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Horizontal Gene Transfer: A Vehicle for the Dissemination of Resistance and Virulence Determinants during Colonization and Disease.

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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. In addition, 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. In addition, 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 affirms 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.

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. Horizontal gene transfer (HGT) provides the basis for the expansion of previously unsuccessful clones (Kelly et al., 2009). Furthermore, interspecies transfer of SCCmec 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 SCCmec carrying methicillin resistance determinants *mec*A ushers 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. Furthermore, 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 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. In addition, 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 to that of clonal complex CC398 found in Pigs. In addition, nasal colonization was also 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.). Higher carriage rates of about 25% to 50% were observed 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). Kluytmans et al. (1997) revealed 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. The author 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.

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). 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. In addition, In the United states, studies have shown that these strains were replacing the hospital acquired MRSA (Chua et al., 2014) . Community- acquired 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). This review is designed to show the role of virulence determinants in MRSA during colonization and disease.

Horizontal Gene Transfer in Methicillin Resistant *Staphylococcus aureus* (MRSA)

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). 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 SCCmec carrying methicillin resistance determinants *mec*A has greatly increase research interest in the study of MSSA (Lindqvist, 2014). 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 (Lindsay et al., 2014; Corvaglia et al 2010; Lee, 1995). 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 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). 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

Horizontal Gene Transfer and Emergence of a new MRSA clone

In the past decades, major changes were observed in the epidemiological characteristic, resistance development, host and environmental interaction of MRSA. Until recently, infection with MRSA was restricted to hospital setting however, the emergence of a new strain of MRSA with a unique epidemiology, microbial and clinical identity to the hospital associated MRSA 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). 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. In addition, isolation of MRSA have been a 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 & Markey, 2008; Saleha and Zunita (2010). Luzzago et al. (2014) reported that t1328 and ST22 isolate obtained from the liver of the chamois kid was a 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 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 SCCmec designed as SCCmecC.

Pathogenesis of Methicillin Resistant *Staphylococcus aureus* (MRSA)

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 immune response (Gordon & 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. However, recently increase in intensity of such research has been witnessed. This is because due to the emergence of a highly pathogenic community associated MRSA strain with an exceptionally rapid resistance spread and virulence ability. 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 association 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. In addition, it was also observed that 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). Virulent species of MRSA causes severe disease, which can be fatal (DeLeo FR., et al., 2010) with a high morbidity and mortality.

The Role of Virulence Factors in the Pathogenesis of 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. In addition, it is considered as a potential zoonosis (Saleha and Zunita, 2010). The diversity of pathogenic methicillin resistant *S. aureus* is due to its' ability to rapidly acquire resistance and virulence determinants. This characteristic is what gives MRSA the potentials of causing a wide range of infections in the host in different habitats worldwide. 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). In addition, 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 the virulence determinants working synergistically (Peacock et al., 2002).

Colonization is the basis for initiation of disease process, MRSA are frequent colonizers of the nostrils (Gordon and Lowy, 2008). Nasal carriage provides the basis for dissemination 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 & 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, as 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). In addition, MRSA can evade killing by neutrophils 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 Role of Accessory Gene Regulator in the Pathogenesis of MRSA

The accessory gene regulator forms the basis for the expression of genes in MRSA (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 of 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 downregulate the expression of surface binding protein (Liu, 2009; Cheung et al., 2011) specific to certain set of virulence determinants. In addition, the use of animal models have enhanced our 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. Similarly, 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* was observed (Balaban et al., 1998). Furthermore, 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 has been shown 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 shown 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 proof 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* has been shown 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 are 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 SCCmec type have been shown to have decreased expression of cytolytic toxins as compared to MRSA with type IV SCCmec (Stewart et al., 2001). Hence the indication that specific SCCmec 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 (Stewart et al., 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.

Methicillin Resistant *Staphylococcus aureus* (MRSA) colonization in Animals

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) . 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; Peton and Le Loir, 2014; Zunita et al., 2008). 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 rejection (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)

Impact of MRSA infection

Methicillin resistant *S. aureus* is a major cause of severe life-threatening infection in humans as well as in animals (Bosch et al., 2015). 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. Currently, MRSA is the leading cause of death among infants and adult individuals in the United States (Chua et al., 2014). 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). 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

In conclusion, horizontal gene transfer of resistance and virulence determinants forms the basis for the successful expansion of several clones of MRSA. In addition, 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. In addition, expression of these virulence determinants are under the control of a quorum sensing system called the accessory gene regulator (agr).

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References

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.

Balaban, N., L. V. Collins., J. S. Cullor., E. B. Hume., E. Medina-Acosta., O. Vieira da Motta., R. O'Callaghan., P. V. Rossitto., M. E. Shirtliff., L. Serafim da Silveira., A. Tarkowski., and J. V. Torres. (2000). Prevention of diseases caused by *Staphylococcus aureus* using the peptide RIP. Peptides 21:1301– 1311.

Balaban, N., A. Giacometti., O. Cirioni., Y. Gov., R. Ghiselli., F. Mocchegiani., C. Viticchi., M. S. Del Prete., V. Saba., G. Scalise., and G. Dell'Acqua. (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.

Balaban, N., T. Goldkorn., Y. Gov., M. Hirshberg., N. Koyfman., H. R. Matthews., R. T. Nhan., B. Singh, and O. Uziel. (2001). Regulation of *Staphylococcus aureus* pathogenesis via target of RNAIII-activating protein (TRAP). *J. Biol. Chem*. 276:2658–2667.

Balaban, N., T. Goldkorn., R. T. Nhan., L. B. Dang., S. Scott., R. M. Ridgley., A. Rasooly., S. C. Wright., J. W. Larrick., R. Rasooly., and J. R. Carlson. (1998). Autoinducer of virulence as a target for vaccine and therapy against *Staphylococcus aureus*. *Science* 280:438–440.

Balaban, N., Y. Gov., A. Bitler., and J. R. Boelaert. (2003). Prevention of *Staphylococcus aureus* biofilm on dialysis catheters and adherence to human cells. *Kidney Int*. 63:340–345.

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

Booth, M. C., Cheung, A. L., Hatter, K. L., Jett, B. D., Callegan, M. C., & Gilmore, Michael S. (1997). Staphylococcal accessory regulator (sar) in conjunction with agr contributes to *Staphylococcus aureus* virulence in endophthalmitis. *Infection and immunity*, *65*(4), 1550-1556.

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.

Chambers, H. F. (2001). The changing epidemiology of *Staphylococcus aureus*? *Emerging infectious diseases*, *7*(2), 178.

Cheung, G.Y.C., Wang, R., Khan, B. A., Sturdevant, D.E., & Otto, Michael. (2011). Role of the accessory gene regulator agr in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. *Infection and immunity*, *79*(5), 1927-1935.

Chiu, Y-K., Lo, W-T., & Wang, Chih-Chien. (2012). Risk factors and molecular analysis of Panton-Valentine leukocidin-positive methicillin-susceptible *Staphylococcus aureus* colonization and infection in children. *Journal of Microbiology, Immunology and Infection*, *45*(3), 208-213. doi: http://dx.doi.org/10.1016/j.jmii.2011.11.011

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

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.

Cuny, C., & Witte, W., MRSA in equine hospitals and its significance for infections in humans. *Veterinary Microbiology*. doi: http://dx.doi.org/10.1016/j.vetmic.2016.01.013

de Boer, E., Zwartkruis-Nahuis, J. T. M., Wit, B., Huijsdens, X. W., de Neeling, A. J., Bosch, T., . . . Heuvelink, A. E. (2009). Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *International Journal of Food Microbiology*, *134*(1–2), 52-56. doi: http://dx.doi.org/10.1016/j.ijfoodmicro.2008.12.007

Fux, C. A., Costerton, J. W., Stewart, P. S., & Stoodley, P. (2005). Survival strategies of infectious biofilms. *Trends in Microbiology*, *13*(1), 34-40. doi: http://dx.doi.org/10.1016/j.tim.2004.11.010

García-Álvarez, L., Holden, M. T. G., Lindsay, H., Webb, C. R., Brown, D. F. J., Curran, M. D., . . . Holmes, M. A. (2011). Meticillin-resistant *Staphylococcus aureus* with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study. *The Lancet Infectious Diseases*, *11*(8), 595-603. doi: http://dx.doi.org/10.1016/S1473-3099(11)70126-8

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. *International journal of antimicrobial agents*, *23*(3), 268-272.

Gordon, R. J, & Lowy, F. D. (2008). Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. *Clinical Infectious Diseases*, *46*(Supplement 5), S350-S359.

Kelly, B. G., Vespermann, A., & Bolton, D. J. (2009). Horizontal gene transfer of virulence determinants in selected bacterial foodborne pathogens. *Food and Chemical Toxicology*, *47*(5), 969-977. doi: http://dx.doi.org/10.1016/j.fct.2008.02.007

Kennedy, A. D, & DeLeo, Frank R. (2009). Epidemiology and virulence of community-associated MRSA. *Clinical Microbiology Newsletter*, *31*(20), 153-160.

Kluytmans, J., Van Belkum, A., & Verbrugh, H. (1997). Nasal carriage of *Staphylococcus aureus:* epidemiology, underlying mechanisms, and associated risks. *Clinical microbiology reviews*, *10*(3), 505-520.

Köck, R., Schaumburg, F., Mellmann, A., Köksal, M., Jurke, A., Becker, K., & Friedrich, A. W. (2013). Livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) as causes of human infection and colonization in Germany.

Kwon, N. H., Park, K.T., Jung, W. K., Youn, H. Y., Lee, Y., Kim, S. H., . . . Kim, Jun Man. (2006). Characteristics of methicillin resistant *Staphylococcus aureus* isolated from chicken meat and

hospitalized dogs in Korea and their epidemiological relatedness. *Veterinary microbiology, 117*(2), 304-312.

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 journal of clinical microbiology & infectious diseases, 31*(6), 1279-1284.

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

Lee., J. C. (1995). Electrotransformation of staphylococci *Electroporation protocols for microorganisms* (pp. 209-216): Springer

Lee, J.H. (2003). Methicillin (oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. *Applied and Environmental Microbiology, 69*(11), 6489-6494.

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. (2014). Evolution of *Staphylococcus aureus* and MRSA during outbreaks. *Infection, Genetics and Evolution, 21*, 548-553.

Lindqvist, M. (2014). Epidemiological and molecular biological studies of multi-resistant methicillin-susceptible *Staphylococcus aureus*.

Liu, G. Y. (2009). Molecular pathogenesis of *Staphylococcus aureus* infection. *Pediatric research, 65*, 71R-77R.

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. *Veterinary Microbiology, 170*(1–2), 157-161. doi: http://dx.doi.org/10.1016/j.vetmic.2014.01.016

Maree, C. L., Daum, R. S., Boyle-Vavra, S., Matayoshi, K., & Miller, L.G. (2007). Community-associated methicillin-resistant *Staphylococcus aureus* isolates and healthcare-associated infections. *Emerging infectious diseases, 13*(2), 236.

Morgan, M. (2008). Methicillin-resistant *Staphylococcus aureus* and animals: zoonosis or humanosis? *Journal of Antimicrobial Chemotherapy, 62*(6), 1181-1187.

Noto, M. J., Kreiswirth, B.N, Monk, A. B, & Archer, G. L. (2008). Gene acquisition at the insertion site for SCCmec, the genomic island conferring methicillin resistance in *Staphylococcus aureus*. *Journal of bacteriology, 190*(4), 1276-1283.

O'Mahony, R., Abbott, Y., Leonard, F.C., Markey, B.K., Quinn, P.J., Pollock, P.J., . . . Rossney, A.S. (2005). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. *Veterinary microbiology, 109*(3), 285-296.

Oliveira, D.C., Tomasz, A., & de Lencastre, H. (2002). Secrets of success of a human pathogen: molecular evolution of pandemic clones of meticillin-resistant *Staphylococcus aureus*. *The Lancet infectious diseases, 2*(3), 180-189.

Pantosti, A., Sanchini, A., & Monaco, M. (2007). Mechanisms of antibiotic resistance in *Staphylococcus aureus*.

Peacock, S.J., Moore, C. E, Justice, A., Kantzanou, M., Story, L., Mackie, K., . . . Day, Nicholas PJ. (2002). Virulent combinations of adhesin and toxin genes in natural populations of *Staphylococcus aureus. Infection and immunity, 70*(9), 4987-4996.

Peton, V, & Le Loir, Y. (2014). *Staphylococcus aureus* in veterinary medicine. *Infection, Genetics and Evolution, 21*, 602-615.

Petersen, A., Stegger, M., Heltberg, O., Christensen, J., Zeuthen, A., Knudsen, L. K, . . . Larsen, J. (2013). Epidemiology of methicillin-resistant *Staphylococcus aureus* carrying the novel mecC gene in Denmark corroborates a zoonotic reservoir with transmission to humans. *Clinical Microbiology and Infection, 19*(1), E16-E22.

Plata, K., Rosato, A. E., & Wegrzyn, G. (2009). *Staphylococcus aureus* as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. *Acta Biochimica Polonica, 56*(4), 597.

Price, J. R., Didelot, X., Crook, D. W., Llewelyn, M. J., & Paul, J. (2013). Whole genome sequencing in the prevention and control of *Staphylococcus aureus* infection. *Journal of Hospital Infection, 83*(1), 14-21. doi: http://dx.doi.org/10.1016/j.jhin.2012.10.003

Raffa, R. B., Iannuzzo, J. R., Levine, D. R., Saeid, K. K., Schwartz, R. C., Sucic, N. T, . . . Young, J. M. (2005). Bacterial communication ("quorum sensing") via ligands and receptors: a novel pharmacologic target for the design of antibiotic drugs. *Journal of Pharmacology and Experimental Therapeutics, 312*(2), 417-423.

Rapacka-Zdonczyk, A., Larsen, A. R., Empel, J., Patel, A., & Grinholc, M. (2014). Association between susceptibility to photodynamic oxidation and the genetic background of *Staphylococcus aureus*. *European Journal of Clinical Microbiology & Infectious Diseases, 33*(4), 577-586.

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.

Salgado, C. D., Farr, B. M., & Calfee, D. P. (2003). Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. *Clinical Infectious Diseases, 36*(2), 131-139.

Shaw, L., Golonka, E., Potempa, J., & Foster, S. J. (2004). The role and regulation of the extracellular proteases of *Staphylococcus aureus*. *Microbiology, 150*(1), 217-228.

Stewart, P. S., & William Costerton, J. (2001). Antibiotic resistance of bacteria in biofilms. *The Lancet, 358*(9276), 135-138. doi: http://dx.doi.org/10.1016/S0140-6736(01)05321-1

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.

Toh, S-M., Xiong, L., Arias, C. A., Villegas, M. V., Lolans, K., Quinn, J., & Mankin, A. S. (2007). Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant *Staphylococcus aureus* resistant to the synthetic antibiotic linezolid. *Molecular microbiology, 64*(6), 1506-1514.

van Cleef, B. A. G. L., Monnet, D. L., Voss, A., Krziwanek, K., Allerberger, F., Struelens, M., . . . Cuny, C. (2011). Livestock-associated methicillin-resistant *Staphylococcus aureus* in humans, Europe. *Emerging infectious diseases, 17*(3), 502.

Yarwood, J.M., Bartels, D.J., Volper, E.M, & Greenberg, E.P. (2004). Quorum sensing in *Staphylococcus aureus* biofilms. *Journal of bacteriology, 186*(6), 1838-1850.

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