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Review *Zoonoses and public Health*

Genomic Overview into the Evolving Epidemiology of Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT

Emerging infections represent an enormous challenge to both human and veterinary medicine. Identification of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in various species and in food has raised concerns about the roles of animals in the epidemiology of MRSA. MRSA are a group of gram-positive bacteria, distinct from other strains of *S. aureus* in that this pathogen is resistant to methicillin, oxacillin, and all beta-lactam antibiotics. The severity of infections caused by MRSA depends on the strain responsible for the infection and can vary from soft tissue infections to bacteremia and sometimes pneumonia. MRSA strains are divided into clones, based on their genetic makeup. According to the setting of infection, MRSA are divided into three epidemiological types: hospital acquired (HA-MRSA), community acquired (CA-MRSA), and livestock acquired (LA-MRSA) (ie. Transmitted from animal carriers). The epidemiology of HA-MRSA, CA-MRSA, and LA-MRSA is blurred as different recent genetic studies have revealed significant overlap of identical clones between HA-MRSA and CA-MRSA, and the significant increase of human infection caused by LA-MRSA. Furthermore, the animal-human and animal-animal transmission of LA-MRSA has prompted further investigation to study the origin of this epidemiological type and the transmission dynamics. The genetic and virulence profiles of different types of MRSA vary widely, where community acquired and livestock acquired strains are more virulent than hospital acquired strains. This review sheds light on three epidemiological groups of MRSA (HA-MRSA, CA-MRSA, and LA-MRSA), and their most prevalent clonal clusters, that can consequently allow better understanding of their evolution, emergence, transmission, and global dissemination.

Keywords: Methicillin-Resistant *Staphylococcus aureus,* HA-MRSA, CA-MRSA, LA-MRSA, Epidemiology

INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is one prominent cause of nosocomial infections. Up until the 1960s, MRSA infections were hospital acquired and highly prevalent between individuals who have been admitted to an intensive care unit or nursing homes [1]. In 1980, the first community-acquired MRSA infection (CA-MRSA) was identified in the US [1], with a steadily increasing prevalence of infection in adults and children in the decades following its discovery. The CDC defines CA-MRSA as a MRSA infection in a patient with no history of any of the predisposing factors, who has not been hospitalized or admitted to a nursing home in the previous year [2]. All MRSA types are resistant to oxacillin, methicillin and all beta-lactam antibiotics, with some strains being multi-resistant to other classes of antibiotics as well [2-4]. Resistance to all β-lactam antibiotics is mediated by the *mecA* gene that is located on a genetic island known as the Staphylococcal Cassette Chromosome (SCC) [3,5]. The *mecA* gene codes for an alternate penicillin binding protein (PBP) known as PBP2a. This modified transpeptidase has low affinity to the beta-lactam ring found in all antibiotics of the β-lactam class and binding of this protein to the ring makes the bacteria resistant [2,5,6]. Predisposing risk factors associated with MRSA infections include recent hospitalization or surgery, intravenous drug use, presence of any indwelling medical device that passes through the skin to the body, dialysis, close contact with a MRSA infected patient, low socioeconomic status, crowdedness, malignancy or misuse of antibiotics [1-6].

Annually, around 1.2 million people acquire invasive MRSA infections during hospitalization, resulting in MRSA to account for 20% of all nosocomial infections [7]. According to the CDC report in 2017, around 120,000 *S. aureus* infections and 20,000 associated deaths were recorded in the US alone [8]. Additionally, the WHO priority pathogen list for 2017 reported

MRSA as a high priority pathogen (level 2) [9], despite the fact that the numbers of MRSA infections have been declining over the past few years [7]. In addition to the drastic consequences of a MRSA infection on individuals, healthcare facilities are often financially challenged by the rather high prevalence rates of MRSA. A US study across 55 hospitals estimated the cost per MRSA infection to be $12,197 while other studies in France indicate a mean cost per infection of $39,500 [10]. Even among lower-middle-income developing countries, the total health expenditure in Egypt is rather insufficient. Only 4.75% of GDP is spent on providing inadequate healthcare to citizens [11], which highlights the urgency of enhancing our knowledge of the organism and designing plans to combat its spread.

In Egypt, reports have described a prevalence rate of 50-82% among hospitalized patients in Cairo and Alexandria but a lower rate of 24% in Minia [12]. Other reports of community acquired MRSA (CA-MRSA) indicated a prevalence rate of 19-47% [1]. It has also been reported that *S. aureus* isolates are primarily responsible for wound and surgical site infections at Minia University Hospital [13]. However, the lack of a national surveillance system makes it challenging to extrapolate the data from the separate cities to give nation-wide data.

The aim of this review is to provide a conclusive overview of different epidemiological groups of MRSA (HA-MRSA, CA-MRSA and LA-MRSA), highlighting the possible threat of these groups on the Egyptian population. This is in accordance with the national multi-sectorial plan of the World Antibiotic Awareness week forum [14] held in Cairo University in November 2017, which entails; 1) increasing awareness of antimicrobial resistance through trainings, education and communication 2) improving incidence of infection via better prevention and control methods 3) monitoring and optimizing the use of antibiotics in animal and human health, and 4) encouraging and supporting research and innovation to strengthen knowledge nation-wide.

Application of Multi-locus sequence typing to study the clonal population structure of MRSA

Multilocus sequence typing (MLST) is a typing technique that is used to identify and characterize bacteria unambiguously, usually based on fragments of 7 house-keeping genes [15-18]. A DNA sequencer is used to sequence around 450-500 base pairs of the internal fragments of each of these genes [15]. Different sequences (polymorphisms) found in each gene within a species are identified as different alleles and those different alleles are then defined as a sequence type (ST) [15-17]. Therefore, 7 integers correspond to the alleles found at the 7 loci of the house-keeping genes that were sequenced [16,17]. Different sequences in the same isolate are given different allele numbers regardless of how different they are (ie. whether they differ in one or more nucleotide) [17-19]. This process usually gives billions of different allele profiles because most bacterial species exhibit enough variation within those genes, providing several alleles per locus. The allelic profile is defined by the alleles found at each locus. Given that many potential alleles can be found at each locus, it is highly unlikely that identical allelic profiles exist by chance [18]. Isolates with the same allelic profiles are classified as members of the same clonal complex (CC). Strains that share almost identical allelic profiles are referred to as sequence types while those that share only some of the alleles are related and therefore referred to as clonal complexes [15,19]. MLST is usually used to investigate the serotypes, geographical origins and relatedness of different strains without establishing phylogenetic relationships (ie. they only show descent and emergence but complementary sequence information is needed to establish phylogenetic relationships) [15,19]. All MLST data that were collected have been grouped into one online library, publicly available at http://www.pubmlst.org [17].

MLST has been previously used in different epidemiological studies as an efficient tool of monitoring the evolution and changes in the population structure of microbes, especially pathogens, in epidemiological studies [19]. MLST has several advantages including high reproducibility, portability, easy interpretation of data and that access to live bacterial isolates is not required [17,19].

Genetic insights into HA-MRSA and CA- MRSA

Differences in the SCCmec type are used to distinguish between HA-MRSA and CA-MRSA. HA-MRSA strains usually have SCCmec types I, II and III while CA-MRSA have the smaller types IV and V [3,6]. A significant percentage of CA-MRSA carry genes for the exotoxin Panton-Valentine leukocidin (PVL) which is lethal to leukocytes [3,20-25]. Genetic and molecular studies support the hypothesis that PVL genes, along with other exotoxins, are responsible for the enhanced virulence of CA-MRSA over HA-MRSA, leading the former to cause necrotic skin lesions and necrotizing pneumonia more frequently [3,4]. Multiple insertion sequences were identified in the mec region of CA-MRSA and are speculated to contribute to the resistance of multi-drug resistant (MDR) strains against quinolones, clindamycin, erythromycin, trimethoprim, and gentamicin [5,26,27]. Severe invasive diseases are more associated with CA-MRSA than HA-MRSA, as CA-MRSA was found to infect healthy young individuals, possibly with no identified risk factors or comorbidities [3,28]. CA-MRSA is predominantly isolated from skin and soft tissue infections (SSTIs), which are usually mild but can occasionally result in hospitalization and mortality [1,3,28]. Despite the above differences and the distinct phenotypical features of both strains, HA-MRSA and CA-MRSA are becoming increasingly difficult to distinguish from one another. As reported in [28], clonal complexes CC1, CC5, CC8 (ST8), CC22, CC30 (ST30), and CC59 (ST59) have been identified in isolates from community and healthcare settings [23,24].

Clonal relation between CA-MRSA and LA- MRSA

MRSA isolates of animal origin are referred to as livestock acquired (LA). Clonal complexes CC8, CC15, CC22, CC30, CC45, CC80, and CC152 are mainly associated with human isolates [29-33,34]. Of CCs isolated from domestic animals, CC130 and CC398 have low host specificity and are therefore frequently isolated from human patients [22-24,29,33,35]. Furthermore, CC97, CC133, CC522 and the lineage ST151 are mainly found in ruminant isolates while the lineage ST385 is present in poultry isolates [23,29,34,36]. Other clonal lineages such as CC1, CC5, CC59, CC9, ST425 were identified, some of which were isolated from companion animals such as dogs, cats, and horses [22-25,29,35,36].

Comparative analyses of CC398 revealed that even though it currently represents poultry and pig isolates, it originally descended from an ancestral population in humans while ST91 originated from ruminants [33,34,37]. The two most widely disseminated lineages in livestock are CC398 and CC9 [5,6,19,23,24,29]. CC398 currently includes 43 different sequence types such as ST541, ST1965, ST1966, ST1967, and ST1968, of which ST398 is the main colonizer in pigs [22,23,33-36,38].

Marked genetic similarities were found between CA-MRSA and LA- MRSA as both clades share the lineages ST239 and ST398, both contain SCCmec IV and V, while exhibiting little differences in their staphylococcal protein a (*spa*) types [22-24,35,37,39]. This suggests that dissemination occurs in a bidirectional manner; from animals to humans and vice versa, with specifically high prevalence of LA-MRSA between veterinarians, farmers and those in close contact with animals [23,25,39].

Growing evidence currently suggests an overlap between CA- and LA- MRSA exists [22]. It was found in a recent study that MRSA ST398 isolated from slaughter pigs, bovine mastitis and farm personnel shared the same genetic virulence gene profiles [20, 22-24, 30, 36,40]. Some CC398 isolates of human origin are more virulent than others due to the production of an alpha-toxin and the ability to lyse human polymorphonuclear leukocytes [6,40]. In addition, in Japanese pigs, CA-MRSA strain ST9 was identified [41].

SCCmec usually contain other resistance genes and genes responsible for combating harmful chemicals such as heavy metals [6]. MRSA strains can acquire this genetic island by means of horizontal gene transfer [6] [20,21,26]. A primitive species of staphylococci, *S. scuiri*, usually found on the skin of domestic animals has been suggested to be the species of origin for the evolution of SCCmec [6,40]. This is supported by findings that the PBP2a of MRSA is very similar to the native PBPs of *S. scuiri* [6]. Some studies suggest that *mecA* was already present in primitive *S. aureus* gene pools even before the introduction of methicillin, given the rapid emergence and prevalence of MRSA and the observation that key events in the evolution of the *mecA* gene were found to be directly linked to time points when antibiotics were introduced in veterinary and human clinical settings [6,42]. The *mecA1* is a ubiquitous homologue to *mecA* and was identified in primitive *S. scuiri,* suggesting that the former is the precursor of the *mecA* gene [6,42]. This is supported by the finding that certain strains resistant to β-lactams possess this quality due to altered promoter region of that gene [42,43]. Further evidence that supports the hypothesis is that *mecA1* gene was able to exhibit β-lactam resistance when experimentally induced into an *S. aureus* strain by producing a protein with similar properties to MRSA PBP2a [42].

One interesting finding is that antibiotic resistance levels of clinically isolated MRSA are widely variable [6] [20,26], as some MRSA strains were only very slightly resistant to methicillin while other strains were highly resistant. Evidence suggests that elevated resistance levels may be attributed to elevated levels of expression of PBP2a, by up-regulation of the *mecA* gene [6]. Other studies propose that auxiliary factors, such as cell division proteins, known as the "femfactors" also determine resistance and virulence [4] [44]. Nonetheless, the exact mechanism by which resistance levels vary so widely is poorly understood. Further investigations might open new windows for treatment of MRSA [4]. In addition, whole genome sequencing (WGS) of the 2 predominant lineages; CA-MRSA ST93-IV and LA-MRSA ST398-V, suggests that anthropozoonotic and zoonotic transmissions occurred and that the movement of ST93-IV from humans to pigs then back to humans, in presence of the MDR ST398-V strain, has led to the acquisition of multiple antibiotic resistance determinants to tetracycline, clindamycin and chloramphenicol [23,25,32,45]. Such determinants were originally absent from ST93-IV [45]. Another study suggests that a host jump of CC398 occurred from humans to animals, followed by acquisition of the SCCmec [38].

A study conducted by Huijsdens et al in 2006 found a possible correlation between pig-farming and contracting MRSA in the Netherlands [6]. Using pulsed field gel electrophoresis (PFGE) with the restriction enzyme *SmaI* and *S. aureus spa* typing for identification, it was found that pig-MRSA isolates were also found in pig-farmers and their families with no known history of exposure to predisposing risk factors [4,38]. This strain of LA-MRSA was not found in other non-pig-farming individuals of the same community [4]. What is even more alarming is the finding that those identified PFGE non-typeable (ie. Livestock acquired) MRSA strains are not only transmitted from pig to human but from human to human as well [4,38].

Another study that demonstrates the ease of transmission of LA-MRSA was conducted by Voss et al, [45]. This study found a 760-fold higher MRSA carriage rate among pig farmers compared to the general Dutch population. A direct correlation

was found between the proportion of MRSA-positive animals, intensity and duration of human-to-animal contact, and CC398 human colonization [45]. Mutters et al. [41] and Sahibzada et al. [45] found that CC398 can survive in the environment, which contributes towards further dissemination.

The most prominent clonal complexes of LA-MRSA are CC398, CC9, CC97, among other lineages. One of the most prominent virulence factors of LA-MRSA CC398 is its ability to acquire foreign DNA, such as the PVL gene [38,45]. Although contrary to information reported in [39], CC398 that produce enterotoxins, adhesion factors, proteases and superantigen-like proteins have also been identified in pig, poultry, and bovine samples [38,45]. Another piece of evidence that supports that CC398 might have been of human origin is the finding that certain strains carry immune evasion cluster (IEC) [21,22,27,33,38] . This cluster is usually absent in animal isolates [38]. CC9 is the major LA-MRSA strain in Asia and was first identified in Europe in 2008 [38]. However, the main strain in Europe is ST398. CC9 strains are generally MDR and carry genes against lincosamides and PHLOPSA (drug classes phenicol, lincosamides, oxazolidinones, pleuromutilins and streptogramin A) [38]. CC97 is a global leading cause of bovine mastitis that is occasionally found small ruminants, pigs, and humans [22,23,32,36,38]. It is suggested that a host jump occurred from bovine to human subjects around 40 years ago [38]. Several other lineages of LA-MRSA have been identified in Africa such as the human-associated ST5-SCCmec IV and ST88-SCCmec IV in pigs from Senegal, and ST153-SCCmec NT from healthy sheep in Tunisia [38]. Human-associated *S. aureus* lineages have also been identified in chimpanzees, possibly due to humanosis [4,38].

Evolving epidemiology of MRSA

Several factors influence prevalence of MRSA such as geographic area, animal origin and the environment harboring the human-animal interaction. The most prevalent strain in Europe and USA is CC398 while that in Asia is CC9 [38]. Aquatic products, raw beef and poultry, pork and dairy products are all possible food-borne sources of infection [35,46]. CC398 has also been infrequently identified in horses and veal calves [38]. Contracting MRSA from any of the above sources can occur by consumption or poor handling of contaminated sources [29,38]. Egypt is the largest African dairy producer and among the leading cheese producers in the Mediterranean region, with household animals producing around 80% of cow and buffalo milk [47]. Being a developing country, safety practices necessary to limit transmission of MRSA via dairy products are usually overlooked which poses considerable threat to the public health [47].

MRSA clones capable of colonizing several livestock animals at once have also been identified that are capable of infecting humans either via direct contact or consumption of their contaminated products (meat, milk, etc.) by humans [39]. A study conducted in 2019 in China revealed that 7.4% of *S. aureus* isolated from contaminated food were MRSA while in [29], 75% of isolates from broiler-chicken were LA-MRSA. In the same study, the prevalence rates of LA-MRSA in turkey, turkey meat, broilers, and chicken meat were 36%, 13%, 5%, and 5% respectively. In another study, two chicken samples tested positive for MRSA strains USA300 and USA500 [29]. Both strains are CA-MRSA which suggests a host jump occurred from humans to the chickens. A study by Mutters et al. [41] estimates that 85% of all farms had MRSA-positive animals. Another study provided further evidence that suggests that the MRSA strain CC398 can be transmitted between humans by direct contact [40]. In that study, isolated CC398 were divided into two different clades; a pig clade and a human clade. Among the pig clade, it was found that some isolates originated from humans without pig contact, which supports the hypothesis that CC398 could be transferred via human-to-human contact [40]. CC398 causes a wide spectrum of illnesses in humans that ranges from minor to invasive infections that may require hospitalization. The low prevalence of CC398 among humans not in usually in frequent contact with animal reservoirs can be attributed to the low transmissibility of the strain, which lowers the clinical significance of CC398 infections [45]. ST398 is a more threatening strain because despite being less transmissible than other strains, it is co-resistant to non β-lactam antibiotics used in medical and veterinary practice [45]. Poor hygienic practices between individuals working in animal husbandry and food processing, along with improper storage are the primary risk factors in food-borne MRSA transmission [29,38,40]. This poses major health risks because some LA-MRSA produce multi-drug resistant enterotoxins [29,39,40].

Key epidemiological differences between CA-MRSA, HA-MRSA and LA-MRSA

Though epidemiological and molecular typing techniques have discriminated MRSA into HA, CA and LA, overlaps between these three epidemiological groups, due to convergent and divergent evolution, are observed. For example, nosocomial infections by CA-MRSA strains were identified, LA-MRSA strains were isolated from hospital environments and LA- and HA-strains were isolated in community acquired infection setting [39]. In other words, isolates from different sources with the same ST were identified [20,21,26,27,39]. Recently, evidence is accumulating that CA-MRSA strains can shuttle between hospitals and the community, suggesting that such strains might replace the traditional, often less virulent, HA-MRSA in healthcare settings [23,24,32,39]. Another interesting finding is that HA-MRSA strains investigated in this study were more resistant but less virulent, contrary to CA- and LA- strains which are usually more virulent but more susceptible to antibiotics [39]. A recent study highlighted the association between human infections and MRSA ST398 in the general population

[39]. Evidence also suggests that the genetic diversity of MRSA is correlated with the rapid spread of CA-MRSA, which acts as a genetic carrier between HA-MRSA and LA- MRSA [39].

Application of genomics in understanding the evolving epidemiology of MRSA

Molecular typing is of utmost significance in tracking emergence, spread and evolution of pathogens, assessing efficacy of outbreak control measures and identifying possible origins of outbreak [48]. Though conventional targeted molecular typing methods, such as pulsed-field gel electrophoresis (PFGE) and MLST can provide useful insight into the epidemiology of a certain pathogen, Whole Genome Sequencing (WGS) significantly enhances resolution [48,49]. This is because the latter negates the assumption that isolates with similar genotypes that are linked by epidemiological data represent linked cases of infection [48]. In other words, in MLST, it is usually assumed that isolates with similar genotypes have similar epidemiology which is often misleading, especially with the observed overlap between different clones of the different sources of MRSA. However, it should be noted that whole genome and core genome MLST schemes have enabled comparison of test sequences with large curated sets of predefined genes for a particular species [48]. With WGS readily available on a large scale, a more detailed study of patterns of spread, transmission and evolution of MRSA is now available [48,49]. It is predicted that with the continuously decreasing costs of WGS and wider availability of the technology, WGS will no longer be confined within research and reference laboratory facilities but will be available in routine clinical labs to identify infection, prevention and control tactics in real time [48]. To highlight the significance and accuracy of WGS in MRSA detection, Humphreys and Coleman [48] reported a study where isolates from 17 pediatric MRSA patients in a neonatal unit outbreak were typed using PFGE, *spa* typing and WGS. The study reported that using WGS, 12 of the 17 isolates were genetically unique and therefore could not have contributed to the outbreak [48]. This finding was critical in such case as it suggests that hospital personnel abided by infection prevention methods [48]. The finding that all isolated strains were USA300 suggests that multiple independent introductions of the strain have occurred [48]. WGS has also been used to investigate commonality, colonization and spreading of CA-MRSA strains [48,49]. The authors in [48] stress that WGS has significantly expanded our understanding of the epidemiological features of HA- and CA- MRSA, evolution patterns of resistance and transmission [44,48]. WGS can confirm low, usually undetected, level ongoing clusters that could be cross-transmitted, as is the case with LA-MRSA [48,49]. The available data can provide guidance for designing infection control schemes to prevent future outbreaks.

Conclusion

MRSA infections can often go undiagnosed because the skin lesions caused resemble spider bites. This leads to an underestimation of the number of CA-MRSA cases. The increasing overlap between different strains and origins of MRSA makes it even more challenging to distinguish between the strains and therefore design suitable action plans to combat infection in the variable settings and strongly advice for applying One Health approach to track MRSA, especially LA-MRSA. Although currently uncommon in community and hospital settings, immediate monitoring of the prevalence of the strain ST398 is necessary to avoid outbreaks [39]. Lack of conclusive data in Egypt and the gap created by the individual, unlinked and unrelated studies make it difficult to draw accurate conclusions.

Conflict of Interest

All authors declare no conflict of interest.

Ethical approval: Not required.

Authors' contribution

Y.E. wrote the paper with some additional edits and supervision from S.F.M and M.E.

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