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Review

Contribution of whole-genome sequencing to understanding of the epidemiology and control of meticillin-resistant *Staphylococcus aureus*

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SUMMARY

In recent years, approaches to tracking the spread of meticillin-resistant *Staphylococcus aureus* (MRSA) as part of outbreak management have used conventional DNA-based methods, including pulsed-field gel electrophoresis and *spa* typing. However, when a predominant clone is present, these methods may be insufficiently discriminatory. A literature search was undertaken to highlight how whole-genome sequencing (WGS) has revolutionized the investigation of outbreaks of MRSA, including intrahospital spread and MRSA in the community, and to review its future potential. WGS provides enhanced isolate discrimination, as it permits the entire genomic DNA sequence of isolates to be determined and compared rapidly. Software packages used for the analysis of WGS data are becoming increasingly available. To date, WGS has been more sensitive in confirming outbreaks, often persisting for prolonged periods, previously undetected by conventional molecular typing. The evolving dynamic of spread from the community to hospitals, within and between hospitals, and from hospitals to the community is only becoming clear with WGS studies, and is more complex and convoluted than widely appreciated. Also, WGS can exclude cross-transmission, when isolates are different. The challenges now are to make WGS technology more amenable for routine use, and to develop an evidence-based consensus for sequence difference thresholds for isolates that are deemed to be part of the same outbreak, including protracted outbreaks. Using such data in a timely way will provide increased sensitivity in detecting cross-transmission events at an earlier stage, with the potential to prevent outbreaks, and have a positive impact on infection prevention and control.

Background

Staphylococcus aureus is a common cause of healthcare-associated infection (HCAI). In a recent European study in children, *S. aureus* was responsible for 11% of infections and was second only to coagulase-negative staphylococci as a cause

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[1]. In a four-country prevalence survey of HCAI in the UK and Ireland, meticillin-resistant *S. aureus* (MRSA) was responsible for 15.8% of all HCAIs [2]. In addition to causing serious infection in acutely ill patients in hospital, MRSA can spread to and between residents in long-stay residential units (LSRUs) and day units, as increasingly the elderly population is managed outside acute hospitals.

Infections caused by MRSA have been prevalent in many countries for decades, although the prevalence of some serious infections, such as bloodstream infections (BSIs), has declined in recent years [3,4]. Data from the UK and Northern Ireland from 2010 to 2014 derived from mandatory reporting of *S. aureus* BSI showed that the proportion of BSI due to *S. aureus* decreased from 16.2% to 8.9%, with a downward trend in all four countries [3]. Molecular typing is important to track spread, indicate the possible origins of outbreaks, confirm the efficacy of outbreak control measures, and track the emergence or evolution of new clones in the outbreak and endemic setting.

Conventional molecular typing of MRSA

Routes of MRSA transmission and spread in healthcare settings have traditionally been investigated using targeted DNA-based typing methods, including pulsed-field gel electrophoresis (PFGE), *spa* typing, conventional multi-locus sequence typing (MLST) and, more recently, the application of DNA micro-array technology [5–7]. In many cases, these approaches provide valuable insights into the relatedness of clinical isolates when combined with relevant epidemiological data and the expertise and insights of infection prevention and control staff. However, conventional molecular typing approaches frequently struggle to discriminate between isolates in the healthcare setting, where a particular or limited number of MRSA clonal lineages predominate. In many countries, MRSA responsible for HCAIs frequently belong to a relatively small number of clones; for example, in Ireland and the UK, multi-locus sequence type (ST) 22 has predominated among MRSA responsible for BSI and carriage for almost two decades [7–9]. Isolates belonging to this lineage are particularly recalcitrant to discrimination by conventional molecular typing approaches, making detection of all or even major patterns of spread problematic [7,10,11]. Consequently, there is a need for more discriminatory methods to distinguish clonal isolates to track spread.

Brief overview of whole-genome sequencing of nosocomial pathogens

Next-generation sequencing

Table I provides a list and explanations of commonly used terms relating to whole-genome sequencing (WGS) and its analysis. Over the last decade, the development of next-generation sequencing (NGS) to determine the entire genetic sequence of microbial pathogens, especially bacterial species, has revolutionized molecular epidemiology. In contrast to Sanger sequencing, NGS permits the high-throughput and rapid determination of whole-genome sequences of pathogens at an affordable cost. A variety of NGS approaches have been developed that utilize innovative sequencing chemistry methods in tandem with small footprint automated bench top sequencers [12–15]. These approaches involve fragmentation of genomic DNA into short segments of a few hundred nucleotide bases in length, tagging of the fragments with adapters to generate genome fragment libraries, immobilization of the libraries on a solid interface, followed by polymerase chain reaction (PCR) amplification of the DNA fragments. The DNA sequence of each fragment is determined during complementary strand synthesis using a variety of approaches depending on the NGS platform being used. This allows millions to billions of DNA fragments to be sequenced in parallel.

Two of the most widely used NGS platforms, also known as second-generation sequencers or short read sequencers, include the Illumina sequencing systems (Illumina, Eindhoven, The Netherlands) and the ion semiconductor systems (Thermo Fisher Scientific, Waltham, MA, USA) [12,13,16]. In recent years, most studies reporting NGS of bacterial pathogens have used Illumina short read sequencers. In contrast, third-generation DNA sequencers yield much longer sequencing reads (i.e. >10 kb) and can sequence single DNA molecules without the requirement for DNA amplification. Examples of third-generation sequencing platforms include the Single Molecular Real-Time sequencing platforms manufactured by PacBio (Menlo Park, CA, USA) and the nanopore MinION sequencers manufactured by Oxford Nanopore Technologies (Oxford, UK) [12]. The error rates generated by third-generation sequencers are relatively high compared with second-generation sequencers, but the long read lengths generated by the former are very advantageous for sequencing entire genomes and plasmids. However, PacBio platforms are currently relatively expensive and are not suitable for high-throughput rapid analysis or the processing of many clinical isolates. In contrast, Oxford Nanopore sequencers are inexpensive and can yield very long sequence reads, but bioinformatics analysis of sequence data is more challenging. Nonetheless, a growing range of nanopore bioinformatics tools is being developed, which will soon make this technology more readily applicable to clinical isolates [17]. A combination of short read sequences generated by NGS platforms such as Illumina MiSeq and long read sequences generated by nanopore sequencers can be used to rapidly and accurately map relatively large genomic regions, such as the staphylococcal cassette chromosome *mec* (SCC*mec*) region, and other large genomic elements such as the arginine catabolic mobile element in *S. aureus* and coagulase-negative staphylococcal species. Typically, the DNA sequences of such elements are spread across several contiguous sequences in genome assemblies generated from short read sequences, and usually require extensive PCRs to accurately refine the assemblies. This can be overcome by generating hybrid assemblies of both short and long read sequences [18,19]. A number of recent reviews have provided comprehensive overviews of WGS platforms and technology, and applications for microbial epidemiology [12–15].

Genome assembly and bioinformatics

The volume and complexity of data generated by WGS platforms requires the application of a variety of bioinformatics tools to determine the quality of the sequence data, and to transform unrefined sequencing read data into more useful or meaningful forms. Software algorithms are used to clean up and organize sequence data, to assemble genome sequences from overlapping sequence reads, and to identify

Table I
List of commonly used terms relating to whole-genome sequencing (WGS) and analysis

Term	Explanation
Next-generation sequencing	The high throughput and rapid determination of whole-genome sequences. Millions to billions of DNA fragments are sequenced in parallel
Sequencing platform	DNA sequencing system consisting of sequencing equipment (i.e. sequencer) and methodology to sequence target DNA. Examples include the Illumina MiSeq, the PacBio Single Molecular Real-Time and the Oxford Nanopore MinION sequencing platforms
Sequence read	A continuous DNA sequence determined from a target organism (e.g. bacterium)
Short sequence reads	A continuous DNA sequence determined from a target organism ranging between approximately 100 and 600 bp. For example, the Illumina MiSeq sequencing platform yields short sequence reads
Long sequence reads	A continuous DNA sequence determined from a target organism generally >10 kb. For example, the PacBio SMRT sequencing platform yields long sequence reads
Sequence read error rate	The proportion of sequence reads containing sequencing errors
Contigs	Contiguous sequences assembled from overlapping smaller sequence reads that represent a consensus region of DNA
Single nucleotide variation	A variation in a single nucleotide base that occurs at a specific position in the genome of an organism of interest without implying how often this variation occurs in a population
SNP	A variation in a single nucleotide base that occurs at a specific position in the genome of an organism of interest and is relatively common in a population
SNP analysis	Strain typing by mapping SNPs in sequence reads or assembled contigs against a reference genome(s). Reference genomes that are closely related to the sequenced samples are best
Core genome	A set of conserved genes present in virtually all isolates of a species.
Accessory genome	A variable set of genes present in some but not all isolates of a species. Examples in *Staphylococcus aureus* and other staphylococci include SCC*mec* and arginine catabolic mobile elements
MLST, also known as conventional MLST	A method that determines the sequences of internal fragments of up to seven housekeeping genes. The different sequences within a bacterial species are assigned as distinct alleles and, for each isolate, the alleles at each of the loci define the allelic profile or sequence type. Curated MLST databases exist for many bacterial species including *S. aureus*. Conventional MLST has largely been replaced by WGS-based MLST schemes for *S. aureus* and other important bacterial pathogens due to significantly enhanced resolution
wgMLST	A typing method that uses WGS data from the core genome and accessory genome to perform MLST on a genome-wide basis and permits gene-by-gene comparisons of very large numbers of genes amongst a group of isolates. The presence/absence of each target locus is determined from WGS data, as are allelic variants. Very high resolution can be achieved
cgMLST	A typing method that uses WGS data to perform MLST on a predefined set of conserved genome-wide core genes (can be >1000 genes) that are present in the vast majority of isolates of a bacterial species. Very high resolution can be achieved across large groups of isolates by core genome comparisons. The approach is highly reproducible across data sets
Genome assembly	The entire DNA sequence of an organism can be re-assembled from overlapping sequence reads which are first assembled into larger contigs. Any remaining gaps between contigs can then be closed yielding the reassembled genome. A wide variety of bioinformatics software tools and algorithms can be used for genome assembly

MLST, multi-locus sequence typing; SNP, single nucleotide polymorphism.

genomic variants for genotyping and phylogenetic analysis. For the non-bioinformatician, the terminology and application of bioinformatics is daunting in this rapidly developing field. A recent review by Carriço *et al.* (2018) provides an excellent overview of bioinformatics as applied to WGS data for the non-expert [20]. Some of the more frequently used bioinformatics approaches and software for microbial genome assembly and subsequent analysis have been reviewed recently [12,20,21]. Many software packages used for the analysis of WGS data are freely available, and several commercially available and easy-to-use software packages including BioNumerics (Applied Maths, Ghent, Belgium) and SeqSphere (Ridom GmBH, Münster, Germany) are widely used for this purpose [12,14,16].

Applications of whole-genome sequencing data for epidemiology

WGS data can be used for a variety of purposes in investigating outbreaks of infection and in tracking the sources and spread of infection in hospitals, as well as in investigating more regional and global aspects of the emergence of specific clones of particular pathogens. Conventional targeted molecular typing of pathogens (e.g. PFGE or conventional MLST) generates a genotype barcode or molecular fingerprint of each isolate based on a relatively small portion of the genome [7,12,14]. Isolates with identical or similar genotypes linked by epidemiological data are presumed to represent linked cases of infection. In contrast, WGS enables the entire genome of isolates to be compared, which enhances resolution significantly. As with conventional molecular typing, the genomes of isolates recovered from an outbreak or cluster of infections are likely to be closely related.

Single nucleotide variation analysis

All micro-organisms accumulate changes in their genomes over time, often through random mutations. These include alterations to single nucleotide bases [single nucleotide variations (SNVs)]. When an SNV becomes fixed within a population, it is referred to as a 'single nucleotide polymorphism' (SNP). Other types of changes to the genome include gene acquisition by horizontal transfer (e.g. plasmid acquisition or lysogenization with a bacteriophage), small insertions and deletions, gene duplication and genome re-arrangements.

SNV analysis is frequently used to type isolates based on WGS data in an outbreak scenario, and has been used extensively using data generated by short read sequencers such as those manufactured by Illumina [12—15,22—24]. This approach involves mapping sequence reads or larger contiguous sequences (contigs) assembled from overlapping short read sequences to a reference genome by core genome alignment. Specific software filtering tools can be used to exclude sequence stretches of high variability. Curated collections of reference genomes for particular species (including *S. aureus*) are available for SNV analysis. However, SNV analysis yields the best results when a reference genome that is closely related to the samples under investigation is used. In the case of an outbreak, isolates are likely to be closely related, and one isolate sequence can be used as a reference against which other outbreak isolates' sequences can be mapped. The alignment of isolate sequences can then be used for phylogenetic analysis to determine the relationships between the isolates based on the identification of bases that differ in the test samples relative to the corresponding bases in the reference genome.

Extended multi-locus sequence typing

The genetic relatedness of isolates can also be investigated using extended versions of conventional MLST. Conventional MLST typically involves the sequencing of segments of a small number of selected housekeeping genes that accumulate genetic changes relatively slowly because the encoded proteins are functionally constrained [25]. Such genes encode segments that are amplified by PCR using specific primers and the amplimers sequenced by conventional Sanger sequencing. Combinations of allelic variants in the selected genes are used to define an ST for a particular micro-organism. The advent of WGS has enabled the establishment of whole genome (wg) and core genome (cg) MLST schemes that enable comparison of test sequences with large curated sets of predefined genes for a particular species [26,27]. Publicly available curated gene sets can include hundreds to thousands of genes depending on the scheme, and analysis can be undertaken with easy-to-use software packages such as BioNumerics and SeqSphere [14]. The use of curated cgMLST schemes facilitates good inter-laboratory reproducibility.

Thresholds of isolate relatedness

Establishing SNV and cgMLST thresholds of relatedness for a micro-organism can be problematic. Genomic variability increases over time, and it is vital that this consideration is borne in mind when attempting to assess the relatedness of isolates based on WGS data. There are no definitive rules for determining isolate relatedness or what constitutes a significant difference between isolates. Judgements on similarity or significant differences should be made separately according to the facts of each case. Meaningful thresholds of relatedness can be developed by investigating epidemiologically linked and unrelated isolates. However, proposed thresholds should always be interpreted in conjunction with epidemiological data.

A recent study that investigated several outbreaks of *S. aureus* using WGS suggested that, in an acute short-term outbreak, there will be insufficient time for diversity to accumulate [23], and therefore establishing thresholds in this scenario should be less problematic. Schürch *et al.* (2018) recently detailed a list of current suggested SNV and cgMLST relatedness criteria for some representative clinically significant bacterial species [14]. It is worth bearing in mind that clonality thresholds may vary within particular clones of a particular pathogen.

Use of whole-genome sequencing for investigating MRSA outbreaks

The more widespread availability of WGS in recent years has enabled more detailed study of patterns of spread, including the detection of previously undocumented transmission, as well as the overall and detailed evolution of strains of MRSA [28,29]. A study of Danish isolates using *spa* typing and Sanger sequencing found 97% agreement between these methods and WGS [30]. As the technology becomes increasingly available and costs reduce, WGS will no longer be confined to research or reference laboratory facilities, but will become increasingly available in routine clinical laboratories to inform infection

Table II
Studies using whole-genome sequencing (WGS) of meticillin-resistant *Staphylococcus aureus* (MRSA) in the neonatal/paediatric setting

Country (reference)	Study details	Main conclusions	Comment
Germany (35)	Retrospective case–control study of neonates and staff	28 isolates formed a predominant strain with two other strains present. Staff involved in transmission	Only one isolate per neonate/staff analysed and greater variability may have been present
Germany (36)	Outbreak of toxic shock syndrome and enterotoxin-A-producing MSSA	26% of neonates positive; 19.5% of staff. 21 related isolates confirming PFGE analysis. 2.8×10^{-6} mutations per nucleotide per year	Prolonged duration of outbreak suggests that staff member may have been reservoir as far back as two to three years ago
UK (37)	Retrospective investigation of outbreak in neonatal intensive care unit	Distinct cluster of outbreak isolates and clear separation with non-outbreak isolates.	Value of WGS in real time for MRSA control in hospitals
UK (38)	Outbreak in neonatal unit with isolates compared with other clinical strains in the hospital or community	11 MRSA infants identified. ST2371 predominated. Similar isolates in emergency department, outpatients and general practice	Mother-to-mother transmission outside hospital and staff carriage allowed outbreak to persist
USA (39)	Retrospective analysis of isolates from screening neonates, 2008–2010	Colonized infants had lower birth weight, gestational age and length of stay. 70% of colonization due to events within the NICU. Transmission varied by strain	Useful longitudinal analysis of strains with evidence of significant within-unit spread and dynamic changes in strain predominance
USA (40)	Temporal cluster of USA300 identified on PFGE and analysed with WGS and *spa* typing	17 neonates acquired MRSA in unit. 12 unique isolates with five in two clusters	WGS excluded cross-transmission in most cases, suggesting multiple and independent introduction of MRSA strains
Italy (41)	Isolates from putative outbreak in NICU and PICU	10/12 isolates ST625; 2xST8. A maximum 1.7% phylogenetic distance amongst ST625. A staff member isolated with ST625	Genetically related isolates but temporal analysis of isolates did not confirm an outbreak
Ireland (42)	Isolates from two outbreaks of MRSA ST78 in an NICU	Considerable homogeneity between 28 isolates with likely importation from abroad and involvement of healthcare worker	Highlights the importance of travel in the spread of MRSA, and the value of WGS in tracking local spread and determining the origins of clones
France (43)	Isolates from two geographically separate NICUs	41 CC isolates from Limoges and Bordeaux with difference <22 SNPs	WGS is useful to determine relatedness but also to track bacterial evolution

MSSA, meticillin-susceptibile *Staphylococcus aureus*; PFGE, pulsed-field gel electrophoresis; NICU, neonatal intensive care unit; PICU, paediatric intensive care unit; CC, clonal complex; SNP, short nucleotide polymorphism; ST, sequence type.

prevention and control strategies, as well as outbreak management, in real time [30—32].

In 2013, Price *et al*. outlined the potential of WGS, discussed some recent applications and highlighted its potential for the future [33]. The present authors undertook to update that and highlight important findings relating to the detection of outbreaks, their evolution over time, interhospital spread, and how this could potentially benefit preventative measures for MRSA in the community. The authors did not set out to cover aspects of WGS and MRSA as they relate specifically to antimicrobial resistance and global molecular epidemiology. A literature search was undertaken of articles in PubMed, Embase, the Cochrane Library and Web of Science for articles up to the end of August 2018. Search terms included MRSA, WGS, outbreaks, clinical, infection, prevention and control. In total, 588 items were found, duplicates were removed, and all titles and abstracts were reviewed for relevance (e.g. excluding those related purely to the veterinary setting). The cited studies focus on the value and relevance of WGS to MRSA in terms of prevention and control, and outbreak investigation. Studies that focused solely on clonal evolutionary trends and were not germane to infection prevention and control practitioners were excluded. What follows is a discussion of the transmission of MRSA, especially outbreak management. Studies that refer to and/or include meticillin-susceptible *S. aureus* (MSSA) are included where MRSA isolates were also included, and/or where the findings might equally apply to outbreaks or settings involving MRSA.

Young *et al*. looked at the evolutionary dynamics of *S. aureus* from carriage to disease (i.e. a patient who carried *S. aureus* in the nose and who subsequently developed BSI) [34]. Just eight mutations accompanied the transmission from carriage to infection [34]. However, during an outbreak which occurs over a matter of weeks or even months, much of the literature seems to agree that up to approximately 30 SNPs may be allowed between isolates before the isolates are considered different (i.e. transmission may have occurred between two patients if their isolates differ by fewer than 30 SNPs) [11,14]. Such conclusions are predicated on the assumption that the epidemiological findings are supportive.

Epidemiology and control

Neonatal and paediatric units

The occurrence of MRSA amongst neonates has potentially devastating consequences. Hence, many studies of WGS and MRSA have focused on neonatal or paediatric units because of the serious clinical consequences. WGS has been used to track the spread of MRSA and to assist in early intervention measures [26,35—43].

Köser *et al*. investigated a putative MRSA outbreak in the UK, and WGS revealed a distinct cluster with clear separation between outbreak and non-outbreak isolates amongst a collection of ST22 isolates [37]. When using less-discriminatory methods of typing, the extent of an outbreak may be exaggerated. In a neonatal unit outbreak involving 17 neonates, *spa* typing, PFGE and WGS were used. All 17 isolates were USA300 isolates according to PFGE. However, while five isolates were involved in recent transmission events, 12 (70.5%) represented genetically unique isolates according to WGS, and were therefore believed not to be part of the outbreak [37]. This finding is important as it suggests that there was no obvious deficiency in infection prevention measures by healthcare staff because there may have been multiple independent introductions of USA300.

Earls *et al*. investigated two protracted outbreaks (2009—2011 and 2014—2017) in the neonatal intensive care unit of an Irish hospital involving clonal complex (CC) 88-MRSA isolates belonging to *spa* types t186 and t786 [42]. Isolates were recovered from 20 separate neonates during the outbreaks, together with two isolates recovered two years apart from the same healthcare worker (HCW). WGS and subsequent wgMLST analysis revealed that both outbreaks were caused by the same CC88/ST78-MRSA-IVa strain. All the isolates formed a large cluster, exhibiting one to 71 pairwise allelic differences in a wgMLST-based minimum spanning tree (MST). The maximum distance observed between any two directly linked nodes was 32 alleles, detected between two t186 isolates which were recovered almost three years apart during the first outbreak. All other directly linked isolates exhibited one to 19 allelic differences [42]. This indicated a high degree of relatedness between all isolates within the cluster network. There were no apparent subclusters based on *spa* type, and the one direct link within the MST between *spa* type t786 isolates and *spa* type t186 isolates corresponded to an allelic difference of 18. The two t786 isolates recovered from a HCW two years apart exhibited 20 allelic differences, and differed from other t786 isolates by 10—21 and nine to 37 allelic differences, respectively, indicating the involvement of the HCW in the outbreak transmission. Unfortunately, no information on whether the HCW was persistently or transiently colonized with the CC88/ST78-MRSA-IVa strain during the two-year period was available, or if attempts to decolonize the HCW were undertaken. This study also demonstrated the spread of the ST78-MRSA-IVa strain to two other Irish hospitals. A cgMLST-based comparison with international comparator isolates showed that the outbreak strain was most likely imported from Australia, where it is among the prevalent MRSA clones.

The French national staphylococcal reference laboratory used WGS to retrospectively investigate MRSA isolates amongst four separate CCs (CC1, CC5, CC8, CC30) involved in community and hospital outbreaks, which included 41 CC5 isolates from newborns [43]. Even though the *spa* type was different, isolates from Limoges and Bordeaux, which are separated by approximately 180 km, differed by fewer than 22 SNPs, suggesting that despite the geographical difference, they could be part of the same transmission pattern [43]. The value of WGS in this setting is outlined in Table II.

Other hospital outbreaks

Studies have shown the value of WGS in teasing out some of the subtleties of general and hospital outbreak evolution and development [11,16,23,44—50]. The isolates from three separate outbreaks were studied, including one in a hospital, resulting in a total of 42 isolates; 15 of 16 isolates from a burns unit formed a single cluster, but 12 isolates from a postsurgical unit were more diverse. The authors concluded that those with fewer than eight SNPs should be considered to be related, and those with nine to 29 SNPs should be considered to be possibly unrelated [16].

Previously, the authors assessed the variability amongst MRSA isolates collected from patients and the environment in a

prospective study that involved 41 patient and environmental isolates that were ST22 [11]. The isolates were traced in terms of the geographical location and the time when they were recovered. Far more combinations of isolates (i.e. patient–patient or patient–environment) indicating potential transmission links were detected by WGS compared with conventional molecular typing using *spa*, *dru* and/or PFGE typing or a combination of all three [11]. The *dru* region is a non-coding DNA segment consisting of imperfect 40-bp variable-number tandem repeats located in the hypervariable region between *mecA* and IS431*mec* of SCC*mec* [7,11].

In a study on a Dutch oncology ward involving an asymptomatic nasal colonized HCW, WGS MLST showed similarities between MSSA and MRSA isolates involved in an outbreak, and the authors hypothesized that a fusidic-acid-resistant isolate of MSSA acquired a SCC*mec*, and subsequently caused an MRSA outbreak [46]. Miller *et al*. were concerned about the patterns of MRSA BSIs in a specific hospital in England involving a clonal variant of EMRSA-16. Isolates causing BSI between 2000 and 2001, and 2006 and 2007 were investigated [48]. The clonal variant was largely confined to that specific hospital, unlike isolates causing BSIs acquired elsewhere, and infections caused by this clone were significantly associated with increased peripheral white cell and neutrophil count, suggesting increased virulence [48]. Similarly, a retrospective review of isolates in 2004–2014 was undertaken in Switzerland to reconstruct transmission pathways [49]. Tracking the geographic locations of patients who were colonized or infected together with WGS data enabled the researchers to assess patterns of spread, which included a network of hospitals and overlapping periods of hospitalization. In one case, an outbreak lasted several months in an orthopaedic ward, but was only detected retrospectively using WGS. Looking at isolates from the same patient over time, the authors concluded that there was one SNP every 8.9 weeks or 0.016 per day [50].

Tong *et al*. (2015) used WGS to investigate the genetic diversity of ST239 MRSA isolates from patients over a three-month period in two intensive care units (ICUs) of a 1000-bed hospital in Thailand where transmission was common [22]. Phylogenetic analysis revealed a flux of distinct ST239 clades (or groups of isolates) over time in each ICU. Analysis of WGS data confirmed intra- and interward transmission events, and revealed that one patient in each ICU was the source of numerous transmission events. The mean pairwise SNP differences between the five ST239 clades identified was $\geq$197 SNPs, indicating that each clade was distinctly different.

Community and wider patterns of spread

While the focus of the hospital infection prevention and control team is to largely prevent and analyse outbreaks within the hospital, these may sometimes arise outside the hospital (i.e. from patients admitted from other hospitals or from LSRUs, as well as potentially from patients who have been abroad, especially if hospitalized there). Hence, the analysis of a wider range of isolates can inform preventative strategies and highlight the innate capacity of *S. aureus* to spread and evolve.

The ST8 USA300 MRSA clone emerged shortly after 2000, and subsequently became the leading cause of skin and soft tissue infections in the USA. The origin of USA300 in Pennsylvania and its subsequent range expansion was recently investigated in detail using genome sequences from 357 isolates from 22 states and territories and seven other countries [51]. USA300 is now common internationally. Fluit *et al*. compared one well-characterized strain from the USA with those from Europe [52]. There was a difference of 144 SNPs between the US isolate and those from Europe, the gene content showed 21 regions of difference, and the European strains were resistant to fewer antibiotics. However, the SNP data suggested a common ancestor around two decades ago [52]. This clone is an important pathogen internationally, although not as common in Europe as in the USA, but that could change with spread via ongoing international travel.

An outbreak of ST97-IVa involving 25 patients, originating from a surgical ward, over a four-year period in Denmark, a country with low prevalence of MRSA, was investigated by WGS [53]. Eighteen patients had been admitted to the surgical ward, of which 13 overlapped in terms of admission periods. Two HCWs and two patient family members were also involved in the outbreak. All except two isolates were *spa* type t267 and belonged to ST97. In this outbreak, WGS linked nine initial isolates to 16 previous isolates, resulting in 23 patients being involved, with the suggestion that a HCW with undetected carriage may have caused the outbreak [53]. The authors noted that with shorter lengths of stay, patients may not be identified as being colonized with MRSA carriers while in hospital, but on follow-up in the community after hospital discharge [53].

Modern health care requires patients to be transferred between hospitals as specialist and tertiary facilities are centralized. A comparison of EMRSA-15 within the UK and Ireland using WGS showed that the hospitals within the same referral regions had similar MRSA populations, but transmission within a hospital arose from patients having been transferred from another hospital [54]. Furthermore, frequent patient admissions to multiple hospitals results in ward-based transmission within a hospital, as detected by a study in two National Health Service hospital groups and a district general hospital in South-East London involving ST22 MRSA isolates [55].

As the age of the population increases, patients are discharged more quickly than before from acute hospitals to LSRUs, such as nursing homes. Sometimes these patients require re-admission and there is constant flux between the acute and long-stay sector. Many studies have highlighted this dynamic in terms of the acquisition and transmission of MRSA between these sectors [56–62]. Furthermore, there has been interest in the role of carriage, including enteric carriage [44,50]. In a long-term outbreak involving 1600 patients where WGS was used to determine the origin, a single clonal variant of ST228 was responsible, and this clone was more frequently recovered from the groin and rectal swabs [50].

In Singapore, where 1700 hospital patients and LSRU occupants were screened for MRSA over a six-week period, the prevalence of MRSA was lowest in acute healthcare facilities (11.8% compared with 29.95 and 20.4% for intermediate and long-term care facilities, respectively) [56]. Furthermore, LSRUs had the greatest diversity of MRSA clones. Stine *et al*. examined the transmission of MRSA from resident to resident in LSRUs using WGS. Multiple sites from residents were screened over a 12-week study [59]. Isolates from multiple body sites were usually closely related, and many residents living together often harboured closely related strains [59].

Following a protracted hospital outbreak between June 2013 and June 2016 caused by multi-drug-resistant ST1-MRSA-IV isolates belonging to *spa* type t157, a collection of 89 isolates

from the outbreak hospital, 16 other hospitals and four other healthcare facilities and the community in Ireland were investigated by WGS [61]. Fifty of the isolates, including 40 from the outbreak, exhibited high-level mupirocin resistance mediated by an *iles2*-encoding plasmid conjugative plasmid [61]. Pairwise SNVs exhibited by healthcare- and community-associated isolates indicated recent transmission of ST1-MRSA-IV within and between multiple hospitals, healthcare facilities and communities in Ireland [60]. This has implications for current MRSA prevention and control guidelines, which are very much focused on measures in the acute hospital sector. More recent studies have identified the multi-drug-resistant ST1-MRSA-IV clone as a novel CC1-MRSA-IV clone that has recently emerged in several European countries (see section on the identification of emerging MRSA clones below).

When developing guidelines, the question of what measures to take within households where there is an MRSA-positive individual often arises. Current Irish recommendations are to highlight personal standards of hygiene and cleanliness, and to minimize disruption/inconvenience in a home, as intrafamilial spread is not considered common [63]. A retrospective study of isolates collected between 2008 and 2010 in Chicago and Los Angeles found very little genetic variation amongst USA300 isolates within households, but that transmission did occur where the index patient had skin and soft tissue infection [64]. In England, where USA300 is less common than in the USA, a 12-month prospective observational study of 2283 screening and clinical isolates from 1465 patients collected between April 2012 and April 2013 from the community and hospitals found USA300 in only 24 cases (1.6%). There were also three groups of closely related isolates with a maximum genetic distance of six, 59 and nine SNPs, respectively, amongst epidemiologically linked cases [65]. The authors concluded that international travel may have played a role in the introduction of this clone into England.

Over the last decade, it has been increasingly recognized that some patients without a recent history of healthcare contact who develop MRSA may have done so via contact with livestock [i.e. livestock-associated MRSA (LAMRSA)]. This has been particularly well described in those countries with a low background prevalence of hospital MRSA, such as Denmark. Larsen *et al*. analysed human cases of LAMRSA between 2010 and 2015 in Denmark [66]. Seventeen cases of BSI, 700 cases of skin and soft tissue infection, and 76 cases of other infections due to LAMRSA were studied. Overall, 32% of the LAMRSA were from individuals with no contact with livestock. Whole-genome sequence analysis suggested that most isolates were closely related to Danish pig isolates [66]. A study of CC398, commonly LAMRSA, was assessed in human and pig isolates in Norway [67]. A human case was identified in 2009, but by the end of 2014, there were a total of 84 human cases. Epidemiological links placed these individuals in three clusters, and all farms had farm workers originating from other European countries where MRSA is more prevalent [67]. Interestingly, while the farm workers and other possible human carriers may have been non-Norwegian, none of the farms had imported pigs from abroad, and the transmission of this CC may have been by human introduction via migration rather than through the importation of pigs. The results of this study would seem to justify the Norwegian control strategy of targeting the screening of personnel before working in pig herds as part of national surveillance [65]. Contact with livestock is therefore a potential risk factor to be considered, and not just in countries with low prevalence of MRSA in acute hospitals.

National surveillance systems together with key performance indicators increasingly drive reductions in serious infections due to MRSA, particularly BSI. In the UK, a 'zero tolerance' approach has been advocated, but there may be a portion of MRSA BSIs that are not preventable. In one instance, WGS assisted in determining if an outbreak was preventable through infection prevention and control measures. A study in Cambridge, UK focused on a cluster of five MRSA BSIs between September 2011 and August 2012. The researchers used detailed epidemiological methods and WGS analysis of isolates, and found that there were varying degrees of overlap in admission to the wards of these cases [68]. A comparison of isolates indicated that each patient was infected by their own carriage isolate. Amongst four of the patients, isolates differed by between 122 and 168 cgSNPs [68]. A wider analysis of all patients with MRSA, whether colonized or infected, was undertaken, including the cases of MRSA BSI. Three of the five BSI episodes were associated with skin conditions, and two were attributable to intravascular catheters. From an analysis of the WGS data, it was concluded that these cases were not due to interpatient transmission [68]. In this setting, WGS suggested that there was no deficiency of infection prevention and control services in preventing these five cases of BSI, and that some or all of these cases of BSI were probably not preventable.

Identification of emerging MRSA clones

A study by Earls *et al*. (2019) used WGS to investigate the recent emergence of multi-drug-resistant Panton-Valentine leucocidin (PVL)-negative CC1-MRSA-IV isolates in multiple Irish hospitals and the community, and in two hospitals in the German city of Regensburg between 2016 and 2018 where it was also identified in the community [69]. Phylogenetic analysis grouped the isolates into a large clade, where no isolate differed from any other isolate by more than 130 cgSNVs. Clade isolates harboured an SCC*mec* type IVa element with a characteristic 4710 nucleotide insertion in the downstream constant segment (*dcs*) adjacent to *orfX*, and harboured the same allelic variants of the SCC*mec* genes, *ccrA2* (1350 bp) and *ccrB2* (1629 bp). Overall, clade isolates exhibited genotypic characteristics which differed comprehensively from those associated with other previously well-characterized CC1-MRSA-IV clones including Western Australia MRSA-1 and USA400. Five MRSA isolates recovered in a Romanian hospital between 2010 and 2012, and 10 CC1 MSSA isolates recovered in the same Romanian hospital between 2009 and 2012 also grouped into the novel CC1 clade. Earls *et al*. (2019) designated the multi-drug-resistant MRSA isolates as a novel European clade of CC1-MRSA-IV, and hypothesized that this clade likely recently emerged from CC1 MSSA in Romania or a neighbouring country [69]. Interestingly, isolates of this emerging European CC1-MRSA-IV clone were also recently identified in an Italian paediatric hospital [24].

Routine applications

Having data interpreted within two to three days or less will enhance infection prevention and control measures, and may

prevent the use of disruptive measures such as the closing of units or wards. Eyre *et al*. used rapid benchtop sequencing to investigate two outbreaks of *S. aureus* within five days of a positive culture result [70]. One involved 10 MRSA isolates from eight patients in an ICU, and the other involved six patients over three months in the south of England with PVL-positive MRSA. Within clusters or outbreaks, most isolates were indistinguishable, and all were within three SNVs [70]. On a broader public health front, WGS may be used to track community and hospital-acquired isolates within and between countries. A total of 308 invasive isolates collected across Europe identified predominant clones (e.g. CC5 and CC22, with the latter representing EMRSA-15 originally from the UK) [71].

In conclusion, the application of WGS has greatly expanded our knowledge of clinical and epidemiologic aspects of MRSA infection and colonization, including transmission, the commonality of clones in the community, as well as the evolution of resistant determinants. Subsequent studies will further inform our understanding of transmission patterns, and guide which interventions are most important and when they should be applied. The use of WGS in day-to-day practice will be facilitated by improvements in our capacity to interpret the data and apply it appropriately, in a timely manner. In many instances, this may confirm low level but ongoing clusters and cross-transmission of MRSA, which, if acted on, may assist in preventing larger outbreaks. The availability of WGS data may also suggest considerable patient-to-patient transmission of a single clone, or that an outbreak is due to the simultaneous emergence of different clones even if patients are temporally and geographically linked. Alternatively, an outbreak may arise from MRSA being carried into a hospital from another hospital or an LSRU.

Future priorities include agreeing parameters for deciding whether isolates are closely related or otherwise, enhanced data analysis, and ensuring that information technology systems can share WGS data between hospitals, between countries and further afield. As this field continues to mature and evolve, WGS may have a significant role in informing measures to prevent transmission through the provision of critically important molecular epidemiological information in real time.

Conflict of interest statement

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