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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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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.

Title: Genomic Insights into the emergence and spread of international clones of methicillin-resistant *Staphylococcus aureus*

Highlights:

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

1 Genomic insights into the emergence and spread of international clones
2 of healthcare, community and livestock-associated meticillin-resistant
3 *Staphylococcus aureus*: blurring of the traditional definitions
4

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38 **Abstract**

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

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52 **Keywords:** MRSA clones, MRSA epidemiology, Whole genome sequencing

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60 **Introduction**

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

78

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

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

87

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

90

91 ***The emergence of MRSA***

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

106

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

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

117

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

135 tetracycline resistant and are strongly associated with pig farms, although they are
136 also found in a range of other farm animals. Like the HA-MRSA and CA-MRSA,
137 evolution of meticillin resistance appears to have occurred independently;
138 phylogenomic analysis of representatives of the LA-MRSA CC398 population
139 suggests this lineage has descended from a human MSSA ST398 clone, which after
140 a host jump acquired tetracycline and meticillin resistance. In this process the LA-
141 MRSA lineage lost the ϕ Sa3 phage carrying the human evasion genes *sak*, *scn* and
142 *chp* (9). LA-MRSA marks a paradigm shift in the epidemiology as humans hitherto
143 have been the dominant reservoir, tackling of LA-MRSA requires a One Health
144 approach with collaboration of veterinarians, farmers, doctors and environmentalists.
145 The epidemiological definitions for CA-MRSA, HA-MRSA and LA-MRSA (Table 1)
146 may not be fit for purpose in future. For example, Folden and colleagues reported
147 the variation in estimating the proportion of CA-MRSA infections using
148 epidemiological definitions: use of one set of definitions classified 5% of isolates as
149 CA-MRSA while using another set led to as many as 49% of infections to be
150 classified as CA-MRSA (10). In order to avoid such misclassification, the Centers for
151 Disease Control and Prevention (CDC) has provided a definition for CA-MRSA
152 (Table 1) (11). However, at the same time as CA-MRSA becomes common in the
153 community, changes in hospital practice such as reduced lengths of stay, day-only
154 admissions and hospital-in-the-home or community hospitals make application of
155 traditional definitions difficult as facilities traditionally available in hospitals are
156 increasingly delivered in the community in a more integrated manner. Miller and
157 colleagues failed to label any MRSA bacteraemia (n= 57) presenting at the Oxford
158 Radcliffe Hospital between 2003 and 2006 as caused by CA-MRSA (12). Problems
159 are also encountered when using antibiotic susceptibility pattern or traditional genetic

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

173

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

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

189

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

194

195 ***Hospital-associated MRSA (HA-MRSA)***

196 One of the most globally prolific clones of HA-MRSA in recent years is the epidemic
197 MRSA 15 (EMRSA-15) clone, which belongs to the ST22/CC22 lineage. EMRSA-15,
198 first described in 1991 in England, from which point it spread rapidly all over the UK,
199 such that 15 years later, two-thirds of all MRSA bacteraemia episodes were caused
200 by this clone. Phylogenomic studies have proposed that pandemic MRSA CC22

201 emerged in the mid 1980s in the UK Midlands, and coincided with the introduction of
202 fluoroquinolone antibiotics in the UK. The acquisition of fluoroquinolone resistance
203 by the EMRSA-15 clone through mutation was a seminal event in its emergence.
204 This resistance pre-dated the clinical licensing of fluoroquinolones, but occurred at a
205 time when fluoroquinolones were used in clinical studies in the UK. In support of the
206 importance of fluoroquinolone resistance in selecting for HA-MRSA, a decline in
207 prescribing of fluoroquinolones in the UK has been followed by the decline of MRSA
208 in hospitals (19, 20).

209

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

225

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

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

236

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

246

247 Other widespread HA-MRSA clones include CC5 (predominately ST5-MRSA-II [New
248 York/Japan MRSA/USA100] and ST5-MRSA-VI [Paediatric clone]), CC8 (ST247-
249 MRSA-I [EMRSA-17/ Iberian MRSA]), CC30 (ST36-MRSA-II [EMRSA-16/USA200]),
250 and CC45 (ST45-MRSA-IV [Berlin MRSA/USA600]) (33). The ST5 MRSA clone has
251 been predominant in the hospitals in America (34). Data from the Active Bacterial
252 Core Surveillance from the CDC indicate that USA100 (ST5) is the predominant
253 strain type although from 2004 onwards the more virulent type USA300 (ST8)
254 isolates are increasingly recognized as a cause of hospital-associated bacteraemia
255 (35-37). Indeed, the USA300 epidemic peaked in 2004 leading to the mathematical
256 predictions that the USA300 CA-MRSA strains will eventually displace the HA-MRSA
257 strains due to the survival advantage of the former as a result of smaller and fewer
258 genes leading to enhanced fitness (38). While USA300 continues to be a

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

267

268 ***Community-associated MRSA (CA-MRSA)***

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

282

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

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

322

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

332 In keeping with their geographical isolation, the epidemiology of CA-MRSA in
333 Australia and New Zealand is unique. One of the earliest reports of CA-MRSA was
334 from this region when infections caused by ST8 MRSA clone were reported amongst
335 indigenous communities in Western Australia (51). Later, ST30 CA-MRSA infections
336 were reported from New Zealand and more recently non-pigmented CC75 from
337 Northern Australia. CC75 is sufficiently different from the other *S. aureus* lineages to
338 an extent that a new species name has been proposed: *S. argenteus* (52, 53). The
339 other CA-MRSA lineages identified in the Australian survey include ST93-MRSA-IV,
340 ST30-MRSA-IV, ST1-MRSA-IV, ST45-MRSA-IV, ST78-MRSA-IV, and ST5-MRSA-IV
341 (denoted by sequence type followed by SCC*mec* carriage). The CA-MRSA type that
342 now predominates in New Zealand is the fusidic-acid resistant ST5-MRSA-IV clone.
343 (54).

344

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

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

360

361 ***Livestock-associated MRSA (LA-MRSA)***

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

380

381 While CC398 MRSA is the overwhelmingly dominant lineage in livestock in Europe,
382 CC9 MRSA is the predominant type in Southeast Asia. In addition to these two
383 clonal complexes, a number of other MRSA lineages including CC1, CC5, CC97,
384 CC121, CC130, and ST425 have been reported from livestock (58). MRSA
385 belonging to CC130 and ST425 are meticillin resistant due to the recently described
386 *mecC* gene instead of the *mecA* gene. The *mecC* gene only has approximately 70%
387 similarity to the *mecA* gene (59). As a result, the molecular diagnostic assays need
388 to encompass primers for both types. CC97, a widely disseminated human CA-
389 MRSA type, is also interesting as it descended from bovine MSSA and has acquired
390 SCC*mec* after the bovine-to-human host adaptation. This is in contrast with the
391 livestock clade of CC398 that acquired SCC*mec* after jumping from humans to pigs
392 (9, 60).

393

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

403

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

406 dependant both on contact time and contact intensity. In Denmark, 70% of all new
407 cases found in 2013 reported direct contact with pigs, and an additional 17% were
408 household members of persons with close contact with live pigs. Of the remaining
409 13% with no pig contact, the majority of cases lived in rural areas with high pig
410 density whereas few occurred in urban areas. This indicates that transmission
411 occurs via local spill over from persons working at livestock farms or through contact
412 with the farm environment itself rather than through a generalized spread in the
413 community (63). The relative contribution of transmission via the environment or via
414 humans in contact with pigs is unknown. However, based on knowledge of
415 transmission of *S. aureus* in other settings, transmission through human to human
416 contact is likely to predominate (64).

417

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

429

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

440

441 ***Application of genomics in epidemiological settings and outbreak***

442 ***investigations***

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

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

474

475 WGS has also been used for understanding of epidemiology and transmission
476 dynamics of MRSA by creating a sequencing database. Based on the numbers of
477 single nucleotide polymorphism (SNP) and their dispersion within the genome it is
478 possible to discriminate between the timing of acquisition and the relationship
479 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

490 **Conclusion**

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

507

508 **Declarations**

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513 **Ethical approval:** Not required

514

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757 **Table 1:** Definitions of community-associated, hospital-associated and livestock
 758 associated MRSA
 759

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.

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