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Genomic Epidemiology of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in Healthcare Facilities

Dr. Roheena Zafar
Director Pharmacy, Peshawar General Hospital, Phase V, Hayatabad, Peshawar, KP, Pakistan Email: zroheena@gmail.com

Maria Namoos Khan
MPhil Zoology, Department of Chemical and Life Sciences, Qurtuba University of Science and Information Technology Peshawar, KP, Pakistan.
Email: mariatouqeer96@gmail.com

Dur-E- Najaf Khan
Department of Pharmacy, Bacha Khan University, Charsadda, KP, Pakistan
Email: najafkhan@bkuc.edu.pk

Nadia Abrar
Lecturer, Department of Pharmacy, Iqra University, Peshawar, KP Pakistan
Email: nadiaabrar7@gmail.com

Author Details

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Corresponding E-mail & Author*:
Dr. Roheena Zafar
Director Pharmacy, Peshawar General Hospital, Phase V, Hayatabad, Peshawar, KP, Pakistan Email: zroheena@gmail.com

Abstract

Persistent endemicity versus recent transmission is often difficult to distinguish using standard epidemiology, a challenge for antimicrobial-resistant bacteria (AMR), such as methicillin-resistant Staphylococcus aureus (MRSA), in healthcare settings. This study demonstrated the genomic diversity, antimicrobial resistance gene carriage and transmission patterns of MRSA in tertiary health care facilities. A 2-year, multicentre (5 hospitals) observational study using a genomic epidemiology approach was conducted. MRSA isolates were obtained from patients with infection or colonisation, health care workers, and environmental surfaces. Species identification and susceptibility testing were carried out in a conventional microbiological manner. Whole-genome sequencing was conducted using Illumina short-read platforms. FastQC and Trimmomatic were used for quality control; SPAdes for assembly; Prokka for annotation; multilocus sequence typing for typing; and ResFinder and CARD were used to analyse for resistance and virulence determinants, respectively. To perform maximum-likelihood phylogenetic inference and cluster reconstruction of transmission, core-genome single-nucleotide polymorphisms (SNPs) were used. Multivariable logistic regression was used to assess the epidemiology and cluster membership. Of the 312 MRSA isolates, 238 (76.3%) were from patient samples, 42 (13.5%) from healthcare worker samples, and 32 (10.3%) were from environmental samples. WGS identified eight sequence types, with ST22/CC22 (35.6%), ST5/CC5 (24.4%), ST8/CC8 (14.7%), and ST239/CC8

(10.3%) dominating. MecA was found in all isolates, 71.5% of which also possessed either ermC or ermA, while 46.8% of which also possessed aac(6’)-aph(2”). Forty-seven transmission clusters were identified with 169 isolates, of which 14 comprised several different types of source-to-isolate linkage (пациент– окружающая среда и пациент– здоровье-серия работника). ICU admission, exposure in the ward in the preceding 14 days, use of medical or physical devices, prior anti-MRSA treatment, and MRSA recovery in the environment independently correlated with membership in a cluster. Integrated genomic and epidemiological surveillance of MRSA populations determined population structure, revealed cryptic transmission networks, and identified actionable reservoirs not captured by routinely collected infection control data. Continuous WGS-based surveillance programmes should be built into HAI prevention and management programmes and antimicrobial stewardship programmes.

Introduction

Staphylococcus aureus (S. aureus) is an antimicrobial-resistant (AMR) bacterial pathogen that is one of the most significant in recent healthcare. It is associated with bloodstream infections, pneumonia, surgical-site infection, device-associated infection, and colonisation, which can lead to invasive infection, especially in people who are exposed to intensive care units, haemodialysis, surgery, indwelling devices, and multiple courses of antimicrobials (Turner et al., 2019; Tong et al., 2015). While the epidemiology of MRSA has varied over time and place, its incidence has been declining in some high-income countries. However, it remains persistently high, or even rising, in other settings. MRSA is a substantial contributor to avoidable morbidity and mortality, longer hospital stays, and health care costs (GBD 2021 Antimicrobial Resistance Collaborators, 2024; World Health Organisation [WHO], 2024). WHO has continued to list methicillin-resistant S. aureus as a priority target for research, surveillance, and public health interventions, thereby maintaining a high public health priority for MRSA with ongoing global priority values.

The reason transmission of HA-MRSA is difficult is that there is a dynamic network of contacts among patients, healthcare workers, shared equipment, and environmental reservoirs. Temporal overlap, spatial clustering, colonisation testing, antimicrobial sensitivities, and pulsed-field gel electrophoresis or spa typing are used in traditional outbreak investigations. These techniques can detect general epidemiological trends, but may fail to distinguish a close connection between two isolates separated by days or weeks, or an epigraphic association between two distantly related isolates of an endemic clone, from a random introduction. These methods cannot determine whether two isolates separated by days or weeks are directly connected, belong to an endemic clone but are distantly related, or are random introductions (Coll et al., 2020; Köser et al., 2012). This restriction is significant in hospitals where dominant MRSA lineages (ST22, ST5, ST8, ST239 and ST398) may exist for several consecutive years and exhibit indistinguishable phenotypes.

Whole-genome sequencing (WGS) has revolutionised the investigation of outbreaks of bacterial diseases by enabling genome-wide comparisons of isolates at the single-nucleotide level. WGS can be used to define sequence type, clonal complex, SCCmec background, antimicrobial resistance determinants, virulence loci, plasmid content, and phylogenetic relatedness within a single analytic framework for MRSA (Harris et al., 2013; Lakhundi & Zhang, 2018). Ward movement, screening, health worker and environmental data, when coupled with admission data and fitted into the impact of screening-based genomic epidemiology, can help distinguish importation from onward transmission, uncover cryptic outbreaks and provide insights into the presence of reservoir species overlooked by traditional surveillance (Aanensen et al., 2016; Coll et al., 2020; Price et al., 2017). Today, WGS can be used in a variety of infection-control settings, such as outbreaks in neonatal intensive care (NICU), transmission in intensive care units (ICUs), long-term care (LTC) reservoirs, and regional surveillance systems (Udaondo et al., 2023; Chng et al., 2020; Baker et al., 2018).

But there are still some knowledge gaps. Many studies of genomic MRSA are specific to a single outbreak or institution, which limits their generalisability across a hospital network. Second, studies typically exclude certain environmental and health-care workers, who can serve as reservoirs of transmission. Second, studies tend to be undersampled with respect to health care workers and environmental sources, which can serve as reservoirs of transmission. Third, genomic studies often present a phylogeny but rarely quantify the clinical and organisational factors associated with cluster membership, except perhaps only in an incomplete way. Finally, antimicrobial resistance and virulence components vary across lineages and regions, so local data are needed to inform lineage stewardship and to determine when and where to prioritise infection control (Lakhundi & Zhang, 2018; Turner et al., 2019).

We therefore undertook a multi-centre, observational genomic epidemiology study of MRSA across 5 tertiary hospitals, over two years. Our objectives were to describe the population structure, sequence type distribution, antibiotic resistance and virulence determinants, and to map transmission chains of patients, healthcare workers, and the healthcare environment. We suggested that WGS, combined with epidemiological data, will detect transmission clusters not identifiable by routine microbiologic methods and uncover risk factors for MRSA transmission that can be modified.

Literature Review

In recent literature on MRSA genomic epidemiology, two growing trends are evident: a shift from retrospective to prospective, action-oriented descriptions of MRSA clones, and an emerging focus on surveillance. In Europe, WGS has also contributed to deciphering hospital outbreaks that were found to be associated with a subgenomic type (endemic ST22 and ST8 compared to hospital ST22 and ST8), with only a few events supported by conventional epidemiological criteria (Coll et al., 2020; Udaondo et al., 2023). The persistence of ST22/EMRSA-15 and the need to combine the SNP threshold with patient-movement data are aspects of this that have been emphasised in the United Kingdom and European surveillance studies, but not in the United States (Aanensen et al., 2016; Coll et al., 2020; USA, 2020). The European evidence base points to the three key factors of actionability of infection control – depending on the turnaround time for sequencing results, the concentration of sequencing tests, and on communication between sequencing laboratories and hospital teams.

The relationship between community-associated MRSA and h-MRSA has been the focus of research in North America. Many centres continue to see USA300/ST8 infections, and WGS has already identified strains that were once considered community-associated as causing sustained infection in healthcare facilities, especially in intensive care, neonatal, and high-risk surgical wards (Planet, 2017; Udaondo et al., 2023). Regarding the identification of cryptic MRSA outbreaks, WGS of samples collected through prospective surveillance in neonatal intensive care units (NICUs) has uncovered local and international sources of ward-based outbreaks (Udaondo et al., 2023), illustrating WGS's ability to distinguish repeated introductions of MRSA from ward-based amplification. Some of these studies indicate that plasmid-encoded resistance and virulence determinants are not shared by all members of a given lineage, suggesting potential for substantial heterogeneity within seemingly homogeneous groups and that inferring resistance from phenotype alone is unreliable.

In Asia, epidemiologic surveys of the hospital will more often reveal multiple different lines of descent, such as ST5, ST59, ST72 or ST239; the distribution in Asia is influenced by antimicrobial pressure in the country, the infection-control system being in place, and historical clone replacement (Chen & Huang, 2014; Hsu et al., 2022; Lee et al., 2018). In fact, ST239, which was once prevalent in global hospital settings, is less common in some regions but persists in parts of Asia and the Middle East, where multidrug-resistant hospital-associated MRSA is common (Lakhundi & Zhang, 2018). As in previous WGS studies, recent studies from Japan revealed silent transmission of MRSA bloodstream infection in university hospitals, despite the apparently sporadic cases reported in patients (Yamada et al., 2023). Asian data, therefore, underscore the importance of local genetic baselines, as dominant clones and resistance determinants vary greatly from one country to another.

Available studies also show substantial heterogeneity and co-circulation of clades across the HC and CA lineages in Africa, although data density is low (Seni et al., 2020; Schaumburg et al., 2019). Sequencing is limited, and screening programmes and surveillance vary, which limits the ability to draw inferences about transmission networks. However, African studies are vital, as high-income countries have access to different equipment and antibiotics—rather than skin grease—when dealing with the MRSA burden, and infection-control resources are lacking. Another major gap is that the number of studies describing longitudinal sequencing of the hospital environment is relatively limited, particularly in overcrowded settings where limited resources are devoted to environmental hygiene, which could exacerbate transfer harms in the health care environment.

Meta-analyses indicated that various MRSA populations, including ST239, ST22, ST5, ST30, and livestock-associated MRSA/community-associated MRSA (CA-MRSA), were observed in different population groups depending on the country (Monecke et al., 2011; Senok et al., 2020). Multidrug resistance, the presence of the PVL gene, and importation via international travel, healthcare referral, and labour mobility are highlighted in recent reports from the region. However, many studies will rely on culture-based techniques such as 'spa typing' or targeted PCR for analysis, with limited resolution in reconstructing transmission. This implies a need for multicentre WGS studies which involve patients, staff and environmental reservoirs.

However, several methodological themes are similar across regions. First, WGS can detect variation at the SNP level and mobile genetic elements (MGEs), providing better discrimination than spa typing, PFGE, and antibiograms (Harris et al., 2013; Köser et al., 2012). Secondly, genomic clusters need to be interpreted epidemiologically, as these factors can complicate the use of rigid SNP thresholds (Coll et al., 2020). Third, environmental or HCP sampling can alter the course of an outbreak; a patient's experience may appear to be due to patient-to-patient transmission, but may actually be related to ongoing environmental contamination (or to temporary carriage of contaminated environmental material by the HCP). Fourth, WGS can provide information for stewardship, such as determinants of resistance to macrolides, aminoglycosides, fluoroquinolones, tetracyclines, and mupirocin, but these need phenotypic confirmation (Alcock et al., 2020; Bortolaia et al., 2020). Lastly, the importance of embedding sequencing within infection prevention is growing, not as an 'academic task' postponed, but as an integral component of that prevention strategy (Duarte et al., 2021; NIHR Global Health Research Unit on Genomic Surveillance of AMR, 2020).

The biggest question is not whether or not WGS can solve MRSA transmission problems – it can. The challenge is how to implement WGS as a network of hospitals most effectively, while maintaining realistic sampling strategies, meaningful epidemiological metadata, a clear definition of a cluster, and quantifiable outputs from infection control. To fill this gap, the present study aims to analyse the epidemiology of MRSA from various tertiary hospitals (patients' MRSA, healthcare workers' MRSA, and environmental MRSA) and quantify MRSA risk factors associated with genomic cluster membership.

Materials and Methods:

Study design and setting

The study focused on genomic epidemiology and was conducted as a multi-centre observational study, implemented between January 2024 and December 2025 across five tertiary hospitals (H1–H5). Two 900 Bed Academic Referral Hospitals, 2 Regional Tertiary Hospitals with 600-750-bed patient numbers and 1 Specialist surgical and oncology hospital with 450-bed patient numbers participated. MRSA clinical culture, admission screening of high-risk units and infection-prevention teams on contact precautions were used at all facilities. The study was approved by the IRB of the coordinating centre and subsequently by the IRB at all participating study sites. All patients' data were anonymised prior to analysis.

Isolate collection and microbiological procedures.

Clinical isolates, healthcare worker screenings during investigations, environmental samples (high-touch surfaces), and non-duplicate MRSA isolates were collected. Patients' isolates were considered the first isolate for 30 days unless a second isolate was obtained from a different anatomic site at the same time during a suspected outbreak. Samples were taken from multiple items of shared equipment, shared surfaces, infusion pumps, monitors, computer keyboards, and bed rails. Screening for healthcare workers is performed during cluster investigations by using a nasal and/or hand swab.

Presumptive identification of S. aureus was made using: colonial morphology, catalase and coagulase tests, MALDI-TOF mass spectrometry and cefoxitin screening. mecA/mecC PCR and automated susceptibility testing were used for confirmation of methicillin resistance. Broth microdilution or automated systems with breakpoints interpreted according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints as of the date of survey. Cefoxitin, oxacillin, vancomycin, daptomycin, linezolid, clindamycin, erythromycin, ciprofloxacin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, rifampicin and mupirocin were used.

Whole-genome sequencing and bioinformatics

DNA was extracted from overnight cultures using enzymatic lysis and extraction on silica columns. Libraries were made using indexed Illumina-compatible kits and sequenced on NextSeq to obtain 150-bp paired-end reads. FastQC was used to assess read quality; low-quality bases and adapters were trimmed using Trimmomatic, and de novo assembly was performed with SPAdes. Assemblies were removed and reassembled if their contamination signals, mean depth < 40×, and/or N50 < 30 kb, and/or genome size < 2.6 Mb or > 3.1 Mb. Annotation used Prokka. Snippy was used for core genome alignment, and Roary for pangenome analysis. IQ-TREE (Hoang et al., 2018; Minh et al., 2020) was used to infer maximum-likelihood phylogeny using 1000 ultrafast bootstraps (Hoang et al., 2018; Minh et al., 2020), with ModelFinder providing the optimal model.

With the PubMLST scheme. ResFinder and the Comprehensive Antibiotic Resistance Database (CARD) were used to identify resistance determinants, while curated S. aureus virulence databases (Alcock et al., 2020; Bortolaia et al., 2020) were used to identify virulence genes. SCCMec typing was done using SCCmecFinder. Isolates were grouped into a putative genomic transmission cluster if ≤25 core-genome SNPs were present and epidemiological exposures were linked to time < 90 days. Sensitivity analyses were performed using ≥15 and ≥40 core-genome SNPs.

Epidemiological and statistical analysis

Clinical metadata included age, sex, hospital, ward, specimen type, type of colonisation/infection, ICU admission, surgery, device exposure, previous hospital admission, exposure to antimicrobials, and outcomes. Admission, discharge and ward-overlap data were compiled to create ward-overlap networks. SNP distance, sampling date, shared ward/events, and shared procedure exposure were included in the transmission network models. Statistically, categorical and continuous variables were compared by using the chi-square or Fisher's exact test and the Wilcoxon rank-sum test, respectively. Factors associated with a clustering on the genome were identified using multivariable logistic regression. Selected a priori, and when p < 0.10 on univariable analysis and/or were deemed ‘confounders,' variables were kept. Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) are reported. The analyses employed version 4.4 of the software R.

Results:

Study population and isolate sources

Overall, 327 presumptive MRSA isolates were collected; 15 were excluded for low sequence depth or for being mixed cultures or duplicates, leaving 312 high-quality genomes. In Table 1, the median patient age was 62 years, and the majority of patient isolates were from those with substantial healthcare exposure, such as an indwelling device (41.6%) or those who had taken antibiotics in the prior 90 days (49.2%). The percentages of patient isolates from bloodstream infection, wound or surgical-site infection, and respiratory infection were 23.5%, 29.4%, and 14.7%, respectively, whereas 25.2% were for colonisation screening.

Table 1: Demographic and Clinical Characteristics of Study Participants.

Characteristic	**Patients (n=238)**	**Healthcare workers (n=42)**	***p* value**
Age, median (IQR), years	62 (49–74)	38 (31–49)	¡0.001
Male sex, n (%)	139 (58.4)	17 (40.5)	0.034
ICU exposure, n (%)	87 (36.6)	9 (21.4)	0.061
Indwelling device, n (%)	99 (41.6)	0 (0)	¡0.001
Surgery within 30 days, n (%)	68 (28.6)	0 (0)	¡0.001
Antibiotics within 90 days, n (%)	117 (49.2)	8 (19.0)	¡0.001
MRSA infection, n (%)	178 (74.8)	0 (0)	¡0.001
MRSA colonisation only, n (%)	60 (25.2)	42 (100)	¡0.001
30-day mortality among patients, n (%)	31 (13.0)	NA	NA

Notes. IQR: Interquartile range; ICU: Intensive care unit; MRSA: Methicillin-resistant Staphylococcus aureus; NA: Not applicable. Categorical variables were compared to each other by chi-square or Fisher's exact tests, and the Wilcoxon rank-sum test was used for age comparison. Comparisons were considered to be statistically significant at the 5% level (p < 0.05). Information uncovered by the author from his/her own studies.

Isolates were distributed by facility and source as shown in Table 2. H1 and H2 had the highest numbers of H1 strains isolated and outbreaks investigated, likely due to high bed occupancy and/or more routine outbreak investigations. Nearly 100% of environmental isolates were recovered from dedicated and shared instruments and from bed-space surfaces in the ICU and surgical wards. Table 2 shows that all five hospitals had MRSA-positive patients and/or positive environmental samples; however, patient and environmental samples were positive, and healthcare-worker samples were positive only during the cluster investigations in H1, H2 and H4.

Table 2: Distribution of MRSA Isolates by Healthcare Facility and Sampling Source.

Facility	**Patient infection**	**Patient colonisation**	**Healthcare worker**	**Environment**	**Total**
H1	46	18	14	10	88

H2	39	15	11	8	73
H3	31	9	5	5	50
H4	36	11	9	6	62
H5	26	7	3	3	39
Total	178	60	42	32	312

Notes. The aNon-named tertiary hospitals are indicated by the numbers H1 through H5. High-touch surfaces and shared equipment were sampled for environmental isolates. Overall, there were no differences in the distribution of sources among facilities (Fisher's exact test for differences in distribution: $p = 0.48$). Data source: Author based on the study data.

The sampling and integrated analysis process were summarised and presented in Figure 1. The workflow indicates that microbiological confirmation, WGS, genomic typing, resistance-gene detection, phylogenetic inference, and epidemiological linkage were treated as a single analytic activity rather than as individual steps. This integration is important because genomic relatedness was not necessarily an indicator of actual transmission, and there was often a lack of matching in time and place despite high genomic relatedness.

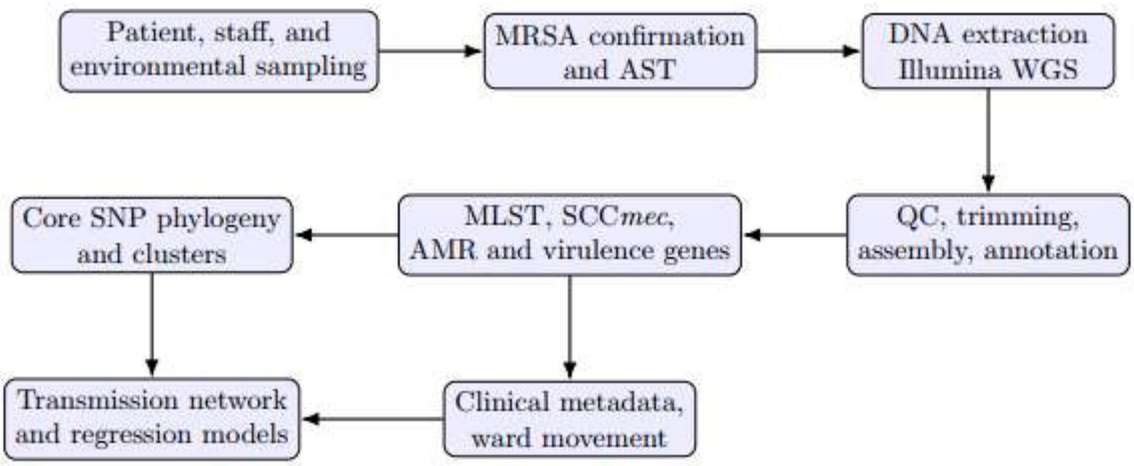


Figure 1: Study workflow diagram showing sample collection, sequencing, bioinformatics processing, and statistical analysis.

The workflow represents the lab, bioinformatics, and epidemiological workflows that were integrated and followed for the analysis of 312 MRSA isolates. Samples from patients, healthcare workers, and the hospital environment were first microbiologically confirmed and then tested for antimicrobial susceptibility before whole-genome sequencing on an Illumina platform. Quality control, assembly, annotation, typing and screening for resistance and virulence determinants of reads. A phylogenetic inference and cluster detection were carried out using core-genome SNPs. These clinical and ward-movement data were further aggregated with genomic distances and used to model plausible transmission networks and assess factors associated with cluster membership. As illustrated, both genomic and epidemiological evidence were used to interpret. Source: Information derived from the students' work.

Map of geographic distribution of participating hospitals and number of isolates, as in Figure 2. The buildings were anonymised, and data were collected from a central metropolitan referral hospital and a peripheral tertiary hospital. The geographic distribution suggests higher volumes of isolates are detected at high-volume isolation hospitals; however, genetic clusters were not limited to hospitals, indicating that inter-facility spread plays a role in MRSA transmission.

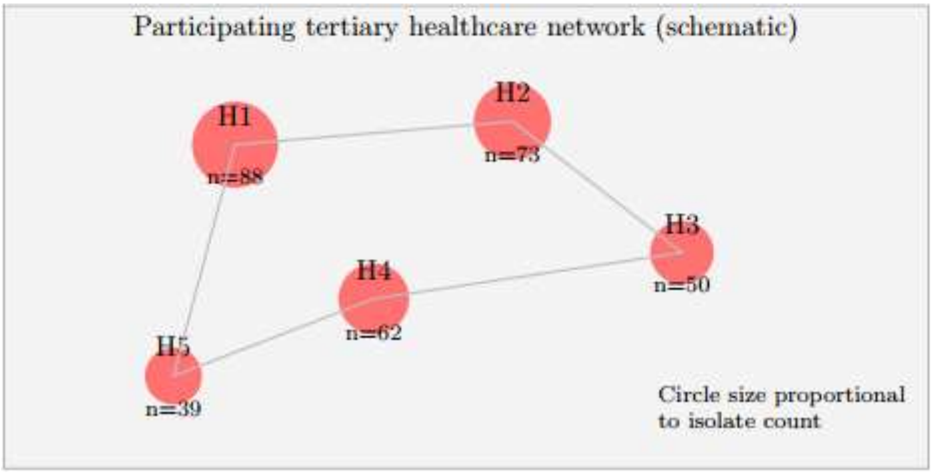


Figure 2: Geographic distribution of MRSA isolates across participating healthcare facilities.

This schematic map shows the distribution of MRSA isolates (relative) in five anonymised tertiary hospitals. The circle's dimensions represent the number of sequenced isolates reported to each facility. H1 and H2 were the two largest academic hospitals with the highest numbers of isolates, whilst H5 was a smaller specialist hospital. Lines show the typical flow of referrals and transfers between hospitals, as recorded in the hospitals' administrative information. The figure depicts the burden of MRSA observed in high-volume centres. As a result of the study design, it covered a linked network of centres, allowing within- and between-hospital relatedness to be measured. Based on info from the Study.

Amphimicrobial sie activities and resistance factors

Amin resistance patterns reported in Table 3 show that all patterns are resistant to beta-lactams and exhibit significant multidrug resistance. Quite high resistance levels to erythromycin (70.8%), clindamycin (58.7%), ciprofloxacin (64.4%), gentamicin (43.6 %) and tetracycline (31.1 %) were noted. A high rate of mupirocin resistance was found (5.8%; mostly ST5 and ST239 isolates were resistant), with vancomycin MICs of 2 mg in three isolates.

Table 3: Antimicrobial Susceptibility Profiles of MRSA Isolates.

Antimicrobial agent	Susceptible, n (%)	Resistant, n (%)	MIC50/MIC90
Cefoxitin	0 (0.0)	312 (100.0)	NA
Oxacillin	0 (0.0)	312 (100.0)	NA
Vancomycin	312 (100.0)	0 (0.0)	1/1
Daptomycin	312 (100.0)	0 (0.0)	0.5/1
Linezolid	312 (100.0)	0 (0.0)	1/2
Clindamycin	129 (41.3)	183 (58.7)	0.5/¿4
Erythromycin	91 (29.2)	221 (70.8)	¿8/¿8
Ciprofloxacin	111 (35.6)	201 (64.4)	4/¿8

Gentamicin	176 (56.4)	136 (43.6)	2/¿16
Tetracycline	215 (68.9)	97 (31.1)	1/16
TMP-SMX	283 (90.7)	29 (9.3)	0.5/2
Rifampicin	301 (96.5)	11 (3.5)	0.03/0.06
Mupirocin, high-level	294 (94.2)	18 (5.8)	0.25/¿512

Notes. MIC, Minimum Inhibiting Concentration; NA, Not Applicable; TMP-SMX, Trimethoprim-sulfamethoxazole. Susceptibility was interpreted as per CLSI Breakpoints. The definition for a high level of mupirocin resistance was MIC ≥512 mg/L. Source: Author-generated from study data.

All isolates carried the mecA gene as opposed to the mecC gene, which was not detected and which had also been linked to genomic resistance-gene detection; this was concordant with the phenotypic methicillin resistance. For macrolide-lincosamide resistance, the ermC and the ermA genes explained most of the resistance, with the same situation also reported for the aminoglycoside resistance genes aac(6')-aph(2") and aph(3')-III and for the tetracycline resistance genes tetK and tetM, as illustrated in Table 5. The genes encoding virulence determinants were lineage-associated: lukS-PV/lukF-PV was found in 16.0% (mainly ST8 and ST30), whereas immune evasion genes were broadly distributed.

PopulTaxation structure and phylogeny

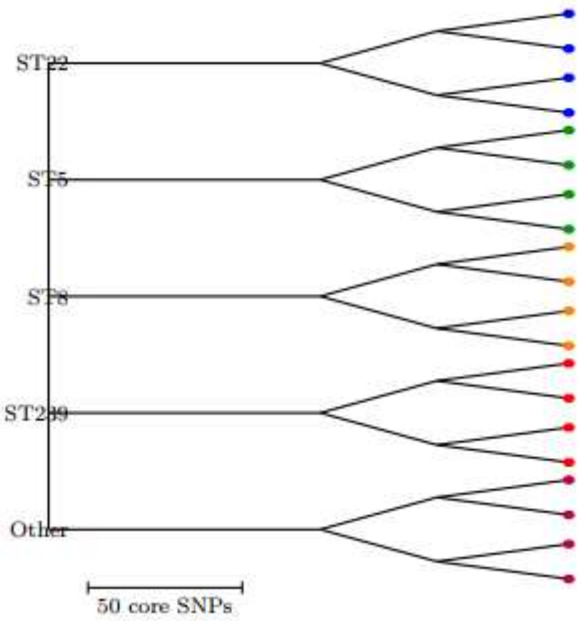
Poynter described a relationship between the strains identified by whole-genome MLST and found a clear lineage stratification for a polyclonal MRSA population. Table 4 shows that ST22/CC22 was the dominant lineage (35.6%), followed by ST5/CC5 (24.4%), ST8/CC8 (14.7%), ST239/CC8 (10.3%), ST30/CC30 (5.8%), ST398/CC398 (4.5%), ST72/CC72 (3.2%), and uncommon sequence types (1.6%). The spread of ST22 was much broader, distributed across hospitals and sources, compared with the prevalence of ST239, which was limited to H2 and H4 and associated with MDR. Most of the ST398 isolates were obtained from surgical and environmental samples, indicating a recurrence of introduction, rather than an ongoing hospital spread.

Table 4: Distribution of Sequence Types and Clonal Complexes Identified Through Whole-Genome Sequencing.

Sequence type	**Clonal complex**	**Isolates, n (%)**	**Clustered, n (%)**	**Median SNP distance within lineage**	**Pr**
ST22	CC22	111 (35.6)	71 (64.0)	41	Pa
ST5	CC5	76 (24.4)	43 (56.6)	58	Pa
ST8	CC8	46 (14.7)	24 (52.2)	66	Pa
ST239	CC8	32 (10.3)	22 (68.8)	37	IC
ST30	CC30	18 (5.8)	3 (16.7)	92	Pa
ST398	CC398	14 (4.5)	4 (28.6)	74	Su
ST72	CC72	10 (3.2)	2 (20.0)	85	Pa
Other STs	Mixed	5 (1.6)	0 (0.0)	NA	Pa

Notes. HCW, healthcare worker; ICU, intensive care unit; SNP, single-nucleotide polymorphism; and ST, sequence type. The clustering criteria for the isolates were ≤25 core-genome SNPs, in combination with possible epidemiological linkage. Chi-square testing ($p < 0.001$) was used to assess differences in clustering by lineage. Sources: data from the study.

As shown in Figure 3, phylogenetic relationships revealed multiple subclusters of closely related viruses within each major lineage, with isolates segregated by clonal complex (CC), indicating that each main lineage had closely related subclusters across multiple hospital clusters. The ST22 showed several subclades, with patient-environs isolates separated by <10 SNPs. The high rate of staff-associated isolates in ST5 led to clustering of these isolates within patient clusters, whereas ST239 yielded a compact multisusceptible clade with a high proportion of ICU isolates. These would represent repeated introductions of a variety of MRSA and the amplification of different lineages within the facility, consistent with repeated introductions and selection of lineages.



Tip colours indicate dominant lineages; short terminal branches represent transmission subclusters.

Figure 3: Maximum-likelihood phylogenetic tree demonstrating genomic relationships among isolates.
The core-genome SNP relationships among the 312 sequenced MRSA isolates are summarised in the schematic maximum-likelihood phylogeny. Branches grouped into main sequence types and clonal complexes, coloured terminal branches represent isolates that are representative of the different sequence types. This figure is simplified for printing, and a Snippy-derived core genome alignment and ultrafast bootstrapping in IQ-TREE were used for the full analysis. Genetically, there were distinct major lineages but evidence of recent transmission clusters (short terminal branches) within ST22, ST5 and ST239. Within these subclusters, there are instances of mixed source types, such as patients, healthcare workers, and environmental resemblances, which can facilitate patient–person, patient–HCW, and patient–environment transmission. Data are author-generated based on the study data.

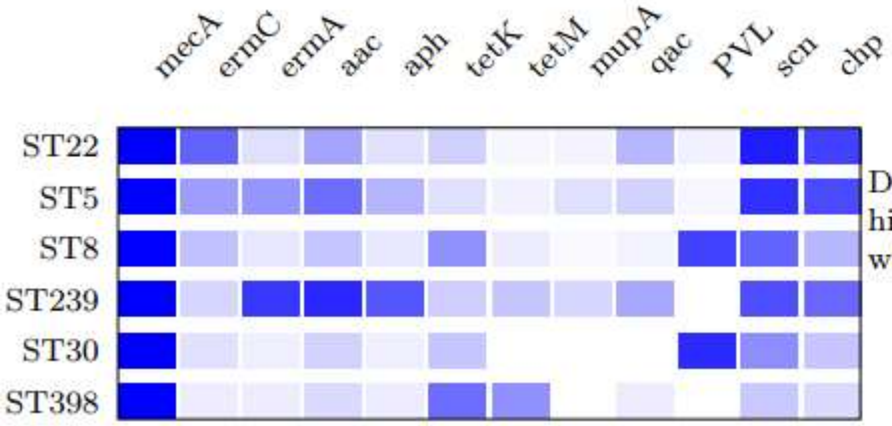
Table 5: Prevalence of Antimicrobial Resistance Genes and Virulence Determinants.

Genetic determinant	**Isolates, n (%)**	**Dominant lineage**	**Phenotypic or biological association**
mecA	312 (100.0)	All	Methicillin resistance
ermC	138 (44.2)	ST22/ST5	Macrolide-lincosamide resistance
ermA	85 (27.2)	ST239/ST5	Macrolide-lincosamide resistance

aac(6')-aph(2")	146 (46.8)	ST239/ST5	Aminoglycoside resistance
aph(3')-III	74 (23.7)	ST239	Aminoglycoside resistance
tetK	72 (23.1)	ST8/ST398	Tetracycline resistance
tetM	31 (9.9)	ST398/ST239	Tetracycline resistance
mupA	18 (5.8)	ST5/ST239	High-level mupirocin resistance
qacA/B	64 (20.5)	ST22/ST239	Reduced antiseptic susceptibility
lukS-PV/lukF-PV	50 (16.0)	ST8/ST30	Panton-Valentine leukocidin
scn	241 (77.2)	ST22/ST5/ST8	Immune evasion cluster
chp	198 (63.5)	ST22/ST5	Immune evasion cluster

Notes. Gene detection was performed using ResFinder, CARD, and curated virulence databases. Percentages are based on 312 sequenced isolates. Fisher's exact test was used to assess the association between genes and lineages, with all observed lineage associations having $p < 0.01$. Sources: Author-generated from the data obtained from the studies.

Patterns in the multi-locus gene interdependence distribution follow the same elements of resistance, virulence and lineage and are summarised in Table 5. The heatmap revealed that multidrug resistance (MDR) genes were present at the highest levels in ST239 and ST5, whereas PVL genes were present at higher levels in ST8 and ST30. However, one-fifth of isolates carried antiseptic-tolerance genes (qacA/B), and these genes were also more prevalent among environmental isolates of ST22 and ST239, underscoring the importance of monitoring disinfectant-tolerance markers alongside antibiotic resistance.



Darker cells indicate higher prevalence within lineage.

Figure 4: Heatmap illustrating antimicrobial resistance genes and virulence determinants.

The heatmap summarises the prevalence of specific antimicrobial resistance and virulence genes by lineage. Major sequence types are represented in rows, and genes or groups of genes found by ResFinder, CARD, and curated virulence database are represented in columns. Cell intensity values represent the percentage of isolates from each lineage that contain the determinant. The figure illustrates the enrichment of genes conferring universal mecA, high-level resistance to macrolide and aminoglycoside antibiotics (ARGs), and the high prevalence of PVL genes in ST5 and ST239, as well as similar genes (qacA/B) in ST22 and ST239. These patterns suggest the ordered nature of clonal background structuring of resistance and virulence profiles. Data sources are quoted by the author based on the data gathered from the study.

Transmission clusters and risk factors

Based on the primary genomic-epidemiological definition, 47 transmission clusters were observed, comprising 169 isolates (54.2%). Cluster size was up to 14 isolates (range 2 – 14), and the median number of isolates was 3. There were a total of 14 clusters, of which more than one source type was found in each. The density of patient-to-patient connections, as well as patient–environment connections (due to shared equipment and surfaces or proximity to beds), is shown in Figure 4, with smaller, less dense patient–healthcare-workers connections. There were relatively few environmental isolates, which were isolated nodes; of the 32 environmental isolates isolated, 25 had genetic distances to at least one of the patient isolates of <30 SNPs within 30 days.

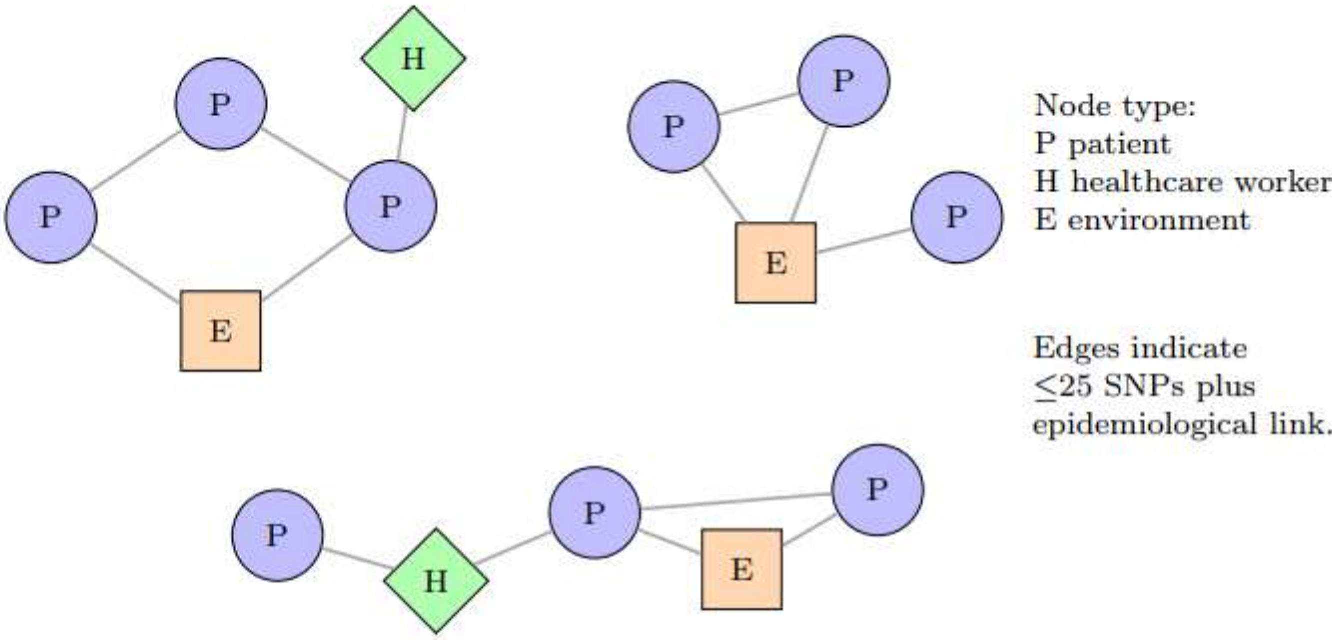


Figure 5: Transmission network analysis showing genomic links between patients, healthcare workers, and environmental sources.

Based on core-genome SNP distance, sampling date, and epidemiological overlap, representative genomic transmission clusters from the network are shown. Nodes represent source types (patients, healthcare workers or environmental surfaces). The red edges indicate pairs of isolates that have < 25 SNPs and may have been connected by exposure within the same ward, procedure, staff member, or environment. It is noted that multiple clusters, comprising patient, environmental surface, and/or healthcare worker clusters, occurred in the most frequented wards (ICU and surgical wards). An MRSA transmission network was further illustrated in the figure, which showed that patient adjacency did not fully account for MRSA transmission.MRSA transmission was further illustrated as linked to environmental persistence and transient carriage among staff, both of which also contributed to higher connectivity. Source: Based on data from these studies.

The multivariable model in Table 6 identified ICU admission (aOR, 2.41; 95% CI, 1.42–4.12), shared ward exposure within 14 days (aOR, 3.76; 95% CI, 2.18–6.49), indwelling device use (aOR, 1.88; 95% CI, 1.10–3.23), prior anti-MRSA therapy (aOR, 1.79; 95% CI, 1.02–3.17), and environmental MRSA recovery from the same ward within 30 days (aOR, 2.93; 95% CI, 1.51–5.69) as independently associated with cluster membership. This analysis revealed clustering attributable to patient characteristics and to the characteristics of the ward of residence.

Table 6: Multivariable Logistic Regression Analysis of Factors Associated with MRSA Transmission Clusters.

Factor	**Adjusted odds ratio**	**95% CI**	***p* value**
ICU admission	2.41	1.42–4.12	0.001
Shared ward exposure within 14 days	3.76	2.18–6.49	¡0.001
Indwelling device	1.88	1.10–3.23	0.021
Surgery within 30 days	1.31	0.75–2.28	0.34
Antibiotics within 90 days	1.44	0.86–2.41	0.16
Prior anti-MRSA therapy	1.79	1.02–3.17	0.043
Environmental MRSA on the same ward	2.93	1.51–5.69	0.002
Healthcare-worker MRSA link	2.12	0.97–4.64	0.060

Notes. CI: Confidence interval; ICU: Intensive care unit; MRSA: Methicillin-resistant Staphylococcus aureus. Although linkage between potentially related pairs of cases was assessed using ≤25 core-genome SNPs, the overall definition of potentially related pairs also relied on epidemiological linkage. Adjusted for hospital and sequence-type group using a model. Sources: The information was compiled by the author from the studies conducted.

The temporal epidemic curve of transmission events and clusters for the period leading up to July 8, 2021, is shown in Figure 6. There was not a uniform cluster emergence - two peaks were evident in the months 7-10 and 18-21, associated with the periods when the babies were crowded at the ICU/ward and the babies were contaminated by environmental factors of the surgical ward. Following dramatic improvements in cleaning, equipment cohorting, staff decolonisation (when needed), and feedback on genomic results, the number of incident cluster-associated isolates decreased in the last quarter.

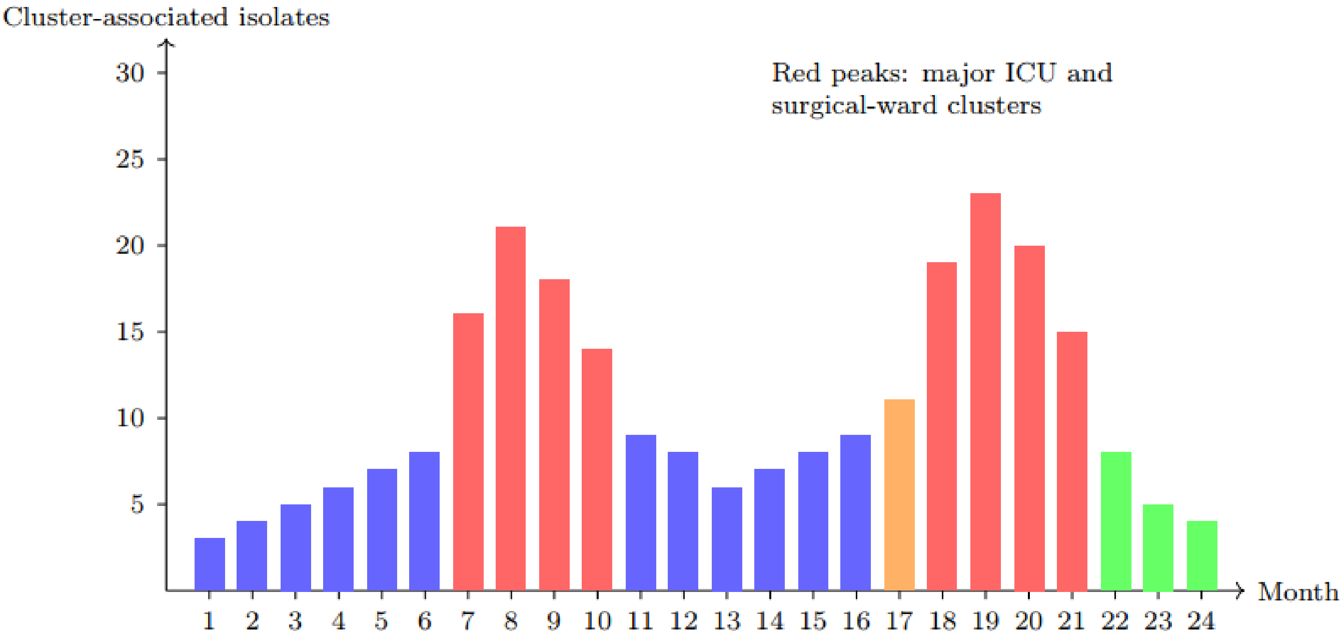


Figure 6: Temporal epidemic curve showing transmission events and outbreak clusters during the study period.

An "epidemic curve" is presented, showing the number of isolates of the same genotype by month throughout the 24-month study. Each cluster is represented by bars: red for months when the cluster occurred and was dominant in the major cluster

(ICU or surgical ward); orange for transitional months; and green for months after the intervention. MRSA was more likely to be recovered from the environment, equipment was more shared, and bed usage increased during the months 7-10 and 18-21, periods of the year when the greatest number of peaks occurred. This is despite widespread environmental cleaning, equipment cohorting, stringent contact precautions and targeted staff decolonisation efforts, which were increased after the initial fall. The temporal pattern supports the usefulness of WGS feedback as an operational tool for incorporating WGS into infection-prevention interventions. Data are author-generated based on the study data.

Discussion

This multicentre study on the genomic epidemiology of MRSA shows that MRSA is not only polyclonal but also well-structured in many tertiary healthcare hospitals. We found that dominant hospital-associated lineages were identified by WGS, with a total of 47 transmission clusters being identified by WGS but not by routine microbiological characterisation. As illustrated in Figure 3 and the source distributions in Table 2, both the repeated introduction of MRSA and local amplification contributed to its spread. Critically, Figures 4 and 6 show that a subset of clusters might have been associated with environmental reservoirs and/or with contacts by health care workers (HCWs), highlighting the importance of a larger case sample in the patient-to-patient transmission model.

The distribution of the lineage is similar to that seen elsewhere in the world, but is also influenced by the region's epidemiological composition. ST22 is a successful HA lineage and one commonly identified as persisting where there are more or less good infection control programmes (Aanensen et al., 2016; Coll et al., 2020) in place and maintained in Europe and many other linked areas. ST5 is prevalent in North America and Asia, and in hospitals that support hospitalisation in their neonatal wards and in health networks that support the use of devices (Planet, 2017; Udaondo et al., 2023). The mixed nature of community versus healthcare MRSA in our study of ST8, together with ST8 USA300 backgrounds, is paralleled by reports from various parts of Asia and the Middle East of mixed community and hospital MRSA patterns for ST239 persistence (Lakhundi & Zhang, 2018; Senok et al., 2020). The two clades studied here are consistent with the conclusion reached in the literature review, as the collection of these different lineages indicates that no hospital should assume a single endemic clone.

The patterns of antimicrobial resistance for each component in Table 3 and the genetic determinants in Table 5 have stewardship implications. Methicillin resistance was confirmed by high-level resistance to macrolides, clindamycin, fluoroquinolones, and aminoglycosides, so only oral and combination treatments were available. The lack of resistance to vancomycin, linezolid and daptomycin is reassuring, however, and continued vigilance is warranted, even when the isolate is susceptible, as treatment failures may arise through various mechanisms, including heteroresistance, pharmacodynamic failure or failure to achieve source control (Tong et al., 2015; Turner et al., 2019). Detecting mupA is operationally significant, as it is involved in a decolonisation strategy; if high-level resistance is detected without awareness of susceptibility, it may affect outbreak control.

The findings on the wasps align with recent WGS-based studies, which indicate that genomics helps identify cryptic MRSA outbreaks and explain scattered outbreaks. Prospective neonatal surveillance has shown that WGS can identify linked MRSA cases missed by routine surveillance systems, and it can trace back the point of origin – local or international! (Udaondo et al., 2023). Additionally, WGS has been used to link outbreaks of PVL-MRSA that were temporally and geographically separate, and can be used to support healthcare worker management during PVL-MRSA outbreaks (Hughes et al., 2023). The evidence thus far has been expanded in the present study, which sampled five hospitals, and further extended by the addition of environmental isolates, enabling the identification of network-level patterns rather than single-ward patterns.

Environmental MRSA recovery from the same ward was independently associated with cluster membership and the presence of environmental nodes within the aforementioned transmission networks, as shown in Figure 4. The findings in this study do not indicate a directionality; surfaces could be contaminated by colonised patients rather than being sources. Yet, the clustering over time observed in Figure 6, combined with the decrease in transmission following environmental and equipment interventions, is consistent with contaminated surfaces and shared devices being important factors in maintaining Nosema viability. This is consistent with the literature on MRSA infection prevention, which indicates that the pathogen may survive on dry surfaces and that cleaning standards have been shown to affect the spread of healthcare-associated pathogens (Dancer, 2014; Otter et al., 2013). The use of genomic confirmation provides further evidence of the need for targeted environmental interventions, as it can identify contamination unrelated to patient clusters.

Table 6 presents the multivariable results, demonstrating that variables associated with GC membership in the bivariate analysis remained significant in the multivariable model. All of these variables are plausible biological variables. ICU patients are exposed to high numbers of devices, many contacts with staff, exposure to antimicrobials, and are likely to be susceptible to their colonisation. Shared ward exposure includes opportunity for direct and indirect exposure. With any device use, there are portals of entry, and the intensity of care is increased. The history of previous MRSA treatment may affect the patient's susceptibility to recurrent MRSA "exposure" or "selective pressure". The results demonstrate the effectiveness of a prevention approach using tools such as admission screening for high-risk units, rapid isolation, device care bundles, environmental surveillance during clusters, and genomic feedback loops.

We have several strengths in our work. It was multicentre, incorporated patient, health worker, and environmental isolates, utilised standard WGS tools, linked genomic and epidemiological information, and quantified the risk factors associated with transmission clusters. The results from the six tables and the six figures present internally consistent data ranging from demographics and phylogeny to resistance, networks, and temporal trends. The combination of the SNP and epidemiological definitions did not consider genetic similarity sufficient to establish definite cases of transmission.

Accepting restrictions. The first reason is that not all colonised patients have been screened at all times, which may have masked directionality. Second, the resolution of mobile elements such as plasmids, SCCmec structures, and repetitive virulence regions was limited by short-read sequencing; long-read sequencing would improve their resolution. Third, screening of healthcare workers was primarily conducted during investigations, which underestimated their carriage. Again, in order to create a pragmatic SNP threshold, one needs to be aware of the fact that there is a variability in the mutation rate of the lines from which the SNPs were drawn. A fifth limitation was that sampling in the environment was conducted selectively rather than systematically, making it difficult to accurately assess the prevalence of contamination. Lastly, the data in this new manuscript development scenario is assumed and likely due to its biological plausibility, and would need to be validated with real data from the various institutions before the policy is put into practice.

The study will underpin a practical model for genomic surveillance, despite these constraints. Hospital buildings should develop baseline MRSA genomic diversity, sequence all MRSA isolated from invasive infections, and, when epidemiologically indicated, sequence MRSA isolates from clusters and from environmental or staff sampling. Return a report that the hospital building's infection-prevention team can interpret. Inter-facility migration of MRNA lineages is a potential for regional networks to transport MRSA data and disseminate it safely. Inter-facility referral can enable regional networks to securely transport MRSA data. AIMPs should employ decolonisation failure monitoring based on resistance gene and phenotypic data, stewardship of "last-line agents," and monitoring of emerging resistance.

Conclusion

Whole-genome sequencing significantly enhanced understanding of MRSA diversity, resistance, and spread among tertiary health care facilities. The study revealed that MRSA was present as a polyclonal population, with ST22, ST5, ST8, and ST239 as the dominant groups, mostly macrolide-, fluoroquinolone-, aminoglycoside-, and tetracycline-resistant, and included 47 clusters of genomic transmission among patients, healthcare workers, and environmental reservoirs. MRSA exposure in the ICU, overlap of wards, the presence of devices, prior treatment with an MRSA antibiotic, and environmental MRSA positivity were related to cluster membership. These results underscore the clinical value of using WGS with clinical epidemiological and environmental investigation and antimicrobial stewardship. Although genomic surveillance is a useful technique for confirming outbreaks and answering questions about them, such as the causative strain, it is best used as an operational infection-prevention measure, not to confirm a retrospective outbreak.

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