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Title: Genomic insights into the emergence and spread of international clones of healthcare, community and livestock-associated meticillin-resistant Staphylococcus aureus: blurring of the traditional definitions

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Keywords: MRSA clones, MRSA epidemiology, Whole genome sequencing

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Abstract: The evolution of meticillin-resistant S. aureus (MRSA) from meticillin-susceptible S. aureus (MSSA) has been a result of accumulation of genetic elements under selection pressure from antibiotics. The traditional classification of MRSA into healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) is no more relevant as there is significant overlap of identical clones between these groups with an increasing recognition of human infection caused by livestock-associated MRSA (LA-MRSA). Genomic studies have enabled us to model the epidemiology of MRSA along these lines. In this review, we discuss the clinical relevance of genomic studies, particularly whole genome sequencing, in investigations of outbreaks. We also discuss the blurring of each of the three epidemiologic groups (HA-MRSA, CA-MRSA, and LA-MRSA) demonstrating the limited relevance of this classification.

***Highlights**

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1. The epidemiology between community-associated MRSA, healthcare-associated MRSA and livestock- associated MRSA is blurring
2. Genomic studies have helped understand the genetic changes associated with the evolving epidemiology
3. Genomics based diagnostic tools such as whole genome sequencing are useful in providing rapid information in relation to epidemiology and outbreaks

Genomic insights into the emergence and spread of international clones of healthcare, community and livestock-associated meticillin-resistant *Staphylococcus aureus*: blurring of the traditional definitions

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Abstract

The evolution of meticillin-resistant *S. aureus* (MRSA) from meticillin-susceptible *S. aureus* (MSSA) has been a result of accumulation of genetic elements under selection pressure from antibiotics. The traditional classification of MRSA into healthcare-associated MRSA (HA-MRSA) and community-associated MRSA (CA-MRSA) is no more relevant as there is significant overlap of identical clones between these groups with an increasing recognition of human infection caused by livestock-associated MRSA (LA-MRSA). Genomic studies have enabled us to model the epidemiology of MRSA along these lines. In this review, we discuss the clinical relevance of genomic studies, particularly whole genome sequencing, in investigations of outbreaks. Wealso discuss the blurring of each of the three epidemiologic groups (HA-MRSA, CA-MRSA, and LA-MRSA) demonstrating the limited relevance of this classification.

Keywords: MRSA clones, MRSA epidemiology, Whole genome sequencing

Introduction

Staphylococcus aureus is associated with a variety of diseases in humans including superficial infections, deep-seated infections, acute sepsis, respiratory infection and toxin mediated illnesses. The first clone of meticillin-resistant *S. aureus* (MRSA) was identified in 1961. Molecular epidemiological evidence suggests MRSA have evolved on multiple occasions from lineages of meticillin-susceptible *S. aureus* (MSSA) isolates. Although MRSA was initially a healthcare-associated pathogen dominated by distinct lineages and often associated with multi-drug resistance, the emergence of genetically distinct community-associated MRSA (CA-MRSA) infections in the last two decades has led to a significant clinical impact outside the hospital setting. Moreover, CA-MRSA has also become established in healthcare settings and it has been suggested that these clones will replace the healthcare-associated MRSA (HA-MRSA) clones over time. In addition, CA-MRSA is slowly acquiring resistance to other antibiotics and as a result phenotypic distinctions between HA-MRSA and CA-MRSA are blurring (1). Livestock-associated MRSA (LA-MRSA) are genetically distinct from CA-MRSA and HA-MRSA, and have their main reservoir in farm animal(2). Recent reports also suggest a blurring of this epidemiology and possible transmission between of LA-MRSA humans.

Genomic studies, particularly using whole genome sequencing (WGS), enhance our understanding of the key features of each MRSA type and the genetic changes associated with changing epidemiology. They also allow us to map global, national, and regional spread of variants and investigate the selective pressures that shape the population. The introduction of WGS technology into routine diagnostics in healthcare provides promise of rapid access to resistance, virulence, host-adaptation

and outbreak information, improving patient management, infection control and biosecurity.

This review is a summary of the discussions that took place at the 5th International Society of Chemotherapy MRSA consensus conference (Verona in May 2014).

The emergence of MRSA

MRSA emerged from MSSA lineages with acquisition of the staphylococcal cassette chromosome (SCC) element that carries either *mecA* or *mecC* (SCC*mec*). SCC elements are large segments of DNA that carry variable arrays of genes, which can include genes that encode resistance to antibiotics other than meticillin. Diverse SCC*mec* types (SCC*mec* types I to XI) have been described (3). Acquisition of other mobile genetic elements (MGEs) carrying virulence genes and other antibiotic resistance determinants may lead to further adaptation of MRSA lineages (4). MGE encoded virulence factors such as enterotoxins, Panton-Valentine leucocidin (PVL), other bi-component leucocidins, toxic shock syndrome toxin, and staphylokinase, are carried by bacteriophages or *S. aureus* pathogenicity islands (SaPI) (5). A wide range of antimicrobial resistance genes can also be carried on plasmids and transposons (6). These genes can confer resistance to penicillins, macrolides, aminoglycosides, tetracyclines, chloramphenicol, fusidic acid, mupirocin, linezolid, and biocides.

Molecular typing techniques such as multi locus sequence typing (MLST) have defined the *S. aureus* population structure (7). MLST assigns isolates to a sequence type (ST) based on the allelic profile of sequence within seven housekeeping loci.

Isolates that share five out of the seven alleles may be grouped together into clonal complexes (CCs) that describe lineages. MRSA commonly associated with human infections worldwide is due to a limited number of lineages including CC1, CC5, CC8 (and related ST239), CC22, CC30, CC45, CC59 and CC80 (4). Lineages are genetically very distinct from each other with substantial variation in genes encoding for surface proteins and regulators, although they originally shared a common ancestor prior to SCC*mec* acquisition (8).

The epidemiology of MRSA in the four decades following the identification of the first MRSA was principally associated with spread in hospitals. A handful of dominant clones account for the majority of HA-MRSA worldwide *e.g.* ST22 and ST36 in the United Kingdom (UK), ST239 in Asia and Australia, and ST5 in North America and in Japan and Korea. These clones were able to establish themselves by their competitive advantage in the presence of intensive antibiotic use, most notably but not restricted to β-lactams. In the late 1980s, MRSA began to emerge in the community. CA-MRSA has evolved independently of the HA-MRSA clones. In the beginning, this was mostly confined to closed communities, for example, Australian aborigines, but around the late 1990s CA-MRSA emerged worldwide in the general population. CA-MRSA clones typically possess SCC*mec* type IV or V elements and are often positive for the PVL toxin. Like HA-MRSA, the distribution of the predominant clones tends to be geographically distinct: ST80 in Europe and Northern Africa, ST59 in the Far East, ST93 and ST1 in Australia, and ST1 and ST8 in the US. Since 2005, MRSA from animals (LA-MRSA) have increasingly been recognized as a cause of human infections. LA-MRSA is predominantly due to the ST398 clone in Europe, whereas ST9/t899 dominates in Asia. ST398 and ST9 are

tetracycline resistant and are strongly associated with pig farms, although they are also found in a range of other farm animals. Like the HA-MRSA and CA-MRSA, evolution of meticillin resistance appears to have occurred independently; phylogenomic analysis of representatives of the LA-MRSA CC398 population suggests this lineage has descended from a human MSSA ST398 clone, which after a host jump acquired tetracycline and meticillin resistance. In this process the LA-MRSA lineage lost the φSa3 phage carrying the human evasion genes *sak, scn* and *chp* (9). LA-MRSA marks a paradigm shift in the epidemiology as humans hitherto have been the dominant reservoir, tackling of LA-MRSA requires a One Health approach with collaboration of veterinarians, farmers, doctors and environmentalists. The epidemiological definitions for CA-MRSA, HA-MRSA and LA-MRSA (Table 1) may not be fit for purpose in future. For example, Folden and colleagues reported the variation in estimating the proportion of CA-MRSA infections using epidemiological definitions: use of one set of definitions classified 5% of isolates as CA-MRSA while using another set led to as many as 49% of infections to be classified as CA-MRSA (10). In order to avoid such misclassification, the Centers for Disease Control and Prevention (CDC) has provided a definition for CA-MRSA (Table 1) (11). However, at the same time as CA-MRSA becomes common in the community, changes in hospital practice such as reduced lengths of stay, day-only admissions and hospital-in-the-home or community hospitals make application of traditional definitions difficult as facilities traditionally available in hospitals are increasingly delivered in the community in a more integrated manner. Miller and colleagues failed to label any MRSA bacteraemia (n= 57) presenting at the Oxford Radcliffe Hospital between 2003 and 2006 as caused by CA-MRSA (12). Problems are also encountered when using antibiotic susceptibility pattern or traditional genetic

signatures as an indicator of epidemiological origin. In the UK, ciprofloxacin susceptibility is used widely in the clinical laboratories as a marker for CA-MRSA but resistance pattern is variable in America where levels of ciprofloxacin resistance approaches 80% in some high-risk groups (13). Similarly, PVL detection should not be used as a sole marker for CA-MRSA (14). Genetic definitions that use the *SCCmec* type IV element as a marker for CA-MRSA are flawed as they potentially include the EMRSA-15 (ST22) and USA800 (ST5) clones, which are epidemiologically HA-MRSA. As the distinction between HA-MRSA and CA-MRSA becomes increasingly blurred a classification based solely on the SCC*mec* type IV element is no longer helpful. The identification of markers associated with different epidemiological niches becomes valuable when combined with stable genetic markers identifying the origin of a successful clone such as CC type and SCC*mec* type.

Each MRSA clone has emerged as a result of genetic variation such as acquisition of resistance, virulence and host-adaptation genes, coupled with selective pressures such as antimicrobial usage that allow it to expand in healthcare, community and animal husbandry niches. As the number and quantity of antibiotics being used increases, so does the selective pressure exerted on the *S. aureus* population to develop and maintain resistance. Such conditions favour horizontal transfer of novel mobile resistance elements, and consequently have the potential to drive the emergence of a new “super bug” with a fully multi-drug resistance spectrum. For example, gene transfer from vancomycin-resistant enterococci led to the emergence of vancomycin-resistant *S. aureus* (15). Fortunately, the relative fitness of these isolates is thought to be compromised (16). Also alarming is the emergence of

linezolid-resistant *S. aureus* (LRSA) belonging to the USA300 clone possessing the *cfr* resistance gene. Three such isolates were identified as part of routine surveillance in the New York region (17). An outbreak of LRSA has also been reported in Spain (18).

In the remainder of the paper we will further discuss the three epidemiological types of MRSA with particular reference to the contribution of genomic information to our understanding of their emergence, spread and global dissemination, virulence, and antibiotic resistance.

Hospital-associated MRSA (HA-MRSA)

One of the most globally prolific clones of HA-MRSA in recent years is the epidemic MRSA 15 (EMRSA-15) clone, which belongs to the ST22/CC22 lineage. EMRSA-15, first described in 1991 in England, from which point it spread rapidly all over the UK, such that 15 years later, two-thirds of all MRSA bacteraemia episodes were caused by this clone. Phylogenomic studies have proposed that pandemic MRSA CC22 emerged in the mid 1980s in the UK Midlands, and coincided with the introduction of fluoroquinolone antibiotics in the UK. The acquisition of fluoroquinolone resistance by the EMRSA-15 clone through mutation was a seminal event in its emergence. This resistance pre-dated the clinical licensing of fluoroquinolones, but occurred at a time when fluoroquinolones were used in clinical studies in the UK. In support of the importance of fluoroquinolone resistance in selecting for HA-MRSA, a decline in prescribing of fluoroquinolones in the UK has been followed by the decline of MRSA in hospitals (19, 20).

Fluoroquinolone resistance is almost universal in EMRSA-15 isolates (21). However, this lineage is also capable of carrying a variety of different genes located on mobile genetic elements that confer resistance to aminoglycosides, macrolides, chloramphenicol, trimethoprim-sulphamethoxazole, fusidic acid, mupirocin, tetracycline and antiseptics, highlighting the selection pressure placed on EMRSA-15 (20). Evidence of how regional prescribing regimes generate regional adaptation can be found in the genetic determinants associated with clindamycin resistance. Holden et al. examined EMRSA-15 from Germany, where clindamycin use is high, and found that the majority of isolates contain multiple independent mutations to the *ermC* leader peptide region rendering them resistant to clindamycin as well as erythromycin. In contrast, in the UK where the use of clindamycin is limited, all EMRSA-15 *ermC* leader peptides were intact, therefore making these isolates susceptible to clindamycin. The human EMRSA-15 epidemic has also spread into the companion animal population (22, 23), with veterinary hospitals also being healthcare settings that may experience high levels of transmission (24).

A noticeable feature of EMRSA-15 is its propensity to spread, expand and replace the dominant HA-MRSA. EMRSA-15 overtook the previously successful CC30 HA-MRSA epidemic MRSA 16 (EMRSA-16; ST36- II) clone in the UK in the early 2000s. The replacement of resident HA-MRSA clones by EMRSA-15 MRSA has been well documented in Portugal, Singapore and Australia (25-27). One feature that may contribute to its success is the SCC*mec* IV element that this clone carries, which has lower fitness cost than the larger SCC*mec* elements such as SCC*mec* types II and III which were prevalent in earlier HA-MRSA clones (28). In addition, some variants of

CC22 HA-MRSA have acquired the PVL-associated genes, causing outbreaks among neonates in Australia (29), and in the UK (30).

Another interesting pandemic HA-MRSA clone is ST239-MRSA-III, widespread in Australian, Asian and South American hospitals. Epidemiological studies using WGS of global populations clearly showed that isolates were clustered into regional groups indicating localized evolution, but that transmission to other continents occurred sporadically (31). There were clear associations between isolates from countries with close cultural links such as Portugal and Brazil. There was also evidence of direct introduction of Asian isolates to Australia (32), UK and Denmark. However, these MRSA failed to become established in hospitals in the UK and Denmark (20).

Other widespread HA-MRSA clones include CC5 (predominately ST5-MRSA-II [New York/Japan MRSA/USA100] and ST5-MRSA-VI [Paediatric clone]), CC8 (ST247-MRSA-I [EMRSA-17/ Iberian MRSA]), CC30 (ST36-MRSA-II [EMRSA-16/USA200]), and CC45 (ST45-MRSA-IV [Berlin MRSA/USA600]) (33). The ST5 MRSA clone has been predominant in the hospitals in America (34). Data from the Active Bacterial Core Surveillance from the CDC indicate that USA100 (ST5) is the predominant strain type although from 2004 onwards the more virulent type USA300 (ST8) isolates are increasingly recognized as a cause of hospital-associated bacteraemia (35-37). Indeed, the USA300 epidemic peaked in 2004 leading to the mathematical predictions that the USA300 CA-MRSA strains will eventually displace the HA-MRSA strains due to the survival advantage of the former as a result of smaller and fewer genes leading to enhanced fitness (38). While USA300 continues to be a

predominant clone, there are significant regional differences in the proportion of bloodstream infections caused by USA300 clone. Jenkins and colleagues found that the proportion of USA300 clone ranged from 19% to 62% of the bloodstream MRSA isolates even within Denver (39). The incidence of USA300 in invasive infections has declined since the peak in 2004 (40). Thus, while different countries and regions have different dominant clones, they also have different rates of MRSA infection, different prescribing practices, and healthcare systems, each providing unique selective pressures that have a profound effect on strain dynamics.

Community-associated MRSA (CA-MRSA)

CA-MRSA has been circulating in Europe since the 1990s (41-43). In the beginning, the CC80 lineage predominated the epidemiological group. The CC80 clones possess the PVL-associated genes which encode the PVL toxin. Phylogeographical analysis reveals CC80 emerged in sub-Saharan Africa in the 1980s before rapidly disseminating into Europe, most likely via coastal areas of Guinea, as a result of human migration. This imported lineage then spread throughout Europe within ten years followed by another secondary spread a few years later. At the time it was introduced into Europe the CC80 lineage contained the PVL-associated genes. Genes encoding PVL were subsequently acquired as it spread across continents. In terms of resistance to antibiotics, the African ancestor lineage was generally susceptible except for tetracycline while fusidic acid resistance was acquired following introduction into Europe where topical use of fusidic acid is widespread in some regions (44).

The American story of CA-MRSA is strikingly different. In the United States CA-MRSA was first documented in children during the late nineties. The isolates belonged to a single clone, designated USA400 (belonging to CC1), and caused sepsis with pneumonia associated with high lethality. The USA400 clone has subsequently been replaced by USA300, a member of CC8, which is now the most common CA-MRSA in America. Accompanying this shift in the MRSA population was a change in the epidemiology of CA-MRSA disease; CA-MRSA are typically associated with skin and soft tissue infection. Amongst the MGEs USA300 isolates carry, is a type I arginine catabolic mobile element (ACME) containing the *speG* gene. The product of this gene, spermidine *N*-acetyl transferase, degrades host polyamines (*e.g.* spermidine) that are lytic to the bacteria, thus enhancing the fitness of the bacteria to survive within the host (45). Within the CC8 lineage, nine clades have been identified, designated clade CC8-A to CC8-I (46). This lineage has undergone a multiclonal expansion in contrast to the European CA-MRSA CC80, which has given rise to a single successful clone with a clear geographical pattern of emergence and spread. Some clades within the CC8 (clades CC8-A, CC8-C, CC8-D and CC8-E) population appear to be geographically diverse, whereas others are geographically restricted to a single area (*e.g.* clade CC8-B in Europe). The multiclonal expansion is reflected in the diverse antibiotic resistance pattern of the CC8 lineage. Some clones are fully susceptible while others are multi-resistant, particularly those belonging to CC8-E. Phylogenetic analysis reveals a stepwise incremental gain in antibiotic resistance genes. The older representatives of CC8-E population harbour resistance to as many as nine antibiotics while the newer clades such as clade CC8-A are pauci-resistant (resistance often limited to two antibiotics).

This appears to support the hypothesis that USA300may be on the same trajectory as CC8-E was at one point in its evolutionary history, and could in the future become multi-resistant. Clade CC8-A also carries the PVL-associated genes and contains isolates that belong to the USA300. In a study investigating the genomics of USA300, Uhlemann et al. found evidence for at least five different acquisitions of the *lukSF*-carrying prophage into the ST8 population, but only a single event into USA300 clone (47). From their phylogenomic analysis the authors concluded that the acquisition of the *lukSF*-carrying prophage coincided with the acquisition of ACME, and occurred between 1970 and 1993, resulting in the emergence of USA300. The USA300 clone has spread to other continents including Europe and South America (48). The expansion of USA300 in Europe may have occurred as a single point introduction followed by spread or by multiple introductions over a long period of time followed by limited spread on each occasion. WGS data on the French isolates reveal that following multiple introductions, the lineage has stabilized and may even decline (49).

Another clinically relevant gene associated with USA300 clone is the *msrA* gene encoding resistance to macrolides and streptogramins. This gene is carried on the *rep16* plasmid, and at least eight *rep* family plasmids have been identified within the CC8 lineage. The *rep16* plasmid was acquired more recently, when it replaced the *rep20* plasmid typical of the older isolates within clade A (46). A feature of the CA-MRSA in the US is high levels of genetic variation. Carpaij and colleagues found great diversity related to acquisition of genes among 13 subtype USA300-0114 isolates isolated over a short period of one month from one single location, which suggests ongoing evolution of the clone (50).

In keeping with their geographical isolation, the epidemiology of CA-MRSA in Australia and New Zealand is unique. One of the earliest reports of CA-MRSA was from this region when infections caused by ST8 MRSA clone were reported amongst indigenous communities in Western Australia (51). Later, ST30 CA-MRSA infections were reported from New Zealand and more recently non-pigmented CC75 from Northern Australia. CC75 is sufficiently different from the other *S. aureus* lineages to an extent that a new species name has been proposed: *S. argenteus* (52, 53). The other CA-MRSA lineages identified in the Australian survey include ST93-MRSA-IV, ST30-MRSA-IV, ST1-MRSA-IV, ST45-MRSA-IV, ST78-MRSA-IV, and ST5-MRSA-IV (denoted by sequence type followed by SCC*mec* carriage). The CA-MRSA type that now predominates in New Zealand is the fusidic-acid resistant ST5-MRSA-IV clone. (54).

The full epidemiological picture in Asia and Africa is unclear as data are lacking from many regions and from prior to the turn of the century. The dominant Asian clones include ST59-MRSA-IV/V in China, Taiwan, Singapore, and Hong Kong, ST72-MRSA-IV in Korea, ST30-MRSA-IV in Japan and the Western Pacific, ST80-MRSA-IV in the Middle East, and the PVL positive Bengal Bay clone ST772-MRSA-V in India. The latter clone has since been reported from various countries in Europe, Middle East, and Australasia. Closely related to the CC1 lineage with, the ST772 has several distinguishing features such as resistance to multiple antibiotics, different *agr* group (group II), and capsule type 5 rather than 8 (55). The African origin of the European CA-MRSA clone has been well described, and CA-MRSA has been reported from Egypt, Mali, Algeria, and Nigeria. Sampling of individuals from the remote Babongo tribes in Gabon found a high percentage (55%) of PVL positive

clones of MSSA, but MRSA was not detected in any of the samples. The MSSA clones found in this study include the common clonal lineages CC1, CC5, CC30, and CC80, despite the remote location of this tribal population (56).

Livestock-associated MRSA (LA-MRSA)

Infections with LA-MRSA can occur in people who have direct contact to farm animals in factory farms (especially pig and poultry production systems). This affects, for example, farmers, veterinarians or slaughterhouse employees. MRSA was first described in animals in 1972 when it was found in a cow (57). However, it was not until 2005 when CC398 MRSA was reported from pigs in both France and The Netherlands that livestock was shown to be an important zoonotic reservoir for MRSA. Pigs are the main reservoir for CC398 but this clone has also been found in veal calves (especially in The Netherlands and Belgium), poultry, horses, and to a lesser extent in dairy cows. In addition, CC398 has been found in pets such as dogs and cats, as well as in rodents. There is a general consensus that the prevalence of CC398 is increasing rapidly worldwide. However, precise information on the true prevalence of CC398 in animals is difficult to obtain. In The Netherlands it is estimated that >80% of all farms are MRSA positive. In Denmark, a recent surveillance study showed that 60-70% of all pig herds are positive, whereas in Norway only very few farms have been found positive. However, in most other countries no systematic surveillance on CC398 or other types of LA-MRSA is performed in animals (the same holds true for the surveillance of these strains in humans).

While CC398 MRSA is the overwhelmingly dominant lineage in livestock in Europe, CC9 MRSA is the predominant type in Southeast Asia. In addition to these two clonal complexes, a number of other MRSA lineages including CC1, CC5, CC97, CC121, CC130, and ST425 have been reported from livestock (58). MRSA belonging to CC130 and ST425 are meticillin resistant due to the recently described *mecC* gene instead of the *mecA* gene. The *mecC* gene only has approximately 70% similarity to the *mecA* gene (59). As a result, the molecular diagnostic assays need to encompass primers for both types. CC97, a widely disseminated human CA-MRSA type, is also interesting as it descended from bovine MSSA and has acquired SCC*mec* after the bovine-to-human host adaptation. This is in contrast with the livestock clade of CC398 that acquired SCC*mec* after jumping from humans to pigs (9, 60).

The risk factors for transmission between herds are not fully elucidated. Trade of MRSA positive pigs is the most important risk factor. However, several herds have been found positive without acquisition of new animals for many years prior to the detection of MRSA CC398. In these cases, introduction from MRSA-positive humans (such as veterinarians or new employees), or from rodents, are possible transmission routes. Within herds, use of antimicrobials, particularly β-lactams and tetracyclines, and trace metals including zinc are important selective pressures (61). The latter is substantiated in the dominant SCCmec V (5C2&5) cassette which includes the *crzC* gene (62).

By far the most important risk factor for LA-MRSA in humans is repeated direct occupational contact to animals positive for MRSA. The extent of the risk is

dependant both on contact time and contact intensity. In Denmark, 70% of all new cases found in 2013 reported direct contact with pigs, and an additional 17% were household members of persons with close contact with live pigs. Of the remaining 13% with no pig contact, the majority of cases lived in rural areas with high pig density whereas few occurred in urban areas. This indicates that transmission occurs via local spill over from persons working at livestock farms or through contact with the farm environment itself rather than through a generalized spread in the community (63). The relative contribution of transmission via the environment or via humans in contact with pigs is unknown. However, based on knowledge of transmission of *S. aureus* in other settings, transmission through human to human contact is likely to predominate (64).

As MRSA has been found on meat in 10-20% of samples tested on many occasions, there have been reports in the lay press on the risk of acquisition of MRSA via the food chain. The epidemiology on LA-MRSA, however, clearly shows that meat is not an important route of transmission. If it were the case, one would see a very different geospatial distribution among cases not associated with pig contact (63). Further evidence against this route of transmission is provided by the low incidence of LA-MRSA found in slaughterhouse workers. There may be some risk of acquisition of MRSA as a result of handling of meat, rather than ingestion, but this risk is not significant and clearly has not been associated with wide-spread transmission. Washing hands immediately after handling food is a very effective way to block transmission.

The increasing reservoir of LA- MRSA in pigs, and in humans in direct contact with pigs, results in increasing number of cases in the general community, particularly in immunocompromised individuals. Therefore, unless this epidemic is contained, it is likely that we will see increasing numbers of severe infections due to LA-MRSA. Köck et al. reported that 16 out 194 (8%) MRSA bacteraemia episodes in Germany diagnosed during years 2008-2012 were due to MRSA CC398 (65). Furthermore, the greater the human carriage of LA-MRSA clones, the greater the risk that these clones will undergo adaptation enhancing human-to-human transmissibility. Measures to counteract the expanding reservoir in pigs are thus urgently needed, as are measures to lower the bacterial burden in the farm environment.

Application of genomics in epidemiological settings and outbreak investigations

Modern genetic tools have greatly expanded our knowledge of the epidemiology of MRSA infection, and allowed a closer examination of outbreaks in the hospital and in the community. Classical typing methods or antibiogram may not have sufficient resolution power to link MRSA clones during outbreak investigation. For example, WGS has been used retrospectively in an outbreak in Special Care Baby Unit in a Cambridge hospital (66). Of the 17 cases in the unit over a six-month period, 12 appeared to be linked based on identical or near-identical antibiotic susceptibility pattern. However, WGS data revealed that 14 of the 17 isolates belonged to a new type, ST2371 (a single locus variant of ST22 that possesses the PVL-associated genes). Thus, only three of the five isolates excluded as a result of antibiogram mismatch were correctly excluded: the remaining two were in fact associated with the outbreak. Also, the sequencing data identified additional cases, both

retrospectively and prospectively, linked to the outbreak but beyond the parameters of the outbreak as defined in the traditional infection control investigations. Following a new case more than two months after the outbreak was thought to have ceased, screening of staff identified a single MRSA carrier. WGS of colonies of *S. aureus* from this individual identified genetic overlap in the colonising population of the staff member and the outbreak population. The application of WGS for the near real time analysis of outbreaks has great utility. Moreover, the technique encompasses the information provided by the more traditional genotyping tools such as sequence typing coupled with detection of specific genes such as those encoding PVL toxin and other virulence determinants. The rapid detection of genes associated with pathogenicity, transmission and resistance may help in limiting spread within hospitals by timely adherence to strict infection control protocols, and may also help in characterizing atypical clones that pose a threat. These clones may be misreported in routine clinical laboratories. Recent sequencing work on four clones from Scotland identified several single nucleotide substitutions in the transpeptidase domains of PBPs 1, 2 and 3 which can explain resistance to β-lactamase-stable penicillins (67). Transmission of MRSA in the setting of deceased donor liver transplant was confirmed by using WGS in a recent report (68). The diagnostic value of WGS cannot be overemphasized.

WGS has also been used for understanding of epidemiology and transmission dynamics of MRSA by creating a sequencing database. Based on the numbers of single nucleotide polymorphism (SNP) and their dispersion within the genome it is possible to discriminate between the timing of acquisition and the relationship between isolates. Bartels and colleagues found 59 SNP differences between two

480 isolates belonging to the ST80 clone from the same household which indicates long
481 term carriage based on the fact that SNPs are acquired at a predictable rate (69).
482 Application of WGS in human infections with MRSA carrying the *mecC* gene
483 identified two distinct clusters with transmission confined between individuals and
484 their livestock with only small differences in SNPs between the animal and the
485 human isolates, which supports zoonotic transmission. MLST, pulse-field gel
486 electrophoresis and multilocus variable number tandem repeat analysis were unable
487 to distinguish between the two clusters (70).
488
489

Conclusion

The story of MRSA is one of dramatic recent success driven by the widespread use of antibiotics. *S. aureus* clones have co-evolved with humans, and MRSA have emerged from the population on multiple occasions. The rapid spread of MRSA has been aided by human migration, which itself has reached a level never seen before in the history of mankind. MRSA has adapted to the environments and conditions that humans have created and where they have thrived. Clones have jumped from one host species to another, have amplified in the niche areas, and then gone back to colonize the population where it emerged while still retaining a strong foothold in specialized environments. There is significant overlap between clones across the traditional groups (HA-MRSA and CA-MRSA) and also some blurring of the groups themselves as healthcare is increasingly delivered in the community. Newer technologies like WGS will lead to a better epidemiological understanding of MRSA. Use of antibiotics in humans and livestock has given this highly adaptive species a survival advantage, as it is easily able to acquire resistance. *S. aureus* has not lost its virulence either, as it continues to be a primary human pathogen with significant mortality associated with invasive disease.

Declarations

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Table 1: Definitions of community-associated, hospital-associated and livestock associated MRSA

MRSA	Definition and/or salient features
HA-MRSA	Identified more than 48 hours after admission to a healthcare facility or MRSA identified in an individual with history of MRSA infection or colonization, admission to a healthcare facility, dialysis, surgery or insertion of indwelling devices in the past year.
CA-MRSA	Identified in the outpatient setting or within 48 hours following hospital admission in an individual with no medical history of MRSA infection or colonization, admission to a healthcare facility, dialysis, surgery or insertion of indwelling devices in the past year.
LA-MRSA	No formal definition. Usually belong to CC398 lineage in Europe but often CC9 in Asia. Acquired via occupational contact with livestock.