasal epithelium is mediated by an aggregation of bacterial protein molecules called the Microbial surface component recognizing adhesins macromolecules (MSCRAM), clumping factor B and cell wall associated teichoic acid (Liu, 2009). Furthermore, it is important to note that colonization does not necessarily mean infection. Previous studies have shown that colonization of the epithelium leads to the activation of a quorum sensing system called the accessory gene regulator (*agr*) which regulates the expression of the virulence gene (Liu, 2009). Methicillin resistant *S. aureus* can evade neutrophilic-killing by secreting two molecules, Chemotaxis Inhibitory Protein (CHP) and Extracellular adherence protein (Eap). These molecules helps to block neutrophil recognition of chemotactic factors and neutrophil binding to endothelial adhesion molecule ICAM-1 which subsequently leads to leukocyte adhesion, diapedesis, and extravasation from the bloodstream to the site of infection (Liu, 2009). Other virulence factors involved in disease pathogenesis includes the exotoxins such as the Panton valentine leucocidin toxin, alpha and beta hemolysin, superantigens and enzymes (Plata et al., 2009).

The pathogenesis of infection with MRSA begins with an initial colonization of host skin and mucosal surfaces. It involves bacterial attachment to host cells often through components of the extracellular matrix MSCRAMMS (microbial surface components recognizing adhesive matrix molecules) and the ability of the organism to evade the host immune response (Gordon and Lowy, 2008). The pathogenesis is mostly associated with expression of several virulence determinants such as the Panton-valentine leukocidine (PVL) a bi-component cytolytic toxin of the synergomenotropic family whose lytic activity is restricted to polymorph nuclear cells, monocytes and macrophages in humans and animals and multiple resistances to antimicrobials, mostly carried on a carried on a mobile genetic element (MGEs) on the MRSA genome (Pantosti et al., 2007). The past decades have witnessed an intense research activity in the aspect of molecular studies of MRSA virulence. This is due to the emergence of a highly pathogenic community associated MRSA strain with an exceptionally high rate of resistance spread and virulence. Similarly, the past decade also witnessed the identification of another unrecognized virulence factor the phenol soluble modulins (Rasigade and Vandenesch, 2014) as well as the characterization of the pathogenic role of long-known toxins such as the Panton-Valentine leucocidin (PVL). The clinical significance of PVL producing strain of community acquired MRSA is associated with skin and soft tissue infection occurring mostly in immune-competent individuals with relatively high morbidity. In addition, Furunculosis, skin abscesses, and severe necrotizing pneumonia were some of the disease conditions associated with PVL toxin (Chiu et al., 2012).

In the hospital setting, MRSA causes infection mostly in immunocompromised patients as opposed to the community acquired strains which were observed to cause disease in apparently healthy individuals without prior association with the hospital. It was reported that, the inability of HA-MRSA to cause infection in healthy individuals was probably due to the low expression of phenol soluble modulins (Psm) peptide and the presence or absence of accessory gene regulator (*agr*) . Thus, indicating the persistent nature of the HA-MRSA in the health care setting. The formation of biofilms by some strains of MRSA deficient in *agr* was also observed to have exacerbated the magnitude of infection within the biofilm matrix; MRSA can withstand the therapeutic effect of some antibiotics and also evade the host defence system by adhering to indwelling catheters, implants, and prosthetic heart devices through the formation of biofilm (Liu, 2009; Doulgeraki et al., 2017). Virulent species of MRSA causes severe disease, which can be fatal (DeLeo FR et al., 2010) with a high morbidity and mortality.

The accessory gene regulator forms the basis for the expression of genes inMRSA (Balaban et al., 2000). The *agr* locus is made up of two divergently distinct transcribed operon identified as RNAII and RNAIII. However, it is the RNAII operon which contains the *agr* BDCA gene coding for signal transducers (AgrC) and response regulator (AgrA), AgrB and AgrD that are involved in the generating signal molecules that initiates Quorum sensing (Balaban et al., 2003). In addition, the survival of *S. aureus* has been observed to be dependent upon the *agr* system regulating quorum sensing and promotes initiation of cellular adhesion necessary for the formation of biofilm. The expression of these virulence determinants are under the control of a quorum sensing system called the accessory gene regulator (*agr*) (Booth et al., 1997; Cheung et al., 2011). The accessory gene regulator system (*agr*) of *S. aureus* is made up of a series of genes whose product build up quorum-sensing regulatory mechanisms that is growth dependent. At a certain stage of growth, the *agr* systems triggers a pronounced changes in the expression of genes called the quorum sensing (Balaban et al., 2001). In addition, the *agr* system also helps in the upregulation of virulent determinants such as the protease, nucleases and lipases and down regulate the expression of surface binding protein (Liu, 2009; Cheung et al., 2011) specific to certain set of virulence determinants. The use of animal models have enhanced the knowledge on the role of *agr* system in the pathogenesis of disease. For example, *agr* mutant strains of MRSA have been demonstrated to produce an attenuated virulence. The authors also reported that, rapid burst in the *agr* within 3 hours of *S. aureus* infection in the

subcutaneous tissue and abscess formation in the presence of *agr* – positive *S.aureus* (Balaban et al., 1998). The quorum sensing of S. *aureus* have been reported to allow for successful adaption and dissemination of closely related progeny while inhibiting the spread of non-related strains. This finding could explain the existence of the concept of gene transfer between those closely related strains allowing for the propagation of such cell. In addition, quorum sensing have been reported to enhance the establishment of a specific ecological niche for each *S. aureu*s strain, in that sense the dissemination of virulence characteristics within such groups of *S. aureus* will be maintained.

In another development, since *agr* quorum sensing has been shown to be responsible for the initiation of biofilm formation. Studies have reported the role of biofilm in facilitating the spread of antibiotic resistance and virulence determinants through horizontal gene transfer (Fux et al., 2005). This finding was however argued by some school of thoughts whose assertions were that the resistance characteristics of *S. aureus* in biofilm is independent of antibiotics resistances acquisition due to plasmids, transposons and insertion sequence or efflux pump system. But rather due to the nature of the metabolites they elicit and the heterogenic nature of the pathogens that made up the biofilm. This they were able to prove by harvesting the organisms in the biofilm and testing them with the same antibiotics they were resistant to in the biofilm. Furthermore, in another study, down-regulation of the *agr* locus or *agr* mutant strains of *S. aureus* have also been reported to persist in their resistance characteristics (Fux et al., 2005). A possibility that the loss or down regulation of the *agr* locus compensate for resistance development. Other authors were of the opinion that since *agr* is known to regulate gene expression, it is likely that it also helps to regulate the spread of resistance. While others believed that since the whole concept of quorum sensing is to initiate cell to cell contact that will ultimately lead to the creation of a multicellular niche. It can be argued out that this close association will enhance gene transfer processes like transformation and conjugation.

It has been established that majority of severe diseases caused by biofilm-associated *S. aureus* infection were under the of *agr* quorum sensing system (Balaban et al., 2003). In addition, studies have also revealed the relationship between persistent bacteraemia and resistance to methicillin (Raffa et al., 2005). Furthermore, loss of *agr* activity has been linked to resistance to methicillin and vancomycin indicating a kind of compensatory mechanism between loss of virulence and resistance (Balaban et al., 1998; Raffa et al., 2005). Similarly, studies carried out in MRSA with type II SCC*mec* type have been shown to have decreased expression of cytolytic toxins as compared to MRSA with type IV SCC*mec* (Stewart et al., 2001). Hence the indication that specific SCC*mec* elements regulates the expression of toxins. In addition, other studies carried out indicate that expression of penicillin binding protein results in decrease expression of *agr* operon. Furthermore, deletion of *mec*A in a strain of MRSA leads to restoration of the *agr* activity (Tseng and Stewart, 2005). Even though the above studies did not directly portrays the role of *agr* operon in the dissemination of antibiotic resistance. It can be inferred that the trade-off between loss of *agr* locus and gain of resistance and virulence determinants is as a result of the transfer and loss of resistance initiated by the quorum sensing mechanism.

Mrsa in Animals and its Public Health and Economic Implications

Methicillin resistant *S. aureus* (MRSA) is an important aetiology of a wide range of infections in humans and animals. Recently higher rate of colonization with MRSA in animals have been reported (Kwon et al., 2006; Lee, 2003). Although the first report of MRSA isolation in animals dates back to the early 1970s when MRSA was isolated from a case of mastitis in cows (O'Mahony et al., 2005). Since then, MRSA colonization has been reported in various species of animals such as dogs and cats, pigs and rabbits, chicken, cattle, sheep and horses (De Boer et al., 2009; Larsen et al., 2012; Lee, 2003, Bitrus et al., 2016; Bitrus et al., 2017). Depending on the study design, the estimated carriage rate of *S. aureus* in chickens is about 90%, while 42% in pigs, 29% in sheep, 14% to 23% in cows and up to 35% in heifers with the teats and the muzzle being the most colonized site (Peton and Le Loir, 2014). The spectrum of infections caused by MRSA in animals includes; mastitis in cows, pyaemic dermatitis in dogs, cats and rabbits, arthritis and septicaemia in chickens, botryomycosis in mares, cows and dogs and urinary tract infection associated with the production of extracellular toxins and enzymes (Lee, 2003; Zunita et al., 2008; Peton and Le Loir, 2014). The colonization of animals by MRSA have severe clinical consequence. This is because studies have revealed colonized animals are serving as potential carriers of MRSA infection to humans. In addition, low milk yield is reported in cows and sheep with clinical mastitis. Furthermore, *S. aureus* mastitis have been reported to be of great public health significance. This is because studies have revealed that more than half of *S. aureus* strains isolated from case of clinical mastitis harbours the staphylococcal superantigen (Peton and Le Loir, 2014). Thus, indicating that milk from mastitic animals is a good medium for to staphylococcal enterotoxins. In rabbits, *S. aureus* causes abscess, foot infection, dermatitis in young does, respiratory and reproductive problems as well as mastitis (Goni et al., 2004). While in poultry it causes a range of infection from localized infection of the skin to a more severe cases of synovitis, yolk sac infection, bumble foot, green coloration of the liver in turkeys which leads to carcass rejec-

tion (Peton and Le Loir, 2014). In small animals such as dogs and cats, *S. aureus* is rarely isolated, however, cases of human transmission to dogs and cats have been reported (Morgan, 2008).

The isolation of MRSA on animal origin have changed the narrative on the epidemiology and clonal spread of MRSA. The emergence of MRSA clone belonging to sequence type 398 in 2005 from healthy pigs and farmers in Netherlands and France stimulated a renewed interest in the study of MRSA of animal origin (Armand-Lefevre et al., 2005; Voss et al., 2005; Bitrus et al., 2016; Ge et al., 2017). Since then, a number of studies have reported the occurrence of livestock-associated MRSA (LA-MRSA) in food animals such as calves, pigs and broilers and in people with frequent contact with animal (Fitzgerald, 2012; Kwoji et al., 2017). For instance, LA-MRSA-398 accounted for more than 20% of the cases of human infection in the Netherlands, this is however, considered as low (Van Rijen et al., 2008). Molecular typing of MRSA have showed that LA-MRSA 398 actually originated from humans as *S. aureus* strain that is susceptible to methicillin. The report further showed that due to sustained usage of antibiotics in an intensively reared livestock farm, this strain later acquired resistance determinants for methicillin and tetracycline (Price et al., 2012). Thus, indicating the role of frequent and indiscriminate use of antibiotics in the transfer of resistance determinants. Additionally, since this strain was isolated from human cases, revealed the potentials of this pathogen as a zoonotic pathogen. The most predominant MRSA clone isolated in Asia among pigs is the ST 9 (Cui et al., 2009). Other lineage related to livestock associated MRSA included ST1, ST5, ST97, ST130 and ST425 and more recently ST 177 in horse (Fitzgerald, 2012; Zunita et al., 2017). Similarly, a number of studies have revealed the occurrence of ST398, ST5 and ST 9 in the United States of America, this could be attributed to movement of people and meat products especially those coming to Asia as tourist (Molla et al., 2012; Smith et al., 2009; Frana et al., 2013). Livestock associated MRSA is now a major public health issue, stakeholders are becoming increasingly worried because of its potential as a zoonotic pathogen that can be transmitted through the food chain.

Methicillin-resistant *S. aureus* is a major cause of severe life-threatening infection in animals (Bosch et al., 2015; El-Gendy et al., 2017). The economic implication of disease caused by MRSA is hinged on its ability to rapidly acquire resistance and virulence determinants, cost of treatment, prevention and control and production of antibiotics (Bitrus et al., 2016). Currently, MRSA is the leading cause of death among infants and adult individuals in the United States (Chua et al., 2014). The development of antibiotic resistance occurred immediately after the introduction of antibiotics, this is classically exemplified in resistance development to penicillin and methicillin in 1940s and 1960s respectively (Chambers and De Leo, 2009; Bitrus et al., 2017). Over the years, antibiotic resistance development represented a relatively less challenging situation, since newer classes of effective antibiotics were developed to counteract the threat posed by antibiotic resistant bacteria. However, the recent years saw a significant decrease in the development of new antibiotics with regards to the emergence of multidrug resistant strains of bacteria (Silver, 2011). The gap created, coupled with the absence or lack of improvement of new and effective antibiotics lead to the emergence of highly resistant strains and ultimately increase the rate of spread of antibiotic resistance globally (WHO, 2012). Recently in 2013, the World Economic Forum identified antimicrobial resistance as one of the greatest risk to human health worldwide. The economic implication of disease caused by MRSA result in drastic reduction in productivity and mortality in humans and animals, a classic case of mastitis caused by MRSA in cows will result in severe reduction in milk yield.

CONCLUSION

Horizontal transfer of resistance and virulence determinants forms the basis for the successful expansion of several clones of MRSA. Colonization of the nasal epithelium enhances the dissemination of MRSA to other body parts. Furthermore, it was also observed that colonization of the skin and mucous membrane is the first line of events in the initiation of a disease. Similarly, successful adaptation and difference in pathogenic potentials of MRSA was largely observed to be due to the presence of a variety of coordinated virulence determinants and toxins. Finally, it was also observed that the survival of MRSA is dependent upon the *agr* system regulating quorum sensing and promoting the initiation of cellular adhesion necessary for the formation of biofilm. Expression of these virulence determinants are under the control of a quorum sensing system called the accessory gene regulator (*agr*).

ACKNOWLEDGEMENTS

This research review is supported by Rachadapisek Sompot Fund for Postdoctoral Fellowship, Chulalongkorn University. The authors also wishes to acknowledge the support of the Research unit Microbial Food safety and Antimicrobial resistance, Faculty of Veterinary science, Department of Public health, Chulalongkorn University.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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 manuscript.

REFERENCES

- Abbasi M, BaseriSalehi M, Bahador N, Taherikalani M (2017). Antibiotic Resistance Patterns and Virulence Determinants of Different SCC*mec* and Pulsotypes of *Staphylococcus aureus* Isolated from a Major Hospital in Ilam, Iran. Open Microbiol. J. 11 : 211. https://doi.org/10.2174/1874285801711010211
- Aires-de-Sousa M (2017). Methicillin-resistant *Staphylococcus aureus* among animals: current overview. Clin. Microbiol. Infect. 23(6): 373-380. https://doi.org/10.1016/j.cmi.2016.11.002
- Al-Talib H, Yean CY, Hasan H, Nik Zuraina NM, Ravichandran M (2013). Methicillin-resistant *Staphylococcus aureus* nasal carriage among patients and healthcare workers in a hospital in Kelantan, Malaysia. Polish J. Microbiol. 62(1): 109-112.
- Armand-Lefevre L, Ruimy R, Andremont A (2005). Clonal comparison of Staphylococcus aureus isolates from healthy pig farmers, human controls, and pigs. Emerg. Infect. Dis. 11(5): 711–714. https://doi. org/10.3201/eid1105.040866
- Balaban N, Giacometti A, Cirioni O, Gov Y, Ghisell RI, Mocchegiani, F, Viticchi C, Del Prete MS, Saba V, Scalise G, Dell'Acqua G (2003). Use of the quorum-sensing inhibitor RNAIII-inhibiting peptide to prevent biofilm formation *invivo* by drug-resistant *Staphylococcus epidermidis*. J. Infect. Dis. 187:625–630. https://doi.org/10.1086/345879
- Balaban N, Collin LV, Cullor JS, Hume EB, Medina-Acosta E, Vieira da Motta O, O'Callaghan R, Rossitto PV, Shirtliff ME, Serafim da Silveira L, Tarkowski A, Torres JV. (2000). Prevention of diseases caused by *Staphylococcus aureus* using the peptide RIP. Peptides. 21:1301– 1311. https://doi.org/10.1016/S0196-9781(00)00272-2
- Balaban N, T Goldkorn, RT Nhan, LB Dang, S Scott, RM Ridgley, A Rasooly, SC Wright J, Larrick W, Rasooly R, Carlson JR (1998). Autoinducer of virulence as a target for vaccine and therapy against *Staphylococcus aureus*. Science. 280:438–440. https://doi.org/10.1126/science.280.5362.438
- Balaban N, Goldkorn T, Gov Y, Hirshberg M, Koyfman N, Matthews HR, Nhan RT, Singh B, and Uziel O. (2001). Regulation of *Staphylococcus aureus* pathogenesis via target of RNAIII-activating protein (TRAP). J. Biol. Chem. 276:2658–2667. https://doi.org/10.1074/jbc.M005446200
- Balaban N, Gov Y, Bitler A, Boelaert, JR. (2003). Prevention of *Staphylococcus aureus* biofilm on dialysis catheters and adherence to human cells. Kidney Int. 63:340–345. https://doi.org/10.1046/j.1523-1755.2003.00733.x
- Bitrus AA, Zakaria Z, Khairani-Bejo S, Othman S, Tijjani AN (2016). Molecular Epidemiology: A Valuable Tool for Determination of Emerging and clonality of Methicillin Resistant *Staphylococcus aureus* (MRSA). Pertanika J. Scholar. Res. Rev. 22: 121-134
- Bitrus AA, Zunita Z, Bejo SK, Othman S, Nadzir NAA. (2017). In vitro transfer of methicillin resistance determinants mec A from methicillin resistant *Staphylococcus aureus* (MRSA) to methicillin susceptible *Staphylococcus aureus* (MSSA). BMC Microbiol. 17(1): 83. https://doi.org/10.1186/s12866-017-0994-6
- Bloemendaal ALA, Brouwer EC, Fluit AC. (2010). Methicillin resistance transfer from *Staphylocccus epidermidis* to methicillin-susceptible *Staphylococcus aureus* in a patient during antibiotic therapy. PLoS One. 5(7): e11841. https://doi.org/10.1371/journal.pone.0011841
- Booth MC, Cheung AL, Hatter KL, Jett BD, Callegan MC, Gilmore MS (1997). Staphylococcal accessory regulator (*sar*) in conjunction with agr contributes to *Staphylococcus aureus* virulence in endophthalmitis. Infect. Immun. 65(4): 1550-1556.
- Bosch T, Verkade E, van Luit M, Landman F, Kluytmans J, Schouls LM. (2015). Transmission and Persistence of Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* among Veterinarians and Their Household Members. Appl. Environ. Microbiol. 81(1): 124-129. https://doi.org/10.1128/AEM.02803-14
- Cafini F, Le Thuy NT, Román F, Prieto J, Dubrac S, Msadek T, Morikawa K (2017). Methodology for the Study of Horizontal Gene Transfer in *Staphylococcus aureus*. JoVE. J. Visual. Exper. (121): e55087-e55087.
- Chambers HF (2001). The changing epidemiology of *Staphylococcus aureus*? Emerg. Infect. Dis. 7(2): 178. https://doi.org/10.3201/eid0702.010204
- Chambers HF, DeLeo FR (2009). Waves of resistance: Staphylococcus aureus in the antibiotic era. Natur. Rev. Microbiol. 7(9): 629-641.
- Cheung GYC, Wang R, Khan BA, Sturdevant DE, Otto M (2011). Role of the accessory gene regulator *agr* in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. Infect. Immun. 79(5): 1927-1935. https://doi.org/10.1128/IAI.00046-11
- Chiu Y-K, Lo W-T, Wang C-C (2012). Risk factors and molecular analysis of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* colonization and infection in children. J. Microbiol. Immunol. Infect. 45(3): 208-213. doi: http://dx.doi.org/10.1016/j.jmii.2011.11.011.
- Chua KYL, Howden BP, Jiang J-H, Stinear T, Peleg AY (2014). Population genetics and the evolution of virulence in *Staphylococcus aureus*. Infect. Genet. Evolut. 21(0), 554-562. doi: http://dx.doi.org/10.1016/j.meegid.2013.04.026.
- Coelho C, Torres C, Radhouani H, Pinto L, Lozano C, Gómez-Sanz E, Poeta P (2011). Molecular detection and characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from dogs in Portugal. Microb. Drug Resist. 17(2): 333-337. https://doi.org/10.1089/mdr.2010.0080
- Corvaglia AR, François P, Hernandez D, Perron K, Linder P, Schrenzel J (2010). A type III-like restriction endonuclease functions as a major barrier to horizontal gene transfer in clinical Staphylococcus aureus strains. Proceedings of the National Academy of Sciences. 107(26): 11954-11958.
- Cui S, Li J, Hu C, Jin S, Li F, Guo Y, Ran L, Ma Y (2009). Isolation and characterization of methicillin-resistant Staphylococcus aureus from swine and workers in China. J. Antimicrob. Chemother. 64: 680e683
- Cuny C, Abdelbary MMH, Köck R, Layer F, Scheidemann W, Werner G, Witte W (2016). Methicillin-resistant Staphylococcus aureus from infections in horses in Germany are frequent colonizers of veterinarians but rare among MRSA from infections in humans. One Hlth. 2:11-17.

- Cuny C,Wolfgang W (2017) "MRSA in equine hospitals and its significance for infections in humans." Vet. Microbiol. 200: 59-64. doi: http://dx.doi.org/10.1016/j.vetmic.2016.01.013.
- Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W (2009). Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs. PloS one, 4(8), e6800. https://doi.org/10.1371/journal.pone.0006800
- Cuny C, Strommenger B, Witte W, Stanek C (2008). Clusters of infections in horses with MRSA ST1, ST254, and ST398 in a veterinary hospital. Microb. Drug Resist. 14(4): 307-310. https://doi.org/10.1089/mdr.2008.0845
- De Boer E, Zwartkruis-Nahuis JTM, Wit B, Huijsdens XW, de Neeling AJ, Bosch T, Heuvelink AE (2009). Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. Intl. J. Food Microbiol. 134(1–2):52-56. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2008.12.007.
- DeLeo FR, Otto M, Kreiswirth BN, Chambers HF (2010) Community-associated meticillin-resistant Staphylococcus aureus. Lancet. 375(9725) 1557e68.
- Doulgeraki AI, Di Ciccio P, Ianieri A, Nychas GJE. (2017). Methicillin-resistant food-related *Staphylococcus aureus*: a review of current knowledge and biofilm formation for future studies and applications. Res. Microbiol. 168(1): 1-15 https://doi.org/10.1016/j.resmic.2016.08.001.
- El-Gendy MMAA, El-Bondkly AMA, Keera AA, Ali AM (2017). Incidence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Microbial Community of Cancer Patients and Evaluation of Their Resistant Pattern. Arabian J. Sci. Engin. 1-10.https://doi.org/10.1007/s13369-017-2670-4
- Fitzgerald JR (2012). Livestock-associated Staphylococcus aureus: origin, evolution and public health threat. Trends in Microbiol. 20(4): 192-198.
- Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, Ramirez A, Smith TC (2013). Isolation and characterization of methicillin resistant Staphylococcus aureus from pork farms and visiting veterinary students. PLoS One 8: e53738. http://journals.plos.org/plosone/article?id¼10.1371/journal.pone.0053738.
- Fux CA, Costerton JW, Stewart PS, Stoodley P (2005). Survival strategies of infectious biofilms. Trends Microbiol. 13(1): 34-40. doi: http://dx.doi.org/10.1016/j.tim.2004.11.010.
- García-Álvarez L, Holden MTG, Lindsay H, Webb CR, Brown DFJ, Curran MD, Holmes MA (2011). Meticillin-resistant *Staphylococcus aureus* with a novel *mec*A homologue in human and bovine populations in the UK and Denmark: a descriptive study. Lancet Infect. Dis. 11(8): 595-603. doi: http://dx.doi.org/10.1016/S1473-3099(11)70126-8.
- Ge BL, Mukherjee S, Hsu C-H, Davis JA, Tran TTT, Yang Q, Abbott JW, Ayers SL, Young SR, Crarey ET, Womack NA, Zhao SH, McDermott PF (2017). MRSA and multidrug-resistant Staphylococcus aureus in U.S. retail meats, 2010–2011. Food Microbiol. 62: 289–297.
- Goni P, Vergara Y, Ruiz J, Albizu I, Vila J, Gomez-Lus R (2004). Antibiotic resistance and epidemiological typing of *Staphylococcus aureus* strains from ovine and rabbit mastitis. Intl. J. Antimicrob. Agents. 23(3): 268-272. https://doi.org/10.1016/j.ijantimicag.2003.07.016
- Gordon RJ, Lowy FD (2008). Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. Clin. Infect. Dis. 46(Supplement 5): S350-S359. https://doi.org/10.1086/533591
- Haaber J, Penadés JR, Ingmer H (2017). Transfer of Antibiotic Resistance in *Staphylococcus aureus*. Trends Microbiol. 25 (11): 893-905. https://doi.org/10.1016/j.tim.2017.05.011
- Kelly BG, Vespermann A, Bolton DJ (2009). Horizontal gene transfer of virulence determinants in selected bacterial foodborne pathogens. Food Chem. Toxicol. 47(5): 969-977. doi: http://dx.doi.org/10.1016/j.fct.2008.02.007
- Kennedy AD, DeLeo FR (2009). Epidemiology and virulence of community-associated MRSA. Clin. Microbiol. Newsletter: 31(20): 153-160. https://doi.org/10.1016/j.clinmicnews.2009.09.004
- Kluytmans J, Van Belkum A, Verbrugh H (1997). Nasal carriage of *Staphylococcus aureus:* epidemiology, underlying mechanisms, and associated risks. Clin. Microbiol. Rev. 10(3): 505-520.
- Köck R, Schaumburg F, Mellmann A, Köksal M, Jurke A, Becker K, Friedrich AW (2013) Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany. PloS one. 8(2):e55040. https://doi.org/10.1371/journal.pone.0055040
- Kwoji ID, Tambuwal FM, Abubakar MB, Yakubu Y, Athliamai AB (2017). Occurrence of methicillin resistant *Staphylococcus aureus* in chickens and farm personnel in Sokoto, North-western Nigeria. J. Adv. Vet. Anim. Res. 4(3):255-260. https://doi.org/10.5455/javar.2017.d220
- Kwon NH, Park KT, Jung WK, Youn HY, Lee Y, Kim SH, Kim JM (2006). Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and hospitalized dogs in Korea and their epidemiological relatedness. Vet. Microbiol. 117(2): 304-312. https://doi.org/10.1016/j.vetmic.2006.05.006
- Lamy B, Laurent F, Gallon O, Doucet-Populaire F, Etienne J, Decousser J-W (2012). Antibacterial resistance, genes encoding toxins and genetic background among *Staphylococcus aureus* isolated from community-acquired skin and soft tissue infections in France: a national prospective survey. European J of Clin. Microbiol. Infect. Dis. 31(6) :1279-1284. https://doi.org/10.1007/s10096-011-1441-5
- Larsen J, Imanishi M, Hinjoy S, Tharavichitkul P, Duangsong K, Davis MF, Skov RL (2012). Methicillin-resistant *Staphylococcus aureus* ST9 in pigs in Thailand. PloS one. 7(2): e31245. https://doi.org/10.1371/journal.pone.0031245
- Lee JH (2003). Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl. Environ. Microbiol. 69(11): 6489-6494. https://doi.org/10.1128/AEM.69.11.6489-6494.2003
- Lee JC (1995). Electrotransformation of staphylococci Electroporation protocols for microorganisms. Springer. : 209-216. https://doi.org/10.1385/0-89603-310-4:209
- Lefebvre SL, Waltner-Toews D, Peregrine AS, Reid-Smith R, Hodge L, Arroyo LG, Weese JS (2006). Prevalence of zoonotic agents in dogs visiting hospitalized people in Ontario: implications for infection control. J. Hosp. Infect . 62. 458–466.
- Leibler JH, León C, Cardoso LJ, Morris JC, Miller NS, Nguyen DD, Gaeta JM (2017). Prevalence and risk factors for MRSA nasal colonization among persons experiencing homelessness in Boston, MA. J. Med. Microbiol. 66(8): 1183-1188. https://doi.org/10.1099/jmm.0.000552
- Leonard FC, Markey BK (2008). Meticillin-resistant *Staphylococcus aureus* in animals: A review. Vet. J. 175(1) : 27-36. https://doi.org/10.1016/j.tvjl.2006.11.008
- Lin Y, Barker E, Kislow J, Kaldhone P, Stemper ME, Pantrangi

M, Foley SL (2011). Evidence of multiple virulence subtypes in nosocomial and community-associated MRSA genotypes in companion animals from the upper midwestern and northeastern United States. Clin. Med. Res. 9(1): 7-16. https://doi.org/10.3121/cmr.2010.944

- Lindqvist M (2014). Epidemiological and molecular biological studies of multi-resistant methicillin-susceptible *Staphylococcus aureus*. https://doi.org/10.3384/diss.diva-103679
- Lindsay JA (2014). Evolution of *Staphylococcus aureus* and MRSA during outbreaks. Infect. Genet. Evolut. 21: 548-553. https://doi.org/10.1016/j.meegid.2013.04.017
- Liu GY (2009). Molecular pathogenesis of *Staphylococcus aureus* infection. Pediat. Res. 65: 71-77. https://doi.org/10.1203/PDR.0b013e31819dc44d
- Loeffler A, Kearns AM, Ellington MJ, Smith LJ, Unt VE, Lindsay JA, Lloyd DH (2009). First isolation of MRSA ST398 from UK animals: a new challenge for infection control teams?. J. Hosp. Infect. 72(3): 269-271. https://doi.org/10.1016/j.jhin.2009.04.002
- Luzzago C, Locatelli C, Franco A, Scaccabarozzi L, Gualdi V, Viganò R, Battisti A (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. Vet. Microbiol. 170(1–2): 157-161. doi: http://dx.doi.org/10.1016/j.vetmic.2014.01.016
- Malik S, Coombs G W, O'brien FG, Peng H, Barton MD (2006). Molecular typing of methicillin-resistant staphylococci isolated from cats and dogs. J. Antimicrob. Chemother. 58(2): 428-431. https://doi.org/10.1093/jac/dkl253
- Mainous AG, Hueston WJ, Everett CJ, Diaz VA (2006). Nasal carriage of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus in the United States, 2001–2002.. Annals Fam. Med. 4(2): 132-137.
- Maree CL, Daum RS, Boyle-Vavra S, Matayoshi K, Miller LG (2007). Community-associated methicillin-resistant *Staphylococcus aureus* isolates and healthcare-associated infections. Emerg. Infect. Dis. 13(2): 236-242 https://doi.org/10.3201/eid1302.060781
- Molla B, Byrne M, Abley M, Mathews J, Jackson CR, Fedorka-Cray P, Sreevatsan S, Wang P, Gebreyes WA (2012). Epidemiology and genotypic characteristics of methicillin-resistant Staphylococcus aureus strains of porcine origin. J. Clin. Microbiol. 50: 3687e3693
- Morgan M (2008). Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? J. Antimicrob. Chemother. 62(6): 1181-1187. https://doi.org/10.1093/jac/dkn405
- Noto MJ, Kreiswirth BN, Monk AB, Archer GL (2008). Gene acquisition at the insertion site for SCC*mec*, the genomic island conferring methicillin resistance in *Staphylococcus aureus*. J. Bacteriol. 190(4): 1276-1283. https://doi.org/10.1128/JB.01128-07
- O'Mahony R, Abbott Y, Leonard FC, Markey BK, Quinn PJ, Pollock PJ, Rossney AS (2005). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. Vet. Microbiol. 109(3): 285-296. https://doi.org/10.1016/j.vetmic.2005.06.003
- Obaidat MM, Salman AEB, Roess AA (2017). High prevalence and antimicrobial resistance of *mec*A S*taphylococcus aureus* in dairy cattle, sheep, and goat bulk tank milk in Jordan. Trop. Anim. Hlth. Prod. 1-8. https://doi.org/10.1007/s11250-017-1449-7
- Oliveira DC, Tomasz A, de Lencastre H (2002). Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant *Staphylococcus aureus*. Lancet Infect. Dis. 2(3): 180-189. https://doi.org/10.1016/S1473-3099(02)00227-X
- Pantosti A, Sanchini A, Monaco M (2007) Mechanisms of antibiotic resistance in *Staphylococcus aureus*. Futur. Microbiol. 2(3):323-34. https://doi.org/10.2217/17460913.2.3.323
- Peacock SJ, Moore CE, Justice A, Kantzanou M, Story L, Mackie K, Day NPJ. (2002). Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus*. Infect. Immun. 70(9): 4987-4996. https://doi.org/10.1128/IAI.70.9.4987-4996.2002
- Petersen A, Stegger M, Heltberg O, Christensen J, Zeuthen A, Knudsen LK, Larsen J (2013). Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel *mec*C gene in Denmark corroborates a zoonotic reservoir with transmission to humans. Clin. Microbiol. Infect. 19(1): E16-E22. https://doi.org/10.1111/1469-0691.12036
- Peton V, Le Loir Y (2014). *Staphylococcus aureus* in veterinary medicine. Infect. Genet. Evolut. 21: 602-615. https://doi.org/10.1016/j.meegid.2013.08.011
- Plata K, Rosato AE, Wegrzyn G (2009). *Staphylococcus aureus* as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. Acta Biochim. Polonica. 56(4): 597.
- Price JR, Didelot X, Crook DW, Llewelyn MJ, Paul J (2013). Whole genome sequencing in the prevention and control of *Staphylococcus aureus* infection. J. Hosp. Infect. 83(1): 14-21. doi: http://dx.doi.org/10.1016/j.jhin.2012.10.003.
- Raffa RB, Iannuzzo JR, Levine DR, Saeid KK, Schwartz RC, Sucic NT,Young JM (2005). Bacterial communication ("quorum sensing") via ligands and receptors: a novel pharmacologic target for the design of antibiotic drugs. J. Pharmacol. Exper. Therap. 312(2): 417-423. https://doi.org/10.1124/jpet.104.075150
- Rahimzadeh G, Gill P, Rezai MS (2017). Characterization of methicillin-resistant *Staphylococcus aureus* (MRSA) phages from sewage at a tertiary pediatric hospital. Archives Ped. Infect. Dis. 5(1).http://dx.doi.org/10.5812/pedinfect.39615.
- Rapacka-Zdonczyk A, Larsen AR, Empel J, Patel A, Grinholc M (2014). Association between susceptibility to photodynamic oxidation and the genetic background of *Staphylococcus aureus*. Euro. J. Clin. Microbiol. Infect. Dis. 33(4): 577-586 https://doi.org/10.1007/s10096-013-1987-5.
- Rasigade J-P, Vandenesch F (2014). *Staphylococcus aureus*: A pathogen with still unresolved issues. Infect. Genet. Evolut. 21(0): 510-514. doi: http://dx.doi.org/10.1016/j.meegid.2013.08.018
- Sakwinska O, Kuhn G, Balmelli C, Francioli P, Giddey M, Perreten V, Riesen A, Zysset F, Blanc DS, Moreillon P (2009). Genetic diversity and ecological success of Staphylococcus aureus strains colonizing humans. Appl. Environ. Microbiol. 75(1):175-183.
- 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. https://doi.org/10.3923/javaa.2010.1094.1098
- Salgado CD, Farr BM, Calfee DP (2003). Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of

prevalence and risk factors. Clin. Infect. Dis. 36(2): 131-139 https://doi.org/10.1086/345436.
- San Sit P, Teh CSJ, Idris N, Sam IC, Omar SFS, Sulaiman H, Ponnampalavanar S (2017). Prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) infection and the molecular characteristics of MRSA bacteraemia over a two-year period in a tertiary teaching hospital in Malaysia. BMC Infect. Dis. 17(1): 274.https://doi.org/10.1186/s12879-017-2384-y
- Shaw L, Golonka E, Potempa J, Foster SJ (2004). The role and regulation of the extracellular proteases of *Staphylococcus aureus*. Microbiol. 150(1): 217-228. https://doi.org/10.1099/mic.0.26634-0
- Silver LL (2011). Challenges of antibacterial discovery. Clin. Microbiol. Rev. 24(1): 71-109.
- Springer B, Orendi U, Much P, Höger G, Ruppitsch W, Krziwanek K, Mittermayer H (2009). Methicillin-resistant *Staphylococcus aureus:* a new zoonotic agent? Wiener klinische Wochenschrift. 121(3-4): 86-90. https://doi.org/10.1007/s00508-008-1126-y
- Stanczak-Mrozek KI, Laing KG,Lindsay JA (2017). Resistance gene transfer: induction of transducing phage by sub-inhibitory concentrations of antimicrobials is not correlated to induction of lytic phage. J. Antimicrob. Chemother. 72(6): 1624-1631. https://doi.org/10.1093/jac/dkx056
- Stewart PS, William CJ (2001). Antibiotic resistance of bacteria in biofilms. Lancet. 358(9276): 135-138. doi: http://dx.doi.org/10.1016/S0140-6736(01)05321-1
- Smith TC, Male MJ, Harper AL, Kroeger JS, Tinkler GP, Moritz ED, Capuano AW, Herwaldt LA, Diekema DJ (2009). Methicillin-resistant Staphylococcus aureus (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS One 4: e4258. http://journals.plos.org/plosone/article?id¼10.1371/journal.pone.0004258.
- Tang Y, Larsen J, Kjeldgaard J, Andersen PS, Skov R, Ingmer H (2017). Methicillin-resistant and-susceptible Staphylococcus aureus from retail meat in Denmark. Intl. J. Food Microbiol. 249: 72-76. https://doi.org/10.1016/j.ijfoodmicro.2017.03.001
- Toh S-M, Xiong L, Arias CA, Villegas MV, Lolans K, Quinn J, Mankin AS (2007). Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant *Staphylococcus aureus* resistant to the synthetic antibiotic linezolid. Molecul. Microbiol. 64(6): 1506-1514. https://doi.org/10.1111/j.1365-2958.2007.05744.x
- Tseng CW, Stewart GC (2005). Rot repression of enterotoxin B expression in Staphylococcus aureus. J. Bacteriol. 187(15). 5301-5309.
- Van Cleef BAGL, Monnet DL, Voss A, Krziwanek K, Allerberger F, Struelens M, Cuny C (2011). Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. Emerg. Infect. Dis. 17(3): 502-505. https://doi.org/10.3201/eid1703.101036
- Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M (2005). Methicillin-resistant Staphylococcus aureus in pig farming. Emerg. Infect. Dis. 11(12): 1965.
- Wedley AL, Dawson S, Maddox TW, Coyne KP, Pinchbeck GL, Clegg P, Williams NJ (2014). Carriage of *Staphylococcus* species in the veterinary visiting dog population in mainland UK: molecular characterisation of resistance and virulence. Vet. Microbiol. 170(1): 81-88. https://doi.org/10.1016/j.vetmic.2014.01.015
- Witte W, Strommenger B, Stanek C, Cuny C (2007). Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. Emerg. Infect. Dis.13 (2): 255-258 https://doi.org/10.3201/eid1302.060924
- World Health Organization (2012). Global action plan to control the spread and impact of antimicrobial resistance in Neisseria gonorrhoeae. http://apps.who.int/iris/bitstream/10665/44863/1/9789241503501_eng.pdf (Accessed 18th January, 2018)
- Yarwood JM, Bartels DJ, Volper EM, Greenberg EP (2004). Quorum sensing in *Staphylococcus aureus* biofilms. J. Bacteriol. 186(6): 1838-1850. https://doi.org/10.1128/JB.186.6.1838-1850.2004
- Zunita Z, Bashir A, Hafizal A (2008). Occurrence of Multidrug Resistant *Staphylococcus aureus* in horses in Malaysia. Vet. World. 1(6): 165-167.
- Zunita Z, Bejo SK, Bitrus AA, Othman S, Nadzir NAA (2017). Antimicrobial resistance and the emergence of *Staphylococcus aureus* sequence type ST 177 isolated from horse wounds. International Conference on Antimicrobial resistance (IC²AR, 2017 Book of Proceedings) page 148-149.http://www.ic2ar2017.com/index.php/scientific-program/oral-contributions/ (Accessed 18th December, 2017)